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Pengaruh Suplemen Organik Terhadap Regenerasi Tunas Pisang Barangan *Musa acuminata* Colla. Secara *In Vitro*

Effect of Organic Growth Supplements on In Vitro Shoot Regeneration of Banana cv. Barangan *Musa acuminata* Colla.

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INTISARI

Pisang merupakan salah satu tanaman buah terpenting di dunia. Kultur jaringan tumbuhan memberikan beberapa keuntungan, diantaranya laju multiplikasi yang tinggi, keseragaman fisiologis, dan eliminasi penyakit. Penelitian ini bertujuan untuk mengetahui kemampuan suplemen organik dalam mendukung regenerasi tunas pisang barangan *Musa acuminata* Colla. Propagasi *in vitro* dilakukan dengan menggunakan eksplan bonggol pisang yang dikulturkan pada media Murashige and Skoog (MS) dengan suplemen organik diantaranya air kelapa dengan konsentrasi 5; 10; 15 dan 20%, ekstrak kecambah kacang hijau dan ekstrak jagung manis pada konsentrasi 50; 100; 150 dan 200 g/L. penambahan suplemen organik pada media menunjukkan hasil yang lebih baik pada semua parameter yang diamati dibandingkan dengan kontrol. Penelitian ini menunjukkan potensi suplemen organik dalam mendukung propagasi pisang barangan *Musa acuminata* Colla. secara *in vitro*. Diantara suplemen organik yang diuji, MS + 200 g/L ekstrak kecambah kacang hijau terbukti memberikan hasil terbaik terhadap multiplikasi tunas (7.11 ± 0.19), panjang tunas (2.00 ± 0.22), dan jumlah daun (4.00 ± 0.38).

Kata kunci : barangan, air kelapa, ekstrak kecambah kacang hijau, ekstrak jagung manis, kultur jaringan

ABSTRACT

Banana is one of the world's most important fruit crop. Plant tissue culture provided excellent advantages, including a high multiplication rate, physiological uniformity, and disease elimination. The research purpose to find out the ability of organic growth supplements in supporting the shoot regeneration of banana cv. barangan *Musa acuminata* Colla. In vitro propagation method has been conducted in banana plants using the sword sucker explants cultured on Murashige and Skoog (MS) medium with organic growth supplements such as coconut water (CW) 5; 10; 15 and 20%, green bean sprout extract (GBSE) and sweet corn extract (SCE) at 50; 100; 150 and 200 g/L. The addition of organic growth supplements to the medium showed better results on all parameters of observed than control. These results indicated the potential of organic growth supplements for supporting of banana cv. barangan *Musa acuminata* Colla. propagation in vitro culture. Among these tested organic growth

supplements, MS + 200 g/L bean sprout extract proved beneficial for multiplication shoots (7.11 ± 0.19), shoot length (2.00 ± 0.22), and number of leaves (4.00 ± 0.38).
Keyword**:** barangan, coconut water, green bean sprout extract, sweet corn extract, tissue culture

INTRODUCTION

Bananas are large perennial herb (*Musa* spp.) belong to the monocotyledonous family Musaceae. Banana is an important and widely grown fruit crop in the tropical and subtropical regions of the world, both as a staple food as well as a major export commodity (Darvari *et al.,* 2010; Rahman *et al.,* 2013; Qamar *et al.,* 2015). Banana is one of the horticultural crops that is important for improving the nutrition of the community. As a source of vitamins, minerals and carbohydrates, banana are easily digested (Hapsari and Lestari, 2016). Banana cv. barangan cultivar *Musa acuminata* Colla. have a high commercial value and opportunity to be developed. It has excellent nutrient content and rich in minerals such as potassium, magnesium, phosphorus, iron, and calcium, contains vitamin C, B complex, B6 and serotonin that are active as neurotransmitters in brain function (Sunyoto, 2011).

Banana is conventionally propagated by sword sucker. Although propagation by sucker retains all characters of the parent but virus diseases can be transmitted from infected parent through suckers or from infested soil around banana plant roots. Regeneration through *in vitro* culture provided alternate method to conventional one to produce diseases free planting material within a short time of period (Suman, 2017).

