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OPTIMIZING SUCROSE AND BAP CONCENTRATIONS FOR *IN VITRO* MICRORHIZOME INDUCTION OF *Zingiber officinale* Rosc. 'Tambunan'

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ABSTRACT

In vitro microrhizome induction is considered as an effective tool for high yielding rhizomatous crops. In this study, the effect of sucrose and BAP was examined to establish a suitable protocol for *in vitro* microrhizome production of *Zingiber officinale* Rosc. 'Tambunan'. The *in vitro* derived plantlets were used as explants and cultured on Murashige and Skoog (MS) medium treated with a combination of sucrose and BAP at various concentrations and maintained at 25 ± 2°C with 16 hr of photoperiod. After three months of culture, explant responded well on MS medium supplemented with 60 g/L of sucrose and 6 mg/L of BAP compared to other treatments. This treatment had significantly promoted the highest number of microrhizomes (seven) with a total weight of 2.90 g and a total number of 35 buds. Acclimatization of this microrhizome showed 88% of survivability rate after 21 days with a formation of new shoot and root. The current finding revealed the potential of microrhizomes for large-scale production of healthy planting material to support the ginger industry in this region.

Key words: 6-Benzyladenine (BAP), ginger, microrhizome, sucrose, Zingiberaceae

INTRODUCTION

Ginger, also botanically known as *Zingiber officinale* Roscoe, is one of the world most important and value plant that consumed as a delicacy, medicine and spices. In Malaysia, ginger is cultivated commercially in Pahang, Johor, Selangor, Sabah and Sarawak (Suhaimi *et al*., 2012). *Zingiber officinale* Rosc. 'Tambunan' is a popular cultivar of ginger in Sabah and it is mainly cultivated in Tambunan and Keningau areas. Locally known as Tambunan ginger, it was believed that this cultivar was originally from Bentong, Pahang. However, application of biotechnology approaches such as metabolic fingerprinting (Mahdi *et al*., 2010) and microsatellite DNA (Mahdi *et al*., 2013) able to differentiate between Malaysian ginger cultivars including Bukit Tinggi, Tanjung Sepat and Sabah cultivars. *Zingiber officinale* Rosc. 'Tambunan' has gained its popularity for its flavor and aroma, thus contribute to high market demand. However, the planting areas and production of gingers in Sabah declined from 467 hectares (5,848.4 tonnes) in 2006 to only 197 hectares (1,341.2 tonnes) in 2016 (Department of Agriculture Sabah 2006; 2016) and it was due to attack of soil-borne bacterial wilt disease that caused by *Ralstonia solanacearum* (Cosmas *et al*., 2016). This incident had caused a significant decrease in ginger yield as well as the production of ginger rhizomes as planting materials.

Microrhizome is a miniature rhizome induced via *in vitro* condition and possesses similar characteristics as normal rhizome in anatomical features such as starch grains, well-developed oil cells and fibers (Maiti & Yepthomi, 2015). The technique of *in vitro* microrhizome induction has been identified as a solution to supply high quality planting materials especially for rhizomatous crops (Archana *et al*., 2013; Nayak & Naik, 2006). Besides, it has advantages of commercial value where it is easier to transport, store and can be used in germplasm conservation (Chirangini & Sharma, 2005). The induction of *in vitro* microrhizome of many Zingiberaceae species was reported to be influenced by several factors such as photoperiod (Abbas *et al*., 2014; Archana *et al*., 2015; Swarnathilaka *et al*., 2016), plant growth regulators

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(Rout *et al*., 2001; Archana *et al*., 2013), nutrient composition (Zheng *et al*., 2008) and culture vessel (Singh *et al*., 2014). Previously, a protocol for *in vitro* mass propagation of *Z. officinale* Rosc. 'Tambunan' was successfully established using rhizome bud as explant (David *et al*., 2016). Therefore this study was conducted to determine and optimize the sucrose and 6-Benzyladenine (BAP) concentrations for *in vitro* microrhizome production of this ginger cultivar.

