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Micro-propagation of *Colocasia esculenta* (cv. Bolosso I) from corm and sprout tip explants

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Taro (*Colocasia esculenta* L.) is a perennial, aquatic and semi-aquatic species, member of the family Araceae grown for its edible tuberous root. The application of conventional propagation techniques for taro could not produce high demand and quality planting materials due to its low productive capacity and diseases transmission. Therefore, this study was carried out to develop micro-propagation protocol for *Colocasia esculenta* (cv. Bolosso I) from corm and sprout tip explants. Explants were collected from Areka Agricultural Research Center and were sterilized using different concentration of sodium hypochlorite solutions with different exposure times. Murashige and Skoog (MS) media supplemented with different types and concentrations of auxins and cytokinins were used for culture initiation, shoot multiplication and root induction experiments. Two percent NaOCl exposures for 15 and 20 min were found to be optimum for sterilization of sprout tip (83.33) and corm (66.63%) explants. Highest culture initiation was obtained on MS medium supplemented with 8 mg/L 6-benzyl amino purine (BAP) (81.33 and 76.67% for corm and sprout explants, respectively). A maximum average number of shoots (8.53/corm and 5.8/sprout explants) were obtained on MS+8 mg/L BAP and 3 mg/Lindole-3-acetic acid (IAA). The highest mean root number (6.9) and root length (11.25 cm) per plantlet were recorded on half strength MS media supplemented with 0.5 mg/L IAA and 1.5 mg/L IAA, respectively. Eighty percent survival efficiency was observed on the soil mix ratio of 1:2:2 (red soil: sand: coffee husk, respectively) without any sign in morphological variation to the mother plant during acclimatization. These result can be used for the development of mass propagation protocol which enable large scale production of this highly demanded cultivar true-to-type and provide a foundation for further studies to generate genetically improved *C. esculenta* and related cocoyam species.

Key words: Explants, Murashige and Skoog (MS) media, plant growth regulators, sterilants, Taro plantlet.

INTRODUCTION

Taro [*Colocasia esculenta* (L.)Schott] is a perennial, aquatic and semi-aquatic herbaceous species that belongs to the family Araceae, originated in North Eastern India and Asia and then gradually spread worldwide (Macharia et al., 2014). Taro is the second most important root staple crop in terms of consumption

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while it is ranked fourth after sweet potato, yam, and cassava in terms of its production by weight with an estimated annual yield of over 229,088 tones worldwide (Singh et al., 2012).

Like any other root crops, taro corms are high in moisture (63- 85%), carbohydrate in the form of starch (47-74%) protein (3-7%), potassium (higher than banana) and low in fat (Adane et al., 2013). Its starch granule is 98.8% digestible, by which it is ideal for people those with digestive problems (Hanson and Imamuddin, 1983).

In Ethiopia, taro is cultivated in high rainfall areas of south and southwestern parts of the country as a potential food security crop (Tewodros, 2013). During 2016/2017 production year, taro production reached 48, 087.35 ha with a total production of 1, 217, 916.45 tons of which 81.2% is used for human consumption (CSA, 2016).

Even though taro has many roles, its propagation and ultimate production is adversely affected by biotic stresses and nature of the plant itself (Singh et al., 2012). Lack of improved varieties, and need of large planting materials (Onokpise et al., 1999), rare natural flowering and seed setting (Mbouobda et al., 2007), transmission of pathogens particularly dasheen mosaic virus (DsMV) during vegetative propagation which can cause yield loss up to 90% or may cause loss of genotypes (Reyes et al., 2006; Nassar et al., 2007; Beyene, 2012) are major constraints of taro and its relate, tannia production. Beyene (2012) reported that 36.9% of the taro samples found to be positive to DsMV specific antibodies out of 295 symptomatic and non-symptomatic taro leaf samples which were collected from major growing areas of south and southwest Ethiopia.

The use of biotechnological approaches in particular tissue culture techniques is the best option to overcome these constraints and improve its production and productiveness. Because, tissue culture provides a means of rapidly producing large quantities of healthy, identical propagate for large scale cultivation within a short period and limited space without seasonal dependency in contrast to conventional propagation (Abraham, 2009; Deo et al., 2009). Moreover, Kebede and Abera (2015) suggested that each variety needs its own *in-vitro* regeneration protocol. Hence, the present study aimed to develop an efficient micro-propagation protocol for this particular Ethiopian taro variety [*Colocasia esculenta* (L.) Schott] cv. Boloso I (ARC/064/96) using sprouted tip and corm explants.

MATERIALS AND METHODS

Plant material

Healthy corms of *C. esculenta* were obtained from Root Crops Research Division, Areka Agricultural Research Institute (AARI) located in Areka town, Bolosso Sore district of Wolaita zone, South Nations Nationalities and Peoples' Regional State (SNNPRS). The corms were then planted and grown on sterilized soil that had a mixture of red soil, coffee husk and sand at a ratio of 1:2:2, respectively under greenhouse conditions of Natural Science College, Jimma University. The established mother stock-plant was daily watered with tap water and sprayed with 0.3% Mancozeb at eight days interval to control fungal infection.

