JOURNAL OF AGROBIOTECHNOLOGY 2021, VOL 12(1S):51-61
e-ISSN: 2180-1983
http://dx.doi.org/10.37231/jab.2021.12.1S.270
https://journal.unisza.edu.my/agrobiotechnology/index.php/agrobiotechnology/index

Effect of 2,4-Dichlorophenoxy Acetic Acid and Activated Charcoal on Callus Induction of *Cocos Nucifera* L. Hybrid MATAG Inflorescence

Dhiya Dalila Zawawi [a,*], Mohd Fahmi Abu Bakar [a] and Siti Nurkhalida Abd. Kadir [a]

[a] Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200, Besut, Terengganu, Malaysia

***Corresponding author: ddalila@unisza.edu.my**

ABSTRACT

Cocos Nucifera Linn. Var. MATAG is a Dwarf coconut variety that had high demand in Malaysia but low supply. Vegetative propagation of high-yielding MATAG coconut by using *in vitro* cloning must be considered in contributing to increase coconut productivity. Thus, attempts were made to develop a protocol that would enhance callogenesis as a first preliminary step towards a protocol for mass propagation of *C. nucifera* L. var. MATAG. The anther isolated from immature inflorescence was used as explants and cultured on modified Eeuwens Y3 media in different concentrations of 2,4-dicholorophenoxy acetic acid (2,4-D) and activated charcoal. The highest callus induction percentage (31.25 ± 12.18) was observed in 20 mg/L 2,4-D. However, 2,4-D at any level tested were not statistically significant. Callus induction media supplemented with 0.5 mg/L activated charcoal gave the highest callusing percentage (25.89 ± 13.59 %) indicating a positive effect of activated charcoal on callusing even though the result obtained not significant compared to control (15.95 ± 6.76 %). But, activated charcoal supplemented in media produced a significant effect compared to control in reducing the percentage of browning. In conclusion, media supplemented with activated charcoal produced a higher rate on callus induction and preventing tissue browning in explant. Besides that, the anther and ovule explant may serve as an efficient explant to study the callus induction of *C. nucifera* L. var. MATAG and as a basis to screen the potential useful plant growth regulators for somatic embryogenesis.

Keywords: *Cocos nucifera* L. var. MATAG, 2,4-dicholorophenoxy acetic acid, activated charcoal, immature inflorescence

INTRODUCTION

Cocos nucifera Linn. (coconut) is a monoecious perennial monocotyledon in the family Aracaceae (Palmaceae). It is indigenous to the coastal regions of Malaysia, Indonesia, Philippines and Melanesia and is cultivated as a plantation crop (Chan & Elevitch, 2006). The coconut palm is popularly known as tree of life because of its various economic uses (Batugal et al., 2009) and has attained an important socio-economic role in the local communities where it is produced (Persley, 1992). Plant parts such as endosperms were extracted for coconut milk or oil (Adkins et al., 2005) which were then used as cooking oil, hair oil and lamp oil and as an essential ingredient in soap making (Chidambaram et al., 2013) The immature nut's water is consumed as a beverage,

husk fibres are made into ropes or other construction material and the shells are used for fuel as a source of energy (Chan & Elevitch, 2006). The anti-inflammatory, antipyretic and wound healing activities of coconut fresh juice and kernel extract had also been detected (Zakaria et al., 2006).

In Malaysia, coconut is the fourth important industrial crop after oil palm, rubber and paddy in terms of total planted area (Sivapragasam, 2008). The Malayan Tall variety is the most widely cultivated in Malaysia (92.2 %), followed by the MATAG variety (4.3 %), MAWA (1.7 %), Pandan (1.7 %), and Malayan Dwarfs (0.2 %). In coconut product market, the constant problem is a scarcity of raw resources, both edible (oil, copra) and non-edible (wood, shell, and husks) (Sivapragasam, 2008). This is mainly because of low productivity level along with the aging traditional Malayan Tall variety, that becoming too old for optimal fruit production. Therefore, replanting of unproductive and old coconut palms with a high-yielding MATAG are a few strategies.

Cocos Nucifera Linn. Var. MATAG is a dwarf coconut variety originated from Malaysia. Their tree size is smaller, with immature nuts' water and the jelly-like kernel is consumed as a beverage.
MATAG variety is a hybrid of Malayan Yellow Dwarf (MYD) (female parent) with Tagnanan Tall (TNG) (male parent). This variety showed an early bearing with commencing flowering at 36 months and the young nuts can be harvested at 48 months after planting. MATAG variety can produce very high nut yield, range from 25,000 to 30,000 nuts per hectare per year. The most recent propagation method of MATAG is via seed-nut production (United Plantations Berhad, 2013). The hybrid process was started with the male florets of Tagnanan Tall were dried for pollen. Then, the emasculation process of male spikelets of the Malayan Yellow Dwarf, followed by the pollination of the emasculated inflorescence of Malayan Yellow Dwarf. Tagnanan pollen is used as a male source whereas MRD/MYD for the female receiver.

