IN VITRO SHOOT REGENERATION FROM RHIZOME BUD OF NATIVE GINGER IN BORNEO, *ETLINGERA COCCINEA*

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ABSTRACT

A reproducible protocol for *in vitro* shoot regeneration was established for edible native ginger species of Borneo, *Etlingera coccinea* using rhizome bud. Intact rhizome buds in 1.0±0.5 cm length were cultured on Murashige and Skoog medium supplemented with 0.1 to 2.0 µM Thidiazuron(TDZ), 6-benzyalaminopurine (BAP) and Kinetin. Among the cytokinins tested 1.0 µM TDZ gave the best response with 100% of explants producing 23.0±0.43 adventitious shoots per bud after 20 weeks of culture following which explant size and sectioning methods were also investigated by excising the sprout buds into various sizes (~1.0-5.0 cm) and sectioned into half or three slices. As compared to other explant size tested, the explant size of ~1.0 ± 0.5cm long was favorable for 100% shoot regeneration and producing 20.0 ± 1.38 shoot per explant after 20 weeks of culture. However, sectioning of explant did not have beneficial effect on shoot regeneration. Sliced explant produced lower shoot regeneration percentage and lower number of shoots, and took up to 13 weeks for regeneration to occur as compared to 9 weeks for intact bud. The regenerated shoots were then transferred to shoot elongation medium containing 2.5 to 20 µM BAP. Regenerated shoots developed well in MS medium supplemented with 5.0 µM BAP by promoting an average of 5.5 ± 0.25 proliferated shoots per explant, 3.0 ± 0.61 cm shoot length, and 6.5 ± 0.35 leaves per explant. Root initiation from the shoot cluster (~1.5 ± 0.5cm long) started a week after being transferred from the elongation medium, and the best rooting was obtained on MS fortified with 1.0 µM indole-3-butyric acid (IBA) (90 ± 2.24% response, with 3.5 ± 0.08 cm root length). Thus, the results obtained in the study suggests a possible regeneration protocol to be exploited for commercial cultivation or large-scale production of *E. coccinea*.

Key words: *Etlingera sp.*, micropropagation, shoot regeneration, rhizome bud explants

INTRODUCTION

Etlingera coccinea is a species native to Borneo, belonging to Zingiberaceae and locally known as *'Tuhau'*. The shades of pink, red bracts and flowers make the plants a very attractive species. *E. coccinea* is a herb which can grow 3 to 4 meters tall and has a long fibrous rhizome around 30 to 60 cm long. The plant is cultivated for its young leafy shoots. The leafy shoots with stout rhizome produce stingy smell when it is crushed. In Sabah, Malaysia, *E. coccinea* is used by indigenous ethnics especially *Kadazan-Dusun* to flavor local dishes. Nowadays, *E. coccinea*-based products such as *'sambal tuhau'* (paste), *'jeruk tuhau'* (pickles) and '*serunding tuhau'* (floss) have been commercially produced for local market. The *Etlingera* species is also reported to possess antimicrobial and antioxidant properties, and contain high phenolics as compared to other species in Zingiberaceae (Chiang et al. 2010; Chan et al. 2007). Major components of essential oils in other *Etlingera* species has also been reported (Tachai et al. 2014).

Propagation of *E. coccinea* in nature is through rhizomes or seeds. However, both systems are slow in growth and population spread is limited (Mendez et al. 2004) to accommodate demand for commercial production. Both systems are also faced with high risk in disease infection common to Zingiberaceae such as rhizome rot caused by *Phytium* and leaf spot caused by *Coleotrichum* (Abdelmageed et al. 2011; Keng & Hing 2004). In addition, *E. coccinea* plants are very poor in