Explants collection and sterilization

After growing a healthy and vigorous plant materials, the apical shoot of mature plants between 4 and 6 months old (1.5-2.0 cm) and newly sprouted axillary shoots (1.5-2.0 cm) segments were used as a source of explants. The excised explants materials were thoroughly washed with tap water and outer leaves are removed until inner cleaner section appeared. Explants were then surfaced-wiped with 70% ethanol for a maximum of one minute. Outer leaves were separated from the dome in a circular fashion using a sterile surgical blade under a laminar flow hood cabinet. They were then washed in four different levels of sodium hypochlorite (NaOCl), namely 1.0, 1.5, 2 and 2.5% with an exposure duration of 5, 10, 15 and 20 min. To maximize the efficacy of the sterilants chemical, some drops of Tween-60 (as a wetting agent) was added into all the sterilants solutions prior to treatment. Then, the treated explants were rinsed repeatedly with autoclaved distilled water under aseptic condition. Finally, the explants which lack any sign due to the effects of the sterilants were then transferred to culture jars and cultured in a plant growth regulator free conditioning medium of full strength MS medium (Murashige and Skoog, 1962) supplemented with 30 g/L sucrose, and solidified with 8 g/L agar-agar. Thus, this experiment was laid in a 4×4×2 combination in CRD (Complete Randomized Design). The four factors evaluated in this experiment were four concentrations (1, 1.5, 2 and 2.5% active chlorine) of sterilants solution, four durations (5, 10, 15 and 20 min), and two types of explants (corm and sprout tip).

Culture medium and growth regulators stock solution preparation

Different growth regulators stock solution such as 6-benzyl amino purine (BAP), α-naphthalene acetic acid (NAA), indol-3-butyric acid (IBA) and indol-3-acetic acid (IAA) were prepared by weighing and dissolving the powder in double distilled water at the ratio of 100 mg:100 ml or in one to one ratio. For dissolution process, 3 to 4 drops of 1 N NaOH, 1 N HCl and 96% ethanol were used based on the requirement of the growth regulators (NaOH/96% ethanol for auxins, HCl for cytokinins). Then, the volume was adjusted by adding autoclaved distilled water. Finally, all the growth regulators stock solutions were stored in a refrigerator at a temperature of 0 to +4°C.

Culture medium was prepared by taking the proper amount of MS powder (Murashige and Skoog medium, 4405.19 g/L conc.) solidified with 8 g/L agar and then 3% sucrose (w/v) as carbon source was added at 5.7 pH. The pH of the medium was adjusted by using some drops of 0.1 N HCl or 0.1 N NaOH, gelled with 0.8% (w/v) agar (Sigma, St Louis MO, USA) and then sterilized by autoclaving at a temperature of 121°C with a pressure of 15PSi for 15 min. Then, the culture media were incubated in the dark at 25 ± 2°C to minimize the effects of light.

Culture initiation

Sterilized corm base and sprout axillary bud segment explants were inoculated in 8 to 12 cm culturing jars in full MS media supplemented with BAP (0, 4.0, 6.0, 8.0 and 10.0 mg/L), kinetin (0, 1.0, 2.0 and 3.0 mg/L). Each treatment consisted of six explants (one explant per jar) and was replicated four times. The cultures were incubated in the light condition for 2 to 3 weeks. Then, all

explants were transferred to fresh media in order to increase the initiation capability of the culture and incubated for another three weeks under light conditions. Data on the percentage of initiated shoots per explants were recorded after 3 to 4 weeks of culture. So, there were 4×3×2 combinations in CRD including free growth regulator (0) as control.

Shoot multiplication

Shoot buds derived from those explants that had responded well to the initiation media were transferred singly onto a shoot multiplication medium. For this experiment, the shoot multiplication media used were composed of MS basal medium supplemented with 30 g/L sucrose and different concentrations of BAP (0, 2, 4, 6, 8, 10 and 12 mg/L) each alone and interacting at each concentration with 0, 1, 2 and 3 mg/L IAA. The experiment was thus being arranged in a 6×3×2 combination in CRD. After 3 to 4 weeks of culture average numbers of shoots per explants were recorded. Then, from cultures, each proliferated shoot (sprouts) were divided and subculture separately on to a fresh medium of similar composition for further growth. Then, after 2 to 3 weeks average height (cm) of the micro shoots were recorded.

Rooting

For root induction experiment, three auxin types (IAA, NAA and IBA) were tested. Healthy and well developed micro-shoots with a minimum length of 2.0 cm were transferred on a fresh rooting media. For this experiment, half strength MS basal medium with 3% sucrose was supplemented with different concentrations of IBA, IAA and NAA. Therefore, the experiment was laid with the treatment of three concentrations for each of auxin (0, 0.5, 1 and 1.5 mg/L). Thus, the treatment combination for this experiment was 3×3×3×2 combination in CRD. Whereas, 3-concentration of NAA, IAA and IBA and 2-explants.

Acclimatization

After completing the root induction experiment, all the plantlets which successfully induced root were removed from the rooting media for acclimatization. For the case, all the roots were carefully washed with running tap water to remove the gelling agent raised from the *in-vitro* media and the plantlets were then planted into pots (4.5 cm x 3.5 cm) filled with sand, decomposed coffee husk and red soil mixes in the ratio of 2:2:1; 1:2:2 and 1:1:1, respectively. Each pot was covered with a polyethylene bag and kept in the greenhouse. The cover bags were removed after two weeks. Finally, the survived plantlets percentage from each soil ratio was recorded after four weeks and the overall survival percentage within the three soil ratio was evaluated after eight weeks.

Statistical analysis

Collected data from each experiment was analyzed using SAS computer software (version 9.2) and ANOVA was constructed followed by mean separation using Ryan-Elinot-Gabriel-Welch Multiple Range Test (REGWQ) at α = 5% probability.

