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Indirect organogenesis in ginger

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ABSTRACT

Indirect organogenesis was attempted in two ginger cultivars *viz.*, Maran and Rio-de-Janeiro. Rhizome buds and various parts of *in vitro* adventitious bud regenerants *viz.*, pseudostem, shoot tip, folded and unfolded leaves were used as explants. Shoot tip was identified as the best explant for callusing. Half-strength MS medium supplemented with 1.00 mg l^{-1} 2,4-D recorded the highest callusing (56.37%) and callus growth (CI - 55.11). Half-strength MS medium supplemented with 3.0 mg l^{-1} BAP was found ideal for shoot morphogenesis (38.46%) and rooting. The cultivar Rio-de-Janeiro recorded higher callusing (44.01%), callus growth (CI - 38.59), early (26 days) and high shoot morphogenesis (39%) and high rate of shoot proliferation. The cultivar Maran was found better for root characters like number and length.

Key words: Indirect organogenesis, ginger, shoot morphogenesis, somaclonal variation.

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), one of the oldest and popular spice crops, is esteemed for its aroma, pungency and medicinal properties. It is much valued as a spice, medicine and vegetable since ancient times. Ginger is used as carminative, stimulant, anti-inflammatory, antiemetic, antirheumatic and aphrodisiac in Ayurvedic system of medicine. As the crop is exclusively propagated vegetatively through underground rhizomes, the natural variability available for exploitation is too low. Moreover, due to shy flowering nature and absence of natural seed set, crop improvement through selection and hybridization is difficult in the crop. Broadening the genetic base through *in vitro* techniques and exploitation of somaclonal variation for isolation of desirable plant types with high yield, quality and tolerance / resistance to diseases are of great significance in ginger crop improvement programmes. Standardisation of indirect organogenesis in ginger was hence attempted and response of two cultivars Maran and Rio-de-Janeiro.

MATERIALS AND METHODS

Seed rhizomes of the two cultivars of ginger were collected from Regional Agricultural Research Station, Ambalavayal, Kerala Agricultural University, Kerala, India. Rhizome buds and various parts of adventitious bud regenerants *viz.*, pseudostem (base, middle and top), shoot tip, folded and unfolded leaves were used as explants. Adventitious bud regenerants of ginger were produced from rhizome buds as per the protocol reported in ginger by Shylaja *et al.* (13).

Murashige and Skoog medium (7) at half-strength was used for callusing and morphogenesis in the present investigations. Growth regulators like 2,4-D (0.5, 1.0, 2.0, 3.0 and 4.0 mg l^{-1}) and BAP (0.5 and 1.0 mg l^{-1}) were incorporated at various levels to MS medium singly or in combinations for inducing calli from different explants. The cultures were incubated both under dark (10 h) and light conditions (14 h) in a culture room maintained at a temperature of 26±1°C and relative humidity of 60±10 per cent. Growth of calli was scored visually based on its spread and a maximum score of four was given to those calli that have occupied the whole surface of the medium in the culture tubes. Callus Index (CI) was worked out as CI = P × G, where P is percentage of cultures initiating calli and G is the growth score.

Growth regulators like 2,4-D, NAA and BAP and supplements like activated charcoal and $AgNO_3$ were incorporated to basal MS medium at half-strength for shoot morphogenesis in ginger. The calli induced from shoot tip explants in half-strength MS medium supplemented with 1.0 mg l^{-1} 2,4-D were inoculated to shoot regeneration medium to study the response. Observations on number of days taken for shoot morphogenesis, percentage regeneration and average number of shoots produced per culture were recorded. Regenerated cultures were sub-cultured at one month interval onto MS medium supplemented with BAP (3.0 mg l^{-1}) for shoot proliferation and rooting. Number of shoots proliferated in each sub-culture cycle was observed. Root characters like number of

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Table 1. Effect of explants on callusing and callus growth in ginger.

