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# EFFECTS OF DIFFERENT ORGANIC ADDITIVES ON *IN VITRO* ASYMBIOTIC SEED GERMINATION OF *ARUNDINA GRAMINIFOLIA* (D. DON) HOCHR., AN EXQUISITE RARE ORCHID

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**Abstract**

Immature seeds of *Arundina graminifolia*, procured 24 wap (wks after pollination ) having 92% viable seeds were cultured in liquid Mitra *et al.* (1976; M) and Knudson C (1946; KC) basal medium with 2% sucrose and fortified with different concentrations of organic additives such as casein hydrolysate (CH), yeast extract (YE), peptone (P) and coconut water (CW). Amongst two different nutrient media tested for seed germination, better germination percentage and subsequent protocorm development was achieved in M medium. M medium containing CW (20%) was found be the best additive and supported 95% seed germination and protocorm development followed by CH (90%) and YE (88%). An average of 4.2 mg fresh weight/protocorm and 2.02 mm diameter of protocorm was obtained in Mitra medium containing 20% CW after 60 days of culture. The protocorms raised in organic additives showed a tendency to proliferate when the protocorms were transferred to solid medium. The proliferated protocorms with emerging shoots and roots after 2nd subculture lasting 60 days were transferred to Mitra medium containing different concentrations of banana pulp (2.5-10%). After 60 days in medium having banana pulp. Well developed seedling with healthy shoots and roots were obtained. After 3rd subculture, healthy seedlings were deflasked and transferred to community pots and 67% establishment rate was observed.

## Introduction

ORCHIDS ARE grown primarily as ornamentals and are valued as cut flowers not only because of their exotic beauty but also for their long shelf life. Though these plants are grown primarily as ornamentals, some are also employed as medicines and food (Arditti, 1992). According to the IUCN Action plan (1999), orchids are amongst the world's most diverse and widely distributed plants. They belong to one of the most diverse plant family known to man; have complex life cycle, require mycorrhizal association and specific pollination syndrome. It is a family of considerable economic importance particularly in horticulture and forestry. The widely diverse climatic regions of India are reflected in the wide diversity of its orchid flora. India with 1129 species in 184 genera is one of the major orchid habitats of the world (Karthikeyan, 2000). The Western Ghats, one of the richest floristic regions in the country is having about 4000 flowering plant species which is about 30% of the flowering plants, in the country (Gopalan and Henry, 2000). Western Ghats harbour 288 species of orchids in 76 genera (Mohanan and Sivadasan, 2002). Many orchid species are threatened globally by over collection pressures from their natural habitats for horticultural and other uses. Plant tissue culture is an effective tool to conserve plant germplasm and the guaranteed survival of endangered and over exploited genotypes is due mainly to the fact that it requires small units (cell and tissue) without sacrificing the mother plant, reduces the pressure of waning wild populations and makes available large number of identical copies of plants for reintroduction and wide distribution.

*Arundina graminifolia* is an exquisite rare orchid, commonly known as *Bamboo orchid*. It occurs among rocks in open grasslands and is distributed from Indo-Malaysia to Pacific Island. It is a terrestrial plant with a height of 1-3m, rigid and woody stem, and pale green glaucous leaves (5-8cm long, 2cm wide). *Inflorescence* terminal raceme; *Flowers* are purple violet in colour; *Lip* trumpet shaped enclosing the column (Figs.1-2). The objective of the study was to assess the effects of different organic additives on asymbiotic seed germination of *Arundina graminifolia* (D.Don) Hochr., an exquisite rare orchid and develop an appropriate propagation method using green pod culture technique for its mass multiplication.

## Material and methods

Unripened green capsules (4.2 cm length and 2.1 cm width) of *A. graminifolia* collected approximately 24 wap (wks after pollination) were used for embryo culture experiments (Fig.3). Microscopic assessment revealed 92% embryonate seeds (Fig.4). The capsules were washed in neutral detergent (Teepol) and surface sterilized by immersing in 0.1% (w/v) $HgCl_2$ solution for 15 min followed by rinsing three times in sterile distilled water. Subsequently, the capsules were then

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Fig.1-9. Asymbiotic seed germination of *Arundina graminifolia* (D. Don) Hochr.: 1, Plant in bloom; 2, Flower close up; 3, Green capsules; 4, Seeds; 5, Protocorms in liquid medium; 6, Protocorms on solidified medium; 7, Rooted seedlings on solid medium; 8, Healthy seedlings in BP containing medium; 9, Seedlings in a community pot.