RESULTS

Effects of NaOCl concentrations and exposure time on sterilization of *C. esculenta* explants

The highest rate of clean survived culture 83.33±0.85 and 76.67±0.92% was obtained from treatment concentration of 2% active chlorine in NaOCl solution with fifteen and ten minute exposure duration respectively for sprouts tip culture. For the culture of corm explants, maximum rate of clean culture was obtained from treatment concentration of 2% active chlorine with 20 min exposure duration with a mean average result of 66.63±0.51% followed by the same concentration with time duration of 15 min with mean average value of 60.00±1.00% (Table 1).

Effect of BAP and kinetin on shoot induction

Maximum shoot induction percentages were achieved on MS medium supplemented with 8 mg/l BAP alone with mean initiation percentage of 81.33±0.59 and 76.67±0.48 for corm explants and sprouts culture, respectively (Table 2 and Figure 1A and B). The culture medium devoid of phytohormones (control) failed to respond to the bud break of sprout tip explants. Of all combination experiments, the highest shoot induction percentage was observed on MS media supplemented with 8 mg/L BAP and 2 mg/L Kinetin with mean present of initiation 65.00±0.55 and 55.00 ± 0.69 from corm and sprout tip explants, respectively. From all given combination, the minimum rate of shoot induction was recorded on MS medium containing 10 mg/L BAP and 3 mg/L Kinetin with an average percentage of 28.33 ± 0.84 and 25.00± 0.47% for corm and sprout tip explants, respectively (Table 2).

Effect of different concentration and combination of BAP and IAA on shoot multiplication of *C. esculenta*

In this study, culture media devoid of phytohormones (control) showed minimal shoot multiplication rate with mean number of shoots per explants 1.93±0.3 and 1.63±0.06 from corm and sprout tip culture, respectively. Of all combination tested, the highest multiplication results were found on MS media containing 8 mg/L BAP and 3 mg/L IAA, with a mean number of shoots 8.53±0.07 and 5.80±0.39 from corm and sprout tip explants respectively (Table 3 and Figure 1C and D).

With regard to length of shoots, longest shoots (6.7±0.10 cm) were also observed on the same media. The second best multiplication media was achieved by MS medium supplemented with 8 mg/LBAP and 2 mg/L IAA with a mean shoot number (7.67±0.14) with an average length of 5.87±0.14 cm from corm explants. For sprout tip explants, an average shoot (5.13±0.38) and mean length (5.27±0.03 cm) was recorded on the same media (Table 3 and Figure 1E).

Effect of different concentrations of auxins on rooting

Shoots inoculated on hormone free (medium lacking

Table 1. Effect of different concentrations sodium hypochlorite with various time of exposure on sterilization of corm base and sprout tip explants of *C. esculenta*.

Concentration (%)	Time (min)	Clean a live culture (%)		Contamination (%)		Tissue death (%)	
		Corm	Sprout tip	Corm	Sprout tip	Corm	Sprout tip
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
0	0	0.00±0.00 ^{m}	0.00±0.00^{m}	100.00±0.00^{a}	100.00±0.00^{a}	0.00±0.00^{l}	0.00±0.00^{j}
1	5	13.33±0.75 ^{l}	20.00± 0.20^{l}	86.66±70.76^{b}	80.00± 0.26^{b}	0.00±0.00^{l}	0.00±0.00^{j}
1	10	18.15±0.10 ^{k}	26.66 ±0.76^{k}	81.15±0.40^{c}	73.33±0.45^{c}	0.00±0.00^{l}	0.00±0.00^{j}
1	15	26.66±0.81^{j}	33.33 ±0.98^{j}	73.33±0.65^{d}	66.66±1.15^{d}	0.00±0.00^{l}	0.00±0.00^{j}
1	20	33.33±0.61^{i}	40.00±0.10^{i}	66.86±0.23^{e}	60.00±0.26^{e}	0.00±0.00^{l}	0.00±0.00^{j}
1.5	5	35.33±0.61^{h}	43.33 ±0.58^{h}	64.00±0.91^{f}	56.66±0.49^{f}	6.66±0.20^{k}	0.00±0.00^{j}
1.5	10	40.00±0.80^{g}	46.66 ±0.61^{g}	46.66±0.41^{g}	53.33±0.40^{g}	10.00±0.18^{j}	0.00±0.00^{j}
1.5	15	46.34±0.57^{f}	53.33 ±0.61^{f}	40.00±0.43^{h}	43.33±0.61^{h}	13. 66±0.28^{i}	3.33±0.15^{i}
1.5	20	50.00 ±0.17^{e}	60.26 ±1.51^{e}	33.33±0.35^{i}	33.33±0.35^{i}	16.00±0.50^{h}	6.66±0.30^{h}
2	5	53.33 ±0.40^{d}	66.66 ±0.92^{d}	26.66±0.57^{j}	23.26±0.64^{j}	20.00±1.00^{g}	10.00±0.36^{g}
2	10	55.00 ±1.20^{c}	76.67 ±0.92^{b}	20.00±0.40^{k}	10.00±0.87^{k}	25.00±1.14^{f}	13.33±0.45^{f}
2	15	60.00 ±1.00^{b}	83.33 ±0.85^{a}	13.33±0.40^{l}	3.33± 0.66^{l}	26.66±0.28^{e}	13.33±0.45^{f}
2	20	66.63 ±0.51^{a}	73.33 ±0.51^{c}	6.66±0.57^{m}	3.33±0.66^{l}	26.66±0.28^{e}	23.33±0.40^{e}
2.5	5	53.33 ±0.40^{d}	60.00 ±0.32^{e}	6.66±0.57^{m}	0.00± 0.00^{m}	40.00±0.50^{d}	40.00±0.57^{d}
2.5	10	50.10 ±0.17^{e}	52.00 ±0.26^{f}	3.33±0.57^{n}	0.00± 0.00^{m}	46.66±1.52^{c}	48.00±1.65^{c}
2.5	15	46.66±0.57^{f}	43.67±0.35^{h}	0.00±0.00^{o}	0.00±0.00^{m}	53.34±0.75^{b}	56.33±0.75^{b}
2.5	20	40.00 ±1.80^{g}	33.34±0.15^{j}	0.00±0.00^{o}	0.00±0.00^{m}	60.00±1.00^{a}	66.67±1.33^{a}
CV		1.81	1.41	1.27	1.46	3.13	3.96

