International Journal of Agricultural Technology 2018 Vol. 14(7): 1755-1764
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

Colchicine and UV radiation treatment on somatic embryo formation of hybrid oil palm sub-PSU variety

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Ramasoot, S., Nuannut, W. and Khawniam, T. (2018). Colchicine and UV radiation treatment on somatic embryo formation of hybrid oil palm sub-PSU variety. International Journal of Agricultural Technology 14(7): 1755-1764.

Abstract Colchicine at 0, 0.1, 0.2, 0.3 and 0.5 % for 12, 24 and 48 hour or UV-C irradiation at doses of 0, 1.8, 3.6, 7.2 and 9.0 kJ/m^2 were used to treat embryogenic callus (EC) of oil palm cv. SUP-PSU, followed by culturing on Murashige and Skoog (MS) medium supplemented with 0.1 mg/l dicamba and 200 mg/l ascorbic acid. The results showed that the LD_{50} of colchicine treatment was 0.3% for 24 hour after culturing for one month. Colchicine at 0.1% for 48 hour gave the highest proliferation of EC proliferation (98.33%), average number of EC (16.88 ECs/culture), somatic embryo (SE) induction (5.00%) and average number of SE (0.75 SE/culture), significant difference ($p \leq 0.05$) after 3 months of culture. The LD_{50} of UV-C irradiation was 1.8 kJ/m^2. ECs irradiated without of UV-C gave the highest proliferation of EC induction and SE induction at 38.88% and 16.25% respectively, significant difference ($p \leq 0.05$) after 3 months of culture.

Keywords: oil palm, colchicine, UV-C irradiation, somatic embryo

Introduction

Oil palm (*Elaeis guineensis* Jacq.) is the source of the most sought-after edible oil in the world market (Jones, 1991) and their used food nutrition, cosmetics, pharmaceuticals, and industry of biofuels (Boari, 2008; Pádua *et al*., 2018). In Thailand, recently a growing amount of oil palms have been used for the production of biodiesel. For biodiesel production, a high yield crop is needed, and the large amount of oil produced by the oil palm fruit makes this species highly suitable (Thawaro and Te-chato, 2010). Currently, all major commercial oil palms are F1 hybrids between selections with small or no kernels (pisifera) and large thick kernels (dura).

Hybrid oil palm sub-PSU variety has a high economic and social impact illustrated by several factors, including reduced reliance on imported seeds,

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high-value yields, a 30% increase in productivity per area and income, all culminating in increased industrial production for the country. All these factors create confidence in oil palm production and in the self-sufficiency economic aspect for famers and private operators. Seed propagation is further hampered by the long time required for germination (approximately 3 years) and the low germination rate of 30% of seeds sown (Luis *et al*., 2010; Martine *et al*., 2009). In addition to time and production rate limitations, production of oil palm seedlings from seeds has the drawback that genetic segregation of relevant traits is required, because parent plants are allogamous, with high heterozygosity (Chanprasert *et al*., 2012; Martine *et al*., 2009; Myint *et al*., 2010). Commercial propagation of oil palm by plant tissue culture technique is widely used and effiency (Thawaro and Te-chato, 2010). Tissue culture technique has proven to be effective tools for breeding programs of several plant species. The method for improving plant quality and quantity by inducing polyploid is of great important in many plants. Polyploidy often generates variants that may possess useful morphological character and also provide a wider germplasm base for breeding studies (Thao *et al*., 2003). Duplication of chromosome using by colchicine has long been used in plant breeding program. Colchicine, a compound that effectively arrests mitosis at the anaphase stage, has been found to have a significant effect on polyploid induction (Samala and Te-chato, 2012). The ultraviolet (UV) radiation may cause two adjacent pyrimidine residues (cytosine or thymine) to form a dimer. During DNA replication, both strands are used as templates to synthesize new strands. The cytosine dimer could cause adenine (instead of the normal guanine) to be incorporated into the new strand. Subsequent DNA replication will produce CC to TT mutation (Mobio, 2018). UV light is electromagnetic radiation with a wavelength from 400 nm to 10 nm, UV is traditionally divided into three wavelengths. UV-C (280-200 nm) is extremely harmful to living organisms, but not relevant under natural conditions of solar irradiation. UV-B (320-280 nm) is of particular interest because although this wavelength represents only approximately 1.5 % of the total spectrum, it can have a variety of damaging effects in plants. UV-A (400-320 nm) represents approximately 63% of the incoming solar radiation and is the least hazardous part of UV radiation (Hollosy, 2002).

