- Short communication

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In vitro Shoot Regeneration in Elephant Foot Yam, *Amorphophallus campanulatus* Blume

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Callus induction and shoot regeneration were obtained from petiole explant in MS with different concentrations and combinations of BAP and NAA in elephant foot yam, *Amorphophallus campanulatus* Blume Highest (75%) callus induction was observed in MS with 0.5 mg/l BAP + 2.5 mg/l NAA followed by 65% containing 0.5 mg/l BAP + 3.0 mg/l NAA. In most cases, calli were white, brownish and white pinkish. Highest shoot regeneration (60%) was obtained in MS containing 4.0 mg/l BAP + 1.5 mg/l NAA. The highest number of shoots per callus was 3.00 ± 0.273. The profuse root development was found in MS with 0.5 mg/l Kn + 1.0 mg/l NAA. Regenerated plantlets were transferred to plastic pots containing organic loamy soils and after acclimated, the plants showed normal growth and development.

Elephant foot yam (*Amorphophallus campanulatus* Blume), also called Olkachu in Bengali, is a popular vegetable tuber crop grown in the tropics and belongs to Araceae. It is a stout herbaceous plant with under ground hemispherical, dark brown corm and comparatively low quantity roots and cormels. The genus *Amorphophallus* consists of more than 100 species (Brown 1988) world-wide of which 47 occur in Malaysia, Australia and tropical Western Pacific (Hay et al. 1995). The crop is widely distributed in the Philippines, India, Malaysia, Indonesia, Sri Lanka and South East Asia. In Bangladesh, it is a choiceable vegetable to the hilly people of Chittagong Hill tracts (Rasid 1983) and its whole rhizome is edible in mature stage and pseudo-stem and leaves, in tender stage. Tubers can be utilized both as animal feed and for human consumption (Sastrapradja et al. 1981).

Olkachu fibres contain a good amount of carotene (Rasid 1999) and are a chief source of carbohydrates. Olkachu is rich in minerals and vitamin A and B,

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but poor in protein (Bose and Som 1986). Its tuber contains fat (0.1%), oxalic acid (1.3%), minerals (0.8 %), calcium (50.0 mg/100 g), phosphorus (34 mg/100 g), iron (0.6 mg/100 g), thiamine (0.06 mg/100 g), riboflavin (0.017 mg/100 g), and vitamin A (434 I.U./100 g) (Joshi 2000). It is also a highly potential economic crop widely used in food, medicine and chemical industries due to its high glucomannan content in subterranean corms (Cescutti et al. 2002). A number of medicinal properties are associated with *Amorphophallus* spp. such as glucomannan has been found to reduce the serum cholesterol level in rats and is an ingredient in various ayurvedic medicines (Chopra et al. 1956, Ghani 2003). It is called "Arshoghna" meaning killing piles (Biswas and Ghosh 1976). Due to the absence of natural seed sets, there is little scope of improving this crop by sexual means. Moreover, some high yielding cultivars do not produce cormels resulting in a shortage of propagating materials. With the presence of the biochemical composition and potential medicinal values, an *in vitro* regeneration system is efficient for the production of plants because field grown plants may be subjected to seasonal and somatic variations, infestation of bacteria, fungi and insects as well as environmental pollution that can affect the medicinal value of the harvested tissues. The main objectives were to identify suitable explant source for *in vitro* propagation and to develop an indirect regeneration protocol for disease free rapid multiplication of plantlets within a short period.

Elephant foot yam accessions, collected from Bangladesh Agricultural Research Institute (BARI), Joydevpur and different local cultivated areas of Bangladesh, were maintained in the experimental farm of the Institute of Biological Sciences, Rajshahi University, Bangladesh and were used in this study for *in vitro* propagation.