Means followed by the same letters within a column were not significantly different from each other by Ryan - Elinot Gabriel - Welsch Multiple Range Test (REGWQ) at 5% probability.

growth regulators) had no rooting response. MS medium containing IBA at higher concentration (1.5 mg/L) resulted in lowest root initiation percentage (38.75±4.78%). From all root inducing hormone (auxins), IAA at lower concentration (0.5 mg/L) showed the highest rooting response (86.25±2.5%) with a mean number of 6.90±0.09 roots per plantlets (Table 4 and Figure 1F). The second highest response (78.75±2.5%) was recorded from ½MS media supplemented with 1 mg/L of NAA with average root numbers of 5.80±0.23 per cultured explants.

In case of root length and shoot heights, the longest root (11.25±0.5 cm) were obtained from a medium supplemented with 1.5 mg/L IAA followed by 1 mg IAA with average root length of 9.63±0.48 cm. Smallest mean root length (2.30±0.26 cm) was obtained from medium supplemented with 1.5 mg/L of IBA.

For the height of the regenerated shoots on the rooting media, ½ MS media augmented with 0.5 mg/L IAA showed longest shoots with a mean height of 6.20±0.16 cm followed with 1 mg/L of IAA with an average shoot height of 5.50±0.18 cm after a month of culture.

Acclimatization

All *in-vitro* plantlets that were transferred to external environment (Figure 1G) and planted on plastic pot with different soil mixes grew actively during acclimatization process without any stress symptoms. Soil media containing sand, red soil and coffee husk in the ratio of 2:1:2 showed highest survival rate (80%) as compared to the other ratio of the same soil type. After acclimatization of two months in the greenhouse, a total of 76% of potted *C. esculenta* plantlets survived (Figure 1H).

DISCUSSION

Effects of NaOCl concentrations and exposure time on sterilization of *C. esculenta* explants

The analysis of ANOVA showed that statistically high significant effect ($P<0.0001$) among interaction of active chlorine concentration in NaOCl and time of exposure (time*NaOCl). This indicated that the degree of contamination and clean culture was highly influenced by the concentration of sterilants and its exposure duration. This means that in higher concentration of NaOCl with longer duration of exposure, there were better removal of contaminates while in lower concentration of NaOCl with shorter time of exposure there were highly contaminated cultures.

As indicated in Table 1, 1.5% NaOCl and 2% NaOCl

Table 2. Effects of different concentrations of BAP combined with Kinetin on shoot induction from corm base and sprout tip explants of *C. esculenta.*

Concentration of PGRS		Explant	
BAP (mg/L)	Kinetin (mg/L)	Corm (Mean ± S.D.)	Sprout tip (Mean ± S.D.)
0	0		
0	1	20.83±0.83^{r}	16.67±0.63^{s}
0	2	21.66 ±0.41^{r}	18.33±0.83^{r}
0	3	23.33±0.48^{q}	20.00±1.58^{q}
4	0	66.67±0.44^{c}	63.33±0.46^{c}
4	1	43.33±2.31^{l}	31.67±0.48^{n}
4	2	45.00±0.57^{k}	38.33±0.84^{k}
4	3	41.66 ±1.22^{m}	35.00±0.63^{m}
6	0	78.33±0.64^{b}	71.67±0.67^{b}
6	1	55.00±0.82^{i}	45.00±0.62^{i}
6	2	56.67 ±3.15^{h}	46.67±0.81^{h}
6	3	53.33±0.23^{j}	43.33±0.80^{j}
8	0	81.33±0.59^{a}	76.67±0.48^{a}
8	1	63.33±2.51^{e}	50.00±0.82^{f}
8	2	65.00±0.55^{d}	55.00±0.69^{d}
8	3	61.00±0.81^{f}	48.33±0.43^{g}
10	0	58.33±0.82^{g}	51.67±0.88^{e}
10	1	40.00±1.11^{n}	36.66±0.31^{l}
10	2	33.33±0.66^{o}	30.00±0.55^{o}
10	3	28.33±0.84^{p}	25.00±0.47^{p}
CV		1.24	2.38

Means within a column followed by the same letters are not statistically significant at p< 0.01 by Ryan - Einot - Gabriel - Welsch Multiple Range Test (REGWQ).

with time exposure of 15 to 20 min, in all the cases, reduce the presence of contaminants in the *in-vitro* culture media with higher percentage of clean alive culture. In the other case, NaOCl with higher concentration (2.5%) with long exposure duration 15 and 20 min did eliminate all the surface contaminants because of its mechanism of action causes biosynthetic alterations in cellular metabolism and phospholipids destruction in the cell wall of bacteria and fungi (Seetohul and Puchooa, 2005). However, it was toxic to cellular tissue of Taro. On the other hand, NaOCl (1.5 and 2%) did not eliminate all the contaminants as some bacteria and fungi were observed in the culture media but, the survival rates, as well as the percentage of green buds that developed shoots after two to three weeks of culture from both explants, were highest. In lower concentration of NaOCl (1%), with lower exposure time (five and ten minutes), the highest percentage of contamination were observed while shoot death is highly low in both explants. This is due to the insufficiency of sterilants concentrations and exposure time to kill surface contaminants mainly fungi and bacteria those are strongly associated to the cultured explants.