Explant	Days taken for callusing			Callusing (%)			Callus index		
	Maran	Rio-de-Janeiro	Mean	Maran	Rio-de-Janeiro	Mean	Maran	Rio-de-Janeiro	Mean
Pseudostem base	39.36	31.36	35.36	33.74	55.98	44.86	33.04	42.60	37.82
Pseudostem middle	39.86	32.17	36.02	23.25	28.44	25.85	21.91	26.59	24.25
Pseudostem top	42.82	35.24	39.03	19.58	36.01	27.80	18.36	25.00	21.68
Folded leaf	34.58	40.76	37.67	42.63	40.55	41.59	40.22	31.67	35.95
Rhizome bud	30.71	30.82	30.77	26.96	26.32	26.64	30.73	28.95	29.84
Shoot tip	25.55	29.67	27.61	78.30	76.74	77.52	76.09	76.74	76.42
Mean	35.48	33.34	-	37.41	44.01	-	36.73	38.59	-
$CD_{0.05}$	5.05	3.88	3.62	13.81	14.11	9.68	14.55	13.24	10.29

Table 2. Effect of growth regulators on callusing and callus growth in ginger.

Half-strength MS supplemented with growth regulators (mg l^{-1})	Days taken for callusing			Callusing (%)			Callus index		
	Maran	Rio-de-Janeiro	Mean	Maran	Rio-de-Janeiro	Mean	Maran	Rio-de-Janeiro	Mean
2,4-D (1.0)	32.98	34.49	33.74	48.60	64.13	56.37	49.56	60.66	55.11
2,4-D (2.0)	41.28	29.63	35.46	35.83	52.52	44.17	30.05	39.83	34.94
2,4-D (3.0)	36.73	33.80	35.27	37.11	46.60	41.86	37.11	32.54	34.83
2,4-D (4.0)	42.57	35.76	39.17	23.55	29.58	26.57	21.73	27.08	24.41
2,4-D (3.0) + BAP (0.5)	35.90	35.47	35.69	46.81	54.06	50.44	45.21	47.29	46.25
2,4-D (0.50) + BAP (1.0)	28.67	30.50	29.59	34.46	29.59	32.03	37.39	29.91	33.65
2,4-D (1.00) + BAP (0.5)	30.25	33.71	31.98	35.54	31.58	33.56	36.06	32.86	34.46
Mean	35.48	33.34	-	37.41	44.01	-	36.73	38.59	-
$CD_{0.05}$	5.46	4.19	3.91	14.92	15.24	10.46	15.72	14.30	11.11

roots produced and length of roots were observed at the time of plant out.

The experiments were done in a completely randomized design with three replications at the rate of 12 tubes / replication. Experiments were repeated thrice. Data were subjected to analysis of variance, critical difference was calculated as per Panse and Sukhatme (9).

RESULTS AND DISCUSSION

Different explants tried for callusing showed significant variation with respect to callusing and callus growth in the two cultivars studied (Table 1). Time taken for callusing in various explants tried varied from 28 to 39 days, percentage callusing varied from 25.85 to 77.52 and callus index recorded varied from 21.68 to 76.42. Shoot tip explants responded better than other explants registering early (27.61 days) and higher callusing (77.52%) and higher callus index values (76.41) (Fig. 1). Next to shoot tip, pseudostem base recorded highest callusing and callus growth followed by folded leaf and rhizome bud explants. Callusing was not observed in unfolded leaves of the both cultivars. The better response of shoot tip explants to callusing may be due to its juvenile nature and the presence of more metabolically active cells in the explant. Moreover,

Table 3. Response of cultivars to shoot morphogenesis and proliferation in ginger.

Cultivar	Mean No. of days taken for shoot morphogenesis	Shoot morphogenesis (%)	Mean No. of shoots in base culture	Mean No. of shoots proliferated in each subculture cycle			Mean total No. of shoots regenerated at the end of IV sc
				I sc	II sc	III sc	
Maran	29.80	38.46	5.00	+4.25	+16.25	+17.75	121.25
Rio-de-Janeiro	26.25	39.39	3.25	+10.00	+30.25	+58.50	162.00

Medium = Half-strength MS + BAP (3.0 mg l^{-1}); sc = sub-culture.

shoot tip being the seat of active synthesis of auxins, could be induced to multiply at an accelerated rate and hence the response was better. Similar response of shoot tip explant to callusing and callus growth was reported by several workers in ginger (Malamug *et al.*, 4; Palai *et al.* 8; Rout and Das, 10). Poor response of unfolded leaves to callusing was reported in ginger by Babu (1).