The aims of this study were to induce somatic embryo formation obtained from colchicine and UV-C irradiation treated embryogenic calluses (ECs) of hybrid oil palm sub-PSU variety.

Materials and methods

Plant material

ECs of hybrid oil palm sub-PSU variety obtained zygotic embryo culturing were cultured modified MS medium added 0.1 mg/l dicamba and 200 mg/l ascorbic acid (Thawaro, 2010). These culture media was adjusted pH to 5.7 with 0.1 N KOH before adding 0.7% agar, then autoclaved at 1.05 kg/cm^2, 121^{o}C for 15 min. The cultures were placed under light conditions of 3,000 lux illumination for 14 h photoperiod at 25±2^{o}C and subcultured every 4 weeks on the same medium component for 3 months.

Effect of colchicine on surivival rate and somatic embryo formation

ECs were treated with different concentration of colchicine at 0 0.1 0.2 0.3 and 0.5% and kept at room temperature on a shaker at 110 rpm for the periods of 12, 24, and 48 hours. Then treated ECs were rinsed with distilled water for three times and they were transferred to MS medium added 0.1 mg/l dicamba and 200 mg/l ascorbic acid. The culture condition was maintained under light conditions of 3,000 lux illumination for 14 h photoperiod at 25±2^{o}C.

Effect of UV-C irradiation on surivival rate and somatic embryo formation

ECs were treated with different UV-C irradiation at doses 0, 1.8, 3.6, 7.2 and 9.0 kJ/m^2. Then treated ECs were transferred to MS medium added 0.1 mg/l dicamba and 200 mg/l ascorbic acid. The culture condition was maintained under light conditions of 3,000 lux illumination for 14 h photoperiod at 25±2^{o}C.

Data analyses

Colchicine treatments were used 5 × 3 Factorial in completely randomized design (CRD) with 4 replicates. UV-C treatments were used CRD with 4 replicates. ECs of oil palm were initiated using 25 ECs. Each EC was inoculated in 25x125 mm test tubes containing MS medium supplemented with 0.1 mg/l dicamba and 200 mg/l ascorbic acid for 3 months. The survival rate of ECs and somatic embryo (SE) formation were recorded and statically analyzed by ANOVA (analysis of variance) and significant difference among the treatments was separated using Duncan's multiple range test (DMRT) at p=0.05.

Results

Effect of colchicine on surivival rate and somatic embryo formation

The effect of colchicine on the survival of ECs after immersing and culture for 1 month depended upon concentrations of colchicine and duration times of treatment. The survival percentage decreased with increasing concentration of colchicine. ECs survived in 12 h treatment with 0.1% colchicines. Survival rate obtained from 0.3% colchicine treatment for 12, 24 and 48 h were 44.17%, 50.42% and 47.50 %, respectively (Table 1 and Figure 1-3). Morphological characters of those ECs were quite different in both shape and size of ECs (Figure 4). These characters could be further used in breeding program. In this present study, the most effective concentration and duration time treatments for the LD_{50} of colchicine treatment was 0.3% (w/v) for 24 h.

Table 1. Effect of different concentrations of colchicine and during time on survival rate of ECs hybrid oil plam sub-PSU variety after 1 month of culture

Colchicine (%)	Survival rate (%)			Average*
	12 h	24 h	48 h	
0	77.08	77.08	77.08	77.08a[1/]
0.10	82.50	80.83	75.00	79.44a
0.20	60.00	62.92	67.50	63.47b
0.30	44.17	50.42	47.50	47.36d
0.50	57.08	55.42	58.75	57.08c
Average [ns]	64.17	65.33	65.17	
F-test		ns		
C.V. (%)		39.60		

[1/]Different letters indicate significant difference at $p \leq 0.05$ (DMRT)

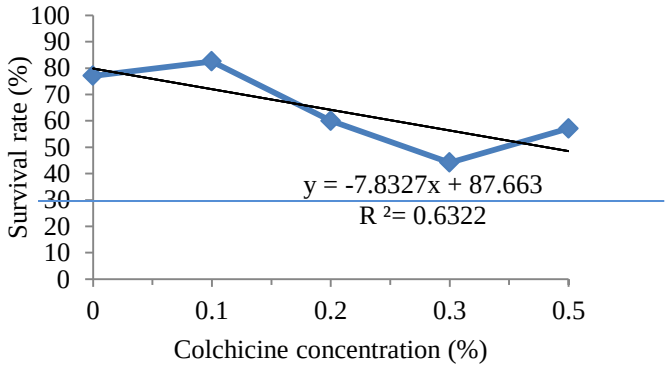

Figure 1. Percentage survival rate of ECs hybrid oil plam sub-PSU variety treated with different concentration of colchicine based on LD_{50} for 24 h after 1 month of culture