flowering and seed set posing problems for species conservation and sustainability. Therefore, it is necessary to develop an efficient *in vitro* propagation system to produce plantlets for farming. The advantages of tissue culture are production of disease-free planting materials in mass, homogenous in growth and consistently produced throughout the year especially for commercial cultivation. Tissue culture protocols and *in vitro* study for others *Etlingera* species have been reported by few researchers such as for *in vitro* multiplication (da Silva Júnior 2015), embryogenic callus induction (Gomes-Dias et al. 2014), *in vitro* mutagenesis (Yunus et al. 2013), *in vitro* propagation (Yunus et al. 2012), micropropagation using axillary buds (Abdelmageed et al. 2011) and shoot tip culture (Mendez et al. 2004). However, there is no report for *E. coccinea* tissue culture. Successful tissue culture depends on various factors such as media, explants preparation and plant growth regulators. In most, those factors are species dependent. Therefore, the present study was conducted to investigate the effect of rhizome bud explant and plant growth regulators on shoots regeneration for *E. coccinea*.

MATERIALS AND METHODS

Plant material and explant preparation

Rhizome of *E. coccinea* which consisted of sprout buds were collected from Kg. Kemburongoh, Ranau, Sabah, Malaysia. All experiments were conducted in Plant Tissue Culture Laboratory, Faculty of Science and Natural Resources, Universiti Malaysia Sabah. For explant preparation, the sprout buds were excised from the rhizome and thoroughly washed under running tap water for 1 hour. These buds were sterilized in the laminar air flow chamber with an aqueous solution of 100% (v/v) Clorox® (5.25% (v/v) Sodium hypochlorite) and 2 drops of Tween-20 for 20 min followed by three rinses with sterilized distilled water to remove traces of chlorine. Under the aseptic condition, the outer scales of the buds were trimmed to remove dead tissues affected by chlorine from the sprout with a sterilized scalpel blade. Single bud consisting of a shoot tip with a small portion of the rhizome was used for culture initiation.

Media preparation

The basal media consisted of full strength MS medium (Murashige & Skoog 1962) supplemented with 3% (w/v) of sucrose (Sigma, USA) and solidified with 0.8% (w/v) of agar powder Phytagel (Sigma, USA). The pH of the media was adjusted to 5.8 prior to autoclaving at 121 ºC and 15 psi for 20 min. The medium was allowed to cool and 25 ml medium poured into each sterile 90 x 15 mm culture dish (GQ, China).

Effect of cytokinins on shoot regeneration and proliferation

Different concentrations (0.1, 0.5, 1.0, 1.5 and 2.0 µM) of TDZ (thidiazuron) (Sigma, USA), BAP (6-benzylaminopurine) (Sigma, USA) and kinetin (6-furfurylamino purine) (Sigma, USA) were tested on MS medium to study the effect of cytokinins on shoot regeneration from rhizome bud explant. Hormone-free medium served as a control. The sprout buds with approximately 1.0 ± 0.5 cm in size were used as explants. Explants were then cultured vertically on shoot regeneration medium. The regeneration percentage, number of shoots per explant, and time to shoot production from the explants were observed weekly and recorded up to 20 weeks of culture. In this experiment, data collected included shoot regeneration percentage, number of shoots per explant, shoot length and time taken to produce shoot. Changes were observed weekly up to 20 weeks of culture.

Effect of explants size and explants sectioning on shoot regeneration

Three different sizes (~1.0±0.5cm, ~3.0±0.5cm and ~5.0±0.5cm in length) of sprout buds were aseptically excised from the rhizome and cultured vertically on the optimal medium for shoot regeneration (MS+1.0 µM TDZ). After the best explant size were selected (1.0±0.5cm), the effect of explant vertical sectioning were investigated by preparing the sprout buds into three different

sectioning which were intact bud, bud cut into half slices and bud cut into three equal slices. All explants were cultured on MS medium containing 1.0 µM TDZ with 3% (w/v) of sucrose (Sigma, USA) and solidified with 0.8% (w/v) of agar powder (Sigma, USA). In this experiment, data collected included shoot regeneration percentage, number of shoots per explant and time to shoot production. Data were observed weekly up to 20 weeks of culture

Effect of BAP concentration on shoot elongation

Explant with multiple shoots from all treatment approximately 0.7 ± 0.5cm in length were transferred to fresh MS basal medium treated with various concentrations (2.5, 5.0, 10, 15 and 20 µM) of BAP to promote shoot elongation and leaves formation. The medium was prepared in conical flasks (Schott Duran, 100ml) with 30ml medium in each flask. Flask mouth was covered with two layers of aluminum foil. Data such as number of shoot, shoot length and number of leaves per explants were observed weekly and recorded after 6 weeks of culture.

