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Callus and hairy root induction of *Melaleuca cajuputi* Powell

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Abstract It is stated that the lowest percentage of contamination at 49.63% and highest percentage of survival at 37.95% after sterilized with 70% ethanol for 1 min and 1.8 % NaOCl for 30 min. The bud explants were cultured on Murashige and Skoog (MS) medium supplemented with 0, 0.02, 0.04, 0.06, 0.08 mg/l 2,4-Dinitrophenylhylhydrazine (2,4-D) combination with 0, 0.02, 0.04, 0.06, and 0.08 mg/l Thidiazuron (TDZ) for callus induction. The results showed that the explants were cultured on MS medium supplemented with 0.06 mg/l TDZ resulting to be suitable for callus induction with biggest size at 2.56x1. 26cm of width and length. Culturing buds on MS medium supplemented with 0.02 mg/l TDZ had the highest averaged shoot number. The roots were cultured in liquid MS medium supplemented with 0.04 mg/l TDZ, 0.04 mg/l 2.4-D gave the highest weight of hairy roots.

Keywords: Callus induction, Hairy root, Thidiazuron, 2,4-Dichlorophenoxyacetic acid

Introduction

Melaleuca cajuputi Powell is a large perennial plant that reaches 35 meters high and stem wide 1.2 meters. It is an important economic tree in southern of Thailand. Melaleuca genus has about 250 species by about 220 species found in foreign countries. For in Thailand have the survey found only one type that is *M. cajuputi*. There are 3 subspecies, Cumingiana (Turcz) Barlow, Platyphylla Barlow and Cajuputi supsp. For type found in Thailand is in the subsp. Cumingiana (Turcz) Barlow. Which is the same type found in Myanmar, Vietnam and Malaysia (Craven and Barlow, 1999; Oyen and Dung, 1999; Southwell and Lowe, 1999). There is vernacular name that call differently according to local for instance the central region is called a Samet, Southern region is a called med. It is a multipurpose tree that every part is usable and the local people have been recognized its usefulness for a long time. Stems of cajuput tree are used for structural post, fuelwood, charcoal

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production, fence, platform, fishing rod, agricultural pole and stake etc. Wood is used for construction and bark is for siding, roofing, boat-sealing material and dying etc. Leaves are used as medicinal purposes and sources of cajuput oil, to use to make drugs, insecticide and kill microorganisms that cause acne. This plant provides good quality and the price is higher than the oil extracted from the eucalyptus leaves (Thinh, 1997) and bring the leaves to boil for drinking instead of tea and to help treat aches, jaundice, asthma, anthelmintics, cough and drink helps to the uterus of women after childbearing into a garage quickly. The cajuput tree forest as a keep source *Boletus griseipurpureus.* Corner and planted as a shade plant, planted as a fence and windbreak. Which is considered a wood that can be used to benefit every part of the plant. *M. cajuputi* is able to grow in a wide range of environment disadvantages including high acid soil, saline soil, arid soil and water-locked soil. This plant has a special ability to adaptation well and is the fastest growing plant when comparing other plants. Especially in the swampy areas along the edge, the peatland can grow very well. Mostly distribute in large groups in dense swamp forests makes sometimes. Therefore, considered that *M. cajuputi* forest is a replacement forest that occurred after the original forest was destroyed. From the survey in Thailand found that the swampy areas along the edge, the peatland which is generally distributed over a total area of more than 300,000 acres (Nuyim and Benjachaya, 2007). The current conditions of most of these areas are being left uncultivated areas, where other agricultural plants and forest plants grow poorly and yielding that are not worth but the area where the *M. cajuputi* can grow very well. Therefore, it is considered to be a high potential area for the public and government agencies to choose to grow cajuput tree to develop into an area of economic forest plantation. All of the benefits mentioned above in the various properties. It is necessary for the propagate species of *M. cajuputi* to conservation the plant species and continue to use the benefits. Moreover, the propagation by tissue culture method is convenient and saves space as well as being able to propagate species large quantity and quickly as well. The objective was aimed the effects of TDZ and 2,4-D growth regulators on the somatic embryo formation in *M. cajuputi* using plant tissue culture techniques.

