

## Direct regeneration of plantlets from immature inflorescence of ginger (*Zingiber officinale* Rosc.) by tissue culture\*

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### ABSTRACT

Immature inflorescences of ginger when cultured on MS revised medium supplemented with  $10 \text{ mg l}^{-1}$  BA and  $0.2 \text{ mg l}^{-1}$  2, 4-D resulted in the conversion of floral buds into vegetative buds and these later developed into plantlets directly without intervening callus phase. About 38% of the buds produced multiple shoots ranging from 5-25. When individual flowers were cultured separately on the same medium plantlets developed from the ovary in 45% of cases. These plantlets were made to develop good root system on MS medium with  $1 \text{ mg l}^{-1}$  NAA. These rooted plantlets established easily in the soil. In some of the cultures the ovaries developed into fruits.

Key words: direct plant regeneration; ginger; immature inflorescence, *Zingiber officinale*.

### Abbreviations:

BA:  $\text{N}^6$  - benzyladenine  
2,4-D : 2,4-dichlorophenoxy acetic acid  
NAA :  $\alpha$ -naphthalene acetic acid

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## Introduction

Ginger is an important tropical spice used all over the world. It is an herbaceous, rhizomatous perennial but cultivated as an annual, the rhizomes of which constitute the spice. Ginger is propagated only vegetatively using sprouted rhizome bits as planting material. The plant is totally sterile. Two major constraints that limit ginger production are the rhizome rot caused by *Pythium* spp. and the bacterial wilt caused by *Pseudomonas solanacearum*. Often the infected rhizomes are the primary sources of inoculum, and hence the use of disease free planting material is a prerequisite for containing the spread of the disease. Micropropagation by tissue culture is an ideal method in achieving this objective. The use of plant parts other than the rhizomes is significant in this context because it is always the rhizome that is the target of infection by the pathogens. Inflorescence culture is an ideal alternative under these circumstances.

Though micropropagation of ginger using vegetative buds was reported by various workers (Hosoki and Sagawa 1977, Pillai and Kumar 1982, Ilahi and Jabeen 1987, Bhagyalakshmi and Singh 1988, Noguchi and Yamakawa 1988, Balachandran *et al* 1990) reports on the use of floral tissues for this purpose are not available. This is the first report of direct plant regeneration from immature inflorescences of ginger. Earlier reports of plantlet formation from inflorescence cultures through callus and embryogenesis are found in other crops like rice (Ling *et al* 1983), wheat (Ozias, Akins and Vasil 1982), sugarbeet (Gilles *et al* 1983) Sorghum (Brettell *et al* 1980), pearl millet (Vasil and Vasil 1982) etc. However, reports of direct plantlet formation from inflorescence cultures are rare and

they were in rice (Ling *et al* 1983) and cardamom (Kumar *et al* 1985).

## Materials and methods

Immature, 1-10 days old, inflorescences of ginger cultivar 'Maran' were collected during the flowering season (July-September) and were washed thoroughly in running water followed by a detergent 'Teepol' solution. The inflorescences were surface sterilized with 0.1%  $\text{HgCl}_2$  solution for 15 minutes and were then washed in 4-5 changes in sterile water. The outer bracts were removed under aseptic conditions and the remaining inflorescence was transferred to the culture medium. Similarly individual flowers were dissected out from the inflorescence in aseptic conditions after surface sterilization and were cultured separately.

The culture medium standardized at this Institute for plant regeneration in ginger i.e., revised MS medium (Murashige and Skoog 1962) supplemented with  $10\text{mg l}^{-1}$  BA and  $0.2\text{ mg l}^{-1}$  2,4-D was used in the present study. The medium was solidified with  $6\text{ g l}^{-1}$  bacteriological grade agar. All the cultures were maintained at  $25 \pm 2^\circ\text{C}$  with 16 hr photoperiod. The cultures were transferred to fresh medium at monthly intervals. MS liquid medium with  $1\text{ mg l}^{-1}$  NAA was used for improved rooting before transplanting the plantlets to the soil. The composition of the soil used was garden soil, river sand and powdered farm yard manure in equal proportions.

