academicJournals

Vol. 13(49), pp. 4508-4514, 3 December, 2014
DOI: 10.5897/AJB2014.14141
Article Number: 2BB75DB48923
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Optimization of *in vitro* regeneration and microcorm induction in elephant foot yam (*Amorphophallus paeoniifolius*)

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Received 31 August, 2014; Accepted 28 November, 2014

Elephant foot yam (*Amorphophallus paeoniifolius)* is a vegetatively propagated stem tuber crop. In this investigation we describe a highly competent and reproducible *in vitro* propagation of the plant from corm bud, petiole and young leaf explants. Friable callus was initiated from all the explants on modified MS medium (half the concentration of NH_4NO_3 and KNO_3) supplemented with 0.5 mg l^{-1} each of benzyl amino purine (BAP), α-naphthalene acetic acid (NAA) and 2,4-dichloro phenoxy acetic acid (2,4-D). Shoot regeneration from calli was optimal on modified MS medium supplemented with 5.0 mg l^{-1} BAP and 1.0 mg l^{-1} NAA. Microcorms, capable of producing micro shoots all over the surface, were induced from the callus at a frequency of 90% on shoot regeneration medium supplemented with 5% sucrose. Rooting was 100% on modified liquid MS medium augmented with 5.0 mg l^{-1} Indole 3- butyric acid (IBA). A 100% survival rate of plantlets on transplantation to soil: sand: coir pith mixture was recorded.

Key words: Callus, elephant foot yam, *in vitro*, microcorms, regeneration, somatic embryo.

INTRODUCTION

Elephant foot yam or *Amorphophallus paeoniifolius* (Dennst.) Nicolson is a tropical tuber crop of South-East Asian origin belonging to the family *Araceae*. The plant is distributed throughout India, Australia, Philippines, Madagascar and Sri Lanka (Chandra, 1984). The plant is cultivated as an intercrop with banana, turmeric and coconut (Ravi et al., 2009). It is a cash crop due to its high production potential (50 to 60 t ha^{-1}), farm income and export potential (Misra et al., 2001; Srinivas and Ramanathan, 2005). The corms and pseudostems of elephant foot yam are used popularly as vegetables. The tubers are used for preparing ayurvedic medicines as they are anti-inflammatory, anti-haemorrhoidal, astringent, haemostatic, digestive, appetizer, anodyne, rejuvenating and tonic (Misra et al., 2002). They are used in the treatment of tumors, elephantiasis, inflammations, cough, asthma, vomiting, flatulence, colic, dyspepsia, constipation, fatigue and anaemia (Nair, 1993). The plant starch is easily extractable and is with good viscosity, stability and suitability for many applications in food industry (Moorthy et al., 1994). The high incidence of mosaic disease, corm dormancy and non-availability of quality planting materials are the major production constraints in elephant foot yam (Khan et al., 2006; Venkatram et al., 2007). The use of

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Table 1. Effect of PGRs on callusing response of different explants of elephant foot yam.

PGRs in modified MS media (mg l^{-1})			Days to callus induction			% Callusing		
BAP	NAA	2,4-D	Corm bud	Petiole	Leaf	Corm bud	Petiole	Leaf
0.0	0.0	0.0	0	0	0	0	0	0
0.5	0.0	0.0	0	0	0	0	0	0
0.0	0.5	0.0	0	0	0	0	0	0
0.0	0.0	0.5	25.43[a]	0	0	56[d]	0	0
0.5	0.5	0.0	21.52[b]	29.00[a]	59.00[a]	72[b]	40[c]	20[c]
0.5	0.0	0.5	25.46[a]	0	0	52[e]	0	0
0.0	0.5	0.5	25.84[a]	0	0	52[e]	0	0
0.25	0.25	0.25	21.35[b]	29.35[a]	50.70[b]	60[c]	56[b]	48[b]
0.5	0.5	0.5	13.91[c]	15.56[b]	30.40[c]	88[a]	72[a]	60[a]

[a]Means with a same letter within a column do not differ significantly according to the LSD test (p≤0.05) following ANOVA using the SAS system. Plant growth regulators (PGRs).

disease free planting material can be a cultural measure of immediate success as proved in many previous studies (Hollings et al., 1965). High quality planting material of elephant foot yam can be obtained through tissue culture but the method, specifically the process of regeneration is far from being routine due to its recalcitrant nature (Mukherjee et al., 2001).

