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PROTOCOL OPTIMAZTION FOR *IN VITRO* PROPAGATION OF SWEET POTATO (*Ipomoea batatas L.)* VARIETY USING BUD EXPLANT

[1]Belachew Beyene*, [2]Gizachew Haile, [3]Temesgen Menamo

[1]Ethiopian Institute of Agricultural Research (EIAR) Debre zeit, P.O. Box 32, ***ETHIOPIA***
[2] Wachamo University, College of Agriculture and Veterinary Medicine, P.O Box 667, ***ETHIOPIA***
[3]Jimma University, College of Agriculture and Veterinary Medicine, P.O Box, 307, ***ETHIOPIA***

Abstract

Conventional propagation methods of sweet potato (Ipomoea batatas L.) through stem cutting require large amount of materials and space for propagation and an extended period to produce plants and there is also a high risk of disease transmission from generation to generation. In vitro propagation is the best alternative to overcome such limitations of conventional propagation. Thus, the study was conducted with the aim of optimize a protocol for in vitro mass propagation of sweet potato variety. The result revealed that Growth regulators free media was resulted in a maximum (66.67%) shoot initiated. Best shoot multiplication was obtained in MS medium supplemented with 1 mg/l BAP which showed that 5.00shoots/explants, 7.82±0.02 cm shoot length and 6.33leaves /shoot. Growth regulator free medium showed that 100% rooting in ½ strength MS medium. Among all plantlets planted in the glasshouse, 86% for in vitro rooted shoots were survived. It could be concluded that in vitro initiation of variety Beletech supplemented with BAP free MS media was the optimal concentrations.MS supplemented with 1mg/l BAP was the optimum concentration for shoot multiplication. For in vitro rooting, PGR free ½ MS medium were optimal. However, further studies will be required for protocol improvement using different hormone combinations with aim of increasing multiplication efficiency and cost reduction.

Keywords: Variety, Optimization, Micro propagation, Bud culture.

Corresponding Author:

Belachew Beyene

Ethiopian Institute of Agricultural Research (EIAR)

Debre zeit, P.O. Box 32, ***ETHIOPIA***

E-mail: *Belachew2145@gmail.com*

Phone: *+251919130793*

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INTRODUCTION

Sweet potato (Ipomoea batatas (L.) Lam.) is a dicotyledonous plant which belongs to the family Convolvulaceae, genus Ipomia and species batatas. Some of the species have fleshy roots, only sweet potato is edible. The cultivated Ipomoea batatasis a hexaploid of 2n = 6x = 90 with basic chromosome number x = 15 and is grown throughout the tropics with wide diversity (Jones, 1964).

It is the seventh most important food crop in the world following wheat (Triticumaestivum), rice (Oryza sativa), maize (Zea mays), potato (Solanumtuberosum), barely (Hordeumvulgare), and cassava (Manihotesculenta) (FAOSTAT, 2012). Globally it is grown in an area of about 8 million hectares with production of 104.45 million tons; average yield being13tons/ hain 2014 (FAOSTAT, 2016). In Ethiopia sweet potato ranks the first in total production (42.84%) and the second in area coverage (25.43%) next to Irish potato from root and tuber crops cultivated. The total area under sweet potato cultivation in Ethiopia is 0.06 million ha with production of 2.7million tons; average yield being 45tons/ha in 2015 (CSA, 2015).

In some of the world's poorest nations, sweet potato is important part of food security packages (Dagneet al., 2014). It is mainly cultivated for its expanded edible roots which contain high carbohydrate, minerals, vitamins, antioxidants and beta carotene to a large sector of the global population (Kapingaet al., 2011; Shongaet al., 2013).

Sweet potato is traditionally multiplied mainly by stem cuttings which are a slow process, and diseases may accumulate in the vine cuttingsfrom generation to generation which could result in declining of root yield and loss of superior genotypes. In general, it requires large area, incurs high cost, consumes time and thus it is wasteful practical system. Despite many efforts, the underlying problem in these procedures is low frequency of regeneration, long periods of culture and frequent media changes (Gosukondaet al. 1995).

Micro propagation of sweet potato offers significant advantages in the production of a very large number of clonal propagules within a short time, disease free plant material with the possibility of eliminating viral, bacterial and fungal infection and the production of high quality and uniform plantlets (Neja, 2009;Tekalignet al., 2012).

