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IN VITRO REGENERATION OF SWEET POTATO (*IPOMOEA BATATAS* (L.) LAM.) CONVOLVULACEAE, FROM LEAF AND PETIOLE EXPLANTS

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ABSTRACT: Biotic stresses are the major constraints that result in total loss of sweet potato (*Ipomoea batatas* (L.) Lam.) yield. The establishment of *in vitro* regeneration protocol for this plant is essential to improve it through tissue culture and genetic engineering. The objective of this study was to establish *in vitro* regeneration protocol for sweet potato varieties Beletech and Awassa-83 using leaf and petiole explants. MS medium containing different concentrations of 2,4-D and kinetin were used to evaluate their effect on callus induction whereas different concentrations of BAP were used to evaluate its effect on shoot regeneration from callus. BAP alone or in combination with GA_3 was used to evaluate their effect on shoot multiplication whereas IBA was evaluated for its effect on rooting. The highest percentage of callus induction (93%) was obtained from petiole explants of both varieties on MS medium containing 0.05 mg/l 2,4-D in combination with 0.5 mg/l kinetin. The highest percentage of shoot regeneration from leaf explants was obtained on growth regulators-free MS medium while the best regeneration from petiole explants was obtained on MS medium supplemented with 1.0 mg/l BAP in both varieties. The highest number of shoots per node (2.40 ± 0.11) was obtained on MS medium containing 1.0 mg/l BAP from shoots originally obtained from callus of leaf explants of Awassa-83. All shoots cultured on MS media containing different concentrations of IBA and the growth regulators-free medium resulted in 100% rooting. After one month of acclimatization, more than 90% of plantlets survived.

Key words/phrases: Callus, Growth regulators, Shoot regeneration, Sweet potato.

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is a gamopetalous dicot crop plant that belongs to the family Convolvulaceae. It is ranked the seventh most important crop in the world (Mmasa and Msuya, 2012). It is a tuber-bearing species and an economically important crop in tropics, sub-tropics and warm temperate regions (Sihachakr *et al*., 1997). It can adapt to a wide

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range of environmental conditions and can grow on marginal areas with poor soils of limited fertility and low moisture.

The productivity of sweet potato is adversely affected by biotic stresses including insect pests, fungal and viral infections. It is highly susceptible to viral diseases, which causes extensive yield reduction of about 80% to total loss (Karyeija *et al*., 2000; Odame *et al*., 2002). Conventional breeding efforts and genetic improvement in sweet potato have been seriously limited by difficulties in sexual crosses mainly due to self incompatibility and male sterility within species, hexaploid nature, dichogamy, seed dormancy, abnormal seed and seedling development (Martin, 1970; Sihachakr and Ducreux, 1993).

Tissue culture techniques have opened a new frontier in agricultural science by addressing food security through biotechnological methods for genetic improvement of sweet potato. The production of somaclonal variants, application of genetic transformation and somatic hybridization requires reliable *in vitro* regeneration protocol. However, each variety requires its own regeneration protocol (Gonzalez *et al*., 1999; El Far *et al*., 2009). Therefore, developing *in vitro* regeneration protocol for each variety is a prerequisite to improve this crop using *in vitro* screening and genetic engineering procedures. Success of any genetic transformation depends largely upon the regeneration capability of the target explants (Chugh and Khurana, 2003). Sweet potato is one of recalcitrant species to *in vitro* regeneration and this recalcitrance has been a bottleneck for the application of genetic engineering in this crop (Liu *et al*., 2001). Plant regeneration protocols through organogenesis and somatic embryogenesis have been developed for few genotypes of sweet potato using different explants such as meristem, leaf, stems, petioles, storage roots, anthers and ovaries (Gong *et al*., 1998). Despite many efforts, the underlying problems in these procedures are low frequency of regeneration, long periods of culture and the need for changes of media frequently (Gosukonda *et al*., 1995). To the best of our knowledge, *in vitro* regeneration protocol has not been developed for any sweet potato variety in Ethiopia. Hence, the objective of this study was to develop *in vitro* regeneration protocol for two farmers-preferred varieties of sweet potato in Ethiopia using leaf and petiole explants.

MATERIALS AND METHODS

Plant material

Young leaf and petiole explants were obtained from about six-week-old *in vitro* grown shoots of Beletech and Awassa-83 sweet potato varieties that were derived from meristem culture. The shoot cultures were maintained by subculturing the shoots every four weeks on fresh MS (Murashige and Skoog, 1962) medium containing 1.0 mg/l 6-benzylaminopurine (BAP) supplemented with 3% sucrose and 7 g/l agar. The medium was adjusted to pH 5.8 and autoclaved at 121°C for 15 min. The cultures were maintained at 27 ± 1°C under white fluorescent light of 40 $\mu molm^{-2}s^{-1}$ light intensity and 16 h photoperiod. Unless and otherwise indicated, this was the culture condition used throughout all the experiments.

