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Regeneration in *Chlorophytum borivilianum* through Somatic Embryogenesis

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ABSTRACT

Chlorophytum borivilianum, an important medicinal plant belonging to the Lilliaceae family is valued for its dried fasciculated roots, which have aphrodisiac properties and also form an important ingredient of various herbal medicines. A high frequency regeneration protocol for rapid multiplication of *C. borivilianum* through the induction of somatic embryos was attempted. Microshoots along with a part of the stem disc were cultured on Murashige and Skoog (MS) medium supplemented with a low concentration range (0.25, 0.5, 0.75 and 1.0 mg l^{-1}) of 2,4-Dichlorophenoxyacetic acid (2,4-D) alone, and in combination with different concentrations of cytokinins like 6-benzylaminopurine (BAP) and kinetin (Kn). One hundred per cent callus induction and embryogenic response was noticed at all low concentrations of 2,4-D. The number of days required for callusing (29.8), the number of embryos (67.2), the diameter of embryogenic callus (2.28 cm) was more with1.0 mg l^{-1} 2,4-D, the average fresh (1.073 g) and dry (0.100 g) weights of callus were more when 1.0 mg l^{-1} 2,4-D was used. The callus resulting from these treatments was friable, glossy and creamish-yellow in appearance. After 60 days of culture in induction media, 100% maturation was achieved on plain MS and on MS medium with 0.1 mg l^{-1} abscisic acid (ABA). Embryos derived from treatments with 2,4-D did not show 100% embryo maturation, which could, however be achieved if these were added to cytokinin-supplemented medium. Sixty two per cent of matured embryos formed shoots on media devoid of plant growth regulators but these took nine days to germinate unlike the precocious germination (5 days) demonstrated by matured embryos treated with 0.5 mg l^{-1} gibberellic acid (GA_3). Shoot induction formed in 60% of embryos treated with 0.5 mg l^{-1} TDZ and 0.5 mg l^{-1} Kn but most shoots showed malformation during growth. Stereo and fluorescence microscopy study confirmed all the different developmental stages of embryos, including the suspensor.

Keywords: aphrodisiac, embryoid, fluorescence microscopy, germination, maturation, safed musli, secondary embryos, somatic embryo
Abbreviations: ABA, abscisic acid; **AH**, adenine hemisulfate; **BAP**, 6-benzylaminopurine; **ELS**, embryo-like structure; **Kn**, kinetin; **TDZ**, thidiazuron

INTRODUCTION

Chlorophytum borivilianum, belonging to the family Liliaceae, is an important medicinal herb of commercial importance (Bordia *et al*. 2003). The crop is highly valued for its dried fasciculated tuberous roots. The dried tubers are used as a health tonic, anti-ageing agent, aphrodisiac and herbal substitute for chemical-based Viagra® (sildenafil citrate). Apart from that it is valued as a muscular tonic, supposed to be equivalent to shilajeeth and the famous tonic plant *Panax ginseng* (Ajay 2003). The natural regeneration of this herb is through tuberous roots that have become scarce in nature because of indiscriminate collection of wild material (Jat and Bordia 1990).

Plant tissue culture has been successfully used to micropropagate several medicinal plants and other members of the Lilliacae (Ramawat *et al*. 1998; Mokshin *et al*. 2006). Direct regeneration of plants through shoot tips attached to the stem disc as explant (Purohit *et al*. 1994; Pudake and Dhumale 2003) and shoot regeneration from immature floral buds along with the inflorescence axis have also been reported in *Chlorophytum* (Sharma and Mohan 2006).

The present study describes regeneration of *C. borivilianum* plantlets through somatic embryogenesis induced by different plant growth regulators (PGRs).

