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*Full Length Research Paper*

# Micropropagation of *Etlingera elatior* (Zingiberaceae*)* by using axillary bud explants

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**Axillary bud explants of *Etingera elatior* were cultured on Murashige and Skoog (MS) basal medium, supplemented with various concentrations of cytokinin (BAP) or auxin (IAA) as plant growth regulators. The addition of 22.2 µM BAP to the MS medium produced the highest mean number of shoots (3.67) per explants as compared to other concentrations. The best shoots length (4.20 cm) was obtained from the medium containing 26.6 µM of BAP. For *in vitro* rooting of shoot, MS medium supplemented with 11.4 µM IAA produced the highest number of root, whereas 34.2 µM IAA gave the longest roots in the medium. The proliferated shoots were green and healthy in appearance. The Plantlets with well developed roots were hardened, acclimatized and planted out successfully with a survival rate of 75%.**

**Key words:** Etlingera elatior, axillary bud, medicinal plant, micro propagation, plant growth regulator, *Zingiberaceae*.

## INTRODUCTION

Torch ginger (*Etlingera elatior*), which is locally known in Malaysia as “kantan” is a species native to Sumatera, Indonesia and widely cultivated throughout the tropics. In Peninsular Malaysia, the plant is cultivated for its young flower shoots, which can be eaten raw and used to flavor local dishes (Abdelmageed et al., 2011). A decoction of the fruits has been used to treat earache and the leaves are used to clean wounds (Burkill, 1966; Ibrahim and Setyowati, 1999). In Australia and Costa Rica, it is cultivated for its inflorescences as cut flowers (Larsen et al., 1999). Leaves of *E. elatior*, mixed with other aromatic herbs in water, are used by post-partum women for bathing to remove body odour (Chan et al., 2009). The mature fruits of *E. elatior* are edible but sour, and are reputed to have antihypertensive activity (Habsah et al., 2005). The same authors reported that the species possessed antimicrobial, antioxidant as well as antitumour promoting activities. Moreover, Chan et al. (2008) reported that, of 26 ginger species, *Etlingera* species had the highest phenolic content and radical activity compared to other *Zingiberaceae* species. Further, Chan et al. (2007) stated that the leaves of *E. elatior* had the most outstanding antioxidant properties among five *Etlingera* species investigated.

*E. elatior* is normally propagated by its rhizome with low proliferation rate, and therefore the risk of spreading systemic infections is high (Mendez et al., 2004). In addition to that, *Zingiberaceae* plants are frequently infected with pathogens (Keng and Hing, 2004), such as rhizome rot caused by *Phytium* species and the leaf spot due to *Coleotrichum* species. These pose problems for the conservation and storage of germplasm of *Zingiberaceae* species, like *E. elatior*. To overcome these problems, it is necessary to produce disease-free seed rhizomes with rapid multiplication by using tissue culture technique. This study was conducted to determine the effect of different hormone concentrations on micropropagation of *E. elatior* and to develop a tissue

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**Abbreviations: BAP,** 6-benzylaminopurine; **IAA,** indole 3-acetic acid.

**Table 1.** Influence of different BAP concentrations on the mean number of shoots, number of leaves and shoots length per explant of *E. elatior* cultured on MS basal medium after 8 weeks of culture.

| BAP (µM) | Shoot number/explant | Shoot length/explant (cm) | Leaves number/shoot |
|---|---|---|---|
| 0.0 | 1.30 [b]* | 3.20 [b] | 3.00[a] |
| 4.4 | 2.33 [a, b] | 3.46 [a, b] | 3.00[a] |
| 8.9 | 2.00 [a, b] | 3.52 [a, b] | 3.07[a] |
| 13.3 | 1.33 [b] | 3.55 [a, b] | 3.07[a] |
| 17.8 | 1.67 [b] | 3.60 [a, b] | 3.13[a] |
| 22.2 | 3.67 [a] | 3.85 [a, b] | 3.40[a] |
| 26.6 | 2.33 [a, b] | 4.20 [a] | 3.40[a] |

* Means followed by the same letter(s) within each column are not significantly different at P ≤ 0.05, according to Duncan's multiple range test.

culture system for the mass production of this potentially promising species.

