

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

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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 1.5ppm to 4.5ppm. After 4 weeks, the callus were weight under sterilize condition. Treatment consisted of MS+3ppm picloram+ 3ppm 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. About 1ml 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 ($5.49 \times 10^{-3}h^{-1}$) was observed at day 5 to day 10.

Key words: Callus, Cell suspension, dry cell weight (DCW), Inoculum density, Picloram.

Ficus deltoidea 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 property¹. The leaves extract of the plant is used traditionally in treating diabetes, high blood pressure and gout². 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 α -tocopherol and its derivatives, steroid and triterpenoids and alkaloids^{3,4}. Flavonoids have several pharmacological activities such as anti-hypertensive, anti-inflammatory, antispasmodic, antimicrobial and antioxidant⁵. 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

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is its ability to produce secondary metabolites 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 numbers⁶, production of artificial seeds⁷, cryopreservation⁸, genetic manipulations⁹ and rapid production of bioactive compounds¹⁰. 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 Zainal Abidin (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°C ± 2°C 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°C, 15psi for 15 minutes. Once the friable callus was added into 100mL 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°C 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 tissue¹¹⁻¹². 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 calluses¹³. 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 another¹⁴. 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 kinetin¹⁵. However, long term cultures supplemented with 2,4-D or other strong auxins can increase the occurrence of somaclonal variation¹⁶.

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 stimulate percentage of half anther producing callus and number of half anthers producing callus of *Anthurium andreaeanum* significantly compared to Swallow agar at concentration of 7g/l¹⁷. The explant growth on the solid media are affected by

the gel mineral composition, mineral availability, inhibitory compounds, water availability and the gel strength itself¹⁸. 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

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

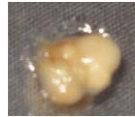








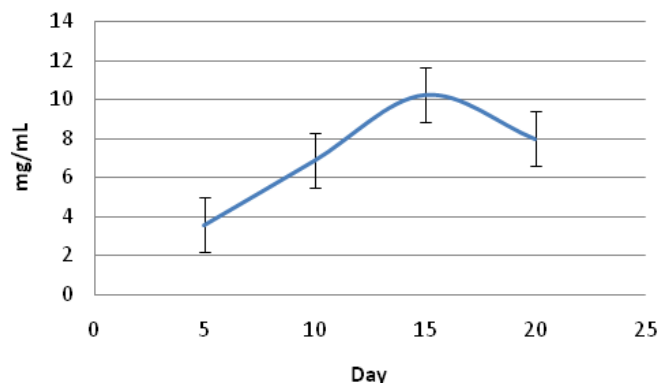
Treatment	Picloram (ppm)	2,4-D (ppm)	Callus Weight (mg)	Photo of callus after 4 weeks
1	1.5	3.0	45.9 + 14.82	
2	1.5	1.5	63.66 + 23.46	
3	1.5	4.5	44.9 + 11.99	
4	3.0	4.5	41.9 + 8.12	
5	3.0	1.5	38.4 + 11.9	
6	3.0	3.0	68.8 + 21.25	
7	4.5	4.5	48.2 + 14.47	
8	4.5	3.0	61.5 + 25.03	
9	4.5	1.5	38.2 + 6.66	

Table 2. Specific growth rate of *F. deltoidea* cell suspension from day 5 to day 20

Day	D _{10/5}	D _{15/10}	D _{20/15}
Specific growth rate, μ (h ⁻¹)	5.49×10^{-3}	3.34×10^{-3}	-2.08×10^{-3}

oxidized phenolics generally contribute negative effect to *in vitro* proliferation¹⁹. Oxidized phenolic compounds may inhibit enzyme activity thus resulting in culture medium darkening and subsequent lethal browning of the explants²⁰. However, the phenolic compounds in the callus of *F. deltoidea* var. *trengganuensis* may be 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 phenylpropanoid²² and phenylpropanoid-phytoalexins²³. Another factor which may contribute to the formation of phenolic browning is the addition of picloram as suggested by Taylor and co-workers²⁴, where suspension culture added with high picloram produced more phenolic compound compared to when 2,4-D was added into

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

the growth media. As an addition, increasing concentration of 2,4-D 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 *Ficus deltoidea* 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 its 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 ($5.49 \times 10^{-3} \text{ h}^{-1}$) while from day 15 to day 20 the specific growth rate is $-2.083 \times 10^{-3} \text{ h}^{-1}$ suggesting a death phase from day 15 to day 20th.

CONCLUSIONS

In this study, MS media supplemented with 3ppm picloram + 3ppm 2,4-D showed highest weight of callus and produced a friable callus after 4 weeks of culture. The growth kinetics of *Ficus deltoidea* 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).

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