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Research Article

IN VITRO PROPAGATION OF *ARUNDINA GRAMINIFOLIA* D. DON. HOCHR – A BAMBOO ORCHID

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ABSTRACT

Arundina graminifolia (D. Don)Hochr. Known as bamboo orchid and it is an endangered orchid (Jain and Sastry, 1980; Chowdhery and Murti, 2000).Here a protocol was established for *in vitro* propagation of bamboo orchid. For micropropagation different concentrations of growth regulators were used and nodal segments were used as explants.MS media supplemented with NAA at different concentration (0.5mg/L,1.0mg/L,1.5mg/L) and Kinetin (1.0mg/L,2.0mg/L,2.5mg/L) was used for Shoot proliferation. Two types of auxin (NAA, IAA) were used to know the effects of both auxin in root induction.MS media supplemented with 1.0mg/L NAA+ 2.5mg/L KN showed highest shoot proliferation with root length 3.50 cm. MS media when supplemented with 3.0mg/L IAA induce root with highest root length4.7cm. 87% micropropagated plants survived after field transfer.

Keywords: Micropropagation, *in vitro,* growth regulators, Endangered.

INTRODUCTION

Among angiosperms orchidaceae is one of the largest families. Orchidaceae family includes 800 genera and 25000 species [1].Orchids are well known for their economic importance and ornamental beauty.

Arundina comes from Latin word arundo which means reed and *graminifolia* means grass like leaves. *Arundina graminifolia* is commonly known as bamboo orchid. Bamboo orchid is a terrestrial perennial orchid with erect stem, forming into large clumps growing to a height between 70cm to 2m. The leaves are long and narrow, with a length of 9cm- 19cm and a width of 0.8 to 1.5. The apex is acuminate. The flowers are oval-shaped and like all orchid flowers, have an outer whorl consist of 3 sepals and an inner whorl with 3 petals. The top sepal is narrow or smaller than the 2 side petals. Flowers are purple red or white tepals (petals and petals like sepals), magenta edged labellum (lip) with a yellow center. The short lived , scented flowers last for about 3 days many flowers bloom at a time. Seed capsules are 6-ribbed when it split it looks like a bird cage and release minute dust like seed in air.

The orchid blooms in summer and autumn, showing rather open clusters of open terminal flowers. They bloom in succession on the terminal racemes, which are 7cm-16cm long. These flowers are 5cm-8cm in diameter. The occasional fertilized seed pods contain minute powdery seeds.

The genus considered to possess activities of detoxification, antiarthritis and abirritation. *Arundina graminifolia* contain benzyldihydrophenanthrene, arundinaol, stilbenoid, arundian and phenanthrene[2]. *Arundina graminifolia* is an endangered orchid [3-4]. In present study we tried to formulate an effective protocol for *in vitro* propagation of bamboo orchid which help to conserve this endangered plant.

MATERIALS AND METHODS

Explant Source

Field grown plants of *Arundina graminifolia* D. Don Hochr. were used as source of explants for *in vitro* culture. Nodal segments about 2cm long used as explants.

Sterilization

The nodal segments were washed in tap water for some time and disinfected with 0.1%(w/v) mercuric chloride ($HgCl_2$) for 5 minutes followed by thorough rinsing in autoclaved distilled water for at least 7- 8 times. The surface sterilized explants sized to 0.5 – 1cm length containing single node.

Culture Medium and Condition

MS medium was selected for in vitro culture of explants, the pH of the medium was adjusted with 1N HCl and 1N NaOH solution between 5.7- 5.8. After adjusting the pH agar powder was mixed with the medium and boiled for sometime to obtain clear solution. After that 40ml of medium was taken in each 100ml screw capped bottle autoclaved at 121°c at 15lbs/sq inch for 20 min.

The screw capped bottles containing the medium were then allowed to cool for 24 hours in the culture laboratory. The surface sterilized explants were placed vertically on the MS media with and without growth regulators. Various concentration of α napthalene acetic acid (0.5,1.0, 1.5, 2.0 and 2.5mg/L) and kinetin (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) was used to observe its effect on axillary bud proliferation, shoot initiation, root initiation.

