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In vitro micropropagation of *Alpinia zerumbet* Variegate, an important medicinal plant, through rhizome bud explants

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An ideal Micropropagation method of *Alpinia zerumbet* has been developed using rhizome bud explants. Basal MS medium supplemented with 3% sucrose (w/v) different concentrations of BAP in combination with 0.5 mg / L of kinetin. Highest percentage (95%) of explants for shoot induction and multiple shoot (7.9 per explants) production were observed in the combination of 1.5 mg / L of BAP, 0.5 mg / L of kinetin. In this case, all the inoculated explants induced multiple shoots within 6-7 weeks of inoculation. Rooting was induced in a medium having half strength MS supplemented with 0.5mg / L of IBA. Most of the generated shoots were successfully transferred to soil under field conditions.

Keywords: *Alpinia zerumbet*, rhizome bud, shoot multiplication.

Alpinia zerumbet commonly known as shell ginger is a 4-8 foot tall herbaceous plant of zigiberaceae family. It is a rhizomatous, evergreen clump forming perennial, propagated vegetatively through rhizome. This plant is a native of eastern Asia which had both commercial and medicinal value. This plant is used in landscape for its attractive foliage and shell like flowers. The leaves of this plant are green and yellow variegated and are quite striking. Leaves and flowers are attractive in flower arrangement. The plant produces fleshly rhizomes much like ginger that have a ginger like aroma. This beautiful tropical plant is becoming a popular tropical house plant as well as a landscape plant in warmer climates. It can be used for the treatment of intestinal and cardiovascular diseases including arterial hypertension (Leal-Cardoso *et al*., 2006). It is also used as diuretic, antihypertensive and antiulcerogenic (de Moura *et al*., 2005). Its rhizome may be utilized in food stuffs as a cheap source of natural antioxidant (Abdelnaser *et al*., 2006).

Alpinia zerumbet is vegetatively propagated through rhizome, because the seeds are rarely formed. *In vitro* culture can produce multiple shoots from single rhizome (Chinnasamy Selvakkumar *et al*., 2007 and Borthakur *et al*., 1998). The present study is to establish a protocol for the invitro micropropagation of *Alpinia zerumbet* variegate.

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Materials and Methods

The rhizomes were collected from NGM college campus. The unnecessary parts like roots and other residual were removed from the rhizome bud. Then the explants were thoroughly wiped with 70% alcohol to remove loose contaminants and reduce the size by removing the outer layer. Then the explants were taken in conical flask containing 0.25 % of sodium hypochlorite and washed further by vigorous shaking for about 12 minutes. This was followed by washing 5 times with sterile distilled water. Finally inside the laminar air flow cabinet, surface sterilization was done by putting the explants in 70 % alcohol for 30 seconds. After the explants were treated with 0.12 % mercuric chloride for 12 minutes followed by sterile distilled water wash. Then the explants were finally dissected. The material is again treated with 0.1 % mercuric chloride for 2 minutes. Immediately wash the explants with sterile distilled water to remove the traces of mercuric chloride.

After surface sterilization just trim the base of the rhizome and were inoculated on MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and different concentration of BAP in combination with 0.5 mg / L of kinetin. The pH of the media was adjusted to 5.8 prior to the gelling with 0.8% agar, dispensed into the culture tubes and sterilized by autoclaving (121˚C for 15 minutes). The culture was maintained in the culture room under a regime of 16 hours photoperiod at 25˚C.

Results and Discussion

The multiple shoots were cultured from rhizome bud explants of *Alpinia zerumbet* on MS solid medium supplement with different concentration of BAP in combination of 0.5 mg / L of kinetin. All these treatment best response was observed in 1.5mg/L of BAP and 0.5mg / L of kinetin (Table 1). In this combination almost 95% of the inoculated explants showed regeneration with in 6-7 weeks of inoculation and the average number of shoots per explants was 7.9 (Figure 1). The second highest response was observed in MS with 2mg / L of BAP and 0.5 mg /L of Kinetin. In this supplement 75% of the inoculated explants showed regeneration with in 7-8 weeks of inoculation and in average, about 6.25 shoot buds were regenerated from each explants. Multiplication can be continued by transferring each divided shoot explants to the same medium. Satisfactory root development was observed in half strength MS medium supplemented with 0.5 mg /L of indole-3-butyric acid.

Complete plants thus obtained were transferred to soil: vermiculate (1:1) in paper cups and covered with polythene covers to maintain humidity. After 3-4 weeks the plants are transferred to the field. Almost 96% of the regenerated plants survived and showed a plant survived and showed a vigorous growth of rhizome and roots without any morphological variations.

