The combination effect of *Naphthalene Acetic Acid* (NAA) and *Benzyl Amino Purine* (BAP) in micro propagation of castrol oil plant (*Jatropha curcas* L.)

Meutia Zahara[1], Zairin Thomy[2] and Essy Harnelly[2]

[1]Department of Biology. Muhammadyah Aceh University. Banda Aceh 23245. Indonesia;
[2]Department of Biology. Syiah Kuala University. Banda Aceh 23111. Indonesia.
Corresponding Author: teeya_razali@yahoo.co.id

Abstract. The fossil energy reservation decreases day by day, this situation impacted to the energy crises especially fuel energy which is increasing in the price on over the world. The scientists/researchers are trying to develop the renewable energy (renewable fuel) as the energy alternative, one of these called biodiesel. Taking the benefit from *Jatropha curcas'* seed as raw material for the fuel is one of the alternative ways to reduce the fuel demand and also can save the countries' foreign exchange. *J. curcas'* oil is renewable fuel and non edible oil, so that it will not compete with oil consumption, such as palm oil, corn oil, etc. The combination between *Naphthalene acetic acid* (NAA) (1-2 ppm) and *Benzyl Amino Purine* (BAP) (0,5 and 1 ppm) for micro propagation of Castrol oil plant (*Jatropha curcas* L.) was tried to induce the callus formation by using the meristem shoot as the explants. The result showed that the combination of NAA 1.5 ppm and BAP 0,5 ppm gave the best result for the callus formation.

Key words: Castrol oil plant (*Jatropha curcas* L.), callus, NAA and BAP

Introduction

Energy is a fundamental for the quality of life in the earth. Meeting the growing demand for energy sustainably is one of the major challenges of the 21st century. Indonesia is a developing country and the world's fourth most populous nation. Total annual energy consumption increased from 300,147 GWh in 1980, 625,500 GWh in 1990, 1,123,928 in 2000 and to 1,490,892 in 2009 at an average annual increase of 2.9%. Presently, fossil-fuel-based energies are the major sources of energy in Indonesia. During the last 12 years, Indonesia has recorded the most severe reduction in fossil fuel supplies in the entire Asia-Pacific region. This reduction has stimulated promoting the usage of renewable energy resources capable of simultaneously balancing economic and social development with environmental protection. Biodiesel is an alternative and environmentally friendly fuel that will participate in increasing renewable energy supply. Jatropha curcas is one of biodiesel resources that offer immediate and sustained greenhouse gas advantages over other biodiesel resources. Globally, J. curcas has created an interest for researchers because it is non-edible oil, does not create a food versus fuel conflict and can be used to produce biodiesel with same or better performance results when testing in diesel engines (Silitonga, et. al. 2011).

J. curcas belongs to family Euphorbiaceae which potent plant of great economic value, it is a drought resistant and received global attention due to its seed oil that contains up to 35-40% oil easily convertible into biodiesel (Rajore and batra, 2007), its potential to reclaim wasteland with positive effects on ecology and socio-economic development. The numbers of critical lands in Indonesia are almost 20 million hectares, half of that are located out of the forest areas which are still not function optimally, even abandoned. By finding the potential of J. curcas that is easy to grow, we can develop it as the biodiesel source on the special land that can promise a new hope for the agricultural business (Maryadi, 2005). According to Sheety (2005), J. curcas (Castrol oil plant) has high growth potential and can adapt to every kind of critical lands.

Plant micro propagation in tissue cultures technique is one of the ways to multiply the plants which have a lot of advantages, it can save the work and time. A large number of plants can be produced with good quality and the same trait as the mother plants in a short time compared to the conventional propagation system (Mineo, 1990). Auxins (NAA, IBA, IAA, etc) and cytokinins (Zeatin, BAP, BA, etc.) are the most widely used plant growth regulators in tissue culture and are usually combine together (Anonymous, 2003).

Materials and Methods

The research had been done in Cell biology and molecular laboratory department of Biology, Sciences Faculty of Syiah Kuala University Banda Aceh, NAD-Indonesia from March until August 2006 by using the young shoot of *Jatropha curcas* L as the materials.

