JOURNAL OF PURE AND APPLIED MICROBIOLOGY, May 2014. Vol. **8**(Spl. Edn. 1), p. 833-838

Study on Callogenesis and Growth Kinetics of *F. deltoidea* Var. Trengganuensis Cell Suspension Culture

TajulAfif Abdullah[1]*, Azura Amid[2], Noor Illi Muhamad Puad[2], Parveen Jamal[2] and Hafsah Jaafar[1]

[1]Department of Biotechnology, Faculty of Agriculture and Biotechnology, Universiti Sultan ZainalAbidin, Kampus Gong Badak, 21300 Kuala Terengganu, Terengganu.
[2]Bioprocess and Molecular Engineering Research Unit (BPMERU), Department of Biotechnology Engineering, Kuliyyah of Engineering, International Islamic University Malaysia, Jalan Gombak, 53100 Kuala Lumpur, Malaysia.

(Received: 08 January 2014; accepted: 24 March 2014)

Active compounds such as secondary metabolite from plants can be produced via its regeneration of organ or callus or from cell suspension culture. Thus, the aim of this study is to establish the protocol for callus regeneration and cell suspension culture of *Ficusdeltoidea* var. trengganuensis. Callus regeneration study was conducted on solid media consisted of 9 treatments with combination of picloram and 2,4-D ranging from 1.5 ppm to 4.5 ppm. After 4 weeks, the callus were weight under sterilize condition. Treatment consisted of MS + 3 ppm picloram + 3 ppm 2,4-D was found to form the highest weight of callus formation (68.8 ± 21.25 mg). Cell suspension was then established where 4 weeks old of soft and friable callus of *F.deltoidea* were used as an inoculum at 2% (w/v) in a 100mL of media. About1ml media was collected at 5 days interval to determine its dry cell weight. The cell suspension culture established in this study showed an increase in its dry cell weight (mg/mL) until day 15th where the density of the biomass were observed to decrease at day 20th. The highest specific growth rate (5.49 × 10^{-3} h^{-1}) was observed at day 5 to day 10.

Key words: Callus; cell suspension; dry cell weight (DCW); inoculum density; picloram.

Ficusdeltoidea which falls under *Moraceae* family is found in tropical and subtropical countries. Many parts of the plants are used in treating various kinds of ailments. The aqueous extract of *F. deltoidea* was reported to show a post prandial mild diabetic property [1]. The leaves extract of the plant is used traditionally in treating diabetes, high blood pressure and gout [2]. The biological activity of *F. deltoidea* extracts comes from its secondary metabolites from various parts of the plant. Moreover, studies have found that the *Ficus* species contains secondary metabolites such as flavonoid and steroids á-tocopherol and its derivatives, steroid and triterpernoids and alkaloids [3,4]. Flavonoids have several pharmacological activities such as anti-hypertensive, anti-inflammatory, antispasmodic, antimicrobial and antioxidant [5]. However, many of the bioactive compounds from the plant extract cannot be synthesized economically due to the fact that most of the secondary metabolites often have a complex stereo structure with many chiral centres. However, flavonoids can be produced using different biotechnological approaches, such as callus cultures, cell suspension cultures and/or organ cultures.

The important property of cell suspension is its ability to produce secondary metabolites

* To whom all correspondence should be addressed.
Tel:+603-61964429; Fax:+603-61964422;
E-mail: azuraamid@iium.edu.my

concomitantly with growth. Suggesting that, there is a possibility to get a continuous source of secondary metabolites from actively growing cells. Thus, plant cell suspension cultures possessed the potential for production of secondary metabolites, which are valuable in pharmaceutical as well as in food industries. Cell suspension culture can be used in producing plant embryogenic cells under controlled environment in which it would be a platform of plant production in large numbers [6] production of artificial seeds [7] cryopreservation [8] geneticmanipulations [9] and rapid production of bioactive compounds [10]Thus, this study aims to establish *F. deltoidea* var. trengganuensis callus regeneration protocol and cell suspension culture using the friable callus. Treatment which produced the highest weight of the callus after 4 weeks of culture were determined and the growth kinetic of *F. deltoidea* cell suspension were observed for 20 days.

MATERIALS AND METHODS

Establishment of friable callus

Callus of *F. deltoidea* var. trengganuensis were supplied by University Sultan ZainalAbidin (UniSZA) and were used as a stock culture. Callus were cultured onto solid media consist of MS media supplemented with 30g/l of sucrose and combination of 2 types of auxins which are picloram and 2,4-D ranging from 1.5ppm to 4.5ppm (Table 1). Gelrite agar was added at 2.5g/l and its pH was adjusted at 5.7 using 0.1M NaOH or 0.1M HCl. The media were autoclaved at 121˚C for 15 minutes before it is poured into petri dishes under sterilize condition. The callus were weight around 0.025g under sterilized condition before cultured onto solid medium in petri dish consist of 10 replicates of each treatment. In this study, all the cultures were maintained under 16 hours light and 8 hours darkness photoperiod at 26 ºC ± 2 ºC with the light intensity provided by white fluorescent tubes. After 4 weeks, the callus was taken out and weight using analytical balance under sterile condition.

