Shadang et al., Vegetos 2016, 29:4
10.5958/2229-4473.2016.00099.9

Vegetos- An International Journal of Plant Research

Research Article A SCITECHNOL JOURNAL

# Regeneration Potential of *Ascocentrum ampullaceum* (Roxb.) Schlter through Inflorescence culture

**Shadang R[1], Dwivedi P[2*] and Hegde SN[1]**

## Abstract

The paper deals with the in vitro regeneration response of immature inflorescence segments (buds, nodes and internodes) of *Ascocentrum ampullaceum* explants. The nutrient medium used played a key role in induction and multiplication of callus, protocorm like bodies and shoot. Inflorescence buds cultured in V&W medium supplemented with 15% coconut milk and 2% sucrose produced highest (80%) protocorm like bodies formation without any growth regulator. Inflorescence node culture in MKC medium supplemented with only 15% CM, and 15% CM and 1.0 mg $l^{-1}$ NAA produced highest callus (70%) and plbs (50%) formation, respectively. Incorporation of 15% CM in all the media proved best for formation of callus and plbs. Our results suggest that *Ascocentrum ampullaceum*, which regenerates poorly in nature, can be micropropagated using inflorescence based explants.

**Keywords**

*Ascocentrum ampullaceum*; Callus; Inflorescence; *In vitro* culture; Nutrient media; Protocorm like bodies; Shoot formation

## Introduction

The north-east Indian region is home to several rare and endemic orchids. *Ascocentrum ampullaceum* (Roxb.) Schlter is one such species of orchid which being monopodial in habit faces difficulties in the production of seedlings and flowers because of its long gestation period for flowering. This species needs to be propagated as its natural populations are receding fast. The shoot-meristem culture has emerged as an important technique for mass multiplication of desired genotypes. This technique, however, requires the sacrifice of the entire new growth or the only growing point and has a limited utility in monopodium taxa where it endangers the survival of the mother plant. While tissue culture techniques have given new dimensions to plant propagation, the importance of identifying an alternate but equally effective explant whose excision does not endanger the survival of mother plant has often been stressed [1-4]. The paper reports *in vitro* regeneration potential of inflorescence segments in *Ascocentrum ampullaceum* with the aim to develop an efficient regeneration system for the species using various nutrient compositions.

***Corresponding author:** Padmanabh Dwivedi, Department of Plant Physiology, Institute of Agricultural Sciences, Baranas Hindu University, Varanasi, India, Tel: 0542-6701112; E-mail: pdwivedi25@rediffmail.com

**Received:** August 31, 2016 **Accepted:** September 29, 2016 **Published:** September 30, 2016

## Materials and Methods

Young inflorescence buds (3-5 cm length), excised from Ascocentrum ampullaceum plants grown in the SFRI Greenhouse, Itanagar were used to prepare explants. Healthy shoots were cut, leaving 2 leaves with the donor plant. Leaves and sheathing bases were removed carefully from the shoot. The explants were washed under running tap water for 30 min followed by washing in Teepol solution for 5 min, scrubbed gently with soft brush to remove all the external debris. These were rinsed with double distilled water, then dipped the plant material in 70% ethyl alcohol for 30 sec under laminar hood for surface decontamination, after which the plant materials were immersed in freshly prepared 20% household bleaching solution for 5 min, followed by three times rinsing with sterile distilled water. The inflorescence bracts and bracteoles were removed with sterile surgical blade and placed over sterile filter paper in order to absorb remaining water. Inflorescence was cut into 0.5-1.0 cm with the help of sterile surgical blade and used as direct explants, whereas the longer ones were segmented into nodes, internodes and buds. Explants thus obtained were inoculated into various nutrient media such as Vacin & Went [5], Modified Knudson C [6], half strength Murashige and Skoog [7] having 2% sucrose as well as 15% Coconut Milk (CM) supplemented with α-naphthalene acetic acid (NAA), benzyl amino purine (BAP), kinetin (Kin) of various strengths, singly and in combination. The cultures were incubated at 25°C ± 1°C under 14 h photoperiod of 50 µmol $m^{-2}s^{-1}$ light intensity. The experiments were repeated thrice with several replicates per treatment, and observations recorded. Statistical analysis was done for one way and two way ANOVA following SYSTAT 10 package.

