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In vitro Shoot Multiplication of Curcuma xanthorrhiza with Coconut Water and Banana Extract Nutrient

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Abstract*—*Curcuma xanthorrhiza* is a medicinal plant, commonly used for spices, growth by rhizome, which is as its main product. Its rhizome demand both quantity and quality are increasing every year. Tissue culture techniques can achieve the efforts for providing plant materials massively in the short-term time with free from pest. In vitro, multiplication is an alternative production method. However, it is still limited by high chemical cost and plant growth regulators. Moreover, various natural materials can be used as a substitute for plant growth regulators, such as coconut water extract and various organic materials. The purpose of this study is to assess the response of *Curcuma xanthorrhiza* explants with coconut water, and Cavendish bananas extract by in vitro. The experiments conducted in February until May 2016 in the Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, Sebelas Maret University Surakarta. A completely randomized design was applied with two factors: the first factor was the four concentrations Cavendish banana fruit extract: 0 g/l, 50 g/l, 100 g/l and 150 g/l. The second factor were the 4 concentrations coconut water: 0 ml/l, 100 ml/l, 150 ml/l, and 200 ml/l. The observed variables were the period of shoots to appear, the period of leaves to appear and the period of roots to appear, the number of shots, the number of leaves, the number of roots, the height of shoots, the length of leaves and the length of roots. Observed data analyzed by descriptive and variance analysis continued with Duncan Multiple Range Test (DMRT). Research results showed that there were some effects on coconut water and Cavendish banana extract treatment toward the average time of shoots appear, number of shoots, number of leaves and length of leaves. The best treatment for this research is a combination of coconut water at 150 ml/l and 100 g Cavendish banana extract.

***Keywords*— Curcuma *xanthorriza*; organic material; tissue culture method.**

I. INTRODUCTION

Curcuma xanthorrhiza is one kind of medicinal plants [1] initially from Indonesia with the local name is "temulawak". *C. xanthorrhiza* rhizome has many benefits, among others, for deys, drinks and food, anti-cholesterol, anti-inflammatory, anemia medication, anti-oxidants, cancer prevention, anti-microbial, and components in various rhizomes can be used as food ingredients such as pulp and starch. *C. xanthorrhiza* also can be used to develop in the pharmaceutical field. [2] The total production of *C. xanthorrhiza* is 113.101 tons, which is exported partially. There are some commodities in export market, fresh *C. xanthorrhiza* and dried rhizome. Currently, the cultivation of *C. xanthorrhiza* is only on a limited scale, so it is low in quantity and quality. Temulawak has many benefits, including being a potent antioxidant. The active component that acts for antioxidant activity in Temulawak is curcumin [3], [4]. Drugs from natural materials or commonly called the herbal medicine must fulfill requirements that include quality, safety, and efficacy [5]. The tendency of people to use natural products has increased. The medicinal plant commodities in Central Java are indicating a strong comparative advantage in global markets. About 70% of herbal medicines on the traditional market contain about 70% of *C. xanthorrhiza*, and *C. xanthorrhiza* from Indonesia is exported [6].

These conditions provide the opportunities for farmers as providers for *C. xanthorrhiza* raw materials. Increasing demand of rhizomes has encouraged the growing demand for *C. xanthorrhiza* seed production. The tissue culture technique is a technology that is very helpful in the production of quality seeds in a short time, so that needs the number of *C. xanthorrhiza* rhizome through tissue culture techniques that can be met. In vitro propagation is affected by various factors, including the type of primarily used media, appropriately applied plant growth regulators, and environmental conditions. Various natural materials can be used as a substitute for plant growth regulators such as

coconut water. Coconut water is a natural substance that has an activity of cytokinin for boosts cell division and encourages the formation of organs. Cytokinin plays a role in cell enlargement, promoting, and morphogenesis [7]. Previous research showed that 50% coconut water addition could induced bud's formation [8]. In the manufacture of tissue culture, media can be added some complex organic materials such as banana extracts. Banana extract is an alternative medium source for tissue culture. Banana has several nutrient contents [9], such as carbohydrate, polyphenol, and flavonoid. The utilization of banana extract as additives in the media has different effects on plants. Banana extract, as additional materials to the media, has been tried to the in vitro culture of *Dendrobium canayo* [10]. The results of previous studies showed that coconut water and banana extract could be used as an adjunct in the medium of in vitro. Coconut water and banana extracts as a growing medium in this study are expected to increase the multiplication *C. xanthorrhiza* growth by in vitro. The purpose of this study is to assess the response of *C. xanthorrhiza* explants with coconut water and Cavendish bananas extract by in vitro.

