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# HIGH FREQUENCY SHOOT MULTIPLICATION OF *ALPINIA GALANGA* (L.) WILLD. USING RHIZOME BUDS

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**ABSTRACT:** A successful rapid shoot multiplication system developed via rhizome buds of *Alpinia galanga,* a valuable medicinal plant. The cytokinins such as BAP and TDZ were used for shoot multiplication. In the present study, BAP was found to be a greater influence than TDZ. The medium supplemented with 0.75 mg/l BAP produced maximum number of shoots (7.33±0.33) and shoot length (11.0±0.28 cm). The regenerated plants were treated with auxins like IAA and IBA. 1.0 mg/l IBA gave the highest number of roots (15.66±1.2) and greatest root length (11.66±0.88 cm) after four weeks. Regenerated plants were successfully transferred to plastic cups containing mixture of sand, soil and vermiculite for the primary hardening. 75% survival rate observed after secondary hardening. The present findings can be a basis for large scale multiplication and the *in vitro* conservation of *A. galanga*.

**KEYWORDS:** *Alpinia galanga,* rhizome, shoot multiplication, rooting, hardening.

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## 1.INTRODUCTION

*Alpinia galanga* (L.) Willd. is a perennial aromatic herb with a showy flower and an important medicinal plant of family Zingiberaceae. This species mainly found in India and Southeast Asia. The use and value of *A.galanga* is very high in pharmacological and food industry. *A.galanga* is used to cure diseases such as throat infections, ulcers, bad breath, fever, whooping cough, bronchial catarrh, rheumatism [1]. The rhizomes have been reported to have the antibacterial [2], antifungal [3], anti-inflammatory [4], Anti Diabetic [5] and Anti-Oxidant [6] activities. Phytochemical

components of *A.galanga* are used for antiulcer [7] and antitumour activity [8]. Camphor, α-fenchylacetate, myrcene, camphene, 1,8-cineole, α-fenchol, guaiol, carotol are the major phytochemical components responsible for odour and medicinal properties [1]. The vegetative propagation method of *A.galanga* is by rhizomes and it is not sufficient to meet its commercial demand. The conventional breeding method is very difficult in rhizomatic plants due to lack of seed set and vegetative propagation by rhizomes results in spread of various diseases such as bacterial wilt and rhizome rot [9]. The *in vitro* culture techniques and procedure described in this study can help to overcome these major problems in breeding and propagation. Tissue culture studies of organogenesis has already reported in a number of Zingiberaceae including *Zingiber officinale* [10], *Curcuma longa* [11], *C.aromatica* [12], *C.anguistifilia* [13], *C.attenuata* [14] and *A.calcarata* [15]. The regeneration protocols of *A.galanga* via direct and indirect organogenesis have been reported earlier [16,1]. However these results remain inefficient for large scale multiplication of this plant. Hence present research was designed to understand high frequency multiple shoot development and plant regeneration using different plant growth regulators (PGRs) including 6-Benzylaminopurine (BAP), Thidiazuron (TDZ), Indole-3-acetic acid (IAA) and Indole-3-butyric acid (IBA). Further, plantlets were transferred to natural field condition for hardening.

## 2. MATERIALS AND METHODS

### Plant materials

Mature rhizomes of *A. galanga* were collected from medicinal plant garden of Unani Medical College, Kozhikode, Kerala and germinated in Jamal Mohamed College garden. The rhizomatic buds were used as the explant and sectioned approximately into 2.0 – 2.5 cm pieces. They were washed under running tap water for 20 min and then immersed in detergent (teepol) for 5 min. After rinsing explants with sterile distilled water, all were surface disinfested by using 70 % (v/v) ethanol for 1 min. Further it was soaked in 0.1% (w/v) aqueous mercuric chloride solution for 5 min and subjected to thorough rinsing with sterile distilled water for three times.

### Culture medium and condition

MS medium [17] with 3 % sucrose and 0.8 % agar were used for *in vitro* culture. The pH was adjusted between 5.6 to 5.8 and autoclaved at 121ºC for 20 minutes and 104 kpa pressure. The culture jars were maintained under 16/8 h (light/dark) photoperiod provided by white fluorescent tubes at 24 ± 2ºC.

### Multiple shoot induction

The rhizome buds explants were cultured on MS medium supplemented with different PGRs such as BAP and TDZ at various concentrations (0.25 to 1.0 mg/l). MS medium without growth regulators considered as control. After 4 weeks of culture, the shoot length, number of shoots per explant and the percentage of responsive explants were recorded.

***In vitro* rooting**

The elongated shoots (>3.0 cm length) with fully expanded leaves were transferred to MS medium supplemented with IBA or IAA at various concentrations (0.5 to 1.5 mg/l) for rooting. After 4 weeks the percentage of rooting and the mean number of roots per plantlet were recorded.