The objective of this study was to optimize protocol for mass propagation of sweet potato variety under in vitro condition using lateral bud as an explant. Furthermore, the optimum concentrations of growth regulators for shoot induction, multiplication and rooting have been worked out for

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sweet potato micro propagation and also the efficiency bud culture have been evaluated and compared.

MATERIALS AND METHODS

Plant material

The study was conducted using sweet potato variety, Beletech. This variety was obtained from Hawassa Agricultural Research Centre, Southern Nations Nationalities and Peoples' Regional State, Ethiopia. Vine cuttings of about 20 cm long were planted and grown in greenhouse at the College of agriculture and veterinary medicine, Jimma University, Ethiopia. The mother plants was irrigated twice per day and allowed to grow for one month.

Media Preparation

Stock Solution and Plant growth regulators preparation

MS media (Murashige and Skoog, 1962) supplemented with various plant growth regulators were used. Stock solutions of the macro salts, micro salts, vitamins, iron source and plant growth regulators (1mg: 1ml) were prepared and stored at +4^{o}C in refrigerator for immediate use (Appendix 4). Plant growth regulators, IBA was dissolved using a drop of ethanol and cytokinins (BAP) by NaOH before making up the final volume with distilled water. The dissolved solution was poured into labeled volumetric flask to be fully dissolved and finally stored in refrigerator for later use.

Culture Medium Preparation

The culture medium was prepared from their respective stock solutions, contained with the appropriate amount of 30 g/l sucrose and plant growth regulators (BAP and IBA) were added to the medium as required at various concentrations. The mixture was stirred using magnetic stirrer and the volume was adjusted using ddH_2O. Then, pH was adjusted in all cases to 5.8 using 1MNaOH and 1M HCL. Finally, 8.0 g/l agar was added and heated to melt throughout the experiment. Before autoclaving, the media were dispensed into sterilized culture jars. The media were steam sterilized using autoclave machine at a temperature of 121^{o}C with a pressure of 0.15 Kpa for 15 minutes and transferred to the culture room and stored under aseptic conditions until for later use.

Sterilization and Initiation of the Cultures

Healthy vines shoot tip of Beletech sweet potato variety was collected as an explant. The explants were then washed with soap solution with distilled water until the foam rejected. Then the

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explants were taken into sterilized laminar airflow cabinet, and dipped in 70% ethanol for 1min in a sterilized jar and washed using sterile distilled water three times for 5 min. They were then sterilized with 1% (v/v) commercial bleach (Berekina) solution containing3–4drops of Tween-20 for 15 minutes and rinsed 4 times with sterile double distilled water each for 5min with gentle shacking to remove the chemical residue under aseptic laminar air condition. The damaged parts were excised off using a sterile scalpel and about 1–1.5cm long explants were introduced into the nutrient media. The cultures were maintained at room temperature with 16/8h light and dark photoperiod respectively and used cool white fluorescent lamps in the growth room.

Experiment 1. Effect of Different levels of BAP on Shoot Induction

The sterilized explant was cultured on basal MS medium supplemented with various concentrations of BAP (0.1, 0.5, 075 and1.0mg\l).Murashige and Skoog medium without PGRs were used as control for establishment of shoot initiation from nodal explants. The experiment was laid down in completely randomized design in factorial with nine regenerated shoots per treatment. After 3 weeks, data on percent of explants regenerates per shoot and shoot length were recorded.

Experiment 2. Effect of Different Concentrations of BAP on Shoot multiplication

For shoot multiplication experiment the initiated shoots were taken after 3 weeks of first culture and then cultured on hormone free MS basal medium for two weeks to avoid carry over effects for the next circumstances. Medium supplemented with different concentrations of BAP were used for shoot multiplication. In this experiment, BAP (0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0mg\l) were used. The experiment was replicated three times arranged in completely randomized design with 15 regenerated shoots per treatment. The cultures were placed in white florescent light room adjusted at 16/8 hrs light/dark at room temperature. Data on shoot number shoot length and leave number were recorded after 6 weeks of culture.

