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In vitro propagation of *Zingiber officinale* through rhizome and effect of plant growth regulators

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Abstract
Ginger (*Zingiber Officinale* Rosc.) is a perennial herb. It belongs to the family Zingiberaceae and commercially cultivated in most tropical regions of the world. In the present study, An efficient simple micro propagation method was developed for *Ginger (Zingiber Officinale Rosc.)* using fresh rhizome sprouting bud in semisolid culture media. Sprouting buds were sterilized and cultured onto MS medium supplemented with different concentrations and combinations of BAP (6-benzyl- amino-purine) and NAA (α- Naphthalene acetic acid) for shoot and root induction. Explants cultured on MS basal medium supplemented with 2.5 mg/l BAP + 1 gm/l NAA showed the highest rate of shoot multiplication.

Keywords: Ginger, Napthalene acetic acid, Explant, Micro propagation

Introduction
Ginger (*Zingiber officinale*) is a monocot plant belongs to the family *Zingiberaceae*. *Zingiber Officinale* Rosc. (Ginger) is an important tropical horticultural plant, values all over the world as an important spices for its medicinal properties.
Ginger is planted in the tropics for its edible rhizomes serving culinary and medicinal purposes (Portnoi *et al*., 2003) [5]. Rhizome part of the ginger plant is commonly used as condiments in food preparation to contribute to the taste and flavor (Larsen *et al*., 1999) [7]. Apart from the flavor, ginger is loaded with bioactive phytochemicals, mainly gingerols, shagols as well as volatile oils such as sesquiterpenes (β-bisabolene and (−)-zingiberene) and monoterpenes (geranial and neral) (Ursell, 2000; Chevallier, 1996) [8, 9]. The rhizome bark was reported to accumulate anthocyanin and tannin (Li *et al*., 2001) [10]. It is rich in secondary metabolite such as Oleoresin (Sakamura *et al*., 1986) [11]. Breeding of ginger is seriously handicapped by poor flowering and seed set. It is propagated vegetatively through rhizome. The germplasm collections in clonal repositories are also seriously affected by fungal diseases. Moreover since pathogenic fungi, bacteria or viruses are readily transmitted through traditional practices, it was deemed important to develop a micro propagation technique and to make available for commercial use to the pathogen free ginger germplasm. *In vitro* regeneration of auxiliary and adventitious shoots from shoot tips has already been attempted by callus culture (Hosoki and Sagawa, 1977) [4, 13] and clonal multiplication methods through meristem tip culture have also been reported by (Pillai and Kumar, 1982; Smith and Hamill, 1996) [14, 15]. However in these methods, the propagation rate was not shown high enough to obtain disease free quality micro plantlets for commercial use, and the acclimatization of the plantlet was very slow and unsatisfactory. Slow multiplication rate limited availability of high yielding genotypes extensive field maintenance of planting material, high susceptibility to rot diseases that necessitates application of tissue culture techniques as a solution to these problems (Nayak and Naik, 2006) [16]. *In vitro* propagation has long been recognized as an efficient means for rapid clonal multiplication and conservation of important taxa. However *in vitro* culture is the best method as a continuous source of supply of disease free planting material for commercial utilization. The utility, the various method of propagation includes efficient cost, effective method of *in vitro* multiplication is essential for improvement of ginger. The most important role of *in vitro* propagation is to conserve the genetic variation and evolutionary process in viable populations of ecologically and commercially viable varieties/ genotypes in order to prevent their potential extinction. The rhizomes were planted in the nursery bed for sprouting. The young fresh buds of sprouting rhizomes were used for *in vitro* propagation. In present study reports a rapid micropropagation of the two elite cultivar (cv-Suprava and Suruchi) of *Zingiber Officinale* using fresh rhizome sprouting bud as an explant which is not included in the earlier studies. As these two cultivar has high potential demand with good market value.

The main purpose of the study was to develop a technique for more rapid and more convenient clonal propagation of ginger in a cost effective manner for obtaining large scale diseases free planting material for off season and year round cultivation for the benefit of the farmers.

Materials and methods

Explant source

Healthy sprouts with active buds were collected from the rhizome. The healthy sprouts were cut in to 1.5 to 2.0 cm length with active buds intact. These rhizome pieces were rinsed several times with running tap water followed by distilled water and then buds were washed with 5% (v/v) detergent solution Tween-20 for 25 minute and rinsed several times with distilled water. These rhizome sprouting bud cuttings were surface sterilized with bavistin 0.3% followed by chlorompenocole 0.1% for 30 minute and then washed with sterile distilled water and transferred to laminar air flow cabinet. In the laminar chamber the sprouting bud cuttings were again dipped with 70% alcohol for one minutes followed by another treatment in 0.1% (w/v) mercuric chloride ($HgCl_2$) for another 8 minutes. Finally, the sprouting bud cuttings were washed 3 to 4 times throughly with sterile distilled water. Finally, the sterilized rhizome buds were then aseptically trimmed to 1-2 cm long pieces respectively under sterile laminar flow with the sterilized scalpel and forceps. Those pieces used as explants for *In vitro* cultures before the inoculation in sterilized nutrient agar media pre-packed in culture Jars.

