The browning of adventitious buds was observed inhibiting adventitious shoot formation. To prevent this, frequent subculturing on a fresh medium was necessary every 5–6 days.

Shoot and root regeneration: Adventitious buds were individually isolated once and cultured on SH medium containing BAP (0–2 mg/l) for shoot development. Shoot elongation was observed in 70% of the buds on SH medium with no hormonal supplement (table 2). Shoot elongation started from adventitious buds after 2 weeks (figure 2).

For the induction of roots, well-developed shoots were cultured on SH medium containing 0.1 mg/l NAA. Root initiation was observed after 2 weeks (figure 3). Fifty per cent of the shoots developed roots. Ishii[10] induced rooting in about 70% of shoots developed from adventitious buds of *Chamaecyparis obtusa*.

Conclusions: 1) SH medium is better than DCR medium for the induction of adventitious buds. 2) Shoot elongation from adventitious buds is highest on SH medium without growth hormones. 3) Root induction in the regenerated shoots is achieved on SH medium. 4) Direct adventitious bud induction, without formation of callus, may be applicable for clonal propagation of forest trees. However, more work is needed to study different types of variation among the regenerated plantlets.

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INFLUENCE OF CARBON, NITROGEN AND PHOSPHORUS NUTRITION ON GROWTH OF *DIOSCOREA DELTOIDEA* CALLUS AND PRODUCTION OF DIOSGENIN AND STEROLS

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THE influence of nutritional factors on the growth of plant cells and secondary product formation has been a subject of intense study[1,2]. It is now realized that a separate media need to be devised for the growth of plant cells and metabolite production. The importance of carbon-to-phosphate ratio, in the nutrient medium, on secondary metabolite formation in microbes is well known[3,4]. However, only limited attention has been paid towards this aspect in plant cell cultures[2].

In this communication we report the influence of carbon (C), nitrogen (N) and phosphorus (P) nutrition on the growth and diosgenin production in callus tissues of *D. deltoidea*.

Seeds of *D. deltoidea* collected from the Kashmir valley were aseptically germinated. The callus was initiated from hypocotyls of 10-day-old seedlings on modified MS medium[5] supplemented with 1 mg/l 2,4-D and 3% sucrose and maintained on the same medium by regular subcultures of 45 days interval. Cultures were incubated at 25 ± 2°C in continuous light (3000 Lux).

The growth of callus was measured in terms of dry weight, after drying the tissue in hot air oven at 60°C to constant weight.

The harvested tissue was dried and powdered. One gram dry powder was soxhlet-extracted with petroleum ether (30–50°) for 24 h. The extract was evaporated and examined for free sterols[6]. The residual cells were hydrolysed by refluxing with 10% HCl for 2 h, cooled and filtered. The residue was washed first with water and then with 0.1 N NaOH and finally with water to neutrality. The residue was then dried at 60°C, powdered and soxhlet-extracted with $CHCl_3$ for 48 h. The $CHCl_3$ extract was evaporated and the diosgenin and bound sterols were quantified by comparing OD values with the standard curve constructed by preparative TLC method[7].

It is clear from figures 1A,B that diosgenin synthesis in *D. deltoidea* callus is restricted to the stationary phase of growth. Hence, the growth and