Growth kinetics of F. deltoidea cell suspension culture

Friable and white callus were induced on solid media supplemented with plant growth hormone (PGH) from above study. Four weeks old callus were taken and weight for approximately 2.00g. A full strength of Murashige and Skoog medium (1962) was used in this study while sucrose as a carbon source was used at 3% (w/v). pH was adjusted at 5.7 using either 0.1M NaOH or 0.1M HCl. The media were autoclaved at 121 ºC, 15psi for 15 minutes. Once the friable callus was added into 100 mL liquid media supplemented with PGH as mentioned before. The cultures were incubated at 25 +1°C with a regular photoperiod of 16 hours light and 8 hours dark. Experiments were carried out in triplicates. At five (5) days interval, 3 mL of the cell culture were collected and filtered using pre-weight Whatman paper and placed in an oven at 80 ºC for 12 hours before weight.

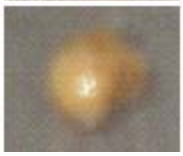
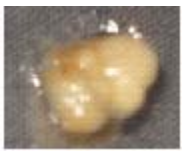
RESULTS AND DISCUSSION

Callus regeneration

Table 1 shows MS + 3.0mg/L picloram and 3.0mg/L 2,4-D is the best media formulation to generate friable callus. Plant growth regulator at an optimum level plays a vital role in inducing subsequent cell division as well as morphogenic response of anther wall and connective tissue [11,12] In this study, white and friable callus were selected and cultured on the solid medium. Picloram plays a role in induction of calluses and calluses maintenance as well as cell suspension or to induce the formation of embryogenic calluses [13]. Plant growth regulator such as 2,4-D was known to be extremely effective in formation of callus, however the optimum concentration applied in the media varied from one plant to another[14.]Some plant requires a combination of 2,4-D with other plant growth regulator in calluses induction such as *Theobroma cacao* cultivated on MS media with 2,4-D and kinetin[15]. However, long term cultures supplemented with 2,4-D or other strong auxins can increase the occurrence of somaclonal variation [16]

The type and content of agar also plays a significant role in affecting the callus formation. Gelrite at the concentration of 2.0g/l was found to stimulated percentage of half anther producing callus and number of half anthers producing callus of *Anthurium andreanum* significantly compared to Swallow agar at concentration of 7g/l [17.] The explant growth on the solid media are affected by the gel mineral composition, mineral availability,

Table 1. Treatment consist of combination of picloram and 2,4-D and its response on production of callus after 4 weeks culture

Treatment	Picloram (ppm)	2,4-D (ppm)	Callus Weight (mg)	Photo of callus after 4 weeks
1	1.5	3.0	45.9 + 14.82	
2	1.5	1.5	63.66 + 23.46	
3	1.5	4.5	44.9 + 11.99	
4	3.0	4.5	41.9 + 8.12	
5	3.0	1.5	38.4 + 11.9	
6	3.0	3.0	68.8 + 21.25	
7	4.5	4.5	48.2 + 14.47	
8	4.5	3.0	61.5 + 25.03	
9	4.5	1.5	38.2 + 6.66	

inhibitory compounds, water availability and the gel strength itself [18]. A number of the callus turned brown after 4 weeks of culture (Treatment 7) suggesting a content of phenolic compounds in the callus. Phenolic compounds specifically oxidized phenolics generally contribute negative effect to *in vitro* proliferation [19]. Oxidized phenolic compounds may inhibit enzyme activity thus resulting in culture medium darkening and subsequent lethal browning of the explants [20] However, the phenolic compounds in the callus of *F. deltoidea* var. trengganuensis may contributed by its antioxidant metabolites such as flavonoid. Ong and co-workers, [21] identified three flavonoids which are rutin, quercetin and naringenin respectively in extracted cell of *F. deltoidea* cell suspension. Phenolic acid are intermediates from metabolism of phenylpropanoid[22] and phenylpropanoidphytoalexins[23] Another factor which may contributed to the formation of phenolic browning is the addition of picloram as suggested by Taylor and co-workers[24], where suspension culture added with high picloram produced more phenolic compound compare to when 2,4-D was added into the growth media. As an addition, increasing concentration of 2,4-D it self above its optimum level would also affect negatively on the compactness of the callus and produce the browning of the callus [25].