dipped in 70% ethanol and flamed for a few seconds. The capsules were then cut vertically into two halves; the seeds were scraped out and released into 20 ml of sterile distilled water. Uniform seed suspensions were inoculated into 250 ml Erlenmeyer conical flasks containing 60 ml Mitra *et al.* (M, 1976) and Kundson C (1946, KC) liquid nutrient media supplemented with 2% sucrose. The nutrient media were also fortified with various organic growth additives *i.e.*, casein acid hydrolysate (CH), yeast extract (YE), peptone (P) at various concentrations (0.01-0.09%) and coconut water (CW, 5-25%). The inoculations were done under sterile conditions. All the cultures were incubated at 25 ± 2°C under 12 hr photoperiod using cool-white fluorescent tubes and constant agitation at 80 rpm (Orbitek gyratory shaker). Observations were made

at regular intervals. After 60 days of culture, samples of developing protocorms were collected for determining their size and fresh weight. Then, the protocorms were transferred to solidified nutrient media containing same concentration of additives. The proliferated protocorms with emerging shoots and roots after 2nd subculture lasting 60 days were then transferred to Mitra medium containing different concentrations of banana pulp (2.5-10%). After 60 days of subculturing, well developed seedlings with healthy shoots and roots were obtained. After 3rd subculture, healthy seedlings were deflasked, washed in tap water to remove traces of agar and dipped in 1% Indofil M 45 fungicidal solution. The seedlings with 2-4 leaves and 3-5 roots were planted in clay pots containing sand and garden soil in the ratio 1:1. The potted plants were maintained in a nursery under diffuse sunlight and were watered once a day.

## Results

The percentage of seed germination in *Arundina graminifolia* varied with different concentrations of CH, P, YE and CW in Mitra *et al.*, (1976, M) medium. Amongst the different growth additives used, CW (20%) showed the maximum seed germination (95%) (Fig.5; Table 1). Swelling of embryo was noticed within 12 days of inoculation. M medium containing different additives showed better germination as compared to KC medium (Table 2). M medium with CW (20%) was selected as the best nutritional combination for supporting maximum percentage (95%) of germination and protocorm growth followed by its combination with CH and YE. A maximum of 4.2 mg fresh weight and 2.02 mm diameter of the protocorms were obtained in medium containing CW (20%) (Table.2). Amongst the different concentrations of CH, YE, P used, maximum seed germination percentage *i.e.*, 90% was noticed in CH, followed by YE (88%) and P (50%) each at 0.05% conc. in M medium (Table 2). The protocorms raised in organic growth additives showed a tendency to proliferate when these were transferred to solidified nutrient medium (Fig.6). Well developed shoots and roots were produced during the second subculture passage (Fig.7). Individual shoots with roots after second subculture were transferred to different concentrations (2.5-10%) of banana pulp in the medium. Well developed seedlings with healthy shoots and roots were obtained within 60 days (Table 3) (Fig.8). After 3rd subculture, the healthy rooted seedlings with 5-6 leaves and 3-4 roots were deflasked and transferred to community pots (Fig. 9). Under nursery condition about 67% seedling survived upon transfer to community pots.

## Discussion

*In vitro* asymbiotic seed culture of a number of species including tropical epiphytes, tropical lithophytes, tropical terrestrial and temperate terrestrials has been attempted with varied levels of success (Arditti and Ernst, 1984; Pathak *et al.*, 2001). Among tropicals, the seeds of terrestrials are more difficult to germinate and may require special nutrient media (Ernst, 1980). Presently, immature seeds of *Arundina graminifolia* successfully germinated during *in vitro* asymbiotic culture. The propagation of orchids through *in vitro* germination of seeds has been emphasized by many workers (Arditti, 1967; Arditti *et al.*, 1981, 1982; Clements, 1973; Hossain *et al.*, 2009, 2010, 2012; Mitra, 1971; Pathak and Vij, 2007, 2012; Pathak *et al.*, 1992, 2001, 2011; Piri *et al.*, 2012; Vij and Pathak, 1988, 1989, 2010). In general, immature seeds are more preferred not only for the ease of surface sterilization but also for obtaining higher germination percentage and the technique is widely used for multiplication of various orchid species and hybrids (Arditti *et al.*, 1982; Pathak *et al.*, 2001).