Effect of IBA and NAA concentrations on rooting of shoots

Shoots (>1.8 cm) obtained from shoot elongation medium were transferred on MS (Murashige and Skoog 1962) basal medium supplemented with 0.5, 1.0, 1.5, 2.0 and 2.5 µM of IBA (indole-3-butyricacid) or IAA (indole-3-acetic acid) to test their efficiency on root formation. The medium was prepared in conical flask (Schott Duran, 250 ml) with 30 ml medium in each flask. Flask mouth was covered with two layers of aluminum foil. Rooting percentage, number of roots per explant, root length, time for all explants produces root and root appearance were observed weekly and recorder after six weeks of culture.

Culture conditions and statistical analysis

All cultures were maintained in a plant growth chamber (SANYO, MLR-350H) at 25 ± 2ºC with 16h-photoperiod provided by 36 µmol m^2s^{-1} cool light fluorescent tube (SANYO, Japan). Experiments were carried out in completely randomized design (CRD) with 5 replicates and 5 explants in each dish resulting in total of 25 explants per treatment. Data were subjected to Analysis of Variance (ANOVA) and the mean values were compared using Duncan's Multiple range test (DMRT) at significant value of $p<0.05$ SPSS (Statistical Package for Social Science) Ver. 12.

RESULTS AND DISCUSSION

Effect of cytokinins on shoot regeneration and proliferation

Results in Table 1 showed the effect of various cytokinin concentrations on shoot regeneration of *E. coccinea* rhizome buds after 20 weeks of culture. The first response observed at 2 to 4 weeks of culture on medium containing cytokinins was enlargement or swelling of the tissue with color changes from creamy white to green (Fig.1D-F). Sprouting of rhizome buds followed by shoot regeneration was observed at 9 to 15 weeks of culture (Fig. 1G-J). It is noted that the presence of exogenous cytokinins is important for shoot regeneration in *E. coccinea* since no shoot regeneration was observed from rhizome bud in the absence of cytokinins. Addition of TDZ at relatively lower concentration played a significant role in promoting adventitious shoot regeneration from rhizome bud. Explants cultured on the medium supplemented with 1.0 and 1.5 µM TDZ successfully resulted in 100% of explants producing 23.0 ± 0.43 and 21.0 ± 0.61 adventitious shoots per explant, respectively.

Shoot regeneration medium supplemented with 1.0, 1.5 and 2.0 µM BAP, and 1.5 µM Kinetin also produced 100% shoot regeneration, but with less number of shoots (7.0 - 8.0 shoots per explant). Furthermore, duration for shoot induction was also delayed up to 10-14 weeks as compared to 9 weeks in medium containing TDZ . Several studies have also reported on the superior effect of TDZ to other cytokinins for shoot regeneration in the Zingiberaceae family (Zhang et al. 2011; Lo-

apirukkul et al. 2012; Verma & Bansal 2014). This behavior is believed to be due to its capability of fulfilling both the cytokinin and auxin requirement of various regenerative responses (Jones et al. 2007) and the ability of TDZ to increase the biosynthesis of endogenous adenine-type cytokinins (Huettman & Preece 1993; Hare & Van Staden 1994). It was also reported that TDZ promotes the activities of peroxidase (POD), a multifunctional enzyme that involved in growth regulation, development and organogenesis (Mamaghani et al. 2010).