MATERIALS AND METHODS

Explants and growth conditions

Microshoots (mother plants maintained *in vitro*) obtained from direct regeneration along with part of the stem disc were used to induce embryogenic callus. Explants were treated with 0.1% (w/v) mercuric chloride and 1% (v/v) sodium hypochlorite. Explants were then washed with 3-4 changes of sterile distilled water. Explants were cultured on MS media supplemented with lower concentrations of 2,4-D (Extra pure, Himedia Laboratories Pvt. Ltd., Mumbai, India) (0.25, 05, 0.75 and 1.0 mg l^{-1}) alone and with different concentrations and combinations with 6-benzylaminopurine (BAP) or kinetin (Kn) (Extra pure, Himedia Laboratories Pvt. Ltd., Mumbai, India). Explants were observed every alternate day up to the 60th day to record days required for callusing, per cent callusing and number of somatic embryos.

The cultures were incubated in a growth room at 25 $\pm$ 2°C under a 16-h photoperiod (16 h light, 8 h darkness) at a light intensity of 1200 lux by fluorescent tubes.

The following growth and culture parameters were assessed:

i) Percent callus response, measured on the 60th day, was expressed by the following equation:

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$$\text{Percent response to callusing} = \frac{\text{Number of explants showing callusing}}{\text{Total number of explants inoculated}} \times 100$$

ii) Color of callus: Based on visual observation, the color of the callus was recorded on the 60th day as either greenish, light green, greenish white, creamish yellow, glossy or creamish yellow.
iii) Type of callus: This parameter classified callus produced as being either compact, friable or friable to compact, and was assessed on the 60th day.
iv) Days to callusing: The number of days taken for explants to initiate callus within different treatments.
v) Number of somatic embryos: The number of somatic embryos or embryoid-like structures (ELSs) formed from each explant, measured on the 60th day.
vi) Percent embryogenic callus: Percent embryogenic callus was calculated on the 60th day by using the following formula:

$$\text{Per cent embryogenic callus} = \frac{\text{Number of embryogenic calli}}{\text{Total number of explants inoculated}} \times 100$$

vii) Diameter of embryogenic callus: The diameter of the callus obtained from different treatments was measured on the 60th day with the use of a scale and expressed in cm.
viii) Fresh weight of callus: The amount of calli induced from individual explants was recorded on the 60th day, and represented in terms of fresh weight (g).
ix) Dry weight of callus: Calli used for recording fresh weight were dried in an oven at 65°C to a constant weight, the dry weight, measured on the 60th day.

Maturation of embryoids

Embryogenic callus obtained on induction media was transferred to maturation media containing 0.25 or 0.5 mg l^{-1} thidiazuron (TDZ) (Extra pure, Himedia Laboratories Pvt. Ltd., Mumbai, India) or PGR-free media.

Germination/sprouting of embryos

Matured embryos were transferred to media containing different concentrations (1, 2, 3 and 4 mg l^{-1}) of BAP, 0.5 mg l^{-1} (indole-3-butyric acid; IBA), 0.5 mg l^{-1} gibberellic acid (GA_3) (Extra pure, Himedia Laboratories Pvt. Ltd., Mumbai, India) and PGR-free media. The number of embryos that germinated/sprouted was expressed as a percentage of the number of embryos inoculated.

Shoot induction

Germinated/sprouted embryos were transferred on to different shoot induction media, as they failed to develop shoots, even after 45 days culture on germination media. Sprouted embryos were cultured on shoot induction media containing different cytokinins: adenine hemisulfate (30, 60 and 90 mg l^{-1}), TDZ (0.1, 0.5 and 1 mg l^{-1}), Kn (0.5, 1, 2 and 3 mg l^{-1}) and BAP (0.5, 1, 2 and 3 mg l^{-1}). The per cent shoot induction was calculated by counting the number of embryos producing shoots to the number of embryos inoculated. Morphological observations like variations in shoot and root characters were also recorded.

Experimental design

The experiment was laid out in a Completely Randomized Design (CRD). Values in percentages were subjected to arcsine transformation to ensure homogeneity. The other statistical values obtained were transformed by using the formula $\sqrt{x} + 0.5$ for statistical analysis. Test of significance was conducted and Critical Difference (CD) values were used for means comparison.