Callus induction and shoot regeneration

For callus induction, young leaf and petiole explants were excised from six-week-old *in vitro* grown shoots of Beletech and Awassa-83 sweet potato varieties and cultured on MS medium containing different concentrations of dichlorophenoxyacetic acid (2,4-D) (0.0, 0.05, 0.1, 0.5 mg/l) in combination with kinetin (0.0, 0.5, 1.0, 1.5, 2.0 mg/l). After autoclaving, 25 ml of medium was dispensed into 90 mm diameter sterile Petri dishes. The explants were cultured in such a way that the abaxial surface of the leaf explants were contacted with the medium and petioles were cut into about 2.0 cm long segments before culturing. Eight explants were cultured per Petri dish and a total of five Petri dishes per treatment were used as replication.

After three weeks of leaf and petiole culture, the calli obtained from these explants were transferred to shoot regeneration medium. The shoot regeneration medium was MS medium containing different concentrations of BAP (0.0, 0.5, 1.0, 2.0 mg/l). Eight explants were cultured per Petri dish and a total of five Petri dishes per treatment were used as replication.

Shoot multiplication

Stem segments with three nodes from shoots obtained from regeneration experiment were cultured horizontally on MS medium containing different concentrations of BAP alone (0.0, 0.5, 1.0, 1.5, 2.0 mg/l) or in combination with gibberellic acid (GA_3) (0.0, 0.5, 1.0, 2.0 mg/l) in Magenta GA-7 culture vessels containing 60 ml of the medium for multiplication. Five stem segments were cultured per culture vessel and three culture vessels per treatment were used as replication. After six weeks, the number of shoots

produced per node and shoot height was recorded.

Rooting and acclimatization

About 3.5 cm long shoots from multiplication experiment were cultured on MS medium containing different concentrations of indole-3-buryric acid (IBA) (0.0, 0.1, 0.5 mg/l). The shoots were excised and cultured in Magenta GA-7 culture vessels containing 60 ml medium. Five shoots were cultured per Magenta culture vessel and three vessels per treatment were used as replication. After four weeks, about 9.0 cm plantlets were removed from the Magenta GA-7 culture vessels and agar was washed under running tap water. After root length and root number per plantlet were recorded, the plantlets were transferred to pots containing red soil, sand and compost in the ratio of 1:2:1 respectively, in glasshouse. The pots were covered with transparent plastic bags and watered every day. The plastic covers were removed partially after a week and completely removed after two weeks. The number of survived plants was recorded. A total of 120 plantlets were planted in glasshouse for acclimatization.

Statistical analyses

The data were analyzed using SPSS version 16.0 software. The mean separation method (LSD) was used to compare means between treatments. Mean homogeneity analysis was carried out using Tukey's B homogeneity test. For all experiments, a probability level of $p \leq 0.05$ was considered as statistically significant.

RESULTS

Callus induction and shoot regeneration

After three weeks of culture of leaf and petiole explants, callus was induced from leaf explants of Beletech and Awassa-83 varieties (Fig. 1A, 1B, 2A and 2B). The dark red coloured petiole explants of Beletech were changed to yellowish callus. The rate of callus induction was significantly different among different concentrations of growth regulators, explants and varieties. The percentage of callus induction was influenced by the concentrations of 2,4-D and kinetin (Table 1). More callus induction was observed at the cut surface of both the leaf and petiole explants. The highest percentage of callus induction was obtained from explants cultured on medium containing 0.05 mg/l 2,4-D in combination with 0.5 mg/l kinetin for both explants and varieties. On this medium, 90% of petiole and 83% of leaf explants of variety Beletech resulted in callus induction. Similarly, callus induction was observed from 93% of petiole and 87% of leaf explants in the MS medium

that received the same 2,4-D and kinetin treatment for the variety Awassa-83. No callus induction was observed on growth regulators-free medium.

Table 1. Percentage of calli induced from petiole and leaf explants of Beletech and Awassa-83 sweet potato varieties, values given as mean % ± SE.