However, this method has some constraints derived since coconut is a genotype dependence and high heterozygocity that produce such as great variability among progeny (Vidhanaarchci et al., 2012). The high degree of genetic variation among seedlings that exist with a long-life span is a serious problem in the establishment of uniformly performing offspring (Verdeil et al., 1999). Furthermore, the fidelity of selected characters of MATAG cannot be confirmed until the seedling reaches maturity after three to four years of growth. The propagation of high-performing individuals MATAG coconut by using *in vitro* cloning must be considered to eliminate this variability.

This strategy has been possible clonal propagation method available for coconut plants and provides a way out to produce uniform planting materials and multiplication of high-yielding plant material for massive replanting (Magdalita et al. 2010). Cloning of coconut *in vitro* has been attempted using various tissues including immature inflorescences (Verdeil et al., 1994) and leaf (Raju *et al.,* 1984, Karunaratne et al., 1991), zygotic embryos (Koffi et al., 2013), plumule (Chan et al., 1998; Pérez-Núñez et al., 2006), anthers (Perera et al., 2008a) and ovule (Perera et al., 2009a). Zygotic tissue such as immature embryos (Fernando & Gamage, 2000) has also been tested for *in vitro* plant regeneration. However, the callusing frequency depended on the embryo developmental stage. Immature embryos are not suitable for clonal replication because they generate palm clones with uncertain performance due to heterogeneity among seedling after cross-pollination.

The ovary can be a potential explant for somatic embryogenesis in coconut cloning (Perera et al., 2007). This shown by the mean percentage of calluses with an unfertilized ovary is from 41 % (Perera et al., 2009b) till 76 % (Perera et al., 2009a). The production of coconut haploids via anther culture was reported by Perera et al., (2008a) with the highest embryo induction was achieved from 100 μM 2,4-D.

However, protocols of micropropagation for MATAG variety have not been developed yet. Thus, inflorescence was chosen as explant to cloning MATAG variety in the present study. The selection of coconut inflorescences as an explant; as it contains meristematic tissue, which induced callus by supplementing auxin to the culture medium and the inflorescences from mature palms could be excised non-destructively from the palms (Oropeza et al., 2005).

On the other hands, explant browning is linked to inhibit callus development due to the physiological age and may be severe (Sukamto, 2011). To avoid tissue browning and remove unwanted chemicals, thus improve anticipated morphogenic responses of explants, activated charcoal will be applied in current research (Sáenz et al., 2009). Since 2,4-D is the most efficient auxin for inducing callus growth in coconut culture, it will be used to induce callus growth in palm (Sukamto, 2011).

Fungus and bacteria are two of the biggest obstacles in *in vitro* propagation. Establishment of aseptic techniques for sterilization is therefore required to get high-rate of sterile explants (George et al., 2008). Thus,

the objective of this study was to determine the optimal concentration of activated charcoal to prevent explant browning, to induce a high rate of callusing from the inflorescence culture and to optimize the sterilization methods for better aseptic culture condition.

The development of haploid plants and protocols to produce homozygous plants had a significant impact on the agricultural system (Perera et al., 2014). Plantation productivity could be significantly increased if homozygous materials obtained by cloning high-yielding plants were available. Therefore, the anther and ovary which become one of the several methods to obtain haploids were tested as a source of explants in this study. Attempts were made to develop a protocol that would enhance callogenesis using those explants as the first step towards a protocol for mass micropropagation of *Cocos Nucifer*a Linn. Var. MATAG, a growing need of the industry.

MATERIALS AND METHODS

Plant Materials

The *Cocos Nucifera* Linn. Var. MATAG inflorescence was collected from the Department of Agriculture Terengganu's farm at Hulu Terengganu, Terengganu.

Anther surface sterilization

The un-open inflorescence (Fig. 1) in the crown containing male and female flowers was collected from the field. The inflorescence (Fig. 1A) was swapped with 70 % ethanol and then put under ultraviolet light (UV) in laminar flow for 20 minutes. After that, the spathe that was enveloping inflorescence was cut off (Fig. 1B) revealing a spadix (a bunch of flowers). Then, the spadix branch bearing large numbers of male flowers with a female flower at the base (Fig. 1C and 1D) were excised from the spadix. The excised spadix branch (about 5 to 10 cm) that contained male flowers than were surface sterilized by immersion in 70 % ethanol for two minutes and followed by 20 % Chlorox solution supplemented with four drops of Tween® 20 for 20 minutes. Then, the male flowers were cleansed with sterile distilled water five times and allowed to air dry under sterile conditions. After that, the sterile male flower bud (Fig. 1E) was cut from the spadix branch. The male flower petal (Fig. 1F) was removed and anther was next excised from the filaments (Fig. 1G). The isolated anthers (Fig. 1H) will then be used as explants in this experiment.