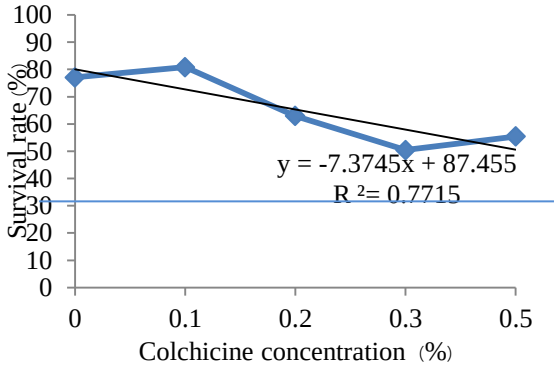

Figure 2. Percentage survival rate of ECs hybrid oil plam sub-PSU variety treated with different concentration of colchicine based on LD_{50} for 24 h after 1 month of culture

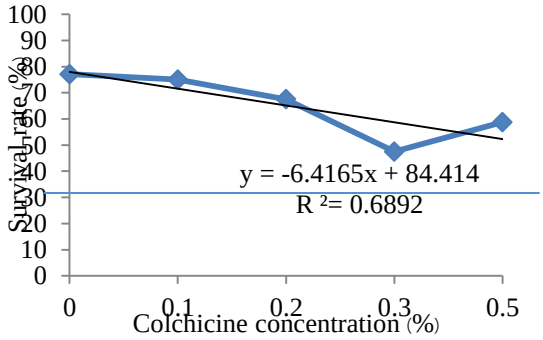

Figure 3. Percentage survival rate of ECs hybrid oil plam sub-PSU variety treated with different concentration of colchicine based on LD_{50} for 48 h after 1 month of culture

For the effect of colchicine on EC proliferation and somatic embryo (SE) formation, the result found that ECs were immersed at 0.1% (w/v) colchicine for 48 h gave the highest EC proliferation at 93.33% and average number of EC at 16.88 ECs/culture, significant difference ($p \leq 0.05$) after 3 months of culture. ECs were immersed at 0.2% (w/v) colchicine for 24 h gave the highest SE induction at 8.33% and average number of SE at 2.00 SEs/culture (Table 2).

Effect of UV-C irradiation on surivival rate and somatic embryo formation

ECs were treated with UV-C irradiation at doses 0, 1.8, 3.6, 7.2 and 9.0 kJ/m^2. The results showed that the LD_{50} of UV-C irradiation was 1.8 kJ/m^2 (Figure 5). ECs irradiated without of UV-C gave the highest proliferation of EC induction and SE induction at 38.88% and 16.25% respectively, significant difference ($p \leq 0.05$) after 3 months of culture (Table 3 and Figure 5).