Concentrations of PGRs (mg/l)		**Percentage of induced calli**			
		Beletech		**Awassa-83**	
		Petiole	**Leaf**	**Petiole**	**Leaf**
2, 4-D	Kinetin				
0.00	0.00	0.00±0.00[l]	0.00±0.00[l]	0.00±0.00[m]	0.00±0.00[l]
0.05	0.50	90±0.00[a]	83±0.00[a]	93±0.00[a]	87±0.00[a]
0.05	1.00	77±0.00[b]	67±0.00[c]	77±0.035[c]	63±0.00[c]
0.05	1.50	63±0.00[c]	60±0.00[d]	73±0.00[d]	60±0.00[d]
0.05	2.00	53±0.00[d]	50±0.00[e]	57±0.00[e]	53±0.00[e]
0.10	0.50	47±0.00[e]	47±0.00[f]	50±0.00[f]	53±0.00[e]
0.10	1.00	43±0.00[f]	40±0.00[g]	43±0.00[g]	47±0.00[f]
0.10	1.50	40±0.00[g]	33±0.00[i]	37±0.00[i]	43±0.00[g]
0.10	2.00	33±0.00[h]	30±0.00[j]	33±0.00[j]	40±0.00[h]
0.50	0.50	77±0.00[b]	73±0.00[b]	83±0.00[b]	80±0.00[b]
0.50	1.00	33±0.00[i]	37±0.00[h]	40±0.00[h]	37±0.00[i]
0.50	1.50	30±0.00[j]	30±0.00[j]	27±0.00[k]	30±0.00[j]
0.50	2.00	23±0.00[k]	20±0.00[k]	17±0.00[l]	17±0.00[k]

Means in each column followed by the same letter (a-m) are not significantly different at 5% probability level.
2,4-D - dichlorophenoxyacetic acid

The frequency of shoot regeneration using different concentrations of BAP was influenced by 2,4-D and kinetin used in callus induction medium. Shoot regeneration was observed only on those calli induced on MS medium containing 0.05 mg/l 2,4-D in combination with kinetin (0.5 1.0, 1.5 mg/l) (Fig. 1C, 1D, 2C and 2D). The highest percentage of shoot regeneration from leaf explants was obtained on growth regulators-free MS medium after four weeks of culture (Table 2). On this medium, 80% and 77% of shoot regeneration was obtained from calli derived from leaf explants of Beletech and Awassa-83, respectively. However, the highest percentage of shoot regeneration from petiole explants (70% and 71%) was obtained on MS medium containing 1.0 mg/l BAP from Beletech and Awassa-83 explants, respectively (Table 2).

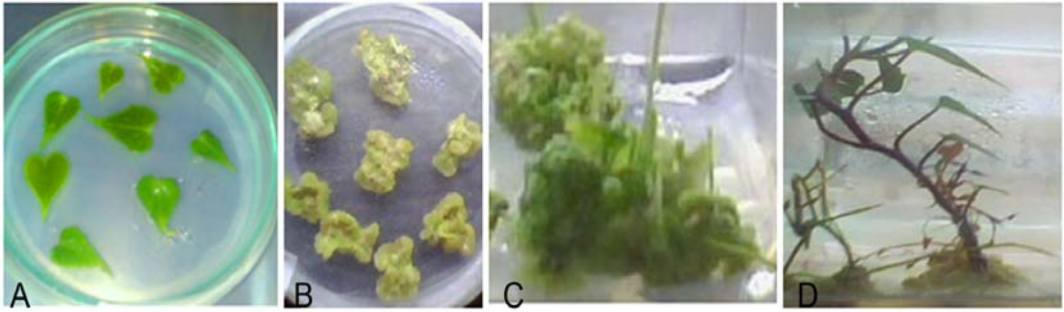

Fig. 1. Shoot regeneration from leaf explants of Beletech variety. (A) Leaf explants cultured on MS medium containing 0.05 mg/l 2,4-D in combination with 0.5 mg/l kinetin; (B) Calli induced from leaf explant after three weeks of culture; (C) Shoot regeneration from six-week-old calli on growth regulators-free MS medium; (D) Three- month-old regenerated shoots on medium without growth regulators.

