The role of cysteine in improving somatic embryos of salak Sidempuan (Salacca sumatrana Becc.)

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Abstract. In vitro study of Salak propagation has been conducted, with the aim to obtain the effect of some amino acids in inducing somatic embryos formation. The experimental design used was Completely Randomized Design, with treatment by varying cysteine concentration (0, 5, 10, 15, 20 and 25 mg. L\(^{-1}\)). The results showed that explants successfully proliferated and lead to callus with high potential to be cultured. All explants showed a fairly high survival rate close to 100%. Early callus formation also varied, the fastest growing callus was observed from 10.14 days of culture with the addition of 10 mg. L\(^{-1}\) cysteine. Callus formation in the form of embryogenic callus has high potential to grow into somatic embryos. In conclusion, the best medium formulation to form somatic embryos was obtained from cysteine-containing media with a concentration of 10 mg. L\(^{-1}\).

1. Introduction
Indonesian fruits contain a high nutrient and can also be used as a health therapy, one of which is Salak. Salak is one of Indonesian native plants [1]. There are two well-known Salak species with a relatively high economic value namely Salacca sumatrana Becc. and Salacca zalacca [2]. Salak cultivation in the local farmers or community is still considered conventional, supporting the low production of the fruits [1]. One alternative technique to propagate Salak is tissue culture in laboratory [3]. In which, somatic embryogenesis is known as the most advantageous in vitro technique for many high-value species [4].

The success of embryogenesis technique is influenced by several factors, e.g. (1) plant genotypes, (2) physiological condition of the plants, (3) type and physical condition of medium, (4) growth regulator and (5) culture environment [5, 6]. Environmental factor mentioned before is like the type of culture medium. The medium is a mixture of water, compactor, sugar, growth regulators and amino acids. Amino acids are generally added to enhance callus induction, regeneration and growth of shoots [7]. The addition of amino acids to the media can increase the success forming of embryogenic callus [8].

The proper concentration of amino acids may possess a positive effect on the success of culture. The study of one or many types of amino acids supplementation to culture medium had been reported extensively. A study reported that 20 mg. L\(^{-1}\) increased the dry weight of Stemona sp. cultured from planlet along with further increasing yields of the secondary metabolites [9]. Another study reported
the use of cysteine as much as 0.25 mM, showed significant effect to somatic embryogenesis (94%) from sugarcane culture as compared to non-amino acid medium [10]. Based on that, we see the prospect of supplementing the culture medium with certain amino acid to increase the quality of tissue culture.

Production of Salak is still considered low, because they are not grown from seeds or the seeds are inferior to grow. The conditions have become problem to local farmers in maximizing the cultivation of Salak in North Sumatera. Therefore, our study reported the prospect use of Cysteine in tissue culture that increased the incidence of somatic embryogenesis of Salak. The results then may reflect the potential of producing superior seeds of Salak in shorter time to solve the locals’ problem in stock availability.

2. Methods

2.1. Induction and propagation of somatic embryos
Embryogenic callus were induced from embryo of Salak Sidempuan. Sterilization of explants is done by washing the seeds, then soaked the seeds within solution containing 1% Sodium hypochlorite and four drops of Tween 80 for 30 minutes [11]. The seeds were rinsed using sterile distilled water for 5 minutes, then soaked in 0.1% HgCl₂ solution for 30 minutes and rinsed again with sterile distilled water for 5 minutes in three times rinsing. The embryonic explants isolated from salak seeds, were cultured into Murashige & Skoog (MS) medium supplemented with 2,4-D, Kinetin 1 mg. L⁻¹ and varying concentrations of cysteine (0, 5, 10, 15, 20 and 25 mg. L⁻¹). Cultures formed after 3 months were sub-cultured onto new medium. The culture was later incubated at 25 °C for 2 months. The experiment was replicated seven times.

2.2. Histological analysis
Somatic embryos were then investigated for its microstructure by using Johansen’s paraffin method. The fixative dye used was Safranin and Fast-Green dyes. The callus was observed for the embryogenesis stages occurring from samples.

2.3. Data analysis
The data on average days required to grow from each cultures were analyzed using Analysis of Variance (ANOVA) at 5% confidence level followed by a post-hoc test using Duncan’s at 5% confidence level. The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) ver. 21.0. Other data were analyzed descriptively.

