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The 2nd International Symposium on Temulawak

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

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

LAY-OUT

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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.

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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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The Effectivity of Indol-3-Butyric Acid (IBA), 6-Benzyl Amino Purin (BAP), and Sucrose to Improve *In Vitro* Multiplication of *Temulawak* (*Curcuma xanthorrizha* Roxb.)

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ABSTRACT

Temulawak is one of the most important medicinal plant. Superior propagule is needed to improve productivity and quality of *temulawak*. Tissue culture or *in vitro* culture is an alternative way to produce superior and healty propagule. Application of plant growth regulator and energy source are very important for *in vitro* plant regeneration and growth. The objective of this reseach has been to study the effect of IBA, BAP and sucrose consentrations added to MS medium on *in vitro* regeneration of *temulawak*. This research consists of two factorial experiments. The first experiment was to study the effect of IBA (0, 0.25, 0.5, 0.75 mg/L) and BAP (0, 1, 2 ,3 mg/L). The second experiment was to study the effect of BAP (0, 1, 2 and 3 mg/L) and surose (30, 40, 50 and 60 g/L). Experiments were arranged in Randomize of Completely Block Design. The result of first experiment showed that combination of IBA 0.75 mg/L with/without BAP gave the lowest shoot number, while appllication of 2 or 3 mg/L BAP with 0.0, 0.25 or 0.50 mg/L IBA can produce highest number of shoot. There were interaction between IBA dan BAP concentration on the number of shoot, leaves, and plantlet height. On the second experiment, result showed that BAP significantly increased the number of shoot but there was no significant different between BAP 1, 2 and 3 mg/L. BAP more than 1.0 mg/L reduced plantlet height, while sucrose at 40 g/L gave the highest plantlet height.

Keywords: regeneration, propagule, medicinal plant, number of shoot, plantlet

INTRODUCTION

Temulawak (*Curcuma xanthorrhiza* Roxb.) of the family *Zingiberaceae* is a perennial rhizomatous medicinal plant. Indonesia is the center of origin of *temulawak*. *Temulawak* is one of the most importan ingredient of "Jamu", an Indonsian famous herbal medicine. The main active ingredients of *temulawak* are the yellow compound class of curcuminoids and volatile oil of xanthorrizol. Curcuminoids has positive effect on neutralizing toxins, relieving pain, lowering cholesterol and triglycerides levels of blood, while xanthorrizol possibly has positive effect on controlling breast cancer, lung, ovarian, antibacterial prevention of tooth enamel damage (Sidik 2006).

Conventionally, *temulawak* is propagated through underground rhizomes which has several constraints in temulawak production sistem including slow multiplication rate of rhizome, expensive maintenance of planting material, yield depend on the size of planted rhizome, susceptibility to various diseases and pests. The use of large rhizome as propagule to obtain high yields causes less rhizome to sell, and in term will reduce farmers income. So, to improve quantities and qualities of rhizome to sell, a more efficient protocol for temulawak propagation system is needed.

Tissue culture technique is one of possibility since tissue culture is not only for multiplication but also as vehicle for biotechnology-baed crop improvement. George and Sherrington (1984) noted that in vitro regeneration of explants need media consisting of anorganic salts, organic compounds and plant growth regulator. The two most imortant plant growth regulator in tissue culture are auxin and cytokinin. Auxin, for instance Indol-3-Butyric Acid (IBA), in tissue culture is used to improve callus, shooth and root growth (Pierik, 1987). Cytokinin also improve callus formation and improve growth and development of shoot because cytokinin physiologically support cell multiplication, morfogenesis or cytokinesis, retard senescence and abscici. 6-Benzyl Amino Purin (BAP) is one of cytokinin. BAP is effective for *in vitro* multiplication of turmeric (Nasirujjaman *et al.*, 2005; Naz *et al.*, 2009; Roopadarshini, 2010). Energy source is very important in in vitro culture since it has no photosynthetic capacity yet. Sucrose is one of the most common energy sources in tissue culture. Khan *et al.* (2006) reported that sucrose at concentration 40 g/L is optimum for sugarcane shoot regeneration, and can also be used for shoot multiplication. However, there is no report on efectivity of IBA, BAP and sucrose on in vitro culture of temulawak yet.

The aims of this research was to study the effect of IBA, BAP and Sucrose concentration on in vitro multiplication of Temulawak.

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MATERIALS AND METHODS

The experiments were done at laboratory of BPPT, Bogor, Indonesia during October 2006-February 2007. The basal media for the experiment was MS (Murashige dan Skoog) suplemented with 30 g/L sucrose (experiment 1), 8 g/L agar, and plant growth regulator IBA and BAP based on treatments. Other materials were including aluminum foil, plastics, tissue paper, and others. The pH of medium was adjusted to 5.7 ± 0.1 before adding agar. Medium was autoclaved at 17.5 psi for 30 min at 121ºC. Each botle of experimental unit contain 20 ml of media.

