TRANSACTIONS ON SCIENCE AND TECHNOLOGY

In Vitro Propagation of *Zingiber officinale* Rosc. 'Tambunan'

Devina David[1]*, Teoh Yan Ji[1] & Jualang Azlan Gansau[2]
[1]Faculty of Sustainable Agriculture, Universiti Malaysia Sabah,
Locked Bag No.3, 90509 Sandakan, MALAYSIA.
[2]Faculty of Science and Natural Resources, Universiti Malaysia Sabah,
Jalan UMS, 88400 Kota Kinabalu, MALAYSIA
*Co-responding author. E-Mail: ddevina@ums.edu.my; Tel: +6089-248100; Fax: +6089-220703

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Abstract
Rhizome buds of ginger (*Zingiber officinale* Rosc. 'Tambunan') were sterilized and cultured on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of NAA and BAP hormones (1-3 mg/L) to induce shoot multiplication and rooting formation. Shoot formation was first observed on treatment of 3.0 mg/L BAP + 1.0 mg/L NAA after 7 days of culture. This treatment also promote the highest number of proliferated shoots, 6.14 ± 0.91 shootlets per explant, with an average shoot length of 1.69 ± 0.17 cm observed after 10 weeks of culture. Rooting of ginger plantlets were significantly initiated on medium supplemented with 2.0 mg/L NAA. This treatment induced up to 34.40 ± 1.81 roots per explant with an average length of 4.52 ± 0.20 cm after 10 weeks of culture. Plantlets were successfully acclimatized in pot containing medium mixture of sand and clay (1:4) with 64% of survivality after transplanted for 3 weeks.

Introduction

Ginger (*Zingiber officinale* Roscoe) is belongs to the family of Zingiberaceae. This plant is an important tropical horticultural plant, valued throughout the countries as an important spice for its nutritional and medicinal properties. Ginger is vegetatively propagated through underground rhizomes and mainly cultivated in many countries including India, China, Japan, Indonesia, Australia, Nigeria and West Indies (Ravindran and Nirmal, 2005). In Malaysia, about 30-40 species of Zingiberaceae have long been used as traditional medicine as it was reported to have active biological compounds with antioxidant, antimicrobial, anti-inflammatory and anti-tumor properties (Rahmani *et al.*, 2014). As in Sabah, a large number of species from the ginger family has been cultivated for use as food, medicine and ornamentals (Kulip, 2007). *Zingiber officinale* Rosc.'Tambunan' which is mainly cultivated in Tambunan and Keningau Sabah was among the popular cultivar of ginger in Malaysia. However, the main problem in ginger cultivation is high infestation by pest and disease such as, *Phyllosticta* leaf spot, storage rots and bacterial wilt or rhizome rot that caused by soilborne bacteria *Ralstonia (Pseudomonas) solanacearum* (Nirmal Babu *et al.*, 2005). This problem has led to the degradation of ginger supply in Sabah. The application of plant tissue culture technique to produce a free disease plant has long been practised for many crops. For ginger, several studies had successfully demonstrated the application of tissue culture technique on this plant (Kambaska and Santilata, 2009;

Abdelmageed *et al*., 2011; Mohamed *et al*., 2011). Therefore the current study was conducted to establish an efficient method for mass propagation of *Zingiber officinale* Rosc. 'Tambunan' through *in vitro* culture of ginger rhizome bud. This study will demonstrated the effects of single and combination of NAA and BAP hormones on *in vitro* shoot multiplication, rooting and regeneration of this plant.

Methodology

Plant material and explant preparation

Zingiber officinale Rosc.'Tambunan' were collected from Tambunan, Sabah and brought to Plant Tissue Culture Laboratory at Faculty of Sustainable Agriculture, Sandakan. To induce bud sprouting, ginger was incubated at 27±2°C for two weeks (Thingbaijam *et al.*, 2014). Buds rhizomes about 0.5-1.0 cm were cut and used as explant (Kavyashree, 2009). The explants were cleaned with 2% (v/v) Clorox and washed thoroughly under running tap water for 20 minutes to remove adhering soil particles. For bud sterilization, explants were washed with 70% (v/v) ethanol for 2 minutes and later immersed in 50% (v/v) Clorox with 2 drops of Tween 20 for another 15 minutes and finally washed for 7 times with sterilized distilled water (Mohamed *et al*., 2011). The sterilized axillary bud was then dissected to remove the outer few layers of leaf sheaths (Abdelmageed *et al.*, 2011).