Experiment 3.. Effect of Different Concentrations of NAA on root formation

Then shoots of one cm or more long were transferred to ½ MS medium containing, 0.0, 0.1, 0.5, 0.75 and 1.0 mg/l NAA. Completely randomized design (CRD) factorial arrangements with three replications of 15 shoots per treatment were used for *in vitro* rooting. After one month, mean number of roots, mean length and percentage of rooted plantlets were recorded for *in vitro* treatments.

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Acclimatization

Plantlets with well-developed root and leaf systems were washed with tap water to remove adhering media and sucrose attached on the roots of plantlets. fifty plantlets was transferred to plastic pots in green house containing hardening medium composed of soil, compost and sand (1:1:2) ratio,- respectively. The plant was placed in pots covered with transparent plastic bags (in order to keep humidity) and irrigated using sprayer every day. Plastic cover were removed partially after a week and completely removed after two weeks. Finally after one month, the survival rates of the plantlets were evaluated by counting the number of successfully acclimatized.

Data Analysis

SAS software (SAS, 2008 9.2 version) was used for data analysis of variance and for significantly different treatments, mean separation was done with Least Significance Difference (LSD) at or below the probability level of 0.01 (at 1% significance level).

RESULT AND DISSCUSSION

Shoot induction from shoot tip

Analysis of variance revealed that the interaction effects of variety and BAP were highly significant ($p<0.01$) for percentage of shoot initiation and shoot length (Appendix 1). Similarly, the analysis of variance indicated that the effect of BAP concentrations were highly significant different both for percentage of shoot initiation and shoot length ($p<0.01$).

The maximum (88.78±0.23) percentages of explants showing shoot initiation with average shoot length of 4.57±0.11cmat MS media supplemented with 0.5 mg/l BAP (Table1, Fig1). An average percentage of shoot initiation (66.66±0.00) was observed at growth regulators free medium, while, the minimum percent of shoot initiation (33.33±0.00) was observed at 1mg/l BAP. Though no significant difference was observed in variety between hormone free, 0.5 and 1.0mg/l of BAP on percent of shoot induction. Therefore, hormone free media could be taken as the best option economically as it reduces the cost of the growth regulator.

In current study, it was found that increase in the concentration of BAP (0.5-1mg/l) increased the percentage of explants showing shoot induction from 66.67±0.00 to 88.78±0.23, respectively. However, it starts declining as the concentrations kept increasing to 1mg/l. Shoot initiation of this variety were significantly low at the maximum concentration of BAP. This is due to high concentration of PGRs that leads to metabolic inhibition. Gosukonda*et al*. (1995) found that different sweet potato variety respond differently to *in vitro* shoot induction media.

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The reduction in the shoot length with the increased in BAP concentration in the culture medium may be as a result of inhibitory effect of BAP at higher concentration on shoot elongation. According to George *et al*. (2008) higher concentration of BAP inhibits shoot elongation.

The current result is in conformity with the finding of Sowal*et al*. (2002) who reported the effectiveness of low concentration of BAP to result in rapid shoot initiation due to the activation of tRNA cytokinins resulting in rapid proliferation of shoot primordial. In similar study, Khalafalla*et al.* (2007) also reported that BAP at the concentration of 5mg/l gives low number of shoot regeneration of sweet potato explants and they concluded that shoot initiation and shoot lengths decreases as increase of BAP concentrations.

Table 1: Effect of BAP on Shoot Initiation of Beletech variety

Note = **, highly significant at 0.01 level of probabilities. Var= variety, MS = Mean square, DF = Degree of freedom, CV = Coefficient of Variation.

Variety	BAP Conc.(mg/l)	% of Shoot	Shoot length (cm) (Mean±SD)
	0	66.66^{ab}±0.00	2.88^{c}±0.10
	0.1	88.78^{a}±0.23	4.57^{a}±0.11
Beletech	0.5	56.56^{ab}±0.39	3.40^{b}±0.07
	0.75	56.56^{ab}±0.39	2.16^{d}±0.07
	1	33.33^{b}±0.00	2.00^{d}±0.07
CV	5.7		2.8

Appendix1: ANOVA for the effect of 6-BAP on shoot initiation

Source	DF	% of initiation	Shoot length
		MS	MS
Var	0	0.05	3.46**
BAP	4	0.66**	3.36**
Var*BAP	4	0.18**	2.08**

Note: BAP= Benzyl Amino Purine. Means with the same letter are not significantly different at 0.01 probability level, CV= Coefficient of Variation.

