VOL. 7, NO. 10, OCTOBER 2012 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science
©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.

www.arpnjournals.com

THE EFFECT OF BAP AND THIDIAZURON ON *IN VITRO* GROWTH OF JAVA TURMERIC (*Curcuma xanthorrhiza* Roxb)

Suci Rahayu and Widiati Hadi Adil
Indonesian Agency for Agricultural Research and Development
Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Jl. Tentara Pelajar 3A, Bogor, West Java
E-Mail: uchie_sr@yahoo.com

ABSTRACT

Java turmeric (*Curcuma xanthorrhiza* Roxb), a natural medicinal crop, has potential to be developed due to a great demand of raw material for Jamoe industry in Indonesia. A technology for rapid propagation such as *in vitro* propagation is required to meet the need of this crop. The aim of this research was to ascertain the effect of BAP either alone or in combination with Thidiazuron on the growth of Java turmeric through *in vitro* culture. The research was conducted at the Laboratory of Cell Biology and Tissue Culture Division, the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Bogor from January to June 2009. The research was arranged in a Fully Randomized Design with 10 treatments and 4 replications. The treatments consisted of MS medium enriched with BAP (0, 1, 3, 5, and 7 mg/l) either alone or in combination with 0.1 mg/l Thidiazuron. The results showed that 5 mg/l BAP was the best media for all growth components in terms of shoots number, shoot height, roots number, root length and leaves number. No effect of Thidiazuron was shown on shoot regeneration of Java turmeric. These findings suggested that a further research should be conducted to find out the appropriate concentration of Thidiazuron for inducing the growth of Java turmeric.

Keywords: *Curcuma xanthorhiza* Roxb, micropropagation, BAP, thidiazuron, *in vitro*.

INTRODUCTION

Java turmeric (*Curcuma xanthorrhiza* Roxb) is one of the raw materials of traditional medicine possessing function of anti-inflammatory and anti tumor (Sugaya, 1992). This commodity plays an important role in supporting the development of the traditional medicine industry in Indonesia. From very intensive studies worked by Indonesian and overseas researchers it had been resulted various products such as standard herbs, snack and soft drink, mints, even toothpaste and shampoo. As a result, the demand for this crop steadily increases and in turn the majority of Java turmeric from the main production areas such as Central Java and East Java can be absorbed by industries (Purwakusumah *et al*., 2008).

A great demand upon Java turmeric has impact on the continuous, ample availability of planting material. On the other hand an efficient technology of Java turmeric propagation is so limited that it needs to be developed and attempted. This seems due to most of the studies more emphasizing on the crop function and marketing rather than agricultural aspect. One of the propagation methods that may be able to assure the sufficiency of planting materials is *in vitro* tissue culture. Through this technique, seedlings can be produced in a great number and short time. In addition, the seedlings produced from *in vitro* culture can be free from pests and diseases.

On *in vitro* propagation, Benzyl Adenine Purine (BAP) is the most common cytokinin plant growth regulator used to induce cell differentiation. Another plant growth regulator that is also commonly used for *in vitro* propagation is Thidiazuron (TDZ). It is able to stimulate cell segregation and commonly used to induce shoots and somatic embryo (Kern and Meyer, 1986; Thomas and Katterman, 1986; Huetteman and Preece, 1993; Kanakis and Demetriou, 1993; Lu, 1993; Chand, *et al*., 1999).

Hence, supplementing BAP and Thidiazuron either singly or in combination is expected to be able to trigger shoot growth of Java turmeric. According to Nielsen *et al*. (1995), a medium containing two different kinds of cytokinin could improve quality and number of produced shoots compared with that containing only one kind.

The objective of this study was to ascertain the effect of plant growth regulator BAP and Thidiazuron on *in vitro* shoot propagation of Java turmeric. The findings of this study are expected to be guidance in developing *in vitro* regeneration of Java turmeric.