II. Materials and Method

This study was conducted on February - May 2016 at the Laboratory of Biotechnology and Tissue Culture Faculty of Agriculture, Sebelas Maret University. Materials for this research were coconut water, banana extract, MS medium, BAP, and rhizome's shoots of *C. xanthorrhiza* with at least 5 cm in length. The explants for in vitro multiplication was from Karanganyar regency. The in vitro multiplication used MS medium with coconut water and banana extract for nutrient enrichment. *C. xanthorrhiza* planting material was its stem with 5 cm long. Before planting in MS medium, the rhizomes were sterilized through wash it using soap, then soaked in a solution of Dithane 2g / 200ml for 45 minutes.

Furthermore, the rhizomes thoroughly washed using distilled water, then soaked in 30% (60ml / 200ml) solution of Clorox for 10 minutes then soaking in 15% (30ml / 200ml) solution of Clorox for 10 minutes. *C. xanthorrhiza* rhizome will slightly brown colored because of absorption of Clorox solution into the tissue stem or rhizome. Section that will be planted was peeled from the absorbing section and cut Clorox solution also peel the stems up to 2 cm from the rhizome. After the rhizomes are clean from Clorox solution, the rhizomes passed over a Bunsen burner and then planted into the planting medium.

This study used a completely randomized design consisting of two factors with three replications. Both four coconut water concentration (0, 100ml/l, 150ml/l, and 200ml/l) and 4 fruit extract of banana concentrations (Cavendish) (0, 50g/l, 100g/l, 150g/l) were applied. Materials used for adjusting were the pH (HCL and KOH) and BAP 1ppm. The data were analyzed by using analysis of variance with F test 5%, if there is significant difference, it was continued with Duncan Multiple Range Test (DMRT) at 5% level. The variables were the period of shoots appear, the period of leaves appear, the period of roots appears, the number of shoots, the number of leaves, the number of roots, the length of sprouts, the length of leaves, and the length of roots.

III. Result and Discussion

Research data analyzed by using analysis of variance to compare treatment effect on each observed variable. Coconut water is a natural plant growth regulator that is widely used in in vitro propagation. Coconut water is often used for in vitro engineering applications. Nutrient content of coconut water includes Ca, Na, Fe, K, P, and C vitamin, so it is likely to be developed as a substitution of macro and micronutrients [11]. Moreover, banana extracts able to stimulate the formation of the shoot in both uniform plantlets and large quantities production [12].

A. The Period of Shoots Appear

The growth of shoots in the explant started from 1 to 2 Weeks After Planting (WAP). Based on the results of the analysis showed that the coconut water on the media are significantly affected by the average time to shoots appear of *C. xanthorrhiza* on the in vitro (Table 1).

TABLE I
Effect Of Coconut Water At The Time Appeared Shoots

Coconut water (A)	Appears shoots (days average)
A0	4.33 a
A1	5.50 a
A2	8.66 b
A3	4.25 a

Description: A0: coconut water 0ml/l; A1: coconut water 100ml/l; A2: coconut water 150ml/l; A3: coconut water 200ml/l; Figures followed by the same letter in the same column are not significantly different at 5% level DMRT.

The effect of coconut water with 150ml/l to the growing media significantly affected on the period of shoots appear. The Nutrient content of coconut water includes Ca, Na, Fe, K, P, and C vitamin [11], so it is likely to be developed as a substitution of macro and micronutrients. The sufficiency of cytokinins in concentrations greater than auxin is presumed to help the growth of the shoot of *C. xanthorrhiza*. [13] showed that the addition of coconut water concentration of 15% and synthetic plant growth regulator BAP1.5mg/l can respond in vitro growth of *C. xanthorrhiza* shoots up to 3.4 shoots in 2 months of planting.