The cultures were maintained at 25°c±1°c under 16 hour photoperiod provided by white fluorescent tubes. After every two week the culture materials were transferred into new medium for better growth of plants.

Sterilization of the potting mixture: The earthen pots containing the potting mixture containing brick bats, charcoal, dried moss, leaf molds, sand were autoclaved at 20lbs/sq inch, pressure for 30 minutes. After sterilization the pots were allowed to cool down for 48 hours and then kept in room temperature in a well ventilated room.

Transplantation: Plants were taken out from culture bottles and washed thoroughly with water to remove culture medium sticking to the plants. After that plants were dipped in 2% Diethane 45 (fungicide) for 30 seconds and transplanted in pots containing sterile mixture and covered with polythene bags. The pots were kept in tissue culture laboratory. The seedlings were regularly sprayed with MS solution up to 15days of transfer and during the next 15 days these were sprayed with liquid MS solution without agar and sugar.

After one month the plants were transferred to acclimatization media and medium comprised of pre sterilized Brick bats + charcoal + dried moss + leaf molds + sand (1:1:1:1:1).

Parameters Studied

1. Days required to axillary bud proliferation
2. Shoot number per explants

3. Shoot length
4. Days required to root initiation
5. Root length
6. Root number per explants
7. Percentage of explants response

RESULTS

In vitro multiplication of *Arundina graminifolia* was investigated during this study and for this purpose a series of experiment was performed.

Effect of Sterilants : Mercuric chloride (different concentration) was used for explants sterilization. Nodal segments were first washed with tap water and then treated with 0.1% (w/v) for 5 minutes. Explants treated with less than 0.1% concentration not respond properly.

Axillary bud proliferation : MS media supplemented with NAA (0.5mg/L, 1mg/L,1.5mg/L,2.0mg/L,2.5mg/L) used for axillary bud proliferation and for this purpose sterilized nodal segments were inoculated in the medium.

Media containing 1.0mg/L showed best result. Explants cultured on this medium proliferated within 40-42 days which one was the minimum time period required for bud proliferation.50% explants showed positive response cultured on this medium.

Among the other treatments media containing 1.5mg/L NAA required 48-50 Days for bud proliferation which one was the second minimum time period.

Multiple shoot proliferation: In this series of experiments different media formulation was tried for multiple shoot initiation. After bud proliferation explants were transferred to the media containing different concentration of NAA (0.5mg/L,1.0mg/L,1.5mg/L) and kinetin (1.0mg/L, 2.0mg/L, 2.5mg/L).

Among the different media treated media supplemented with 1.0mg/L NAA and 2.5 mg/L KN produced highest shoot length (3.50±0.17) with average 4 number of shoot. More concentration of NAA and kinetin reduce shoot length as well as shoot number.

Media supplemented with 0.5mg/L NAA and 2.0mg/L also showed significant results and produced shoot with shoot length 3.13cm (mean).

***In vitro* Rooting:** Experiments were also done in order to optimize the rooting medium. The number of root formed per shoot and time required for root initiation was significantly different among the different treatments.

To study the effect of auxins on root initiation two types of auxins (IAA,NAA) was added with half strength MS medium. IAA in different concentrations (2.0mg/L, 2.5mg/L, 3.0mg/L) combined with half strength MS medium. 3.0mg/L IAA when combined with half strength MS medium produced highest root length 4.7 cm with root number 5.

MS medium supplemented with NAA in different concentrations (0.5mg/L, 1.0mg/L,1.5mg/L, 2.0mg/L) and this medium was used for root induction. Media with 0.1mg/L NAA produced root with root length 3.7cm (mean).

Acclimatization Well developed plants were transferred in the sterile vermiculite mixture for one month. Cultured plants were transferred to the potting mixture containing Brick bats+ leaf molds +charcoal +Dried moss+ sand in 1:1:1:1:1ratio. After one month of field transfer 87% plants survived.

Table1: Effect of various concentration of NAA on axillary bud proliferation (Data collected after 70 days, 10 replicates for each treatments, repeated thrice).