Materials and Methods

Sterilized explants

The young branches of cajuput were collected from the forestry in Chumphon province. The young branch and shoot tip without leaf about 10-12

cm were washed in running water for 1 hour, rinsed in 70% ethanal for 1 min, surface sterilization in 1.8 % NaOCl for 30 min, and followed by 5 min washing with distilled water for 3 times. The lateral bud was used as explant with the size of 1 cm. Murashige and Skoog medium was supplemented with 8 g/l agar as the basal medium for all of the experiments. The media were into baby jar containing 15 ml of medium, adjusted to pH 5.6 before autoclaved at 121˚C for 20 min. The cultures were incubated at 25 ± 2˚C with a 16-h photoperiod under white fluorescent lamps and sub-cultured every 4 weeks.

Callus induction

Callus was initiated by culturing the node of explants 1 cm on MS media containing combinations of plant growth regulators (PGRs) 0, 0.02, 0.04, 0.06, 0.08 mg/l 2,4-D combination with 0, 0.02, 0.04, 0.06, 0.08 mg/l TDZ. The experimental design was performed in completely randomized design (CRD) 25 treatments, each treatment consisted of 5 cultures and each experiment was repeated three times. Data were statisticaly analysed by Dancan's multiple rang test.

Root culture

The hairy root length of 1 cm. was cultured in 50 ml of MS liquid medium supplemented with 0.04 mg/l TDZ, 0.04 mg/l 2,4-D in 120 ml Erlenmeyer flask and MS medium free hormone was a control. The explant was cultured on the 90 rpm shaker and under dark condition at temperature 25±2 ℃ for 30 days. The experiment was performed in completely randomized design (CRD) 3 treatments, each treatment consisted of 5 cultures.

Statistic analysis

The experiment was repeated three times. All data were analyzed by analysis of varience (ANOVA) and means were compared using the least significant difference (LSD) at 5% probability level and Duncan's multiple range tests.

Results

Induction of callus

After 8 weeks of culture, the callus was obtained from node explants culturing in MS medium contained with TDZ and 2,4-D. The control MS

medium without hormone was found shoots induction. The root induction was obtained in MS medium with 2,4-D and without TDZ (Fig.1). The largest callus was obtained in culturing on MS medium supplemented 0.06 mg /l TDZ. The calli occured the most width and length dimensions of 2.56 and 1.26 cm, respectively (Fig. 1 and Fig. 2).