Ovary surface sterilization

Unfertilized ovaries were obtained from immature female flowers of coconut palms. The female flowers that were attached at the basal part of spadix branch (Fig. 2A) were surface sterilized with 70 % ethanol for three minutes. The sterile step continued with 20 % Chlorox solution added with five drops of Tween® 20 for 20 minutes. The female flowers were then rinsed five times with sterile distilled water and let it dry. Then, the ovaries from female flowers were excised (Fig. 2B) with the sepal and petals in each female flower were removed (Fig. 2C and Fig. 2D) and the ovary was dissected out (Fig. 2E) under laminar air flow. The isolated ovary was cut in half and cultured in callus induction medium.

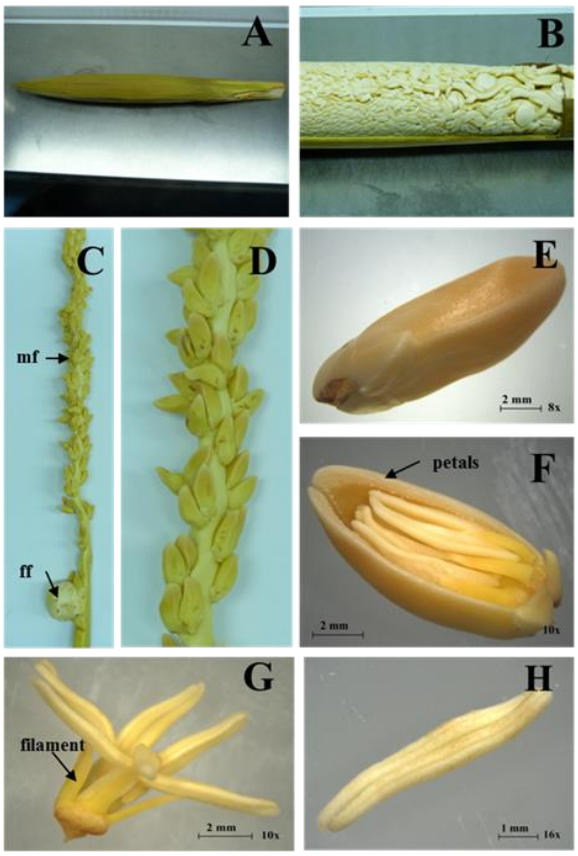

Fig. 1. Isolation of anther from MATAG male flowers; (A) inflorescence; (B) spathe that enclosed the inflorescence were cut off; (C) spadix branch with male flower (mf) and female flower (ff); (D) close-up of the male flowers attached to the middle part of spadix branch; (E) isolated male flower; (F) anthers are covered with the petals (G) anthers attached to the filaments; (H) excised anther

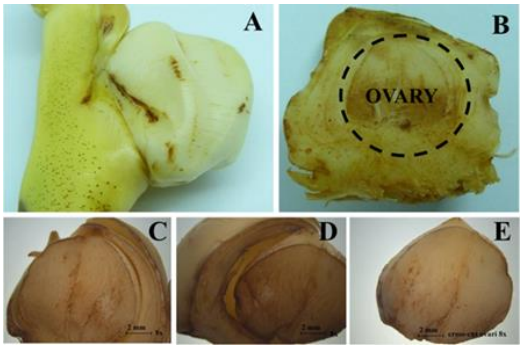

Fig. 2. Isolation of ovaries from MATAG inflorescence; (A) female flower at the bottom of spadix branch; (B) cross-section showed that ovary is situated at the middle of female flower; (C) and (D) the perianth segment (sepal and petals) were removed and ovary was dissected out; (E) isolated ovary.

Media Compositions and Growth Conditions

Effect of 2,4-Dichlorophenoxyacetic acid (2,4-D) on callus induction from anther

Modified Y3 Media (Eeuwens, 1976) with various concentrations of 2,4-D (0, 10, 20, 30 and 40 mg/L) supplemented with 90 g/L sucrose, 0.1 % activated charcoal and solidified with 4.0 g/L gelrite were tested for callus induction. The pH of each media was adjusted to 5.8. The medium was autoclaved at 121°C for 20 min. The cultures then were incubated under dark at 25 ± 2 °C for 3 months.

Effect of activated charcoal on browning and callus induction from cultured anther

Modified Y3 Media (Eeuwens, 1976) containing various concentrations of activated charcoal (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) supplemented with 20 mg/L 2,4-D, 90 g/L sucrose and 4.0 g/L gelrite. The pH of each media was adjusted to 5.8 before it was autoclaved at 121 °C for 20 min. The anther cultures were then incubated under dark at 27 ± 2 °C for 3 months.

Effect of thidiazuron (TDZ) on browning from ovary cultured

Modified Y3 Media (Eeuwens, 1976) with different level of TDZ (0, 2, 4 and 6 mg/L) (Table 3) supplemented with 20 mg/L 2,4-D, 40 g/L sucrose, 0.1 % activated charcoal and 4.0 g/L gelrite were tested for callus induction. The pH of each media was adjusted to 5.8. These cultures then were incubated under dark at 27 ± 2 °C for 3 months.

