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Clonal Propagation of Ginger through Shoot Tip Culture

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Abstract

In vitro response for multiplication of ginger *(Zingiber officinale* Rosc.) from shoot tip was studied. Shoot tips (3-5 mm) were used as explants. Murashige and Skoog salts and vitamins were used as basal medium. Benzylaminopurine and Naphthalene acetic acid constituted the growth regulators. Maximum shoot multiplication with well developed roots and good plant height was achieved on medium containing 2 mg/l BAP and NAA. After four weeks, plants were transferred to the pots in the green house.

Introduction

Ginger *(Zingiber officinale)* is mostly propagated by vegetative means through seed ginger or seed pieces obtained from the current crop. Since ginger is heavily attacked by systemic diseases like wilt disease *(Pseudomonas solamacearum), Fusarium* yellow disease *IFusarium oxysporum* f.) and soft rot *(Pythium)* which are difficult to eliminate through conventional methodologies, it is necessary to tap alternate propagation methods. Tissue culture is one such technique which can be efficiently and conveniently used to acquire a disease free stock of source material. It is estimated that a three fold increase in the production of rhizome could be possible by the effective control of diseases (Hosoki and Sagawa, 1977). During the year 1996-97 ginger was cultivated in Pakistan over an area of 76 hectares which produced 26 tonnes of ginger while 23,000 tonnes was imported which was worth 375.4 million rupees (Anonymous, 1997). The area under cultivation as well as per hectare production can be increased by providing disease free and high yielding source material.

There are reports on *in vitro* multiplication of ginger using shoot tip (Choi and Kim, 1991; Inden *et al.,* 1988) and regeneration from callus has been also achieved (Babu *et al*., 1992). *In vitro* formation of plants from anther and immature inflorescence of ginger has been achieved by Ramachandran and Nair (1992) and Babu *et al*. (1992). Present study was planned to optimize media formulations to produce disease free ginger clones.

Materials and Methods

The source material was acquired from a local variety *Zingiber officinale* Rosc. The rhizomes were washed thoroughly with running tap water and incubated in moist absorbent cotton at room temperature to initiate sprouting. A week old sprouted buds furnished the explant source for the initiation of the experiment. An antioxidant solution containing 100 mg/l ascorbic acid and 150 mg/l citric acid for 2 hours was used prior to sterilization to control browning. Asepsis was administered with 0.1 percent $HgCl_2$ solution for 10 minutes followed by several washings with autoclaved water. Shoot tips approximately 3.0-5.0 mm were carefully trimmed to initiate the cultures for which 250 ml culture flasks were used. The cultures were maintained at a temperature of $25 \pm 2^{o}C$ with 16 hours photoperiod with a light intensity of 1600-2000 lux. Solid M.S medium enriched with vitamins and varying concentrations of BAP and NAA (Table 1) was tested (Murashige and Skoog, 1962). First subculturing was possible after 3 weeks after which regular subculturing was done after every 4 weeks on the same medium. The rooted plantlets were acclimatized in a soil mixture containing red soil, cattle dung and sand in an approximately 1:1:1/ratio in glasshouses and humidity level was maintained by covering pots with polythene bags for two weeks.

Results

For shoot proliferation different combinations of growth regulators were tested. In the first experiment M.S medium enriched with BAP (0.0-4.0 mg/l) was used. Results after one month of culturing are shown in Table 2. It is obvious from the results that, when shoot tips were cultured on plain M.S medium no shoot proliferation was observed. Only single shoots grew into complete plants. Rooting was started after two weeks of shoot initiation and after four weeks plant height increased to 2.6 cm. Maximum number of shoots from single explants were 3.33 when medium contained 3.0 mg/l BAP while maximum plant height during the same period was on medium number 3 M containing 2.0 mg/l BAP and shoot number was also relatively better (3.11). With the increase in BAP level (4.0 mg/l) number of shoots as well as plant height also decreased whereas callus like swelling was observed at the pseudostem of the plant.

In the 2nd experiment different levels of BAP in combination with NAA (0.5 mg/l) was tested (Table 2). Results of shoot multiplication rate varied from 1.44 to 5.44 according to the level of BAP used. Maximum number of shoots were achieved at 1.5 mg/l BAP and maximum plant height (5.05 cm) was also on the same medium.

