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Rapid *in vitro* Micropropagation of *Alpinia officinarum* Hance, An Important Medicinal Plant, Through Rhizome Bud Explants

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Abstract: An efficient multiple shoot has been developed for the medicinal plant *Alpinia officinarum* Hance (Zingiberaceae) using rhizome buds as explants. Multiple shoots originated when the rhizome buds were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of growth regulators. Optimum shoot multiplication was observed on MS medium containing 3% (W/V) sucrose and 3.0 mg L^{-1} kinetin (Kn) and 1.0 mg L^{-1} Naphthalene Acetic Acid (NAA). Each rhizome bud gave rise to an average of 11 shoots per explant. Rooting experiments with half-strength Murashige and Skoog medium revealed that 0.5 mg L^{-1} Indole-3-butyric acid (IBA) was more suitable for root induction when compared to IAA and NAA. Healthy *in vitro* rooting plantlets were transferred to pots containing a mixture of vermiculite and soil (1:1) for acclimatization for a period of three-four weeks and 93% of plantlets survived under field conditions.

Key words: *Alpinia officinarum*, rhizome bud, shoot multiplication

INTRODUCTION

Alpinia officinarum Hance (Lesser galangal) belonging to the family Zingiberaceae is an aromatic perennial herb distributed throughout the tropical and the subtropical Asian region. The name *Alpinia officinarum* was given to this herb, as the source of Galangal is less when compared to *Alpinia galangal.* It can grow up to 3-5 feet in height and flowers are small, white with red streaks. The rhizomes are reddish brown and about 2 cm in diameter. They are widely used in India, China and Asia for a wide range of diseases like respiratory tract infections, arthritis, Cancer, microbicidal and gastro intestinal disorders (Uehara and Yasuda, 1987; Sakai and Miyazaki, 1989; Lin and Hsu, 1998; Tewari and Pant, 1999; Wu and Larsen, 2000). It has been used in Europe as a spice for over a thousand years, having probably been introduced as a medicinal plant by the Arabian or the Greek physicians. The active molecule (5-hydroxy-7-(4'-hydroxy-3'-methoxyphenyl)-1-phenyl-3-heptanone) was found to possess significant inhibitor of pancreatic lipase activity in hyperlipidemic mice (Shin *et al*., 2004). One of the active principles of the plant, diarylheptanoids have been reported to inhibit the pro inflammatory mediators like the Nuclear Factor kappa B (NF-κB), phosphorylation of MAPK, P44/42 and also potential inhibitors of leukotriene (LT), biosynthesis and prostaglandin synthase enzymatic activity and also suppression of inducible nitric oxide synthase expression level in RAW 264.7 cell line (Prem *et al*., 2003; Matsuda *et al*., 2006; Lee *et al*., 2006). However, there are many species of Zingiberaceae family having antioxidant properties (Vankar *et al*., 2001). Six diarylheptanoids were elucidated from the root material which was determined quantitatively by HPLC of *Alpinia officinarum* (Liu *et al*., 2005; An *et al*., 2006). *Alpina officinarum* is widely used in the pharmaceutical industry for therapeutic purposes and it provides the essential raw material for many ayurvedic preparations. Razing of *Alpinia officinarum* is a necessity in order to meet pharmaceutical needs and also to prevent the plants from becoming endangered or extinct (Peter, 2006). Several Zingiberaceae species have been investigated through *in vitro* rhizome bud multiplication which is an easy and safe method for obtaining uniformity and it also assures the consistent production of true-to-type plants within a short span of time (Balachandran *et al*., 1990; Borthakur *et al*., 1999; Rahman *et al*., 2004) To our knowledge there is no work reported on *Alpinia officinarum* which include multiple shoot formation from rhizome buds and micro propagation from seedlings. Hence the aim of the present study was to develop efficient protocols for *Alpinia officinarum* multiple shoot formation from rhizome bud explants. This technique would facilitate an alternative method for rapid large-scale clonal production and successful outdoor establishment of this medicinal species.

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MATERIALS AND METHODS

Young rhizome buds of *Alpinia officinarum* were collected in October 2005 from TAMPCOL Medicinal garden (Tamil Nadu Medicinal Plant Corporation Limited), Kolli Hills, South India. The rhizome buds were initiated for micro propagation at Tissue Culture and Drug Discovery Laboratory, Centre for Biotechnology, Anna University, Chennai, India. The explants used for the study were washed with 5% (V/V) commercial bleach (Tween 20) and then rinsed in running tap water for 10 min. The cleaned buds were sterilized by soaking it with intermittent agitation in 0.2% (W/V) mercuric chloride for 5 min. The rhizome buds were subsequently washed three times with sterile double distilled water and were cut into appropriate sizes and inoculated onto the appropriate sterile media.