## MATERIALS AND METHODS

### Plant material

The axillary buds of *E. elatior* were collected from healthy plants grown at the Agricultural Conservatory Park, Universiti Putra Malaysia, Malaysia. The explants were washed thoroughly under running tap water to remove adhering soil particles. To control the microbial contamination, a composite approach of washing the explants first with 60% Clorox added with 6 to 7 drops of Tween 20 for 30 min was employed. Subsequently, the explants were thoroughly washed once with sterilized distilled water. Sterilized explants were then dissected to remove one outer layer of leaf sheath under aseptic conditions. Then, the excised explants were immersed again in 20% Clorox added with 6 to 7 drops of Tween 20 for another 15 min and thoroughly washed 7 times with sterilized distilled water. The sterilized rhizomes were then dissected again to remove the outer few layers of leaf sheaths.

### Basal medium and incubation conditions

All the media used in this study were based on Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) supplemented with 30 g/l sucrose. The medium was adjusted to pH 5.8 before solidifying with 4.5 g/l Gelrite and autoclaved at 121°C for 20 min. All cultures were kept under 16 h photoperiod at a photosynthetic flux of 150 µmol $m^{-2}$ $s^{-1}$, provided by cool fluorescent lamps and maintained at 25 ± 2°C. The explants were cultured on MS medium supplemented with BAP (0.0, 4.4, 8.9, 13.3, 17.8, 22.2, and 26.6 µM) for shoot regeneration and multiplication. Plant growth regulators combinations treatments were excluded. According to Balachandran et al. (1990), BAP alone was adequate for the shoot regeneration and multiplication of *Curcuma domestica*, *C. aeruginosa* and *Z. officinale.* In addition, Prakash and Staden (2007) found that the supplementation of MS medium with different concentrations of BA enhanced the morphogenic potential of explants and favoured the proliferation of healthier shoots of *Hoslundia opposita*. For rooting of *in vitro* regenerated shoots, the shoots were sub cultured on MS medium supplemented with different concentrations (0.0, 5.7, 11.4, 17.1, 22.8, 28.5, 34.2 µM) of IAA. Plantlets with well developed roots were removed from the medium, then washed thoroughly under running tap water to remove adhering solid MS medium, and transplanted to plastic pots containing sterilized peat moss soil and kept in a 50% shaded net house. The plants were covered by a transparent perforated polyethylene bags in order to maintain high humidity and to avoid plant dehydration by water loss. The plants were frequently watered to keep high level of humidity. The polyethylene bags were then removed after 7 days and the plantlets were acclimatized for another 3 weeks. Then, the percentage of survival rate was taken.

### Data collection

After 8 weeks of culture, the following parameters were recorded; number of shoot, number of leaves, number of roots, shoots and root length and survival rate was calculated.

### Statistical analysis

Collected data was analyzed using the SPSS statistical package software version 10.0 (Chicago, USA). Analysis of variance (ANOVA) with mean separation by Duncan's multiple range test ($P < 0.05$) was used to compare means of different treatments. For each treatment, 28 replicates were used and each experiment was repeated three times.

## RESULTS AND DISCUSSION

Different concentrations (0.0, 4.4, 8.9, 13.3, 17.8, 22.2, and 26.6 µM) of BAP were used to determine their influence on the shoot proliferation. All the concentrations of BAP used were able to produce multiple healthy shoots. Generally, an increase in the BAP concentration led to an increase in the number and length of shoots. Of the different concentrations of BAP used for shoot multiplication, the best response was obtained in the MS basal medium supplemented with 22.2 µM. Up to 3.67 shoots per explants were produced, but not significantly different from 4.4, 8.9 and 26.6 µM concentrations (Table 1). The best shoot length was observed on the medium containing 26.6 µM of BAP. These results imply that BAP as a plant growth regulator played an important role in shoot multiplication (Table 1). Numerous adventitious shoots were observed near the basal portion of the shoot cluster, when sub cultured (Figure 1C). In *E. elatior,* application of BAP 22 µM + IAA 5 µM was found to be the best treatment for multiplication of shoots (Colombo et al., 2010). Similarly, Rescarolli and Zaffari (2009) observed that the highest number of shoots were

