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In Vitro Microrhizome Formation of Temulawak (*Curcuma xanthorrhiza* Roxb.)

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ABSTRACT

The aims of this research were to find the influence of the physical form of media (liquid and solid), composition of media (full and half strength concentration Murashige and Skoog (MS) media), benzylaminopurine (BAP) and sucrose concentration to the growth of explants and formation of micro rhizome. Research consists of three experiments that used factorial design. Cultures of all experiments were incubated in the dark for 16 hours day⁻¹. The results showed that microrhizomes of Temulawak (*Curcuma xanthorrhiza* Roxb.) was formed in liquid half and full strength MS. Interaction BAP and sucrose had significant effect on rhizomes formation at 18 weeks. The rhizomes formation frequency was higher on media enhanced with BAP 1 and 2 gL⁻¹ and higher sucrose concentration. More and larger micro rhizomes produced obtain from small size shoots.

Keywords: temulawak, liquid MS media, BAP, sucrose, microrhizome

INTRODUCTION

Temulawak (*Curcuma xanthorrhiza* Roxb.) is a medicinal plant native to Indonesia. Various studies have revealed the efficacy of this plant for human health. Peculiar efficacy of its rhizomes are mainly caused by the yellow compound, curcuminoids and volatile oil, xanthorrhizol. The efficacy of curcuminoids are to neutralize toxins, relieve pain, increases bile secretion, lowering blood levels of cholesterol and triglycerides, while xanthorrhizol used to treat breast cancer, lung, ovarian, and antibacterial prevention of tooth enamel damage (Sidik 2006).

Nowdays, seed propagation of temulawak still apply in conventional technique by using quite old rhizome. The higher weight of seed rhizomes planting will increase the yield (Djakamihardja *et al.* 1985). Per plant production of temulawak from seed weighing 40 g rhizome higher than plant from the lower-weighted rhizome seedling (Kasiran 2008). The using of large rhizome as seed to obtain high yields causes tendly large amount of seed need, thus reducing the amount of rhizome for consumption or for processing.

In vitro technique is a technology that can be applied to overcome the seed needs in the form of micro rhizomes. Tyagi *et al.* (2006) and Anisuzzaman *et al.* (2008) stated that micro rhizome can be planted directly without acclimatization process before. Storage and transport of micro rhizome as seed will be easier. Micro rhizome can be used for germplasm conservations also so that more efficient and longer time storage.

Induction micro rhizome process is called organogenesis. This process occurs when cells of explant divide and then differentiate to form an organ. Cells plant regeneration is determined by genotype, type and age of explants, plant growth regulators, medium, temperature and radiation. In the plant, formation of tuber or rhizome organs will take place on certain conditions (Wattimena 2006). Salisbury and Ross (1992) stated that in vitro formation of plant storage organs would be induced by sucrose and plant growth regulators that were added to the media. Several studies have been conducted to induce micro rhizome formation such on ginger plant (Rahmawati *et al.* 2003, Tyagi *et al.* 2006), temuputih (Anisuzzaman *et al.* 2008), turmeric (Shirgurkar *et al.* 2001), but has not been done on temulawak. So, it is necessary to conduct in vitro micro rhizome formation studies on temulawak. The purposes of this research were to find the influence of the physical form of media (liquid and solid), composition of media (full and half strength concentration MS media), BAP and sucrose concentration to the growth of explants and formation of micro rhizome.

MATERIAL AND METHODS

These researches were conducted at Laboratorium Pengembangan Teknologi Industri Agro dan Biomedika (LAPTIAB) BPPT, Puspiptek Area, Serpong lasted from October 2008 until May 2010. This study consists of three series of experiments:

- a) Experiment I: Effect of physical forms of media and sucrose to growth and formation of temulawak micro rhizome

- b) Experiment II: Effect of the composition of MS media and sucrose to growth and formation of temulawak micro rhizome
- c) Experiment III: Effect of BAP and sucrose to growth and formation of temulawak micro rhizome

Experiment I: Effect of Physical Form of Media and Sucrose Concentration

The media used in the experiments was MS composition media. This experiment used factorial design. Factor A was the physical form of medium that consists of 2 levels: solid and liquid. Factor B was the sucrose concentration with 4 levels of concentration were 30, 60, 90 and 120 gL⁻¹. There were 8 treatment combinations (in a complete randomized design) with 10 replications per treatment so that there were 80 units experiments. An experimental unit was a bottle containing one explants. Solid medium was made by adding agar powder (8 gL⁻¹ medium) into solution. Cultures maintained in the dark for 16 hours every day.

