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RESEARCH ARTICLE

INVITRO PROPAGATION OF *KAEMPFERIA GALANGA* USING RHIZOME

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ABSTRACT

A protocol was standardized for the rapid propagation *Kaempferia galanga* using rhizome. The medium used was MS medium with auxin (IAA, IBA) and cytokinin (BA). Of these maximum shoots were produced when cultured with MS medium containing 1.0mg/l BA and 0.1mg/l IAA. Maximum shoots were produced by sub culturing in two weeks of sub culturing in the same medium. Regenerated plants were acclimatized and established on soil with eighty five percent success.

INTRODUCTION

Kaempferia galangal belongs to *Zingiberaceae* family with a common name "kacholam" in Malayalam and "black thorn" in English. It is a rhizomatous medicinal plant, widely used as medicine for its volatile oil and aromatic compounds It is very important because the rhizome is having the carminative, diuretic properties and widely used in manufacturing of medicines for cough, stoppage of nasal, block etc...The common method of reproduction is vegetative reproduction through the rhizomes, but there is susceptibility to disease and slow growth. More than that the demand of this plant is increased day by day and the price is also high. Because of these reasons it is necessary to find another method for the rapid propagation of these plants. *Invitro* propagation is most suitable for this. Rhizomatous plants like ginger, curcuma etc. can be grown by this method so invitropropagation to this plant is also important. A lot of invitropropagation methods were develop for zingiberaceae plants by using rhizome. Khatun *et al.,* 2003, methods of culture initiation and multiple shoot regeneration *Z. officinale* and almost similar protocol is effectively used for *Curcuma* species (Tyagi *et al.,* 2004; Das *et al.,* 2010). A study on in vitro multiplication and rhizome formation for *Z. officinale*. Was conucted uner the effect of of different growth regulators and culture conditions on was studied by Rout *et al.* (2001). A major problem in rhizomatous plants during initiation and successful establishment of aseptic cultures is contamination (Borthakur *et al.,* 1999).

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The time of collection is important regarding the responding percentage and the contamination rate in *invitro* studies of *Zingiber* species. Rainy season, is the most favorable time for initiation of culture because the buds are in actively growing state adventitious shoots developed from 80 % of the explants and rate of contamination was also less. Stanly and Keng 2007 reported in vitro seasonal effect on bud growth in *Z. zerumbet* and *Curcuma zedoaria* and *Curguligo orchioides* (Wala and Jasrai 2003). A widely used as a standard carbon source for plant tissue culture is Sucrose, and different concentrations and different osmotic environments have been used (Das *et al.,* 2010). Reports were there stating that higher concentration of sugar source is ideal for in vitro micro rhizome production in *Z. officinale.* Although explants showed a fair response to individual cytokinins used, the combinations of two regular cytokinins (BA and Kn) were found to be ideal for shoot multiplication. Similar results were found by Anish *et al.* (2008) found out that cytokinins (BA and Kn) were found to be ideal for shoot multiplication. in *Bosenbergia pulcherrima*, a threatened ginger. Genetic purity of in vitro raised plants using proved to be an efficient tool for many plant species (Rout and Das 2002; Hussain *et al.,* 2008). The explants source and mode of regeneration are known to play a major role in determining the presence or absence of variation. Using rhizomatous buds as explants for micropropagation lowers the risk of genetic instability as the organized meristem is generally more resistant to genetic changes that might occur by indirect regeneration (Salvi *et al.,* 2002).

Objectives

In this present work an effort was taken to do the invitropropagation of Kaempferia galanga using rhizome as the

explants. We are also aiming to develop a fast and large scale multiplication of the plant by using the same explants.

MATERIALS AND METHODS

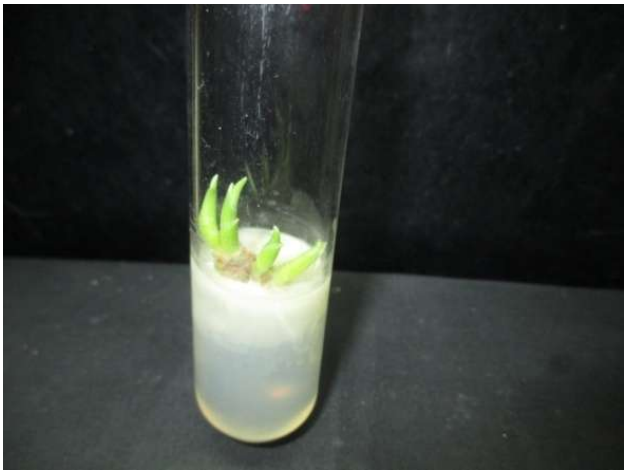
Rhizome explants were collected from the field grown plants from various places of Thrissur District Kerala. They were brought to the laboratory and surface sterilization was performed by excising the rhizome, washing it thoroughly under running water, for 20 minutes, then with Teepol for 20 minutes and again with Bavistine (Fungicide) for 20 minutes and then with distilled water for 10 minutes. Then they were taken to the laminar air flow chamber and treated with .01% $HgCl_2$ for 3 minutes and rinsed with water for 5 times to remove the traces of $HgCl_2$. After surface sterilization the rhizome were trimmed into appropriate size and inoculated in the MS medium for shoot multiplication. The basal medium used was Murasige and Skoog medioum containing all salt and vitamins, 30 g/l sucrose, 8g/l Agar. The media were variously supplemented with Benzyl amine individually and in combination with Indole 3 Acetic Acid (IAA) and Indole- 3 butric acid (IBA). Regenerated micro shoots were placed in rooting medium containing half strength MS supplemented with various concentration of auxins (IAA,IBA) singly for rooting. P^H of the medium was adjusted to 5.7+ 1before adding the agar and autoclaved 1.1 Kg cm^{-2} for 20 minutes at 120^0 C. Cultures were incubated at 25±1 ^{0}C with a photoperiod of 16 hours with photon flux density of about 70 µmol m^{-2} provided with a white fluorescent light.