The presence of TDZ at higher concentration (2.0 µM) significantly reduced shoot regeneration percentage, number of shoots per explant and shoot length. Although the shoot regeneration percentage and number of shoots were higher at 1.0 and 1.5 µM TDZ, the shoots were shorter and could not be elongated even after prolonged culture.The shoots appeared stunted and showed distortion of leaf formation. BAP or kinetin treatments promote better performance for shoot elongation as compared to TDZ treatments, thus BAP was added into the shoot elongation medium. The longest shoot was obtained at 1.5 µM BAP which reached up to 2.0 cm at 20 weeks of culture. The inhibitory effects of high TDZ concentrations on shoot elongation have been reported previously (Kumar et al. 2010; Kumar et al. 2011) and results obtained in the present study are in agreement with those findings. According to Hare and Van Staden (1994), shoot inhibition occurs because of high cytokinin activity of TDZ and its capacity to inhibit the action of cytokinin oxidase, the only known plant enzyme acting in the inactivation of naturally occurring cytokinins , which in turn may increase the level of endogenous cytokinin.The formation of short-length or miniature shoots on TDZ supplemented medium was also in agreement with Hamirah et al. (2010), Giri and Tamta, (2011), and Verma and Bansal (2014) for *Hedychium spicatum, Zingiber montanum* Koenig, and *Hedychium coronarium*, respectively. In other findings, BAP was found to be beneficial for shoot induction and multiplication of other Zingiberaceae family, *Aframomum corrorima* (Hagos and Geebrhemdhin 2014), *Curcuma xanthorrhiza* Roxb.and *Z. aromaticum* Val (Melati et al. 2014).

Table 1. Effect of BAP, TDZ and kinetin concentrations on shoot regeneration of *Etlingera coccinea* rhizome buds after 20 weeks of culture on MS basal medium supplemented with 3% (w/v) of sucrose, 0.8% (w/v) agar, pH 5.8 and grown at 25 ± 2ºC with 16 h photoperiod.

TDZ (µM)	BAP (µM)	KIN (µM)	Shoot regeneration percentage	Mean number of shoots per explant	Mean shoot length (cm)	Time taken to produce shoots (week)
control			0	0	0	-
0.1			85.0 ± 0.71^{f}	13.0 ± 0.25^{e}	0.5 ± 0.01^{i}	9
0.5			97.0 ± 0.71^{c}	18.9 ± 0.19^{c}	0.7 ± 0.01^{gh}	9
1.0			100 ± 0.00^{a}	23.0 ± 0.43^{a}	0.8 ± 0.02^{fg}	9
1.5			100 ± 0.00^{a}	21.0 ± 0.61^{b}	0.6 ± 0.02^{hi}	9
2.0			98.0 ± 0.35^{b}	18.0 ± 0.41^{d}	0.5 ± 0.01^{i}	9
	0.1		80.0 ± 0.79^{g}	5.3 ± 0.22^{j}	1.3 ± 0.11^{d}	11
	0.5		95.0 ± 0.28^{d}	6.5 ± 0.14^{h}	1.5 ± 0.14^{c}	11
	1.0		100 ± 0.00^{a}	7.0 ± 0.16^{g}	1.8 ± 0.08^{b}	10
	1.5		100 ± 0.00^{a}	8.1 ± 0.16^{f}	2.0 ± 0.23^{a}	10
	2.0		100 ± 0.00^{a}	8.0 ± 0.07^{f}	1.8 ± 0.08^{b}	12
		0.1	79.0 ± 0.35^{h}	4.9 ± 0.29^{k}	0.9 ± 0.04^{ef}	13
		0.5	85.0 ± 0.79^{f}	5.5 ± 0.22^{j}	1.0 ± 0.07^{e}	10
		1.0	91.0 ± 0.71^{e}	6.0 ± 0.07^{i}	1.0 ± 0.14^{e}	10
		1.5	100 ± 0.00^{a}	7.2 ± 0.07^{g}	1.2 ± 0.14^{d}	12
		2.0	79.0 ± 0.35^{h}	6.5 ± 0.16^{h}	1.3 ± 0.04^{d}	14

Mean in each column followed by the same letters did not differ significantly at $p < 0.05$ according to Duncan Multiple Range Test (DMRT).