Effect of NAA and BAP hormones on in vitro propagation

Explants were cultured on medium supplemented with 6-Benzylaminopurin (BAP) (Sigma) at 1, 2 and 3 mg/L or naphthalene acetic acid (NAA) (Sigma) 1, 2 and 3 mg/L in singly and combination treatments and medium devoid of any hormone served as control.

Culture medium and incubation conditions

Murashige and Skoog (Murashige and Skoog, 1962) (MS) was used as basal medium supplemented with 30 g/l sucrose. The medium was adjusted to pH 5.8 before solidifying with 10 g/l of agar and sterilized at 121°C for 20 min. All cultures were kept under 16h photoperiod, provided by cool fluorescent lamps and maintained at 25 ± 2°C.

Acclimatization

Plantlets with well-developed roots was removed from the medium, then washed thoroughly under running tap water to remove adhering solid medium (Abdelmageed *et al*., 2011; Das *et al*., 2012). Plantlets were then transplanted to plastic pots containing sterilized sand and clay at ratio of 1:4. Plantlets were maintained in a semi shaded net house with high humidity (RH 80%) at 28±2°C for hardening (Das *et al*., 2012).

Experimental design and statistical analysis

The experimental design used was Completely Randomized Design with ten replicates for each treatment. The collected data was analyzed using the SAS version 9.4. All data were analyzed by one-

way Analysis of variance (ANOVA), and the difference between means are tested by using Duncan's multiple range test at $p < 0.05$.

Results and discussion

Effect of NAA and BAP on shoot multiplication

The *in vitro* growth and development of *Zingiber officinale* Rosc. 'Tambunan' rhizome buds were depends greatly on the types and concentrations of plant growth regulators applied in the medium. Data obtained after 10 weeks of culture shows that treatment with BAP induced better shoot multiplication respond as compared to NAA, with the number of shoots produced ranged from 3.33±0.49 to 4.00±0.41 and number of leaves ranged from two to four per shoot (Table 1). Treatment of 2 mg/L also had significantly promoted up to 4.70 ± 0.25 leaves per shoot. Several studies have demonstrated the effectiveness of 2 mg/L BAP supplemented in culture medium on shoot multiplication of *Z. officinale* (Keng and Hing, 2004; Rajani and Patil, 2009; Abbas *et al*., 2012). Beneficial effect of BAP treated along with NAA has promoted to the improvement of shoot multiplication in explants. In this study the combination of 3 mg/L BAP and 1 mg/L NAA in culture medium had significantly induced 6.14±0.91 shoots with an average of 1.77±0.14 leaves per shoot and 1.69±0.17 cm shoot length. It was also observed that the first emergence of shoot bud from explant recorded at day 7 which was earlier compared to other treatments (Figure 1b). Several studies have supported that a combination of BAP and NAA at specific concentration were able to increase the shoot proliferation of *Z. officinale* (Nirmal Babu *et al.*, 2005; Kambaska and Santilata, 2009; Thiangbaijam *et al.*, 2013).

Table 1. Effects of different concentrations and combinations of NAA and BAP on shoot multiplication of rhizome bud of *Zingiber officinale* Rosc. 'Tambunan' after 10 weeks of culture.

Treatment		Days to shoot initiation	Percentage of explant producing shoot (%)	Number of shoot per explant (mean ± SE)	Number of leaves per shoot (mean ± SE)	Shoot length in cm (mean ± SE)
BAP (mg/L)	NAA (mg/L)					
0	0	13	60	1.17 ± 0.17^{de}	0.30 ± 0.18^{c}	0.34 ± 0.02^{d}
1	0	13	70	3.71 ± 0.75^{b}	2.19 ± 0.37^{b}	0.99 ± 0.09^{bcd}
2	0	13	40	4.00 ± 0.41^{b}	2.50 ± 0.35^{b}	1.19 ± 0.06^{bc}
3	0	13	60	3.33 ± 0.49^{bc}	4.70 ± 0.25^{a}	0.68 ± 0.06^{cd}
0	1	13	70	1.43 ± 0.30^{cde}	2.30 ± 0.26^{b}	1.46 ± 0.51^{abc}
0	2	13	50	1.20 ± 0.37^{de}	2.83 ± 0.31^{b}	2.15 ± 0.19^{a}
0	3	13	40	0.75 ± 0.25^{e}	0.33 ± 0.33^{c}	0.30 ± 0.06^{d}
1	1	10	80	4.13 ± 0.79^{b}	1.85 ± 0.15^{b}	1.23 ± 0.12^{bc}
2	1	10	80	3.00 ± 0.65^{bcd}	1.71 ± 0.24^{b}	1.42 ± 0.13^{abc}
3	1	7	70	6.14 ± 0.91^{a}	1.77 ± 0.14^{b}	1.69 ± 0.17^{ab}