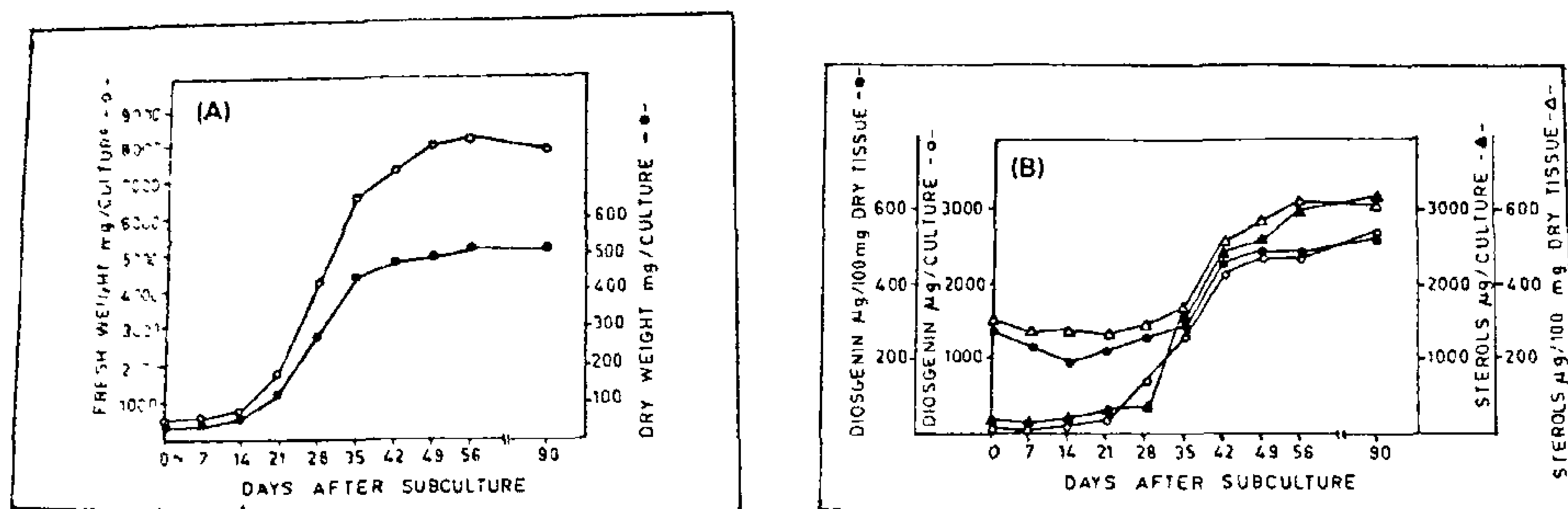

Figure 1A, B. Time course study of growth of callus (A) and production of diosgenin and sterols (B) in *D. deltoidea* on modified MS medium supplemented with 1 mg/l 2,4-D and 3% sucrose.

steroid synthesis in experimental media were recorded during the stationary phase of culture, on day 90.

Carbon (3% sucrose), nitrogen (ammonium nitrate and potassium nitrate totally equivalent to 840 mg/l N), and phosphorus (KH_2PO_4 170 mg/l) were used at 1:1:1 concentration[5] for control (table 1, treatment 1). In treatments, C, N and P ratios were varied as in table 1.

Influence of CNP ratio on growth of D. deltoidea: Elevation of carbon supply in relation to N&P (table 1, treatment 2) nearly doubled the growth, whereas increase of nitrogen to C and P had marginal effect (treatment 3) and increase of phosphate to C and N showed growth inhibition (treatment 4). However, elevation of phosphate and carbon (treatment 6) had a synergistic effect on the growth which was twice as much as control.

Table 1 *Influence of carbon, nitrogen and phosphorus changes on growth of D. deltoidea tissues and steroid production*[a]

Treatment C:N:P	Growth[b] Dry weight (g/culture)	Diosgenin %	Diosgenin µg/ Culture	Sterols %	Sterols µg/ Culture
[c]1:1:1	0.522 (±0.041)	0.520 (±0.035)	2714 (±213)	0.60 (±0.027)	3132 (±246)
2:1:1	0.974 (±0.062)	0.551 (±0.034)	5366 (±331)	1.24 (±0 058)	12077 (±768)
1:2:1	0.687 (±0.056)	0.144 (±0 010)	989 (±80)	1.24 (±0 071)	8518 (±694)
1:1:2	0.427 (±0.025)	0.372 (±0.015)	1588 (±82)	1.06 (±0.055)	4526 (±264)
2:2:1	0.572 (±0.032)	0 620 (±0 026)	3546 (±198)	1.80 (±0 055)	10296 (±576)
2:1:2	1.125 (±0 073)	0.232 (±0.013)	2610 (±169)	0.70 (±0 033)	7875 (±511)
1:2:2	0 416 (±0 027)	0.356 (±0 012)	1481 (±96)	1.44 (±0 086)	5990 (±388)
2:2:2	0.806 (±0 051)	0 609 (±0.023)	4908 (±310)	1 74 (±0 09)	14024 (±887)