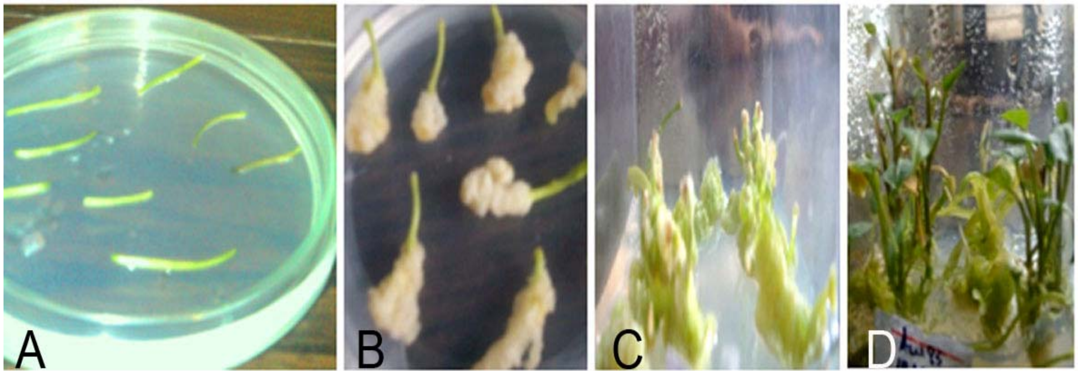

Fig. 2. Shoot regeneration from petiole explants of Awassa-83 variety. (A) Petiole explants cultured on MS medium containing 0.05 mg/l 2,4-D in combination with 0.5 mg/l kinetin; (B) Calli induced from petiole explants after three weeks of culture; (C) Shoot regeneration from six-week-old calli on MS medium containing 1.0 mg/l BAP; (D) Three-month-old regenerated shoots on MS medium containing 1.0 mg/l BAP.

Table 2. Percentage of regenerated shoots from leaf and petiole calli of Beletech and Awassa-83 sweet potato varieties on MS medium containing different concentrations of BAP.

Variety	Explant	Callus induction PGRs (mg/l)		BAP (mg/l)	Shoot regeneration (%)
		2,4-D	Kinetin		
Beletech	Petiole	0.05	0.5	0.00	30^{h}
				0.50	56^{d}
				1.0	70^{a}
				2.0	0^{j}
		0.05	1.0	0.00	35^{g}
				0.5	52^{e}
				1.0	65^{b}
				2.0	0^{j}
		0.05	1.50	0.00	21^{i}
				0.5	42^{f}
				1.0	58^{c}
				2.0	0^{j}
	Leaf	0.05	0.5	0.00	80^{a}
				0.5	68^{b}
				1.0	32^{f}
				2.0	0^{g}
		0.05	1.0	0.00	60^{c}
				0.5	40^{d}
				1.0	0^{g}
				2.0	0^{g}
		0.05	1.50	0.00	39^{e}
				0.5	0^{g}
				1.0	0^{g}
				2.0	0^{g}
Awassa-83	Petiole	0.05	0.5	0.00	35^{e}
				0.5	43^{d}
				1.0	71^{a}
				2.0	0^{f}
		0.05	1.0	0.00	52^{c}
				0.5	65^{b}
				1.0	0^{f}
				2.0	0^{f}
				0.00	0^{f}
		0.05	1.50	0.5	0^{f}
				1.0	0^{f}
				2.0	0^{f}
	Leaf	0.05	0.5	0.00	77^{a}
				0.5	62^{b}
				1.0	46^{c}
				2.0	0^{d}
		0.05	1.0	0.00	42^{cd}
				0.5	42^{cd}
				1.0	0^{d}
				2.0	0^{d}
		0.05	1.50	0.00	0^{d}
				0.5	0^{d}
				1.0	0^{d}
				2.0	0^{d}

Percentages followed by the same letter (a-j for Beletech petiole, a-g for Beletech leaf, a-f for Awassa-83 petiole and a-d for Awassa-83 leaf) are not significantly different at 5% probability level.

There was no significant difference in the number of shoots per callus on the medium containing 0.5 or 1.0 mg/l BAP, and growth regulators-free medium. No shoots were regenerated from calli cultured on 2.0 mg/l BAP (Table 3). The longest shoot (9.1 ± 0.31 cm) was obtained from calli derived from petiole explants of Beletech on growth regulators-free MS medium (Fig. 3).

Table 3. Mean number of shoots per explant at different concentrations of BAP from petiole and leaf calli of Beletech and Awassa-83 sweet potato varieties, values given as mean ± SE.

BAP (mg/l)	**Mean number of shoots per explant**			
	Beletech		**Awassa-83**	
	Petiole	**Leaf**	**Petiole**	**Leaf**
0.00	1.20 ± 0.20^{a}	1.50±0.14^{a}	1.00±0.16^{a}	1.17±0.17°
0.50	1.40 ±0.16^{a}	1.50±0.19^{a}	1.33±0.21^{a}	1.29±0.18^{a}
1.00	1.47 ±0.13^{a}	1.00±0.00^{a}	1.60±0.00^{a}	1.36±0.15^{a}
2.00	0.00 ±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}

Means in each column followed by the same letter (a-b) are not significantly different at 5% probability level.