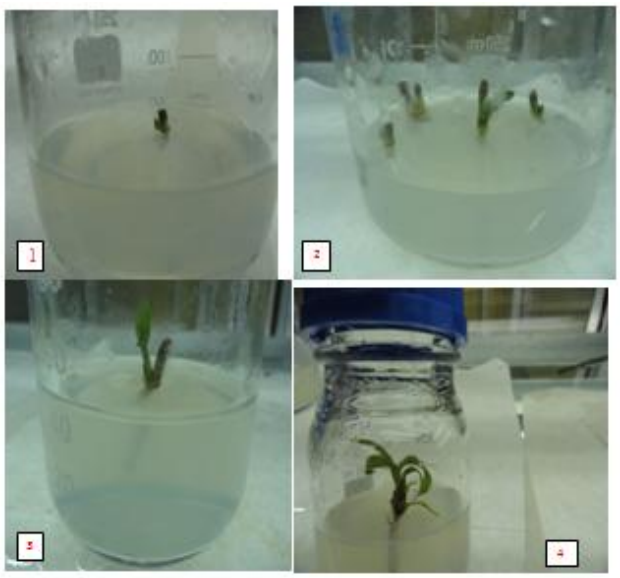
Explant	Treatments	Days to bud break	Percentage of explants response
Nodal segments	MS+ o mg/L NAA	-	-
	MS+0.5mg/L NAA	38-40	16
	MS+ 1.0mg/L NAA	45-47	50
	MS+1.5mg/L NAA	50-52	43
	MS+2.0mg/L NAA	59-61	25
	MS+ 2.5mg/L NAA	68-70	18

Table2: Effect of growth regulators on shoot formation in nodal explants of *Arundina graminifolia* D. Don.Hochr.(Data collected after 3 month, 10 replicates for each treatment, repeated thrice).

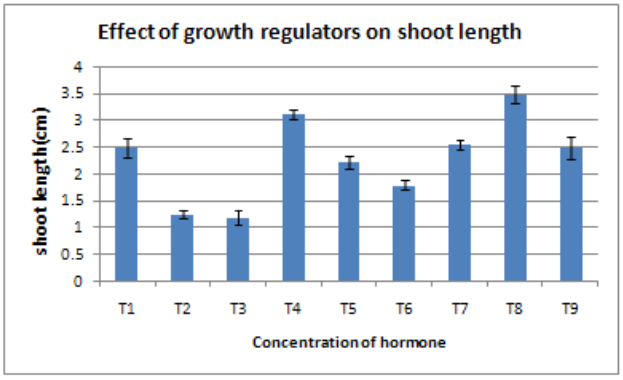
Treatments	Conc. of Hormone NAA	KN	Percentage of explants response	Mean no. of shoot per explants ± SE	Mean Shoot length (cm)±SE
T_1	0.5	1.0	40	1.66±0.35	2.50±0.17
T_2	1.0	1.0	27	1.66±0.35	1.26±0.07
T_3	1.5	1.0	31	2.33±0.26	1.20±0.14
T_4	0.5	2.0	50	4.33±0.71	3.13±0.09
T_5	1.0	2.0	39	3.66±0.71	2.23±0.11
T_6	1.5	2.0	18	3.66±0.26	1.80±0.09
T_7	0.5	2.5	21	4.0±0.46	2.56±0.09
T_8	1.0	2.5	52	5.33±0.26	3.50±0.17
T_9	1.5	2.5	17	1.33±0.31	2.50±0.21

Table3: Influence of different concentration of IAA and NAA on rooting of *in vitro* generated shootlets of *Arundina graminifolia* (Data scored after 4 month of inoculation, 10 replicates per treatment, repeated thrice)