RESULTS AND DISCUSSION

Shoot proliferation

In order to find out an optimum culture medium for the maximum multiple shoot production from the rhizome of *Kaempferia galanga* a number of experiments were conducted.

Initial stage of inoculation

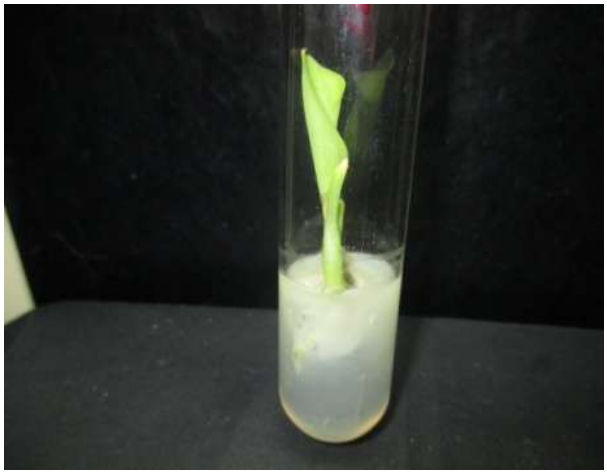


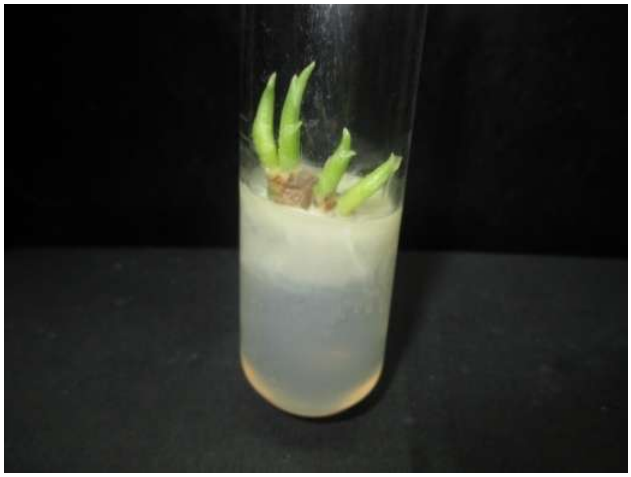
Shoot proliferation from the rhizomes

Multiple shoot development was there in all combinations of growth hormones but maximum was observed with Ms +1 mg/l BA+0.1 mg/l IAA. Multiple shoots were initiated in four week old culture. Number of shoots per culture was 19.36±1.25 and average length of shoot per culture was 6.35±0.25. Similar effect was reported in Alpinia calcarata by Amin *et al* in 2001. The effect of various concentration of BA on shoot initiation and proliferation was also studied here. Best response for shoot proliferation was in 1.0mg/l BA +0.1mg/l IAA supplemented MS medium almost 96% explants showed shoot proliferation at this concentration. Similar results obtained for ginger and turmeric by Blachandran *et al* (1990) and M.M. Rahman *et al* in *Kaempferia galanga* (2005)

Rooting of shoots

Rooting was induced by placing them in half strength MS medium supplemeted with various concentration of IAA, IBA ranging from 01 -1.0 mg/l. The best performance was for 0.2 mg/l of IBA with 905 root at 6th week. These findings are in agreement with the result obtained by Amin *et al* in 2001 for Alpinia calcarata and Blachandran *et al* (1990) for ginger and turmeric and M.M. Rahman *et al* in *Kaempferia galanga* (2005).





Rooting

Rooted plant in pot

Kaempferia galanga

Establishment under *exvitro* condition

The invitro generated plants were transfered to the soil by making their root agar free by continuous flashing of tap water. Then these plants were slowly transfreerd to ice cream pots containing sand, gareden soil and compost in 1:1:1 ratio. 85% of survival was noticed .

Summary and Conclusion

A protocol was developed for invitro propagation of *Kaempferia galanga* using MS medium for shoot multiplication. The basal medium used was Murashigue and Skoog medium containing all salt and vitamins, 30 g/l sucrose, 8g/l Agar which was were variously supplemented with Benzyl amine individually and in combination with Indole 3 Acetic Acid (IAA) and Indole- 3 butric acid (IBA). Rooting was induced by placing in rooting medium containing half strength MS supplemented with various concentration of auxins (IAA, IBA) singly for rooting. We can conserve the red listed medicinal plant Kaempheria galanga by in vitro propagation method. It is very effective, fast and easy method to produce such plants in mass. For this the rzhizome can be used for the best result and MS medium supplemented with 1.0 mg/l BA and 0.1 mg/l IAA is more suitable to provide large number of multiple shoots.

Aknowlegement

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