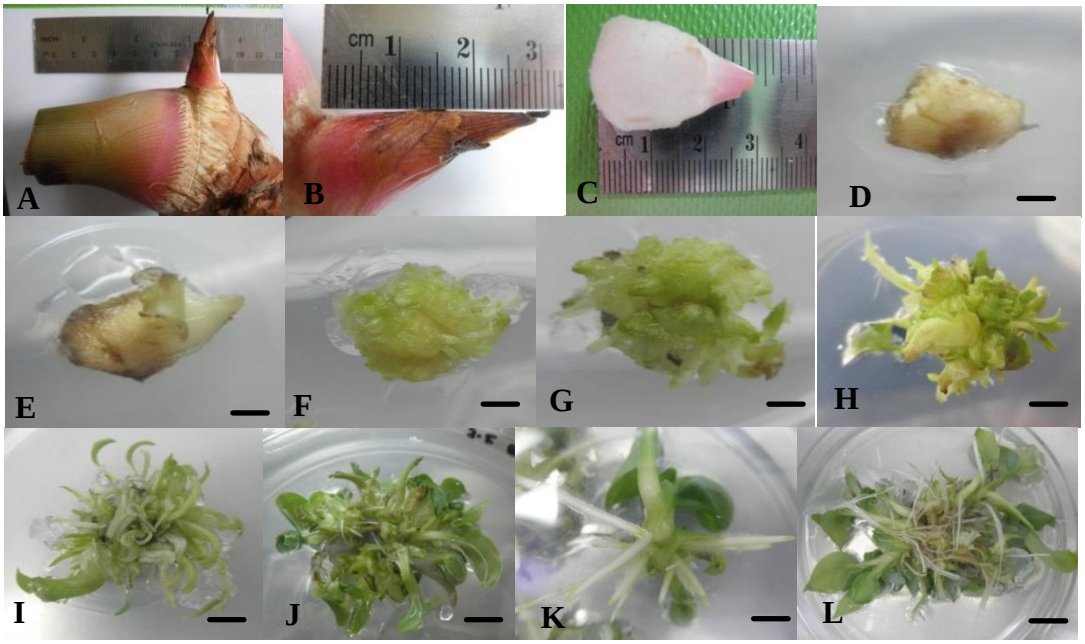

Figure 1. Micropropagation of *in vitro Etlingera coccinea*. (A-C) Rhizome bud explant as plant material; (D-F) Swelling of tissue and initiation of shoot within 2 to 9 weeks of culture in medium supplemented with TDZ (G-I) Shoot development during 10 to 15 weeks of culture; (J) Shoot elongation in MS medium supplemented with BAP (K-L) Rooting of shoot after transfer to MS medium supplemented with IBA. Bar=3mm

Effect of explants size on shoot regeneration

The effect of explant size on shoot regeneration of *E. coccinea* rhizome buds after 20 weeks is shown in Table 2. The explant size of ~1.0 ± 0.5cm is significantly different (p<0.05) in shoot regeneration response (100%) as compared to ~3±0.5 cm and ~5.0±0.5 cm which gave 58% and 43% shoot regeneration, respectively. The explants of ~1.0±0.5 cm also produced higher number of adventitious shoots (20.0±1.58). This result is consistent with other Zingiberaceae family including *Z. officinale*, *C. comosa* and *H. coronarium* for shoot regeneration (Sathyagowri & Seran 2011; Lo-apirukkul et al. 2012; Mohanty et al. 2013). Moreover, the ~1.0±0.5 cm explants survived well due to very low rate of contamination as compared to other treatments (~3.0±0.5 cm and ~5.0±0.5 cm) which showed an endogenous microbial contamination that lead to reduction of shoot regeneration percentage. This may be due to high surface contacts of explant with pathogens during their growth in nature that lead to more microbial activities (Sathyagowri & Seran 2011).

It is also generally accepted that *in vitro* plant is influenced by the developmental age of the tissues or organs that are used as sources of explants (George et al. 2008). In this experiment, ~1.0 ± 0.5 cm long cultured explants produced shoot at 10 weeks of culture. Time to initiate shoot regeneration delayed up to 13 and 15 weeks for ~3.0 ± 0.5 cm and ~5.0 ± 0.5 cm explants, respectively. It was suggested that explants size is related to the age of the explant tissue in terms of its degree of differentiation. Increased size of rhizome bud explants is described as an increase in the plant age. Hence, morphogenesis of older explants reduced its regenerative capacity as compared to juvenile (Pierik 1987). This means, younger and smaller buds of the rhizomes are more likely to grow out than larger proximal basal buds due to the paradormancy (Horvath 2010).