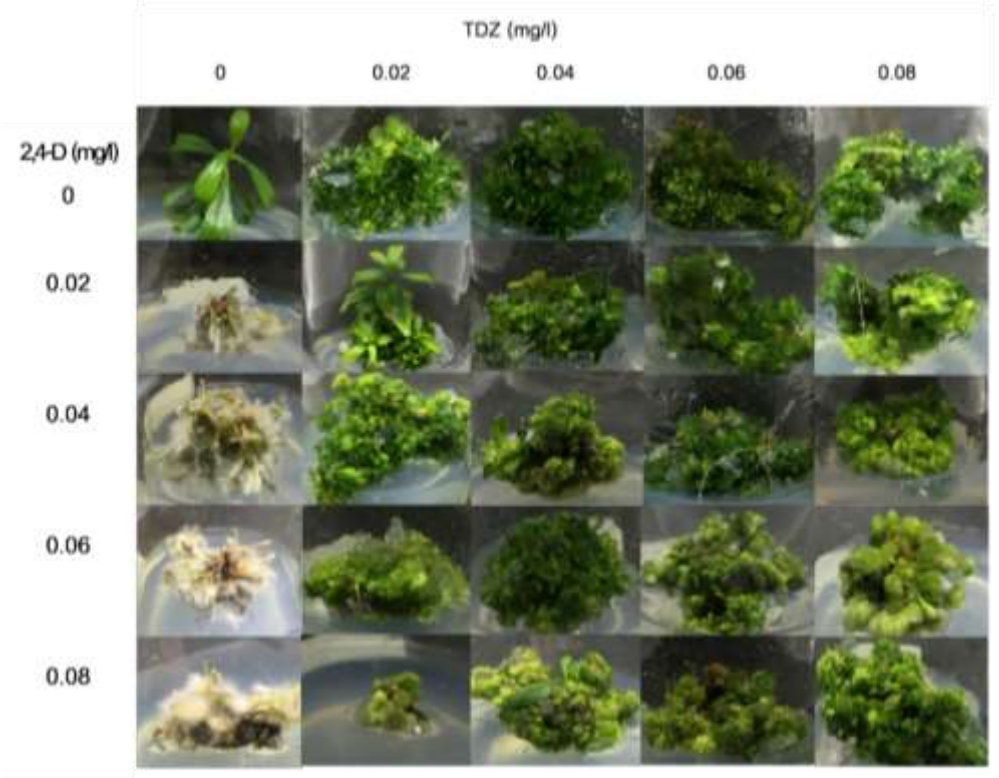

Figure 1. Cullus and shoot induction on MS medium containing TDZ and 2,4-D after 8 weeks

Figure 2. Plant regeneration from node after 8 weeks (A) shoot (B) root formation (C) callus formation

The effect of TDZ combination with 2,4-D on growth the node was studied. The results showed the MS medium supplemented with 0.02 mg/l TDZ. and 0.02 mg/l 2, 4-D gave the highest percentage of shoot formation, shoot numbers were 0.80 shoots/plant after 3 weeks. The node was cultured on MS medium with 2,4-D induced the highest number and length of roots without shoots (Table 1).

Table 1. Effect of TDZ and 2,4-D on callus and shoot induction of cajuput tree after 8 weeks of culture

plant growth regulator		shoot number	shoot length (cm)
TDZ	**2,4-D**		
0	**0**	0.13 ±0.35b	0.30 ±0.80bc
	0.02	0.00 ±0.00b	0.00 ±0.00c
	0.04	0.00 ±0.00b	0.00 ±0.00c
	0.06	0.06 ±025.b	0.40 ±1.54abc
	0.08	0.00 ±0.00b	0.00 ±0.00c
0.02	**0**	0.80 ±1.01a	0.79 ±1.17a
	0.02	0.80 ±1.14a	0.60 ±0.92ab
	0.04	0.00 ±0.00b	0.00 ±0.00c
	0.06	0.00 ±0.00b	0.00 ±0.00c
	0.08	0.00 ±0.00b	0.00 ±0.00c
0.04	**0**	0.20 ±0.56b	0.26 ±0.72bc
	0.02	0.13 ±0.35b	0.14 ±0.41bc
	0.04	0.06 ±0.25b	0.02 ±0.07c
	0.06	0.00 ±0.00b	0.00 ±0.00c
	0.08	0.13 ±0.35b	0.06 ±0.17bc
0.06	**0**	0.20 ±0.56b	0.17 ±0.53c
	0.02	0.00 ±0.00b	0.00 ±0.00c
	0.04	0.00 ±0.00b	0.00 ±0.00c
	0.06	0.00 ±0.00b	0.00 ±0.00c
	0.08	0.00 ±0.00b	0.00 ±0.00c
0.08	**0**	0.00 ±0.00b	0.00 ±0.00c
	0.02	0.00 ±0.00b	0.05 ±0.19c
	0.04	0.13 ±0.51b	0.00 ±0.00c
	0.06	0.13 ±0.35b	0.20 ±0.58bc
	0.08	0.06 ±0.25b	0.04 ±0.18c
F-test		**	**
%cv		19.59	21.53

** Significant different at $P \leq 0.01$; Means within column followed by the same letter are not significant different as determind by Duncan's multiple range test.

Effects of TDZ and 2,4-D on the root culture

Effects of TDZ and 2,4-D on the root induction was studied. The culture in liquid (MS) medium supplemented with 0.04 mg/l TDZ or 0.04 mg/l 2,4-D found that the MS medium supplemented with 2,4-D showed the highest root weight of 2.71 g. after 10 weeks (Table 2). The root was cultured on MS medium without hormone found no alteration (Fig. 3A). The big root characteristic was found in MS medium supplemented with TDZ (Fig. 3B). The highest weight of hairy root was found in MS medium supplemented with 2,4-D.