Experiment II: Effect of MS Media Composition and Sucrose Concentration

The physical condition of the media (solid or liquid) used was determined based on the results of experiment I. This experiment used factorial design. Factor A was the composition of MS media consisting 2 levels: half and full strength MS media. Factor B was the sucrose concentration with 4 levels of concentrations: 30, 60, 90 and 120 gL⁻¹. There were 8 treatment combinations (in a complete randomized design) with 20 replications per treatment so that there were 160 experimental units (a bottle contains 1 explants). During the experiments, the culture maintained in the dark for 16 hours every day.

Experiment III: Effect of BAP and sucrose concentration

The physical condition and composition of MS media used were determined based on the results of experiment I and II. This experiment also used factorial design. Factor A was BAP concentrations, consisting 4 levels: 0, 1, 2, and 3 mgL⁻¹. While factor B was sucrose concentration, consisting 4 levels: 30, 60, 90 & 120 gL⁻¹. There were 16 treatment combinations (in a completely randomized design) and 10 replications in each treatment, so there were 160 units experiment. In all experiments, the parameter of data that was observed: number of rhizome induction, swelling diameter, plantlet height, leaf length, leaf width, and root length.

Statistics Model

The design of experiments I, II and III were completely randomized factorial design. Data analyzing with ANOVA and followed with Duncan Multiple Range Test (DMRT) at level 5% error if the result was significantly different.

RESULTS AND DISCUSSION

Experiment I: Effect of Physical Form of Media and Sucrose Concentration

Physical forms of medium and sucrose concentration interactions hadn't significant effect to micro rhizome formation until the end of the experiment for 16 weeks. Micro rhizome formation induction was influenced by physical forms medium but not influenced by sucrose concentration. In the liquid medium the number of micro rhizome induction higher than in the solid medium (Table 1).

Plantlets with higher size, broader leaves and longer roots on solid medium produced swell-induction of micro rhizome fewer and smaller than the plantlets in liquid medium. Micro rhizome formations in liquid medium have been successfully conducted to *Curcuma aromatica* Salisb. (Nayak *et al.* 2000) and *Curcuma longa* L. (Islam 2004). In liquid medium higher plantlet size produced less number of micro rhizome (correlation -0.99*). Leaf length and leaf width addition were inversely proportional to the addition of the amount of micro rhizomes that was produced.

Table 1. The number of micro rhizome induction and plantlet size (15 WAP*)

Variabel	Treatments	
	Solid Medium	Liquid Medium
Number of Micro Rhizome Induction	0.33	1.22
Swelling Diameter (cm)	0.20	0.53
Plantlet Height (cm)	6.79	4.17
Leaf Length (cm)	3.47	1.99
Leaf Width (cm)	1.17	0.89
Root Length (cm)	1.78	1.02

*WAP : Weeks After Planted

Experiment II: Effect of MS Media Composition and Sucrose Concentration

In the first experiment number of micro rhizome induction occur higher in the liquid media than in the solid media. Because of it, media that was used in the second experiment was liquid media. Until 18 WAP micro rhizome formation was not influenced by single factors and by interaction of both treatments. Although it wasn't significantly different, amount of micro rhizome showed higher on strength half MS medium than on full concentration MS medium.

Formation of turmeric micro rhizome (*Curcuma longa* Linn.) according to Shirgurkar *et al.* (2001) optimum on half strength MS media, whereas according to Islam (2004) the number and size optimum on ¾ MS media. Unlike to that statements, Kenyo *et al.* (2002) stated that the number of in-vitro micro bulblet of two cultivars lily (Avignon and Bergamo) higher on full concentration MS media than on lower concentration media.

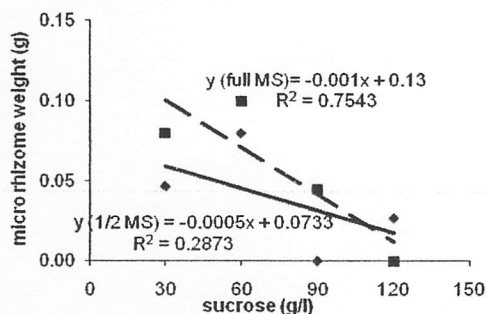


Figure 1. Micro rhizome weight on half strength and full concentration MS media.