B. Number of Shoots

Coconut water concentration, banana extracts concentration and the combination of coconut water and banana extract concentration treatments are not significantly affecting the number of shoots of *C. xanthorrhiza* for 16 weeks of average. Based on Figure 1, it shows that the addition of coconut water and banana extracts are not significantly affected the number of shoots but the average number of shoots is 1 until 7 buds. Furthermore, the addition 50g of banana extracts produced 6 shoots. Growth of shoot in vitro was significantly influenced by banana extract [14]. The results of research done by [15] showed that the treatment with a concentration of coconut water is able to increase the number of shoots regenerated per explant.

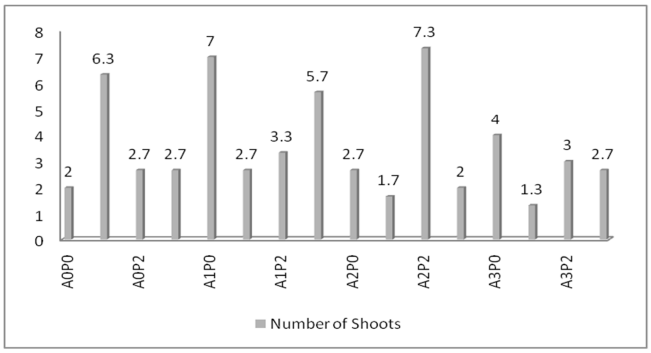

Fig. 1 Effect of coconut water and banana extract on number of shoots

Description:
A0P0: coconut water 0ml/l and banana extract 0g A2P0: coconut water 150ml/l and banana extract 0g
A0P1: coconut water 0ml/l and 50g banana extract A2P1: coconut water 150ml/l and 50g banana extract
A0P2: coconut water 0ml/l and 100g banana extract A2P2: coconut water 150ml/l and 100g banana extract
A0P3: coconut water 0ml/l and 150g banana extract A2P3: coconut water 150ml/l and 150g banana extract
A1P0: coconut water 100ml/l and banana extract 0g A3P0: coconut water 200ml/l and banana extract 0g
A1P1: coconut water 100ml/l and 50g banana extract A3P1: coconut water 200ml/l and 50g banana extract
A1P2: coconut water 100ml/l and 100g banana extract A3P2: coconut water 200ml/l and 100g banana extract
A1P3: coconut water 100ml/l and 150g banana extract A3P3: coconut water 200ml/l and 150g banana extract

C. The Length of Shoots

The study of coconut water addition and banana extract showed that the length of shoots was presumably caused by low synthetic auxin addition. Auxin serves sprouts elongation; lower concentration of auxin might cause shorter sprouts.

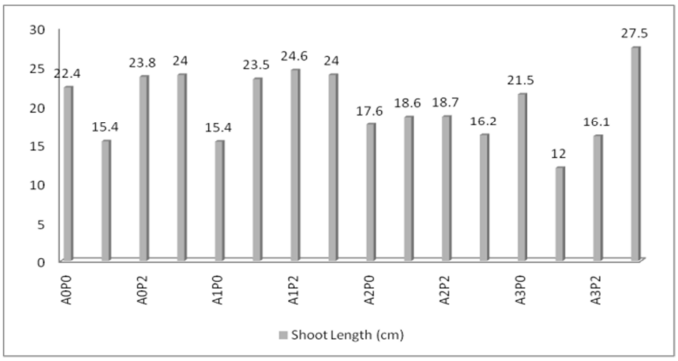

Fig. 2 Effect of coconut water and banana extract on shoots length

Description:
A0P0: coconut water 0ml/l and banana extract 0g A2P0: coconut water 150ml/l and 0g banana extract
A0P1: coconut water 0ml/l and 50g banana extract A2P1: coconut water 150ml/l and 50g banana extract
A0P2: coconut water 0ml/l and 100g banana extract A2P2: coconut water 150ml/l and 100g banana extract
A0P3: coconut water 0ml/l and 150g banana extract A2P3: coconut water 150ml/l and 150g banana extract
A1P0: coconut water 100ml/l and banana extract 0g A3P0: coconut water 200ml/l and 0g banana extract
A1P1: coconut water 100ml/l and 50g banana extract A3P1: coconut water 200ml/l and 50gbanana extract
A1P2: coconut water 100ml/l and 100g banana extract A3P2: coconut water 200ml/l and 100g banana extract
A1P3: coconut water 100ml/l and 150g banana extract A3P3: coconut water 200ml/l and 150g banana extract

Based on Figure 2 it shows that the addition of coconut water and banana extract are not significantly affected the shoots length but the average number of shoots length were 12 cm until 27 cm. Moreover, the growth of explants can be influenced by the height of culture bottle, so the height of shoots will be obstructed or even cannot grow well. The content of K nutrient mineral contained in coconut water and Cavendish banana can affect the elongation of plant growth [16].

