

IN VITRO CULTURE OF *DENDROBIUM WARDIANUM* WARNER : MORPHOGENETIC EFFECTS OF SOME NITROGENOUS ADJUVANTS

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SUMMARY

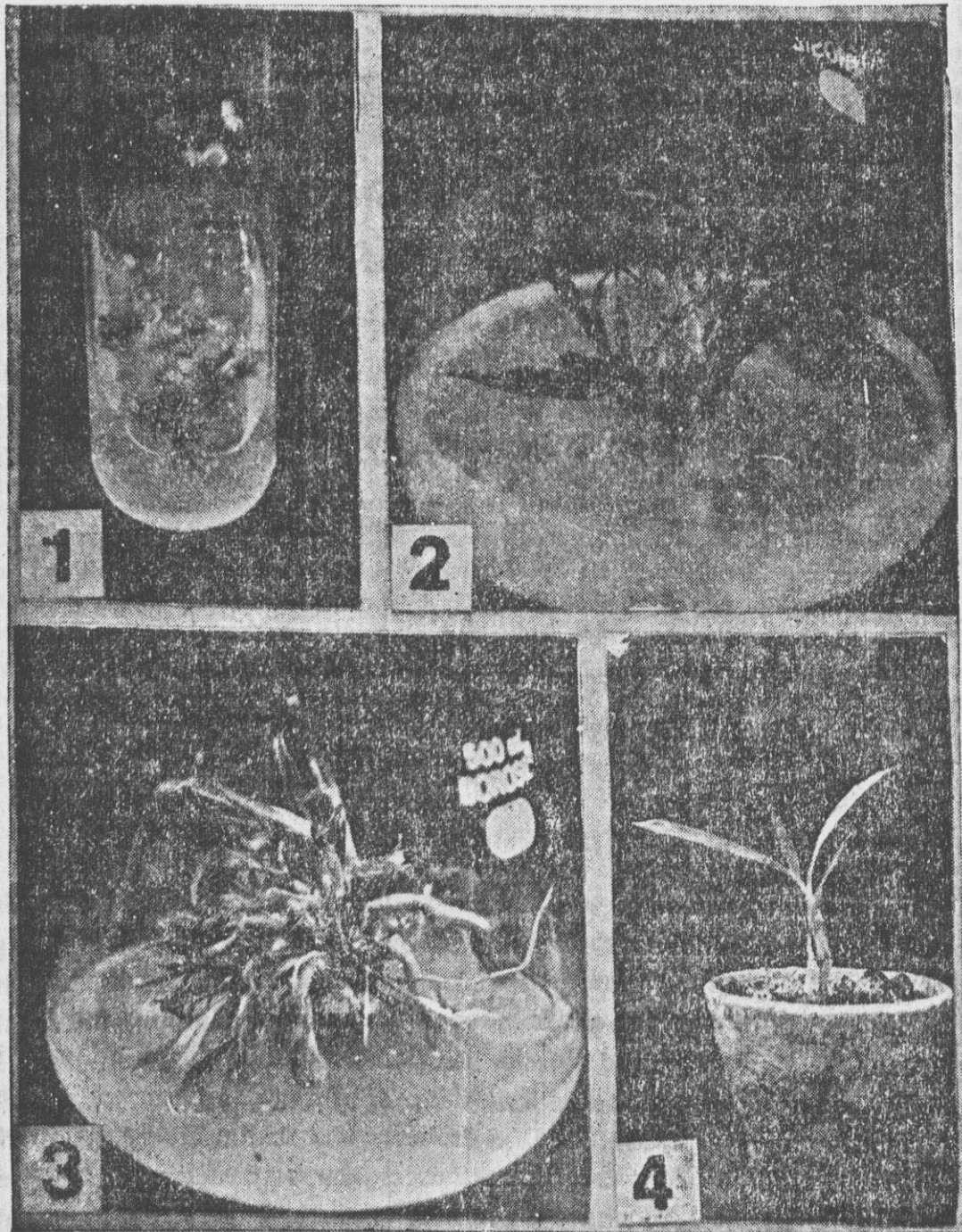
Excised shoot apices of *Dendrobium wardianum*, cultured on MS medium supplemented with inorganic and organic nitrogenous sources, viz., calcium nitrate, ammonium sulphate, urea and amino acids (alanine, glutamine, glycine, leucine and serine) showed varied responses. Calcium nitrate and urea gave rise to direct multiple shooting besides forming protocorm-like bodies. Regenerants obtained by rooting isolated shoots and germinating protocorm-like bodies were successfully transplanted to a substratum containing charcoal pieces, brick bats and coconut fibres. The rate of survival was recorded to be 65% under glass house conditions.