Growth Kinetics of Ficusdeltoidea var. trengganuensis cell suspension

Figure 1 shows that the dry cell weight increased to its maximum (10.27± 1.514 mg/ml) at day 15th . However, at day 20th the cell dry weight decreased to 8.00 ± 2.458 mg/ml. Ong and colleagues [26] found that MS + 3ppm picloram was the best concentration to induce growth of *F. deltoidea* cell suspension with the highest packed cell volume (PCV). In our study we found that the cell suspension entered an exponential stage at 3rd interval (day 15th) compared to study carried out by Ong and colleagues [26] which reached it stationary phase at day 3. This is due to difference in inoculum size used. In the study by Ong and colleagues[26], they used 50 % (w/v) of inoculum density in studying the effect of picloram and 2,4-D on growth measurement of *F. deltoidea* cell suspension. In our study, only 2 % (w/v) of inoculum density were used in establishment of *F. deltoidea* cell suspension. This might explain why exponential phase stopped at (day 15th) in our study. From our study, we found that the specific growth rate was the highest at day 5 to day 10 (5.49 x10^{-3} h^{-1}) while from day 15 to day 20 the specific growth rate is -2.083 x 10^{-3} h^{-1} suggesting a death phase from day 15 to day 20th.

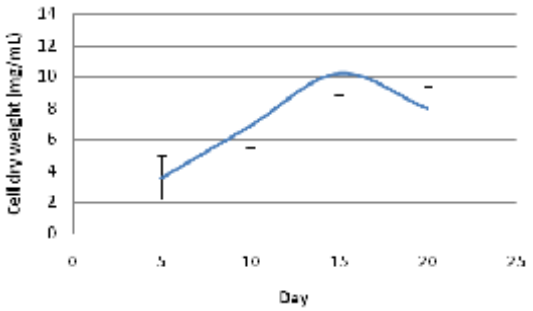


Fig. 1. Dry cell weight of *F. deltoidea* cells from day 5th to day 20th

CONCLUSIONS

In this study, MS media supplemented with 3 ppm picloram + 3ppm 2,4-D showed highest weight of callus and produced a friable callus after 4 weeks of culture. The growth kinetics of *Ficusdeltoidea* var. trengganuensis cell suspension culture reach its maximum dry cell weight at day 15. The specific growth of *F. deltoidea* cell suspension was at the highest from day (5) to day (10).

ACKNOWLEDGEMENTS

This work is supported by Ministry of Higher Education Malaysia under Fundamental Research Grant Scheme (Grant no FRGS12-063-0212) and authors would also thank the Skim LatihanAkademikBumiputra (SLAB) for funding the tuition fees of TajulAfif Abdullah at the PhD level.

REFERENCES

1. Adam, Z., Hamid, M., Ismail, A., Khamis, S. Effect of Ficus deltoidea Aqueous Extract on Blood Glucose Level in Normal and Mild Diabetic Rats. *Jurnal Sains Kesihatan Malaysia*. 2007; **5**: 9-16.
2. Ahmad, F., Holdsworth, D.K. Medicinal Plants of Sabah, East Malaysia -Part I. *Pharm Biol*.