RESULTS AND DISCUSSION

In the present study lower concentrations of the growth regulator 2,4-D alone, or 2,4-D either with BAP or Kn was found to induce embryogenic calli as determined by visual observations. More and better (friable in texture, glossy, creamish-yellow in color and healthy ≈ growing) embryogenic calli were induced in the presence of 2,4-D than by either BAP or Kn (**Table 1; Fig. 1**). Culture of explants with auxin or cytokinin frequently resulted in formation of more than one type of callus, while 2,4-D was shown to be effective in the induction of callus from the explants of several crops (George and Sherrington 1984).

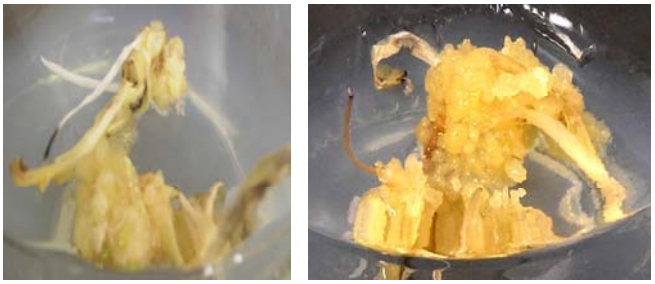

Fig. 1 Embryogenic callus observed with 0.5 mg l^{-1} l 2, 4-D.

Table 1 Qualitative characteristics of calli induced in media with 2,4-D, 2,4-D + Kn and 2,4-D + BAP (60 days after culture).

PGR (mg l^{-1})	Texture	Callus color
2,4-D 0.25	Friable	Glossy and creamish-yellow
2,4-D 0.50	Friable	Glossy and creamish-yellow
2,4-D 0.75	Friable	Glossy and creamish-yellow
2,4-D 1.0	Friable	Glossy and creamish-yellow
2,4-D 0.5 + Kn 0.5	Friable	Light green
2,4-D 0.5 + Kn 1.0	Friable-compact	Greenish-white
2,4-D 1.0 + Kn 1 .0	Friable-compact	Light green
2,4-D 1.5 + Kn 1.5	Friable-compact	Light green
2,4-D 2.5 + Kn 0.5	Friable	Creamish-yellow
2,4-D 2.5 + BAP 1.0	Friable	Greenish-white
2,4-D 2.5 + BAP 2.0	Compact	Greenish
2,4-D 2.5 + BAP 2.5	Compact	Greenish

Number of explants/treatment: 30

Callus induction response and embryogenic callus was obtained in all media containing a lower concentration range (0.25-1 mg l^{-1}) of 2,4-D. Callusing was early (29.8 days) when 2,4-D was used alone and was delayed when a combination of auxin with cytokinin was incorporated in the media (**Table 2**).

When 2,4-D was combined either with BAP or Kn callusing response was low, but this combination resulted in increased fresh weight and dry weight of calli, which could be attributed to the enhanced accumulation of metabolites in embryogenic calli or probably an increase in protoplast contents (George and Sherrington 1984).

The fresh weight (1.073 g) and dry weight (0.100 g) were highest at a low concentration (0.5 mg l^{-1}) of 2,4-D, while the diameter (2.28 cm) and number of somatic embryos (67.2) from embryogenic calli induced from explants also increased with 1.0 mg l^{-1} 2,4-D (**Tables 2, 3**). The fresh weight of callus could be a useful data candidate to estimate the yield of embryoids and subsequently ELSs, which form from the callus.

Stereo and fluorescent microscopy confirmed all the developmental stages of somatic embryos: suspensor, pro-embryo, globular, heart, torpedo, cotyledonary, malformed embryos and many intermediary stages (**Fig. 2**). Different stages of embryos were more abundant when the explants were cultured with 0.5 mg l^{-1} 2,4-D (**Fig. 3**).