Explants sterilization

The explants were washed 3 times under running tap water and continued with the detergent then washed again under running tap water, immersed in *clorox* for 5 minutes and washed thoroughly distillates water inside the laminar air flow cabinet.

Cultivation

The explants were cut in small pieces and cultured on MS medium consist of NAA (1.0, 1.5 and 2.0 ppm) and BAP (0.5 and 1 ppm). The medium were consisted of 8 g/l agar and 30 g/l sucrose, PH were adjusted to 5.8 using 0.1 N NaOH or 0.1 N HCL. Completely randomized design (CRD) with 2 factors and repeated 3 times of each were used. NAA concentration, consisted of three levels i,e. 1 ppm, 1,5 ppm and 2 ppm, BAP concentration, also consisted of three levels i.e. 0,5 ppm, 1 ppm and 1,5 ppm, so that this research had 9 combinations of treatment. The result analyzed by ANOVA, then will be continued with less significant honestly test. Expansion time, callus appearance time and fresh weigh were calculated.

Results and Discussion

Expansion time of the explants

According to the Analysis of Varian (ANOVA), it showed that the combination between NAA and BAP had significant effect for the expansion time of the explants (size was getting bigger) ($P < 0, 01$). The expansion was the beginning of the explants growth by taking up the nutrition from medium then followed by proliferation (cell multiplication). This process assumed that related with the ability of the cell to growth and protect its structure, by expanding the cell wall and plasma lemma step by step through the metabolic process. This process resulted in the water come in and fulfilled the empty spaces, synthesis the cellulose fibre which is the cell wall composer through the spaces formed. This statement is also appropriate with Salisbury and Ross (1992) that mentioned the cellulose fibre synthesized resulted in the expansion of the cell wall and couldn't back anymore to the previous size, so that the growth happened. The turgor pressure was also very important in growth. Auxin and cytokinin were kind of plants growth regulator which were involved in increasing the plant cell wall expansion.

The growth of the explants in each treatment showed the different time for expanding the cell wall in water up taking process. This situation estimated because of the combination of Plant growth regulator in different concentration. The average time for expanding of the explants are shown on the Table 1.

According to the result of our observation, explants that were growing on the MS medium which consisted NAA 1 ppm + BAP 0, 5 ppm and NAA 1 ppm + BAP 1 ppm combination showed the fastest time for explants to expand, NAA 1 ppm was the best concentration to stimulate the cell expansion and cell division. NAA is a type of auxin that produced naturally by plants endogenously, and nowadays some company produced it synthetically. The amount of auxin inside the plants (endogenous) also has different amount, even thought in the same plants, it was very important to stimulate the cell expansion, cell division, and callus growth.

Table 1. The average time for cell wall expansion

No	NAA	BAP	Expansion time (dap)
1	1 ppm	0.5 ppm	4^{a}
2	1 ppm	1 ppm	4^{a}
3	1 ppm	1.5 ppm	$4,66^{ab}$
4	1.5 ppm	0.5 ppm	7^{bcd}
5	1.5 ppm	1 ppm	9^{c}
6	1.5 ppm	1.5 ppm	$7,33^{cd}$
7	2 ppm	0.5 ppm	6^{abc}
8	2 ppm	1 ppm	$6,66^{bcd}$
9	2 ppm	1.5 ppm	$7,33^{cd}$

Callus formation time

Based on the ANOVA result, it showed that the combination between auxin and cytokinin (in this case is NAA and BAP) significantly affected the callus formed time (P < 0, 01). The average time to formed callus in each treatment is shown on the table 2

Table 2. The average time to formed callus

No	NAA	BAP	Time (dap)
1	1 ppm	0.5 ppm	7^{a}
2	1 ppm	1 ppm	9^{ab}
3	1 ppm	1.5 ppm	$8,66^{ab}$
4	1.5 ppm	0.5 ppm	9^{ab}
5	1.5 ppm	1 ppm	12^{c}
6	1.5 ppm	1.5 ppm	9^{ab}
7	2 ppm	0.5 ppm	7^{a}
8	2 ppm	1 ppm	8^{ab}
9	2 ppm	1.5 ppm	10^{bc}

The auxin and cytokinin combination with the concentration that almost balance showed the growth of callus could be optimally. This condition matched also with Campbell and Reece (2002)'s statement, if we put the only cytokinin without any combination with auxin in the tissue culture medium technique, there would be nothing happen except the cell expansion without division, so that no influence in explants growth. But, if we could combine auxin and cytokinin, it could stimulate the cell division optimally. When the concentration among plants growth regulator almost in balance, then the plants cell mass will increase by forming undifferentiated callus. The formed callus could be sub cultured to a new medium with the concentration of cytokinin higher that auxin to stimulate the root formation. Basically, the gene expression could be controlled by manipulated this chemical agent.