Culture media and in vitro growth conditions

Modified Y3 Media (Eeuwens, 1976) medium contained the following macronutrients: 2020 mg/L KNO_3, 294 mg/L $CaCl_2 \cdot 2H_2O$, 120 mg/L $MgSO_4 \cdot 7H_2O$, 1492 mg/L KCl, 535 NH_4Cl mg/L and 276 mg/L $NaH_2PO_4.2H_2O$ while the content of micronutrients was 11.2 mg/L $MnSO_4 \cdot 4H_2O$, 0.16 mg/L $CuSO_4 \cdot 5H_2O$, 7.2 mg/L $Zn._7SO_4 \cdot H_2O$, 3.1 mg/L H_3BO_3, 8.3 mg/L KI, 0.24 mg/L $Na_2MoO_4 \cdot 2H_2O$, 0.24 mg/L $CoCl_2 \cdot 6H_2O$, $NiCl_2.6H_2O$ 0.024 mg/L and 32.5 mg/L FeNa-EDTA. The supplements contents were 100 mg/L myo-inositol, 1 mg/L nicotinic acid, 1 mg/L pyridoxine HCl, 1 mg/L thiamine HCl, 2 mg/L glycine, 100 mg/L L-glutamine, 100 mg/L L-arginine and 100 mg/L L-asparagine.

Data Analysis

Data were started to collect after 4 weeks of culture till 8 weeks. All experiments were set up in a completely randomized design under factorial arrangements. Each treatment consisted of five replicates having ten explants per petri dish. Explants that showed intense browning were excluded from analysis because these type of explants do not growth further in *in vitro* responses. The Callus Induction (CI) percentage was calculated as follows:

$$\frac{\text{No. of explants with callus}}{\text{Total no. of explants}} \times 100 = \text{Percentage of callusing (\%)}$$

Analysis of variance (ANOVA) was applied to test the statistical significance and the significance of differences among means was carried out using Duncan's Multiple Ranges Test (DMRT) at P=0.05 by using the Statistical Package for Social Science (SPSS) Program. The data are presented as mean ± standard error of the mean.

RESULTS AND DISCUSSION

Effect of 2,4-D on anther callogenesis

The effect of plant growth regulators on anther callus induction of MATAG was identify by using different concentrations of 2,4-D. The result is shown in Table 1. In all treatments tested, callus formation was observed. Percentage of callus induction could be evaluate starting from 2 to 3 months of culture initiation. The anther wall will have burst open (Fig. 3A) and the developing callus are visible (Fig. 3A and 3B).

Table 1. Effect of different concentrations of 2,4-D on percentage of callus induction in cultured anther of coconut.

2,4-D (mg/L)	Callus induction (%)
0	30.03±8.12[a]
10	23.67±11.39[a]
20	31.25±12.18[a]
30	30.91±11.38[a]
40	27.9±8.64[a]

All treatments were supplemented with 90 g/L sucrose, 0.1% activated charcoal and 4.0 g/L gelrite with pH 5.8. The cultures were incubated under dark at 25 ± 2 °C for 3 months.

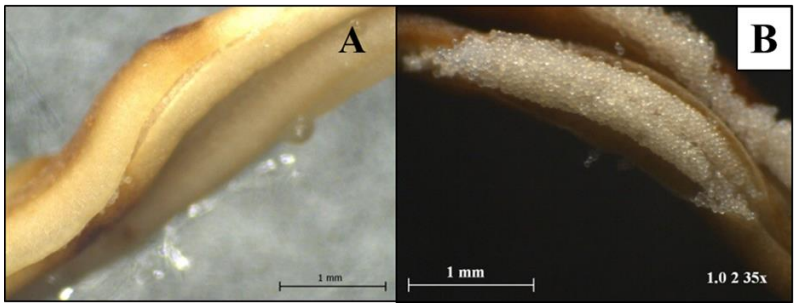

Fig. 3. Callus growth from the anthers of *Cocos Nucifera* Linn. Var. MATAG on Y3 media supplemented with 20 mg/L of 2,4-D after 4 weeks of incubation at 25 ± 2 °C in dark condition.; (A) anther wall breaks open; (B) developing whitish callus from anther.

The obtained callus remains in callus stage although it was subcultured in somatic embryogenesis induction media by gradual reduction of auxin concentration in the culture medium (data did not present) since somatic embryogenesis in *Cocos nucifera* L. is generally induced by low auxin concentration. Fernando and Gamage (2000) stated that other types of PGR such as abscisic acid (ABA) enhanced the production of somatic embryos. Pretreatments of anther either thermal shocks or cold pretreatment should be applied in this study since it has been effective to induce high frequency of callus development to other crops such as in rice (*Oryza sativa* L.) (Kaushal et al., 2014), wheat (*Triticum aestivum* L.) (Rubtsova et al., 2013) and borage (*Borago officinalis* L.) (Eshaghi et al., 2015). Besides adding PGR to produce callus through anthers culture, the other step is to identify and isolate anthers at their optimal stage of development. The anthers containing mid- or late uninucleate microspores are the stages that most responsive to embryogenesis induction for anther culture of *Hibiscus cannabinus* L. (Mahmoud et al., 2014), eggplant (Salas et al., 2012) and *Curcuma attenuata* (Kou et al., 2013).