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Fig. 1: *In vitro* shoot initiation of Beletech variety on hormone free MS medium

Effects of BAP on Shoot Multiplication

Analysis of variance revealed that the interaction effects of variety and BAP were highly significant ($p<0.01$) for number of shoots/explant, shoot length and leaves/shoot (Appendix 2).The effect of BAP on shoot multiplication showed that the best performance of (5.00±0.34) shoots/explant with 7.82±0.02cm average shoot length and 6.33±0.34 leaves/ shoot on MS media supplemented with 1.0mg/l BAP (Table 2; Fig.2).).The minimum 1.00±0.00 shoots number with 2.11±0.12cm shoot length and 2.78±0.19 leaves was formed MS media supplemented with the highest concentrations of 3.0mg/l BAP.

In this finding, it was showed that as the concentration of BAP increased from 0.5-3mg/l, number of shoots/explant, shoot length/explant and leave number also increased from 2.00±0.33to5.00±0.34, and then starts declining 1.00±00 as the concentrations kept increasing to 3mg/l. The result revealed that shoot number, shoot length and leaves numbers /explant was decreased when the concentration of BAP increased, shoot formation of genotype was significantly lower at the highest used concentration of BAP; the effect of BAP at 0.5, 1.0and 1.5 mg/l was significantly higher than its effect at 2.0, 2.5 and 3.0mg/l of genotype showed similar trend to BAP, i.e., higher number of shoot per explants at 0.5, 1.0 and 1.5mg/l and lower number of shoot per explants at 2.0, 2.5 and 3.0mg/l (Table 2).

As the BAP increased (>2.5 mg/l) the shoots showed bushy and distorted growth. This agrees with the previous findings on different varieties of sweet potato by (Geleta and Tileye, 2011; Neja, 2009).The current result is in line with Addisu (2013) who obtained the highest mean number of shoots on MS medium containing 1.0 mg/l BAP and 2.0 mg/l BAP for different sweet potato genotypes. According to Onuoch and Onwubiku (2007), BAP has physiological behavior that exhibit inhibitory effects on shoot elongation and multiplication at higher concentrations.

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Tassew (2012) also reported that as compared to BAP alone and the medium supplemented with combination of the two growth regulators (BAP and GA3), has resulted less number of shoot per node and BAP is most effective on Beletech and Awassa-83 varieties. As the concentration of GA3 increases while maintaining constant BAP, degeneration of shoot to calli was observed instead of shoot during shoot multiplication.

Table 2: Effect of BAP on Shoot Multiplication

variety	BAP Conc.(mg/l)	Shoot number (MEAN±SD)	Shoot length (MEAN±SD)	Leaf number (MEAN±SD)
	0	2.00^{d}±0.00	5.10^{g} ±0.10	3.22^{gh} ±0.19
Beletech	0.5	3.67^{b}±0.33	6.51^{e} ±0.04	3.00^{gh} ±0.33
	1	5.00^{a}±0.00	7.82^{b} ±0.02	6.33^{d} ±0.34
	1.5	2.67^{c}±0.33	6.44^{e} ±0.03	5.11^{e} ±0.01
	2	4.33^{b}±0.00	7.20^{d} ±0.10	8.22^{b} ±0.19
	2.5	2.33^{c}±0.34	5.20^{g} ±0.10	5.44^{e} ±0.20
	3	1.00^{e}±0.00	2.11^{j} ±0.12	2.78^{h} ±0.19
	CV	8.0	0.92	4.72

Note: BAP=Benzyl Amino Purine, Means with the same letter in the same column are not significantly different at 0.01 probability level.