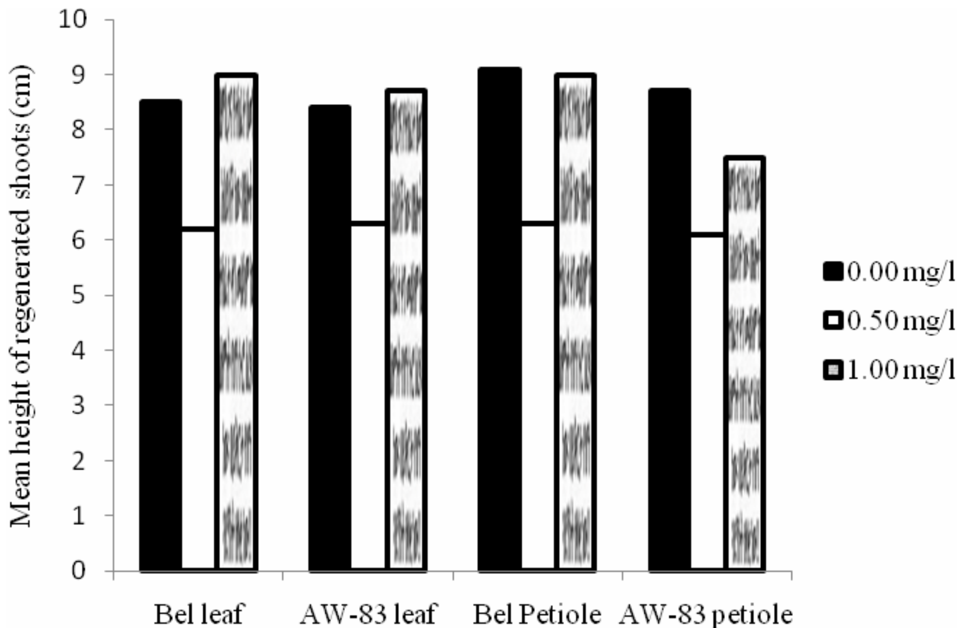

Fig. 3. A graph showing mean height of shoots regenerated from petiole and leaf explants of Beletech and Awassa-83 sweet potato varieties on MS medium containing different concentrations of BAP. Bel= Beletech, Aw-83 = Awassa-83

Shoot multiplication

The highest number of shoots per node (2.40 ± 0.11) was obtained on MS medium containing 1.0 mg/l BAP from shoots originally obtained from callus of leaf explants of Awassa-83 (Table 4). A medium containing 1.0 mg/l BAP in combination with 0.5 mg/l GA_3 resulted in a maximum mean shoot number of 1.70 ± 0.15 per explant from petiole explants of the two varieties (Table 4).

Table 4. Mean number of shoots per node from shoots regenerated from petiole and leaf calli of Beletech and Awassa-83 on MS medium at different concentrations of BAP alone or in combination with GA_3, values given as mean ± SE.

BAP(mg/l)	**GA3 (mg/l)**	**Mean number of shoots per node**			
		Beletech		**Awassa-83**	
		Petiole	**Leaf**	**Petiole**	**Leaf**
0.00	0.00	1.33±0.08^{b}	1.03±0.22^{bc}	1.13±0.10^{bc}	1.10±0.20^{b}
0.50	0.00	1.20±0.07^{b}	1.20±0.18^{bc}	1.27±0.08^{b}	1.07±0.18^{b}
1.00	0.00	2.03±0.03^{a}	2.27±0.12^{a}	2.33±0.09^{a}	2.40±0.11^{a}
1.50	0.00	1.30±0.09^{b}	0.73±0.11^{c}	1.37±0.14^{b}	0.90±0.10^{bc}
0.5	0.5	1.30±0.09^{b}	1.10±0.10^{bc}	1.30±0.15^{b}	1.10±0.20^{b}
0.5	1.0	1.30±0.09^{b}	1.00±0.00^{bc}	1.10±0.10^{bc}	0.90±0.10^{bc}
0.5	2.0	1.20±0.07^{b}	0.70±0.15^{c}	1.00±0.21^{bc}	0.80±0.13^{bc}
1.0	0.5	1.70±0.15^{ab}	1.50±0.17^{ab}	1.70±0.15^{ab}	1.50±0.17^{ab}
1.0	1.0	1.40±0.21^{b}	1.00±0.00^{bc}	1.00±0.21^{bc}	1.10±0.20^{b}
1.0	2.0	1.30±0.09^{b}	1.10±0.10^{bc}	1.30±0.15^{b}	1.20±0.10^{b}
2.0	0.5	0.90±0.21^{bc}	0.60±0.16^{c}	0.50±0.17^{bc}	0.70±0.15^{bc}
2.0	1.0	0.60±0.21^{bc}	0.50±0.17^{c}	0.30±0.15^{c}	0.50±0.17^{c}

Means in each column followed by the same letter (a-c) are not significantly different at 5% probability level.

The longest shoots (11.10 ± 0.38, 11.40 ± 0.31, 10.70 ± 1.0, 11.90 ± 0.31) were obtained from petiole and leaf explants of Beletech and petiole and leaf explants of Awassa-83, respectively, on MS medium containing 1.0 mg/l BAP (Fig. 4).