The shoot multiplication media was composed of MS (Murashige and Skoog, 1962) basal medium containing myo-inositol (100 mg L^{-1}) and sucrose (3% W/V); this media was supplemented with different concentrations of kinetin (Kn) and 6-benzylaminopurine (BAP) alone or in combinations with naphthalene acetic acid (NAA). Individual regenerated shoots were excised and used for rooting. Root induction was carried out on half-strength MS solid medium supplemented with different auxins. The pH of the medium was adjusted to 5.8 with KOH before adding agar (0.7%). Medium without plant growth regulators were used as a control. Cultures were incubated at 25±2°C under a 16/8 h (light/dark) photoperiod with light supplied by white fluorescent tubes at 2,000 lux. Rooted plants were removed from the culture medium, rinsed in water to remove media and transferred to pots containing vermiculite and soil (1:1) then potting plants were transferred to the greenhouse and subsequently to the field. Each experiment with 20 cultures per treatment was repeated three times.

RESULTS AND DISCUSSION

The multiple shoots were cultured from rhizome bud explants of *Alpinia officinarum* on MS solid medium supplemented with various concentrations and combinations of auxin/cytokinins after 4-5 weeks of incubation (Table 1). Hormone free MS medium was used as a control, which induced shoots at a rate of 2.5 shoots per explant. Comparisons between the best individual concentrations for each growth regulator (Kn/BAP) revealed an average of 8.5 and 5.6 shoots and the length was 3.3 and 4.2 cm respectively at the rate of 3.0 mg L^{-1}. A lower number of shoot multiplication and elongation was observed at higher concentration of 4.0 mg L^{-1} of kinetin and 4.0 mg L^{-1} of BAP (Table 1). However, the highest regeneration frequency (100%) and a maximum of 11 shoots per explant were obtained after 4-5 weeks on a medium containing 3.0 mg L^{-1} Kn in combination with 0.5 mg L^{-1} NAA which was most effective for rhizome bud multiplication (Fig. 1A). This was found to be considerably different from all other treatments. Also shoot elongation was more prominent (7.5 cm) in the same treatment (Fig. 1B). Similar results were also observed in rhizome bud explants of *Alpinia galanga* (Borthakur *et al*., 1999). The synergistic effect of cytokinins in combination with a low concentration of

Table 1: Effects of different concentrations of Kn/BAP alone or in combination with NAA on shoot proliferation from rhizome bud explants of *A. officinarum*. There were 20 explants in each treatment and data (mean±SE) were recorded after 5 weeks of culture

Growth regulator (mg L^{-1})					
Kn	BAP	NAA	Response (%)	No. of shoots per node (mean±SEM)	Average length of shoots per culture (cm) (mean±SEM)
Hormone-free MS medium			90	2.5±0.1	2.1±0.2
1.0			70	4.8±0.2	2.6±0.2
2.0			100	6.4±0.2	3.1±0.4
3.0			100	8.5±0.1	3.3±0.3
4.0			80	5.2±0.2	2.7±0.3
	1.0		90	4.9±0.2	2.6±0.3
	2.0		80	3.7±0.1	3.1±0.2
	3.0		70	5.6±0.2	4.2±0.4
	4.0		90	4.3±0.1	2.8±0.2
3.0		0.5	80	2.6±0.1	2.5±0.2
3.0		1.0	100	10.9±0.3	7.5±0.3
3.0		1.5	100	8.4±0.2	5.9±0.3
3.0		2.0	90	7.9±0.2	6.4±0.3
3.0		2.5	80	5.6±0.3	5.7±0.3
	3.0	0.5	100	5.7±0.3	5.1±0.1
	3.0	1.0	70	5.6±0.3	5.4±0.2
	3.0	1.5	80	7.2±0.3	5.8±0.3
	3.0	2.0	80	4.6±0.2	5.6±0.2