D. The Period of Roots Appear

Roots from explants without going through the stage of formation of callus. Based on the results of analysis of variance addition of coconut water, banana extract and interaction with coconut water and banana extract do not significantly affect the period of *C. xanthorrhiza* roots appears. Coconut water and banana extracts addition on the growing media show the average period of root appearance on 6 until 16 days as showed on Figure 3.

The use of auxin for rooting at various concentrations have different effects on the root growth [17]. The combination of MS medium by 150ml/l of coconut water and 100g of banana extract showed a long time for roots growth with an average of 16.7 days.

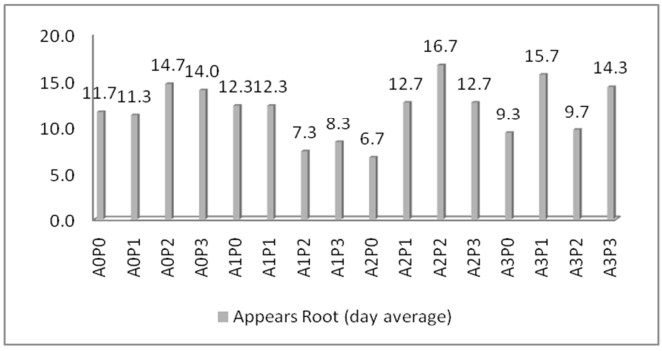

Fig. 3 Effects of coconut water and banana extract on appears root

Description:
A0P0: coconut water 0ml/l and banana extract 0g A2P0: coconut water 150ml/l and 0g banana extract
A0P1: coconut water 0ml/l and 50g banana extract A2P1: coconut water 150ml/l and 50g banana extract
A0P2: coconut water 0ml/l and 100g banana extract A2P2: coconut water 150ml/l and 100g banana extract
A0P3: coconut water 0ml/l and 150g banana extract A2P3: coconut water 150ml/l and 150g banana extract
A1P0: coconut water 100ml/l and banana extract 0g A3P0: coconut water 200ml/l and 0g banana extract
A1P1: coconut water 100ml/l and 50g banana extract A3P1: coconut water 200ml/l and 50gbanana extract
A1P2: coconut water 100ml/l and 100g banana extract A3P2: coconut water 200ml/l and 100g banana extract
A1P3: coconut water 100ml/l and 150g banana extract A3P3: coconut water 200ml/l and 150g banana extract

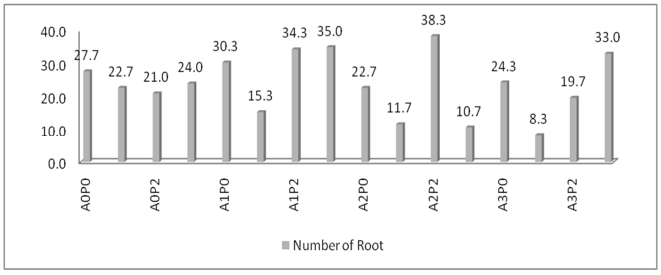

Fig. 4 Effects of coconut water and banana extract on the number of roots

Description:
A0P0: coconut water 0ml/l and banana extract 0g A2P0: coconut water 150ml/l and 0g banana extract
A0P1: coconut water 0ml/l and 50g banana extract A2P1: coconut water 150ml/l and 50g banana extract
A0P2: coconut water 0ml/l and 100g banana extract A2P2: coconut water 150ml/l and 100g banana extract
A0P3: coconut water 0ml/l and 150g banana extract A2P3: coconut water 150ml/l and 150g banana extract
A1P0: coconut water 100ml/l and banana extract 0g A3P0: coconut water 200ml/l and 0g banana extract
A1P1: coconut water 100ml/l and 50g banana extract A3P1: coconut water 200ml/l and 50gbanana extract
A1P2: coconut water 100ml/l and 100g banana extract A3P2: coconut water 200ml/l and 100g banana extract
A1P3: coconut water 100ml/l and 150g banana extract A3P3: coconut water 200ml/l and 150g banana extract

E. The Number of Root

The roots of plant require for the absorption of nutrients from the media and root areas extension used for nutrient absorption [18]. In Figure 4, it shows that even the addition of coconut water and banana extract are not significantly affected the number of root but the number of roots are around 8,3 until 38,3. [19] demonstrated the use of BAP 2mg/l, 150ml/l of coconut water and 50g/l of banana extract indicate the number of the best roots.

