# Proceedings

## The 2nd International Symposium on Temulawak

## The 40th Meeting of National Working Group on Indonesian Medicinal Plant

ISBN NO. 978-979-25-1209-0

# PROCEEDINGS OF THE 2nd INTERNATIONAL SYMPOSIUM ON TEMULAWAK AND THE 40th MEETING OF NATIONAL WORKING GROUP ON INDONESIA MEDICINAL PLANT

**LAY-OUT**

Titis Arifiana, SSi

**December, 2011**

**ISBN No. 978-979-25-1209-0**

ISBN 978-979-25-1209-0
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# *Acknowledgement from Rector of the Bogor Agricultural University*

**RECTOR SPEECH**

The 2nd International Symposium on Temulawak
the 40th Meeting of National Working Group on Indonesian Medicinal Plant

*Assalamualaikum wr. wb.*
Salam sejahtera bagi kita semua
Good morning.

It is our pleasure today that we are all here to attend the important event so called 'Globalization of Jamu Brand Indonesia' which consists of several agenda such as the 2nd International Symposium on Temulawak (*Curcuma xanthorrhiza*); the 40th Meeting of National Working Group on Indonesian Medicinal Plant; Workshops, Business Meeting, Jamu Festival, 'Jamu' Batik Design Competition and Temulawak 'Welcome Drink' Formula Competition here in Bogor, Indonesia.

As most of us may still remember that 3 years ago, in 2008, I attended 'Gelar Kebangkitan Jamu Brand Indonesia' and the First International Symposium on Temulawak. The event was officially opened by the President of the Republic of Indonesia, in *Istana Negara*, Jakarta; while the symposium was held here in the same place, IPB International Conference Center. Now, we are here again to attend the Globalization of Jamu Brand Indonesia. For the two important events, held in 2008 and 2011, multisectors and international stakeholders are involved, and IPB is significantly taking parts. This clearly indicates that continuous improvement to achieve our goal, to make Jamu for the Word Quality of Live, is really our concern.

We all know that Indonesia is the second largest countries in the world regarding biodiversity. The Indonesian people are used to their natural resources with knowledge they inherited from their ancestors. For example, medicinal plants, animals, and microbes are applied as preventive, promotive and curative alternatives. Jamu has long been known and applied by the society; and nowadays, there is also an increasing tendency of using herbal medicine that is popularly known as 'back to nature' for curing diseases worldwide. Jamu business keeps increasing, I heard that almost reach to 10 trillion IDR in 2010; and the support from Indonesian government becomes stronger and real actions have also been conducted, such as: Scientification of Jamu, and Roadmap of Jamu Development will be launched and used as a national guidance for Jamu development. So I believe that through the strong commitment from Jamu stakeholders, our vision that Jamu for the World Quality of Live can be achieved in the near future.



ISBN No. 978-979-25-1209-0

Distinguish Guest, Ladies, and Gentleman,

Bogor Agricultural University (IPB) with its vision to becoming a world class research university with core competences in tropical agriculture and bioscience with entrepreneur character, and one of our mission is to improve the welfare of human beings through the application of developed science and technology are clearly in line with our effort to improve Jamu development.

Within this context, Biopharmaca Research Center IPB keeps focusing its research development and optimizing its efforts to develop qualified biopharmaca products with the support of strong networking with international and national partners. IPB also contributes to the national policy development of Indonesian biopharmaca and committed its existence in education and research development in order to achieve national and international reputation. Currently, IPB is proposing the establishment of Indonesian Biopharmaca Center (IBC) through the support of Japan International Corporation Agency (JICA), and the visibility study of the project is now being conducted. The IBC is expected to be a center of excellence in Biopharmaca Research Development within the country and will contribute to international reputation.

Today as part of Globalization of Jamu Brand Indonesia, we also have an important international event, namely "The 2nd International Symposium on *Temulawak* (*Curcuma xanthorriza*)" which is conducted by Biopharmaca Research Center, IPB in collaboration with Indonesian government institutions, private sectors, and foreign partners. The theme of the symposium is utilization and application of *Curcuma xanthorrhiza* through scientific and technological approach toward better and healthy life.

We know that temulawak is known as one of the Indonesian indigenous herbals, which mostly used as the main ingredient for traditional medicine or 'Jamu'. The popularity of temulawak is increasing along with its commercial use and research result applications. Many scientists have conducted research to reveal the secret of temulawak. Temulawak can be used for various purposes such as for maintaining human health, animal health, and supplement beverages to increase appetite and to keep fresh our stamina. As a continuation of our commitment, IPB is conducting various aspects of research based on temulawak, e.g. brain tonic, cardiovascular diseases, diabetic; further, our research output on avian flu has been registered for patent.

Nowadays, in the middle of modern lifestyle, temulawak is occupying place in our society's heart and with its various benefits, thus temulawak deserves to be Indonesian "ginseng" herbal. IPB fully support the three days activities involving researchers and scientists all over the world to share their experience and experties which will be conducted in IPB International Convention Center.

We do hope that through Globalization of Jamu Brand Indonesia which are involving biopharmaca stakeholders within the country and abroad, modernization of Indonesian medicine/Jamu will be accelerated and generated benefits to increase our health and welfare.

We would like to ask to the Minister of Coordinator of People's Welfare, Republic of Indonesia to officially open The Globalization of Jamu Brand Indonesia.

Finally, thank you to all of ypu who make this precious event possible.