Effect of activated charcoal on anther callogenesis

Activated charcoal is one of the essential components in coconut tissue culture media (Fernando & Gamage, 2000; Fernando et al., 2003 and Perera et al., 2007). The activated charcoal possesses significant adsorptive properties on phenol and other growth inhibitory substances (Fernando et al., 2010). Therefore, it is preventing a high incidence of cultured tissue browning that leads to loss of explants. The result is shown in Table 2.

Table 2. Effect of different concentrations of activated charcoal on percentage of callus induction from anther culture of coconut

Activated charcoal (mg/L)	Callus induction (%)	Browning (%)
0	15.95 ± 6.76[a]	76.4 ± 12.44[a]
0.5	25.89 ± 13.59[a]	23.63 ± 8.72[b]
1.0	22.10 ± 11.39[a]	14.65 ± 9.41[b]
1.5	24.15 ± 12.54[a]	9.55 ± 7.99[b]
2.0	15.69 ± 8.95[a]	12.81 ± 6.95[b]
2.5	22.47 ± 12.99[a]	15.27 ± 11.72[b]
3.0	17.8 ± 8.3[a]	15.48 ± 8.96[b]

All treatments were supplemented with 20 mg/L 2,4-D, 90 g/L sucrose and 4.0 g/L gelrite with the pH 5.8. The cultures were incubated under dark at 27± 2 °C for 3 months.

Although activated charcoal is added into media to remove harmful substances, it has been discovered that it also adsorbs plant growth regulators, particularly 2,4-D or BAP (Ebert, Taylor, & Blake, 1993), ABA (Pullman et al., 2005) and some minerals likes cuprum and zink (Pan & Staden, 1998). These situations can result in undefined culture conditions thus lead to various tissue response and non-reproducible result. Therefore, various concentrations of activated charcoal were added in the basal media and Table 2 showed that at the concentration 1.5 mg/L is suitable to induce a high rate of callus induction percentage (24.15 ± 12.54[a]) plus lower browning explant (9.55 ± 7.99[b]). At the concentration 0.5 mg/L, the callus induction percentage was higher (25.89 ± 13.59[a]) but the browning percentage was higher (23.63 ± 8.72[b]) respectively. The other alternative to avoid tissue browning is by employ polyvinylpyrrolidone (PVP). Sáenz, et al., (2005) identify that PVP did not adsorb the auxin, contrasting with activated charcoal that shown strong 2,4-D adsorption. Besides, that embryogenic calli responses from coconut palm plumules occurred more rapidly compared to medium containing activated charcoal.

Effect of TDZ on ovary callogenesis

Other than anthers, the ovaries of *Cocos nucifera* L. var. MATAG also were studied as an explant for callus induction. Thidiazuron (TDZ) was added in different concentrations to determine the optimized callus induction percentage. Callusing of ovary culture of coconut was improved by the application of TDZ (Perera et al., 2009[a]). At the early phase of culture, the explants turned brown but after 3 months, translucent masses of globules (Figure 4A and 4B) could be observed in all concentrations. The callus induction percentage slightly lower compares to the control (Table 3). Although the highest callusing percentage was observed is 31.67±1.67a % in control, the lowest percentage of explant browning (62.59 ± 10.74[a]) was observed in medium containing 6 mg/L TDZ with 29.34 ± 18.81[a] of callus induction percentage.

Table 3. Effect of different concentrations of TDZ on percentage of callus induction and browning in cultured ovary of coconut

TDZ (mg/L)	Callus induction (%)	Browning (%)
0	31.67 ± 1.67[a]	70.73 ± 1.5[a]
2	29.84 ± 13.17[a]	77.06 ± 11.84[a]
4	25.84 ± 10.84[a]	69.17 ± 4.17[a]
6	29.34 ± 18.81[a]	62.59 ± 10.74[a]

All treatments were supplemented with 20 mg/L 2,4-D, 40 g/L sucrose, 0.1% activated charcoal and 4.0 g/L gelrite were tested for callus induction. The pH of each media was adjusted to 5.8. These cultures then were incubated under dark at 27±2 °C for 3 months.

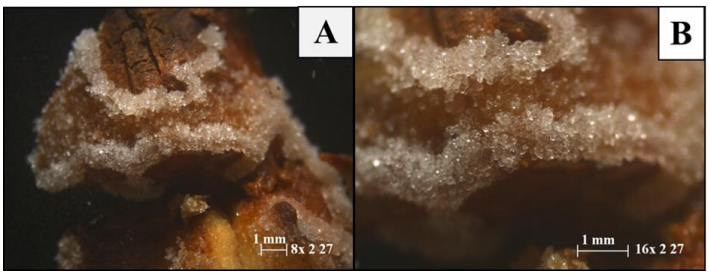

Fig. 4. Callus growth from the ovary of *Cocos Nucifera* Linn. Var. MATAG cultured on Y3 media supplemented with thidiazuron, after 4 weeks of incubation at 25 ± 2 °C in dark. (A) callogenesis from ovary in callus induction medium and (B) close view of the callus grow after 3 months of culture initiation.

