

MICROPROPAGATION OF *ZINGIBER OFFICINALE* L.

IHSAN ILAHI AND MUSSARAT JABEEN

*Department of Botany,
University of Peshawar, Peshawar, Pakistan.*

Abstract

Stem cuttings from 3 month old plants, young buds, rhizome cuttings with shoot bud primordia and juvenile shoots were cultured on 1/2 MS medium supplemented with different combinations of growth hormones. Whereas callus could not be induced on the stem explants, callus was induced on the juvenile shoots which on subculturing to medium with varying concentrations of 2, 4-D and BAP developed bud primordia. These buds on further subculturing grew into small plantlets. Callus induction was also observed on two week old shoot buds which on subculturing turned into green segmented, pouch like structures. Buds were induced on the rhizome explants which after isolation were further propagated into plantlets. The plantlets have been induced to root and transferred to field conditions.

Introduction

Rhizome or underground stem of ginger (*Zingiber officinale*) of the family Zingiberaceae has much medicinal importance and is used as condiments and spices (Harris, 1909). Ginger rhizomes are imported at a cost of about Rs. 20 m from India and Ceylon for home consumption. Cultivation of ginger at elevations of 1300 m – 1500 m in Fatehpur and Thana and other parts of NWFP was not successful due to poor sprouting capacity and short period of growth. Similarly its cultivation in the plains also failed due to high summer temperatures of 38-45°C during June and July (Jalis & Khan, 1982). Tissue culture studies were therefore initiated for micro-propagation, flavour production and acclimatization of the ginger plant since tissue culture is regarded as an ideal method for producing clonal material particularly where normal propagation methods by seed or vegetative cuttings is not possible (Murashige, 1974; Holdgate, 1978).

Micropropagation of plants through tissue cultures in general can provide an unlimited number of plantlets from a single explant. This method represents an important tool in plant breeding because it offers plant material of identical quality with insurance against plant diseases (Murashige, 1978). Tissue culture work in the family Zingiberaceae is very meagre. Only few reports on *Costus speciosus* and the vegetative propagation of *Curcuma longa* and *Zingiber officinale* are available (Nadgauda *et al.*, 1978; Hosoki & Sagawa, 1977). In *C. longa*, through *in vitro* culture plantlet formation was obtained rapidly and throughout the season, while under natural conditions plants grew only during the monsoon (Nadgauda *et al.*, 1978). In the present study experiments were

conducted to establish microplant propagation from ginger buds, juvenile shoots, rhizome explants, stem segments and callus cultures using various concentrations of different growth hormones.

Materials and Methods

Ginger plants were grown in pots and shoot buds, stem segments and juvenile shoots were used as explants. The plant material washed in running tap water was surface sterilized with 1% mercuric chloride solution for 3 minutes followed by 3 washings in sterilized distilled water and then inoculated onto the basal medium. The basal medium used was Murashige & Skoog (1962) inorganic salt solution at half strength containing 5% sucrose, 0.9% agar and adjusted to pH 5.8. Growth hormones used were 1AA, 2, 4-D, NAA, K and BAP. The medium was sterilized at 15 psi, for 15 min. The cultures were incubated in biotrons with 16 h of light photo period in 24 h cycle at $26 \pm 1^\circ\text{C}$.

Results

Use of stem explants: No callus formation was observed when lower portions of stems from 3 month old potted plants were inoculated onto the basal medium for about eight weeks. Addition of different concentrations of growth hormones to the medium did not show callus induction. The explants however, remained alive for about 8 week after which they turned brown and died.

Callus formation on 2-week old shoot buds: Good callus growth was observed within 4 weeks when 2-week old shoot buds were inoculated on a medium supplemented with BAP and 2, 4-D each @ 0.5 mg/l. Callus growth stopped on subculturing to plain basal medium and roots initiated from it, whereas the callus subcultured on a medium containing BAP and 2, 4-D each @ 0.5 mg/l developed into greenish-white compact callus masses. Copious callus growth was also induced on MS containing 0.1 mg/l 2, 4-D and 0.5 mg/l of BAP. Callus produced was hard and dark green in colour with pouch-like structures which on subculturing developed into roots.

Use of rhizome explants bearing shoot primordia: Young shoot buds along with a portion of rhizome when inoculated on MS medium containing 2, 4-D and BAP each @ 0.5 mg/l ruptured and some callus was produced. The callus on further subculturing on media containing 0.1 mg/l 2, 4-D and 0.5 mg/l BAP and 0.1 mg/l both of BAP and 2, 4-D produced lateral buds and typical multiple shoots. Excised single shoot when transferred to a medium supplemented with 0.1 mg/l each of BAP and of 2, 4-D started rooting within 2-3 days.