The results of this study is similar with the study done by Seetohul and Puchooa (2005) who reported 2% active chlorine (NaOCl) with 15 exposure time resulted in highest percentage of contamination free *in-vitro* culture from axillary bud sucker explants of taro after been washed for one hour with running water and treated with 15 min in a solution of benlate at 0.06% on a shaker, while the present study reported developed optimal sterilant concentration with time duration of 2% NaOCl with 15 and 20 min from sprout tips and corm base explants after treated with 70% v/v ethanol alcohol for a minute prior to NaOCl treatment.

Hossain (2012) also similarly reported using meristem and parenchymatous storage tissues of taro explants after treated with 70% ethanol for three minutes followed by soaking in 1.5% chlorine water with few drops of Tween 20 for ten minutes of exposure as effective sterilization combination. The same author, in 2009 also showed that 1.5% NaOCl with time duration of 10 min in the presence of 70% ethanol alcohol is used as best sterilization combination for *C. esculenta* var. Latiraj. Study on related coccame species, *Xanthosoma sagittifolium* (L) Schott by Bari and Paul (2007) used mercuric chloride for surface sterilization. However, mercury chloride is a very toxic chemicals for human,

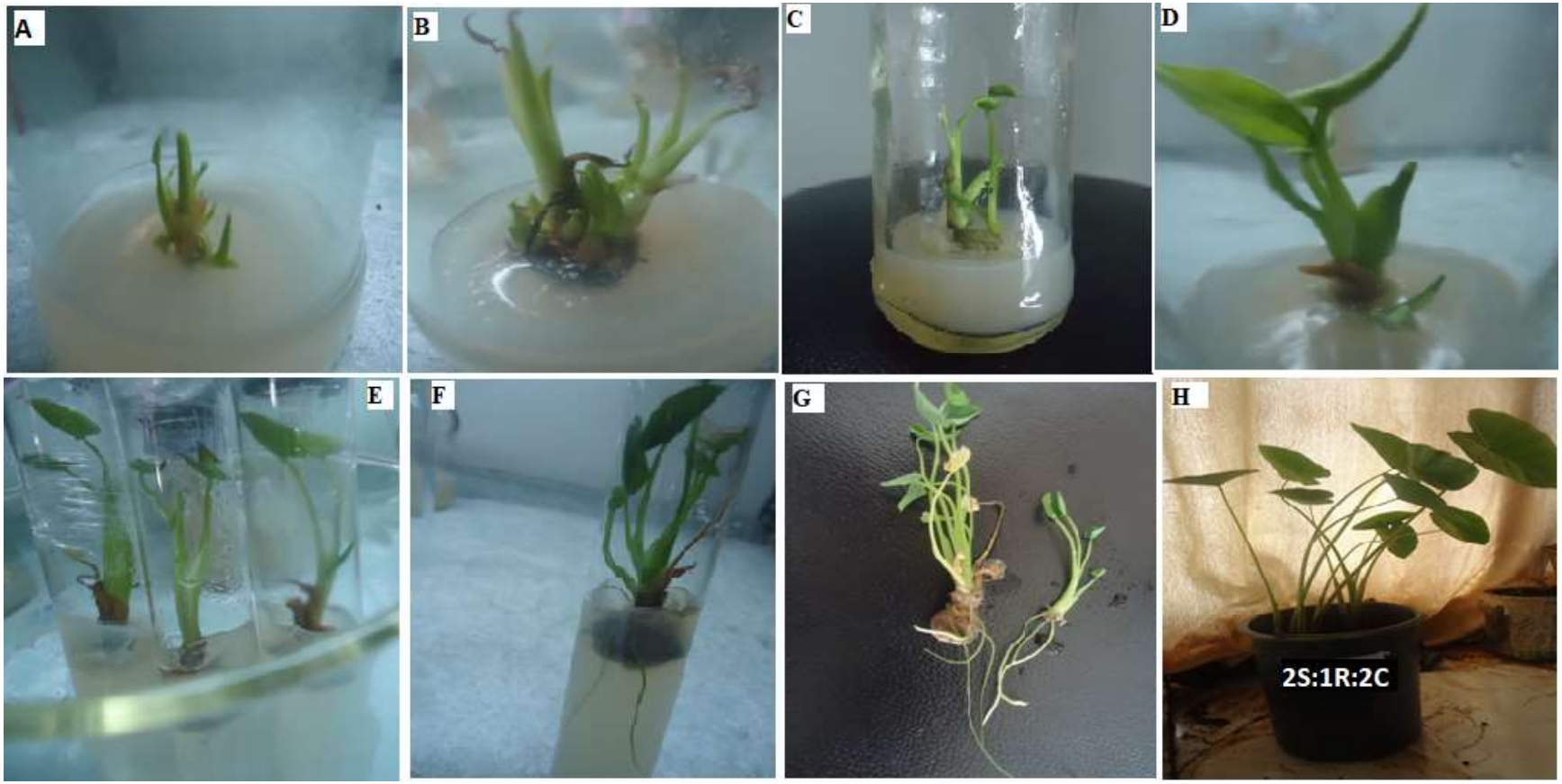

Figure 1. *In-vitro* shoots induction of *C. esculenta* on MS+8 BAP mg/L (A and B); shoots multiplied on 8 mg/L of BAP and 3 mg/L IAA (C and D); regenerated shoots after sub-culturing and ready for rooting experiment (E): root induced on ½ MS + 0.5 mg/L IAA(F); Ex *vitro* plantlets ready for acclimatization (G) and 8 weeks old seedling of taro under greenhouse condition (H).

unsafe and unfriend with the environment and hence difficult to discard after the sterilization process. Thus, it is better to substitute and use with relatively safe and easily available sterilants chemical such as sodium hypochlorite /bleach solution to disinfect cultured explants as used in this study.