This research consists of two experiments. The first experiment was to study the effect of IBA and BAP (6-Benzyl Amino Purine) on temulawak multiplication, consist of a factorial experiment of IBA (0.0, 0.25, 0.50, and 0.75 mg/L) and BAP (0.0, 1.0, 2.0, and 3.0 mg/L). The second experiment was to study the effect of BAP and sucrose concentration consist of a factorial of BAP (0.0, 1.0, 2.0, and 3.0 mg/L) and Sucrose (30, 40, 50 and 60 g/L). The experiments design was completely block design with 3 replications (first experiment) and 4 replications (second experiment). On both experiments, the experiment unit is 3 culture botles containt 1 explant per botle.

Explants used were rhizhome buds collected from the sprouted rhizomes of green house grown temulawak. Explants were sterilized using Dhitane-M45, clorox 20%, tween, sterilled aquades, alcohol, and betadine. After buds were washed with detergen, then washed at running tap water for 30 minutes, then buds were sterilized inside laminar air flow with 15% soap for 15 minutes, and rinsing 3 times with sterilled aquadest. Buds then were dipped in 2 g/L dithane-M45 and 2 drops tween for 20 minutes, and then rinsed 3 times rinsing sterille water. After that, several layers of buds were removed, the buds then sterrilized with 20% clorox for 20 minutes and 3 time rinsing with sterilled water. Another layers then removed and the buds then sterillized as before untill buds clear and ready to plant to media.

Explants then planted on precondition media (MS0; media MS withoud PGR) for 2 weeks to get non-contaminated explants. After 2 weeks, explants then planted onto treatments media. Culture was incubated for 9 weeks at 21 ± 3 °C with continuous light of 1000 lux.

The data obtained in this study were analyzed statistically by one-way analysis of variance (ANOVA) and covariance analysis of *Duncan's Multiple Range Test* at α 5%.

RESULT AND DISCUSSION

Number of Adventive Shoots

There was no significant effect of IBA and BAP on shoot proliferation untill 4 week after treatments (WAT), while at 6 and 8 WAT, there were significant effect of IBA/BAP interaction on shoot proliferation (Table 1). Data at 8 WAT showed that application of 2 or 3 mg/L BAP without IBA have similar result with application of BAP (1, 2, or 3 mg/L) with 0.25 or 0.50 mg/L IBA. In the other hand, application higher concentration of IBA (0.75 mg/L) reduced the capacity of temulawak to proliferate to the lowest number of shoots. So, application of 2 or 3 mg/L BAP is enough to improve proliferation of temulawak bud explant to produce 4.7-5.3 shoots/explant. Several other researchers found that application of 2 mg/L BA on turmeric (*Curcuma longa* Linn) produce 14.5 shoots/explant (Rahman *et al.*, 2004), while combination of BA with NAA or kinentin with NAA was not as effective as BA alone. Nasirrujaman *et al.* (2005) found that application of 4 mg/L BAP and 1 mg/L NAA produced highest number of shoot/explant of turmeric (6.7 shoots). Roospadarshini (2010) also found that BAP is better than kinetin for turmeric *in vitro* multiplication. Using media of Linsmaier and Skoog, application of 3.0 mg/L BAP can produce 36.2 shoots/explant from *in vivo* vegetative buds.

Data of the second experiment showed that there was no interaction between BAP and sucrose concentration on number of shoot. Table 2 also showed that there was no significant effect of sucrose on shoot number. Only BAP concentration levels have significant effect on shoot number. Application of BAP significantly improves proliferation ability of temulawak at 2 – 8 WAT. However, on 6 – 8 WAT there was no significant differences between BAP concentrations (1, 2 or 3 mg/L) (Table 2). Application of BAP at those concentrations can increase the number of shoot up to 3 fold higher than that of control. So, application BAP at 1 mg/L is effective to improve temulawak explants ability to proliferate. However, other researchers also found that the number of shoots/explant of turmeric was incresed by increased BAP concentration up to 5 mg/L (Naz *et al.*, 2009) or up to 3 mg/L (Roospadarshini, 2010).

