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AXENIC GERMINATION OF SEEDS AND RHIZOME-BASED MICROPROPAGATION OF AN ORCHID *ARUNDINA GRAMINIFOLIA* (D. DON.) HOCHR.

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Key words: *Arundina graminifolia*, *In vitro* germination, Micropropagation

Abstract

Seeds of *Arundina graminifolia* were aseptically cultured on MS, PM and MVW basal media. They germinated on MS and PM media but failed to germinate on MVW. The tiny seedlings continued to grow on the same media but their elongation rate was slow. Rapid elongation took place on hormone supplemented media and the highest rate of elongation was achieved on 0.8 % (w/v) agar solidified MS supplemented with 3 % (w/v) sucrose + 2.5 mg/l BAP + 0.1% (w/v) activated charcoal (AC). The seedlings developed strong and stout roots along with rhizomes on half strength MS containing 1.5 % (w/v) sucrose. Addition of AC in the medium stimulated both rooting and rhizome formation. The rhizome segments were used for micropropagation and that produced highest number of multiple shoot buds on 0.8 % (w/v) agar solidified MS fortified with 3 % (w/v) sucrose + 2.0 mg/l BAP + 1.0 mg/l IAA. MS + 3 % (w/v) sucrose + 1.0 mg/l NAA + 0.5 mg/l BAP proved most effective for enhancing elongation whereas rooting was best on half strength MS + 1.5 % (w/v) sucrose. The *in vitro* developed seedlings were finally transferred to pots by successive phases of acclimatization.

Introduction

Arundina graminifolia (D. Don.) Hochr. is an evergreen terrestrial orchid commonly known as 'bamboo orchid'. Generally it grows in plain and hilly areas of Chittagong, Sylhet and North-eastern India. Flowers of this orchid are attractive and persist for about one and a half months and therefore used as cut flower for decorative purposes. Because of ruthless collection and destruction of habitats, this orchid species has become rare in Bangladesh (Huda *et al.*1999). For protection from extinction and to meet up floricultural demand, it is important to develop techniques for rapid propagation of this species. Nagaraju *et al.* (2001) developed a protocol only for *in vitro* germination of seeds of this orchid species. The present study was undertaken with a view to developing protocol for *in vitro* germination of seeds and rhizome based micropropagation, which can support mass scale production of seedlings for commercial use and conservation purpose.

Materials and Methods

A few fruits of *A. graminifolia* were collected from the Orchid House of the Botanical Garden of Chittagong University and washed with running tap water, rubbed with savlon soaked cotton and washed with distilled water. The surface of fruits were sterilized with $HgCl_2$ and washed with savlon and in a laminar airflow cabinet. Surface sterilized capsules were placed on a sterile aluminum slab and dissected longitudinally with a surgical blade. The seeds were scooped with the help of forceps and inoculated on to the surface of germination medium in culture vessels. Three basal media, namely MS (Murashige and Skoog 1962), PM (Arditti 1977) and MVW (Modified after Vacin and Went 1949) were used for germination. Before inoculation of seeds, the culture vessels containing medium were autoclaved and pH of the media was adjusted to 5.8 in case of MS and 5.4 in PM and MVW. The temperature of the culture room was 25 ± 2°C with a cycle of 14 h