F. The Length of The Root

The length of the root affects the explants growth. Long-rooted plants will have a better ability to absorb water and nutrients in the growing media compared with short-rooted plants. The results of analysis of variance addition of coconut water, the treatment of banana extract and the combination with coconut water with banana extract show that there are not affect in the length of *C. xanthorrhiza* root explants.

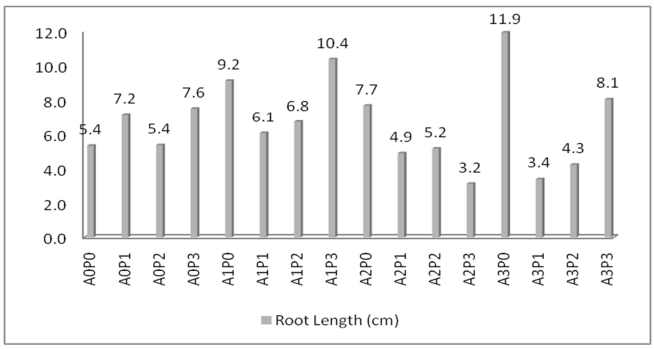

Fig. 5 Effects of coconut water and banana extract on root length

Description:
A0P0: coconut water 0ml/l and banana extract 0g A2P0: coconut water 150ml/l and 0g banana extract
A0P1: coconut water 0ml/l and 50g banana extract A2P1: coconut water 150ml/l and 50g banana extract
A0P2: coconut water 0ml/l and 100g banana extract A2P2: coconut water 150ml/l and 100g banana extract
A0P3: coconut water 0ml/l and 150g banana extract A2P3: coconut water 150ml/l and 150g banana extract
A1P0: coconut water 100ml/l and banana extract 0g A3P0: coconut water 200ml/l and 0g banana extract
A1P1: coconut water 100ml/l and 50g banana extract A3P1: coconut water 200ml/l and 50gbanana extract
A1P2: coconut water 100ml/l and 100g banana extract A3P2: coconut water 200ml/l and 100g banana extract
A1P3: coconut water 100ml/l and 150g banana extract A3P3: coconut water 200ml/l and 150g banana extract

Based on Figure 5, it shows the best root length is affected by the treatment 200ml/l of coconut water with an average root length by 11.9cm and addition of 100ml/l coconut water to 150g of banana extract effect on the root's length with an average by 10.4cm. The addition of coconut water and banana extract may assist in the development of the network. In addition, the provision of auxin and cytokinin should consider the concentration and the ratio in the media. [20] revealed that if the auxin is higher than cytokinin, it caused by the differentiation of the growth of the roots.

G. The Period of Leaves Appear

Based on the results of analysis of variance addition of coconut water, banana extract and the combination of coconut water and banana extract did not significantly affected the time of *C. xanthorrhiza* leaf explants arises. Application 20% of coconut water in the treatment have a shorter time until leaves were appear.