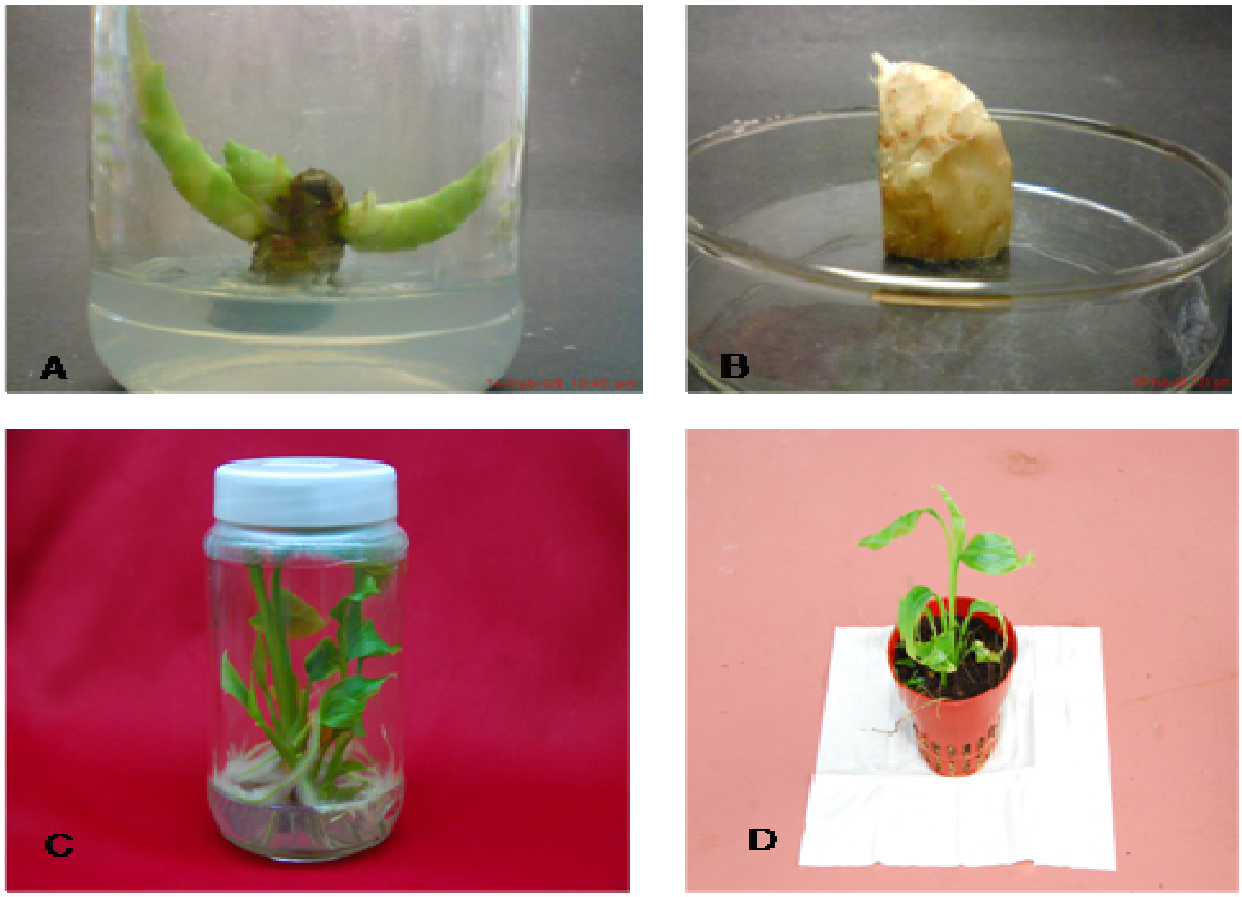


**Figure 1.** Stages of *in vitro* multiplication of *Etlingera elatior.* (a) Axillary bud used as explants; (b) Induction of multiple shoots from axillary buds on MS medium supplemented with 22.2 µM BAP after 8 weeks; (c) Regenerated plants with shoots and roots after 8 weeks of subculture; (d) Established plant following transplantation of regenerated plantlets into plastic pots containing sterilized peat moss under nethouse conditions.

obtained with the use of BAP 4.44 µM, which indicates the importance of BAP growth regulator in the multiplication of *E. elatior*. This result is in accordance with that reported by Singh (1988) who reported that shoot production of *Z. officinale* was stimulated by increasing the concentration of BAP to 22.2 µM and Nayak (2000) and Sharma and Singh (1995) who reported that shoot multiplication and plant regeneration were achieved by using explants of *Curcuma aromatica* and *Z. officinale* from media supplemented with 22.2 µM and 35.5 µM of BAP, respectively.