Shoot induction from explants

As indicated in Table 2, in all cases, shoot bud proliferation increases with an increased level of BAP concentration alone from 0 to 8 mg/L for both explants. However, further addition of BAP concentration alone and in combination showed lower culture initiation percentage with poor bud breaking for both corm and sprout tip explants. Therefore, MS basal media containing 8.0 mg/L BAP alone was found to be optimum media for *in-vitro* shoot initiation of *C. esculenta* for both corm and sprout tip explants.

This indicated that BAP alone is more effective

Table 3. Effect of different concentrations of BAP alone and combined with IAA on average number and length of shoots per explants.

Level of PGR		Corm		Sprout tip	
BAP (mg/L)	IAA (mg/L)	Shoot number (Mean ± S.D.)	Shoot length (Mean ± S.D.)	Shoot number (Mean ± S.D.)	Shoot length (Mean ± S.D.)
0	0	1.93±0.30^{m}	1.57±0.21^{o}	1.63±0.06^{l}	1.30±0.3^{k}
0	1	2.87±0.21^{l}	2.00±0.20^{no}	1.83±0.15^{kl}	1.45±0.09^{k}
0	2	2.93±0.12^{l}	2.34±0.07^{nm}	2.00±0.00^{kjl}	2.24±0.0.24^{ji}
0	3	3.00±0.00^{l}	2.67l±0.15^{mk}	2.20±0.10^{kjl}	2.30±0.17^{ji}
2	0	3.53±0.15^{k}	2.80±0.20^{jimk}	2.27±0.08^{jhi}	2.44±0.80^{ji}
2	1	3.67±0.15^{jk}	3.00±0.22^{jlik}	2.33±0.28^{jhi}	2.50±0.30^{ghi}
2	2	3.43±.12^{k}	3.37±0.20^{jih}	2.33±0.29^{kjhi}	2.87±0.30^{fihg}
2	3	3.73±0.12^{jk}	3.4±0.21^{ih}	2.40±0.00^{jhi}	3.20±0.40^{feg}
4	0	3.83±0.15^{jk}	3.54±0.12^{gih}	2.43±0.15^{jhi}	3.43±0.15^{feg}
4	1	3.87±0.15^{jk}	3.83±0.15^{gfh}	2.43±0.21^{jhi}	3.83±0.16^{de}
4	2	3.87±0.21^{jk}	3.5±0.0.46^{gih}	2.53±0.15^{ghi}	3.5±0.46^{fe}
4	3	4.0±0.16^{jk}	3.8±0.17^{gfh}	2.6±0.09^{ghi}	3.83±0.15^{de}
6	0	4.15±0.11^{ji}	3.6±0.20^{gih}	2.67±0.07^{bhi}	3.52±0.07^{fe}
6	1	4.67±0.29^{hg}	3.8±0.17^{gfh}	2.77±0.05^{gh}	3.47±0.16^{feg}
6	2	5.67±0.15^{f}	4.63±0.15^{dce}	3.90±0.01^{e}	4.6±0.10^{c}
6	3	6.00±0.00^{ed}	5.00±0.24^{c}	4.43±0.12^{d}	4.63±0.15^{c}
8	0	6.13±0.06^{e}	4.20±0.19^{fe}	4.53±0.15^{cd}	4.2±0.53^{dc}
8	1	7.27±0.02^{cb}	4.80±0.20^{dc}	4.93±0.10^{cb}	4.37±0.06^{dc}
8	2	7.67±0.14^{b}	5.87±0.14^{b}	5.13±0.38^{b}	5.27±0.03^{b}
8	3	8.53±0.07^{a}	6.7±0.10^{a}	5.80±0.39^{a}	5.56±0.21^{a}
10	0	3.83±0.20^{jk}	3.2±0.17^{jik}	2.20±00^{kjl}	3.13±0.32^{fhg}
10	1	4.93±0.06^{g}	4.37±0.21^{dfe}	3.37±0.40^{f}	3.4±0.06^{feg}
10	2	6.67±0.21^{d}	3.82±0.14^{gfh}	4.00±0.000^{e}	3.47±0.15^{feg}
10	3	7.07±0.29^{c}	4.07±0.31^{gf}	4.54±0.31^{cd}	2.9±0.10^{fihg}
12	0	2.73±0.25^{l}	2.8±0.2^{jlmk}	2.16±0.15^{jhi}	2.34±0.21^{ji}
12	1	3.53±0.08^{k}	3.13±0.17^{jlik}	2.5±0.10^{gjhi}	2.8±0.26^{jhi}
12	2	3.87±0.15^{jk}	3.0±0.27^{jlik}	2.57±0.03^{ghi}	2.5±0.10^{jhi}
12	3	4.47±0.11^{hi}	2.6±0.27^{lm}	2.97±0.09^{gf}	2.2±0.11^{j}
CV		3.64	5.57	5.59	6.96

Mean within a column followed by the same letters were not statistically significant at P < 0.01 by Ryan-Einot-Gabriel-Welsh Multiple Range Test (REGWQ).

than kinetin alone and in combinations with BAP for *in-vitro* initiation stage of taro.