MATERIALS AND METHODS

Plant material

Four months old of *in vitro* derived plantlets of *Zingiber officinale* Rosc. 'Tambunan' from the previous study (David *et al*., 2016) were selected and used as explants. Each explant was excised into 4 cm height and the root was trimmed into a shorter length. The excised explants were initially cultured in a culture jar containing 30 mL of MS (Murashige & Skoog, 1962) medium without supplementing any growth regulator for two weeks to minimize carryover effect of growth regulators in previous treatments.

Culture treatment

Explants were then transferred to culture jar containing 30 mL of MS medium supplemented with combinations of sucrose and BAP at various concentrations. Three levels of sucrose concentrations (30, 60 or 90 g/L) and two concentrations of BAP (6 or 9 mg/L) were tested in this study with a total of six combination treatments. Medium without any addition of sucrose and BAP was served as control. The pH of the culture media was adjusted to 5.7 before solidifying with 10 g/L of agar and sterilized at 121°C for 20 min. All cultures were maintained at the temperature of 25 ± 2°C with 16 hr photoperiod.

Data collection and statistical analysis

All cultures were observed periodically for 12 weeks. Each treatment consisted of ten replicates of one explant each, arranged in Completely Randomized Design. Data were collected for two main parameters (i) vegetative growth of explant which include the number of leaves, roots, shoot and shoot length (cm); (ii) microrhizome induction which includes a number of microrhizome, fresh weight of microrhizome (g), number of buds and number of buds per microrhizome. Data were analyzed using one-way analysis of variance (ANOVA) through IBM SPSS Statistic Version 25.0 New York, IBM Corp. The significant difference between the means was separated by Tukey's Honestly Significant Different (HSD) test at $p < 0.05$.

Acclimatization

A total of 20 *in vitro* derived microrhizomes were selected for establishment in the nursery to investigate their ability to acclimatize in *ex vitro* condition. For this purpose, microrhizomes were treated with 0.5% (w/v) of fungicide for 20 min before planting in pots containing coco peat. The microrhizomes were initially covered with a polythene sheet for two weeks to maintain high humidity and irrigation was done through misting once a day with tap water. Growth performance of microrhizome was observed and the survival rate was recorded.

RESULTS AND DISCUSSION

Effect of BAP and sucrose on vegetative growth of explant

The vegetative growth of ginger explant was observed and shown in Figure 1. It was observed that after one week of culture, whitish buds were initiated from the explant (Figure 1A) and later changed its color to pale green before developing into shoots within 15 days of culture. The shoots continued to lengthen and then started to form leaf sheaths. The leaf sheaths grew into flat leafy structure after six weeks of culture and the well-developed ginger plantlets with leaves and roots were obtained within 12 weeks of culture (Figure 1B).

Supplementation of sucrose and BAP in MS medium had significantly influenced all vegetative growth parameters of *Z. officinale* Rosc. 'Tambunan' explants. Combination treatments including 30 g/L sucrose + 6 mg/L BAP; 30 g/L sucrose + 9 mg/L BAP and 60g/L sucrose + 6 mg/L BAP significantly produced the maximum number of leaves (28 - 41) after three months of culture (Figure 2A). Treatments of 30 g/L sucrose + 6 mg/L BAP and 60 g/L sucrose + 6mg/L BAP also induced 55 and 53 of new roots, respectively (Figure 2B). The combinations of sucrose at 30 and 60 g/L with 6 and 9 mg/L of BAP enhanced the production of a new shoot, however, increased in sucrose concentration (90 g/L), decreased shoot multiplication as well as shoot length of the explants (Figure 2C and 2D). This event was observed similar in leaves and root production and this finding was in agreement with a study by Kusumastuti *et al*. (2014) that 30 g/L of sucrose was beneficial in promoting shoots and roots multiplication of *Z. aromaticum* as compared to higher sucrose concentration (60 and 90 g/L). Sucrose is the most common carbon source and acts as an energy source to maintain osmotic potential as well as ensuring optimal development during *in vitro* growth of plants (Yaseen *et al*., 2013).