## Results and Discussion

The inoculated explants swelled after 10 days in the basal media but their subsequent behaviour varied with the medium composition and growth stimulus therein. Inflorescence bud cultured in V&W media supplemented with 15% CM + 2% sucrose produced the highest Plbs formation (80%), followed by 40% Plbs in V&W supplemented with 15% CM. Highest (50%) callus and plbs was induced in V&W media supplemented with 15% CM + 0.5 $mgl^{-1}$ Kin, and 50% of callus formation was observed in MKC supplemented with 15% CM (Table 1 and Figure 1A). The inflorescence node culture in MKC medium supplemented with 15% CM induced the highest (70%) callus. MKC medium supplemented with 15% CM + 1.0 $mgl^{-1}$ NAA induced the highest (50%) plbs formation, followed by 30% of Plbs in V&W amended with 15% CM + 0.5 $mgl^{-1}$ Kin and 20% of plbs in V&W media supplemented with 15% CM + 2% sucrose. In the other nutritional compositions, the explants swelled but perished without showing any morphogenetic change (Table 2 and Figure 1B). The regeneration competence of explants representing inter-nodal regions of inflorescence was best demonstrated in MKC medium supplemented with 15% + CM; nearly 50% explants showed a callus mediated plb regeneration, followed by 40% of plbs in MKC medium supplemented with 15% CM + 0.5 $mgl^{-1}$ NAA, 30% of callus and plbs in V&W media supplemented with 15% CM + 0.5 $mgl^{-1}$ Kin + 0.5 $mgl^{-1}$ NAA, 20% of plbs in V&W supplemented with 15% CM + 0.5 $mgl^{-1}$ Kin + 0.5 $mgl^{-1}$ NAA, and finally 10% Plbs in V&W supplemented with 15% CM + 0.5 $mgl^{-1}$ Kin. In other media tried, the explants swelled and died (Table 3 and Figure 1C).

*doi: 10.5958/2229-4473.2016.00099.9*

**Table 1:** Morphogenetic response of *Ascocentrum ampullaceum* floral buds *in vitro*.

| Nutrient Medium | Response frequency of explants (%) | Morphogenetic responses in explants (after Days) | | | |
|---|---|---|---|---|---|
| | | 10 | 20 | 30 | 40 |
| V&W | 100 | Swelling | Unchanged | Died | - |
| V&W + 15% CM | 40 | Swelling | Vigorous elongation | Plbs development | Plbs development |
| V&W + 15% CM + 2% sucrose | 80 | Swelling | Vigorous elongation | Multiples Plbs formation | Multiples Plbs formation |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn | 50 | Swelling | Vigorous elongation | Callusing | Callusing and Plbs development |
| V&W + 15% CM + 1.0 $mgl^{-1}$ Kn | 20 | Swelling | Vigorous elongation | Callusing | Callus |
| V&W + 15% CM + 2.0 $mgl^{-1}$ Kn | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 0.5 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 1.0 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15 % CM + 0.5 $mgl^{-1}$ Kn + 0.5 $mgl^{-1}$NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 1.0 $mgl^{-1}$ Kn + 0.5 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 2.0 $mgl^{-1}$ Kn + 0.5 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn + 1.0 $mgl^{-1}$NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn + 1.0 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 2.0 $mgl^{-1}$ Kn + 1.0 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Died | - | - |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Died | - | - |
| V&W + 15% CM + 2.0 $mgl^{-1}$Kn + 2.0 $mgl^{-1}$NAA | 100 | Swelling | Died | - | - |
| MKC | 100 | Swelling | Unchanged | Died | - |
| MKC + 15% CM | 50 | Swelling | Vigorous elongation | Callusing | Callusing |
| MKC + 15% CM + 1.0 $mgl^{-1}$ NAA | 100 | Swelling | Vigorous elongation | Unchanged | Died |
| MKC + 15% CM + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Vigorous elongation | Unchanged | Died |

**Note: Seed Swell (days)** n = 210, df = 20, F = 316.750, p < 0.001 [p = 0.000]; **Elongations (days)** n = 210, df = 20, F = 60.203, p < 0.001 [p = 0.000]; **Callus development** n = 210, df = 20, F = 71.613, p < 0.001 [p = 0.000]; **Plbs response** n = 210, df = 20, F = 309.960, p < 0.001 [p = 0.000].

**A**

**B**

**C**

**Figure 1:** Multiplication of Plbs in response to various nutrients in the media using inflorescence segments in vitro **(A)** Inflorescence bud cultured in V&W + 15% CM + 2% sucrose: Multiple Plbs formation; **(B)** Inflorescence node cultured in MKC+15% CM+1.0 $mgl^{-1}$ NAA: Multiple Plb development; **(C)** Inflorescence internode cultured in MKC + 15% CM: Multiple Plbs formation