The result of this study is in accordance with the study of Chien-Ying et al. (2008) on MS media containing 8 mg/L of BA for shoot proliferation of Dasheen (*C. esculenta*) whereas slightly in contrast to the study of Ngetich et al (2015), who reported 10 mg/L of BAP as effective media for *in-vitro* initiation of taro while in this study MS media containing above 8 mg/L BAP showed low or poor sign of shoot induction rate for the selected variety of taro. This deviation might be as a result of variation in the genotype of the stock plant used. According to George et al. (2008), the effect of phytohormones used during *in-vitro* multiplication in plant tissue culture varied with plant genotype. Ngetich et al. (2015) also similarly showed that response in shoot proliferation varied within the three taro varieties namely: Dasheen, wild and eddoe. This indicated that shoot initiation performance varied with the particular genotype of the same species.

On the other hand, the lower percentage of shoot induction with increasing level of BAP above optimal concentration is in accordance with the work of other root crops such as anchote (Bekele *et al.,* 2013) and cassava (Beyene et al., 2010). According to these studies, using BAP above a certain optimum level decreased the shoot proliferation rate either by direct inhibition of the produced shoots or by encouraging callusing in the *in-vitro* culture plant. Here, such an effect was observed, when a higher concentration of BAP (above 8 mg/L) alone was used.

Table 4. Effect of various types and concentration of auxins on percentage of rooting, number of roots per shoot, root and shoot length of *C. esculenta.*

Concentration of PGRs (mg/L)			Rooting percent (Mean ± S.D.)	Shoot height (cm) (Mean ± S.D.)	Root number (Mean ± S.D.)	Root length (cm) (Mean ± S.D.)
IBA	NAA	IAA				
0	0	0	0.00±0.00^{h}	2.2±0.28^{g}	0.00±0.00^{g}	0.00±0.00^{j}
0	0	0.5	86.25±2.5^{a}	6.20±0.16^{a}	6.90±0.09^{a}	6.93±0.09^{c}
0	0	1	70.00±4.08^{c}	5.50±0.18^{b}	5.40±0.16c^{b}	9.63±0. 48^{b}
0	0	1.5	57.50±2.88^{e}	5.10±0.24^{cb}	3.85±0.19^{d}	11.25±0.5^{a}
0	0.5	0	61.25±2.5d^{e}	4.15±0.18^{e}	4.95±0.19^{c}	5.90±0.19^{d}
0	1	0	78.75±2.5^{b}	4.25±0.14^{ed}	5.80±0.23^{b}	5.20±0.16^{e}
0	1.5	0	67.5±2.89^{dc}	4.70±0.25^{cd}	5.30±0.35^{c}	4.20±0.18^{f}
0.5	0	0	47.59±6.45^{f}	4.40±0.26^{ed}	3.40±0.33^{e}	3.6±0.32^{g}
1	0	0	45.00±4.08^{gf}	3.50±0.24^{f}	3.15±0.29^{e}	3.10±0.20^{h}
1.5	0	0	38.75±4.78^{g}	3.30±0.27^{f}	2.60±0.26^{f}	2.30±0.26^{j}
	CV		6.6	4.95	5.46	5.38

Data were given as Mean ± S.D., mean within a column followed by the same letters were not statistically significant at α = 5% probability by Ryan-Einot-Gabriel-Welsh Multiple range Test (REGWQ).

Shoot multiplication

The highest shoot multiplication result in terms of shoot number and length per explant from MS media supplemented with 8 mg/L BAP and 3 mg/L IAA. As indicated in Table 3, with increase in the concentration of BAP alone from zero to 8 mg/L, there was an increase in number of shoots per shoot bud cultures. This might be due to the effect of cytokinin (BAP) in releasing lateral bud shoots through breaking apical dormancy by inhibiting the effect of high level of edophytic auxins. This result agrees with the report of Chien-Ying et al. (2008). This is however, slightly in contrast to earlier reports in *C. esculenta* by Ngetich et al (2015). This difference might be because of the genotypic variation of the plant. On the other hand, further increases in BAP concentration to 10 and 12 mg/L, shoots become few in number, very short and starts to develop the abnormal morphological appearance of shoots. This might result from the use of higher level of cytokinins (BAP) above the optimum level. The inhibition of *in-vitro* growth of crop plants with an increase BAP concentration above certain optimal concentration was observed by Bekele et al. (2013) and Beyene et al. (2010). From combination media tested, medium containing 8 mg/L of BAP with 2 and 3 mg/L IAA elicited optimal response in terms of average shoot numbers and length as compared to media containing only BAP, of which, MS media containing 8 mg/L BAP and 3 mg/L IAA showed a relatively highest response in terms of quality shoots, average shoots and length, in which, shoots with very good morphological appearance (reasonable shoot height, stem thickness and leaf structure in comparison with the other combinations) were obtained (Figure 1E).

A comparatively lower response in shoot number, average height and bad-looking shoots were observed when BAP at higher concentration combined with higher auxins concentration. This might be related with the interacting effect of the two growth regulators at this level of concentration or due to the use of supra-optimal concentration of BAP. Here, combination of a relatively lower auxin with a relatively higher cytokinin promote the shoot number and shoot height on this stage.

The average number of shoots and length obtained from the this study were in agreement with the work of Chien-Ying et al*.* (2008) who recorded an average of 5.9 shoots per explants using MS + 8 mg/L of BA + 3 mg/L IAA while in this work, using BAP instead of BA in combination with 3 IAA mg/L an average number of shoots 8.53 with an average length of shoot 6.7 cm was recorded. A medium with free growth regulators and a medium with high concentration of BAP alone and in combination with IAA resulted in low multiplication rate (Table 3). Length of shoots that were obtained from sprout tip explants was slightly shorter than from corm base explants.