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Tabel **1**. Effect of IBA and BAP on Number of Temulawak Shoots

IBA (mg/L)	BAP (mg/L)	Number of Shoots (WAT)			
		2	4	6	8
0	0	0.00	0.00	0.11^{d}	0.11^{e}
	1	0.22	0.33	1.11^{cd}	2.11^{cd}
	2	0.11	0.22	2.67^{ab}	**4.67^{ab}**
	3	0.11	0.66	3.45^{a}	**5.33^{a}**
0.25	0	0.00	0.00	0.11^{d}	0.89^{de}
	1	0.33	0.56	2.11^{bc}	3.44^{bc}
	2	0.11	0.44	2.78^{ab}	**5.22^{a}**
	3	0.11	0.56	3.33^{a}	**4.11^{ab}**
0.50	0	0.00	0.11	0.22^{d}	0.45^{e}
	1	0.11	0.66	2.45^{ab}	**4.33^{ab}**
	2	0.11	0.45	2.45^{ab}	**3.89^{ab}**
	3	0.11	0.89	2.56^{ab}	**4.44^{ab}**
0.75	0	0.00	0.00	0.00^{d}	0.00^{e}
	1	0.00	0.11	0.11^{d}	0.44^{e}
	2	0.00	0.00	0.00^{d}	0.11^{e}
	3	0.00	0.00	0.00^{d}	0.00^{e}
		ns	ns	**	**

Table **2**. Effect of BAP and Sucrose on Number of Temulawak Shoots

Treatments	Number of Shoots (Weeks after Treatments)			
	2	4	6	8
BAP (mg/L)				
0.0	0.21c	0.44c	0.54b	1.12b
1.0	1.08b	1.60b	2.37a	3.07a
2.0	1.27ab	1.91ab	2.39a	3.07a
3.0	1.57a	2.36a	3.00a	3.95a
Sucrose (g/L)				
30.0	0.98a	1.42a	1.85a	2.52a
40.0	1.01a	1.65a	2.17a	3.14a
50.0	0.98a	1.49a	1.94a	2.46a
60.0	1.16a	1.75a	2.34a	3.12a

Number of Leaves

Data of the first experiment (Table 3) showed that there were no interaction between IBA and BAP untill 4 WAT. At 6-8 WAT, there were significant interaction of IBA/BAP on number of temulawak leaves. However, the trend of the effect is not really clear.

Table **3**. Effect of IBA and BAP on Number of Temulawak Leaves

IBA (mg/L)	BAP (mg/L)	Number of Leaves (WAT)			
		2	4	6	8
0	0	0.22	1.89	3.33^{abc}	**4.44^{abc}**
	1	0.33	1.45	2.45^{cde}	3.56^{cd}
	2	0.00	0.89	3.56^{ab}	**4.89^{ab}**
	3	0.00	0.67	1.78^{e}	3.00^{d}
0.25	0	0.45	2.33	3.55^{ab}	**4.56^{abc}**
	1	0.22	1.11	2.33^{de}	3.67^{cd}
	2	0.22	0.89	2.78^{bcd}	**4.11^{abcd}**
	3	0.00	0.78	3.34^{abc}	**4.89^{ab}**
0.5	0	0.44	2.11	3.11^{abcd}	**4.00^{abcd}**
	1	0.22	0.67	3.00^{bcd}	**4.11^{abcd}**
	2	0.11	0.78	3.00^{bcd}	**4.33^{abc}**
	3	0.00	0.33	2.56^{cde}	**4.11^{abcd}**
0.75	0	**0.66**	**2.89**	**4.00^{a}**	**5.11^{a}**
	1	0.22	1.89	3.22^{abcd}	**4.11^{abcd}**
	2	0.00	1.44	2.67^{bcde}	3.78^{bcd}
	3	0.11	1.11	2.78^{bcd}	3.67^{cd}
		ns	ns	**	*

For instance, at 8 WAT, application of BAP at 0, 1, 2, 3 mg/L combined with 0.5 mg/L IBA have the similar number of leaves with those of control. Actually, all combination of treatments of IBA/BAP have higher number of leaves but was not significantly different with those of control. While application of 2-3 mg/L BAP with 0.75 mg/L IBA gave the lower number of leaves but similar to those of application of 1 or 3 mg/L BAP without IBA. So, there is no sharp conclusion can be drawn from those data.

Data of second experiment also showed significant interaction of BAP and sucrose concentration (Table 4) on 2-4 WAT, but the interaction was not significant on 6-8 WAT. Although statistically is not significant, application of 30 or 40 g/L sucrose with 3.0 mg/L BAP gave the best result with about 11 leaves per original explant. Media with normal sucrose concentration (30 g/L) suplemented with 3.0 mg/L BAP is effective enough to increase number of leaves to 10.6 per explant compare to 4.3 – 8.4 leaves on other combination of treatments. The second highest number of leaves is occur by application of 40 g/L sucrose and 1 mg/L BAP that produced 9.2 leaves per explant.