Use of juvenile shoots: Young shoots inoculated on MS medium supplemented with 0.5 mg/l both of BAP and 2, 4-D developed greenish-white hard callus within 4 weeks

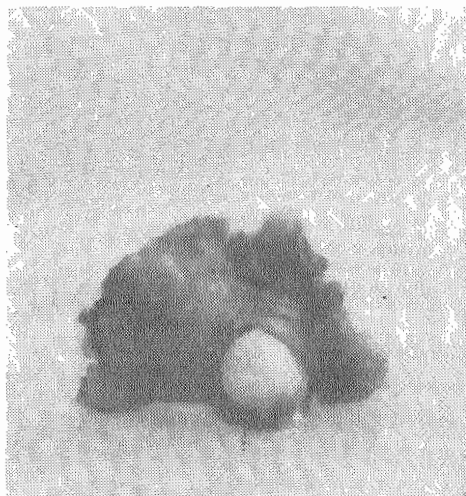


Fig. 1. A young shoot inoculated on MS medium with 0.5 mg/l each of BAP and 2, 4-D. Note initiation of callus after 4 weeks of culture.

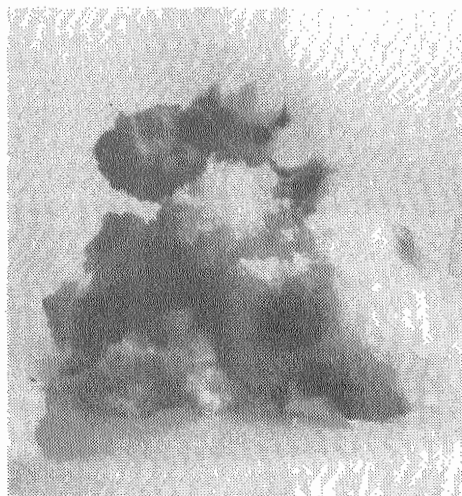


Fig. 2. Further proliferation of the induced callus on MS supplemented with 0.5 mg/l of BAP and 0.1 mg/l 2, 4-D after 4 weeks of culture.

(Fig. 1). These calli when subcultured on a medium containing 0.1 mg/l of 2, 4-D and 0.5 mg/l of BAP produced good callus growth within a week (Fig. 2). Shoot buds were observed after 4 weeks which ultimately grew into small plantlets (10-15 plantlets in each culture) (Fig. 3). Decreasing the concentration of BAP upto 0.1 mg/l in the medium resulted in an increase in root growth with a decrease in shoot formation.

In another experiment, a piece of callus produced on the medium containing BAP and 2, 4-D each @ 0.5 mg/l was transferred to MS medium supplemented with 0.5 mg/l BAP, 0.1 mg/l 2, 4-D and 5, 10, 15 or 20 mg/l Adenine sulphate (AS). No marked difference in callus was observed when AS @ 5 mg/l was used. In AS @ 10 mg/l only small green projections were observed in the callus, but these shoot buds did not develop into plantlets. When AS @ 15 or 20 mg/l was used the callus increased in bulk with no bud formation. At 25 mg/l of AS in the medium, the callus growth was suppressed. The plantlets formed in the above experiments were transferred to field conditions for further growth.

Discussion

Stem explants of *Z. officinale* showed no activity when cultured on the basal medium. Addition of auxins and cytokinins did not help callus induction. The calli formed



Fig. 3. Shoot bud induction and its growth into plants on callus from Fig. 2.

from various tissues were morphologically different. Soft and friable calli developed from shoot primordia, whereas hard and compact calli developed from 2 week old buds on treatment with the same concentrations of hormones. Very young buds could only induce a slight callus and gave rise to multiple plantlets. Callus formation from tissues placed on nutrient agar occurs at widely different rates which suggests that the origin of the explant can be critical.

Since the callus cultured on 1/2 MS containing 0.5 mg/l BAP and 0.1 mg/l 2, 4-D, produced more shoots as compared to 1.0 mg/l of 2, 4-D and 0.5 mg/l of BAP, this would indicate that lowering the auxin level enhances shoot formation and its subsequent development. High cytokinin auxin ratio was found necessary for shoot formation in plants like *Hyacinthus* (Pierek & Post, 1975) and *Crotolaria* (Ramawat *et al.*, 1977).

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