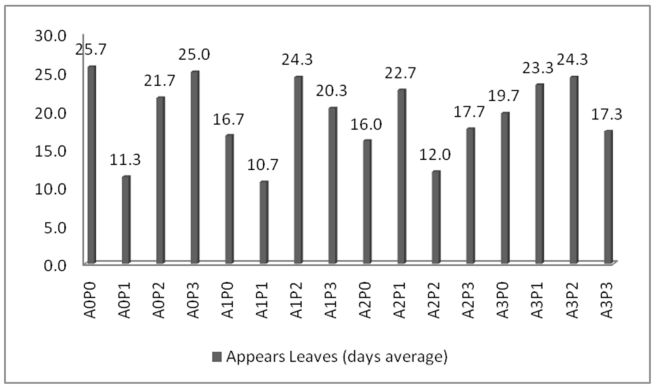

Fig. 6 Effects of coconut water and banana extract on appears leaves

Description:
A0P0: coconut water 0ml/l and banana extract 0g A2P0: coconut water 150ml/l and 0g banana extract
A0P1: coconut water 0ml/l and 50g banana extract A2P1: coconut water 150ml/l and 50g banana extract
A0P2: coconut water 0ml/l and 100g banana extract A2P2: coconut water 150ml/l and 100g banana extract
A0P3: coconut water 0ml/l and 150g banana extract A2P3: coconut water 150ml/l and 150g banana extract
A1P0: coconut water 100ml/l and banana extract 0g A3P0: coconut water 200ml/l and 0g banana extract
A1P1: coconut water 100ml/l and 50g banana extract A3P1: coconut water 200ml/l and 50gbanana extract
A1P2: coconut water 100ml/l and 100g banana extract A3P2: coconut water 200ml/l and 100g banana extract
A1P3: coconut water 100ml/l and 150g banana extract A3P3: coconut water 200ml/l and 150g banana extract

Based on the average time of leaves appearance data in Figure 6, use of coconut water and banana extract was not significantly affected. Treatment combination were able to accelerate the leaves growth, it showed that leaves growth on 10 until 25 days after planting. [21] showed that the use of natural ingredients of coconut water at the concentration of 1.5% and 1mg/l of BAP for shoots multiplication can improve the growth of *Vanilla planifolia*. In addition, [22] suggested that single treatment of coconut water at 250ml/l of concentration was able to produce leaves and roots faster on the in vitro culture of orchids (*Phalaenopsis amabilis* BL.).

H. The Number of Leaves

Based on the analysis of variance result, coconut water treatment affected amount of *C. xanthorrhiza* leaf explants (Table 2). The addition of banana extract and the combination of coconut water and banana extract did not significantly affected the number of leaves explants.

TABLE II
EFFECT OF COCONUT WATER AT THE NUMBER OF LEAVES

Coconut water (A)	Number of leaves
A0	5.75 b
A1	5.58 b
A2	3.50 a
A3	4.91 b

Description: A0: coconut water 0 ml/l; A1: coconut water 100 ml/l; A2: coconut water 150 ml/l; A3: coconut water 200 ml/l; Figures followed by the same letter in the same column are not significantly different at 5% level DMRT.

Based on Duncan Multiple Range Test (DMRT) results, explants with 150ml/l of coconut water treatment showed significant difference compared to other treatments, which it leaves number was the lowest results with only 3.5 average leaves sheets. The effect of coconut water was quite significant if compared to banana extract treatments and combination of coconut water and banana extracts. [13] states that coconut water usage with 15% concentration stimulate the growth of leaves number by 2.2 sheets for two weeks.

I. The Length of The Leaves

Based on the analysis of variance results, coconut water treatments affected the leaves length of *C. xanthorrhiza* (Table 3). Moreover, the addition of Cavendish banana extracts and treatments combination did not affect to the leaf's length of *C. xanthorrhiza* explant.

TABLE III
EFFECT OF COCONUT WATER AT LEAVES LENGTH

Coconut water (A)	Leaves length (cm)
A0	8.70 b
A1	9.44 b
A2	5.86 a
A3	8.66 b

Description: A0: coconut water 0ml/l; A1: coconut water 100ml/l; A2: coconut water 150ml/l; A3: coconut water 200ml/l; Figures followed by the same letter in the same column are not significantly different at 5% level DMRT.

Table 3, Duncan analysis with 5% level test showed that 150ml/l of coconut water was significantly different if compared to the other treatments. Furthermore, the average of the length of the leaves were 5.86cm. [20] states that enhancement length of leaves caused by accelerated of cell division and promote cell differentiation. Cell division requires energy that can be obtained from the auxin or cytokinin nutrients. The length and width of leaves is closely connected to the direction of the division, enlargement, the number and distribution of cells. [20] reveals that on the Mokara orchids used coconut water following the 15% of concentration + 75g of banana + 1 ppm of BAP can be significantly affected the length of the leaves compared to the other treatments.

IV. CONCLUSIONS

Based on research results, it can be concluded that 150ml/l of coconut water is able to accelerate the period of shoots appearance and the period of roots appear in the rhizome's explants. Moreover, the application of 150ml/l coconut water and 100g of banana extract can increase the number of shoots and roots. In addition, the application 200ml/l coconut water and 150g of banana extract treatments could increase the rhizome shoots height and roots length. The use of 100ml/l – 200ml/l coconut water tends to increase the number of leaves, and the length of the roots.

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