In general, the culture of anthers, pollen, ovaries, and ovules produces haploid regenerants as fixed gametoclonal variants resulting from meiosis (Perera et al., 2014). Besides that, immature inflorescence contains numerous meristematic regions and the embryogenic potential that depends on the maturity stage of the inflorescence. The coconut leaf was excluded as an explant since it has low embryogenic potential and seedlings responded compared to inflorescence (Fernando, 2010). Plumule can be a potential explant to form secondary somatic embryos and embryogenic callus. However, it's still has some obstacles, such as the relatively low percentage of embryogenic calli growth and insignificant of somatic embryos formed per callus. In the present study, both anther and ovary explants from immature inflorescence cannot induce a high rate of callus growth, thus alternative approaches were necessary to be applied to trigger callogenesis.

CONCLUSION

The sterilization method for inflorescence of MATAG variety has been optimized. The inflorescence of *Cocos Nucifera* Linn. Var. MATAG has a potential to be suitable explants for callus induction. Y3 media supplemented with 50 mg/L 2,4-D plus 1 g/L of activated charcoal showed optimum condition for callus induction from the anthers. However, it is suggested that further improvement of the callus induction method to get higher rate of callus induction percentage. A combination of 2,4-D with picloram might be considered to increase the quantity of embryogenic callus and somatic embryoids. The addition of high levels of activated charcoal up to 3 g/L caused a decline in the callogenesis of the explant tissue and reduced the 2,4-D availability in the medium thus the media will be added activated charcoal ranged 1.0 to 2.0 g/L for callus formation. The use of anthers as explants has never been reported for the MATAG variety and has only been reported on *Cocos Nucifera* (Tall type). These results provide a foundation for future study towards the development of micropropagation and haploid plant breeding.

ACKNOWLEDGMENTS

The authors would like to acknowledge Universiti Sultan Zainal Abidin (UniSZA) and Ministry of Higher Education Malaysia for the permission to publish this work and financing this project under Research Acculturation Grant Scheme (RAGS) (RAGS/2013/UNISZA/SG05/2) and the Department of Agriculture Terengganu for the supply of *Cocos Nucifera* Linn. Var. MATAG inflorescences.

REFERENCES

Adkins S. W., Samosir Y. M. S., Nikmatullah A. & Ogle, H. (2005). Coconut (*Cocos nucifera*) *in vitro* ecology: Modifications of headspace and medium additives can optimize somatic embryogenesis. II International Symposium on Biotechnology of Tropical and Subtropical Species (Eds. W.-C. Chang, R. Drew), pp. 21-31. International Society for Horticultural Science, Belgium.

Batugal, P., Bourdeix, R. & Baudouin L. (2009). Coconut Breeding. In Jain, S.M., & Priyadarshan, P.M. (Eds), *Breeding Plantation Tree Crops: Tropical Species*. New York: Springer Science Business Media, LLC.

Chan E. & Elevitch C. R. (2006). *Cocos nucifera (coconut), species profiles for pacific island agroforestry. 2.1, 30–50.* Retrieved from www.traditionaltree.org.

Chan, J. L., Saénz, L., Talavera, C., Hornung, R., Robert, M., & Oropeza, C. (1998). Regeneration of coconut (*Cocos nucifera* L.) from plumule explants through somatic embryogenesis. *Plant Cell Reports*, 17(6-7), 515–521.

Chidambaram, S., Singaraja, C., Prasanna, M. V., Ganesan, M., & Sundararajan, M. (2013). Chemistry of tender coconut water from the cuddalore coastal region in Tamil Nadu, India. *Natural Resources Research*, 22(2), 91–101.

Ebert, A., Taylor, F., & Blake, J. (1993). Changes of 6-benzylaminopurine and 2, 4-dichlorophenoxyacetic acid concentrations in plant tissue culture media in the presence of activated charcoal. *Plant Cell, Tissue and Organ Culture*, 33(2), 157-162.

Eeuwens, C.J. (1976). Mineral requirements for growth and callus initiation of tissue explants excised from mature coconut palms (*Cocos nucifera*) and cultured *in vitro*. *Physiol. Plant*, 36, 23-28.

Eshaghi, Z. C., Abdollahi, M. R., Moosavi, S. S., Deljou, A., & Seguí-Simarro, J. M. (2015). Induction of androgenesis and production of haploid embryos in anther cultures of borage (*Borago officinalis* L.). *Plant Cell, Tissue and Organ Culture*, 122(2), 321-329.

Fernando, S.C. & Gamage, C.K.A. (2000). Abscisic acid induced somatic embryogenesis in immature embryo explants of coconut (*Cocos nucifera* L.). *Plant Science*. 151: 193-193.