Also, the plant being a stem tuber and the corm bud being one of the most studied explant, obtaining contamination free starter cultures is the most challenged part in the tissue culture. However, tissue culture of *A. paeoniifolius* has been achieved earlier with limited success (Mukherjee et al., 2009). The *in vitro* regeneration through callus and/ somatic embryogenesis can be employed for effective regeneration thereby mass propagation through tissue culture (Hu et al., 2005; Zhao et al., 2012). In the present study, an efficient *in vitro* callus multiplication and regeneration protocol in elephant foot yam was developed using corm bud, petiole and leaf explants. The callus cultures were used as a source for microcorm induction for enhanced mass propagation of this plant.

MATERIALS AND METHODS

Plant material and establishment of aseptic starter cultures

The source plants of elephant foot yam were grown in open field of ICAR-Central Tuber Crops Research Institute (CTCRI), Kerala, India. 50 explants each of corm buds, petioles and young leaves collected from the field grown plants were used for getting aseptic starter cultures. Apical buds as well as lateral dormant buds of field grown corms of elephant foot yam were scooped out and rinsed under running tap water for 30 min and surface sterilized with 0.1% (w/v) bavistin for 30 min followed by 0.05% (w/v) streptomycin for 1 h, 0.1% (w/v) mercuric chloride for 1 min and 70% (v/v) ethanol for 1 min. Each treatment was followed by washing three times with sterile distilled water. The petiole and leaf explants were surface sterilized with bavistin (0.1% w/v) for 1 h followed by streptomycin (0.05% w/v) for 1 h, mercuric chloride (0.1% w/v) for 5 min and ethanol (70% v/v) for 1 min. As an alternative, one step surface sterilization procedure was done by dipping in absolute ethanol and flaming the unopened leaves covered by cataphylls to get petiole and young leaf explants. The explants were inoculated on modified Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with half the concentration of NH_4NO_3 and KNO_3 supplemented with different plant growth regulators (PGRs).

Effect of different PGRs on callus induction

Modified MS medium supplemented with various combinations of 6-benzylaminopurine (BAP), α-naphthalene acetic acid (NAA) and 2,4-dichloro phenoxy acetic acid (2,4-D) were used for callus induction (Table 1). For each treatment, 25 explants each of corm buds, petioles and young leaves were used. The experiments were repeated thrice for reproducibility and accuracy. All the components of the medium including 3% sucrose and PGRs were mixed and adjusted to pH 5.6 prior to adding 0.7% plant agar for solidifying the media. The cultures were incubated under light intensity of 2500 lux with 16 h light and 8 h dark cycle. The temperature was maintained at 25 ± 2°C throughout the incubation period. Observation on the days to callusing, callusing % and the nature of callus were recorded. The effect of PGRs on Growth Index (GI) was calculated by taking 10 sets of callus cultures obtained from different explants. Calli were cut into pieces to make the initial fresh weight (FW) 100 mg and the GI was recorded after 60 days of growth. The GI was calculated as:

GI = (Final callus FW - Initial callus FW)/ Initial callus FW.

Shoot regeneration, microcorm induction and *in vitro* rooting

Shoot regeneration was optimised by culturing the calli on modified MS medium with various combinations of BAP and NAA in the ratio 5:1 along with control (without PGRs). For each treatment, 24 sets of calli of FW 250 mg were used and the days taken for shoot initiation, number of shoots per mass of calli and percentage response was recorded. A total of 20 callus mass of size 1 cm^2 were inoculated on optimal shoot regeneration medium supplemented with various concentrations of sucrose ranging from 1 to 10% to find its effect on microcorm induction. The experiment was repeated twice. Subculture of microcorms was done in every 60 days. Rooting of the regenerated shoots was attempted on regeneration medium as well as on modified MS medium supplemented with various auxins. Trials with liquid medium instead of agar solidified medium were carried out for rooting.