Treatments	Conc. of hormone mg/L	Days to root initiation	% of explants response	Mean root no.±SE	Mean root length (cm)±SE
T0	MS	-			
T1	MS +2.0mg/LIAA	32-34	50	5.33±0.26	3.0±0.09
T2	MS+2.5mg/L IAA	30-32	52	4.33±0.26	3.53±0.07
T3	MS+3.0mg/L IAA	25-27	67	6.0±0.47	4.70±0.02
T4	MS+0.5mg/LNAA	31-33	43	3.66±0.26	2.53±0.11
T5	MS+1.0mg/LNAA	22-25	58	4.33±0.26	3.70±0.09
T6	MS+1.5mg/LNAA	38-40	62	4.0±0.46	3.2±0.21
T7	MS+ 2mg/L NAA	36-38	49	3.0±0.81	2.9±0.04



1.Inoculated nodal segment.
2.Axillary bud proliferation started.
3.Plant treated with NAA (1mg/L) and Kinetin(1.0mg/L).
4.Regenerated plant (in vitro).DISCUSSION



Nodal segments of *Arundina graminifolia* was used as explants for *in vitro* propagation. MS media was selected as culture media and for axillary bud proliferation MS medium was supplemented with different concentration of NAA (0.5mg/L, 1.0mg/L, 1.5mg/L, 2.0mg/L, 2.5mg/L) and nodal segments were inoculated in it. 50% cultured explants showed positive response on MS medium containing 1.0mg/L NAA within 45-47 days (Table 1). MS medium supplemented with 0.5mg/L NAA required minimum time(38-40 days) for axillary bud proliferation but percentage of explant response was also very minimum(16).

MS medium supplemented with 2.5mg/L kinetin + 1.0mg/L NAA was recorded as best medium for shoot growth and average 3.50 cm long shoots produced in this media composition with average 5.33 number of shoots per explants. George and Ravishankar[5] cultured *Vanilla planifolia* on MS medium containing 2mg/L BA and 1mg/L NAA using axillary bud as explants. Second highest root length (3.13cm) with root number 4.33 obtained in media composition MS+ 2.0mg/L KN + 0.5mg/L NAA.

MS medium supplemented with different concentration of IAA(2.0mg/L , 2.5mg/L,3.0mg/L) and NAA (0.5mg/L, 1.0mg/L, 1.5mg/L, 2.0mg/L)was used for root initiation . Satisfactory result was obtained on MS medium with 3.0mg/L IAA supplementation. Average 4.70cm(Table 3) long roots with 6.0number of roots were observed in MS medium with 3.0mg/L IAA.MS medium containing 1.0mg/L NAA was effective for root growth and it induced root with root length 3.70cm with 4.33 number of root.

Martin [6] propagated *Arundina graminifolia* through protocorm-like bodies (PLBs) using node as explants. Explants were cultured on half strength MS medium + 6.97µM Kinetin effective for sprouting of the axillary bud and half strength MS medium with 13.3µM BA also favoured axillary bud proliferation. In present experiment nodal segments were used for *in vitro* propagation through direct axillary bud proliferation and shoot multiplication, no PLBs formed during this propagation. For root induction we used two types of auxin (IAA, NAA) with MS medium. Martin[6] used half strength MS medium with 1g/L activated charcoal for rooting. Yan *et al* .,[7] cultured *Cypripedium flavum in vitro* using Havais media and reported that BAP was not beneficial for root induction of *C. flavum* In this experiment we used MS media for propagation of *Arundina graminifolia* and our result also support the view that cytokinin was not efficient for rooting because without cytokinin only auxin induce rooting in micropropagated plants.

Sharma and Tandon[8] worked with *D. wardianum* R. Warner and used MS+ 2.5mg/L BAP for PLBs production *in vitro*. Nagaraju and Parthasarathy [9] propagated *Arundina bambusifolia* Lindl. and for shoot proliferation they used Raghavan and Torrey's [10] medium without any growth regulators. The results of this experiment completely differ with the result of the present experiment. *Vanilla planifolia* Andr. was cultured by Kalimuthu *et al*.,[11] using MS+ 1mg/L BAP + 150ml/L CW for shoot and root multiplication.

Here an attempt was made to propagate *Arundina graminifolia* using minimum number of growth regulators. In this investigation an efficient micropropagation technique was derived which may be useful for raising quality plants of *Arundina graminifolia* for commercial purpose in lowest cost. This technique paves the way not only for *ex situ* conservation but also for the restoration of genetic stock of the species.

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