

# Nutritional studies on in vitro raised Protocorms of endangered dendrobe : *Dendrobium wardianum* Warner

Abha Sharma and Pramod Tandon

Sharma Abha, Tandon Pramod 1991 Nutritional studies on *in vitro* raised protocorms of endangered dendrobe : *Dendrobium wardianum* Warner – IBC 8 : 75–78

Protocorm-like bodies obtained by culturing shoot apices of *Dendrobium wardianum* were subjected to various nutrient media with different salt levels for proliferation and plantlet-regeneration. Remarkable differences were observed in growth and development of regenerant in response to various nutrient levels. This study can be of immense importance to nursery industry.

Abha Sharma, Pramod Tandon : Plant Biotechnology Laboratory, Botany Department, North-Eastern Hill University, Shillong 793 014, India.

Studies on the nutrition of plant embryos *in vitro* are of considerable importance in understanding the physiological and biochemical aspects of growth and development in plants. Besides activation of enzymes and hormonal factors, the harmonious development of embryo and functional differentiation within it may result from induction by gradients of nutritional substances (Raghavan, Torrey 1964). Although repeatable regeneration of plants has now been made possible, understanding of the nutritional and hormonal balance is still incomplete. Small variations in conditions for sterilization and culture media can result into qualitative and quantitative variations.

Taxonomically the orchids represent the most highly evolved family among monocotyledons. Unfortunately a large number of orchid species are underway of extinction due to the increasing devastation of forest lands and their overexploitation to cater a great demand in the medical and cut-flower industries. Besides, less than 5% of orchid seeds germinate in nature (Rao 1977) owing to their extremely small size, underdeveloped or lack of endosperm and the requisition of a specific fungi to enter a symbiotic association with. Mass clonal propagation of a number of orchids has been developed in recent years and various protocols developed (Arditti et al 1982). However, only a few studies are carried out for the selection of specific culture media for the maximum regeneration and multiple shooting, for use in nursery. *Dendrobium wardianum* Warner – a beautiful epiphytic orchid – is fast becoming an endangered species. It is one of the commercially important dendrobes. The present communication describes experiments to establish the optimum culture conditions for shoot multiplication, and to maximize the overall nutrient efficiency of *in vitro* regeneration of *D wardianum* Warner.

## Materials and Methods

### Establishment of initial cultures :

shoot tips (ca 2-3 mm) were excised from *Dendrobium wardianum* Warner plants and sterilized with 5% (v/v) NaOCl solution (with 1-3% available chlorine) for 5 minutes, and were inoculated on Murashige and Skoog's (1962) basal medium containing 3% sucrose, 0.8% agar and 2.5 mg l<sup>-1</sup> 6-benzylaminopurine (BAP) (under report). The pH of the medium was adjusted to 5.8 prior to addition of agar and autoclaved for 15 min at 120°C. The cultures were incubated at 24± 2°C with 16 h illumination of 2000 lux. Protocorm-like bodies (plbs) analogous to embryoids were produced after 6 weeks of culture.

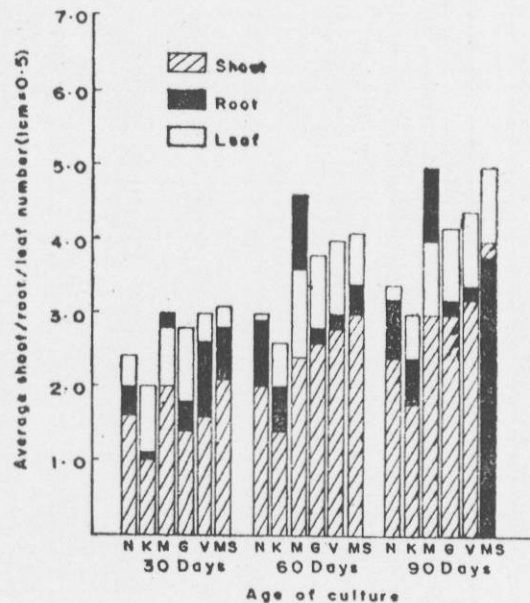


Fig 1. Effect of various nutrient media on shoot, root and leaf number of regenerants. (N, Nitsch; K, Kundson C; M, Mitra et al; G, Gamborg; V, Vacin & Went; MS, Murashige & Skoog)

### Shoot multiplication:

Protocorm-like bodies (plbs) so developed were transferred to fresh medium containing six different basal media: Vacin and Went (1949), Mitra et al (1976), Gamborg et al (1968), Nitsch and Nitsch (1969), Knudson-C (1946), and Murashige and Skoog (MS), and the effects of six macroelement formulae were studied on growth and development of seedlings. To avoid the possibility of a carry over effect of growth regulators from the original mother explants, all the results given in this paper were obtained during the third set of experiment. Each experiment was repeated thrice. The cultures were transferred to a fresh medium every four

weeks. The growth and development of the plbs were studied in terms of fresh weight; number of shoot, root and leaf; and length of shoot and root.