Maturation of somatic embryos

Somatic embryos are actually incomplete in their development, unlike zygotic embryos. They do not pass through the final phase of embryogenesis – embryo maturation – which is characterized by the accumulation of reserve food materials and protein that impart desiccation tolerance to the embryo (Janick *et al*. 1989). In our study when embryoids were transferred to media lacking PGRs or to media with a

Table 2 Effect of 2,4-D, 2,4-D + Kn and 2,4-D + BAP on callus induction from *in vitro*-grown stem discs (60 days after culture).

PGR (mg l^{-1})	% Response	Days for callus initiation	Number of somatic embryos
2,4-D 0.25	100 (89.426) [a]	32.2 (5.718) [a]	25.8 (5.127) [g]
2,4-D 0.50	100 (89.426) [a]	31.0 (5.612) [a]	57.2 (8.227) [a]
2,4-D 0.75	100 (89.426) [a]	30.6 (5.576) [b]	48.0 (6.963) [c]
2,4-D 1.0	100 (89.426) [a]	29.8 (5.504) [b]	67.2 (7.582) [b]
2,4-D 0.5 + Kn 0.5	100 (89.426) [a]	30.6 (5.576) [b]	32.8 (5.770) [e]
2,4-D 0.5 + Kn 1.0	100 (89.426) [a]	31.2 (5.629) [a]	31.8 (5.682) [e]
2,4-D 1.0 + Kn 1.0	80.0 (63.929) [c]	32.2 (5.718) [a]	40.6 (6.410) [f]
2,4-D 1.5 + Kn 1.5	40.0 (39.147) [e]	31.6 (5.665) [a]	27.3 (5.272) [g]
2,4-D 2.5 + Kn 0.5	40.0 (39.147) [e]	30.8 (5.594) [a]	10.6 (3.329) [i]
2,4-D 2.5 + BAP 1.0	60.0 (50.852) [d]	31.6 (5.665) [a]	21.4 (4.678) [h]
2,4-D 2.5 + BAP 2.0	90.0 (74.808) [b]	31.8 (5.682) [a]	31.0 (5.617) [e]
2,4-D 2.5 + BAP 2.5	100 (89.426) [a]	32.4 (5.735) [a]	36.8 (6.060) [d]
Mean	74.489	5.639	1.292
SEM ±	3.054	0.051	0.023
CD (*P*=0.05)	8.914	0.149	0.068

Number of explants/treatment: 30
Means superscripted by the same letters do not differ significantly.
Figures in parentheses indicate transformed values

Table 3 Effect of 2,4-D, 2,4-D + Kn and 2,4-D + BAP on callus induction from *in vitro*-grown stem discs (60 days after culture).

PGR (mg l^{-1})	% embryonic callus	Diameter of embryogenic callus	Fresh weight (g)	Dry weight (g)
2,4-D 0.25	100 (89.426) [a]	1.50 (1.413) [d]	0.472 (0.985) [b]	0.036 (0.732) [c]
2,4-D 0.50	100 (89.426) [a]	1.92 (1.555) [b]	1.073 (1.253) [a]	0.100 (0.774) [a]
2,4-D 0.75	100 (89.426) [a]	0.98 (1.216) [f]	0.624 (1.059) [a]	0.070 (0.755) [b]
2,4-D 1.0	100 (89.426) [a]	2.28 (1.667) [a]	0.830 (1.102) [a]	0.068 (0.750) [b]
2,4-D 0.5 + Kn 0.5	100 (89.426) [a]	1.28 (1.333) [e]	0.480 (0.989) [b]	0.045 (0.773) [c]
2,4-D 0.5 + Kn 1.0	100 (89.426) [a]	0.80 (1.139) [f]	0.380 (0.937) [b]	0.036 (0.732) [c]
2,4-D 1.0 + Kn 1.0	80.0 (63.929) [b]	0.70 (1.094) [f]	0.932 (1.196) [a]	0.097 (0.766) [a]
2,4-D 1.5 + Kn 1.5	80.0 (63.929) [b]	1.40 (1.378) [d]	0.475 (0.986) [b]	0.065 (0.751) [b]
2,4-D 2.5 + Kn 0.5	40.0 (39.147) [d]	1.08 (1.256) [f]	0.310 (0.898) [b]	0.049 (0.740) [c]
2,4-D 2.5 + BAP 1.0	60.0 (50.852) [e]	0.30 (0.893) [g]	0.421 (0.958) [b]	0.054 (0.744) [c]
2,4-D 2.5 + BAP 2.0	80.0 (63.929) [b]	0.77 (1.264) [f]	0.475 (0.987) [b]	0.051 (0.742) [c]
2,4-D 2.5 +BAP 2.5	100 (89.426) [a]	1.56 (1.435) [c]	0.975 (1.214) [a]	0.078 (0.760) [b]
Mean	75.648	1.292	1.047	0.749
SE ±	2.548	0.023	0.822	0.004
CD (P=0.05)	7.438	0.068	2.289	0.011