Table 2. Hairy root induction on MS medium contain TDZ and 2,4-D after culture 10 weeks

Treatment	Root weight (g)	Dry weight (g)
MS	0.03±0.01b	0.00±0.00b
MS+TDZ	0.06±0.02b	0.03±0.00b
MS+2,4-D	2.71±1.56a	0.38±0.11a
F-test	**	**
%cv	10.81	8.09

** Significant different at P ≤ 0.01; Means within column followed by the same letter are not significant different as determind by Duncan's multiple range test.

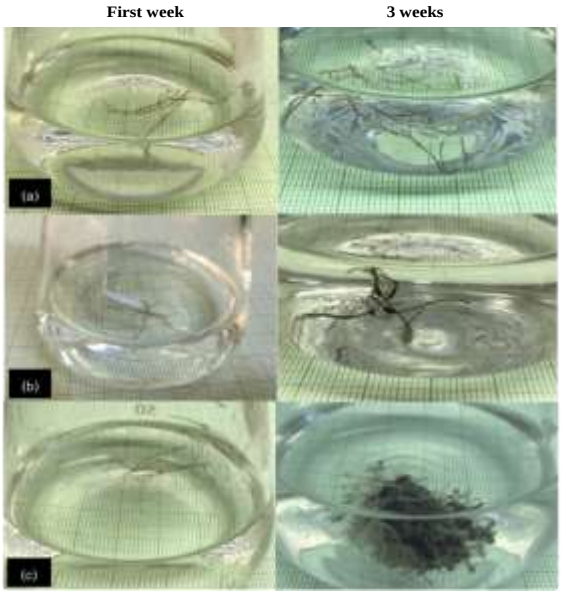

Figure 3. Hairy root cultured in MS medium with (a) without hormone (b) 0.04 mg/l TDZ (c) 0.04 mg/l 2,4-D

Discussion

Effects of TDZ and 2,4-D on friable callus induction

Callus induction from the node explants found that the explants which cultured in all MS medium suppremented with 2,4-D combination TDZ occured callus formation (Table.1). The results showed that using 2,4-D, the node explants developed the roots which related to the growth regulators TDZ and 2,4-D. It was found that auxin and cytokinin can promote the plant growing tissue, differentiated to be callus at the appropriate concentration level. Different plant tissues may have various levels of endogenous plant growth regulators (PGRs), and therefore the type of explants might have an important impact on the callus induction process (Das *et al*., 2012). It was found that on MS medium supplemented with TDZ combination with 2,4-D could induce callus developed into a shoot. The explants were cultured in MS medium supplemented with 0.06 mg/l TDZ showed the compact callus width and length of the callus 2.56 and 1.26 cm, respectively. TDZ is a growth regulator in the cytokinin group that can stimulate calluses the cell division encourages in the growth of stem, lateral meristem, somatic embryos and many shoots (Hutchinson *et al.*,1985; Taji and Williams, 1996). Huetteman and Preece, (1993) reported *Dendrobium formosum* was cultured on medium with a low concentration of TDZ found can better induce shoots than the high concentration of TDZ which inhibited the elongation of shoots.

Effects of TDZ and 2,4-D on the root culture

The roots cultured on MS medium supplemented with 2,4-D induced the hairy root which has more the average fresh and dry weight than other treatments. The hairy roots have industrial applications and used as important research too for elucidation of secondary metabolite. Normaly, the hairy roots are produced when *Agrobacterium rhizogenes*, infects a host plant (Dhiman *et al*., 2018). In this research, we can used 0.04 mg/l 2,4-D for induceing hairy root. Zhang *et al*. (2013) reported a combination of auxin and cytokinin significantly affected the formation of the adventitious root on agar-solided B5 media and a maximal induction rate of 83% in darkness.

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