Embryogenesis Callus Induction of *Carica pubescens* Using Divine Smoke Particulates Containing Amino Acids

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**Abstract.** *Carica pubescens* is one of plants that can live in the highlands which has a potential as medicinal plant to treat many diseases. The plant propagation is still done conventionally. Embryogenic callus culture is the alternative. The success of callus culture depends on the role of auxin growth regulator (2,4-D) and Amino Acids. The aim of this study is to determine the effect of amino acids through Divine smoke particulation to the induction of embryogenesis callus *carica pubescens*. This research is an experimental study with Complete Randomized Design (CRD) method. It has 7 treatments consist of control and the Amino Acids (Threonine, Tryptophan, Serine, Histidine, Asparagine, and Methionine). The result of this study showed a significant effect of amino acids through Divine smoke particulation to the induction of embryogenesis callus *Carica pubescens* which is grown on ½ MS media + 3 mg/L of 2,4-D. Tryptophan is the fastest treatment to induce callus which is grown 24 days after planting (DAP). Serine influences to the percentage of callus area in explants is 98,8% and the fresh weight of callus 0,6733 gr.

The observation of morphology and anatomy embryonic callus color is yellowish white, friable texture and has a large cell nucleus

**Keywords:** Amino Acids, *Carica pubescens*, Divine particulation

1. Introduction

Indonesia is one of the country that has high Biodiversity. One of them is the richness of plant species. One of plants that has the benefits is *Carica pubescens* Lenne & K. Koch. For the people of Indonesia, some of us are not so familiar with this one fruit. *Carica* fruit is hard to find because *Carica* fruit only grows in areas high and has a cold temperature or in the mountains. *Carica* fruit which be found in Bromo, Cangar Batu East Java, and Dieng Plateu Central Java. *Carica* fruit has many functions as a food ingredient because it can be processed into many kinds, like jam, sweets, syrup, chips. his fruit is used as a non-alcoholic soft drink and made into jam. Young fruit is usually dried to be used as a powder material for making skin diseases or cosmetics. Wonosobo residents themselves are making a home industry that process *Carica* fruit and sell it. *C. pubescens* became one of the typical hand of the area, and also export abroad [1]. Besides that, *C. pubescens* has potential as medicinal plant, contain antioxidants, The seed extract of *C. pubescens* can be potentially as larvacide of *Aedes aegypti* mosquitoes [2]. The seed of *C. pubescens* can use as an extract for antibacterial to Enteropathogenic *Escherichia coli* (EPEC) that it causes diarrhea to male mice [3].

The multiplication of *C. pubescens* in Indonesia is still done conventionally. If it grown conventionally, the germination of *C. pubescens* seed needs a long time. It is difficult to germinate because of the type of seeds, genetic factor, and the condition of the environment. Besides, a
conventional germinating caused by the pulp in the aleuron of *C. pubescens* that contains absisic acid. This hormone plays a role as inhibitor to the germination of the seeds. So, if the cultivation of *C. pubescens* is not developed optimally, it is concerned that this germplasm will be increasingly rare and extinct. Therefore it is necessary to do some conservation efforts because it is not cultivated specifically. So, it needs a tissue culture plant. In vitro culture has been proven to produce seedling in large number, in a short time, pathogen free, and does not need a lot of mother plant. Embryogenic callus culture has the potential become a plant through organogenesis and embryogenesis [4]. The embryogenic cells show characteristics common to meristematic cells: high division rates, cells are isodiametric and small with a dense cytoplasm with several starch grains, large nucleus and prominent nucleolus, small vacuoles, thin cell walls, and a higher metabolic activity. The embryogenic callus morphology show characteristics callus color is yellowish white with a friable texture.

Growth regulator 2,4-**Dichlorophenoxyacetic acid** is a type of strong Auxin and can be broken down into the plant’s body [5]. Increasing formation and growth callus can be done by giving Amino Acids. Amino Acids are protein constituent that has a function to plant as transporting other substances, coordinating the activities of organisms, responding cells to stimuli, movement, protection against diseases, and catalyzing chemical reactions selective [6].

Amino Acids can be obtained from Divine smoke processed from tobacco and other syntheses that contain nicotine and tar with Amino Acids additives. The Amino Acids that added into explant plants can be through Divine smoke particulation which are able to penetrate the cells of the desired plant organs, because Divine smoke particulation works in nano molecules [7]. Divine smoke containing complex nanostructures can supply electrons in millivolts. In nano size, there is an electric field force. Divine smoke can function to supply energy while capturing mercury free radicals. even divine smoke supplies energy more efficiently than nutrients. Divine smoke can also be used to improve the quality of plant growth [8].

Tryptophan plays a role in protein synthesis that make the auxin higher. Methionine is one of an amino acids that contains sulfur, the plants need sulfur to form disulfides bond between protein chain, help to protein synthesis and growth plants [8]. Therefore, The aim of this study is to determine the effect of amino acids through Divine smoke particulation to the induction of embryogenesis callus *C. pubescens*.