Number of explants/treatment: 30
* Means superscripted by the same letters do not differ significantly.
Figures in parentheses indicate transformed values.

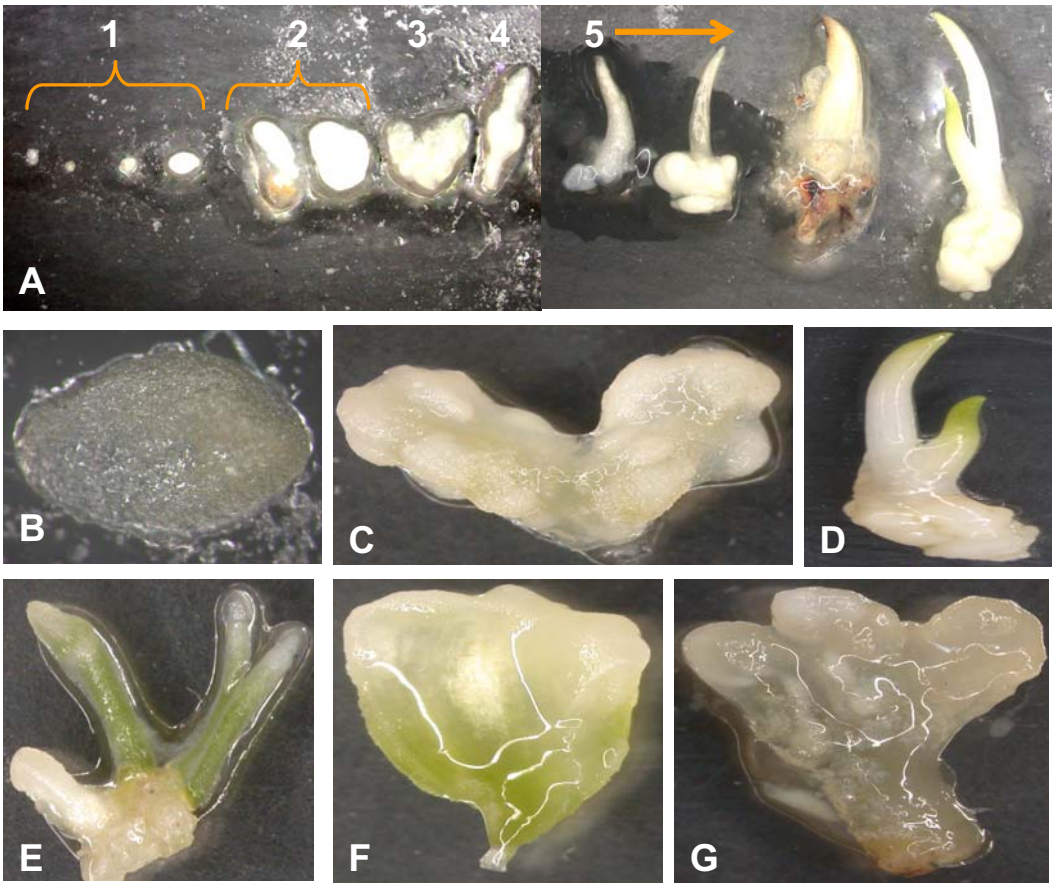

Fig. 2 Different stages of somatic embryogenesis observed with stereo microscope. (**A**) An arrangement of different stages of somatic embryos: 1 = pre-globular; 2 = globular; 3 = heart-stage; 4 = torpedo; 5 onwards = development of shoot. (**B**) Suspensor. (**C**) Multiple globular embryos. (**D**) Shoot emergence. (**E**) Early cotyledonary stage. (**F**) Fused cotyledonary leaves. (**G**) Malformed embryos a cause for degeneration.

Table 4 Effect of different growth regulators on maturation of embryos.

PGR (mg l^{-1})	Maturation (%)
ABA 0.1	100 (89.429) [a]
ABA 0.5	70 (56.997) [b]
MS plain	100 (89.426) [a]
Mean	78.611
SE ±	2.111
CD (*P*=0.05)	7.308

Number of embryos/ treatment -30
* Means superscripted by the same letters do not differ significantly.
# Figures in parentheses indicate transformed values

Table 5 Effect of different growth regulators on sprouting of embryos.

PGR (mg l^{-1})	№ of somatic embryos germinated	Days for germination	№ of shoots	№ of roots, if any	Rooting (%)
BAP 0.5	48.0 (43.837) [a]	8.0 (2.915) [b]	-	-	-
BAP 1.0	50.0 (45.000) [a]	8.0 (2.915) [b]	-	-	-
BAP 2.0	26.0 (30.368) [c]	8.0 (2.915) [b]	-	-	-
BAP 3.0	30.0 (33.002) [b]	8.0 (2.915) [b]	-	-	-
BAP 4.0	32.0 (40.330) [b]	8.0 (2.915) [b]	-	-	-
GA_3 0.5	36.0 (42.673) [a]	5.0 (2.332) [c]	-	-	-
IBA 0.5	50.0 (45.580) [a]	10.0 (3.237) [a]	-	15.0	60.0