**Acclimatization**

Regenerated plantlets with 4-6 fully developed leaves were removed from the culture medium and agar was removed from the plantlets by washing them under running tap water and transferred to sand, soil and vermiculite (1:2:1) mixture in paper cups for primary hardening. The percentage of survival rate was calculated after one month. Further, they were transferred for secondary hardening in earthen pots containing cattle manure, soil and sand (1:2:1).

**Statistical analysis**

All experiments were repeated three times with ten explant for each treatment. The data on various parameters were assessed using Duncan's multiple range test (ANOVA). The results were analysed using SPSS (version 20, IBM, corporation, NY).

## 3. RESULTS AND DISCUSSION

**Shoot multiplication**

The rhizome explant did not show any response of bud break when cultured on phytohormone free medium and failed to induce any shoots even after 4 weeks of culture. Explants cultured on MS medium with various doses of TDZ and BAP for different days showed a discernible response on bud breakage. Multiple shoot induction was observed in the presence of various cytokinins (BAP and TDZ) at different concentration (0.25, 0.5, 0.75, 1.0 mg/l)(Figure 1C). Among the single growth regulator treatments, 0.75 mg/l BAP produced optimum shoot induction (93%) with maximum number of shoots (7.33±0.33cm). BAP was found to have a greater influence than TDZ on the number of shoots and shoot length (Table 1)(Figure 1D). Similarly, shoot bud regeneration potential was significantly enhanced in Zingiberacae species, when explants derived from rhizome segments and treated with BAP in *A.calcarata* [15], *Etlingera coccinea* [18] and *Z.officinale* [19]. However, in some plants of Zingeberaceae like *C.vamana* [20] and *Z.montanum* [21] reported the highest rate of shoot production in the presence of TDZ.

**Table 1: Effect of cytokinins on shoot induction**

| BAP (mg/l) | TDZ (mg/l) | % of response on shoot induction | Number of shoots/explant | Shoot length (cm) |
|---|---|---|---|---|
| 0.0 | - | $0.0^{e}$ | $0.0^{g}$ | $0.0^{e}$ |
| 0.25 | - | $54.33\pm1.85^{c}$ | $1.66\pm0.33^{f}$ | $7.0 \pm 0.5^{d}$ |
| 0.5 | - | $74.33\pm4.37^{b}$ | $3.66\pm0.66^{de}$ | $9.0 \pm 0.5^{bc}$ |
| 0.75 | - | $93.00\pm2.30^{a}$ | $7.33\pm0.33^{a}$ | $11.0 \pm 0.28^{a}$ |
| 1.0 | - | $85.0\pm2.0^{a}$ | $6.00\pm0.57^{ab}$ | $9.66 \pm 0.44^{b}$ |
| - | 0.25 | $44.0\pm2.08^{d}$ | $2.33\pm0.33^{ef}$ | $7.0 \pm 0.28^{d}$ |
| - | 0.5 | $68.0\pm1.52^{b}$ | $5.33\pm0.66^{bc}$ | $8.16\pm0.44^{c}$ |
| - | 0.75 | $69.66\pm3.52^{b}$ | $6.33\pm0.33^{ab}$ | $8.16\pm0.16^{c}$ |
| - | 1.0 | $42.0\pm4.04^{d}$ | $4.0\pm0.57^{cd}$ | $6.5\pm0.28^{d}$ |

Values are expressed as the mean ± SE, taking ten explants in each experiment with three replications. All data were taken after 4-5 weeks of inoculation.Within each group, values with different letters indicate significant difference at $P \geq 0.05$ using Duncan's multiple range test (DMRT)