Leaf petioles were collected from cormel bud explants-derived *in vitro* regenerated plantlet, and were cut into segments of convenient size. Petiole segments from 20 - 25 day-old *in vitro* grown shoots regenerated directly from cormel buds, were also used for shoot regeneration on MS supplemented with 8 g/l agar and 30 g/l sucrose was used as a culture medium to conduct all experiments for callus induction, shoot regeneration, shoot proliferation and root initiation. IAA, 2,4-D and NAA at 0.50, 1.0, 1.5, 2.0 or 2.5 mg/l and BAP and Kn at 0.5, 1.0, 1.5, 2.0 or 2.5 mg/l were used singly or in combinations to investigate the initiation of callus and its subsequent regeneration. The pH of the medium was adjusted to 5.70 ± 0.1 with 0.1N KOH or 0.1N HCl before autoclaving. Culture vessels containing medium were autoclaved at 1.1 kg /cm^2 pressure at 121^o C for 20 min to ensure sterilization. Cultures were grown in the growth chamber illuminated by 40 watt white florescent tubes fitted at a distance of 40 cm from the culture shelves. The cultures were maintained at 27 ± 1^{o}C under the warm

florescent light intensity varied from 2000 - 3000 lux. The photoperiod was maintained at 16 hrs light and 8 hrs dark. The vessels were checked daily to note the explant response for callus induction. Data on days to callus initiation, percentage of callus formation, physical characteristics of calli, shoot regeneration and rooting of regenerated shoots were recorded after 4 - 12 weeks of culture. Experiments were consisted of 15 explants and each of the experiment repeated thrice and mean values were calculated separately for each replication.

Table 1. Effects of different combinations of BAP, NAA and Kn on callus indiction from petiole explants in *A. campanulatus*.

Plant growth regulator (mg/l)	Days to callus initiation	Callus formation (%)	Callus colour	Callus texture	Fresh callus weight (g)	Callus growth
BAP + NAA					Mean ± SE	
0.5 + 0.5	70	30.00	W	F	6.00 ± 0.223	*
0.5 + 1.0	70	50.00	W	F	8.00 ± 0.273	**
0.5 + 1.5	70	60.00	W	F	10.00 ± 0.831	**
0.5 + 2.0	70	60.00	W	F	10.50 ± 0.223	**
0.5 + 2.5	70	75.00	W, P	F	11.00 ± 0.447	***
0.5 + 3.0	70	65.00	W	F	10.00 ± 0.831	**
1.0 + 0.5	70	20.00	W	F	5.00 ± 0.367	*
Kn +NAA						
3.0 + 0.5	70	20.00	W	F	6.00 ± 0.223	*
3.0 + 1.0	70	30.00	W	F	5.00 ± 0.367	*
3.0 + 1.5	70	35.00	W	F	7.00 ± 0.316	*
3.0 + 2.0	70	45.00	W	F	6.50 ± 0.232	*
3.0 + 2.5	70	30.00	W	F	5.50 ± 0.367	*

* poor growth, ** moderate growth, *** profuse growth, W = White, P = Pinkish, F = Friable.

Results obtained on morphogenic response of the cultured explants are shown in Table 1. Frequency of callus formation ranged from 20 to 75%. Highest percentage (75) of callus formation occurred on MS containing 0.5 mg/l BAP and 2.5 mg/l NAA. In most cases, calli were white, brownish or white pinkish. At initiation, calli were white pinkish and protocorm like in structure. The texture of callus was friable and compact. The optimum growth in terms of fresh weight was 11.00 ± 0.45 g on MS with 0.5 mg/l BAP and 2.5 mg/l NAA followed by 10.50 ± 0.22 g on 0.5 mg/l BAP and 2.0 mg/l NAA.

For shoot regeneration, white pinkish friable calli were transferred on MS supplemented with different concentrations and combinations of BAP and Kn alone and in combination with different concentrations of NAA and IAA. Morphogenic potentialities of calli varied with PGR treatments and their results

Table 2. Effects of BAP and NAA combinations on shoot regeneration from *in vitro*-derived petiole explants in *A. campanulatus*.