INTRODUCTION

Orchids are slow growing plants which could be accounted for by their sluggish nitrogen metabolism (Poddubnaya-Arnold, 1967). A major part of nitrogen is required for the production of proteins and formation of chlorophyll and cytochrome. Nitrogen deficiency, after water stress, is the most common limitation on growth. In their initial stage of growth and development, orchids are unable to utilize nitrate but the ability to absorb nitrate ions indicates the appearance of nitrate reductase activity (Raghavan and Torrey, 1964). Dendrobes are splendid orchids important both commercially and/or medicinally. An experiment was designed to investigate the effect of different inorganic nitrogen sources on the morphogenetic potential of cultured shoot apices of an epiphytic, commercially favoured ornamental, *Dendrobium wardianum* Warner.

MATERIALS AND METHODS

Excised shoot apices (ca. 2-3 mm) from *Dendrobium wardianum* Warner plants were sterilized with 5% (v/v) NaOCI solution (with 1-3% available chlorine) for 5 minutes. These were then inoculated on Murashige and Skoog (MS, 1962) basal



- Fig. 1. Proliferation of protocorm—like (plb) from cultured shoot apices.
Fig. 2. Direct multiple shooting from the cultured shoot apex under influence of calcium nitrate (2.5 mg/l).
Fig. 3. Regeneration of plantlets by transferring protocorm—like bodies on MS basal medium.
Fig. 4. Potted plantlet ready for hardening.

medium containing 3% sucrose, 0.8% agar and 2.5 mg/l 6-benzylaminopurine (BAP) for protocorm-like body formation (Sharma and Tandon, 1991), supplemented with different inorganic and organic nitrogen sources, viz. calcium nitrate, ammonium sulphate, urea and aminoacids (alanine, glutamine, glycine, leucine and serine) at a range of concentrations (0.5-5.0 mg/l). Explants cultured on MS medium supplemented with 2.5 mg/l BAP served as control. The pH of the medium was adjusted to 5.8 prior to addition of agar and the cultures incubated from cool fluorescent white lamp at $24 \pm 2^\circ\text{C}$ at 16 h illumination of 2000 lux. Data were collected every 30 day up to 3 months and the experiment repeated thrice.

RESULTS AND DISCUSSION

All the cultures more or less gave rise to globular, green protocorm-like bodies with 0.5 as expected (Fig. 1). Shoot apices cultured on MS medium supplemented and 2.5 mg/l calcium nitrate gave rise to direct multiple shooting thus bypassing the usual intervening protocorm-like body (plb) formation phase (Fig. 2). However, higher concentrations of calcium nitrate resulted in drying of the explants. Response with urea was parallel to that of calcium nitrate at 2.5 and 5 mg/l, (Table 1). Lower concentration of the urea gave rise to plb's which on transfer to basal MS medium regenerated into complete plantlets. Explants inoculated on ammonium sulphate supplemented MS medium gave a poor percentage of plb formation at higher concentrations. Most cultures turned brown within a week and dried ultimately. Addition of individual aminoacids did not show any significant effect.