Table 1: Effect of different concentration of BAP and kinetin in MS on Shoot multiplication from Rhizome bud explants of *Alpinia zerumbet*

Conc. of BAP (mg/L)	Conc. of Kinetin (mg/L)	No of shoots/ explants	% of regeneration
0.5	0.5	3.570	55
1.0	0.5	4.320	70
1.5	0.5	7.900	95
2.0	0.5	6.250	75
2.5	0.5	5.550	65

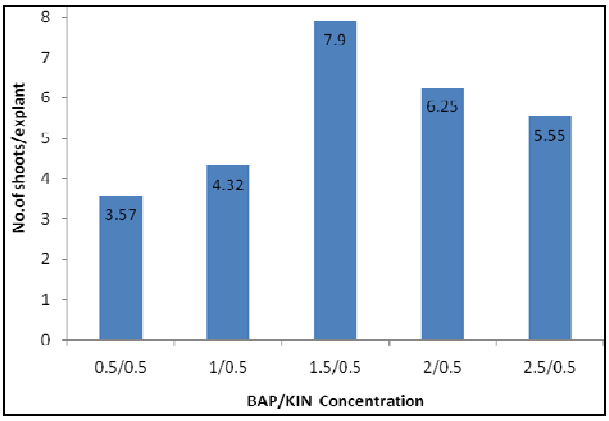

Figure 1. Number of shoots per explant in different contentraction of BAP and Kinetin

The ability of BAP to induce branching is well documented (George, 1993). In general, herbaceous plants are highly responsive to BAP treatment and most cultured herbaceous species produces robust, well-formed shoots suitable for further shoot proliferation (Debergh and Zimmerman, 1999). In ginger a related species of *Alpinia zerumbet*, role of BAP in shoot multiplication was reported (Bhagyalakshmi and Sing 1988, Balachandran *et al*., 1990 and Inden *et al*., 1988). In consistence with these results, our present study also revealed the role of BAP in combination with little amount of kinetin in shoot multiplication of *Alpinia zerumbet*. The regenerated shoots with spontaneously formed roots were successfully transferred to soil.

References

Abdelnaser A, Elzaawely, Tran D. Xuan, and Shinkichi Tawata, 2006. Essential oils, kava pyrones and phenolic compounds from leaves and rhizomes of *Alpinia zerumbet* and their antioxidant activity. *Science Direct*, 2:486-494.

Balachandran, S.M., S.R. Bhat and K.P.S. Chandel, 1990. *In vitro* clonal multiplication of turmeric (*Curcuma* spp.) and ginger (*Z. officinale* Rosc.). *Plant Cell Rep*., 8: 521-529.

Bhagyalakshmi, N. and N.S. Sing, 1988. Meristem culture and micropropagation of variety of ginger (*Zingiber Officinale* Rosc.) with a high yield of Oleoresin. *J. Hortic. Sci*., 63: 321-327.

Borthakur, M., J. Hazarika and R.S.Sing, 1998. A protocol for micropropagation of *Alpinia galanga*. *Plant cell, Tissue and Organ Culture*, 55:231-233.

Chinnasamy Selvakkumar, Arun Balakrishnan and Baddireddi Subhadra Lakshmi, 2007. Rapid *in vitro* micropropagation of *Alpinia officinarum*, an important medicinal plant, through rhizome bud explants. *Asian J. Plant Science*, 1682-3974.

de Moura, R Soares; Emiliano, AF; de Corvalho, LC.R. Marins; Souza, MA. V; Gueds, DC; Teno, T; Resende, AC, 2005. Antihypertensive and endothelium-dependent vasodilator effects of *Alpinia zerumbet*, a medicinal plant. *J. Cardiovascular Pharmacology*, 46(3):288-294.

Debergh, P.C. and R.H. Zimmerman, 1999. Micropropagation Technology and Application. Kluwer Academic Publishers, Dordrecht, the Netherlands.

George, E.F. 1993. Plant Propagation by Tissue Culture. Part 1. The Technology. Exegetics. Ltd., Edington, Wilts, UK.

Inden, H., T. Asahira and A. Hirano, 1988. Micropropagation of ginger. *Acta Hortic.*, 230: 177-184.

Leal-Cardoso JH, Moreira MR, da Morais SM, Lohlou MS, Coelho-de-Souza AN, 2006. Effects of essential oil of *Alpinia zerumbet* on the compound action potential of the rat sciatic nerve. *Phytomedicine* 11(6):549-53.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. *Physiol. Plant* 15:473-497.