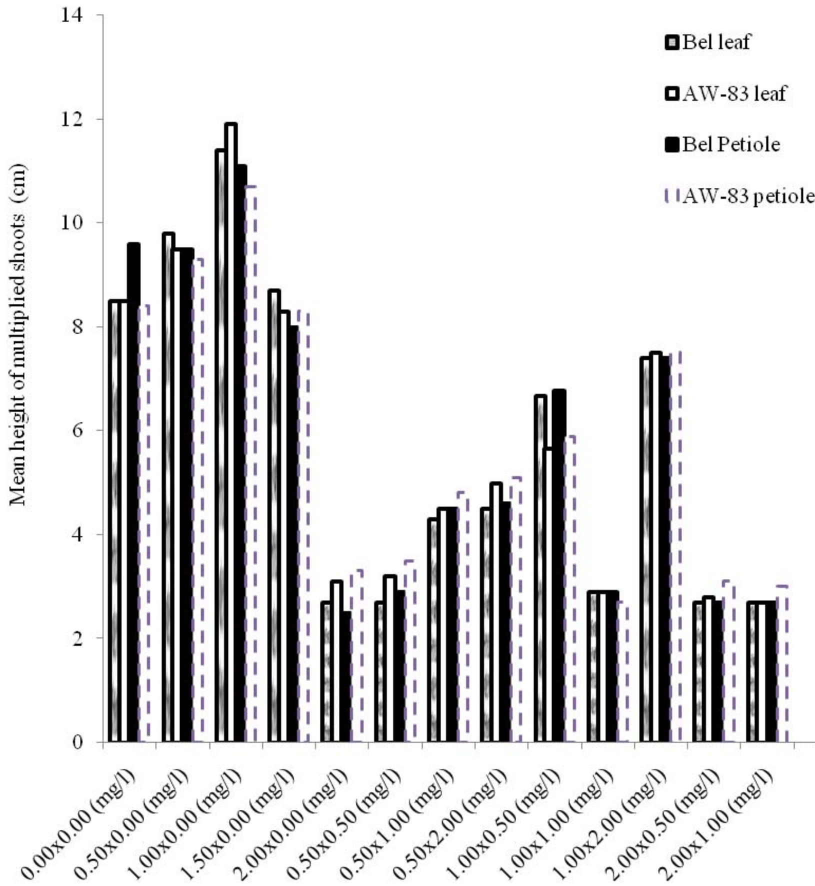

Fig. 4. Mean height of shoots multiplied from node culture of shoots regenerated from leaf and petiole calli of Beletech and Awassa-83 sweet potato varieties on MS medium containing different concentrations of BAP in combination with GA_3. Bel= Beletech, Aw-83 = Awassa-83, x = in combination.

Rooting and acclimatization

After one month of culture on MS rooting medium, all media containing different concentrations of IBA and the growth regulators-free medium resulted in 100% rooting. However, there was significant difference in the number and length of roots on these media. The highest number of roots per plantlet (8.10 ± 0.23 and 8.03 ± 0.21) was obtained on growth regulators-free medium from originally petiole derived shoots of Beletech and Awassa-83, respectively. The best mean shoot height of plantlets (11.10 ± 0.41) was obtained from shoots that were originally derived from petiole explants of Awassa-83 (Table 5).

Table 5. Effect of IBA on number of roots and root length of shoots regenerated from petiole and leaf calli of Beletech and Awassa-83 sweet potato varieties, values given as mean ± SE.

IBA (mg/l)	Mean number of roots per plantlet				Root length (cm)			
	Beletech		Awassa-83		Beletech		Awassa-83	
	Petiole	Leaf	Petiole	Leaf	Petiole	Leaf	Leaf	Petiole
0.0	8.10± 0.23[a]	6.80± 0.37[a]	6.07± 0.17[b]	4.83± 0.17[b]	10.0± 0.39[a]	10.10± 0.58[a]	10.60± 0.40[a]	11.10± 0.41[a]
0.1	6.00± 0.19[b]	5.30± 0.25[b]	8.03± 0.21[a]	6.97± 0.29[a]	8.50± 0.27[b]	9.70± 0.59[a]	10.40± 0.47[a]	8.70± 0.42[b]
0.5	3.97± 0.12[c]	3.80± 0.13[c]	3.73± 0.14[c]	3.40± 0.09[c]	6.90± 0.57[c]	7.70± 0.39[b]	8.80± 0.33[b]	6.60± 0.34[c]

Means in each column followed by the same letter (a-c) are not significantly different at 5% probability level.

After one month of acclimatization in glasshouse, 90% and 100% of plantlets originally derived from shoots of petiole and leaf of Beletech, respectively, survived. Similarly, 80% and 90% of plantlets originally derived from shoots of petiole and leaf of Awassa-83, respectively, survived (Fig. 5A-F).