Culture medium and condition

Sterilized blotted explants were implanted on to the Murashige and Skoog's (Murashige and Skoog's, 1962) agar-gelled medium fortified with various concentrations and combinations of growth hormones. For shoot induction, the medium was supplemented as follows 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mg/l BAP (6-benzyl- amino-purine) and 0.25 and 0.5 mg/l NAA (α-naphthalene acetic acid) either individually or in combination. For root induction in vitro raised shoots measured about 4-5 cm grown in multiplication medium were cultured on half-strength MS medium supplemented with either NAA (α-Naphthalene acetic acid) or IBA (Indole 3-butyricacid) in concentration of 0.25, 0.5, 0.75, 1.0,1.25, 1.5,1.75 and 2.0 mg/l. The pH of the medium was adjusted to 5.8 before autoclaving at 15 lbs pressure and 121 °C temperature for 15 - 20 minute. Molten medium of 50 ml was dispensed into the jar. All cultures were incubated in 14 h light/10 h dark photoperiod (cool, white fluorescent light -30µ mol m-2S-1). The cultures were incubated at 25 ± 3 °C in diffused light under 60 - 70% relative humidity in the culture room. Each treatment had 20 jars and the experiment was repeated thrice. The cultures were maintained by regular subcultures at 30 Days intervals on fresh medium with the same medium compositions.

Acclimatization

Rooted micro-propagules were removed from the jars and the roots were washed under running tap water to removed agar. Then the plantlets were transferred to portrays with sterile cocopeat and place in to poly tunnels and maintained inside the tunnel 70-80% relative humidity and temperature 28 °C. After four weeks were transplanted to poly bags containing mixture of soil + sand + manure (FYM) in 1:1:1 ratio and kept under shade house for a period of six weeks for acclimatization.

Results and discussion

The results of multiple shoot development, growth development, mean number of multiple shoots developed, and effect of different concentration of BAP were observed.

The effect of different concentration of BAP on bud initiation and shoot multiplication were investigated. After the initial bud was sub-cultured, multiple adventitious buds were produced from the base of the explants after 30 days.

Table no. 1 shows the number of multiple shoots developed at different levels of BAP. 2.5 mg/l BAP shows increased average no. of shoots on the other hand 0.5mg/l BAP shows least no. of shoots in all replicants. In the present investigation we studied effect of different concentration of BAP on of *Zingiber Officinale* Rosc, similar study was also confirmed on single cultivar by various workers.

The response of *Zingiber Officinale* Rosc. rhizome with slightly initiating buds as explants cultured on different shoot proliferation media over a period of six weeks. MS medium with growth regulator supplements produced better results in terms of percentage explants response, shoots / explants, average shoot length and average number of nodes produced per shoot. In such media combinations bud break was noticed within 25-30 days of culture (Table 1**).** Of the combination tested MS+BAP (2.5 mg/l) elicited optimal response in which an average of 7.8 ± 0.44, shoot lets (Table 1) with a mean shoot length of 5.2 ± 0.39 cm per explant was recoded the second best shoot multiplication 4.9 ± 0.43 was obtained in the medium MS+BAP (2.0 mg/l) with a mean shoot length of 5.1 ± 0.43 cm. Higher concentration of BAP (3.0 mg/l) showed callusing of the explants with fewer number of shoots. In such cultures shoots were stunted with a mean shoot length of 2.6 ± 0.36 cm. The dependence of cultured explants on bud break response and shoot multiplication has already been established and extensively discussed (Babu *et al.*, 1992) [2]. Recently, it is reported the case of micro propagation of other *Zingiberaceae* like *Curcuma longa* (Balachandran *et al*., 1990) [1], *Zingiber Officinale* (Bhat *et al*., 1994, Hosoki and Sagawa, 1977; Sunitibala *et al*., 2001) [3, 4, 13, 6]. In present study, sprouting rhizome bud of *Zingiber Officinale* Rosc. showed significantly higher response in the medium with the combination of BAP(2.5 mg/l). The quality of shoots and the over all growth response in terms of average shoot length was shown better in this growth regulator combination.

Table 1: Number of multiple shoots developed at different levels of BAP

Explant No : 10	**BAP Conce..**				
	1.0 mg/l	**1.5 mg/l**	**2.0 mg/l**	**2.5 mg/l**	**3.0 mg/l**
No.of Shoots	3.9 ± 0.14	4.0 ± 0.38	4.9 ± 0.43	7.8 ± 0.44	4.1 ± 0.32
Shoot length/cm	4.8 ± 0.34 cm	5.0 ± 0.36 cm	5.1 ± 0.43 cm	5.2 ± 0.39 cm	2.6 ± 0.36 cm

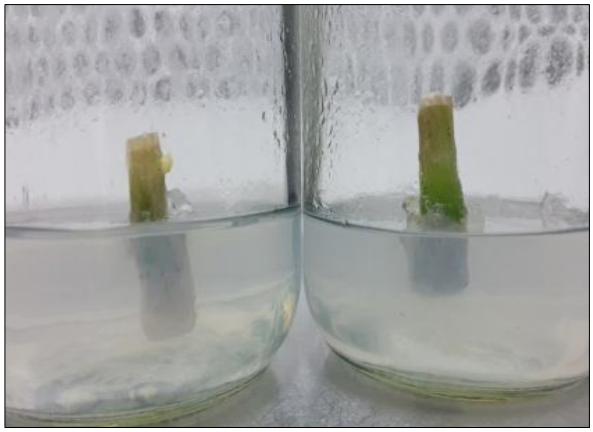

Fig 1: Iinitiation

Fig 2: Multiplication

Fig 3: Rooting

Fig 4: Plantlets

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