* 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

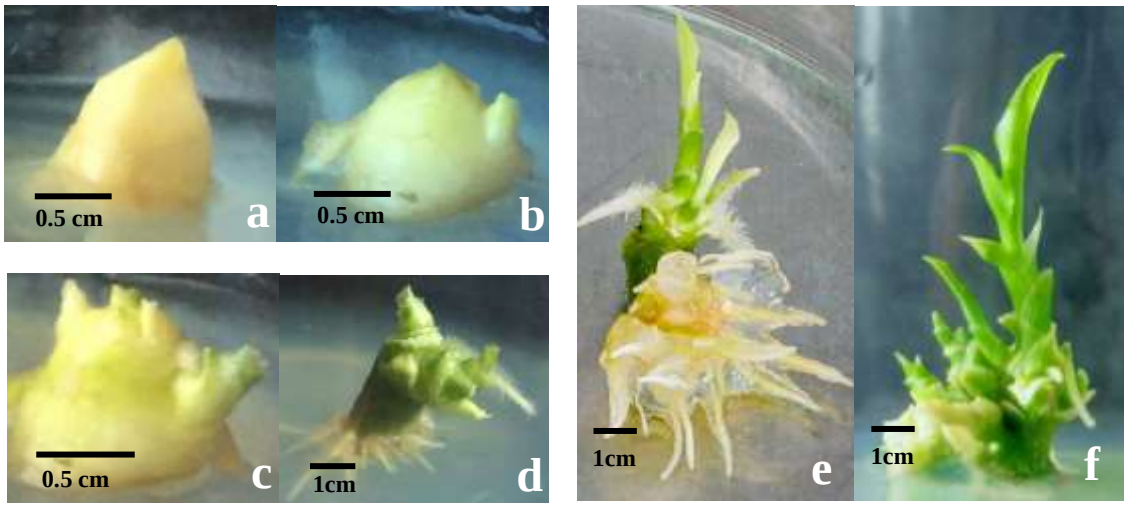

Figure 1. *In vitro* micropropagation of *Zingiber officinale* Rosc. 'Tambunan' through rhizome bud culture. (a) Axillary bud explant on a semi-solid culture; (b) Initiation of shoot bud after the first week on MS fortified with 3 mg/L BAP + 1 mg/L NAA.; (c) Formation of multiple shoots after two weeks on MS fortified with 3 mg/L BAP + 1 mg/L NAA.; (d) Formation of root after two weeks on MS fortified with 2 mg/L BAP; (e) Elongation of shoots and roots after four weeks on MS fortified with 2 mg/L BAP (f) Elongation of shoot and leaves formation at week 10 on MS fortified with 3 mg/L BAP + 1 mg/L NAA.

Effect of NAA and BAP on root formation

The influences of NAA and BAP at different concentrations have important roles in promoting well developed roots. In this study, the highest number of rooting was recorded on medium treated with 2mg/L NAA, followed by 3mg/L BAP and 1mg/L BAP (Table 2). The addition of 2mg/L NAA in medium had significantly induced 34.40±1.81 roots with an average root length of 4.52±0.20 cm. It was also observed that the root initiation started after two weeks in this treatment (Figure 1d). The current finding was in line with Kambaska and Santilata (2009), that the application of 2 mg/L NAA was suitable for *in vitro* root induction of *Zingiber officinale* Rosc. cv Suprava and Suruchi. However, the application of 3 mg/L NAA has promoted to poor rooting formation. According to Abdelmageed *et al*. (2011), auxin promoted the growth of intact roots and excised root sections but only at a relatively low concentration range. If high concentration of auxin applied, it will suppress morphogenesis in cultured plants (Smith, 2013). The current finding was also supported by Kambaska and Santilata (2009) that addition of 3 mg/L NAA reduced rooting in *Zingiber officinale* Rosc. cv Suprava and Suruchi.