The success of plant tissue culture is highly influenced by the growth regulators and nutrition supplied in the media. Growth regulator is one of the most important component for large scale plant micropropagation (Shi, 2014). For cost effective and the quest to improve the *in vitro* techniques research efforts have focused on the growth regulator compounds that there is a good scope toward substituting the expensive chemical nutrient media by low cost natural extracts. Various kinds of organic supplements from natural sources have been used in plant tissue culture to promote the growth of the plants including coconut water, banana homogenate, potato homogenate, corn extract, papaya extract, tomato extract, sweet-lime (Akter *et al.,* 2007; Vora and Jasrai, 2012; Mondal *et al.,* 2012).

The treatments with organic growth supplements proved beneficial for multiplication of regeneration of explants, maximum number of shoots formation and early plantlet development. Numerous researchers have investigated the effect of using plant extracts in different *in vitro* culture media. Organic growth supplements such as sweet-lime (5%) (Vora and Jasrai, 2012); coconut water (100 ml/L) (Kaur and Bhutani, 2012; Mondal *et al.,* 2012; Khatun *et al.,* 2018), bean sprout extracts (100-200 g/l) (Jufri *et al.,* 2014) help in producing higher rate of shoot-multiplication of banana. The objective of this study was to find out the ability of organic growth supplements in supporting the regeneration of banana cv. barangan *Musa acuminata* Colla.

MATERIALS AND METHODS

Preparation of Stock Plants

All experiments were conducted at Research Centre of Agriculture Biotechnology, Universitas Hasanuddin, Makassar, South Sulawesi, Indonesia. Sword suckers used in this experiment were excised from rhizome, rinsed under running tap water for 30 minutes and surface sterilization using sodium hypochlorite (NaOCl) 5% plus 2 drop of Tween-80 solution for 20 minutes and thorough rinsing with sterile distilled water. This was followed by aseptically trimming the buds to have final size of 2 cm and shaking them in the 0,5% NaOCl solution containing Tween-80 for 10 minutes, followed by three-time rinsing with sterile distilled water. Sterile sword sucker of banana cv. barangan *Musa acuminata* Colla. were cultured in basic Murashige and Skoog (MS) medium.

Preparation of Extract

About 50, 100, 150 and 200 g/L of green bean sprout and sweet corn were extracted according to the required concentration. The extract were stored at -20^{0}C deep freezer for long term use, whereas for the coconut water samples were taken directly from young coconut.

Preparation of Culture Media with Organic Growth Supplements

Basic MS medium was used with addition of three different plant that had been prepared beforehand. Extract was added to the solution of basic MS medium and then adjusted to pH 5.8.

Culture Conditions

The culture vessels were transferred to culture room and were allowed to grow in controlled environment at a temperature 26 ± 2^{0}C with relative humidity of 55 ± 5% and were exposed to 16 h photo period.

Data Recording and Analysis

After four weeks in culture, the multiplication shoots, shoot length and number of leaves on each explant were recorded. The mean values and standard deviations were calculated using computer software (Microsoft Office Excel Worksheet).

RESULTS

The efficacy of natural supplements showed positively results (Figure 1-3) on *in vitro* shoot multiplication, shoot length, and leaf number of *Musa acuminata* Colla. Data analysis show that the treatment of bean sprout extract starting from concentrations of 100 to 200 g/L significantly influences shoots multiplication shoots in 21 day after incubation (DAI). Figure 1 shows that the highest average on multiplication of shoots was obtained in the treatment of MS + 200 g/L bean sprout extract (7.11 ± 0.19), significantly different from the treatment of MS + 50 g/L bean sprout extract (2.77 ± 0.20), and MS without addition of bean sprout extracts (3.33 ± 0.34). However, it is insignificantly different with the treatment of MS + coconut water 15% (5.11 ± 0.51) and MS + 200 g/L sweet corn extract (6.00 ± 0.58).

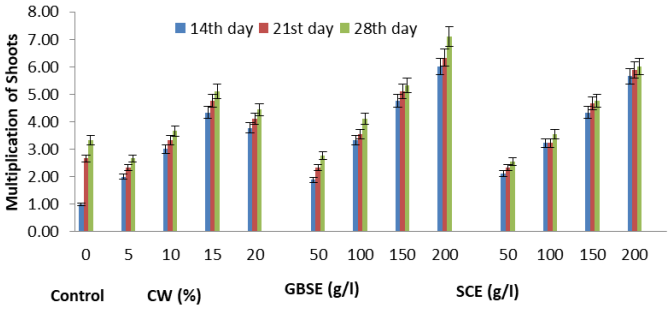

Figure 1. Effect of different natural supplements on shoots multiplication of *Musa acuminata* Colla. 14-28 days after incubation