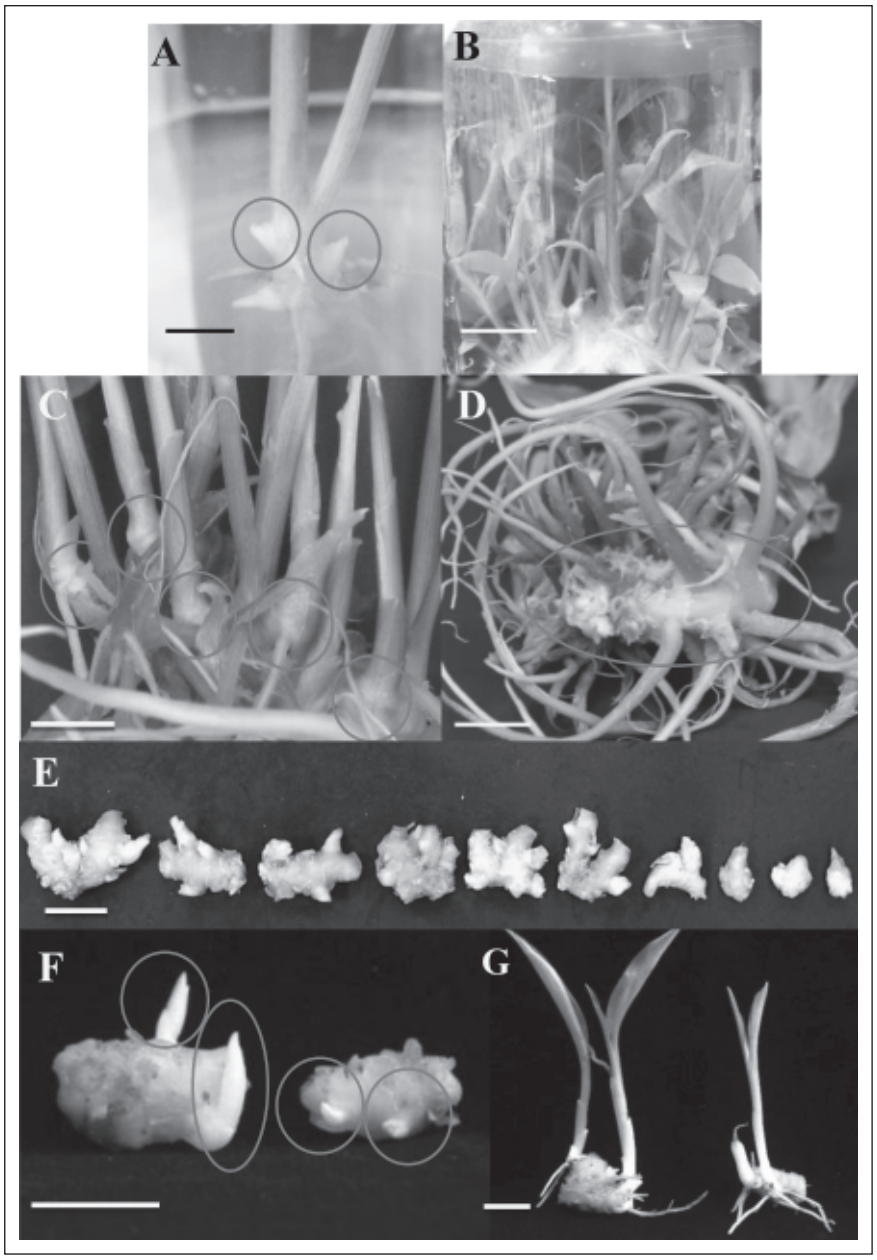

Fig. 1. *In vitro* growth and development of microrhizomes in *Zingiber officinale* Rosc. 'Tambunan'. (A) Initiation of whitish bud after one week of culture (B) Well developed plantets with leaves and roots after 12 weeks of culture (C) Bulging of pseudostem indicate microrhizome induction (D) Swollen of basal roots from the microrhizome (E) Microrhizomes produced in treatment of 60 g/L sucrose and 6 mg/L BAP (F) Buds emergence from acclimatized microrhizome (G) Acclimatized microrhizomes with shoots and roots after 14 days of transplant. Bar (A) 0.5 cm; (B - G) 1.0 cm.

Effect of BAP and sucrose on *in vitro* induction of microrhizome

The initial indication of microrhizome induction was observed when the pseudostem and basal root of ginger plantlets were swollen (Figure 1C and 1D). In this study, the combination of sucrose with BAP in culture medium had significantly influenced *in vitro* microrhizome formation in this ginger. Explants cultured on MS medium treated with 60 g/L of sucrose and 6 mg/L of BAP had significantly produced an average of seven microrhizomes with a total weight of 2.90 g and a total number of 35 buds after 12 weeks of culture (Figure 2E, 2F and 2G). The microrhizome formed through this treatment was characterized as a yellow-orange mini rhizome (Figure 1E), and produced a fresh ginger smell when crushed. The current finding was in agreement with Maiti and Yepthomi (2015), that microrhizome possesses the same characteristics as the normal ginger rhizome.