Acclimatization and hardening

Well rooted plantlets as well as rooted/unrooted microcorms were removed from the culture bottle and were treated with 0.2% bavistin for 5 min. The unrooted microcorms were treated *ex vitro* with 5.0 mg l^{-1} Indole 3- butyric acid (IBA) for 5 min. Transplantation was done on potting mixture [(soil: sand: coir pith, 1:1:1 (v/v/v)]. The plantlets were kept covered in trays/pots using transparent poly covers with holes and were allowed to grow in the net house. After a week, or when the plants started showing signs of healthy growth, the plants were transferred to normal external environmental conditions, but in shade. The plants were thereafter transferred to soil kept in pots or in the field. Survival rate of the *in vitro* hardened plants on monthly interval up to 6 months were recorded.

Statistical analyses

Analysis of Variance (ANOVA) and comparison of the mean value of different treatments using Least Significant Difference (LSD) was performed with the SAS software (SAS, 2010).

RESULTS AND DISCUSSION

Initiation and multiplication of callus from different explants

The method of surface sterilisation followed for corm bud, petiole and leaf explants of elephant foot yam proved to be effective in getting 70% of contamination free cultures. The use of fungicide, mercuric chloride and ethanol in surface sterilisation has proved effective in initiating *in vitro* cultures using explants taken from underground parts (Nongalleima et al., 2014). The method of dipping in ethanol and flaming proved to be effective in getting 75% aseptic starter cultures from leaf and petiole explants, thus avoiding long steps of surface sterilisation normally followed for these explants. The treatment of ethanol dip and flame has been utilised successfully in establishing aseptic starter cultures from difficult to sterilise explants (Sugii, 2011). In the present study, modified MS medium with half the concentration of NH_4NO_3 and KNO_3 was used instead of basal MS medium (with normal nutrient concentration) due to the prominent occurrence of necrosis and yellowing in the initial stages of development observed in preliminary studies, which inhibited the callus induction and further growth. The positive effect of altered concentrations of NH_4NO_3 and KNO_3 on *in vitro* culture growth has been reported in tobacco (Ramage and Williams, 2002), *Gossypium hirusutum* (Ikram-ul-Haq and Zafar, 2004) and *Azadirachta indica* (Srinidhi et al., 2008). In this experiment the tissue culture response of elephant foot yam differed with the explant type as well as with the combinations of PGRs used in the medium. Small sprouts initially appeared from the corm bud explants which then bulged leading to callus initiation (Figure 1a). Enormous bulging followed by callus initiation was observed in petiole and leaf explants (Figure 1b and 1c). Time taken for callus initiation and the percentage of explants responded differed significantly depending on the explant type and the combinations of PGRs used (Table 1). The use of 2,4-D alone in the medium resulted in callus induction in corm bud explant within 56 days while it failed to induce callus from other explants. Callus was induced from corm bud, petiole and leaf explants in 21, 29 and 59 days, respectively, with a callusing percentage ranging from 20 to 72 depending on the explant type on medium supplemented with 0.5 mg l^{-1} each of BAP and NAA. The BA/NAA combination has been used with great success in the callus induction of *Amorphophallus* spp. (Hu and Liu, 2008; Anil et al., 2012). A combination of all the 3 PGRs (BAP, NAA and 2,4-D) in 0.25 mg l^{-1} concentration resulted in increased callusing % in petiole and leaf explants.

However, the combination was not effective in the case of corm buds. The increase in the concentration of all the 3 PGRs from 0.25 to 0.5 mg l^{-1} decreased the callus initiation time and increased the callusing %. Thus, modified MS medium supplemented with 0.5 mg l^{-1} each of BAP, NAA and 2,4-D (Callus Induction or CI medium) was found to be optimal for callus induction as well as callus multiplication and was therefore used in further studies. Yellowish white or pink friable callus was initiated within 15 to 30 days of inoculation from corm bud, petiole and leaf explants on CI medium (Figure 1d). In an earlier report, it took more than 20 to 24 weeks for callus induction from apical bud explants of the crop with limited survival/multiplication rate while the petiole gave rise to callus in a month time (Anil et al., 2012). The effect of PGRs on the nature of callus and GI recorded is shown in Table 2. Irrespective of the explant type, GI as high as 8 was observed on callus induction medium. While on MS medium with half of the concentration of PGRs (0.25 mg l^{-1} each of BAP, NAA and 2, 4- D), the GI was markedly reduced to ~ 3 and did not differ significantly with the explant type. When 0.5 mg l^{-1} of BAP and NAA was used without 2,4-D, the GI decreased to 2.5 in all the 3 different explants. Medium without any of the 3 PGRs either failed to induce callus or if induced showed reduced GI. Hence, for elephant foot yam, irrespective of the explants, the combination of the 3 PGRs (BAP, NAA, 2,4-D) at 0.5 mg l^{-1} concentration is essential for callus induction with enhanced GI. In *Amorphophallus konjac* also friable calli was induced when appropriate combinations of BAP, NAA and 2,4-D was used, although the callus induction rate was as low as 35.5% (Zhao et al., 2012). Subculture of callus was done in 60 days interval on CI medium.