Table 2. Effect of different explants size of *Etlingera coccinea* rhizome buds on shoot regeneration after 20 weeks cultured on MS basal medium supplemented with 1.0µM TDZ, 3% (w/v) of sucrose, 0.8% (w/v) agar, pH 5.8 and grown at 25 ± 2ºC with 16 h photoperiod.

Explant size	Shoot regeneration percentage	Mean number of shoots per explant	Mean shoot length (cm)	Time taken to produce shoots (week)
Control (~1 ± 0.5 cm)	100±0.00[a]	20.0±1.38[a]	1.0±0.25[a]	10
(~3 ± 0.5 cm)	58.0±0.38[b]	9.5±0.07[b]	0.7±0.16[b]	13
(~5 ± 0.5 cm)	43.0±0.51[b]	7.8±0.03[b]	0.5±0.04[b]	15

Mean in each column followed by the same letters did not differ significantly at $p < 0.05$ according to Duncan Multiple Range Test (DMRT).

Effect of explant sectioning on shoot regeneration

Effect of explant sectioning on shoot regeneration is shown in Table 3. Sectioning of rhizome bud into 2 or 3 slices did not have beneficial effect on shoot regeneration because intact rhizome bud had a greater response. Shoot regeneration was maximum in intact bud (100%) and produced 18.0±0.85 shoots per explant after 9 weeks of culture. This result is in agreement with other species such as *Curcuma comosa* (Lo-apirukkul et al. 2012) and *Curcuma caesia* Roxb (Behar et al. 2014). In the present study, it was demonstrated that degree of explant wounding can reduced development capacity. Sectioning of rhizome bud into half and 3 slices induced poor shoot regeneration percentage (48-56%) with lower number of shoots (4.2-6.7 shoots per explant). Moreover, duration for rhizome bud explants producing shoots were extended to 11 and 13 weeks in decreased size (sliced) of explants. This could be due to lack of available nutrient reserves in smaller rhizome fragmented for shoot regeneration therefore more time is required to obtain nutrients from the medium to initiate the shoot bud (Sathyagowri & Seran, 2011). This was strenghthened by Smith (2000) who explained that the explants size has an effect on the response of the tissue. Generally, the smaller the explant, the harder is to culture therefore the culture medium usually have additional required component. Sectioning of explants can also injure the active cell for division in the explant. While, the faster morphogenic response of the larger intact bud explants could be due to the high amount of available nutrient reserves, higher contain of endogenous plant growth regulators and more surface area in contact to surface medium, hence absorbing nutrients more easily (Smith 2000; Sathyagowri & Seran 2011). In contrast, Hamirah et al. (2010) was found that sectioning the bud explant into half is able to double shoots production when compared to intact bud in red ginger (*Zingiber montanum*).

Table 3. Effect of different explant sectioning of *Etlingera coccinea* rhizome buds explant on shoot regeneration after 20 weeks cultured on MS basal medium supplemented with 1.0 µM TDZ, 3% (w/v) of sucrose, 0.8% (w/v) agar, pH 5.8 and grown at 25 ± 2ºC with 16 h photoperiod

Number of sections	Shoot regeneration percentage (%)	Mean number of shoots per explant	Mean shoot length (cm)	Time taken for all explants to produce shoots (week)
Intact bud	100±0.00[a]	18.0±0.85[a]	1.2±0.15[a]	9
2 slices	56.0±2.24[b]	6.7±0.2[b]	0.8±0.08[b]	11
3 slices	48.0±0.38[c]	4.2±0.1[c]	0.5±0.01[c]	13

Mean in each column followed by the same letters did not differ significantly at $p<0.05$ according to Duncan Multiple Range Test (DMRT).