*Billahi taufik wal hidayah, wassalamualaikum wr.wb.*

Bogor, May 26, 2011
Rector of IPB,

Prof Dr Herry Suhardiyanto, MSc



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JAPAN



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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 strenght 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 it's rhizomes are mainly caused by the yellow compound, curcuminoids and volatile oil, xanthorrizol. The efficacy of curcuminoids are to neutralize toxins, relieve pain, increases bile secretion, lowering blood levels of cholesterol and triglycerides, while xanthorrizol 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 strenght 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



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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 strenght 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 contains1 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 proporsional 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.

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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.

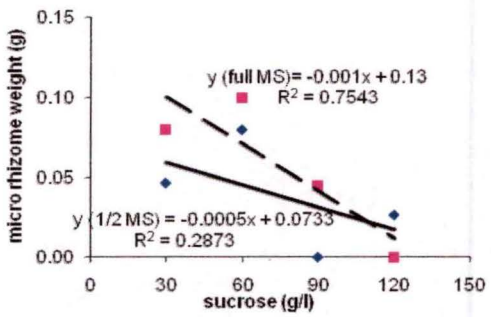



Figure **1**. Micro rhizome weight on half strenght and full concentration MS media.

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 strenght 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 strenght 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.

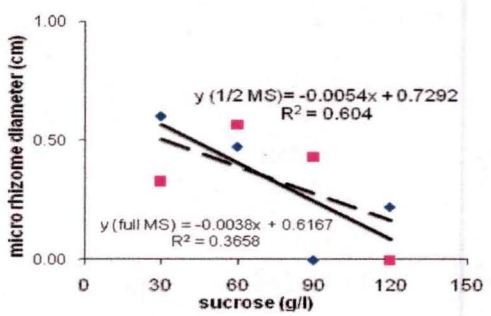
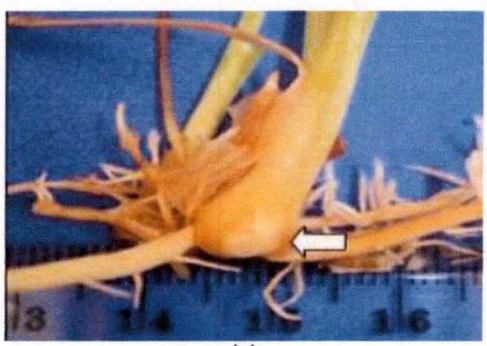


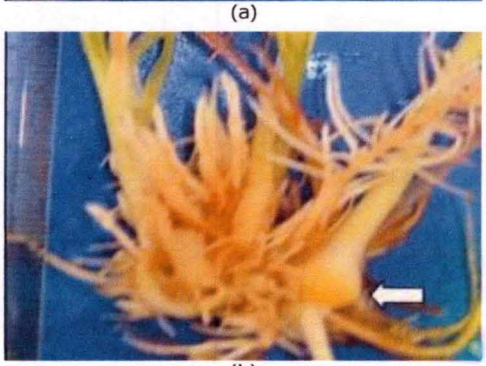

Figure **2**. Diameter of the micro rhizome on half and full strength MS medium

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

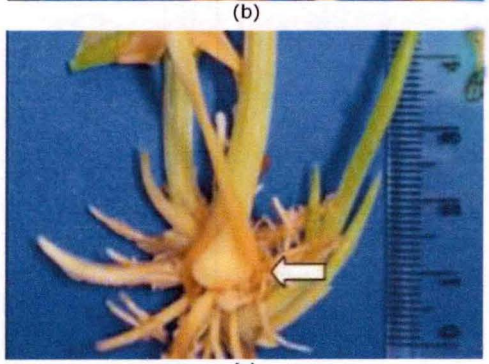
Along with the increasing of sucrose, total micro rhizome formation also increased on media without BAP and with 1, 2 $gL^{-1}$ BAP (Fig 3a, b, c). Otherwise, the number of micro rhizome progressively decreasing on media with 3 $gL^{-1}$ BAP (Fig. 4). According to Rahmawati *et al.* (2003) the amount 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.



(a)



(b)



(c)

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 $gL^{-1}$

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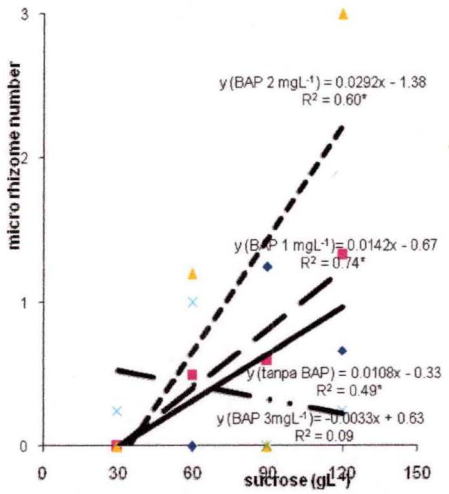


Figure **4**. Number of micro rhizome on medium without BAP and with BAP 1, 2, 3 $mgL^{-1}$

----- linear (without BAP)
-- -- linear (BAP 1 $mgL^{-1}$)
- - - linear (BAP 2 $mgL^{-1}$)
-- - linear (BAP 3 $mgL^{-1}$)
* : significant a α = 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 senescene 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 Senescene 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 α = 5% ,**: highly significant at α = 1%

Table **3**. Correlation between leaf length, number of senescene 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 Senescene 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 α = 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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ISBN No. 978-979-25-1209-0