MATERIALS AND METHODS

The study was carried out in the *in vitro* culture laboratory of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development in Bogor, from January to June 2009. Shoots generated from *in vitro* culture of Java turmeric were used as explants. These explants were cultured on MS media (Murashige and Skoog, 1962) enriched with BAP (0, 1, 2, 3, 5 and 7 mg/l) either with or without Thidiazuron (0.1 mg/l). Sucrose (30 g/l) as carbon source and Gelrite (2.5 g/l) as solidifier were added into all media. As much as 25 ml of media was poured into each jam bottle and then the bottle was covered with aluminum foil. Prior to sterilizing with autoclave, the pH of the media was adjusted to 5.7. Sterilization was performed at 121^{o}C and 110 Kpa for 20 minutes. All cultures were placed on culturing racks in the room with 26 ± 2ºC air temperature and 1217 µmol $m^{-2}s^{-1}$ light intensity using fluorescent lamp for 16 h/day.

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.


The study was arranged in a fully randomized design (FRD) comprising 10 treatments (5 concentrations of BAP with 0.1 mg/l Thidiazuron and 5 concentrations of BAP without Thidiazuron) with 4 replications. Parameters observed were shoot number, shoot height, root number, root length, and leaf number. If the data sets contain some zeroes, the original data is transformed into $\sqrt{x+0.5}$ first before analyzed. With the exception of shoot length data that was analyzed with analysis of covariance, data of the other parameters were analyzed with analysis of variance.

RESULTS AND DISCUSSIONS

Except on the root length, there were no significantly different effects among treatments on the other parameters observed in four weeks after culture (Table-1). In general, supplementing 5 mg/l BAP into media could provide the best effect on all growth components of Java turmeric shoot explant. Among the media containing Thidiazuron, only the media + 0.1 mg/l Thidiazuron seemed to give good effect, especially on root length, root number and leaf number, and even better than that enriched with 5 mg/l BAP. Visually, the green leaves were growing on all treatments after 4 weeks of culture.

Table-1. Effect of BAP and thidiazuron on shoot number, shoot height, root number, root length, and leaf number in regeneration of Java turmeric, four weeks after culture

Treatment (mg/l)	**Shoot number**	**Shoot height** (cm)	**Root number**	**Root length** (cm)	**Leaf number**
0 mg/l BAP (MS only)	0.25 a	1.33 a	0.50 a	0.70 ab	1.00 a
1 mg/l BAP	0.75 a	1.70 a	1.75 a	0.93 ab	2.00 a
3 mg/l BAP	1.50 a	1.63 a	2.25 a	0.56 ab	1.00 a
5 mg/l BAP	2.00 a	2.10 a	2.75 a	1.46 a	1.75 a
7 mg/l BAP	1.00 a	1.00 a	0.50 a	0.21 ab	0.75 a
0.1 mg/l TDZ	1.50 a	1.51 a	2.25 a	1.13 ab	1.75 a
1 mg/l BAP + 0.1 mg/l TDZ	0.25 a	0.75 a	0.00 a	0.00 b	0.25 a
3 mg/l BAP + 0.1 mg/l TDZ	1.00 a	1.65 a	0.00 a	0.00 b	1.25 a
5 mg/l BAP + 0.1 mg/l TDZ	1.50 a	1.89 a	1.00 a	0.98 ab	1.00 a
7 mg/l BAP + 0.1 mg/l TDZ	1.00 a	0.37 a	0.00 a	0.00 b	0.33 a

Means in a column followed by the same letters are not significantly different at 5% level of DMRT

From Table-1, it appears that among media treatments, 5 mg/l BAP medium could produce the greatest number and highest of shoots as well as roots. The same result revealed that among combinations between BAP and Thidiazuron, media containing 5 mg/l BAP + 0.1 mg/l Thidiazuron gave most significant effect on the growth components of Java turmeric. This indicated that concentration of 5 mg/l BAP was the most appropriate concentration to *in vitro* culture of Java turmeric. This result confirmed the result of research worked by Nayak (2000) that 5 mg/l BAP was the optimal concentration for shoot propagation and rooting in *Cucurma aromatica* Salisb. *In vitro* regeneration of shoot is not only influenced by concentration of cytokinin but also by kind of cytokinin used (Tyagi *et al*., 2004). Furthermore, from their research results on 8-wild species of *Curcuma*, Tyagi *et al*. (2004) concluded that BAP was known to have higher effect on regeneration of plantlets compared to cytokinin. The reason why in the present study the use of plant growth regulators either alone or in combination had not yet affected the growth components after 4 weeks of culture was that the cultured explants likely still adapt to those plant growth regulators added in media.