Control	62.0 (52.261) [a]	9.0 (3.079) [a]	-	-	-
Mean	41.631	2.902	-	-	-
SE ±	3.484	0.069	-	-	-
CD (*P*=0.05)	10.447	0.207	-	-	-

Number of embryos/ treatment -30
* Means superscripted by the same letters do not differ
# Figures in parentheses indicate transformed values

Table 6 Effect of different cytokinins and abscisic acid on growth and development of shoot (agar 0.5%).

PGR (mg l^{-1})		Shoot induction (%)	Morphological observation
AH	30	40 (39.147) [c]	Rooting also observed apart from shooting
	60	20 (26.070) [e]	Profuse rooting apart from shooting
	90	0 (0.000) [h]	Failed to convert into plant, malformed
TDZ	0.1	0 (0.000) [h]	Failed to convert into plant, malformed
	0.5	60 (50.852) [a]	Dark green shoots, failed to root
	1.0	20 (26.070) [e]	Dark green shoots
Kinetin	0.5	60 (50.852) [a]	Shoots were observed but they are pale green
	1.0	40 (39.147) [e]	Dark green shoots
	2.0	20 (26.070) [e]	Dark green shoots
	3.0	0 (0.000) [h]	Failed to convert into plant, malformed
ABA	0 5	0 (0.000) [h]	Failed to convert into plant, malformed
	1.0	40 (39.147) [c]	Shoots produced but they were sturdy
	2.0	40 (39.147) [c]	Shoots produced but they were sturdy
	3.0	20 (26.070) [e]	Shoots produced, they were sturdy and dark green
Mean		25.898	-
SE ±		3.190	-
CD (*P*=0.05)		9.241	-

Number of embryos/ treatment -30
Means superscripted by the same letters do not differ significantly.
Figures in parentheses indicate transformed values

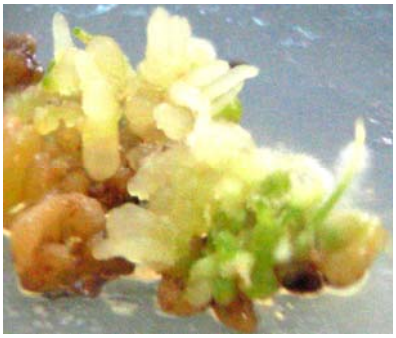

Fig. 3 Different stages of somatic embryos.