The good looking response in shoot numbers and length on MS multiplication media containing IAA also reported on other cocoyam species (*C. esculenta and X. sagittifolium*) by Bari and Paul (2007), Kambaska and Sahoo (2008) and Chien-Ying et al. (2008).

Rooting and acclimatization

As clearly indicated in Table 4, root initiation of the cultured shoot of *C. esculenta* was influenced by the type of auxins and concentration. IAA at a lower level of concentration vigorous root looking with highest shoot number was given. Root initiation percent, as well as number of roots, per culture increased with increase IAA levels zero up to 0.5 mg/L and then ceased. Among the

given concentration of auxins, IAA with lower concentration (0.5 mg/L) resulted in the highest rooting percentages (86.25±2.5%) as compared to higher level of IAA (57.50±2.88%). Maximum average numbers of the root (6.90±0.09) was produced from ½ MS media augmented with 0.5 mg/L IAA (Table 4 and Figure 1F).

Rooting response in terms of root percent and average root number from ½ MS+0.5 mg/L IAA media agrees with the work of Bhuiyan et al. (2011). The result in root initiation and average root numbers per plantlets were higher in this study as compare to the earlier report. This variation in figure might be due to the duration of culture stayed on the rooting media. In the previous study, rooting status has been recorded after 10 days of culture while in this study rooting response was recorded after a month of culture on the rooting media. An increase in root numbers and length per plantlet with duration of time on rooting media has been indicated in micro-propagation work of Yambo and Feyissa (2013) in *Coccinia abyssinica.* The result of this study is also near to the study on other coccame species (*X. sagittifolium)* by Bari and Paul (2007).

The effect of different levels of NAA on root initiation for the selected variety was also tested. Like IAA, NAA also enhanced rooting in *in-vitro* cultures. Table 4 showed that root initiation percent was increased with an increased level of NAA from zero up to 1.0 mg/L, and then it declined. The highest root initiation (78.75±2.5%) was given by the treatment of 1.0 mg/L NAA, whereas, 1.5 mg/L NAA gave a lower percentage of roots (67.50±2.89%). It is clear that auxins are responsible for root initiation, auxins up to a certain level enhanced rooting but excess auxins might work as anti-auxins activity and reduced root initiation percent (Hailu et al., 2013).

Similarly, the number of roots increased with an increase of NAA level up to 1.0 mg/L. The highest average number of 5.80±0.23 roots/culture was obtained from ½ MS medium containing 1.0 mg/L NAA. The highest level of NAA (1.5 mg/L) reduced the number of roots/culture (5.30±0.35). This might be due to the anti-auxin effect of excess auxin (Hartmann et al., 2002). The effects of NAA on rooting status (both rooting percent and number of roots per culture) increased with level of NAA up to 1 mg/L is in accordance with the micro-propagation work of Bhuiyan et al. (2011) in *C. esculenta* var. globulifera. But, it is slightly against the study of Kambaska and Sahoo (2008) in *C. esculenta* cv. Jhankhar, who reported a higher level of NAA (2 mg/L), showed the highest result (95% rooting response) and average roots of 6.4±0.30 per culture. This agreement might be as a result of the particular genotype of the stock plant used (Aggarwal and Barna, 2004).

In this study, root regeneration performance for *C. esculenta* cv. Bolosso I using IBA was also tested. In all case, IBA was not effective and showed the lowest result (47.59±6.45%) on initiation as compared to IAA and NAA. Better effect of both IAA and NAA reported for different cultivars of taro in different authors (Kambaska and Sahoo, 2008; Bhuiyan et al., 2011; Verma and Cho, 2010; Bari and Paul, 2007). In the present study IAA and also NAA at lower concentration (0.5 up to 1 mg/L) showed optimum result whereas concentrations above 1 mg/l is supra-optimal for *in-vitro* root regeneration for micro-propagation of taro (*C. esculenta* cv. Bolosso I). Such protocol in *in-vitro* rooting is important for the successful establishment of regenerated taro plantlets in the soil and finally useful for the improvement of other related root crops through modern biotechnology.

For acclimation, *in-vitro* grown plantlets which developed well in the rooting media (Figure G) were transferred to plastic pots containing the soil mix of sand, red soil and coffee husk in different ratio with a polyethylene cover to overcome high humidity. For this purpose, three soil media were tested to compare survival percentage of *in-vitro* plantlets during the hardening process. The results indicated that taro plantlets could survived on all tested media with a good overall survival percentage (76.67%). Although the survived plantlet performance is good in all cases; the different ratio of those soil types showed various survival percentages. Soil media containing sand, red soil and coffee husk in the ratio of 2:1:2 showed highest survival rate (80%) as compared to another ratio of the same soil type (Figure 1H). This result might be a good indication that taro needs fertile soil as it is cultivated closer to the residence and naturally grown to riverside areas where different compost and sandy soil are available. The requirement of fertile soil (soil with dung) and aerated soil such as sandy soil for hardening of taro during acclimatization is also stated by Chien-Ying et al. (2008) and Fujimoto (2009).

Conclusion

At sterilization stage, 2.0% active chlorine in NaOCl for 15 min with 70% alcohol dip was best for surface sterilization of taro explants. At multiplication stage, the MS medium supplemented with 8 mg/L BAP and 3 mg/L IAA showed highest number of shoots in both explants. At rooting stage, the highest number of roots per shoots was achieved on half strength MS medium containing 0.5 mg/L IAA after 30 days of culture. At acclimatization stage, a soil mix of sand + red soil + coffee husk at the ratio of 2:1:2 respectively showed the highest surviving percentage.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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