Appendix 2. ANOVA for the effect of 6-BAP on shoot multiplication

Source	DF	Shoot number	Shoot length	Leaves number
		MS	MS	MS
variety	0	7.44**	6.26**	16.09**
BAP	6	15.12**	26.80**	32.47**
var*BAP	6	4.46**	3.74**	1.24**

Fig. 2: *In vitro* shoot multiplication of Beletch variety on MS+1.0 mg/l BAP after 6weeks of *culture*

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Effect of NAA on in vitro generated micro-shoots

The analysis of variance indicated that the interaction effect of variety and NAA application were highly significant ($p< 0.01$)for number of roots per shoot and root length of sweet potato variety tested (Appendix 3). The highest (100±0.00) *in vitro* rooting on 1/2 MS media with NAA free and 0.1mg/l NAA, and the minimum *in vitro* rooted shoots (33.33±0.01) on 1/2 MS medium with 1mg/l NAA. The maximum (7.44±0.38) number of roots/shoot with (6.22±0.11)cm average root length on 0.5mg/l IBA and a minimum of 3.33±0.33 and 4.09±0.11cm number of roots and root lengths, respectively, on ½ MS medium supplemented with 1.0mg/l NAA(Table 3). Sweet potato propagated through vegetative means and although it is possible that there were high endogenous auxin concentration in the explanted organ. As the concentration of NAA increased, number of root and the length of roots were significantly reduced for the both genotype. This indicates that rooting was highly influenced by the concentrations of NAA used. Hence, appropriate amounts of auxin in the rooting medium are crucial for root induction. This agrees with the work of Geleta and Tileye (2011) on Awassa-83,Guntute and Awassa local varieties.

Table 3: Effect of NAA on Rooting of Beletech Sweet potato variety

variety	NAA (mg/l)	Rooting % (Mean ±SD)	No.of roots (Mean±SD)	Root length (Mean±SD)
Beletech	0	100^{a}±0.00	1.67^{i}±0.00	8.27^{b}±0.05
	0.1	100^{a}±0.00	4.22^{d}±0.19	7.19^{c}±0.03
	0.5	86.69^{ab}±0.23	7.44^{a}±0.38	6.22^{e}±0.11
	0.75	66.67^{abc}±0.00	5.22^{c}±0.19	4.99^{f}±0.01
	1	33.33^{c}±0.01	3.33^{ef}±0.33	4.09^{g}±0.11
	CV	4.0	5.9	1.3

Note NAA=Naphthalene Acetic Acid. Means with the same letter in the same column are not significantly differentat 0.01 probability level, CV= Coefficient of Variation.

Appendix 2. ANOVA for the effect of NAA on rooting of Beletech Variety

Source	DF	%of rooted shoot	root number	root length
		MS	MS	MS
NAA	5	0.74**	15.08**	27.02**
var	0	0.01	1.93**	5.48**
Var *NAA	5	0.71**	3.17**	0.40**

Note = **, highly significant at 0.01 level of probabilities.Var = variety, MS = Mean square.

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Fig. 3: A) *In vitro* rooting of Beletech variety at hormone free ½ MS medium

Acclimatization of Plantlets

The *in vitro* rooted plantlet was hardened in the green house. After one month of acclimatization, 86% of plantlets was survived and successfully established from *in vitro* experiments of Beletech variety. The current results is in harmony with the finding of Berihu (2014) who reported (81.25% and 70.59 %) successfully survived plantlets, using the mixture of moist red soil, sand soil, and compost in the ratio of 1:2:1. Tasew (2013) also obtained 80% - 90% of plantlets transferred to sterilized soil were acclimatized after one month.

Fig. 4: Acclimatized plantlets of Beletech variety in the green house.

A= Plantlets covered with plastic bags (1stphase of acclimatization)

B = Acclimatized plantlets after one month in greenhouse

CONCLUSION

Among BAP concentrations used for shoot initiation, growth regulators free, 0.5mg/l and 1.0mg/l medium were found to be optimum concentration for Beletech variety. No significance difference

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was observed between growth regulators free, 0.5 and 1mg/l BAP for shoot initiation of variety. Thus, hormone free medium could be as the best option for shoot initiation of this variety economically as it reduces the cost of the growth regulators.

The effects of BAP concentrations on shoot multiplications were also highly significant. The genotype gave a maximum of 5.00±0.00 shoots/explant with 7.82±0.02 cm shoot length and 6.33±0.34leaves/shoot for BAP concentration of 1.0mg/l. For *in vitro* rooting, MS medium with growth regulators free, 0.1mg/l NAA concentration resulted in the highest value for percentages of shoots rooted for this genotype. NAA at 0.5mg/l could be taken as the best performance (7.44±0.38) for number of roots/shoot. About 86% of the *in vitro* rooted plantlets of Beletech variety were acclimatized successfully.

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