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of continuous light and 10 h dark. After germination the seedlings continued their growth on the same medium very slowly. To induce rapid elongation, the seedlings were transferred on to MS, PM and MVW supplemented with different concentrations and combinations of BAP (0.5 - 3.0 mg/l), NAA (1.0 - 2.0 mg/l), Kn (0.5 - 1.5 mg/l), IAA (0.5 - 1.0 mg/l), picloram (0.5 - 1.0 mg/l), casein hydrolysate (2.0 g/l) and zeatin (1.0 mg/l). For induction of strong root system the elongated seedlings were further grown on rooting media: (i) half strength MS + 1.5 % (w/v) sucrose, (ii) MS + 3 % (w/v) sucrose + 0.5 mg/l IAA and (iii) MS + 3 % (w/v) sucrose + 1.0 mg/l IAA + 0.1 % (w/v) AC, where they produced strong and stout root system along with rhizomes. The rhizomes of these *in vitro* seedlings were used as explants for micropropagation. Rhizome segments were cultured on different plant growth regulator (PGR) supplemented MS, PM and MVW media. In some of the media compositions the rhizome segments developed multiple shoot buds. The multiple shoot buds were isolated and individually grown on elongation media as used earlier for elongation of seed originated seedlings. The elongated seedlings were further cultured on rooting media. The seedlings were taken out of the culture vessels and transferred to outside the culture room following successive phases of acclimatization. For this purpose, the culture vessels were kept open for one day in the culture room and then kept outside of the culture room for 6 h and then those were kept outside the culture room for 12 h. Finally the seedlings were taken out of the culture vessels and rinsed with running tap water to free agar attached to the roots. Finally, the seedlings were transplanted in pots containing sandy loam soil. Transplanted seedlings were watered regularly for about one-two months and they adapted well.

Results and Discussion

The seeds germinated on MS and PM (Fig. 1a) but failed to germinate on MVW indicating the existence of species-medium specificity. Barua and Bhadra (1999); Gupta and Bhadra (1998); Bhadra *et al.* (2002); Nagaraju *et al.* (2001) and Vij *et al.* (1989) also reported such species-medium specificity for germination of orchid seeds. The overall results suggest that PM was more effective than MS.

The germinated seedlings underwent elongation when subcultured on the same germination medium but the growth rate was very slow. For enhancement of growth, the tiny seedlings were transferred to different plant growth regulators (BAP, NAA, IBA, IAA, picloram and Kn), casein hydrolysate (CH) and activated charcoal (AC) supplemented media. The highest rate of elongation was achieved on MS supplemented with 2.5 mg/l BAP + 0.1 % (w/v) AC (Fig. 1b) followed by that on PM supplemented with 1.0 mg/l BAP + 0.5 mg/l IAA (Table 1). Similar findings were also reported by Harvais (1973) in *Cypripedium reginae* and Mathews and Rao (1980) in *Vanda* hybrids. They noted the growth promoting ability of AC which generally happens because of adsorption of excess nutrients by AC. A second possibility is that AC adsorbs phytotoxic metabolites, which may be released by growing tissues (Yam *et al.* 1990).

The elongated seedlings produced weak root system on the germination and elongation media. Therefore, it was considered important to induce strong and stout root system in these seedlings for rapid adjustment to outside environment. For this purpose, the elongated seedlings of 2 - 3 cm length were transferred to rooting media. Three different types of 0.8 % (w/v) agar solidified rooting media, namely (i) half strength MS + 1.5 % (w/v) sucrose, (ii) MS + 3 % (w/v) sucrose + 0.5 mg/l IAA and (iii) MS + 3 % (w/v) sucrose + 1.0 mg/l IAA + 0.1 % (w/v) AC were

used (Table 3). The efficiency of the rooting media was evaluated based on the increase in length and number of roots developed per seedling. Half strength MS + 1.5 % (w/v) sucrose proved more effective for the development of better root system (Fig. 1c). This finding indicates that less nutrient containing medium was more effective for induction of strong and stout root system than auxin supplemented full strength media. Similar result was noted by Agarwal *et al.* (1992) in *Vanilla walkeriae*; Gupta *et al.* (1998) in *Dendrobium crepidatum*; Barua and Bhadra (1999) in *Spathoglottis plicata*; Bhadra *et al.* (2002) in *Dendrobium aphyllum* and Sinha and Roy (2004) in *Vanda teres*.