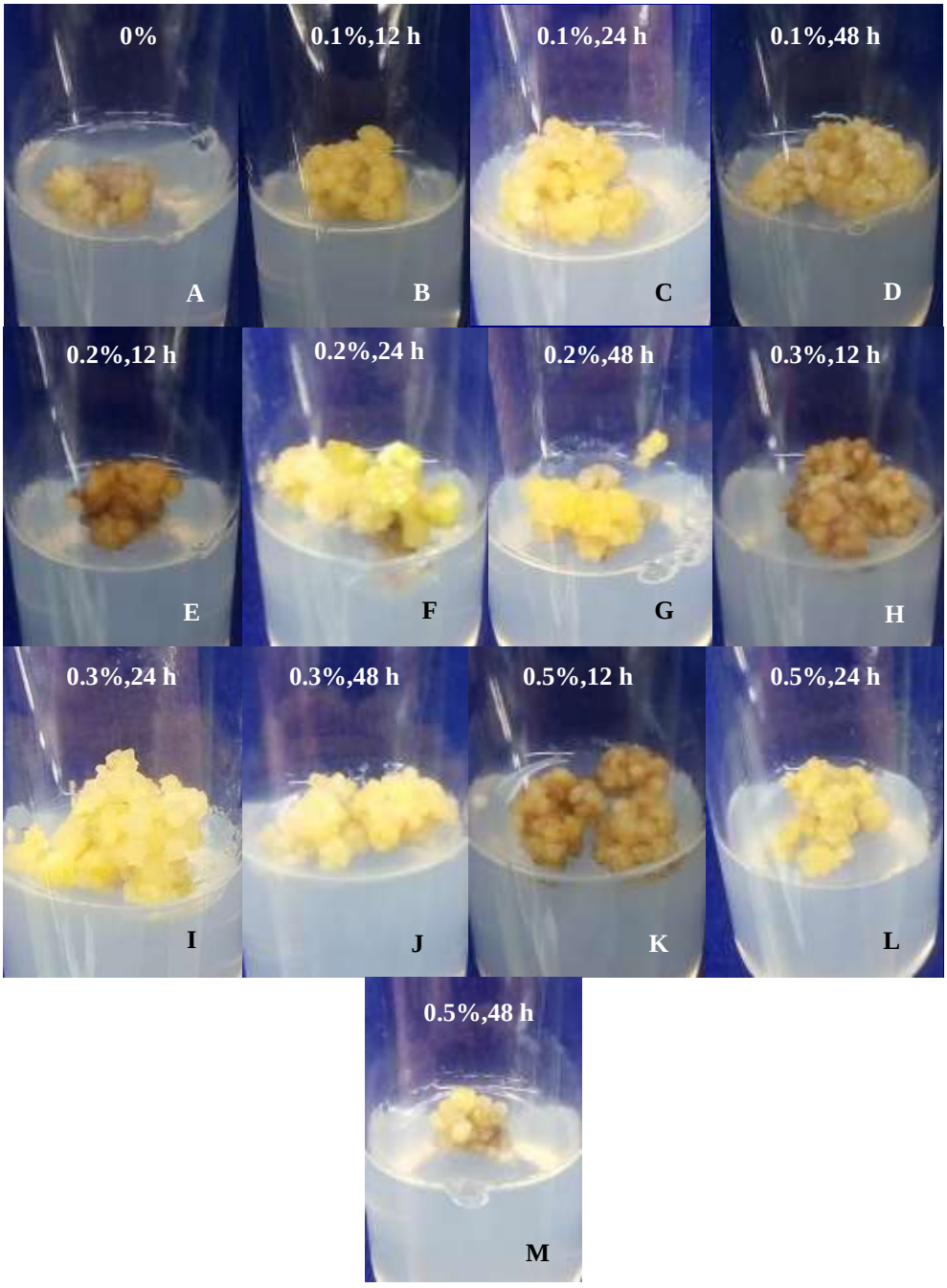

Figure 4. Characteristic of EC proliferation and SE induction immersed different concentration of colchicine were cultured on MS medium added 0.1 mg/l dicamba and 200 mg/l ascorbic acid after 3 months of culture

Table 2. Effect of colchicine and during time on EC proliferation and SE formation after 3 months of culture

Colchicine (%)	During time (h)	EC proliferation (%)	No. of EC (ECs/culture)	SE induction (%)	No. of SE (SEs/culture)
0		33.33d[1/]	10.28cd	1.67b	0.75
0.10	12	74.83bc	14.93ab	2.50ab	0.50
	24	21.58de	16.38a	3.33ab	1.00
	48	98.33a	16.88a	5.00ab	0.75
0.20	12	15.83e	8.33d	4.83ab	0.50
	24	29.17de	16.02a	8.33a	2.00
	48	65.83c	13.83abc	2.50ab	1.00
0.30	12	21.58de	13.95ab	4.83ab	0.50
	24	26.25de	13.31abc	4.16ab	1.25
	48	82.50b	15.94a	1.67b	0.75
0.50	12	29.17de	14.46ab	4.83ab	0.50
	24	24.58de	11.75bcd	2.50ab	0.50
	48	32.91d	13.50abc	1.67b	1.00
F-test		*	*	*	ns
C.V. (%)		47.50	15.73	51.42	50.20

[1/]Different letters indicate significant difference at $p \leq 0.05$ (DMRT)

Table 3. Effect of different UV-C irradiations on EC proliferation and SE formation after 3 months of culture

UV-C (KJ/m^2)	EC proliferation (%)	No. of EC (ECs/culture)	SE induction (%)	No. of SE (SEs/culture)
0	36.88a[1/]	10.28b	16.25a	2.75a
1.8	9.75d	8.33b	5.00b	0.75b
3.6	23.13c	18.31a	5.00b	0.75b
7.2	26.51c	16.45a	3.75b	0.75b
9	30.00b	15.65a	6.25b	1.25b
F-test	*	*	*	*
C.V. (%)	3.76	27.53	62.32	40.00

[1/]Different letters indicate significant difference at $p \leq 0.05$ (DMRT)