Effect of BAP concentrations on shoot elongation

Shoot growth and elongation were directly affected by BAP concentration (Table 4). This results indicates that lower BAP concentration (2.5-5 µM) favors shoot elongation and leaf development of *E.coccinea* adventitious shoots as compared to the higher BAP-fortified media. The culture medium supplemented with 5.0 µM BAP has significantly promoted shoots multiplication (5.5± 0.25 shoots per explant), shoot elongation (3.0±0.61), and number of leaves (6.5±0.35). The ability of BAP in enhancing shoot elongation and multiplication was reported for a number of Zingiberaceae species such as *Etlingera elatior* (Yunus et al. 2012), *Curcuma longa* (Ugochukwu et al. 2013) and. *Hedychium coronarium* (Verma & Bansal 2014). BAP is commonly used in stimulating the growth of axillary and adventitious buds and foliar development of shoot tips cultures (Buah et al. 2010). For some species, a low level of BAP (below 10 µM) has been reported to be optimal for shoot elongation (Prakash et al. 2004; Bharalee et al. 2005; Giri & Tamta 2011).

While, for other Zingiberaceae species, high concentrations of BAP have been used to promote shoot elongation and proliferation (Raihana et al. 2011; Yunus et al. 2012; Ugochukwu et al. 2013; Melati et al. 2014). However, in this study, further increase in BAP concentration to 10, 15 or 20 µM gradually decreased the number of shoots, shoot length and number of leaves per explant. BAP at 20 µM induced shorter shoots (1.8 ± 0.16 cm) and 2.5 ± 0.15 shoots per explant. Thus, it was suggested that BAP at low concentration was sufficient for further development of induced shoot. The inhibitory effect of BAP at high concentration on shoot elongation of *E.coccinea* is in line with previous report for turmeric (Naz et al. 2009) and reduce shoot lengths of others Zingiberaceae species such as *Curcuma amada, Curcuma valeton* and *Curcuma varmana,* (Das et al. 2010; Zhang et al. 2011; Bejoy et al. 2012).

Table 4. Effect of BAP concentrations on elongation and multiplication of shoots after 6 weeks of culture on MS basal medium supplemented with 3% (w/v) sucrose, 0.8% (w/v) agar, pH 5.8 and grown at 25 ± 2ºC with 16 h photoperiod.

BAP (µM)	Number of shoots per explant	Mean shoot length (cm)	Number of leaves per shoot
Control	1.5±0.07^{f}	0.7±0.08^{e}	1.0±0.22^{f}
2.5 µM	4.0±0.10^{b}	2.0±0.01^{cd}	3.5±0.08^{d}
5.0 µM	5.5±0.25^{a}	3.0±0.61^{a}	6.5±0.35^{a}
10 µM	3.8±0.01^{c}	2.5±0.07^{b}	5.0±0.41^{b}
15 µM	3.0±0.16^{d}	2.3±0.16^{bc}	4.5±0.09^{c}
20 µM	2.5±0.15^{e}	1.8±0.16^{d}	3.0±0.63^{e}

Mean in each column followed by the same letters did not differ significantly at $p < 0.05$ according to Duncan Multiple Range Test (DMRT).

Effect of IBA and NAA on rooting of shoots

Results in Table 5 shows that roots were successfully induced from shoot grown on medium containing IBA or NAA, while no root was induced in control treatment. The highest rooting percentage (90.0±2.24 %), number of roots (7.5±0.03) and length of roots (3.5±0.08 cm) were obtained in 1.0 µM IBA. Further increase of IBA concentrations significantly reduced rooting percentage (57.0±1.58), number of roots (3.0±0.08) and roots length (2.0±0.08). The results also indicates that IBA treatments performed better than NAA. Treatment with 1.0 µM NAA induced 73±2.55% rooting, 2.3±0.03 roots and 1.5±0.01 cm roots length. In addition, IBA promotes rapid rooting (1-2 weeks) as compared to NAA (3-4 weeks). Roots obtained in IBA treatments are longer, thicker, white and with heavy root hairs (Figure 1K) whereas in NAA treatment, root induced was white, shorter, less root hairs and few explants induced white callus especially at higher concentration.