In eight weeks after culture all growth components seemed to increase more significant than in four weeks after culture. Complementing 5 mg/l BAP in media still gave the best effect on the growth components of Java turmeric explant. Good performance of several shoots growing vigorously with green leaves and well roots on MS media enriched with 5 mg/l BAP can be seen in Figure-1. The significant differences ($p \leq 0.05$) among effects of the treatments on the number and length of roots are presented in Table-2. The highest number of roots was produced on media containing 3 mg/l BAP (7.25) followed by media containing 5 mg/l BAP (6.0). On media containing Thidiazuron, in fact, only on the medium of 0.1 mg/l Thidiazuron was the roots growing in high enough number (3.25).

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.

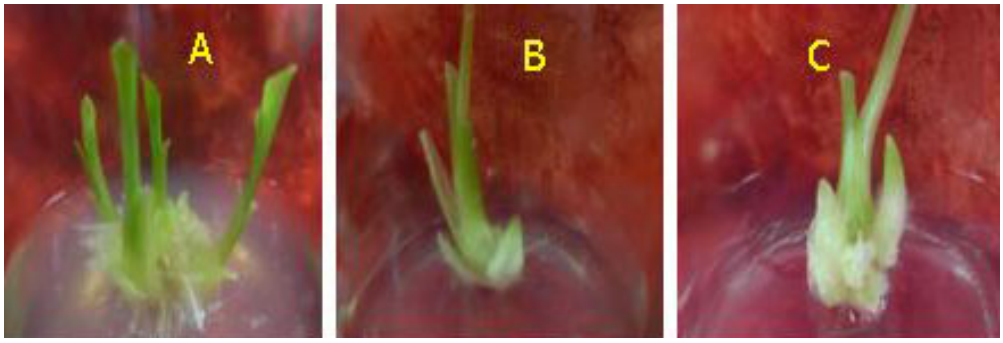

Figure-1. The growth of Java turmeric on the media 5 mg/l BAP (A), 0 mg/l BAP (B), and 5 mg/l BAP + 0.1 mg/l TDZ (C), 8 weeks after culture.

Concerning the root length, media containing 5 mg/l BAP singly could produce the longest root (1.43 cm), while media containing 0.1 mg/l Thidiazuron alone and combined with 5 mg/l BAP could produce 1.05 cm and 1.00 cm, respectively. The formation of the roots on media containing Thidiazuron both alone and in combination with BAP did not mean that the two plant growth regulators were suitable for roots inducing. This is due to the fact that Thidiazuron is a plant growth regulator possessing capability of rooting inhibition (George, 1993; Sango *et al*., 1996). Mutui *et al*. (2005) who worked on geranium proved that Thidiazuron did not only influence on the number and growth of roots but also inhibit the formation of roots. Therefore, the researchers generally used auxins such as IBA and NAA either alone or in combination to induce rooting, for examples, Sango *et al*. (1996) working on nuts crop and Mutui *et al*. (2005) working on geranium.

Table-2. Effect of BAP and thidiazuron on shoot number, shoot height, root number, root length, and leaf number in regeneration of Java turmeric, eight weeks after culture.

Treatment (mg/l)	**Shoot number**	**Shoot length** (cm)	**Root number**	**Root lenght** (cm)	**Leaf number**
0 mg/l BAP (MS only)	0.50 a	1.44 a	2.00 bc	0.32 bc	0.75 a
1 mg/l BAP	2.50 a	1.08 a	1.75 bc	1.10 ab	1.00 a
3 mg/l BAP	2.25 a	2.27 a	7.25 a	1.01 ab	1.36 a
5 mg/l BAP	2.50 a	2.95 a	6.00 ab	1.43 a	1.06 a
7 mg/l BAP	1.00 a	1.41 a	0.50 c	0.30 bc	0.75 a
0.1 mg/l TDZ	2.50 a	1.65 a	3.25 bc	1.05 ab	0.88 a
1 mg/l BAP + 0.1 mg/l TDZ	0.50 a	0.39 a	0.00 c	0.00 c	0.25 a
3 mg/l BAP + 0.1 mg/l TDZ	1.25 a	1.61 a	0.00 c	0.00 c	1.00 a
5 mg/l BAP + 0.1 mg/l TDZ	2.50 a	0.91 a	1.00 c	1.00 ab	0.64 a
7 mg/l BAP + 0.1 mg/l TDZ	1.00 a	0.85 a	0.00 c	0.00 c	0.58 a