Shoot regeneration and microcorm induction from friable callus

Vigorous growth followed by emergence of greenish micro shoot like structures was observed within 25 to 40 days when friable calli were sub cultured on MS medium augmented with various combinations of BAP and NAA in

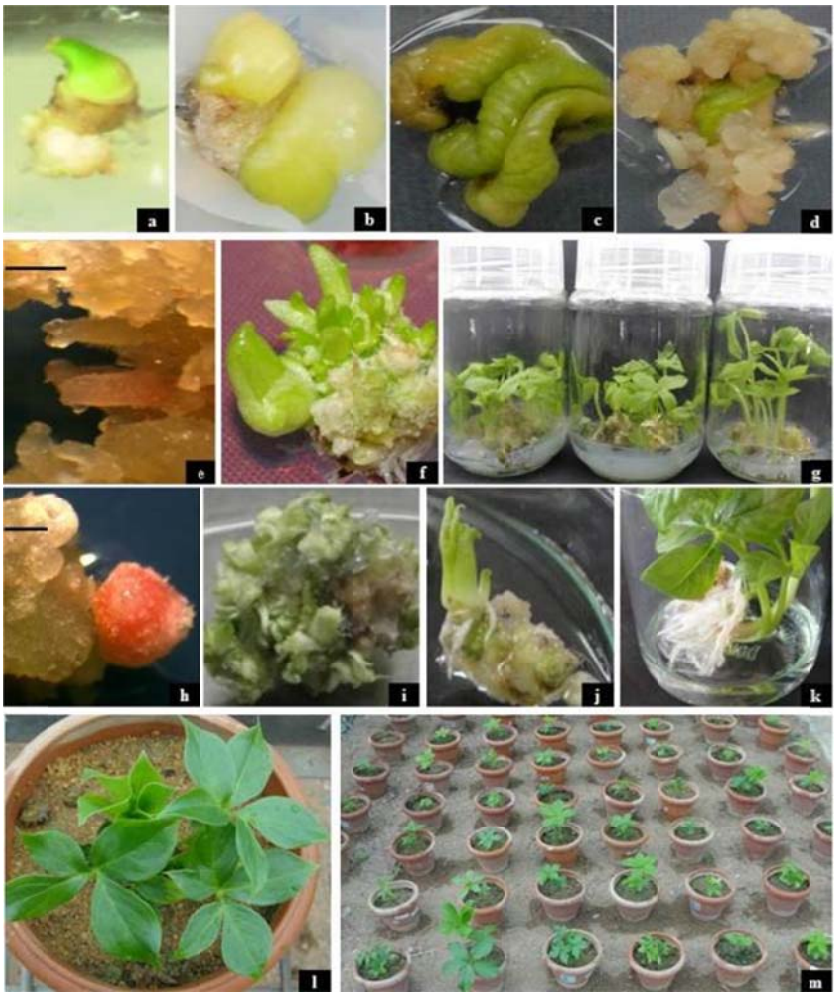

Figure 1. Various stages in the *in vitro* propagation of elephant foot yam. **a.** sprouted corm bud. **b.** bulged petiole. **c.** expanded leaf, **d.** callus. **e.** Somatic embryos. **f.** Shoot initials arising from callus. **g.** Shoot multiplication. **h.** Microcorm arising from callus. **i.** Microshoots arising all over the surface of microcorm. **j.** Rooted microcorm. **k.** *In vitro* rooted plants. **l.** Plant clusters arising from microcorm lacking shoots and roots on transplantation. **m.** Hardened plants kept in net house. Bar = 2 mm.