Fernando, S.C., Verdeil, J.L., Hocher, V., Weerakoon, L.K. & Hirimburegama, K. (2003). Histological analysis of plant regeneration from plumule explants of coconut (*Cocus nucifera* L.). *Plant Cell, Tissue and Organ Culture*. 72: 281-248.

Fernando, S.C., Vidhanaarachchi, V.R.M., Weerekoon, L.K. & Santha, E.S. (2010). What makes clonal propagation of coconut difficult? *Asia-Pacific Journal of Molecular Biology and Biotechnology*. 18(1): 163-165.

Fernando, S.C., Weerakoon, L.K., Perera P.I.P., Bandupriya, H.D.D. & Ambagala I.E. (2004). Genetic fidelity and *ex-vitro* performance of tissue cultured coconut palms. *Proceeding of the International Conference of the Coconut Research Institute, Sri Lanka* (CRISL). 47-57.

George, E.F., Michael, A.H. & Geert, J.D.K. (2008). *Plant Propagation by Tissue Culture*, 3rd edition, (Vol 1, pp. 56-59). Dordrecht, Netherlands: Springer Science and Business Media.

Karunaratne, S., Gamage, C. & Kovoor, A. (1991). Leaf maturity, a critical factor in embryogenesis. *Journal of Plant Physiology*. 139: 27-31.

Kaushal, L., Sharma, R., Balachandran, S. M., Ulaganathan, K., & Shenoy, V. (2014). Effect of cold pretreatment on improving anther culture response of rice (*Oryza sativa* L.). *J Exp Biol Agric Sci*, 2(2S), 233-242.

Koffi, Y., N'Nan-Alla, O., Konan Konan, J.-L., Malaurie, B., & Engelmann, F. (2013). Morphological and agronomical characteristics of coconut (*Cocos nucifera* L.) palms produced from in vitro cultured zygotic embryos. *In Vitro Cellular & Developmental Biology - Plant*, 49(5), 599–604

Kou, Y., Ma, G., da Silva, J. A. T., & Liu, N. (2013). Callus induction and shoot organogenesis from anther cultures of *Curcuma attenuata* Wall. *Plant Cell, Tissue and Organ Culture*, 112(1), 1-7.

Magdalita, P.M, Damasco, O.P. & Adkins, S.W. (2010). Effects of medium replenishment and acclimatization techniques on growth and survival of embryo cultured coconut seedlings. *Philippine Science Letters*. 3(2): 1-9.

Mahmood I, A., Binti Kayat, F., Ermiena Surya Mat Hussin, Z., Susanto, D., & Ariffulah, M. (2014). Determination of suitable microspore stage and callus induction from anthers of kenaf (*Hibiscus cannabinus* L.). *The Scientific World Journal*, 2014.

Oropeza, C., Rillo, E., Hocher, V., & Verdeil, J.L. (2005). Coconut micropropagation. In P. Batugal, V. R. Rao, & J. Oliver (Eds.), *Coconut Genetic Resources*. (pp. 334-348). Serdang, Malaysia: International Plant Genetic Resources Inst., Regional Office for Asia, the Pacific and Oceania.

Pan, M. J., & Van Staden, J. (1998). The use of charcoal in *in vitro* culture–A review. *Plant Growth Regulation*, 26(3), 155-163.

Perera, P. I. P., Hocher, V., Verdeil, J.-L., Bandupriya, H. D. D., Yakandawala, D. M. D., & Weerakoon, L. K. (2008a). Androgenic potential in coconut (*Cocos nucifera* L.). *Plant Cell, Tissue and Organ Culture*, 92, 293–302.

Perera, P. I. P., Vidhanaarachchi, V. R. M., Gunathilake, T. R., Yakandawala, D. M. D., Hocher V., Verdeil, J. L., & Weerakoon, L. K. (2009a). Effect of plant growth regulators on ovary culture of coconut (*Cocos nucifera* L.). *Plant Cell, Tissue and Organ Culture*, 99(1), 73–81.

Perera, P. I. P., Wickremasinghe, I. P., & Fernando, W. M. U. (2008b). Morphological, cytogenetic and genotypic differences between spicata and ordinary tall coconut (*Cocos nucifera* L.) *Plant Cell, Tissue and Organ Culture,* 36, 103–108.

Perera, P. I. P., Yakandawala, D. M. D., Hocher, V., Verdeil, J.-L. & Weerakoon, L. K. (2009b). Effect of growth regulators on microspore embryogenesis in coconut anthers. *Plant Cell, Tissue and Organ Culture*, 96(2), 171–180.

Perera, P. I., Hocher, V., Verdeil, J. L., Doulbeau, S., Yakandawala, D. M., & Weerakoon, L. K. (2007). Unfertilized ovary: a novel explant for coconut (*Cocos nucifera* L.) somatic embryogenesis. *Plant Cell Reports*, 26(1), 21-28.