The response of two ginger cultivars were compared with respect to callusing and callus growth. The explants of cultivar Rio-de-Janeiro was found better for the different parameters studied. Irrespective of the explants, overall callusing percentage observed in cultivar Rio-de-Janeiro was 44.01 as against 37.41 per cent in cultivar Maran. The callus index values recorded was also higher in the cultivar Rio-de-Janeiro (38.59) and Rio-de-Janeiro explants callused earlier than that of Maran. There was not much variation observed in the response of explants like shoot tip and rhizome bud in the two cultivars. The explants from pseudostem registered higher callusing and callus growth in the cultivar Rio-de-Janeiro. With regard to folded leaf explant, the cultivar Maran recorded higher callusing, callus growth and earliness in callusing than the cultivar Rio-de-Janeiro. Similar genotype dependent response on callusing and callus growth was reported earlier by Palai *et al.* (8), Singh *et al.* (14), Shylaja *et al.* and Nair (12), and Shylaja *et al.* (13). The difference in performance of the two cultivars to callusing and callus growth may be due to the difference in endogenous auxin-cytokinin balance of genotypes and difference in *in vitro* uptake of exogenous cytokinins.

Different media combinations incorporating 2,4-D and BAP at various levels differed significantly in callusing and callus growth of explants (Table 2). Half-strength MS medium supplemented with 2,4-D alone (1.0 mg l^{-1}) recorded highest callusing (56.37%) and callus growth (55.11). For earliness in callusing, combination of 2,4-D with BAP was found best. Medium with 2,4-D + BAP combination (2,4-D at 0.50 mg l^{-1} and BAP at 1.00 mg l^{-1}) took only 29.59 days for callusing as compared to 33.73 days in medium with 2,4-D alone (1.0 mg l^{-1}). The favourable effect of 2,4-D in callusing may be due to stimulated cell division in 2,4-D incorporated medium or increased DNA content in cells as observed in tobacco cell suspension cultures (Miyazava *et al.*, 6) or due to accumulation of large amounts of endogenous IAA in cells as reported in carrot callus cultures (Michalczuk *et al.*, 5). It is also possible that 2,4-D acts indirectly in cell cultures by disturbing the endogenous auxin metabolism of cells. The beneficial effect of 2,4-D - BAP combination in callusing was reported in ginger (Palai *et al.*, 8; Samsudeen *et al.*, 11).

A gradual decline in response to callusing was observed with increasing levels of 2,4-D in the medium. The percentage callusing was 56.37 in medium supplemented with 1.0 mg l^{-1} 2,4-D, while it was 26.56 per cent in medium supplemented with 4.0 mg l^{-1} 2,4-D. Similarly, the callus growth was less and the days taken for callusing were more in medium supplemented with higher levels of 2,4-D. In the present investigations, calli induced at lower concentrations of 2,4-D and combinations of 2,4-D and BAP at various levels were hard and compact. But at higher levels of 2,4-D (3.0 and 4.0 mg l^{-1}), the induced calli were friable, loose and watery with root hairs. The friability of induced calli in 2,4-D incorporated medium may be due to the presence of highly vacuolated and elongated cells in the calli. Explants of cultivar Rio-de-Janeiro recorded highest callusing (44.01%) and callus growth (38.59) in the different media combinations tried. Also, in the best medium identified for callusing (half MS with 2,4-D 1.0 mg l^{-1}), response of explants of cultivar Rio-de-Janeiro was better as compared to cultivar Maran. The two culture conditions tried in the investigation *viz.,* incubation in dark and light were found, par with respect to callusing and callus growth in the two cultivars studied.

Shoot morphogenesis was observed in ginger in half-strength MS medium supplemented with only