## Results and discussion

Young inflorescences as well as individual flower buds when cultured on the revised MS medium supplemented with  $10\text{ mg l}^{-1}$  BA and  $0.2\text{ mg l}^{-1}$  2,4-D gave the following morphogenic responses.

### Inflorescence cultures

When immature inflorescence as a whole was cultured, it continued growth and the bracts started to turn red as seen under natural conditions. By the fourth week green vegetative buds start emerging out instead of floral buds from the axils of the bracts in 80% of the cultures (Fig. 1). This was confirmed later when well-developed shoots emerged out of the bracts in most of the cases. In a few cases 1-2 floral buds emerged out from the axils of the outermost older bracts (Fig. 1A). These are the flower buds already differentiated and probably too old for conversion to the vegetative phase. The shoots that developed, grew in to complete plantlets in 7-8 weeks time. These plantlets also had 1-3 roots (Fig 1B). In the majority of the cases only one or rarely two plantlets developed per axil of the bract. But in about 38% of the cultures multiple shoots were also noted (Fig. 1C). These multiple shoots ranged in number from 5-25. In 2% of the cases floral bracts showed abnormal growth and developed in to leaf like structures. The direct plantlet development from the immature inflorescences may be due to the conversion of floral buds into vegetative buds. Such phenomena were earlier reported in rice (Ling *et al* 1983) and in cardamom (Kumar *et al* 1985).

### Single flower cultures

When individual flowers were cultured separately (Fig. 2 A), the growth of the ovaries was conspicuous in 7-10 days (Fig. 2B). The growing ovaries were mostly 3-celled and rarely 2 celled. The ovary wall split open in about 60% of the cases by the fourth week (Fig. 2 E). There was no visible growth of the callus in the cultures. In 40% of the cultures single plantlets were seen emerging directly

from the ovaries by the 7th week (Fig. 2 C & D). These plantlets developed roots simultaneously and later produced tillers.

In 2% of the cultures the ovaries grew and developed into fruits (Fig. 2B). This may be either due to apomixis or rare occurrence of *in vitro* pollination.

The plantlets developed from inflorescences as well as single flowers were transferred to liquid medium with MS basal salts and  $1 \text{ mg l}^{-1}$  NAA (Fig. 1 D). The plantlets produced profuse rooting and they were then transferred to soil and were kept in a humid chamber with 100% relative humidity for 15 days for hardening and establishment. Over 80% of the plantlets got established in the soil.

Thus, it was possible to develop plantlets directly from the inflorescence as well as from single flowers by tissue culture. During this process reversal of the morphogenic sequence takes place under the influence of BA and 2, 4-D. This method is well suited for rapid clonal multiplication of ginger, and compared to the process when underground rhizomes were used as explants, contamination rate is minimal in inflorescence cultures. Ginger does not set seed and this hampers the conventional breeding programmes. By refining the single flower culture system *intra-ovarian* pollination *in vitro* could be tried for a possible seed production in ginger.