**2. Materials And Methods**

This research is an experimental study with Complete Randomized Design (CRD). This study has 7 treatments consist of control and 6 Amino Acids (Threonine, Tryptophan, Serine, Histidine, Asparagine, and Methionine) through Divine smoke particulation with 3 replications, in order to obtain 21 experimental units. The study was done in April until August 2018. The research was done at Tissue Culture Laboratorium and Plant Physiology, Laboratorium Biology Department, Science and Technology Faculty, Islamic State University Maulana Malik Ibrahim Malang.

**2.1 Explant Material and Media**

Explant material preparation was done by taking young embryos from the fruit of *C. pubescens*. Young embryos of *C. pubescens* were soaked in fungicides solution (benlate 0.5 mg/L). Then, it shaked for an hour. After that, it moved to *Laminar Air Flow* (LAF) to soak the young embryos using 30% and 20% chlorox solution for each 10 minutes. Then, continued using 70% alcohol for 10 minutes. The young embryos rinsed three times using sterile aquades for each 10 minutes. The Murashige and Skoog (MS) media that it used by measuring ½ MS media composition, 30 grams of sugar, and 7 grams of agar. The ½ media and the sugar are put into Erlenmeyer that it contains 1000 ml of aquades. Then it homogenized with stirrer. It added by 3 mg/L of growth regulator 2,4-**Dichlorophenoxyacetic acid** (2,4-D). The media measured by pH media until 5,7-5,8. It heated and stirred until boiling. After that, it inserted into a culture bottles about 10 ml for each bottle. The culture bottles which containing media are covered with plastic and tied with the rubber. Then, it sterilized with the autoclave.
2.2 Initiation Phase

2.2.1 Amino Acids through Divine smoke particulation

Amino Acids was given to young embryos of *C. pubescens* through Divine smoke particulation for 30 minutes. Then, these treatments can be sterilized and planted in growth media.

2.2.2 Explant Initiation

The young embryos of *C. pubescens* are grown in embryogenic callus induction media. The young embryos were grown on ½ MS + 3 mg/L of 2,4-D. After that, the bottle cultures that containing explant were incubated at storage room on 23-25ºC and it observed daily.

![Figure 1. Morphology a) Young fruit, b) Young seeds of Carica pubescens Lenne & K.Koch Plants (individual documents, 2018).](image)

2.3 Observation Phase

The observations are about 60 Days After Planting (DAP). Parameters observed were: Time Appeared Callus (TAC), callus fresh weight, callus color and texture, and the percentage of callus area in explants.

2.4 Data analysis

Observation data is qualitative and quantitative data. Qualitative data are the visual observations, include: callus color, callus texture, and callus anatomy. While quantitative data are time appeared callus (TAC), the percentage of callus area in explants, and callus fresh weight. Quantitative data were analyzed using One Way ANOVA statistical test. If there is a mean difference then it proceeds with the Duncan’s Multiple Range Test (DMRT) test at 5% significant level.

3. Results and Discussion

3.1 Time Appeared Callus (TAC)

Swelling in explants is the initial stage of callus formation which indicates cell activity in explants. The appearance of callus begins with the existence of explant parts that grow enlarged or swollen. Then, in that part there are tissues which grow actively and undifferentiated. In this process will initiating callus [9]. This is one of the success propagation in tissue culture. Based on the average of Time Appeared Callus (Figure 2) shows that the giving of Amino Acids through Divine smoke particulation can accelerate the growth of callus.
Figure 2. The average of the Time Appeared Callus (TAC) callus *Carica pubescens* grown in ½ MS + 3 mg/L 2,4-D on 60 DAP.

Figure 2 shows that Tryptophan through the Divine smoke particulation gave the fastest average of time appeared callus at 24.44 DAP, and than serin 25.55 DAP. The amino acid tryptophan synthesizes the endogenous hormone, thus affecting callus growth faster. Tryptophan is one of the Amino Acids that can be synthesized into Indole-3-Acetic acid (IAA). Tryptophan works in protein synthesis and makes Auxin levels higher [10]. So, Tryptophan can induce callus to the explants of *C. pubescens* [11]. Tryptophan plays a role in biosynthetic pathways as precursors to Auxin synthesis [12]. Synthesis of IAA is doing by eliminating the carboxyl group (decarboxylation) from the side ring of Tryptophan to Tryptamine which is oxidized to form IAA [13]. The other pathway of IAA synthesis is from Tryptophan that combines with alpha keto acid through the transaminase reaction to indolepyruvic acid. Then it was induced by indolepyruvic decarboxylation to indoleacetaldehyde which was subsequently oxidized to form IAA [14].