Note : --- linear (full MS)
 - - - linear (1/2 MS)

Micro rhizome that formed on half strength MS media had lower weight than micro rhizome formed on full concentration MS media (Fig. 1). Rhizome diameter was almost same on both media (Fig. 2). Thus, number of micro rhizome on half strength MS media higher with lower weight and smaller size than micro rhizome formed on full concentration MS media.

Experiment III: Effect of BAP and sucrose concentration

Based on the results of two previous trials, the media used was half strength liquid MS media. In this experiment, interaction between BAP and sucrose affected significantly to the number of micro rhizome at 18 WAP. The DMRT results demonstrate that interaction BAP 2 gL⁻¹ and sucrose 120 gL⁻¹ formed the most number of micro rhizome.

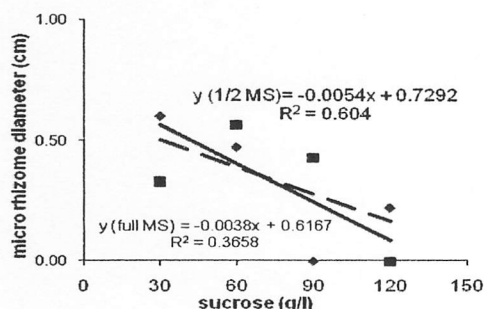


Figure 2. Diameter of the micro rhizome on half and full strength MS medium

Note : --- linear (full MS)
 - - - linear (1/2 MS)

Along with the increasing of sucrose, total micro rhizome formation also increased on media without BAP and with 1, 2 gL⁻¹ BAP (Fig 3a, b, c). Otherwise, the number of micro rhizome progressively decreasing on media with 3 gL⁻¹ BAP (Fig. 4). According to Rahmawati *et al.* (2003) the amount of ginger (*jahe emprit*) micro rhizome increased along with increasing of BAP and sucrose concentration. Explants tend to store excess nutrients from the media in the rhizomes form. In vitro culture of *Curcuma aromatica* Salisb L on medium with 60-90 gL⁻¹ sucrose (Nayak 2000), *Curcuma longa* on medium with 40-80 gL⁻¹ sucrose (Shirgurkar *et al.* 2001) and 60-100 gL⁻¹ (Islam, 2004) were the optimum sucrose concentration for increasing the number and size of micro rhizome. Anisuzzaman *et al.* (2008) reported that 70% of in-vitro shoots of *Curcuma zedoaria* (Christm.) Roscoe forming micro rhizome occurred on medium containing 60 gL⁻¹ sucrose.

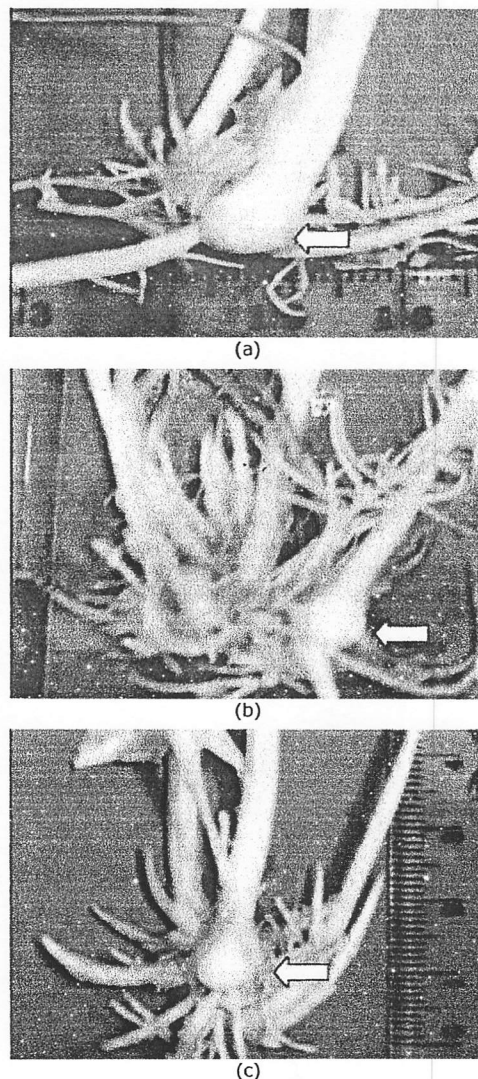


Figure 3. Micro rhizome from treatment 90 g/l sucrose and BAP 0 gL⁻¹(a), 120 g/l sucrose and BAP 1 gL⁻¹ (b), 120 g/l sucrose dan BAP 2 gL⁻¹