Fig. 4 Somatic embryos on maturation media.

lower (0.1 mg l^{-1}) concentration of abscisic acid (ABA) 100% maturation resulted (**Fig. 4**). For the production of vigorous plantlets, a period of embryogenic growth and maturation is needed before germination for the accumulation of sufficient biochemical constituents and embryonic tissue (Ammirato 1983). In the present study, ABA at a higher concentration (0.5 mg l^{-1}) resulted in a lower percentage of maturation (**Table 4**). This is mainly because ABA prevents precocious germination and promotes normal development of embryos by suppression of secondary embryogenesis and plurocotyledonary formation (Ammirato 1983). ABA is known to trigger the expression of genes which normally express during the drying-down phase of seeds and has been shown to increase desiccation tolerance in somatic embryos of celery (Kim and Janick 1990). A reduction in survival percentage (70%) was noticed when ABA concentration increased to 0.5 mg l^{-1} in our study. For the maturation of somatic embryogenesis ABA content should be kept at a lower concentration or the residue may adversely effect germination (Atree *et al*. 1990).

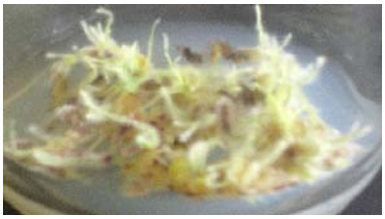

Fig. 5 Sprouting of somatic embryos with 0.1 mg l^{-1} absisic acid.

Fig. 6 Sprouted embryos on shoot induction media. (**Left**) TDZ 0.5 mg l^{-1}. (**Right**) Kinetin 0.5 mg l^{-1}.

Germination/sprouting of somatic embryos

Somatic embryos of many plant species mature and germinate satisfactorily on the induction medium itself. In quite a few cases, it is necessary to transfer the embryos to fresh media with a different concentration and combination of PGRs or to a medium where auxin is omitted (George and Sherrington 1984). In the present study embryogenic tissue exposed to PGR-free medium resulted in maximum sprouting (**Fig. 6**) thus clearly indicating a cessation of repetitive somatic embryogenesis. Medium supplemented with lower concentrations of BAP also resulted in 40-50% sprouting (**Table 5**) and these results are in line with the findings of Arora *et al*. (1999) in *Chlorophytum*. Fewest and most precocious germination was noticed at 0.5 mg l^{-1} GA_3; these germinated embryos dried within a few days, possibly because GA_3 induced ethylene accumulation, as embryos are highly sensitive to ethylene accumulation (George and Sherrington 1984).

Induction of shoots

A lower concentration of Kn (0.5 mg l^{-1}) and TDZ (0.5 mg l^{-1}) resulted in maximum growth and development of shoots. Sixty per cent shoot induction with few malformed shoots occurred and the percentage shoot induction decreased as the concentration of either Kn or TDZ increased (**Table 6**).

Shoots derived by treatment with TDZ were vigorous and were taller than those derived from media with other cytokinins (**Fig. 6**) suggesting that TDZ might have exhibited a duel role of both auxin- and cytokinin-like activity. Similar dual roles have also been put forth by Jayashree and Tulaseedaran (2005) in rubber *in vitro* cultures.

In the present study, all sprouted embryos did not convert into fully developed shoots. Sprouting and full plant recovery has been defined as two different stages during morphogenesis (Sturat and Strickland 1984).

