Embryogenesis and plant regeneration from ovary derived callus cultures of ginger (*Zingiber officinale* Rosc.)[1]

K NIRMAL BABU, K SAMSUDEEN, M J RATNAMBAL[2] & P N RAVINDRAN

Indian Institute of Spices Research
Marikunnu P.O., Calicut - 673 012, Kerala, India.

ABSTRACT

Ovary explants from 1-2 week old flowers of ginger (*Zingiber officinale*) developed profuse callus in Murashige and Skoog medium supplemented either with 2,4-dichlorophenoxyacetic acid (1mgl^{-1}) alone or with 2,4-dichlorophenoxyacetic acid (0.5 mgl^{-1}) and benzyladenine (1mgl^{-1}). The callus later turned embryogenic and produced white globular embryoid like structures when cultured on modified Murashige and Skoog medium supplemented with benzyladenine (10 mgl^{-1}) and 2,4-dichlorophenoxyacetic acid (0.2 mgl^{-1}). The embroyoid formation was more pronounced when growth regulators were removed from the culture medium after initial embryogenesis. Some of the embryoids developed into complete plantlets. The primary embryoids directly produced secondary embryoids in subsequent cultures on growth regulator free medium. The individual embryoids developed into plantlets with better rooting when α-naphthalene acetic acid (1 mgl^{-1}) was added to the culture medium. About 80 per cent of these plantlets were established in soil.

Abbreviations

BA	:	Benzyladenine
2,4-D	:	2,4-Dichlorophenoxyacetic acid
MS	:	Murashige and Skoog medium
NAA	:	α-Naphthaleneacetic acid

Introduction

Diseases like rhizome rot and bacterial wilt and the absence of resistant varieties are major production constraints in ginger (*Zingiber officinale* Rosc., Zingiberaceae), a herbaceous perennial, the rhizomes of which form the spice ginger. The possibilities of developing

[1]Contribution No. 152 of Indian Institute of Spices Research, Calicut - 673 012, Kerala, India.

[2] Present address: Central Plantation Crops Research Institute, Kasaragod - 671 124, Kerala, India.

resistant varieties and rapid multiplication of disease-free planting material utilizing tissue culture techniques assumes paramount importance in ginger since conventional breeding programmes are inapplicable due to lack of seed set. Micropropagation of ginger has been reported earlier by various workers (Hosoki & Sagawa 1977; Pillai & Kumar 1982; Ilahi & Jabeen 1987; Bhagyalakshmi & Singh 1988; Balachandran, Bhat & Chandel 1990). The present study reports embryogenesis and plantlet formation from callus cultures derived from excised ovaries, and further multiplication of these embryoids by adventitious budding in ginger.

Materials and methods

Young 7-15 days old inflorescences of ginger were collected during July-August and disinfected by washing with 5% detergent solution (Teepol) for 20 min followed by distilled water. These inflorescences were then surface sterilized with 0.1% $HgCl_2$ solution for 5-7 min and rinsed 4-5 times with sterile water. Ovaries from individual flowers were excised under aseptic conditions and cultured on MS medium (Murashige & Skoog 1962) containing 30 gl^{-1} 'Qualigens' bacteriological grade agar agar supplemented with 2,4-D (0.2, 0.5, 1.0 mgl^{-1}) and BA (0.2, 0.5, 1.0 mgl^{-1}) in various combinations for callus production. The callus obtained was cultured on MS medium with higher concentrations of BA (0.5, 1.0, 5.0, 10.0 mgl^{-1}) and reduced concentrations of 2,4-D (0.1, 0.2, 0.5 mgl^{-1}) for morphogenesis. The resultant embryoids and plantlets were cultured on MS medium with 1 mgl^{-1} of NAA for further growth and development.

The pH of the medium was adjusted to 5.7 before autoclaving and the cultures were incubated at 25 ± 2°C with 16 h of photoperiod. The well developed plantlets were transferred to polythene bags containing garden soil, sand and farmyard manure in equal proportions and were kept in a humid chamber with 90-100% Relative Humidity for 20 days for hardening and establishment.

Results and discussion

The excised ovary tissues started swelling and produced callus by the 4th week in MS media in all the concentrations of 2,4-D with or without BA. However, the best callus production was obtained with either 1 mgl^{-1} 2,4-D alone or with 0.5 mgl^{-1} 2,4-D and 1 mgl^{-1} BA. The callus could be maintained and multiplied in the same media by monthly subcultures.