Further, in another study conducted on *Curcuma spp.* and *Z. officinale*, addition of 13.32 µM BAP to the MS medium, was found to be optimum for all the species for producing highest number of shoots per explant, which explained that addition of BAP to the medium not only enhanced the multiplication rate, but also favoured the proliferation of healthier shoots (Balachandran et al., 1990). This observation is also in agreement with the recent results of Rajani and Patil (2009) who mentioned that MS medium supplemented with 8.88 µM BAP gave the best response for shoot multiplication of *Z. officinale* and Jagadev et al. (2008) who cultured axillary buds of *Z. officinale* on MS medium supplemented with 13.32 µM BAP. While, Keng and Hing (2004) observed that MS medium supplemented with 8.88 µM of BAP, gave the highest mean number of multiple shoots per explant of *Z. officinale*. In the same trend, Vincet et al. (1992) reported that the highest number of shoots per explant were obtained when cultured axillary buds of *K. galanga* on MS medium supplemented with 2.2 µM of BAP and 13.9 µM Kinetin after 120 days of incubation. The average number of leaves per explants obtained at the different concentrations of BAP were similar, indicating an insignificant effect of BAP on leaf growth and multiplication. In this study, BAP played an important role and had a significant effect on both average number of shoots per explant and shoot length (Table 1). Further, Stanley and Keng (2007) reported that MS medium supplemented with 26.64 µM BAP induced the formation of multiple shoots of Z. zerumbet. The proliferated shoots were green and healthy in appearance (Figure 1C).

**Table 2.** Influence of different IAA concentrations on the number of roots and root length per shoot of *E. elatior* cultured on MS basal medium after 8 weeks of culture.

| IAA (µM) | Root number/explant | Root length (cm) |
|---|---|---|
| 0.0 | 1.00 [b]* | 3.00 [c] |
| 5.7 | 0.67 [b] | 3.00 [c] |
| 11.4 | 3.00 [a] | 3.00 [c] |
| 17.1 | 1.00 [b] | 3.44 [b, c] |
| 22.8 | 1.33 [b] | 3.59 [a, b] |
| 28.5 | 1.33 [b] | 3.67 [a, b] |
| 34.2 | 1.67 [b] | 4.00 [a] |

*Means followed by the same letter(s) within each column are not significantly different at P ≤ 0.05, according to Duncan's multiple range test.

In this study, the IAA hormone was found to have a positive effect on root induction. According to Sharma (2006), IAA and IBA are usually used for easier-to-root herbaceous plants and NAA for more recalcitrant woody plants. The efficacy of different auxins also depend on the explants type and exposure to light.

MS medium supplemented with 11.4 µM of IAA produced significantly highest mean number of roots per explant (Table 2). The longest roots were produced with the addition of 34.2 µM of IAA to the medium. IAA promoted the growth of excised root sections and intact roots, but only at relatively low concentration range. Higher concentration of auxin, in the range that normally stimulates elongation of shoots, causes a significant inhibition of root growth (Hopkins and Huner, 2004). Bejoy et al. (2006) found that the shoot multiplication and root systems were obtained on MS medium supplemented with 4.4 µM of BAP and 2.9 µM of IAA. This result strengthens the present study, which indicated the importance of IAA hormone in the induction of roots. According to Anish et al. (2008) MS medium supplemented with IAA at 2.85 µM in combination with BAP seems to be optimum for rooting of *Boesenbergia pulcherrima*.

Based on these observations, the addition of BAP showed a positive effect on shoot regeneration. The highest number of shoot growth was found in MS medium supplemented with 22.2 µM BAP. MS medium supplemented with 11.4 µM IAA produced the highest number of roots from regenerated *E. elatior* shoots.

Acclimatization of plantlets can be considered as one of the most important phase in tissue culture techniques. In the present investigation, the mortality rate of the plantlets with well developed roots that were acclimatized and hardened was low. On average, 75% of *in vitro* transferred plantlets survived in potted soil and did not show any morphological abnormalities (Figure 1D). In conclusion, the micropropagation system described in this study could form the basis to develop protocols for conservation and large scale production of *E. elatior*, a promising medicinal species by using the axillary bud as explant.

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