The reasons for malformation of sprouted embryos on germination media could be due to the non-availability of apical meristems or epicotyl formation or cotyledonary structures with or without a radical on opposite ends (Maruyama and Ishii 1999). Similar observations were also made by Jayashree and Tulaseedaran (2005) in rubber.

REFERENCES

Ajay (2003) Safed musli. *Remunerative Medicinal Crops* **1**, 19-20

Ammirato PV (1983) Embryogenesis. In: Evans X, Sharp WR, Ammirato PV, Yamada Y (Eds) *Handbook of Plant Cell Culture* (Vol I) Macmillan, New York, pp 82-123

Arora KD, Suri SS, Ramawat GK, Merillion (1999) Factors affecting somatic embryogenesis in long term callus cultures of safed musli (*Chlorophytum borivilianum*), an endangered wonder herb. *Indian Journal of Experimental Biology* **37**, 75-82

Atree SM, Budimir S, Fowke LC (1990) Somatic embryogenesis and plantlet regeneration from cultured shoots and cotyledons of seedling from stored seeds of black and white spruce (*Picea glauca*). *Canadian Journal of Botany* **8**, 30-34

Bordia PL, Joshi Simlot (2003) Safed musli. In: Chadha KL, Rajendra Gupta (Eds) *Advances in Horticulture (Vol 11) Medicinal and Aromatic Plants*, Malhotra Publishing House, New Delhi, India, pp 429-451

George EF, Sherrington PD (1984) *Plant Propagation by Tissue Culture*, Exegetics Ltd., Eversley, UK, pp 612-689

Janick J, Kitto SL, Kim YH (1989) Production of synthetic seed by desiccation and encapsulation. *In Vitro Cellular and Developmental Biology – Plant* **25**, 1167-1172

Jat RD, Bordia PC (1990) Propagation studies in safedmusli (*Chlorophytum* species). Paper presented at the *National Symposium on Advances in Plant Sciences Current Status and Emerging Challenges*. Sukadia University, Udaipur, pp 46

Jayashree KP, Tulaseedharan A (2005) *In vitro* regeneration of *Heavia* somatic embryos: Effect of cytokinins. *Plant Cell Biotechnology and Molecular Biology* **6**, 61-64

Kim YH, Janick J (1990) Synthetic seed treatment in improving desiccation tolerance of somatic embryos of celery. *Acta Horticulturae* **280**, 23-28

Maruyama E, Ishii K (1999) Somatic embryogenesis in big leaf mahagony (*Swietenia macrophylla* King). In: Jain SM, Gupta PK, Newton RJ (Eds) *Somatic Embryogenesis in Woody Plants*, Kluwer Academic Publishers, The Netherlands, pp 45-62

Mokshin EV, Lukatkin AS, Teixeira da Silva JA (2006) Micropropagation of selected cultivars of *Lilium* and *Gladiolus*. In: Teixeira da Silva (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1st Edn, Vol II), Global Science Books, London, pp 533-539

Pudake RN, Dhumale DB (2003) *In vitro* multiplication of *Chlorophytum borivilianum*. *Journal of Maharastra Agricultural University* **28**, 265-267

Purohit D, Dave A, Kukda G (1994) Micropropagation of safed musli: A rare Indian medicinal plant. *Plant Cell, Tissue and Organ Culture*-Plant **39**, 93-96

Ramawat KG, Jain S, Suri SS, Arora DK (1998) Propagation of important medicinal plants. In: Khan IK, Khannum A (Eds) *Role of Biotechnology in Medicinal and Aromatic Plants*, Ukaaz Publications, Hyderabad, India, pp 210-226

Sharma U, Mohan JS (2006) *In vitro* clonal propagation of *Chlorophytum borivilianum* Sant. *et* Fernard., a rare medicinal herb from immature floral buds along with inflorescence axis. *Indian Journal of Experimental Biology* **44**, 77-82

Sturat DA, Strickland SG (1984) Somatic embryogenesis from cell cultures of *Medicago Sativa*. The interaction of amino acids with ammonium. *Plant Science Letters* **34**, 165-174