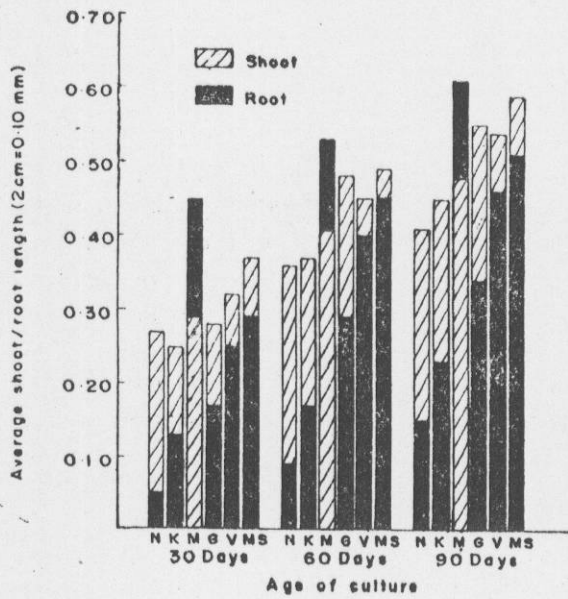


Fig 2. Effect of various nutrient media on shoot and root length. (N, Nitsch; K, Knudson C; M, Mitra et al; G, Gamborg; V, Vacin & Went; MS, Murashige & Skoog)

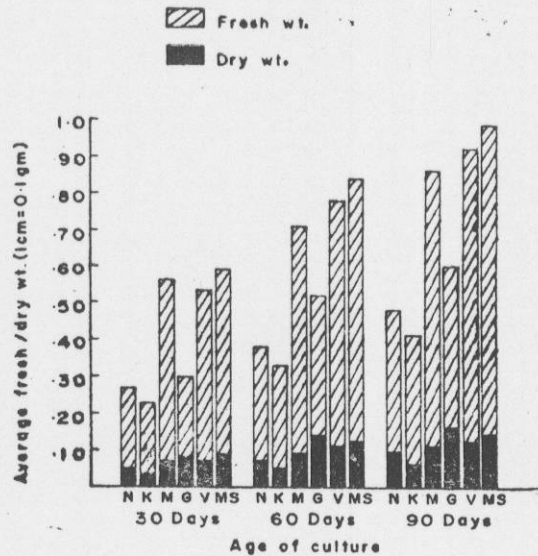


Fig 3. Effect of various nutrient media on fresh and dry weight of regenerants. (N, Nitsch; K, Knudson C; M, Mitra et al; G, Gamborg; V, Vacin & Went; MS, Murashige & Skoog)

## Results

Of the six mineral media tested, best results were obtained with MS medium. Besides resulting into the highest shoot and leaf number, shoot length and fresh weight (0.984 g), it produced dark green stems and long leaves. However, the

highest average root number and root length were observed with Mitra et al medium. Gamborg's medium yielded the highest dry weight; and average shoot, root and leaf number were found to be the lowest on Knudson-C. Growth on both, Knudson-C and Nitsch media was stunted. The cultures grown on Vacin and Went medium were the second best in their growth.

## Discussion

The successful establishment and growth of plant cells *in vitro* generally is determined by the nature of the explant and the composition of the nutrient medium (White 1951). Growth and differentiation are controlled by various components of media and mineral nutrients. It has been reported that seeds/protocorms of orchid are unable to absorb and utilize nitrates during the initial phase of germination and development. However, appearance of nitrate reductase activity has been reported to be correlated with the absorption of nitrates in the later stage (Raghavan 1964). Presence of high amount of ammonium nitrate in MS might favour the growth as  $\text{NH}_4^+$  ions are readily assimilated during the initial phase and  $\text{NO}_3^-$  at later stage of development. Moreover, most tissue culture media are poorly buffered and pH fluctuations that occur may be detrimental to long term survival and to growth of cells at either low density (Caboche 1980) or as single cells (Koop et al 1983).

We derive that the nutrient medium containing high concentration of ammonium nitrate favours luxuriant growth of *D wardianum*, which can be exploited for nursery and orchard developments.

## Acknowledgement

Financial assistance from Department of Environment, Government of India is gratefully acknowledged.

## References

- Arditti J, Clements M A, Fast G, Hadley G, Nishimura G, Ernst R 1982 *In Orchid Biology-Reviews and Perspectives*, II, Cornell Univ Press, USA
- Caboche M 1980 *Planta* 149 7
- Gamborg O L, Miller R A, Ojima K 1968 *Exp Cell Res* 50 151
- Knudson L 1946 *Am Orchid Soc Bull* 15 274
- Koop H U, Weber G, Schweiger H G 1983 *Z Pflanzenphysiol* 112 27
- Mitra G C, Prasad R N, Roychoudhury A 1976 *Indian J Exp Biol* 14 350
- Murashige T, Skoog F 1962 *Physiol Pl* 15 473
- Nitsch J P, Nitsch C 1969 *Science* 163 85
- Raghavan V 1964 *Bot Gaz* 125 260
- Raghavan V, Torrey J G 1964 *Am J Bot* 51 264
- Rao A N 1977 *In Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture*, Springer-Verlag 44
- Vacin E, Went F W 1949 *Bot Gaz* 110 605
- White P R 1951 *Ann Rev Pl Physiol* 2 231