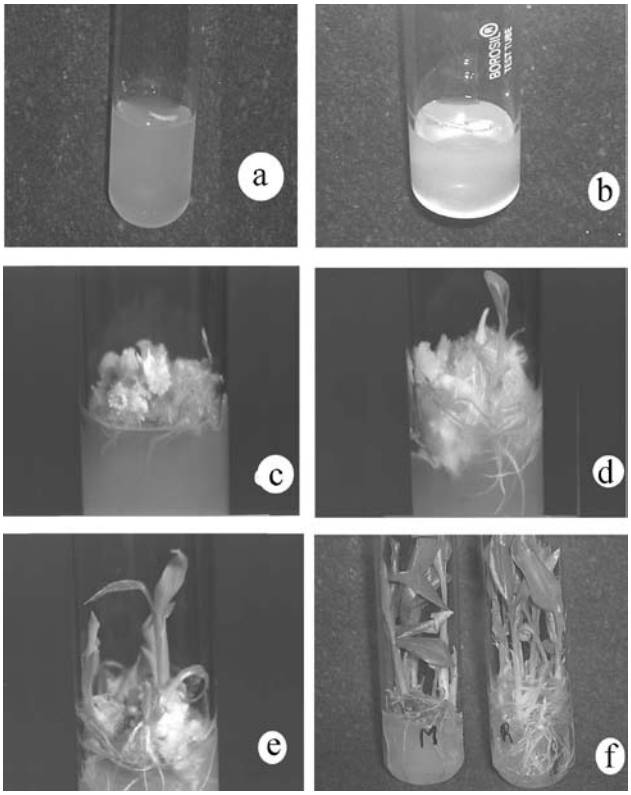

Fig. 1. Indirect organogenesis in ginger. (a) Shoot tip explant, (b) Callusing in shoot tip, (c) Callusing and morphogenesis, (d) Simultaneous shoot and root morphogenesis, (e) Shoot elongation, and (f) Shoot proliferation and rooting.

cytokinin. Media containing auxin and cytokinin failed to give shoot morphogenesis. Similarly, in half-strength basal MS medium and medium with supplements like charcoal or $AgNO_3$, no shoot morphogenesis was observed. Highest shoot morphogenesis (38.46 %) and highest number of shoots per culture (1.63) was recorded in half MS medium supplemented with BAP at 3.0 mg l^{-1} followed by half MS with BAP at 4.0 mg l^{-1}. Morphogenic potential of the calli decreased with increase in concentration of BAP and the regeneration percentage was lowest in half MS medium with higher concentration of BAP, *i.e.* 5.0 mg l^{-1}. The favourable effect of BAP alone to promote callus mediated organogenesis in ginger was highlighted in studies conducted by several workers (Malamug *et al*., 4; Samsudeen *et al*., 11). Regenerated cultures were again sub-cultured to half MS medium supplemented with BAP (3.0 mg l^{-1}) for further proliferation.

In the best medium identified for shoot morphogenesis, the cultures of cultivar Rio-de-Janeiro registered early (26.25 days) and higher shoot morphogenesis (39.39 %) than the cultivar Maran (Table 3). The rate of shoot proliferation was also higher in the cultivar Rio-de-Janeiro recording more number of shoots in first (10.00), second (30.25) and third (58.50) subculture cycles as against 4.25, 16.25 and 17.75 respectively in the cultivar Maran. Mean number of shoots regenerated at the end of fourth subculture cycle was also high in the cultivar Rio-de-Janeiro recording 162 shoots as compared to 121 shoots in the cultivar Maran. The superiority of cultivar Rio-de-Janeiro in shoot proliferation was reported in regenerants of ginger produced through bud culture (Shylaja *et al*., 13).

Shoot and root morphogeneses were simultaneous in *in vitro* cultures of ginger. Root morphogenesis occurred in half strength MS medium supplemented with BAP at 3.0 mg l^{-1}. The simultaneous emergence of shoot and root primordia in the same medium was also reported in ginger by Hosoki and Sagawa (3). The cultivar Maran exhibited better root characters even though the *in vitro* response with respect to callusing and shoot morphogenesis was better in the cultivar Rio-de-Janeiro. Plantlets of cultivar Maran recorded higher number of roots (20.70) and length of roots (8.58 cm) as compared to cultivar Rio-de-Janeiro (17.50, 6.04 cm).

The regenerants were ready for plant out four to five months after culture establishment. Plantlets with well-developed pseudostem and roots were planted in polythene bags filled with sterile sand and hardened for two weeks. After two weeks, the plantlets were transferred to big poly bags filled with potting mixture and kept in the net house for rhizome formation. Regenerants were successfully acclimatized in a shaded net house. Regenerants of cultivar Rio-de-Janeiro exhibited better *ex vitro* establishment (71.21%) as compared to cultivar Maran (65.75%).