Table 2. Effect of PGRs on callus nature and growth index (GI) of different explants of elephant foot yam.

PGRs in modified MS media (mg l^{-1})			Nature of callus			GI after 60 days (100 mg Initial FW)		
BAP	NAA	2,4-D	Corm bud	Petiole	Leaf	Corm bud	Petiole	Leaf
0.0	0.0	0.0	NC	NC	NC	0	0	0
0.5	0.0	0.0	NC	NC	NC	0	0	0
0.0	0.5	0.0	NC	NC	NC	0	0	0
0.0	0.0	0.5	W,C	W, C	W, C	2.25[d]	0	0
0.5	0.5	0.0	W/P, C	W/P, C	W/P, C	2.58[c]	2.38[c]	2.51[c]
0.5	0.0	0.5	W, C/F	W, C	W, C	2.17[d]	0	0
0.0	0.5	0.5	W, Pu/F	W, Pu	W, Pu	1.32[e]	0	0
0.25	0.25	0.25	YW, F	YW, F	YW, F	3.00[b]	2.90[b]	3.08[b]
0.5	0.5	0.5	YW/P, F	YW/P, F	YW/P, F	8.83[a]	8.83[a]	8.47[a]

[b]Means with a same letter within a column do not differ significantly according to the LSD test ($p \leq 0.05$) following ANOVA using the SAS system. NC- Callus not induced, W-White, P-Pink, YW-Yellowish white, C-Compact, Pu-Puffy, F-Friable. Benzyl amino purine (BAP), α-naphthalene acetic acid (NAA), plant growth regulators (PGRs).

Table 3. Effect of combination of PGRs on *in vitro* shoot regeneration in elephant foot yam from callus.

Medium number	PGRs (mg l^{-1})		Days to shoot initiation	% Shooting	Mean number of shoots per cluster	Mean shoot length (cm)
	BA	NAA				
Control	0	0	0	0	0	0
1	1.0	0.2	38.83[a]	16.37[g]	1.33[f]	3.83[c]
2	2.0	0.4	27.67[b]	70.75[d]	7.33[e]	4.25[b]
3	3.0	0.6	27.25[bcde]	69.87[d]	9.00[d]	3.25[d]
4	4.0	0.8	25.33[f]	91.12[b]	18.67[b]	3.67[c]
5	5.0	1.0	24.58[g]	95.87[a]	22.00[a]	6.17[a]
6	6.0	1.2	26.92[e]	75.50[c]	12.67[c]	0.70[e]
7	7.0	1.4	27.08[de]	57.62[f]	12.67[c]	0.29[f]
8	8.0	1.6	27.17[cde]	57.25[f]	12.17[c]	0.27[f]
9	9.0	1.8	27.50[bcd]	60.75[e]	9.00[d]	0.22[f]
10	10.0	2.0	27.58[bc]	61.75[e]	9.00[d]	0.19[f]

[c]Means with a same letter within a column do not differ significantly according to the LSD test ($p \leq 0.05$) following ANOVA using the SAS system. Benzyl amino (BA), α-naphthalene acetic acid (NAA) plant growth regulators (PGRs).