The results are in line with those obtained by Kambaska and Santilata (2009) who reported that rooting is hardly induced in media without auxins, although some plant species such as *Zingiber petiolatum* (Prathanturarug et al. 2004) and *Zingiber montamum* (Hamirah et al. 2010) are capable of inducing roots in hormone-free medium. Loc et al. (2005) reported that IBA and NAA at different levels has significantly affected the number and length of roots on *Curcuma zedoaria* plantlets. In many Zingiberaceae species, IBA was the most favorable root inducer compare to NAA or other auxins (Giri & Tamta 2011; Asha et al. 2012; Bimal et al. 2014). In contrast, addition of either IAA or NAA in the medium was optimum for root initiation in *Etlingera elatior* (Abdelmageed et al. 2011), *Zingiber officinale* (Abbas et al. 2011), *Curcuma aromatica* (Sharmin et al. 2013) and *Hedychium coronarium* (Verma & Bansal 2014). Such a difference in root induction response can be explained by the complex interactions between the endogenous and exogenous levels of hormones during *in vitro* culture (Ditengou et al. 2008). It was also reported that both IAA and IBA are usually used for easier-to-root herbaceous plants and NAA is more for recalcitrant woody plants (Sharma 2006).

Table 5. Effect of IBA and NAA concentrations on rooting of shoots after 6 weeks cultured on MS basal medium supplemented with 3% (w/v) of sucrose, 0.8% (w/v) agar, pH 5.8 and grown at 25 ± 2ºC with 16 h photoperiod.

PGRs (µM)		Morphogenetic responses				
IBA	NAA	Rooting percentage	Mean number of roots/explant	Mean root length (cm)	Time for all explants to produce roots (weeks)	Root appearance (texture/color)
Control	0	0	0	-	-	-
0.5		78.0±0.45^{c}	3.5±0.04^{d}	1.7±0.01^{e}	2	Short thick, white, hairy root
1.0		90.0±2.24^{a}	7.5±0.03^{a}	3.5±0.08^{a}	1	Long thick, white, hairy root
1.5		85.0±0.79^{b}	5.0±0.35^{b}	3.0±0.16^{b}	1	Long thick, white, hairy root
2.0		64.0±1.46^{e}	4.0±0.47^{c}	2.5±0.04^{c}	2	Long thin, white, hairy root
2.5		57.0±1.58^{h}	3.0±0.08^{e}	2.0±0.08^{d}	2	Long thin, white, hairy root
	0.5	62.0±0.79^{ef}	2.0±0.13^{g}	1.0±0.02^{g}	3	Short thin, white, hairy root
	1.0	73.0±2.55^{d}	2.3±0.03^{f}	1.5±0.01^{f}	3	Short thick, white, hairy root
	1.5	58.0±3.53^{gh}	3.0±0.25^{e}	1.0±0.18^{g}	3	Short thin, white, hairy root
	2.0	60.0±0.38^{fg}	3.5±0.06^{d}	0.7±0.03^{h}	4	Short thick, white, hairy root
	2.5	41.0±1.92^{i}	1.8±0.07^{g}	0.5±0.04^{i}	4	Short thin, white, hairy root

Mean in each column followed by the same letters did not differ significantly at $p < 0.05$ according to Duncan Multiple Range Test (DMRT).

CONCLUSION

Shoot regeneration capacity of *E. coccinea* greatly influenced by types and concentrations of cytokinins, explant size and explant sectioning. TDZ was found to be suitable for shoot regeneration compared to BAP and Kinetin. *In vitro* shoot regeneration of *E. coccinea* was possible through rhizome bud culture on MS medium supplemented with 1.0 µM TDZ and further developed in medium supplemented with 5.0 µM BAP. Fully established plantlet was successfully produced by transferring shoot into MS media containing 1.0 µM IBA for rooting. The *in vitro* culture protocol described in this study offer a powerful and potential tool for large-scale plantlets production of this commercially important edible plant species.

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