Means in a column followed by the same letters are not significantly different at 5% level of DMRT

In general, from Tables 1 and 2 it appears that supplementing Thidiazuron has not yet shown a significant effect on the culture of Java turmeric shoot although some researchers explain that Thidiazuron is a good cytokinin for inducing shoot regeneration, even it is known to have biological activity higher than other cytokinin types of adenin (Mok *et al*., 1987; Niedz *et al*., 1989; Huetteman and Preece, 1993). In this study the insignificant effect of Thidiazuron maybe because Thidiazuron concentration given in media was so low that it was not able to stimulate the formation or growth of Java turmeric shoot. Concentration of Thidiazuron that is commonly used in other crops is 0.2-0.3 mg/l, e.g. in *Poncirus trifoliata* L. Raf. (Le *et al*., 1999), *Brassica oleracea* (Cheng *et al*., 2001) and *Brassica napus* L (Jonoubi *et al*. (2004).

Addition of Thidiazuron combined with BAP in MS medium generally gives effect poorer than that of Thidiazuron only. This result was opposite to the statement of Jonoubi *et al*. (2004) and Zimmerman and Scorza (1992) that medium supplemented with Thidiazuron and another cytokinin could effectively improve shoot regeneration. The previous research results indicated that medium enriched with BAP and Thidiazuron could affect the shoot growth of the shoot

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.


explants on peach (Zimmerman and Scorza, 1992), on bread fruit (*Arthocarpus communis* Forst) (Mariska *et al.*, 2004) and star fruit (*Averrhoa carambola* L.) (Supriati *et al*., 2006). On the contrary, the result reported by Prathanturatug *et al*. (2003 and 2005) showed that the use of Thidiazuron alone in MS medium gave better effect on shoot growth of the other curcuma relatives like *Curcuma Longa* L. Hence, those results confirmed the result of the current study. Apparently, synergistic effect between Thidiazuron and other cytokinin types had not yet occurred in those researches.

CONCLUSIONS

Based on the results of this present study it can be concluded as follows:

a) MS media supplemented with 5 mg/l BAP was the most suitable medium for shoot regeneration of Java turmeric; however, its significant effect was found only on root length and root number.
b) Supplementing Thidiazuron in media did not significantly affect on regeneration of Java turmeric, even tended to inhibit the growth of roots both on number and length.

Further study should be addressed to find out the more proper concentration of Thidiazuron so as to induce the formation and growth of shoots. In contrast, in inducing the Java turmeric roots it should not supplement Thidiazuron in the media.

ACKNOWLEDGMENT

We are deeply grateful to Dr. Nurliani Bermawi for providing planting materials used in this research and Dr. Ika Roostika for her comment and critical discussion.

REFERENCES

Chand H., M.N. Pearson and P.H. Lovell. 1999. Rapid vegetative multiplication in *Colocasiaesculenta* (L) Schott (taro). Plant Cell Tiss. Org. Cult. 55: 223-226.

Cheng P. K., P. Lakshmanan and S. Swarup. 2001. High frequency direct shoot regeneration and continuous production of rapid-cycling *Brassica oleracea in vitro*. *In Vitro* Cell. Dev. Biol. Plant. 37: 592-598.

George E.F. 1993. Plant propagation by tissue culture: Part 1 The technology. London: Exegetics Ltd.

Huetteman C.A. and J.E. Preece. 1993. Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell. Tiss. Org. Cult. 33: 105-119.

Jonoubi P., A. Mousavi and J. Majd. 2004. Improved *Brassica napus* L., regeneration from hypocotyls using Thidiazuron and Benzyladenine as cytokinin sources. Pak. J. Bot. 36(2): 321-329.

Kanakis A.G. and K. Demetriou. 1993. *In vitro* shoot regeneration of globe artichoke from shoot apices treated with Thidiazuron and from mature zygotic embryos treated with cytokinins. J. Hort. Sci. 68: 439-445.