the ratio 5:1 (Table 3). Somatic embryos developed within 15 to 20 days on the same medium (Figure 1e) which followed the micro shoot appearance. The length of somatic embryos ranged from 2 to 8 mm. The friable nature of callus was found to be essential for somatic embryo induction. In *Amorphophallus rivieri* and *A. konjac* somatic embryos were induced from compact and incompact callus, respectively (Hu et al., 2005; Zhao et al., 2012). For somatic embryogenesis, no special incubation was required; as against the necessity of dark incubation in many crops (Ozias-Akins and Vasil, 1983; Compton, 1999). There was no significant difference (at $p \leq 0.05$) between the response of calli developed from different explants on further growth and plant regeneration. The medium devoid of any of PGRs (basal medium) failed in shoot regeneration, while there was a steady increase in the shooting % with increase in the concentrations of BAP and NAA in the 5:1 combination from an initial concentration of 1 mg l^{-1} BAP and 0.2 mg l^{-1} NAA. The days for shoot initiation decreased from 38 to 24 with the increase in the BAP: NAA concentration from 1: 0.2 to 5:1. However, a further increase in the concentration did not show any improvement in the shooting %. Thus, modified MS medium containing 5.0 mg l^{-1} BAP and 1.0 mg l^{-1} NAA (Shoot Regeneration or SR medium) was found to be optimum for shoot regeneration (Figure 1f) of up to 95%. On SR medium, a mean shoot number of 22 with an average shoot length of 6 were recorded in 3 months. The maximum shoot regeneration percentage reported so far is 78% in *Amorphophallus albus* (Hu et al., 2004) on medium containing BAP and NAA in 4:1 ratio. In the present study, the number of shoots produced depended on the callus subculture rate rather than the number of explants. Shoots of length 3 to 9 cm developed within a period of 3 months (Figure 1g). The callus from lateral bud explants of *Amorphophallus campanulatus* yielded less number of plantlets as well as shooting percentage even after a prolonged period of culture (Irawati et al., 1986). In elephant foot yam, a maximum of 15 shoots per segmented explant or 2 to 3 shoots per whole explant were obtained through direct organogenesis by Mukherjee et al. (2009). The method explained in this study is efficient in producing about 25 to 30 plants per subculture per 250 mg callus. Thus, the number of planting materials available from a single explant depends on the subculture frequency. The corm bud was found to be the most suitable explant for mass production in elephant foot yam due to its high callusing % and less time for plantlet establishment as against the previous reports on the need of long retention time in medium for positive response (Anil et al., 2012).

The effect of sucrose concentration in SR medium on callus response and microcorm induction was studied. The callus proliferated enormously on SR medium supplemented with 1 and 2% sucrose while there was a tendency towards the plant regeneration with 3% sucrose. Pink, off white or dark brown microcorms were induced as hard globular structures in between friable callus in SR medium with sucrose concentrations between 3 and 7% (Figure 1h). They were induced with a frequency of 90% on SR medium supplemented with 5% sucrose while it was only 10% with 3 or 4% sucrose. With 6% sucrose, the induction % was 70 whereas with 7%, there was a tendency to microcorm enlargement rather than its multiplication. The sucrose concentrations beyond 7% lead to darkening of the callus while 10% sucrose was detrimental to growth. Various studies show the importance of sucrose concentration on microcorm/microtuber induction (Dantu and Bhojwani, 1995; Gopal et al., 1998; Sinha and Roy, 2002). In *Amorphophallus* spp., the concentrations of PGRs were reported to play a role in the formation of corm-like structures (CLS) (Liu et al., 2001; Hu et al., 2006; Anil et

Table 4. Effect of auxins on rooting in elephant foot yam under *in vitro* conditions.

PGRs (mg l^{-1})			Days to root initiation	% Rooting	Number of roots	Mean root length (cm)
BA	NAA	IBA				
5.0	1.0	0.0	60.30[a]	50[c]	8.55[c]	3.81[b]
0.0	5.0	0.0	21.20[b]	80[b]	13.3[b]	3.52[b]
0.0	0.0	5.0	8.65[c]	100[a]	20.9[a]	5.07[a]

[d]Means with a same letter within a column do not differ significantly according to the LSD test (p≤0.05) following ANOVA using the SAS system, plant growth regulators (PGRs).

al., 2012) rather than the sucrose concentration as shown in this study. CLS had been observed on organogenic calli derived from the petioles of *Amorphophallus* spp. *in vitro* (Hu and Liu, 2008; Anil et al., 2012). However, a prolonged retention time in the medium was required for the CLS formation in the callus derived from corm bud explants (Irawati et al., 1986; Anil et al., 2012). The microcorms were separated from the callus and subcultured on the SR medium. They enlarged when kept on the same medium for more than 60 days. The enlargement was enormous when the sucrose concen-tration in the SR medium was increased from 3 to 5%. However for microshoot regeneration, the normal sucrose concentration (3%) remained the best. The large sized microcorms were cut into pieces for further multiplication. They were capable of producing microshoots all over the surface (Figure 1i).