The callus derived from ovary tissues when tested for its morphogenic response by increasing the concentration of BA and reducing the concentration 2,4-D in the culture medium continued to grow in all the combinations tested. However, morphogenic response was not noticed in the first culture cycle, except in the medium without growth regulators, where rhizogenesis was evident. The calli were transferred to fresh media of same compositions in repeated subcultures at monthly intervals. After 4-5 such transfers, indications of morphogenesis were seen in the media supplemented with 10 mgl^{-1} BA and 0.2 mgl^{-1} 2,4-D and also in 5 mgl^{-1} BA and 0.1 mgl^{-1} 2,4-D (Fig. 1a). In both the media white globur embryoid like structures were observed in 60 per cent of the cultures (Fig. 1b). This process continued in the subsequent cultures in the same medium. The embryogenic calli were separated out and used for subsequent cultures. When growth regulators were removed from the culture medium

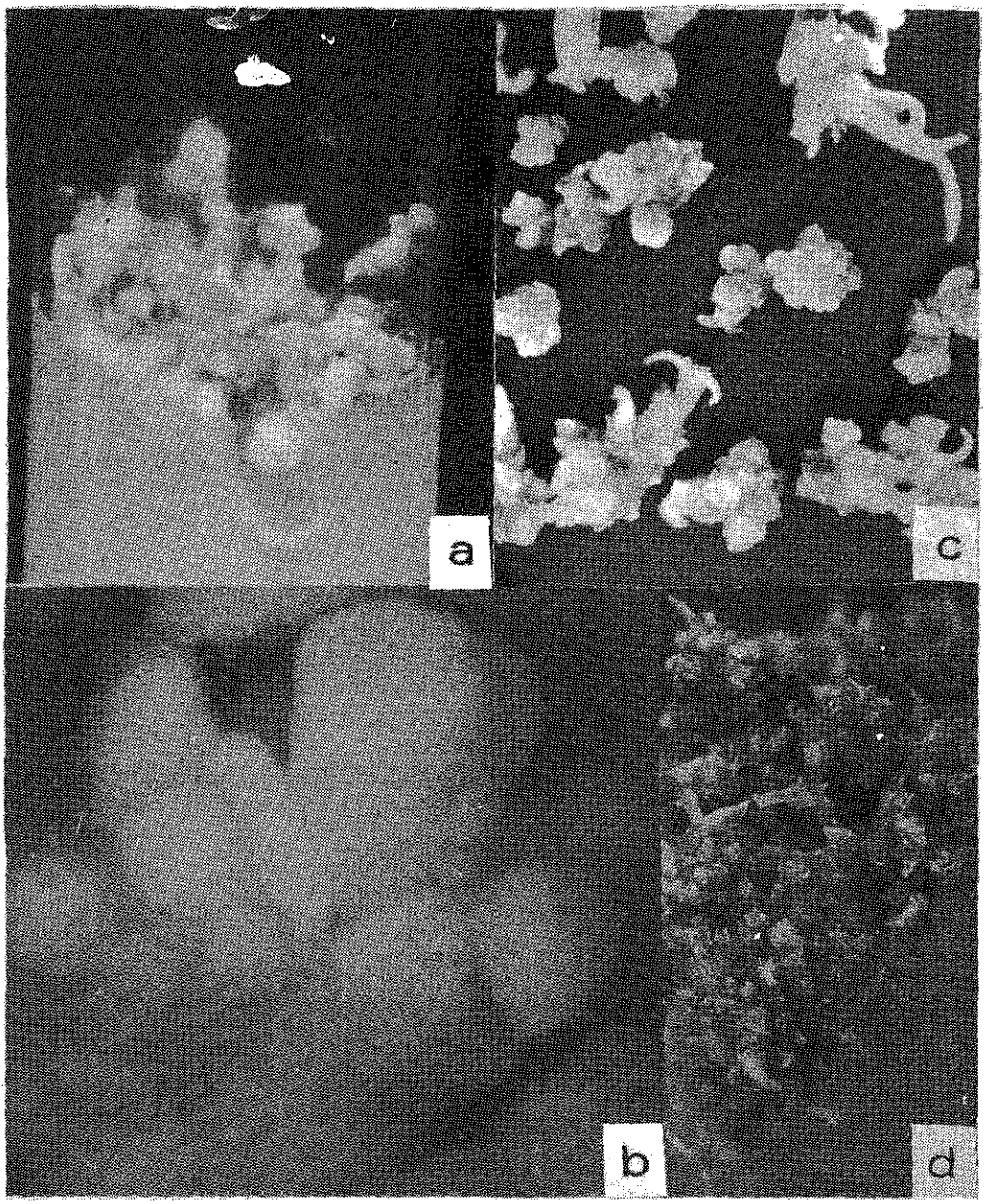

Fig. 1. Embryogenesis and plant regeneration from ovary derived callus of ginger a & b. Embryogenesis c & d. Production of embryoids from primary embryoids