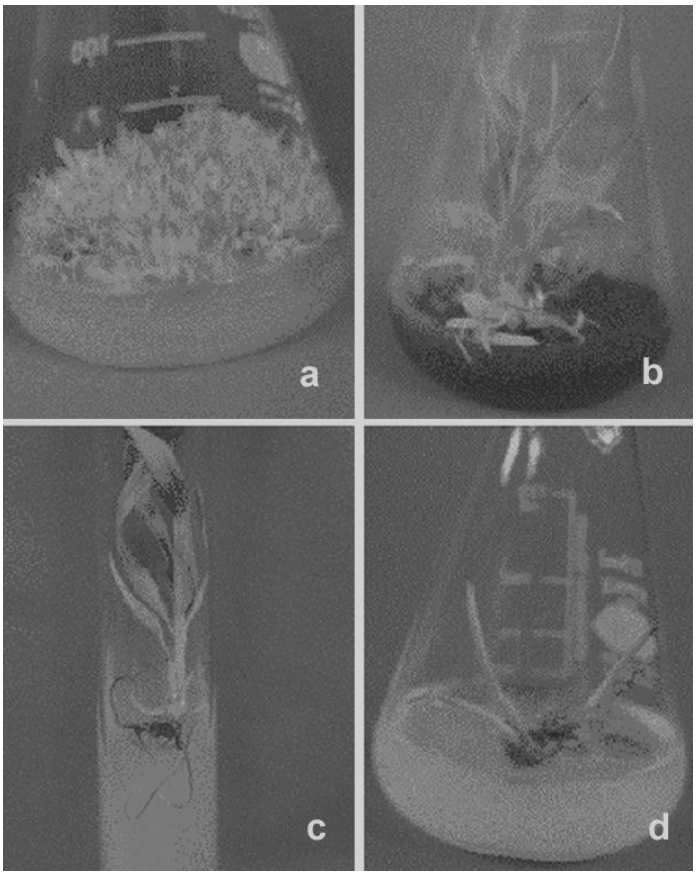

Fig. 1. *In vitro* germination and micropropagation of *A. graminifolia*, a. *In vitro* germination. b, Elongation of germinated seedlings. c. Rooting of elongated seedling. d. Development of multiple shoot buds in rihizome segment.

For *in vitro* micropropagation, rhizome segments (0.5 - 1.0 cm) of the aseptically grown seedlings were cultured on 0.8 % (w/v) agar solidified MS supplemented with various combinations and concentrations of PGRs (Table 2). The rhizome segments underwent direct organogenesis producing multiple shoot buds (Fig. 1d). The number of shoot buds developed per segment was dependent on PGR combinations of the nutrient media. The efficiency of a medium was assessed on the basis of number of multiple shoot buds developed in each rhizome segment.

Maximum number of shoot buds (7 - 9/segment) was developed on MS supplemented with 1.0 mg/l IAA and 2.0 mg/l BAP. Similar result was also noted by Sheelavantmath *et al*. (2000) in *Geodorum densiflorum*; Takahashi (1999) in *Pogonia japonica* and Vij *et al.* (1989) in *Eulophia hormusijii.*

Table 1. Elongation of the germinated seedlings of *A. graminifolia* on 0.8 % (w/v) agar solidified medium supplemented with different kinds of PGRs within 30 days of culture.

Medium composition	Mean increased length (cm) of seedlings* ± S.E.
MS + 3 % (w/v) sucrose + 2.0 mg/l BAP + 2.0 mg/l NAA	3.30 ± 0.19
MS + 3 % (w/v) sucrose + 0.5 mg/l Kn + 1.0 mg/l BAP + 2 g/l CH	3.52 ± 0.28
MS + 3 % (w/v) sucrose + 1.0 mg/l NAA + 0.5 mg/l BAP	3.63 ± 0.22
MS + 3 % (w/v) sucrose + 2.0 mg/l 2,4-D + 1.0 mg/l zeatin	*2.58 ± 0.27*
MS + 3 % (w/v) sucrose + 1.0 mg/l IAA + 0.5 mg/l BAP	2.91 ± 0.20
MS + 3 % (w/v) sucrose + 0.5 mg/l BAP + 1.0 mg/l picloram	3.28 ± 0.24
MS + 3 % (w/v) sucrose + 2.5 mg/l BAP + 0.1% (w/v) AC	3.69 ± 0.22
PM + 2 % (w/v) sucrose + 0.5 mg/l picloram + 2.0 mg/l BAP	3.21 ± 0.18
PM + 2 % (w/v) sucrose + 0.5 mg/l IAA + 1.0 mg/l BAP	3.66 ± 0.18
MVW + 2 % (w/v) sucrose + 1.5 mg/l Kn + 3.0 mg/l BAP + 1.0 mg/l NAA	2.74 ± 0.22

*Based on observations from 50 seedlings taking five at random from each of ten culture vessels.