Acclimatization

Hardened plantlets of *Zingiber officinale* Rosc. 'Tambunan' were found to grow healthily with 64% of survival rate after three weeks of potting with mixture of sand and clay (1: 4). Newly formed shoot was observed after two weeks of transplanting (Figure 2b). Previously, Das *et al*. (2012) reported plantlets that planted in poly-bags containing sand and clay at the ratio of 1:4 found to grow healthily

with 90% of survival rate. Other previous studies that reported to have high survival rate of *Zingiberaceae in vitro* propagated plantlets were Tyagi *et al.* (2006) and Ayenew *et al.* (2012).

Table 2. Effects of different concentrations and combinations of NAA and BAP on root induction of rhizome bud explant of *Zingiber officinale* Rosc. 'Tambunan' after 10 weeks of culture

Treatment		Percentage of explant producing root (%)	No. of root (mean ± SE)	Root Length (mean ± SE) (cm)
BAP (mg/L)	NAA (mg/L)			
0	0	60	4.50 ± 0.99^{e}	0.78 ± 0.09^{e}
1	0	70	13.71 ± 0.87^{c}	2.27 ± 0.08^{bcd}
2	0	40	9.00 ± 1.08^{d}	1.51 ± 0.23^{de}
3	0	60	12.17 ± 2.76^{cd}	1.50 ± 0.10^{de}
0	1	70	17.86 ± 0.77^{b}	4.42 ± 0.19^{a}
0	2	50	34.40 ± 1.81^{a}	4.52 ± 0.20^{a}
0	3	40	3.50 ± 0.50^{e}	0.71 ± 0.05^{e}
1	1	80	4.87 ± 0.55^{e}	1.91 ± 0.13^{cd}
2	1	80	4.38 ± 0.73^{e}	2.66 ± 0.13^{bc}
3	1	70	12.71 ± 1.25^{c}	2.84 ± 0.28^{b}

* 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

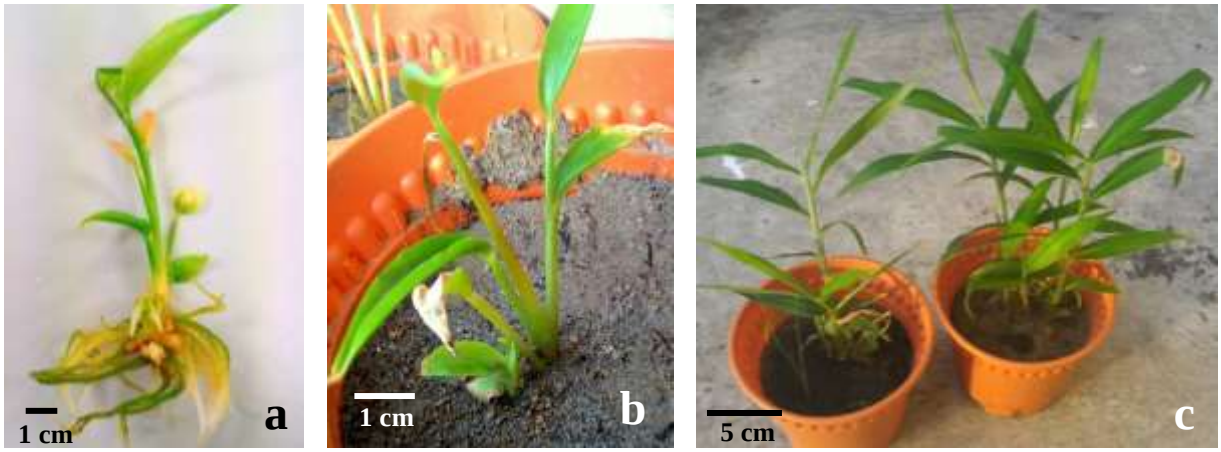

Figure 2. Acclimatization of micropropagated *Zingiber. officinale* Rosc. 'Tambunan' plantlets. (a) *In vitro* culture of actively growing plantlets with profuse roots; (b) 2 weeks after transplanting; (c) 12 weeks after transplanting

Conclusion

This study reports the protocol for *in vitro* propagation of *Zingiber officinale* Rosc. 'Tambunan'. Shoot multiplication from rhizome bud was best initiated on MS medium supplemented with a combination of 3mg/L BAP + 1mg/L NAA. Meanwhile, the application of 2mg/L NAA in culture medium was recommended for plantlet rooting before being acclimatized successfully. This technique is useful for rapid clonal propagation of healthy ginger plants for commercial production.

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