Data of shoot length of *Musa acuminata* Colla on 21 DAI was provided in Figure 2. Data analysis shows that treatment of bean sprout in every concentration influences significantly on the shoot length. Figure 2 also shows that the longest shoot length average is obtained in the treatment of MS+ 200 g/L bean sprout extract (2.00 ± 0.22) and significantly different from MS without addition of bean sprout extract (1.00 ± 0.00). However it is insignificantly different in the treatment of MS + 150 g/L bean sprout extract (1.90 ± 0.41). In the treatment of sweet corn extract, the longest shoot length obtained at a concentration of 200 g/L (1.57 ± 0.20), treatment of MS + 100 g/L sweet corn extract (1.22 ± 0.14) insignificantly different from sweet corn extract at concentration 150 g/L (1.28 ± 0.40). Addition of coconut water on MS medium showed the longest shoot length at a concentration of 20%.

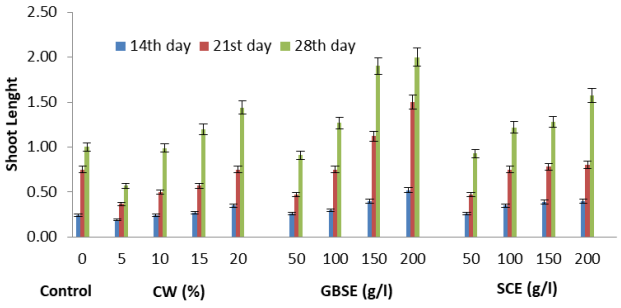

Figure 2. Effect of different natural supplements on shoots lenght of *Musa acuminata* Colla. 14-28 days after incubation

At 21 DAI, number of leaves produced in all medium containing natural supplements showed significant difference with control (1.11 ± 0.19) (Figure 3). Medium containing bean sprout extract concentration 200 g/l (4.00 ± 0.38) showed the highest number of leaves, followed by medium containing sweet corn extract 200 g/l (3.22 ± 0.19) and coconut water 20% (2.55 ± 0.69) respectively.

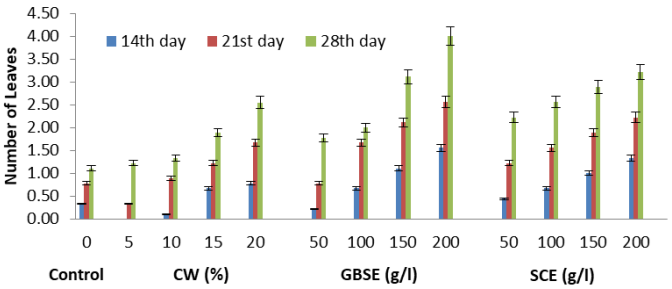

Figure 3. Effect of different natural supplements on number of leaves of *Musa acuminata* Colla. 14-28 days after incubation

DISCUSSION

The regeneration potential of explant was markedly influenced by quality and quantity of organic growth supplements added to the medium. The average of regeneration response was increased by addition of organic growth supplements in the culture in suitable concentration. In our experiments, 15% coconut water proved beneficial for development of multiplication shoots of *Musa acuminata* Colla. The results obtained support the earlier reports of Pervin *et al.* (2013) that the addition of coconut water (200 mg/l) has effective in promoting callus growth and rhizogenesis in banana callus. Increasing the concentration of coconut water in the medium above 15% did not show any significant effect on multiplication shoots. Whereas, at concentration 20% coconut water proved an increase shoot length and number of leaf. Developed shoots showed significant increase in length with increasing coconut water concentration, as well as the number of leaves. The addition of coconut water increased the average of multiplication shoots, shoot length and number of leaves. According to Mondal *et al.* (2012) coconut water considered as a nutrient medium because it is a source of mainly growth hormones and vitamins, zeatin (γ-alyl aminopurine), inositol and reduced nitrogen compounds. Coconut water from fresh green fruits was observed to be a suitable alternative to BAP in the *in vitro* culture of banana plants that produced shoot number and plant height not significant with benzylaminopurine (BAP) (Buah and Agu-Asare, 2014). Whereas according to Agampodi and Jayawardena (2009) that coconut water is a rich supplement that naturally contains plant growth regulators such as indole acetic acid (IAA).