Ionic extra/intra cellular environment has been, besides other factors, found to affect cell elongation, enlargement, division and morphogenesis (Tran Thanh Van and Mutaftschiev, 1990). It is generally believed that reduced forms of nitrogen have value for certain plant tissue cultures. Although ammonium (NH_4^+) is rarely adequate as the sole nitrogen source, small amount of it may be essential for good growth; however, NH_4^+ , in certain cases may be detrimental to survival and growth of plant cells (Ozias-Akins and Vasil, 1985). The browning and drying of explants treated with ammonium sulphate can be attributed to ammonium toxicity which is caused by higher N-accumulation when NH_4^+ is used in higher concentrations (Barker *et al*, 1967; Barker and Maynard, 1972). The form of N supplied to plants has a great influence on the absorption of nutrients. Alan (1989) reported that nitrate generally increased all mineral contents except for N in the tissue whereas ammonium decreased them. Decrease of K, Ca and Mg content of the plant tissues with NH_4^+ have been well documented in other plants (Hartman *et al*, 1986). Urea has been reported to serve as an effective N source for *Cattleya* embryos (Mariat, 1948) but has also been reported to cause necrosis at higher concentrations as it significantly

Table 1 : Effect of different organic and inorganic nitrogen sources on the morphogenetic potential of shoot apices cultured on MS₁ (MS+2.5 mg/l BAP)*

Nitrogen source	Conc. (mg/l)	% Morpho-genetic	Nature of response		Remarks
			Plb	MS** SS***	
M²+No					
N—Source	—	60	✓	— —	Green, globular plb
Inorganic N-Source					
MS ₁ +Calcium Nitrate	0.5	55	✓	✓ —	50% cultures formed 2-3 thick MS
	2.5	60	—	✓ —	4-5 healthy MS
	5.0	50	✓	— —	Green, globular plb
MS ₁ +Ammonium sulphate	0.5	45	✓	— —	-do-
	2.5	35	✓	— —	-do-
	5.0	20	✓	— —	Pale green plbs
Organic N-Source					
MS ₁ +Urea	0.5	50	✓	— —	Green, globular plb
	2.5	55	✓	✓ —	45% cultures formed MS
	5.0	55	✓	✓ —	45% cultures formed 3-4 MS
Amino Acids					
MS ₁ +Alanine	0.5	50	✓	— ✓	40% cultures formed globular plbs
	2.5	55	✓	— ✓	50% cultures gave rise to plbs
	5.0	50	✓	— ✓	40% cultures formed plbs
MS ₁ +Glutamine	0.5	45	✓	— ✓	Green, globular plbs
	2.5	50	✓	— ✓	-do-
	5.0	40	✓	— —	Green, globular plbs
MS ₁ +Glycine	0.5	50	✓	— —	-do-
	2.5	55	✓	— —	-do-
	5.0	40	✓	— ✓	30% cultures formed green plbs
MS ₁ +Leucine	0.5	45	✓	— —	Green plbs
	2.5	40	✓	— ✓	35% cultures gave rise to plbs
	5.0	40	✓	— ✓	-do-
MS ₁ +Serine	0.5	55	✓	— —	Green, globular plbs
	2.5	50	✓	— ✓	40% cultures gave rise to healthy plb
	5.0	50	✓	— ✓	-do-

* Data collected every 30 day for 3 months, Each experiment repeated thrice,

** Multiple shoots

*** Single shoot,

increases the N content and decreases Ca content (Alan, 1989). Individual amino acids or mixtures of amino acids have been found to give no growth stimulation in orchids when added to a medium already supplying inorganic N (Withner, 1959). No specific growth enhancement following the addition of amino acids could be attributed to competitive interactions between the amino acids (Street, 1989).

Isolated shoots, obtained directly from calcium nitrate and urea treated shoot apices (Fig. 2), were transferred to basal MS medium where they grew well developed, stout roots in 2 weeks. Protocorm-like bodies when sub-cultured to the same basal MS media regenerated into complete plantlets (Fig. 3). The regenerated plantlets after attaining a height of 4-5 cms. were transferred to small, earthen pots (Fig. 4) containing charcoal pieces, brick bats and cocount fibres with a layer of moss at the surface and kept under glasshouse conditions. Survival rate was found to be 65%. The results obtained can be immensely important to and exploited by the nursery growers, orchard developers and cut flower industries for rearing the species on mass scale.

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