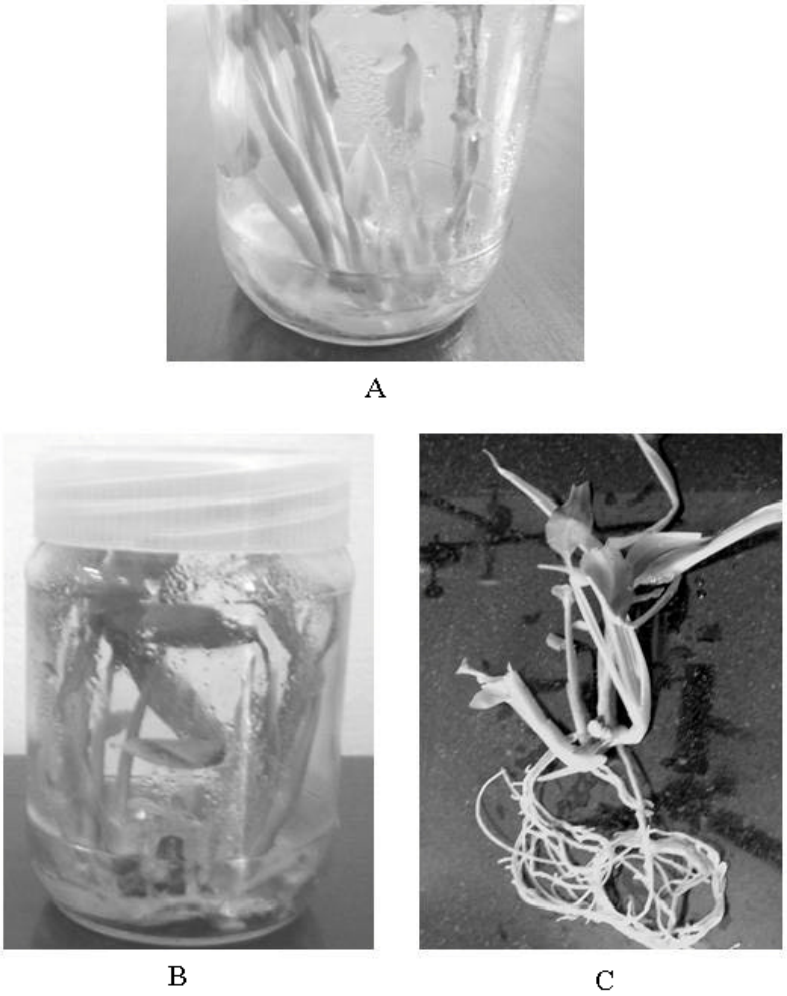

Fig. 1: *In vitro* multiplication of *Alpinia officinarum*. (A) Multiple shoot initiation from rhizome bud explant on MS medium with Kn (3 mg L^{-1}) and NAA (0.5 mg L^{-1}), (B) Root formation in shoot cuttings after 25 days in half strength MS medium containing, IBA 0.5 mg L^{-1} and (C) Rooted plantlets ready for hardening

auxin has been reported earlier for several medicinal and aromatic plant species including *Zingiber officinale* (Hosoki and Sagawa, 1977) *Curcuma longa* (Rahman *et al*., 2004) *Withania somnifera* (Sen and Sharma, 1991). Based on this information, the present study also exemplifies the positive modification of shoot multiplication efficacy by low concentrations of an auxin (NAA) in combination with a cytokinin (Kn). For further multiplication process, the explants excised from sub-culturing shoots were placed on the same fresh medium. During the 5-6 subculture passages the percentage of shoot development as well as the number of shoots per explant retained the same value. Such type of simultaneous production of multiple shoot was reported earlier for a few medicinal plants species, such as *Hemidesmus indicus* (Sreekumar *et al*., 2000) and *Withania somnifera* (Sen and Sharma, 1991).

Excised shoots failed to produce roots on half-strength or full-strength MS medium without growth regulators even after 40 days of culture. The rate of root multiplication of *Alpinia officinarum*, significantly differed based on growth regulators (Table 2), when cultured on half-strength MS solid medium. A maximum rate was achieved at 0.5 mg L^{-1} IBA, each shoot developed an average of 7 roots and the length was 8.5 cm (Fig. 1C) after 20-25 days. The effectiveness of IBA in rooting has been reported for medicinal plants like *Hemidesmus indicus* (Sreekumar *et al*., 2000), *Cunila galioides* (Fracaro and Echeverrigary, 2001) and *Aloe polyphylla* (Abrie and van Staden, 2001). The slow movement and slow degradation of IBA facilitates its localization near the site of application and thus its better function in inducing roots (Nickell and Kirk-othmer, 1982). Rooted shoots of *A. officinarum* were transferred directly

Table 2: Effects of different concentrations of auxins on adventitious root formation from *in vitro* shoots. Data represent the mean of 20 cultures. Growth period was 20-25 days (mean±SEM)

Growth regulators (mg L^{-1})	Response (%)	No. of roots (mean±SE)	Root length (cm) (mean±SEM)
Hormone free MS	80	-	-
Hormone free half strength MS	90	-	-
NAA			
0.1	100	2.7±0.2	3.3±0.1
0.2	90	3.2±0.8	5.0±0.2
0.5	90	3.3±0.2	6.3±0.2
1.0	70	3.0±0.2	5.7±0.2
IBA			
0.1	80	3.1±0.2	4.3±0.1
0.2	100	3.9±0.2	6.1±0.2
0.5	100	7.2±0.3	8.5±0.1
1.0	90	4.5±0.2	6.8±0.2
IAA			
0.1	100	2.8±0.2	5.1±0.1
0.5	90	4.0±0.2	6.6±0.1
1.0	100	5.5±0.3	7.9±0.2
1.5	70	3.7±0.3	7.1±0.2

to small pots filled with vermiculite and soil (1:1) and kept in green house for acclimatization. Whereas, 93% of plantlets survived their transfer into pots all of them survived in the garden. Plants appeared to be morphologically uniform and were successfully adapted to field conditions. It is known that plantlets obtained from organized meristem shoot tissues of vegetative buds exhibit normally no signs of visible morphological variation and are genetically identical (Bajaj *et al*., 1988). The present procedure might be used to produce around 500-600 whole plants per bottle with out any intermediate callus phase within 4-5 weeks. It is important especially for micro propagation of medicinal plants.

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