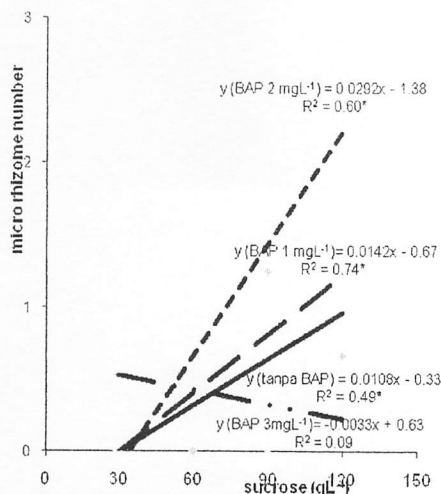


Figure 4. Number of micro rhizome on medium without BAP and with BAP 1, 2, 3 mgL⁻¹

---- linear (without BAP)
 - - - linear (BAP 1 mgL⁻¹)
 - - - linear (BAP 2 mgL⁻¹)
 - - - linear (BAP 3 mgL⁻¹)
 * : significant $\alpha = 5\%$

Sucrose as carbon source can be used for food storage filling. One of food storage organ is micro rhizome. Salisbury and Ross (1992) stated that in the storage organs, carbohydrates accumulate as aminoplas by result of sucrose translocation. Trigiano and Gray (2005) stated that sucrose was the most often used as carbon and energy sources in the in-vitro culture because it was naturally synthesized and transported by plants.

On 18 WAP, with the presence of BAP in the medium, plantlet height and number of shoots significantly correlated with the number and diameter of micro rhizome. In the medium with BAP 1 mgL⁻¹, more shoot would be produced more micro rhizome. The increasing of plantlet height followed by the decreasing of number and diameter of rhizomes. Such was the case, more micro rhizome with large-sized was harvested from small size shoot (Tables 2 and 3).

Table 2. Correlation between leaf length, number of senescence leaves, plantlet height, root length, and number of shoots to micro rhizome number at 18 WAP

Variabel	Number of Rhizomes		
	BAP 0 mgL ⁻¹	BAP 1 mgL ⁻¹	BAP 2 mgL ⁻¹
Leaf Length	0.09	-0.09	-0.41
Number of Senescence Leaves	-0.24	-0.05	-0.13
Plantlet Height	0.05	-0.58*	-0.82**
Root Length	-0.23	-0.27	-0.35
Number of Shoots	-0.23	0.64*	0.32

* : significant at $\alpha = 5\%$, **: highly significant at $\alpha = 1\%$

Table 3. Correlation between leaf length, number of senescence leaves, plantlet height, root length, number of shoots to rhizome diameter micro at 18 WAP

Variabel	Micro Rhizome Diameter		
	BAP 0 mgL ⁻¹	BAP 1 mgL ⁻¹	BAP 2 mgL ⁻¹
Leaf Length	0.21	-0.10	-0.07
Number of Senescence Leaves	-0.24	-0.15	-0.13
Plantlet Height	0.08	-0.54*	-0.52*
Root Length	-0.30	-0.28	-0.16
Number of Shoot	-0.31	0.58*	0.52*

* : significant at $\alpha = 5\%$

Micro rhizome formations in all three experiments were closely related with the concentration of sucrose in the medium. The high sucrose concentration causes medium osmotic pressure higher than in the plant cells so that the water becomes unavailable for plants. These conditions can be interpreted that water stress happen in the plant. Haryati (2003) stated that water stress during vegetative growth would affect the source intensity such as size of canopy, while at the generative growth would affect the size of sinks such as part of plant that were usually harvested (fruit or rhizome).

CONCLUSION AND SUGGESTION

Temulawak micro rhizome formed in liquid half and full strength MS medium. Interaction of BAP and sucrose significantly affected to micro rhizome number that found on 18 MSP. The presence of BAP 1 and 2 mgL⁻¹ in the medium will increase the number of micro rhizome during the increasing of sucrose concentration. More and larger micro rhizome produced obtain from small size shoots.

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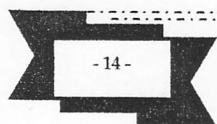
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