In vitro rooting and transplantation of plantlets to soil

In about 50% of the cultures, roots developed on the SR medium itself after 60 days. But the root length as well as the root number was enhanced when a separate rooting media was used. The inclusion of 5 mg l^{-1} NAA or IBA in the modified basal MS medium increased the rooting % to 80 and 100, respectively. The days to root was decreased from 60 to 8 with the supplementation of IBA in the medium. Therefore, modified MS medium augmented with 5.0 mg l^{-1} IBA (Rooting or R medium) was found to be the most potent rooting medium for elephant foot yam (Table 4). A mean number of 21 roots with an average root length of 5 were recorded in 25 days. IBA had been reported to be the most favourable root inducer compared to Indole 3- acetic acid (IAA) and NAA (Caboni and Tonelli, 1999). IBA acts as a slow release reservoir of a more easily metabolized auxin, whereas NAA, blocks the root emergence as it remains in the tissue in free form due to its stable nature (Fogaca and Fett-Neto, 2005). However in *A. konjac*, NAA proved to be efficient in root induction (Zhao et al., 2012). The number of roots obtained in the present study is much better than those reported in previous studies on this plant (Mukherjee et al., 2009). The use of liquid medium in the rooting phase considerably decreased the time required in agar removal and post hardening contamination (due to the presence of poorly removed agar from finely honed root hairs). The microcorms as such or with plantlets rooted within 15 days when kept on liquid R medium (Figure 1j). In *A. albus*, rooting percentage of *in vitro* corm like structures was not significantly affected by the presence of auxins (Hu and Liu, 2008). The plants rooted in liquid medium are shown in Figure 1k. The unrooted microcorms from the SR medium on direct transplantation after dipping in 5 mg l^{-1} IBA gave rise to plant clusters (Figure 1l). It took approximately 7 months from explant inoculation to reach the hardening stage. Poor survival rates on hardening have so far been reported in this plant (Anil et al., 2012). However in the present study, a survival rate of 100% was observed when the plants/microcorms were transferred to sand: soil: coir pith mixture. The hardened plants were maintained in the net house, ICAR-CTCRI (Figure 1 m).

A high frequency *in vitro* mass propagation of elephant foot yam, a stem tuber crop, using different explants was developed. The present study can be extended for virus elimination and transformation for inducing desirable traits in this plant. As the protocol was found efficient in producing large number of plantlets from less number of explants, it can be commercially exploited for medicinal purpose as well as for its value addition.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Director, CTCRI for extending all the facilities to carry out this work, Dr. S. Chandra Babu for his guidance in corm bud excision, Dr. J. Sreekumar for his help with statistical analysis and Council for Scientific and Industrial Research, India for providing Junior Research Fellowship to the first author.

REFERENCES

Anil SR, Siril EA, Beevy SS (2012). *In vitro* propagation strategies for Elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson. J. Root Crops 38:97-108.