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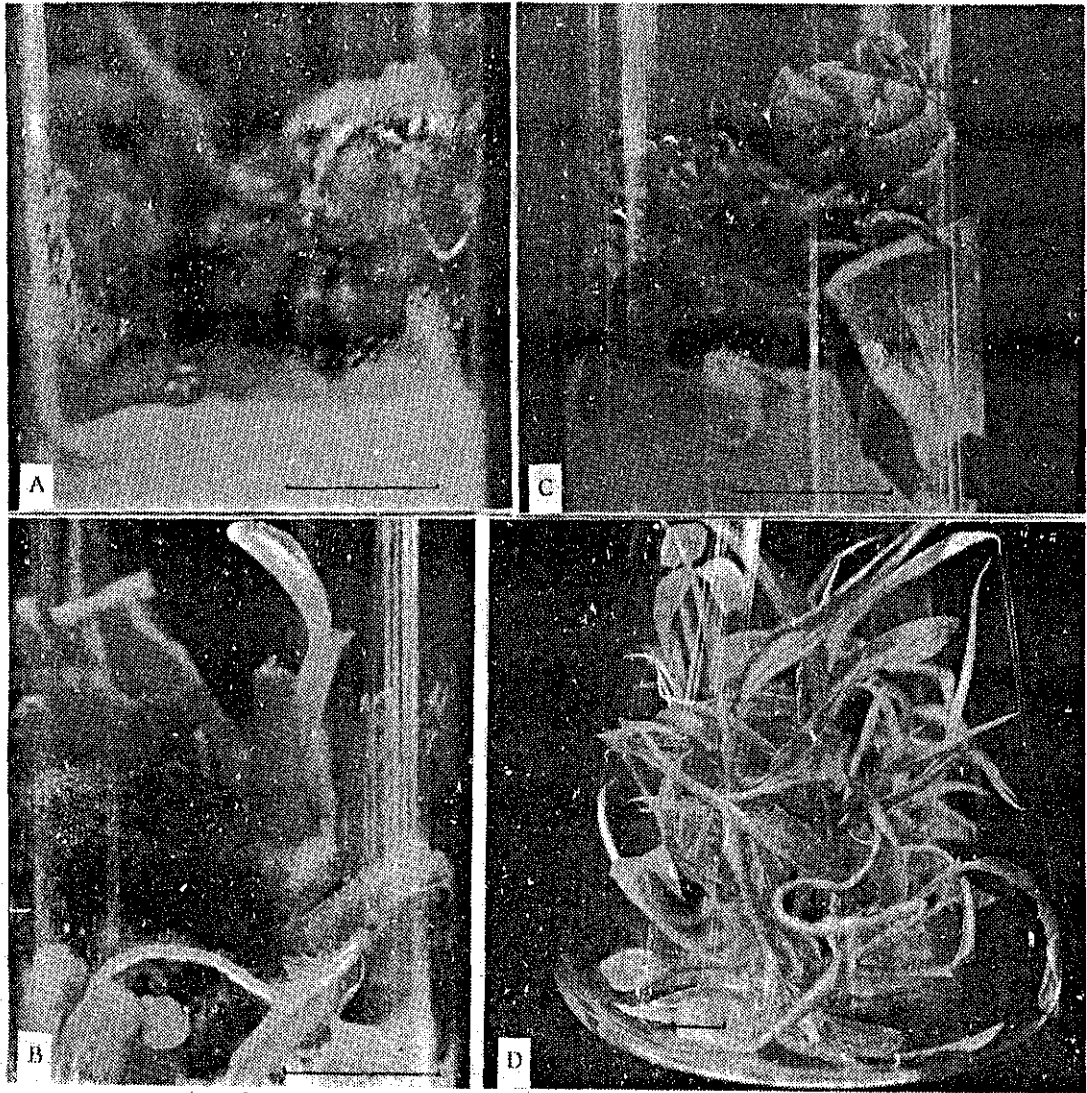


Fig-1 *In vitro* plant regeneration from immature inflorescence of ginger  
(Scale: Bar = 1 cm)

- a) Young inflorescence in culture showing opening of florets. b) Vegetative shoots with roots emerging from the axil of the bracts. c) Formation of multiple shoots. d) Well developed plants with tillers