Presently, in *A. graminifolia*, better germination percentage and subsequent protocorm development were recorded in M medium when compared to KC medium. M medium has been proved beneficial for seed germination in a large number of orchid species. It contains lesser amounts of ammonium salts, nitrates of calcium, potassium and phosphate ions than that of

Table 1. Effect of different concentrations of growth additives on seed germination in *Arundina graminifolia* in Mitra *et al.* (1976) medium.

| CH | | YE | | P | | CW | |
|---|---|---|---|---|---|---|---|
| Conc. ($mgl^{-1}$) | Germination (%) | Conc. ($mgl^{-1}$) | Germination (%) | Conc. ($mgl^{-1}$) | Germination (%) | Conc. ($mll^{-1}$) | Germination (%) |
| 100 | 25 | 100 | 40 | 100 | 15 | 50 | 60 |
| 300 | 30 | 300 | 60 | 300 | 45 | 100 | 65 |
| 500 | 90 | 500 | 88 | 500 | 50 | 150 | 70 |
| 700 | 60 | 700 | 55 | 700 | 25 | 200 | 95 |
| 900 | 50 | 900 | 40 | 900 | 30 | 250 | 65 |

Conc. : concentration; Observations were made after 60 days of culture

KC medium. It also has various minor salts and vitamins. Amongst various organic additives *i.e.*, CH, YE, P and CW when used in the medium, maximum percentage of germination (95%) was achieved in 20% CW supplemented medium. CW is a complex additive which contains many nutrients and hormonal substances (Dix and Van Staden, 1982) and has a marked growth promoting effect on a variety of plant tissues. The promotory effect with regard to morphogenesis is related to its growth regulator content specially cytokinins. Enhancing effect of CW on seed germination has been studied in *Cypripedium*

Table 2. Effect of different growth additives in M and KC media on *in vitro* seed germination in *Arundina graminifolia*.

| Nutrient Medium | Additives | Germination (%) | Protocorm Fresh weight Mean = SD | Diameter of the protocorm (mm) | Remarks |
|---|---|---|---|---|---|
| M | - | 60 | 0.97 ± 0.89 | 0.84 ± 0.98 | White protocorms |
| | 0.05% CH | 90 | 3.10 ± 0.54 | 1.93 ± 0.52 | Pale Green |
| | 0.05% YE | 88 | 2.40 ± 1.43 | 1.78 ± 0.83 | Green |
| | 0.05% P | 50 | 2.42 ± 1.25 | 1.87 ± 0.76 | Pale green |
| | 20% CW | 95 | 4.20 ± 1.64 | 2.02 ± 0.55 | Yellowish green |
| KC | - | 43 | 0.82 ± 1.88 | 0.81 ± 0.78 | Creamy white |
| | 0.05% CH | 87 | 2.31 ± 1.94 | 1.70 ± 0.43 | Green |
| | 0.05% YE | 83 | 2.30 ± 1.97 | 1.73 ± 0.66 | Pale green |
| | 0.05% P | 52 | 2.54 ± 1.48 | 1.93 ± 0.13 | Pale green |
| | 20% CW | 89 | 2.80 ± 1.14 | 1.84 ± 0.85 | Yellow |

Observations made after 60 days of culture

*calceolus* (Chu and Mudge, 1994), *Zeuxine sulcata* (Arekal and Karanth, 1978) and *Acampe praemorsa* (Krishnamohan and Jorapur, 1986).

The seedlings transferred to BP (Banana Pulp) containing medium yielded healthy and robust plants. The growth stimulating effects of banana pulp on seedling growth are well documented (Ernst, 1974, 1975; Hinnen *et al.*, 1989; Pierik *et al.*, 1988). Beneficial effects of CW and Banana homogenate added to medium on orchid seedling growth have been reported by many authors (Decruse *et al.*, 2003; Goh and Wong, 1990; Lo *et al.*, 2004; Seeni and Latha, 2000). Banana pulp is a rich source of natural cytokinins which inhibit culture initiation but promote differentiation and growth of the seedlings (Arditti and Ernst, 1993). Promotory effect of BP on increase in number and growth of seedling have also been indicated earlier by Arditti (1968), Bopaiah and Jorapur (1986), Lo *et al.* (2004), and Vyas *et al.* (2009).

Table 3. Relative growth of seedlings of *A. graminifolia* in different concentrations of Banana pulp (BP)

| Nutrient Medium | BP (%) | Seedlings with |
|---|---|---|
| M | 2.5 | 3 leaves 2 roots |
| M | 5.0 | 6 leaves 2 roots |
| M | 7.5 | 4 leaves 3 roots |
| M | 10.0 | 3 leaves 3 roots |

Observation were made after 60 days of culture

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