Table 2. Number of multiple shoot buds developed from rhizome segments and their increased lengths on PGR supplemented media.

Medium composition	Time (d) required for sprouting of multiple shoot buds	Average number of shoot buds* sprouted in each segment ± S.E.	Average increased length (cm) of shoot buds* within 30 d of culture on elongation medium ± S.E.
MS + 3 % (w/v) sucrose + 2.0 mg/l BAP + 2.0 mg/l NAA	25 - 30	7.14 ± 0.48	3.27 ± 0.18
MS + 3 % (w/v) sucrose + 1.0 mg/l BAP + 0.5 mg/l Kn + 2.0 g/l CH	30 - 35	6.13 ± 0.42	3.34 ± 0.20
MS + 3 % (w/v) sucrose + 1.0 mg/l NAA + 0.5 mg/l BAP	30 - 35	6.98 ± 0.32	3.80 ± 0.16
MS + 3 % (w/v) sucrose + 2.0 mg/l BAP + 1.0 mg/l IAA	25 - 30	8.82 ± 0.46	3.12 ± 0.22
MS + 3 % (w/v) sucrose + 0.5 mg/l picloram + 2.0 mg/l BAP	25 - 30	6.27 ± 0.38	2.97 ± 0.17
MS + 3 % (w/v) sucrose + 1.0 mg/l BAP + 0.1 % (w/v) AC	30 - 35	6.76 ± 0.32	3.56 ± 0.16
PM + 2 % (w/v) sucrose + 1.0 mg/l BAP + 0.5 mg/l IAA	27 - 30	7.77 ± 0.42	3.22 ± 0.24
MVW + 2 %(w/v) sucrose + 3.0 mg/l BAP + 1.0 mg/l NAA + 1.5	35 - 40	7.15 ± 0.52	2.84 ± 0.20

mg/l Kn

*Based on observations from 40 segments and shoot buds.

In order to induce rapid elongation and thereafter rooting, multiple shoot buds developed from rhizome segments were further cultured on elongation and rooting media, respectively (Tables 2, 3). MS + 3 % (w/v) sucrose + 1.0 mg/l NAA + 0.5 mg/l BAP was found most effective for enhancing elongation whereas rooting was best on half strength MS + 1.5 % (w/v) sucrose. The seedlings at a stage of 8 - 10 cm were taken out of the culture vessels and transferred in pots outside the culture room following successive phases of adjustment. Finally, the seedlings were grown in earthen pots containing sandy loam soil. Seventy eight per cent of 300 transplanted seedlings survived and continued normal growth.

Table 3. Mean increased length (cm) and number of roots per seed-originated and rhizome-originated seedling of 30 days of culture on rooting media.

Rooting medium	Average increased length and number of roots per seed derived seedling		Average increased length and number of roots per shoot bud	
	Mean length (cm) ± S.E.	Mean No. of roots/seedling ± S.E.	Mean length (cm) ± S.E.	Mean No. of roots/shoot bud ± S.E.
½ MS + 1.5 % (w/v) sucrose	2.42 ± 0.18	1.56 ± 0.14	3.28 ± 0.33	5.08 ± 0.32
MS + 3 % (w/v) sucrose + 0.5 mg/l IAA	1.58 ± 0.14	0.99 ± 0.08	2.93 ± 0.26	3.24 ± 0.34
MS + 3 % (w/v) sucrose + 1.0 mg/l IAA + 0.1% (w/v) AC	1.41 ± 0.12	0.25 ± 0.08	2.74 ± 0.33	3.08 ± 0.28

*Based on observations from 50 seedlings/shoot buds taking five at random from each of ten culture vessels.

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