3.2 Percent of Callus Area in Explants

The result of *C. pubescens* growth callus are influenced by the type of Amino Acids and 2,4-D. Amino Acids are important for callus growth because they play a role as precursors. Amino Acids have long polymers as a basic unit form in the molecular field. Amino Acids consist of 20 types that play a role in the basic structure and function of the body. There are 20 types of Amino Acids in proteins that become the basic structure and functional in the plant. Carboxyl (-COOH) and (-NH3) are contained in an Amino Acid that has the same bond on the carbon atom [15]. In plants, D-amino acids can synthesized, contained, and absorbed by plants. Plants have ability to metabolise a chemical compound like new nitrogen source [16]. Based on the result of 7 treatments of Amino Acids through Divine smoke particulation which is grown on ½ MS media + 3 mg/L of 2,4-D had a significant effect on the induction of *C. pubescens* embryogenic callus.
Figure 3. The average of the percentage of callus area in explants *Carica pubescens* grown in ½ MS + 3 mg/L 2,4-D on 60 DAP

Figure 3 shows that giving 6 types of Amino Acids through Divine smoke particulation which is grown on ½ MS media + 3 mg/L of 2,4-D affected to the percentage of callus area in explants. Serine through the Divine smoke particulation produces the highest percentage of callus area in explants that is 98.88% if it compared to the other Amino Acids. Methionine through Divine smoke particulation produces 82.22% explant callus.

3.3 Callus Fresh Weight

The 6 types of Amino Acids through Divine smoke particulation which is grown on ½ MS media + 3 mg/L of 2,4-D had an effect on callus fresh weight variables. Chart 3 shows that Serine through Divine smoke particulation produces the highest average of callus fresh weight compared to other Amino Acids.

Figure 4. The average of the callus fresh weight of *Carica pubescens* grown in ½ MS + 3 mg/L 2,4-D on 60 DAP.

Figure 4. shows that Serine through the Divine smoke particulation produces the highest average callus fresh weight of 0.67 g. Then, Methionine through Divine smoke particulation produces an average callus fresh weight of 0.46 g. Whereas, the Amino Acid which produces the lowest average callus weight is Asparagine which is 0.21 g. Serine are known as endogenous ligands in receptor
bonds by mediating calcium flow in plants. In addition, Serine plays a role as a single nitrogen source. Serin is needed as an essential role in cellular processes. Serine is the main source in one carbon unit for the methylation reaction that occurs through the generation of S-Adenosilmethionine. In the metabolic process, serine plays a role as a major contributor and works together with glycine, taurine, cysteine, D-serine, and phospholipid in a form. Serine can induce callus with the highest percentage of callus area in explants and fresh weight callus [17]. Methionine is one of amino acids that contains sulfur, the plants need sulfur to form disulfides bond between protein chain, help to protein synthesis and growth plants [8].

The amount of particulate, performance and function of smoke as a source of energy for the metabolic system can be added to amino acids. Molecular nano complex structures like these are similar to molecular complexes naturally found in cell physiological systems such as chlorophyll which play a role in energy transfer systems. The addition of amino acids to Divine cigarettes, particle size and complex structures of non-chemical compounds resulting from Divine combustion processes are smaller than the process of burning cigarettes without amino acids. It was suspected that the embryogenic callus induction C. pubescens using Divine smoke particulation that contains amino acids was able to move the metabolic system which was added with amino acids [18].

3.4 Colour, Textur and anatomy of callus

The observation result that have been done to knowing the effect of the types of Amino Acids through Divine smoke particulation to the callus color and texture can produce different callus color and texture in each treatment. Callus has a varied color due to the presence of light pigmentation and the origin of explants [19]. Callus pigmentation can be equal on all callus surfaces or it can be distributed only partially on the surface. Callus color can change from white, green, brown, yellowish white, and greenish white [20].

![Figure 5. Embryogenic Callus Using Amino Acids Through Divine smoke particulation grown in ½ MS + 3 mg/L 2,4-D on 60 DAP.](image)
Observation of callus *C. pubescens* color showed a yellowish white. It shows that the yellowish white callus is meristematic (Figure 5). The callus color that produced is affected by the speed of the regeneration or the multiplication cells. The color of meristematic callus is yellowish white as usual because the cells still forming young tissue and regenerate continuously [21]. The texture of *C. pubescens* embryogenic callus is friable. Compared to compact callus, callus which has a friable texture can multiply or regenerate rapidly. One of the friable callus characteristics is the callus cells can separate each other easily. Visually, friable callus has a tenuous bond between cells and when the callus is taken with pinset, the callus will break easily and stick on the pinset. The structure of friable callus shows that the callus is active dividing (meristematic) [12].

Anatomy observations showed a large cell nucleus and cytoplasm with several starch grains (Figure 6). The embryogenic cells show characteristics common to meristematic cells: high division rates, cells are isodiametric and small with a dense cytoplasm with several starch grains, large nucleus and prominent nucleolus, small vacuoles, thin cell walls and a higher metabolic activity [22].

4. Conclusion

The conclusion of this research showed a significant effect of 6 types of amino acids through Divine smoke particulation to the induction of embryogenesis callus *Carica pubescens*, which is grown on ½ MS media + 3 mg/L of 2,4-D. Tryptophan is the fastest treatment to induce callus which is grewed 24 days after planting (DAP). Serine influences to the percentage of callus area in explants is 98.8% and the fresh weight of callus 0.6733 gr. The observation of morphology and anatomy embryonic callus color is yellowish white with a friable texture and has a large cell nucleus and cytoplasm with several starch grains

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6. References

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