Tabel **4**. Efeect of BAP and Sucrose on Temulawak Leaves

Treatments		Number of Leaves (WAT)			
BAP (mg/L)	Sucrose (g/L)	2	4	6	8
0.0	30.0	1.39a	3.07ab	4.18	5.67
	40.0	0.92ab	2.33bcd	4.25	6.25
	50.0	0.75ab	2.00cdef	2.92	4.34
	60.0	0.34b	1.75cdef	3.34	5.75
1.0	30.0	0.92ab	1.42def	3.75	6.75
	40.0	1.00ab	2.17bcde	4.92	9.25
	50.0	0.75ab	1.25ef	3.09	7.00
	60.0	0.42b	1.50def	4.17	8.25
2.0	30.0	0.42b	1.17ef	2.92	5.75
	40.0	1.42a	2.58bc	5.17	8.75
	50.0	0.61b	1.25ef	3.21	6.36
	60.0	0.67b	1.09f	4.08	7.33
3.0	30.0	0.79ab	1.77cdef	5.86	**10.6**
	40.0	1.42a	3.67a	6.84	**11.2**
	50.0	0.42b	1.44def	4.04	7.98
	60.0	0.42b	1.28ef	3.44	8.42

Plantlet Height

Temulawak plantlet height was significanly affected by interaction of IBA and BAP at 9 WAT (at the end of experiment when the plantlet was harvested from the botle). However, the trend of effectiveness was also not really clear. All combinations of IBA/BAP, except combination of 0.5 mg/L IBA with 2 or 3 mg/L BAP, or treatment of 3 mg/L BAP without IBA, gave the similar or non significant difference height range from 6.1 – 7.6 cm (Table 5).

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Application of 3 mg/L BAP alone gave the shortest plantlet of 3.4 cm but was not significantly different with result of application 2 or 3 mg/L BAP with 0.5 mg/L IBA that gave 4.3 - 4.8 cm plantlet height. Data of Table 3a showed that increasing BAP will tend to reduce plantlet height. Nasirujjaman *et al.* (2005) and Naz *et al.* (2009) also found that application of BAP increase the number of shoots/explant but decrease the shoot lenght or plantlet height.

Table **5**. Effect of IBA and BAP on Temulawak Plantlet Height (cm) at 9 WAT

IBA (mg/L)	BAP (mg/L)			
	0	**1**	**2**	**3**
0.0	**7.56^{a}**	6.78^{abc}	7.46^{a}	**3.39^{e}**
0.25	6.69^{abc}	6.20^{abcd}	6.92^{ab}	6.09^{abcd}
0.50	6.01^{abcd}	5.46^{abcd}	**4.76^{cde}**	**4.32^{de}**
0.75	5.21^{bcde}	6.51^{abc}	6.72^{abc}	6.81^{abc}

Data of second experiment showed that there was no significant interaction between BAP and sucrose concentration on plantlet height. However, application of BAP >1 mg/L significantly reduced the plantlet height, while appliation of 40.0 g/L sucrose significantly improved plantlet height (Table 6). The result of the second experiment, that BAP decreased plantlet height is supported by the result of Nasirujjaman *et al.* (2005) and Naz *et al.* (2009) on turmeric. Khan *et al.* (2006) also found that sucrose at concentration 4% was optimum for sugarcane shoot regeneration, and can also be used for shoot multiplication. Increasing the sugar to more than 40 g/L reduce leaf number and plantlet height. Javed and Ikram (2008) noted that increasing the sucrose consentration will reduce growth rate, macro cations (K^{+}, Ca^{2+}, Mg^{2+}) and micro cations (Mn^{2+}, Fe^{2+}) of wheat culture.

Table **6**. Effect of BAP and Sucrose on Temulawak Plantlet Height at 9 WAT

Treatments BAP (mg/L)	Plantlet Height (cm)	Treatments Sucrose (g/L)	Plantlet Height (cm)
0.0	10.16a	30.0	9.48b
1.0	9.90ab	40.0	11.08a
2.0	9.15b	50.0	9.13b
3.0	9.01b	60.0	8.52b

CONCLUSION

Appllication of 2 or 3 mg/L BAP without IBA or in combination with 0.25 or 0.50 mg/L IBA can produce high number of shoot range from 4.1 to 5.3 shoot/esplant. There were interaction between IBA dan BAP concentration on the number of shoot, leaves, and plant height. IBA at 0.75 mg/L inhibited explant multiplication. Effect of IBA/BAP on number of leaves was significant but the trend of effect was not clear. Application of 3 mg/L BAP without IBA reduced plant height, but application of 1-3 mg/L BAP with 0.25-0.75 mg/L IBA retained plantlet height. While on the second experiment, result showed that BAP significantly increased the number of shoot but there was no significant different effect of BAP 1, 2 and 3 mg/L, but at > 1 mg/L it reduced plantlet height. Thus, application of 1.0 mg/L BAP was effective. Sucrose did not have signifiant effect on number of shoot, number of leaves, but at 40 g/L it significantly gave the highest plantlet height.

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