The results showed that the time for explants to expand (Cell enlargement) didn't involved the time for callus appearance in the explants. In some treatments, the explants could expand in a short time, but need a long time to emerge the callus compared to others treatments. Contrary, in some other treatments the explants need a long time for expanding itself, but could be merged and induced the callus in short time, even though it was getting bigger day by day. This phenomenon happened because of the differentiation in

the concentration of plants growth regulator that we put in the MS medium. NAA 1 ppm + BAP 0, 5 ppm combination showed the shortest time among the others combinations. It proved that BAP (cytokinin) played important roles in the cell division, growth and morphogenesis. Callus growth started from the edge of the explants and then continued from wounded parts which were contacted with the medium directly. After 4 weeks (days after growing), the size of the callus showed different percentage in each treatment, we can see the result in the table below:

Tabel 3. The percentage of the callus size in each treatment after 4 weeks

No	NAA	BAP	Repetition		
			I	II	III
1	1 ppm	0.5 ppm	++	++	++
2	1 ppm	1 ppm	++	++	++
3	1 ppm	1.5 ppm	++	++	++
4	1.5 ppm	0.5 ppm	+++	+++	+++
5	1.5 ppm	1 ppm	++	++	++
6	1.5 ppm	1.5 ppm	++	++	++
7	2 ppm	0.5 ppm	+	++	++
8	2 ppm	1 ppm	+	++	+++
9	2 ppm	1.5 ppm	+	+	++

Annotation:
\+ = Small in percentage
++ = Mid in percentage
+++ = Big in percentage

Callus which grows in each treatment has almost the same structures (compacted structure/rounded and harder) with yellow green colors and the growth direction to the center. According to result, it showed us that the combination of NAA 1, 5 ppm + BAP 0, 5 ppm formed the biggest size and keep growing bigger day by day, this phenomenon also correct with the statement of George and Sherington (1984) that the combination of concentration between auxin and sitokynin in balances or nearly balances that accelerate the growth. The callus which formed after 4 weeks shows in the picture below:

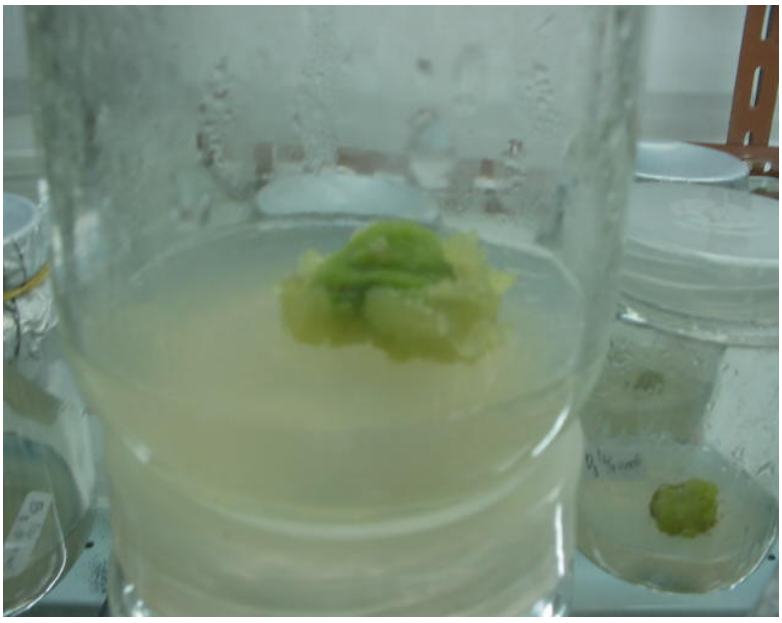

Figure 1. Callus formed after 4 weeks.