*doi: 10.5958/2229-4473.2016.00099.9*

**Table 2:** *Ascocentrum ampullaceum*: inflorescence node culture *in vitro*.

| Nutrient Medium | Response frequency of explants (%) | Morphogenetic responses in explants (after Days) | | | |
|---|---|---|---|---|---|
| | | 10 | 20 | 30 | 40 |
| V&W | 100 | Swelling | Unchanged | Died | - |
| V&W + 15% CM | 100 | Swelling | Vigorous elongation | Unchanged | Died |
| V&W + 15% CM + 2% sucrose | 20 | Swelling | Vigorous elongation | Plbs generation | Plbs generation |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn | 30 | Swelling | Vigorous elongation | Plbs development | Plbs development |
| V&W + 15% CM + 1.0 $mgl^{-1}$ Kn | 100 | Swelling | Vigorous elongation | Unchanged | Died |
| V&W + 15% CM + 2.0 $mgl^{-1}$ Kn | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 0.5 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 1.0 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn + 0.5 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 1.0 $mgl^{-1}$ Kn + 0.5 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn + 1.0 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 1.0 $mgl^{-1}$ Kn + 1.0 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 2.0 $mgl^{-1}$ Kn + 1.0 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Died | - | - |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Died | - | - |
| V&W + 15% CM + 2.0 $mgl^{-1}$ Kn + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Died | - | - |
| MKC | 100 | Swelling | Vigorous elongation | Unchanged | Died |
| MKC + 15% CM | 70 | Swelling | Vigorous elongation | Callusing | Callusing |
| MKC + 15% CM + 1.0 $mgl^{-1}$ NAA | 50 | Swelling | Vigorous elongation | Plbs generation | Plbs multiplication |
| MKC + 15% CM + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Vigorous elongation | Unchanged | Died |
| **Note: Seed Swell (days)** n = 200, df = 19, F = 439.251, p < 0.001 [p = 0.000]; **Elongations (days)** n = 200, df = 19, F = 61.632, p < 0.001 [p = 0.000]; **Callus development** n = 200, df = 19, F = 109.283, p < 0.001 [p = 0.000]; **Plbs response** n = 200, df = 19, F = 86.211, p < 0.001 [p = 0.000]. | | | | | |

**Table 3:** Inflorescence inter-node culture of *Ascocentrum ampullaceum*.

| Nutrient Medium | Response frequency of explants (%) | Morphogenetic responses in explants (after Days) | | | |
|---|---|---|---|---|---|
| | | 10 | 20 | 30 | 40 |
| V&W | 100 | Swelling | Unchanged | Died | - |
| V&W + 15% CM | 100 | Swelling | Unchanged | Died | - |
| V&W + 15% CM + 2% sucrose | 100 | Swelling | Unchanged | Unchanged | Died |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn | 10 | Swelling | Elongation | Plbs generation | Plbs generation |
| V&W + 15% CM + 1.0 $mgl^{-1}$ Kn | 100 | Swelling | Unchanged | died | |
| V&W + 15% CM + 2.0 $mgl^{-1}$ Kn | 0 | Unchanged | Died | - | - |
| V&W + 15% CM + 0.5 $mgl^{-1}$ NAA | 0 | Unchanged | Unchanged | Died | - |
| V&W + 15% CM + 1.0 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Died | - |
| V&W + 15% CM + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Unchanged | Died | - |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn + 1.0 $mgl^{-1}$ NAA | 30 | Swelling | Swelling | Callusing | Plbs generation |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn + 0.5 $mgl^{-1}$ NAA | 20 | Swelling | Swelling | Plbs generation | Multiple Plbs |
| V&W + 15% CM + 2.0 $mgl^{-1}$ Kn + 0.5 $mgl^{-1}$ NAA | 100 | Swelling | Vigorous elongation | Died | - |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn +1.0 $mgl^{-1}$ NAA | 100 | Swelling | Vigorous elongation | Died | - |
| V&W + 15% CM + 1.0 $mgl^{-1}$ Kn + 1.0 $mgl^{-1}$ NAA | 100 | Swelling | Vigorous elongation | Died | - |
| V&W + 15% CM + 2.0 $mgl^{-1}$ Kn + 1.0 $mgl^{-1}$ NAA | 100 | Swelling | Vigorous elongation | Died | - |
| V&W + 15% CM + 0.5 $mgl^{-1}$ Kn + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Vigorous elongation | Died | - |