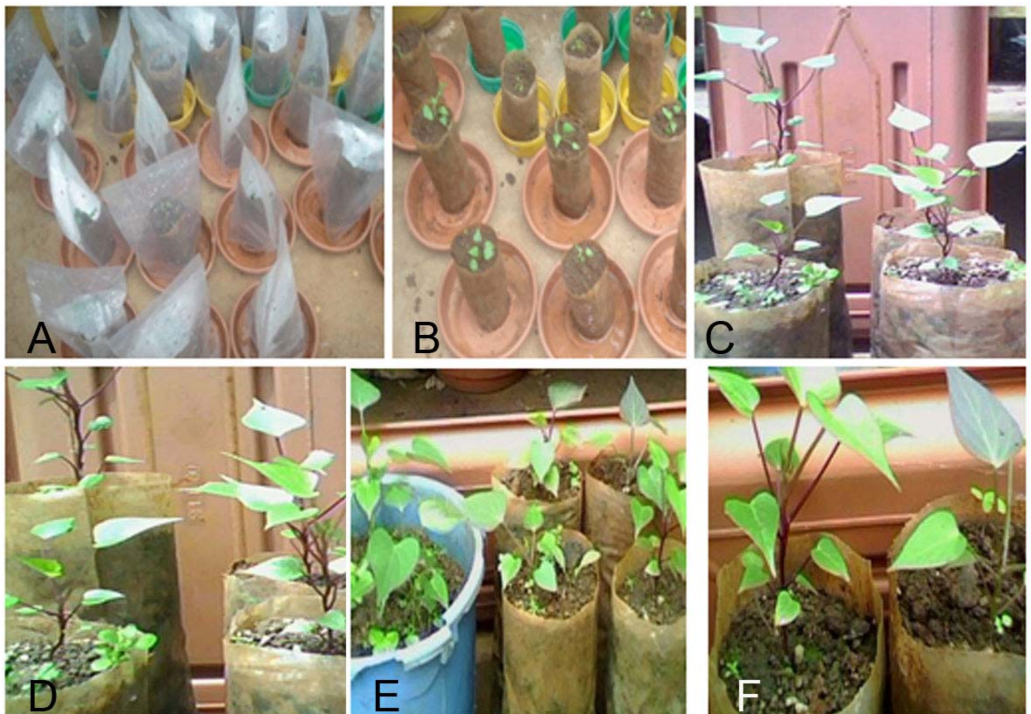

Fig. 5. Acclimatized plantlets of Beletech and Awassa-83 varieties in the glasshouse: (A) Plantlets covered with plastic bags; (B) One-week-old plantlets after planting; (C) Acclimatized plantlets originally regenerated from petiole calli of Beletech variety; (D) Acclimatized plantlets derived from leaf calli of Beletech variety; (E) Acclimatized plantlets derived from petiole calli of Awassa-83 variety; (F) Acclimatized plantlets derived from leaf calli of Awassa-83 variety after about one month in glasshouse.

DISCUSSION

Callus induction and shoot regeneration

Different concentrations of 2,4-D in combination with kinetin were used for callus induction and it was found that the medium that did not contain these growth regulators could not induce callus in sweet potato. However, only low concentrations of these growth regulators were found to be effective in producing morphogenic calli in different plants. Amiri *et al*. (2011) reported that 0.25 mg/l 2,4-D in combination with 0.5 mg/l kinetin resulted in best morphogenic callus induction of *Datura stramonium* leaf explants. In the present study, 0.05 mg/l 2,4-D in combination with 0.5 mg/l kinetin resulted in highest percentage (83-93%) of callus induction from petiole and leaf explants of Beletech and Awassa-83 sweet potato varieties. The percentage of callus induction from petiole explants is significantly higher than that of leaf explants indicating that the rate of callus induction is also dependent on source of explants.

There was significant difference in the rate of shoot regeneration among different concentrations of BAP used for shoot regeneration after one month of callus culture on shoot regeneration medium. Moreover, shoot regeneration was observed only from those calli induced on MS medium containing 0.05 mg/l 2,4-D in combination with kinetin (0.5 1.0, 1.5 mg/l). This indicates that morphogenic calli are induced only on very limited concentrations of these two growth regulators in sweet potato and most calli induced by these growth regulators are not morphogenic. Therefore, the growth regulators used for callus induction medium have strong influences on shoot regeneration. Similar to the present study, Sato *et al*. (1999) reported highest percentage of shoot regeneration from leaf explants of sweet potato variety Genki on growth regulators-free MS medium. Contrary to our study, the same authors reported that calli of petiole origin that had been cultured on 3.0 mg/l BAP resulted in higher regeneration percentage than those cultured on growth regulators-free medium. This difference could be due to the variation in response of genotypes to different concentrations of growth regulators. Otani *et al*. (1996) reported that BAP inhibited shoot regeneration in sweet potato. These authors obtained higher percentage of shoot regeneration of sweet potato from growth regulators-free medium and the rate of shoot regeneration was reduced with an increase in concentration of BAP. In the present study, BAP improved percentage of shoot regeneration from calli originated from leaf explants of varieties Beletech and Awassa-83. However, it was found to be less effective for shoot

regeneration from petiole calli of both varieties. There was no significant difference in the number of shoots per explant on MS medium containing 0.5, 1.0 mg/l BAP and growth regulators-free medium.