Callus fresh weight

According to the data's analysis, it showed that the combination between auxin and sitokynin influenced in callus fresh weight significantly with high standard deviation (55 %). Basically, the callus growth rate in each treatment was good with a big callus formation. In some treatments showed the callus induction in short time (fast), but the growth rate was slow (not maximal), so that it had some differentiation even though the explants come from the same mother plants (Table 4.). This condition assumed that because of the tissues condition and endogenous hormones in the plants was different.

Table 4. Average of the callus fresh weight after treatments.

No	NAA	BAP	Fresh weight (g)
1	1 ppm	0.5 ppm	0,943^{cde}
2	1 ppm	1 ppm	0,735^{bcd}
3	1 ppm	1.5 ppm	0,807^{bcde}
4	1.5 ppm	0.5 ppm	2,501^{f}
5	1.5 ppm	1 ppm	0,653^{bc}
6	1.5 ppm	1.5 ppm	1,122^{e}
7	2 ppm	0.5 ppm	0,606^{ab}
8	2 ppm	1 ppm	0,967^{ab}
9	2 ppm	1.5 ppm	0,351^{a}

The final callus fresh weight was influenced by water absorption then impacted to cell wall enlargement, so that emerged callus formation. This phenomenon also matched with Lakitan's statement (1996) that if we want to increase the cell size, the water absorption must occurs all the time which is influenced by the water concentration lower than water concentration outside of the cell. The water come in to the cell will increase the water concentration in it, so that the water absorption will decrease also. To increase the absorption again, we have to increase the water potential outside if the cell by increasing the solutes content inside the water. The cell wall enlargement also influenced by the hormones/growth regulator activity such as auxins, cytokinins or gibberellins with its own functions

The combination between NAA 1, 5 ppm + BAP 0, 5 pp, gave the highest fresh weight in callus formation. Maybe this is best combination to induce the callus formation for Castrol oil plants (*Jatropha curcas* L.) which was the first step for growing and developing of these plants in the future. The cell growth that will influence the fresh weight at the end; need water and nutrition absorption from the environment (culture medium)

Conclusions

The combination between auxin (NAA) and cytokinin (BAP) were playing important roles for callus growth and development of Castrol oil plants (*Jatropha curcas* L.), this combination also showed the significant effect on the callus appearances in time, final fresh weight and callus expansion. The combination between NAA 1, 5 ppm and BAP 0, 5 pp, showed the best results in Castrol oil plants growth (*Jatropha curcas* L.). Cell growth was influenced by water and nutrition absorption from the environmental (medium), this part was very important.

Acknowledgements

The Authors would like to thanks for Syiah kuala university and DIKTI for had been providing the laboratory, materials and grand in this research.

References

Anonymous. 2003: Plant tissue culture. http://www.oup.com/uk/orc/bin/0199254680/ch02.pdf. June, 12. 2010.

Campbell, N.A., Reece, J.B. 2002. Biology. 6th edition. San Francisco: Benjamin Cummings.

George, F.E., Sherrington, P.D. 1984. Plant propagation by tissue culture. 1st edition. Exegetics limited. England.

Lakitan, B. 1996. Dasar-dasar Fisiology Tumbuhan. Rajawali Press.

Maryadi. 2005. Budidaya Tanaman Jarak (*Jatropha curcas*). Jakarta.

Mineo, L. 1990. Plant tissue culture techniques. Chapter 9. Tested studies for laboratory teaching 11 (C. A. Goldman, Editor). Proceeding of Eleventh Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 151-157.

Rajore, S., Batra, A. 2007. An alternative Source for regenerable organic callus induction in Jatropa curcas L. Indian journal of biotechnology, Volume 9: pp-545-548

Salisbury and Ross. 1992. Plant Physiology. Fourth edition. Belmont, CA: Wadsworth, Inc.

Shetty, A. S. 2005. Energy Plantation Problems and Progress. India. http://www.lablandbiotechs.com/event001.html.

Silitonga A.S., Atabani A.E., Mahlia T.M.I., Masjuki H.H., Badruddin I.A., Mekhilef S. 2011. A review on prospect of Jatropha curcas for biodiesel in Indonesia. Elvisier, Renewable and Sustainable Energy Reviews 15 (2011) 3733– 3756.