| | | | | | |
|---|---|---|---|---|---|
| V&W + 15% CM + 1.0 $mgl^{-1}$ Kn + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Vigorous elongation | Died | - |
| V&W + 15% CM + 2.0 $mgl^{-1}$ Kn + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Vigorous elongation | Died | - |
| MKC | 100 | Swelling | Vigorous elongation | Died | - |
| MKC + 15% CM | 50 | Swelling | Vigorous elongation | Callus mediated Plbs generation | Plbs multiplication |
| MKC + 15% CM + 0.5 $mgl^{-1}$ NAA | 40 | Swelling | Vigorous elongation | Plbs generation | Multiple Plbs |
| MKC + 15% CM + 1.0 $mgl^{-1}$ NAA | 100 | Swelling | Died | - | - |
| MKC + 15% CM + 2.0 $mgl^{-1}$ NAA | 100 | Swelling | Died | - | - |
| **Note: Inter-node Swell (days)** n = 230, df = 22, F = 501.831, p < 0.001 [p = 0.000]; **Elongations (days)** n = 230, df = 22, F = 42.760, p < 0.001 [p = 0.000]; **Callus development** n = 230, df = 22, F = 90.000, p < 0.001 [p = 0.000]; **Plbs response** n = 230, df = 22, F = 46.173, p < 0.001 [p = 0.000]. | | | | | |

The nutrient medium used exhibited a key role in induction and multiplication of callus and plbs. Whereas culture of inflorescence bud responded best in V&W medium, inflorescence node and inter-node culture produced best response in MKC medium in terms of callus and plbs formation. Inflorescence bud in V&W medium supplemented with 15% CM and 2% sucrose produced highest plbs formation, with no growth regulator required. Presence of sucrose along with CM in the V&W medium triggered better proliferation rate of plbs, similar to that recorded in Aranda 'Dedorah' [8]. Inflorescence nodal culture in MKC medium supplemented with 15% CM and 1.0 $mgl^{-1}$ NAA produced highest callus and plbs formation. Bud segments cultured in V&W medium supplemented with 15% CM and Kin (0.5 $mgl^{-1}$) were found to de-differentiate into vigorous plbs. The morphogenetic response of inflorescence segment was found to vary with the developmental stage, position and the nutrient stimulus, similar to that observed in *Rhynchostylis retusa* [9].

Thus, the present investigation indicates the importance of nutrient medium in induction and multiplication of callus and plbs in orchid culture [10-12]. It is also evident from the present study that incorporation of 15% CM in all the media proved best for formation of callus and plbs. Our results thus suggest that *Ascocentrum ampullaceum*, which regenerates poorly in nature, can be micropropagated using inflorescence based explants.

### Acknowledgements

The authors acknowledge the financial assistance from Department of Biotechnology, Govt. of India, New Delhi.

### References

1. Arditti J, Krikorian AD (1996) Orchid micropropagation: the small path from laboratory to commercialization and an account of several unappreciated investigators. Bot J Linn Soc 122: 183-241.
2. Devi YS, Laishram JM (1998) In vitro propagation of Dendrobium hybrids through shoot-tip and axillary bud culture. J Orchid Soc India 12: 79-81.
3. Kanika, Vij SP (1997) Root to shoot transformation in Vanda coerulea Griff. Possibilities in vitro: In: Proc. Natl. Seminar on Development Biology and Commercialization of Orchids, Gangtok, Sikkim, India.
4. Vij SP, Dhiman A (1997) In vitro inflorescence segment culture in Malaxis acuminate D. Don. In: Proc. Natl. Seminar on Development Biology and Commercialization of Orchids, Gangtok, Sikkim, India.
5. Vacin EF, Went FW (1949) Some pH changes in nutrient solutions. Bot Gaz 110: 605-613.
6. Knudson L (1946) A new nutrient solution for the germination of orchid seeds. Amer Orchid Soc Bull 15: 214-217.
7. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol Plant 15: 473-497.
8. Goh CJ, Wong PF (1990) Micropropagation of the monopodial orchid hybrid Aranda 'Deborah' using inflorescence explants. Sci Hortic 4: 315-321.
9. Kaur P, Vij SP (1995) Morphogenetic response of Rhynchostylis retusa inflorescence segments: A study in vitro. J Orchid Soc India 9: 85-90.
10. Shadang R, Dwivedi P, Hegde SN, Ahmed N (2007) Effects of different culture media on seed germination and subsequent in vitro development of protocorms of Hygrochilus parishii (Veith & Rchb.f.) Pfitz (Orchidaceae). Indian J Biotech 6: 256-261.
11. Shadang R, Dwivedi P, Hegde SN (2007) Shoot formation through in vitro culture of axillary buds in Ascocentrum ampullaceum (Roxb.) Schltr. J Orchid Soc India 21: 71-73.
12. Shadang R, Dwivedi P, Hegde SN (2009) Regeneration competence of Hygrochilus parishii inflorescence segments and axillary buds: a study in vitro. J Orchid Soc India 23: 75-78.

### Author Affiliations

Top

[1]*State Forest Research Institute, Itanagar, Arunachal Pradesh, India*

[2]*Department of Plant Physiology, Institute of Agricultural Sciences, Baranas Hindu University, Varanasi, India*