In the present investigations, protocol for indirect organogenesis in ginger was developed and the *in vitro* response of the two cultivars Maran and Rio-de-Janeiro were studied in detail. Shoot tip was identified as best explant for callusing in both cultivars. Half-strength MS medium supplemented with 2,4-D at 1.0 mg l^{-1} was most effective for callusing and callus growth. Incubation of cultures in dark conditions were found on par to incubation under light with respect to callusing and callus growth. For shoot morphogenesis and rooting, half-strength MS medium supplemented with BAP at 3.0 mg l^{-1} was found ideal. The two cultivars of ginger studied varied with respect to their response to callusing and shoot morphogenesis. The cultivar Rio-de-Janeiro recorded higher callusing and callus growth, early and high shoot morphogenesis and high rate of shoot proliferation than the cultivar Maran. Regenerants of cultivar Maran were better for various root characters as compared to cultivar Rio-de-Janeiro.

REFERENCES

1. Babu, K.N. 1997. *In vitro* studies in *Zingiber officinale* Rosc. Ph.D. thesis, University of Calicut, Kerala, India.

2. Davis, J.M. and Keathley, D.E. 1992. Micropropagation of black locust. **In**: *Biotechnology in Agriculture and Forestry. High-Tech Micropropagation II,* (Y.P.S. Bajaj (Ed.). Springer-Verlag, Berlin, New-York, pp. 25-39.

3. Hosoki T. and Sagawa, Y. 1977. Clonal propagation of ginger (*Zingiber officinale* Rosc.) through tissue culture. *HortSci.* **12**: 451-52.

4. Malamug, J.J.F., Inden, H. and Asahira, T. 1991. Plantlet regeneration and propagation from ginger callus. *Scientia Hort.* **48**: 89-97.

5. Michalczuk, L., Cooke, T.J. and Cohen, J.D. 1992. Auxin levels at different stages of carrot somatic embryogenesis. *Phytochem.* **31**: 1097-103.

6. Miyazawa, Y., Kutsuna, N., Inada, N., Kuriowa, H., Kuriowa, T. and Yoshida, S. 2002. Dedifferentiation of starch-storing cultured tobacco cells: Effects of 2,4-dichlorophenoxy acetic acid on multiplication, starch content, organellar DNA content and starch synthesis gene expression. *Plant Cell Rep.* **21**: 289-95.

7. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**: 473-97.

8. Palai, S.K., Rout, G.R., Somantray, S. and Das, P. 2000. Biochemical changes during *in vitro* organogenesis in *Zingiber officinale* Rosc. *J. Plant Biol.* **27**: 153-60.

9. Panse, V.G. and Sukhatme, P.V. 1985. *Statistical Methods for Agricultural Workers*, Second edition, Indian Council of Agricultural Research, New Delhi, India, 325 p.

10. Rout, G.R. and Das, P. 1997. *In vitro* organogenesis in ginger (*Zingiber officinale* Rosc.). *J. Herbs Spices Med. Plants,* **4**: 41-51.

11. Samsudeen, K., Babu, K.N., Divakaran, M. and Ravindran, P.N. 2000. Plant regeneration from anther derived callus cultures of ginger (*Zingiber officinale* Rosc.). *J. Hort. Sci. Biotech.* **75**: 447-50.

12. Shylaja, M.R. and Nair, G.S. 2000. Response of black pepper (*Piper nigrum* L.) variety Panniyur-1 and cultivars to indirect organogenesis. *J. Trop. Agric.* **38**: 21-25

13. Shylaja, M.R., Sanchu, C.R., Paul, R. and Nybe, E.V. 2003. *In vitro* adventitious bud regeneration in ginger (*Zingiber officinale* Rosc.) - Response of cultivars. **In**: *Proc. Nat. Seminar on New Perspectives in Spices, Medicinal and Aromatic Plants.* V.S. Korikanthimath, T.J. Zacharia, K.N. Babu, R.S. Bhai and K. Kandiannan (Eds.), 27-29 November, 2003, ICAR Research Complex, Goa, India. pp. 51-55.

14. Singh, A., Singh, M. and Singh, B.D. 2010. Comparative *in vitro* shoot organogenesis and plantlet regeneration in tomato genotypes. *Indian J. Hort.* **67**: 37-42.

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