Shoot multiplication

The highest number of new shoots per node (2.40 ± 0.11) was obtained on MS medium containing 1.0 mg/l BAP from shoots originally obtained from callus of leaf explants of Awassa-83. There was no significant difference in number of shoots per explant among shoots derived from petiole and leaf explants in both varieties at same concentration of BAP. However, higher concentrations of BAP (more than 1.0 mg/l) was found to be supra-optimal and resulted in callus formation instead of shoot production. This agrees with the work of Geleta Dugassa (2009) on rate of shoot multiplication of shoots obtained from meristem of sweet potato varieties Awassa local and Awassa-83. This could be due to the fact that higher concentration of BAP inhibits shoot elongation and multiplication (Berrie, 1984). The longest shoots (11.10 ± 0.38, 11.40 ± 0.31, 10.70 ± 1.0, 11.90 ± 0.31) were obtained from petiole and leaf explants of Beletech and petiole and leaf explants of Awassa-83, respectively, on MS medium containing 1.0 mg/l BAP indicating that lower concentrations of BAP do not negatively affect shoot elongation.

MS medium supplemented with 1.0 mg/l BAP in combination with 0.5 mg/l GA_3 resulted in a maximum mean shoot number of 1.70 ± 0.15 per explant from petiole explants of the two varieties. As the concentration of GA3 increased, shoots degenerated to calli. This could be due to the reason that higher concentration of GA_3 promotes callus formation (Robbins, 1972).

Rooting and acclimatization

In the present study, 100% rooting was obtained in all rooting media as sweet potato is easy-to-root plant. The highest number of roots per plantlet (8.10 ± 0.23 and 8.03 ± 0.21) was obtained on growth regulators free medium from originally petiole derived shoots of varieties Beletech and Awassa-83, respectively. The highest mean shoot height (11.10 ± 0.41) was obtained from shoots that were originally derived from petiole explants of Awassa-83. Higher concentration of IBA (0.5 mg/l) reduced the number and length of roots as supra-optimal concentration of auxin results in low efficiency of root induction. This is consistent with the previous work of Geleta Dugassa (2009) and Neja Jemal (2009).

During acclimatization in glasshouse, 90% and 100% of plantlets originally derived from shoots of petiole and leaf of Beletech, respectively, survived. Similarly, 80% and 90% of plantlets originally derived from shoots of petiole and leaf of Awassa-83, respectively, survived. This high survival rate of sweet potato is due to the fact that rooting and establishment of this plant is easy as it is easily propagated vegetatively. The difference in survival rate of plantlets in glasshouse might be due to differences in adaptation of the varieties to *ex vitro* environment as observed by Matimati *et al*. (2005) that there was significant interaction between variety and planting material of sweet potato.

CONCLUSION

Among the different concentrations of 2,4-D and kinetin used for callus induction, 0.05 mg/l 2,4-D in combination with 0.5 mg/l kinetin was found to be the best for the leaf and petiole explants of both Beletech and Awassa-83 sweet potato varieties. The highest percentage of shoot regeneration (80% and 77%) was obtained on growth regulators-free medium using leaf explants of Beletech and Awassa-83, respectively. Similarly, the highest percentage of shoot regeneration (70% and 71%) from calli of petiole origin was obtained on 1.0 mg/l BAP of Beletech and Awassa-83, respectively. The highest number of shoots per node (2.40 ± 0.11) was obtained on MS medium containing 1.0 mg/l BAP from shoots originally obtained from callus of leaf explants of Awassa-83. There was no significant difference (at 5% probability level) in number of shoots per explant among shoots derived from petiole and leaf explants in both varieties at 1.0 mg/l BAP. Growth regulators-free MS medium was found to be the best medium for rooting of shoots derived from leaf and petiole explants of variety Beletech, whereas 0.1 mg/l IBA was the best concentration for rooting of shoots derived from leaf and petiole explants of variety Awassa-83. Among all acclimatized plantlets, the highest percentage of survival (100%) was obtained from shoots regenerated from leaf calli of variety Beletech.

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