2003; **41**: 340-6.
3. Diaz, G., Arruda, A.C., Arruda, M.S.P., Muller, A.H. Methoxyflavones from Ficus maxima. *Phytochemistry*. 1997; **45**: 1697-9.
4. Chiang, Y.M., Kuo, Y.H. Two novel alpha-tocopheroids from the aerial roots of Ficus microcarpa. *Tetrahedron Lett*. 2003; **44**: 5125-8.
5. Picerno, P., Mencherini, T., Lauro, M.R., Barbato, F., Aquino, R. Phenolic constituents and antioxidant properties of Xanthosoma violaceum leaves. *J Agric Food Chem*. 2003; **51**: 6423-8
6. Martin KP .Plant regeneration through somatic embryogenesis in medicinally important Centella asiatica L. *In Vitro Cell Dev Biol Plant* 2004; **40**: 586-591
7. Maruyama E, Hosoi Y, Ishii K . Somatic embryo culture for propagation, artificial seed production, and conservation of sawara cypress (Chamaecyparis pisifera Sieb. et Zucc.). *J For Res*. 2003; **8**: 1-8
8. Kong L, von Aderkas P . A novel method of cryopreservation without a cryoprotectant for immature somatic embryos of conifer. *Plant Cell Tiss Organ Cult*. 2011; **106**: 115-125
9. Parimalan R, Akshatha V, Giridhar P, Ravishankar GA.Somatic embryogenesis and Agrobacterium-mediated transformation in (Bixa orellana L.). *Plant Cell Tiss Organ Cult*. 2010; **105**: 317-328
10. Jeong JH, Jung SJ, Murthy HN, Yu KW, Paek KY, Moon HK, Choi YE.Production of eleutherosides in in vitro regenerated embryos and plantlets of Eleutherococcus chiisanensis . *Biotechnol Lett*. 2005; **27**: 701-704.
11. Leguay,J.J., and Guern, J. Quantitative effects of 2,4-dichlorophenoxyacetic acid on growth of suspension cultured Acer pseudoplatanus cells : Influence of 2,4-D metabolism and intracellular pH on the control of cell division by intracellular 2,4-D concentration. *Plant Physiol*. 1977; **60**: 265-270
12. Rodrigues, L.R.,de Forte B.C., Oliveira, J.M.S., Mariath, J.E.A. and Bodanese-Zanettini,M.H. Effect of light conditions and 2,4-D concentration in soybean anther culture. *Plant Growth Regulator*. 2004; **44**: 125-131
13. Stefanello, S., Dal Vesco, L.L., Ducroquet, J.P.H.J., Nodari, R.O., Guerra, M.P. Somatic embryogenesis from floral tissues of feijoa (Feijoa sellowiana Berg). *Sci Hortic-Amsterdam*. 2005; **105**: 117-26.
14. Fu, X.P., Yang, S.H. and Bao, M.Z . Factors affecting somatic embryogenesis in anther cultures of Chinese pink (*Dianthus chinensis* L.). *In Vitro Cell Dev Biol Plant*. 2008; **44**: 194-202
15. Alemanno, L., Berthouly, M., MichauxFerriere, N. Histology of somatic embryogenesis from floral tissues cocoa. *Plant Cell Tiss Org*. 1996; **46**: 187-94.
16. Von Arnold, S., Sabala, I., Bozhkov, P., Dyachok, J., Filonova, L. Developmental pathways of somatic embryogenesis. *Plant Cell Tiss Org*. 2002; **69**: 233-49.
17. Winarto, Budi and da Silva, Jaime A. teixeira. Influence of isolation technique of half-anthers and of initiation culture medium on callus induction and regeneration in Anthurium andreanum. *Plant Cell Tiss Organ Cult*. 2012; **110**: 401-411
18. Cardoso, M.B., Bodanese-Zanettini, M.M.H, De Mundstock, E. and Kaltchuk-Santos, E.K Evaluation of gelling agents on anther culture:response of two soybean cultivars. *Braz Archiv Biol Technol*. 2007; **50**: 933-939
19. Arnaldos, T.L., Munoz, R., Ferrer, M.A., Calderon, A.A. Changes in phenol content during strawberry (Fragaria x ananassa, cv. Chandler) callus culture. *Physiol Plantarum*. 2001; **113**: 315-22.
20. Laukkanen, H., Haggman, H., Kontunen-Soppela, S., Hohtola, A. Tissue browning of in vitro cultures of Scots pine: Role of peroxidase and polyphenol oxidase. *Physiol Plantarum*. 1999; **106**: 337-43.
21. Ong, S.L., Ling, A.P.K., Poospooragi, R., Moosa, S. Production of flavonoid conpounds in cell cultures of Ficus deltoidea as influenced by mediaum composition. *Int J Med Arom Plants*. 2011; **1**: 62-74.
22. Cvikrova, M., Hrubcova, M., Eder, J., Binarova, P. Changes in the levels of endogenous phenolics, aromatic monoamines, phenylalanine ammonia-lyase, peroxidase and auxin oxidase activities during initiation of alfalfa embryogenic and nonembryogenic calli. *Plant Physiol Bioch*. 1996; **34**: 853-61.
23. Kessmann, H., Choudhary, A.D., Dixon, R.A. Stress Responses in Alfalfa (Medicago-Sativa L) .3. Induction of Medicarpin and Cytochrome P450 Enzyme-Activities in Elicitor-Treated Cell-Suspension Cultures and Protoplasts. *Plant Cell Reports*. 1990; **9**: 38-41.
24. Taylor, P.W.J., Ko, H.L., Adkins, S.W., Rathus, C., Birch, R.G. Establishment of Embryogenic Callus and High Protoplast Yielding Suspension-Cultures of Sugarcane (Saccharum Spp Hybrids). *Plant Cell Tiss Org*. 1992; **28**: 69-78.
25. Zaman,M.A., Manjur, A.B.M.K., Ahmed,M. and Islam,M.M.Effect of 2,4-D on callus

Induction and subsequent morphogenesis in mature chickpea (Cicer arietum L.) embryo culture. Plant Tissue Culture and Biotech. Conf. 2010; 53-58.
26. Ong, S.L., Kiong, A.L.P., Hussein, S. Establishment and optimization of growth parameters for cell suspension culture of Ficus deltoidea. *American-Eurasian Journal of Sustainable Agriculture*. 2008; **2**: 38-49.