Caboni E, Tonelli MG (1999). Effect of 1,2-benzisoxazole-3-acetic acid on adventitious shoot regeneration and *in vitro* rooting in apple. Plant Cell Rep. 18: 985-988.
Chandra S (1984). Edible Aroids. Clarendon Press, Oxford, p. 252.
Compton ME (1999). Dark pre-treatment improves adventitious shoot organogenesis from cotyledons of diploid watermelon. Plant Cell Tissue Org. Cult. 58: 185-188.
Dantu PK, Bhojwani SS (1995). *In vitro* corm formation and field evaluation of corm-derived plants of *Gladiolus.* Sci. Hortic. 61:115-129.
Fogaca CM, Fett-Neto AG (2005). Role of auxin and its modulators in the adventitious rooting of Eucalyptus species differing in recalcitrance. Plant Growth Regul. 45: 1-10.
Gopal J, Minocha L, Dhaliwal HS (1998). Microtuberization in potato (*Solanum tuberosum* L.). Plant Cell Rep. 17: 794-798.
Hollings M (1965). Disease control through virus-free stock. Annu. Rev. Phytopathol. 3:367-396.
Hu J, Gao X, Liu J, Xie C, Li J (2004). Plant regeneration from petiole callus of *Amorphophallus albus* and analysis of somaclonal variation of regenerated plants by RAPD and ISSR markers. Bot. Stud. 49: 189-197.
Hu JB, Liu J (2008). Morphogenetic pathway in petiole derived callus of *Amorphophallus albus in vitro*. Acta Physiol. Plant. 30: 389-393.
Hu JB, Liu J, Xie CH, Gao XX (2006). Corm induction and multiplication of *Amorphophallus albus in vitro.* J. Hort. Sci. Biotechnol. 81:859-863.
Hu JB, Liu J, Yan HB, Xie CH (2005). Histological observations of morphogenesis in petiole derived callus of *Amorphophallus rivieri* Durieu *in vitro*. Plant Cell Rep. 24:642-648.
Ikram-ul-Haq, Zafar Y (2004). Effect of nitrates on embryo induction efficiency in cotton (*Gossypium hirsutum* L.). Afr. J. Biotechnol. 3: 319-323.
Irawati, Arditti J, Nyman LP (1986). *In vitro* propagation of the elephant yam, *Amorphophallus campanulatus* Var. hortensis Backer (*Araceae*). Ann. Bot. 57:11-17.
Khan NM, Aswathi LP, Singh PK (2006). Survey on symptomatology and assessment of yield losses due to viral diseases in elephant foot yam (*Amorphophallus paeoniifolius* Blume). J. Root Crops 32: 90-93.
Liu J, Xie CH, Yu ZS, Liu Y (2001). Research on the propagation of *Amorphophallus in vitro*. J. Huazhong Agric. Univ. 20:283-285.
Misra RS, Nedunchezhiyan M, Swamy TMS, Edison S (2002). Mass multiplication technique for producing quality planting material of *Amorphophallus paeoniifolius*. Trop. Sci. 34:371-376.
Misra RS, Shivlingaswamy TM, Maheshwari SK (2001). Improved production technology for commercial and seed crops of elephant foot yam. J. Root Crops 27:197-201.
Moorthy SN, Unnikrishnan M, Lakshmi KR (1994). The physicochemical properties of starch of some accessions of *Amorphophallus paeoniifolius.* Trop. Sci. 34: 371-376.
Mukherjee A, Naskar SK, Misra RS, Das BB, Sahoo BK (2001). *In vitro* propagation of tubercrops: Assessment of axillary shoot proliferation. J. Root Crops 27:82-88.
Mukherjee A, Naskar SK, Nedunchezhiyan M, Rao KR (2009). *In vitro* propagation of elephant foot yam. Indian J. Hortic. 66: 530-533.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473-497.
Nair RV (1993). Indian Medicinal Plants vol 3, Orient Longman, Madras, India. pp.118-122.
Nongalleima K, Singh DT, Amitabha D, Deb L, Devi HS (2014). Optimization of surface sterilization protocol, induction of axillary shoots regeneration in *Zingiber zerumbet* (L.) Sm. as affected by season. Biol. Rhythm Res. 45:317-324.
Ozias-Akins P, Vasil IK (1983). Improved efficiency and normalization of somatic embryogenesis in *Triticum aestivum* (wheat). Protoplasma 117: 40-44.
Ramage CM, Williams RR (2002). Inorganic nitrogen requirements during shoot organogenesis in tobacco leaf discs. J. Exp. Bot. 53: 1437-43.
Ravi V, Ravindran CS, Suja G (2009). Growth and productivity of Elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson): an Overview. J. Root Crops 35:131-142.
SAS (2010). SAS Institute Inc. Cary. NC, USA.
Sinha P, Roy SK (2002). Plant regeneration through *in vitro* cormel formation from callus culture of *Gladiolus primulinus* Baker. Plant Tissue Cult. 12: 139-145.
Srinidhi HV, Gill RIS, Sidhu OS (2008). Micropropagation of adult and juvenile neem (*Azadirachta indica* A. Juss). J. Crop. Improv. 21: 221-232.
Srinivas T, Ramanathan S (2005). A study on economic analysis of elephant foot yam production in India. Agri. Econ. Res. Rev. 18: 241-252.
Sugii NC (2011). The establishment of axenic seed and embryo cultures of endangered Hawaiian plant species: special review of disinfestation protocols. *In Vitro* Cell. Dev. Biol. Plant 47: 157-169.
Venkatram R, Mani K, Saraswathi T (2007). Production and marketing of Elephant foot yam in Salem district of Tamil Nadu. J. Root Crops 33:133-137.
Zhao L, Wu J, Diao Y, Hu Z (2012). Embryogenesis and plant regeneration from unpollinated ovaries of *Amorphophallus konjac*. Afr. J. Biotechnol. 11:13472-13476.