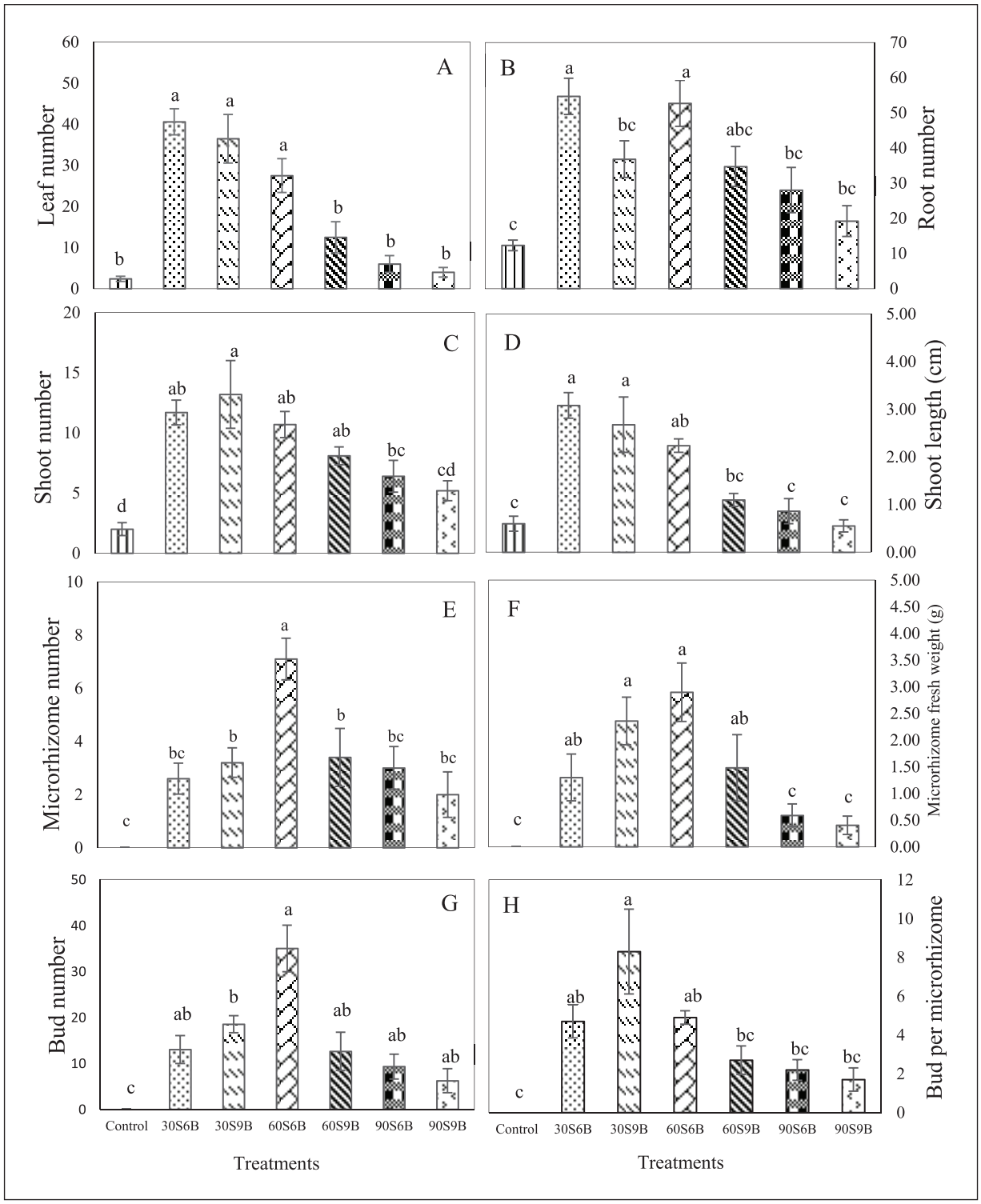

Fig. 2. Effect of sucrose and BAP at various concentrations on *in vitro* growth and development of microrhizome on *Z. officinale* Rosc. 'Tambunan'. (A) Leaf number; (B) Root number; (C) Shoot number; (D) Shoot length; (E) Microrhizome number; (F) Microrhizome fresh weight; (G) Bud number; (H) Bud per microrhizome. Notes: Value are expressed as the mean of ten replicates ± S.E. Mean values followed by different letters are significantly different at p <0.05. S: Sucrose (g/L); B: BAP (mg/L).

In this study, 60 g/L of sucrose was favorable in promoting microrhizome production might be attributed to high carbon energy in sucrose since rhizomes mainly contain carbohydrate and sucrose (Nayak & Naik, 2006). Several studies had demonstrated the effectiveness of sucrose between 60–90 g/L on *in vitro* induction of ginger microrhizome (Rout *et al*., 2001; Zheng *et al*., 2008; Abbas *et al*., 2014; Singh *et al*., 2014). However, the application of 90 g/L of sucrose in the current study showed a detrimental effect. Higher sucrose content might induce osmotic stress in this medium and resulted to slow absorption of nutrient as described previously in Kusumastuti *et al*. (2014).

Acclimatization of microrhizome

A total of 20 harvested microrhizomes were transplanted to a pot containing cocopeat as medium and maintained under a high level of relative humidity. After seven days of transplant, the emergence of buds was observed from the microrhizomes (Figure 1F) and later it developed into the shoot. The appearance of root was observed later after 14 days of transplant (Figure 1G). New shoot continues to grow from the microrhizome and the survival rate was recorded up to 88.30% after 30 days of transplanting. A study by Archana *et al*. (2013) showed that ginger microrhizomes transferred out to a nursery survived at 90–100% of survival rate.

CONCLUSION

This study has successfully established an efficient protocol for *in vitro* microrhizome induction of *Z. officinale* Rosc. 'Tambunan'. This approach could be an alternative to solve the issue in lacking ginger rhizome as planting material for cultivation as well as for consumption. The current finding revealed the highest number of microrhizome formation was observed on treatment of MS medium supplemented with 60 g/L of sucrose and 6 mg/L of BAP. Further study is recommended to examine other factors such as photoperiod, culture media and types of plant growth regulators that might affect the *in vitro* microrhizome induction for this Tambunan ginger cultivar.

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