Bean sprout extract that has been added to the culture medium could be optimally utilized by the *Musa acuminata* Colla. shoot primordial as in figure 4. Figure 2 indicated that increasing the concentration of bean sprout extract causes increased multiplication of shoots, shoot length regeneration and number of leaves. Similar phenomena were reported in previous study in which the addition of bean sprout extract had significant effect on the number and fresh weight of barangan propagules (Latunra *et al.*, 2016). In addition, Jufri *et al.* (2014) observed the dosage of 100 g/L and 200 g/L bean sprout extracts supplemented in medium MS during *in vitro* propagation of Plantain Unti sayang (*Musa paradisiaca* L.) showed best plant height, leaf number, root length, and root number. This is also in agreement with the results of Mastuti *et al.* (2017) where the bean sprout extract has promoted all explants to promote shoot regeneration in *Physalis angulate* L. According to Amilah and Astuti (2006), bean sprout extract contains vitamin and mineral such as vitamin C, thiamin, riboflavin, niacin, vitamin B, β-carotene, vitamin A and vitamin E, whereas mineral contained in bean sprout are calcium (Ca), ferrum (Fe), magnesium (Mg), phosphor (P), sodium (Na), zinc (Zn), copper (Cu) and manganese (Mn). The bean sprout extract is potentially useful in improving explant growth quality of banana by tissue culture. The different response in the multiplication shoots, length and leaves number might be influenced by nutritional content of

each type of organic growth supplements added into the culture medium.

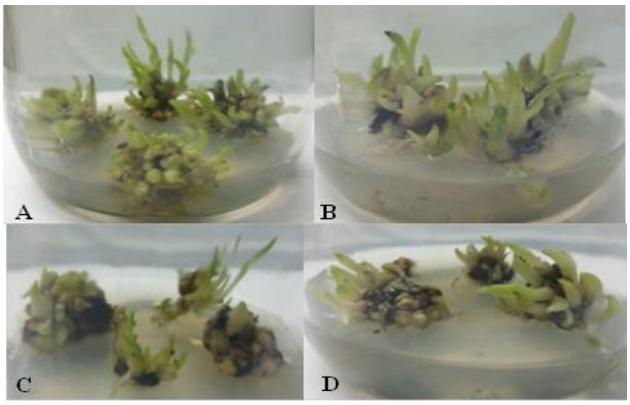

Figure 4. Effect of natural supplements on in vitro shoot multiplication of *Musa acuminata* Colla. (A) MS + 200 g/L bean sprout extract. (B) MS + 200 g/L sweet corn extract. (C) MS + 15% coconut water; (D) MS (control)

At 200 g/L concentration of sweet corn extract, the highest number of shoots, shoot length and number of leaves were obtained compared with other treatment of sweet corn extract. Islam *et al.* (2003) reported that corn extract at 100 mL/L was highly suitable for both callus proliferation and PLB formation from calli. Research conducted by Aiman (2007) confirmed that media supplemented sweet corn extract at 100 g/L produce the higher shoots compared media with the addition of other organic growth supplements (banana extract, bean extract and avocado extract). Similarly, Damiska *et al.* (2015) have reported that medium with corn extract is the best medium for multiplication shoots of *Garcinia mangostana* L. Sweet corn extract is known to contain cytokinin, namely zeatin, zeatin riboside and C-3 (Letham, 1966) with cell division activity in various plant species (Miller, 1967). These compounds may have played an important role in increasing the physiological parameters in the plant.

Increasing the concentration of organic growth supplements tended to be accompanied by an increasing multiplication shoots, shoot length and number of leaves of *Musa acuminta* Colla. Shoot formation is influenced by growth regulators cytokinins. Giving a cytokinin with a low concentration may provide shoot induction because the endogenous cytokinin content is sufficient (Rodinah *et al.*, 2012). There was a difference in the number of shoots production might influenced by the nutritional content in each of the organic material is not the same. All organic growth supplements function by supplying a form of organic nitrogen (a mixture of amino acids) and this would have been a reason for increased protein content in the plant grown on media with organic growth supplements (Swamy *et al*., 2014). The organic growth supplements contain natural vitamins, phenols, fiber, hormones, and also proteins (Gnasekaran *et al.*, 2010). Biomass is significantly affected by the type and concentration of organic growth supplemented to the medium. The organic growth supplements also effectively increased the chlorophyll content, total protein, and total carbohydrate content in the plant (Swamy *et al.*, 2014).

CONCLUSION

Organic growth supplements like coconut water, bean sprout extract, and sweet corn extract can be used in plant tissue culture medium. The best organic growth supplements for multiplication shoots, shoot length and number of leaves is MS basal medium with 200 g/L bean sprout extract.

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