Fig. 1: Shoot initiation on medium containing BAP & NAA 0.5 mg/l

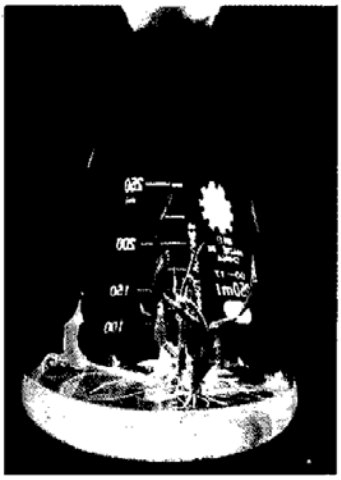

Fig. 2: Shoot multiplication on medium containing BAP & NAA 2.0 mg/l

Fig. 3: Establishment of plantlet in soil

Table 1: Media used for shoot multiplication

Med-No.	NAP (mg/l)	NAA (mg/l
	Experiment 1	
M1	0.0	
M2	1.0	
M3	2.0	
M4	3.0	
M5	4.0	
	Experiment 2	
M6	0.1	0.05
M7	0.5	0.5
M8	1.0	0.5
M9	1.5	0.5
M10	2.0	0.5
	Experiment 3	
M11	0.1	0.1
M12	0.5	0.5
M13	1.0	1.0
M14	1.5	1.5
M15	2.0	2.0

Med: Medium number, BAP: Benzylaminopurine, NAA: Naphthalene acetic acid

Table 2: Effect of BAP and NAA on shoot multiplication and plant height

Med-No.	No. Of shoots /explant* ± S.E.	Plant height (cm) ± SE
M1	1.44 ± 0.17	4.66 ± 0.23
M2	2.11 ± 0.20	5.89 ± 0.26
M3	3.11 ± 0.20	6.00 ± 0.22
M4	3.33 ± 0.23	4.22 ± 0.18
M5	2.22 ± 0.22	4.22 ± 0.26
M6	1.44 ± 0.17	4.11 ± 0.23
M7	1.67 ± 0.23	3.78 ± 0.23
M8	2.23 ± 0.23	3.78 ± 0.26
M9	5.44 ± 1.70	5.05 ± 0.29
M10	4.89 ± 0.26	4.11 ± 0.18
M11	1.33 ± 0.17	5.00 ± 0.26
M12	1.77 ± 0.22	4.83 ± 0.08
M13	1.89 ± 0.11	5.84 ± 0.20
M14	2.78 ± 0.22	5.61 ± 0.24
M15	5.67 ± 0.24	6.33 ± 0.12

*All averages are means of 20 replicates

When medium contained higher level of BAP shoot multiplication rate as well as plant height reduced.

In the third experiment equal concentrations of BAP and NAA were used (ranging from 0.1 - 2.0 mg/l). Results are shown in Table 2. Shoot multiplication rate was highest (5.67) when medium contained highest concentration of BAP and NAA (2.0 mg/l) each. It was also observed that plant height increased with the increase in hormone concentration which varied from 4.83 to 6.33 cm.

Discussion

From the preliminary results it was observed that growth hormones were very essential for shoot tip proliferation as plain M.S medium showed no response. Higher concentration of BAP (5.0 mg/l) in combination with 0.5 mg/l NAA was used by Pandy *et al*. (1996) to initiate the cultures. In the present investigation lower concentrations of BAP (0.5 mg/l) triggered culture initiation. Results also indicated that when shoot tip grew to the size of 1.0 cm (Fig. 1) it developed into a complete plantlet with well developed roots within four weeks of culturing.

Our results indicate that increasing concentrations of benzylaminopurine from 0.0 to a maximum level of 3.0 mg/l was responsible for shoot multiplication. With the increase in concentration of BAP but below' the optimum level i.e 3 mg/l the number of shoots as well as plant height increased showing a positive response (Table 2). Results are comparable to Inden *et al*. (1988), who achieved 4.0 number shoots/explant at 1.0 mg/l BAP but the plant height remained relatively less. From the results it is also clear that levels of BAP exceeding the optimum levels showed reduced number of shoots and a suppressed plant height. Instead callus like swelling were observed at the pseudostem indicating the negative effect of increased concentration of BAP on shoot proliferation.

When medium was enriched with 0.5 mg/l NAA in combination with different concentration of BAP, shoot multiplication rate was increased. A maximum 5.44 plantlets/explant were achieved when medium contained 1.5 mg/l BAP and 0.5 mg/l NAA while 5.0 number of shoots/explant were achieved by Ikeda and Tanabe (1989) when M.S medium contained 4 mg/l BAP with 0.1 mg/l NAA. Results also indicated that maximum number of shoots/explant was 5.6 and maximum plant height was 6.33 cm when medium contained equal concentrations of BAP and NAA (Fig. 2). Complete root system was also established on all the media tested within four weeks of culture initiation. After multiplication the plantlets were shifted to the soil media for establishment in the green house condition (Fig. 3).

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