at this stage, the callus turned green and the rate of morphogenesis increased considerably resulting in many embryoids which could be separated easily with a gentle tap. On subsequent transfer to fresh medium without growth regulators, some (10-20 per cent) of these embryoids developed into complete plants while many others produced secondary embryoids by adventitious embryogenesis resulting in a large number of (100-300) tiny embryoids. Some of these tiny embryoids were compactly arranged while others were loosely arranged (Fig. 1. c & d). The development of these embryoids into complete plantlets was higher when NAA (1 mgl^{-1}) was added to the culture medium which also resulted in good rooting of these plantlets. About 80 per cent of the plantlets established when transferred to polythene bags filled with soil mixture and kept in a humid chamber for 15-20 days with above 90% Relative Humidity.

Though adventive embryogenesis, the development of embryos from cells that are not products of gametic fusion, is a known phenomenon, its occurrence is restricted to intra-ovular tissues. In tissue cultures also floral or reproductive tissues in general, have proven to be an excellent source of embryogenic material (Ammirato 1983). Somatic embryogenesis from ovular tissue have been reported in various plant species like citrus (Kochba & Spiegel Roy 1973), *Paulownia tomentosa* (Radojevic 1979), *Vitis vinifera* (Srinivasan & Mullins 1980), *Carica papaya* (Litz & Conover 1982), etc. This is the first report of somatic embryogenesis from ovary derived callus in *Z. officinale* . The high frequency regeneration system developed in this study has tremendous

Table 1. *In vitro* responses of ovary derived tissues of ginger

Tissue	Hormonal composition# (in MS* basal medium)	Morphogenic response
Excised ovary	0.5 mgl^{-1} 2,4-D+1 mgl^{-1} BA or 1 mgl^{-1} 2,4-D	Callus induction and proliferation
Ovary derived callus	Hormone-free medium 0.2 mgl^{-1} 2,4-D+10 mgl^{-1} BA or 0.1 mgl^{-1} 2,4-D+5 mgl^{-1} BA**	Rhizogenesis Morphogenesis with white globular embryoids
Morphogenic callus with white globular embryoids	Hormone-free medium	Increase in rate of embryoid formation; adventitious production of secondary embryoids and plantlets with well developed roots
Embryoids	1 mgl^{-1} NAA	Well developed plantlets with profuse rooting

Combinations where best response was observed
* Murashige & Skoog (1962)
** After 4-5 repeated transfers into the same medium at monthly intervals

advantages because of the large number of tiny embryoids that are available per culture. This not only gives a high rate of multiplication but also provides an ideal system for *in vitro* polyploidisation as well as *in vitro* mutagenesis for increasing somaclonal variations and utilizing them for crop improvement of ginger including development of disease resistant varieties.

References

Ammirato P V 1983 Embryogenesis. In : Evans D A, Sharp W R, Ammirato P V & Yamada Y (Eds.) Hand Book of Plant Cell Culture Vol. 1 - Techniques for Propagation and Breeding. Macmillan Publishing Company, New York.

Balachandran S M, Bhat S R & Chandel K P S 1990 *In vitro* clonal multiplication of turmeric (*Curcuma* spp.) and ginger (*Zingiber officinale* Rosc.). Plant Cell Rep. 8 : 521-524.

Bhagyalakshmi & Singh N S 1988 Meristem culture and micropropagation of a variety of ginger (*Zingiber officinale* Rosc.) with high yield of oleoresin. J. Hort. Sci. 63 : 321-327.

Hosoki T & Sagawa Y 1977 Clonal multiplication of ginger (*Zingiber officinale* Rosc.) through tissue culture. HortScience 12 : 451.

Ilahi I & Jabeen M 1987 Micropropagation of *Zingiber officinale* Rosc. Pak. J. Bot. 19 : 61-65.

Kochba J & Spiegel Roy P 1973 Effect of culture media on embryoid formation from ovular callus of 'Shamonti' orange (*Citrus sinensis*). Z. Pflanzen Physiol. 69: 156-162.

Litz R E & Conover R A 1982 *In vitro* somatic embryogenesis and plant regeneration from *Carica papaya* L. ovular callus. Plant Sci. Lett. 26 : 153-158.

Murashige T & Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15 : 473-497.

Pillai S K & Kumar N B 1982 Clonal multiplication of ginger *in vitro*. Indian J. Agric. Sci. 52 : 397-399.

Radojevic L 1979 Somatic embryos and plantlets from callus cultures of *Paulownia tomentosa* Stead. Z. Pflanzen Physiol. 77 : 33-41.

Srinivasan C & Mullins M G 1980 High frequency somatic embryo production from unfertilised ovules of grapes. Sci. Hort. 13 : 245-152.