[a] Data represent an average of 5 replicate cultures (90-day-old) & figures in parentheses are standard errors; [b] Initial inoculum 500 ± 25 mg fresh tissue; [c] Control.

Influence of CNP ratio on diosgenin and sterol production: The increase of carbon in relation to N and P (table 1, treatment 2) influenced marginal increase in diosgenin percentage, but the total yield of diosgenin was higher than in the other treatments tested. On this medium sterol synthesis on percentage basis increased two-fold over the control and on absolute basis the yield was four-fold. Such a beneficial effect of raising initial sucrose level on metabolite production has been reported for other plant cell cultures[8]. In the presence of 4% sucrose the cells of Paul's Scarlet Rose accumulate 150% more polyphenols than at 2% sucrose[9].

Increased nitrogen level alone (treatment 3) inhibited diosgenin synthesis but sterol synthesis was promoted. Similar results were obtained with increased phosphate concentration (treatment 4). It is interesting to note that low phosphate to C and N ratio (treatment 5) had a highly beneficial effect on diosgenin and sterol percentage. These results agree with the results of Mantell *et al*[8] for increased nicotine production.

The present results have brought to light the interplay of CNP ratio and also the nutritional requirements for growth of *D. deltoidea* callus (MS salts with double concentration of KH_2PO_4 plus 6% sucrose) and for diosgenin production (MS salts with double concentration of nitrates and 6% sucrose). These results are useful for adoption in the two-stage culture system for *in vitro* diosgenin production.

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CYTOMIXIS IN A MAIZE TRISOMIC

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THE intrusion of chromatin material from one cell to another through cytoplasmic connections — referred to as cytomixis — is observed in mitotic and meiotic cells of many plant species[1]. Cytologically, and hence physiologically and biochemically, imbalanced plants like haploids, triploids, aneuploids, hybrids and apomicts show cytomixis more often than normal cytogenetically balanced and established plants[2,3]. Temperature fluctuations during the growth period are also considered to be a factor leading to cytomixis[4]. In maize (*Zea mays* L.) cytomixis was reported in the triploid and its hyperploid progeny by McClintock[5]. The present paper reports the occurrence of cytomixis in pollen mother cells of maize trisomic for chromosome 5.

The maize stock trisomic for chromosome 5 ($2n = 21$) was obtained from Dr G. G. Doyle, University of Missouri, USA. The young male inflorescence of plants, grown in the field during July–August 1987 at New Delhi, was fixed in 3:1 ethanol–acetic acid. Acetocarmine smear preparations of anthers were made to observe the meiosis in pollen mother cells. The normal diakinesis and metaphase I stages had ten bivalents and one univalent (10 II + 1 I) or one trivalent and nine bivalents (1 III + 9 II) (figure 1). Certain pollen mother cells were found to have double the number of chromosome i.e. 20 II 2 I or 1 III + 19 II + 1 I (figure 2). Some pollen mother cells at anaphase I also had double the number of chromosomes. The increase in chromosome number was due to cytomixis (figure 3). The absence of quadrivalents or multivalents at diakinesis or metaphase I in cells with double the number of chromosomes indicates that cytomixis occurred in early prophase after initiation of chromosome pairing. Chromosome fragmentation occurred in some pollen mother cells at first meiotic division leading to the formation of diads with a high number of chromosome fragments (figure 4). The disturbance in the rhythm of development of the pollen mother cells giving rise to