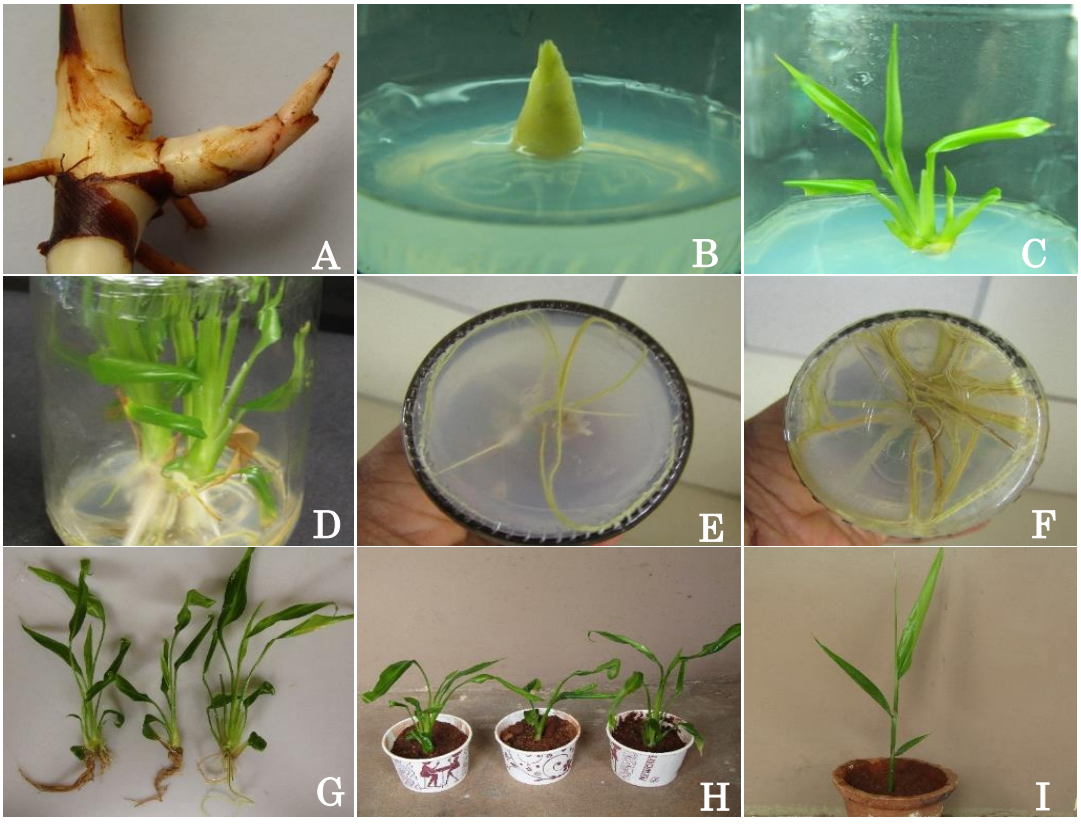


**Fig 1. Shoot multiplication and hardening of *A. galanga.* (A) Rhizome bud used as explant (B) Surface sterilized rhizome buds on MS medium (C) Plantlet formation on shoot induction medium (D) Maturation of plantlets (E&F) Root development in rooting medium (G) Plantlets excised for hardening (H) Primary hardening in paper cups (I) Secondary hardening in earthen pots**

**Rooting**

The highest rate of root initiation occurred 2-3 weeks after the inoculation on the rooting medium. The most efficient auxins for rooting are IAA, IBA and NAA [22]. IAA and IBA are common auxin for root initiation in Zingiberaceae family [23]. In this study, IBA (1.0 mg/l) gave the highest root development (94 %), optimum root length (11.66±0.88 cm) and maximum number of roots per plantlets (15.66±1.2) after 4-5 weeks of culture (Figure 1F) (Table 2). This is in accordance with the previous findings in ***C.caesia*** [24], *A.calcarata* [25] and *A.officinarum* [26].

**Table 2: Effect of auxins on *in vitro* rooting**

| IAA (mg/l) | IBA (mg/l) | Rooting response (%) | Average number of roots per explant | Mean length of roots (cm) |
|---|---|---|---|---|
| 0.0 | - | $13.0 \pm 1.52^{d}$ | $2.33\pm0.33^{c}$ | $4.66\pm0.88^{d}$ |
| 0.5 | - | $32.0\pm1.52^{c}$ | $4.0\pm0.57^{c}$ | $6.33\pm0.33^{cd}$ |
| 1.0 | - | $74.0\pm4.72^{b}$ | $9.66\pm0.88^{b}$ | $9.33\pm0.33^{b}$ |
| 1.5 | - | $76.0\pm2.0^{b}$ | $9.0\pm0.57^{b}$ | $8.0\pm0.57^{ab}$ |
| - | 0.5 | $74.66\pm2.02^{b}$ | $7.66\pm0.88^{b}$ | $6.66\pm0.33^{c}$ |
| - | 1.0 | $94.33\pm1.20^{a}$ | $15.66\pm1.2^{a}$ | $11.66\pm0.88^{a}$ |
| - | 1.5 | $80.0\pm2.64^{b}$ | $14.0\pm1.0^{a}$ | $11.33\pm0.66^{a}$ |

Values are expressed as the mean ± SE, taking ten explants in each experiment with three replications. All data were taken after 4 weeks of inoculation. Within each group, values with different letters indicate significant difference at $P \geq 0.05$ using Duncan's multiple range test (DMRT)

**Acclimatization**

The acclimatization process usually enhances the capacity of regenerated plantlets to withstand waterless condition and will also allow them to adopt survive in the same environmental conditions of its mother plant [27]. In the present study, number of plantlets with 4-6 leaves and roots transferred to plastic cups containing mixture of sand, soil and vermiculate (1:2:1)(Figure 1H). 85% survival was recorded after primary hardening. Survived plants were transferred for secondary hardening. After one month of growth, it was observed that the survival rate was 75% under shade condition. (Figure 1I)

**4. CONCLUSION**

In the present study, we have developed a simple and efficient regeneration method for propagation of *A.galanga*. This study examined the role of different cytokinin and auxin for the shoot and root induction from the rhizome bud explant. This protocol can be used as a prerequisite for mass propagation as well as the conservation of *A.galanga*

**CONFLICT OF INTEREST**

Author has no conflicts of interest

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