Perera, P.I.P., Ordonez, C.A., Bacerra Lopez-Lavalle, L.A. & Dedicova, B. (2014). A milestone in the doubled haploid pathway in cassava. *Protoplasma*. 251, 233-246.

Pérez-Núñez, M. T., Chan, J. L., Sáenz, L., González, T., Verdeil, J. L., & Oropeza, C. (2006). Improved somatic embryogenesis from *Cocos nucifera* (L.) plumule explants. *In Vitro Cellular & Developmental Biology - Plant*, 42(1), 37–43.

Persley, G.J. (1992). *Replanting the tree of life*: Towards an international agenda for coconut palm research. Wallingford, Oxon, UK : CAB International - Australian Centre for International Agricultural Research (ACIAR).

Pullman, G. S., Gupta, P. K., Timmis, R., Carpenter, C., Kreitinger, M., & Welty, E. (2005). Improved Norway spruce somatic embryo development through the use of abscisic acid combined with activated carbon. *Plant Cell Reports*, 24(5), 271-279.

Raju, C. K., Prakash Kumar, P., Chandramohan, M. & Iyer, R. D. (1984). Coconut plantlets from leaf tissue cultures. *Journal of Plant Crops*. 12, 75-91.

Rubtsova, M., Gnad, H., Melzer, M., Weyen, J., & Gils, M. (2013). The auxins centrophenoxine and 2, 4-D differ in their effects on non-directly induced chromosome doubling in anther culture of wheat (*T. aestivum* L.). *Plant Biotechnology Reports*, 7(3), 247-255.

Sáenz, L., Herrera-Herrera, G., Uicab-Ballote, F., Chan, J. L. & Oropeza, C. (2009). Influence of Form of Activated Charcoal on Embryogenic Callus Formation in Coconut (*Cocos nucifera). Plant Cell, Tissue and Organ Culture*, 100(3), 301–308.

Sáenz, L., Souza, R., Chan, J. L., Azpeitia, A., & Oropeza, C. (2005). 14C-2, 4-dichlorophenoxyacetic acid uptake and formation of embryogenic calli in coconut plumular explants cultured on activated charcoal-free media. *Revista Fitotecnia Mexicana*, 28(2), 151-159.

Salas, P., Rivas-Sendra, A., Prohens, J., & Seguí-Simarro, J. M. (2012). Influence of the stage for anther excision and heterostyly in embryogenesis induction from eggplant anther cultures. *Euphytica*, 184(2), 235-250.

Sivapragasam, A. (2008). Coconut in Malaysia: Current Developments and Potential for Re-vitalization. 2nd International Plantation Industry Conference and Exhibition (IPICEX2008), Shah Alam, Malaysia. 18-21, November.

Sukamto, L. A. (2011). Effect of Physiological Age and Growth Regulators on Callus Browning of Coconut Endosperm. *Biotropia*, 18(1), 31–41.

United Plantation Berhad. (2013). MATAG and MAWA, Two Elite Coconut Planting Materials Produced Through Hybridization. [Online]. From: http://www.unitedplantations.com/Products/Coconut_products.asp. Accesed 20 December 2015.

Verdeil, J.C., Huet, C., Grosdemange G. & Buffard Morel, J. (1994). Plant regeneration from cultured immature inflorescences of coconut (*Cocus nucifera* L.): evidence for somatic embryogenesis. *Plant Cell Report*, 13, 218-221.

Verdeil, L. J. L., Hornung, R., Jacobsen, H. J., Rillo, E., Oropeza, C., Bourdeix, R., N'Cho, Y. P., Hocher, V., Hamon, S. and Snagare, A. (1999). Recent progress on coconut micropropagation through a joined effort involving different countries. In Oropeza, C., Verdeil, J. L., Ashburner, G. R., Cardena, R & Santamaria, J.M. (Eds), *Current advances in coconut biotechnology* (pp. 391-405). Dordrecht, Netherland: Kluwer Academic Publisher.

Vidhanaarachchi, V. R. M., Fernando, S. C., Perera, P. I. P., and Weerakoon, L. K. (2012). Application of Un-Fertilized Ovary Culture to Identify Elite Mother Palms of *Cocos nucifera* L. with Regenerative Potential. *Journal of the National Science Foundation of Sri Lanka*. 41 (1), 29–34.

Zakaria, Z. A., Reezal, I., Mat Jais, A. M., Somchit, M. N., Sulaiman, M. R., Marmin, A. H. I., ... & Abdul Rahman, L. (2006). The anti-inflammatory, anti-pyretic and wound healing activities of *Cocos nucifera* L. (MATAG types) fresh juice and kernel extract in experimental animals. *J. Pharmacol. Toxicol*, 1, 516-526.

How to cite this paper:
Zawawi, D.D., Abu Bakar, M.F. & Abd. Kadir, S.N. (2021). Effect of 2,4-Dichlorophenoxy Acetic Acid and Activated Charcoal on Callus Induction of *Cocos Nucifera* L. Hybrid MATAG Inflorescence. Journal of Agrobiotechnology, *12*(1S), 51-61