Kern H.R. and M.M. Meyer. 1986. Tissue culture propagation of *Acer* x freemanii using Thidiazuron to stimulate shoot tip proliferation. Hort. Sci. 21: 1209-1210.

Le B.V., N.T. Ha, T.A. Hong and K.T.T. Van. 1999. High frequency shoot regeneration from trifoliate orange (*Poncirus trifoliata* L. Raf.) using the thin cell layer. Science de la Vie. 322(12): 1105-1111.

Lu C.Y. 1993. The use of thidiazuron in tissue culture. *In Vitro* Cell. Dev. Biol. 29: 92-96.

Mariska I., Y. Supriati dan S. Hutami. 2004. Mikropropagasi sukun (*Arthocarpus communis* Forst), tanaman sumber karbohidrat alternatif. Kumpulan Makalah Seminar Hasil Penelitian BB-BIOGEN hal. 180-188.

Mok M.C., D.W.S. Mok, J.E. Turner and C.V. Muser. 1987. Biological and biochemical effects of cytokinin-active phenylurea derivatives in tissue culture systems. Hort Science. 22: 1194-1197.

Murashige T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant. 15: 473-497.

Mutui T.M., H. Mibus and M. Serek. 2005. Effects of thidiazuron, ethylene, abscisic acid and dark storage on leaf yellowing and rooting of Pelargonium cuttings. J. of Hort. Science and Biotech. 80(5): 543-550.

Nayak S. 2000. *In vitro* multiplication and microrhizome induksi in *Curcuma aromatica* Salisb. Plant Growth Regulation. 32(1): 41-47.

Niedz R.P., S. Smith and K.B. Dunbar. 1989. Factors influencing shoot regeneration from cotyledonary explants of *Cucumis melo*. Plant Cell Tiss. Org. Cult. 18: 313-319.

Nielsen J.M., J. Hansen and K. Brandt. 1995. Synergism of thidiazuron and Benzyladenine in Axillary shoot formation depends on sequence of application in *Mischantus xogiformis* 'Giganteus'. Plant Cell Tiss. Org. Cult. 44: 165-170.

Prathanturatug S., N. Soonthornchareonnon, W. Chuakul, Y. Phaidee and P. Saralamp. 2003. High-frequency shoot multiplication in *Curcuma longa* L. using thidiazuron. Plant Cell Report. 21(11): 1054-1059.

Prathanturatug S., N. Soonthornchareonnon, W. Chuakul, Y. Phaidee and P. Saralamp. 2005. Rapid micropropagation of *Curcuma longa* L. using bud explants

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.


precultured in thidiazuron supplemented liquid medium. Plant Cell, Tissue and Organ Culture. 80(3): 347-351.

Purwakusumah E.D., Y. Lestari, M. Rahminiwati, M. Ghulamahdi, B. Barus dan M. Machmud. 2008. Menjadikan temulawak sebagai bahan baku utama industri berbasis kreatif yang berdaya saing. Pusat Studi Biofarmaka LPPM-IPB. 24 hal.

Sango M.H.M, V.I. Shattuck and J. Strommer. 1996. Rapid plant regeneration of pea using thidiazuron. Plant Cell, Tiss. and Org. Cult. 45: 165-168.

Sugaya A. 1992. Micropropagation of turmeric (*Curcuma domestica* Valet) and other Curcuma species. *In*: Biotechnology in Agriculture and forestry. Vol. 19. High-Tech and Micropropagation III. Y.P. Bajaj (Ed.). Springer-Verlag Berlin.

Supriati Y., I. Mariska dan Mujiman. 2006. Multiplikasi belimbing dewi (*Averrhoa carambola*) melalui kultur *in vitro*. Buletin Plasma Nutfah. 12(2): 50-55.

Thomas J.C. and F.R. Katterman. 1986. Cytokinin activity induced by thidiazuron. Plant Physiol. 81: 631-683.

Tyagi R.K., A. Yusuf, P. Dua and A. Agrawal. 2004. In vitro plant regeneration and genotype concervation of eight wild species of Curcuma. Biologia Plantarum. 48(1): 129-132.

Zimmerman T.W. and Scorza R. 1992. Shoot growth and proliferation of peach under varying environmental regimes. HortScience. 27: 696.