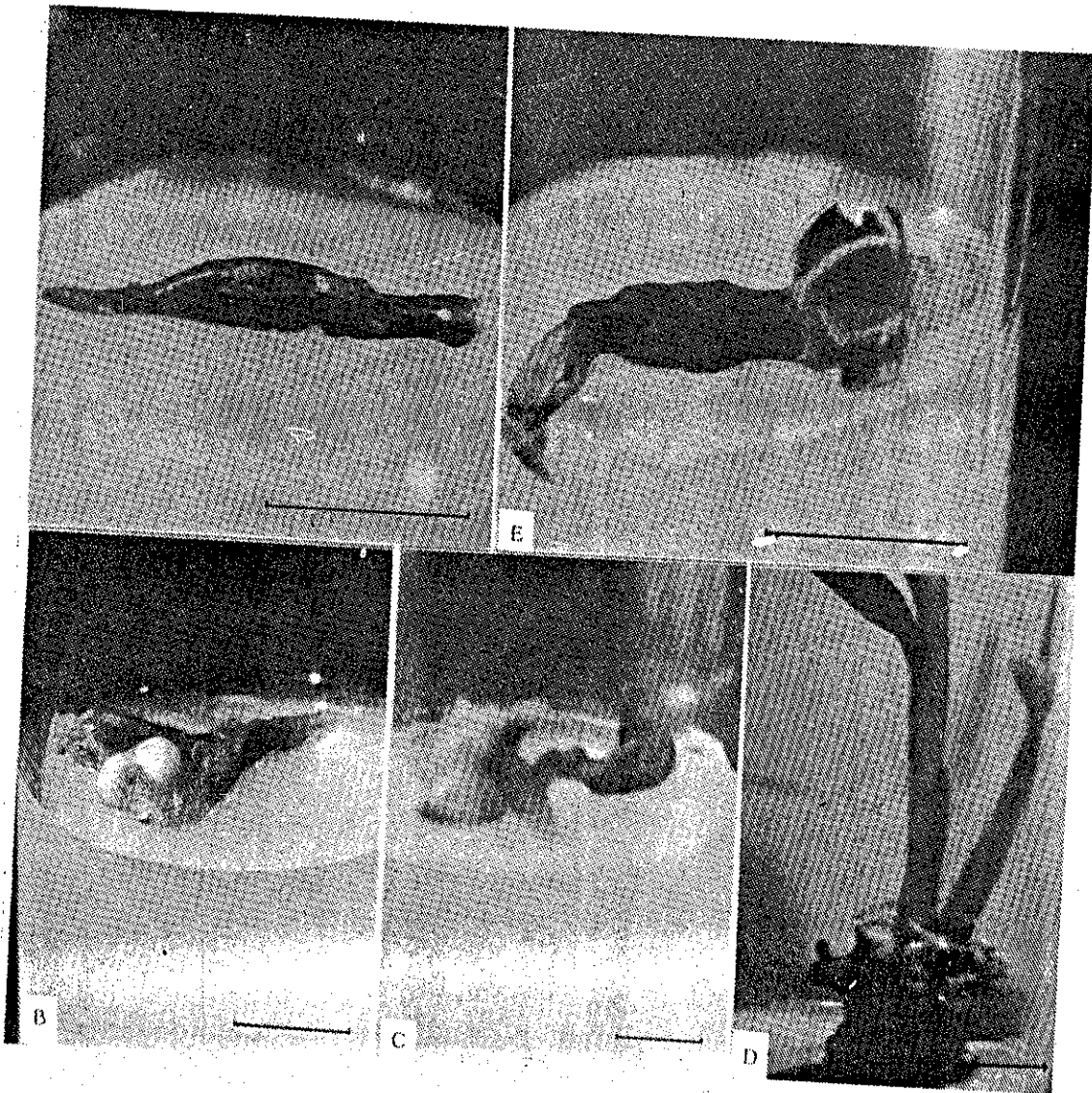


Fig-2 *In vitro* plant regeneration from young flowers of ginger (Scale: Bar = 1 cm)

- a) Young flower with ovary. b) Ovary developing into fruit. c) Developing plantlet from ovary with roots. d) Well developed plantlet from ovary. e) Breaking of ovary walls