Plant gwoth regulator (mg/l)	Days to shoot regeneration from callus	Shoot regeneration (%)	Fresh weight of callus with shoot (g) (Mean ± SE)	Organogenic responses		Intensity of callus	Av. number of shoot/culture (Mean ± SE)	Av. length of shoot/ culture (cm) (Mean ± SE)
				Root	Shoot			
BAP + NAA								
3.0 + 0.5	130	30.00	7.00 ± 0.223	*	*	**	1.00 ± 0.00	3.00 ± 0.273
3.0 + 1.0	130	45.00	7.50 ± 0.223	*	***	**	1.00 ± 0.00	2.50 ± 0.273
3.0 + 1.5	130	40.00	7.00 ± 0.223	*	**	**	1.00 ± 0.00	2.00 ± 0.221
3.0 + 2.0	130	35.00	7.50 ± 0.316	*	**	**	1.00 ± 0.00	2.50 ± 0.221
3.0 + 2.5	130	20.00	7.00 ± 0.223	**	**	**	2.00 ± 0.222	2.00 ± 0.221
4.0 + 0.5	130	45.00	8.5 ± 0.316	**	**	***	2.00 ± 0.0.22	3.0 ± 0.273
4.0 + 1.0	130	55.00	10.50 ± 0.316	***	**	**	3.00 ± 0.316	3.50 ± 0.223
4.0 + 1.5	130	60.00	9.50 ± 0.273	**	***	**	2.00 ± 0.316	3.00 ± 0.273
4.0 + 2.0	130	40.00	9.00 ± 0.223	**	**	***	3.00 ± 0.00	2.50 ± 0.273
4.0 + 2.5	130	35.00	8.00 ± 0.221	**	*	poor	1.00 ± 0.00	2.50 ± 0.273
BAP + NAA								
5.0 + 0.5	130	40.00	7.5 ± 0.273	**	**	**	1.00 ± 0.00	4.50 ± 0.316
5.0 + 1.0	130	42.00	7.00 ± 0.316	**	***	***	1.00 ± 0.00	5.00 ± 0.273

* poor growth ,** moderate growth , *** profuse growth.

are presented in Table 2. Highest percentage (60) of shoot regeneration was recorded on a medium with 0.4 mg/l BAP and 1.5 mg/l NAA. The highest number of shoots per callus was recorded (3.00 ± 0.273) on 4.0 mg/l BAP and 2.5 mg/l NAA. Highest shoot length (5.00 ± 0.273 cm) was recorded on media supplemented with 5.0 mg/l BAP and 1.0 mg/l NAA and with 5.0 mg/l BAP and 1.5 mg/l NAA, while the lowest shoot length was 2.0 ± 0.00 cm on 3.0 mg/l BAP and 2.5 mg/l NAA. Shoot regeneration and proliferation was increased with the increase of callus age but after a few months, calli become blackish and were dead. Corm explants of *Amorphophallus rivieri* Durieu was cultured by Irawati et al. (1986). MS supplemented with 1.0 mg/l NAA and 2.0 mg/l BA was found suitable for corm like structures, white translucent callus, pink translucent compact callus, white opaque callus and brown callus in elephant foot yam by Akhond and Ali (1998). They also obtained highest percentage of plant regeneration on MS with 0.2 mg/l NAA and 1.0 mg/l BA. Callus induction in *Amorphophallus rivieri* was reported by Hu et al. (2005), who used petiole explants on MS supplemented with 5.37 μM NAA and 4.44 μM BAP. They also reported that shoot and corm organogenesis occurred from the compact calli when they were transferred to a medium containing 0.54 μM NAA and 4.44 μM 6-BA.

Organogenic responses of fibrous rootings were highest on MS supplemented with 0.5 mg/l Kn + 1.0 mg/l NAA.

Two-month-old individual plantlets after separating from callus and washing in water were planted in plastic pots containing a mixture of cow-dung and loamy fertile soil. Normal cultural operations were followed to grow the plants. It was observed that most of the plantlets survived and acclimated successfully.

Callus induction and shoot regeneration protocols were developed from *in vitro* grown petiole explant with 0.5 mg/l BAP and 2.5 mg/l NAA after 70 days of culture and shoot regeneration with 0.4 mg/l BAP and 1.5 mg/l NAA after 130 days of culture on MS in elephant foot yam, *Amorphophallus campanulatus* successfully.

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