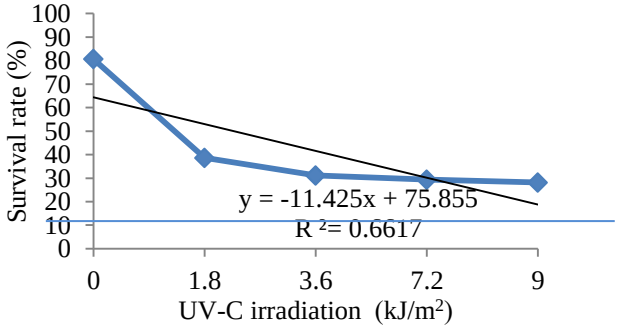

Figure 5. Percentage survival rate of ECs hybrid oil plam sub-PSU variety treated with UV-C irradiation based on LD_{50} for 24 h after 1 month of culture

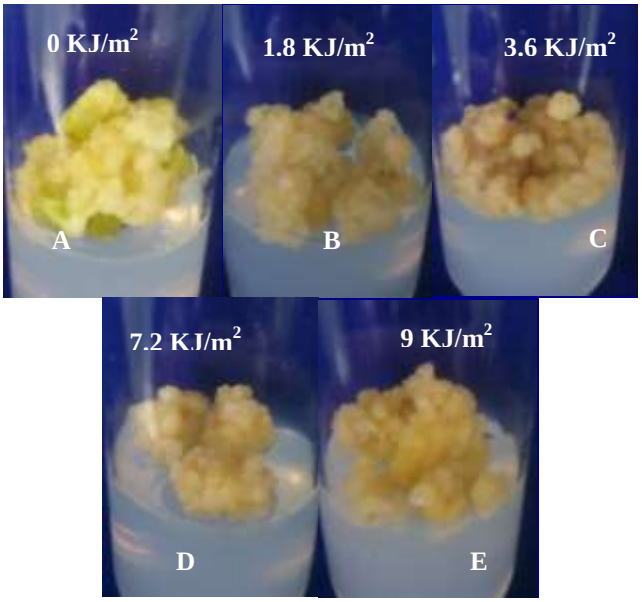

Figure 6. Characteristic of EC proliferation and SE induction treated with UV-C irradiation were cultured on solid MS medium added 0.1 mg/l dicamba and 200 mg/l ascorbic acid after 3 months of culture

Discussion

In this present study, colchicine and UV-C irradiation were studied to be inducing somatic embryo formation. The results showed that the LD_{50} of colchicine treatment was 0.3% for 24 hour after culturing for one month. Colchicine is a toxic chemical and it dosage and during of treatment can be associated with mortality and growth inhibition (Kimbery *et al*., 2006). The result found that ECs were immersed at 0.1% (w/v) colchicine for 48 h gave highest EC proliferation at 93.33% and average number of EC at 16.88 ECs/culture, significant difference ($p \leq 0.05$) after 3 months of culture. ECs were immersed at 0.2% (w/v) colchicine for 24 h gave highest SE induction at 8.33% and average number of SE at 2.00 SEs/culture. It has been reported previously that higher colchicine concentrations and increases in exposure time to this chemical result in decreases in explant survival rates (Chakraborti *et al*., 1998; Väänölä, 2000; Zhang *et al*., 2008) and in the number of regenerated shoots which could be obtained from treated callus tissue (Song *et al*., 1997). However, stimulating (e.g. Petersen *et al*., 2002) or at least non-negative (e.g. Barnabás *et al*., 1999) impacts on culture rates of inhibitors of the karyokinesis spindle have also been observed. In *Allium fistulosum* × *Allium cepa*, a 48-h exposure to 0.1% colchicine enhanced the number of regenerating shoots from

calluses relative to a 36-h exposure (Song *et al*., 1997). For the ultraviolet (UV) radiation experiment, the results showed that the LD_{50} of UV-C irradiation was 1.8 kJ/m^2. ECs irradiated without of UV-C gave the highest proliferation of EC proliferation and SE induction at 38.88% and 16.25% respectively, significant difference ($p \leq 0.05$) after 3 months of culture. The result according to Asma G.O. (2017) reported that cotyledons explant treated with UV-C radiation gave callus induction at 42.2% and callus fresh weight at 82.7 g. Abd ElKadder *et al*. (2014) who reported that ultraviolet radiation significantly affected callus growth in term of fresh weight, they also investigated that irradiation of UV for 30 min lead to increasing the callus growth up to 17.3%. Riksa and Rizkita (2014) reported that UV radiation is a useful technique to enhance secondary metabolites production.

Acknowledgement

The authors are grateful to Nakhon Si Thammarat Rajabhat University the Office of the Higher Education Commission and the National Research Council of Thailand for financial support.

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(Received: 13 September 2018, accepted: 31 October 2018)