3. Results and discussion

3.1. Performance growth of Salak culture
The initial grow of Salak culture was counted manually until reaching 90 days of incubation. In the 8th days of incubation, the cultures started to grow, characterized by the presence of swelling without any callus formation. Average days required to grow from each cultures were 13.29 days for control and 13.04 days for treatments. Treatment with concentration of 10 mg. L⁻¹ cysteine showed the fastest initial grow from Salak culture, which was 10.14 days while the slowest obtained from the higher concentration of cysteine (Figure 1). From the figure, it can be seen that average days to grow Salak culture were within 13 days or 2 weeks.

Initial growth of culture started when some parts of explant contacted with the medium surface. Callus is then formed as response behavior to injury in specific sites. In this experiment, the callus formed a wound-covering tissue that triggered during days of incubation. Also, addition of 2,4-Dichlorophenoxyacetic acid (2,4-D) may also stimulate cell division and growth in explants leading to callus formation [12,13]. Salak cultures were then sub-cultured into fresh medium to trigger more
callus formation. The sub-culturing will sustain the generation of culture as well as keeping the cultures in exponential stages [8].

![Profile of Salak growth culture in MS medium with varying cystein concentration](image1)

**Figure 1.** Profile of Salak growth culture in MS medium with varying cystein concentration

3.2. **Proliferation and callus formation from Salak culture**

The sub-culturing process triggered cultures to proliferate into callus in medium. During sub-culturing process, the friable and nodular callus from growth cultures was later differentiated into embryogenic callus until the end of incubation (**Figure 2**). Types of culture proliferation were different between control and treatments regarding the number from each types (**Table 1**).

![Visual appearance of embryogenic callus from Salak culture during](image2)

**Figure 2.** Visual appearance of embryogenic callus from Salak culture during,
Left (Sub-culture) & Right (End of incubation)
Table 1. Types of culture proliferation from Salak cultures

| Cystein Concentration (mg. L⁻¹) | Proliferation types |
|--------------------------------|---------------------|
|                               | Swelling | Roots | Buds | Callus |
| 0                              | 4        | -     | 1    | 2      |
| 5                              | 1        | 1     | -    | 5      |
| 10                             | -        | -     | 1    | 2      |
| 15                             | 3        | -     | 1    | 2      |
| 20                             | 3        | 1     | -    | 2      |
| 25                             | 5        | 1     | -    | 1      |
| Total                          | 16       | 3     | 2    | 19     |

We obtained a total of 19 callus formation from the treatments. This type of proliferation was the highest among types found in this study. The addition of cystein then was considered as successfully enhance the numbers of callus from Salak culture. Concentration of 10 mg. L⁻¹ cysteine gave the best result by forming 7 callus while control (0 mg. L⁻¹) only formed 2 callus. The result is supported by other study, which found that supplementation of 10 mg. L⁻¹ also enhanced the percentage of callus formation reaching 75% from date palm (Phoenix dactylifera) [14]. The low result from the highest cystein concentration (25 mg. L⁻¹) indicated the inhibitory effect of nutrient to culture.

Cystein supplementation was not giving any considerable formation of root types, with only 3 observed in this study. The abundant amount of nitrogenous compound in medium is not suitable since the amino acids may interfere with the physiological condition of culture in forming roots. The same result was also observed from buds formation, indicating that culture failed to produce any buds in medium with concentration >15 mg. L⁻¹. Specific amino acids are needed to induce buds formation in certain cultured explants and species. A study reported that cysteine was not able to support the buds formation in sugarcane (Saccharum officinarum) culture, and instead arginine and glycine did showed some effects to it [15].

A more higher result in types of proliferation was observed from swelling formations. The failure of explants to produce organ is assumed to be affected by differences among tissues regarding the ability in assimilating nutrients, plant growth regulator, and amino acids in medium. The swelling incidence of culture occurs due to excessive water absorption by tissue cells, causing the whole culture to be enlarged in size [16].

3.3. Profile of embryogenic callus from Salak culture

Cultures were observed visually until reaching the stage of any embryonic callus formation. The total number of somatic embryos obtained in this study was 16 with differences among treatments (Figure 3). The addition of cystein concentration gave different results in the range of 5–25 mg.L⁻¹.
The highest number of somatic embryos was again obtained from 10 mg.L\(^{-1}\) of cysteine concentration while the lowest was from control without any cysteine. During development, not all callus cells are capable of reaching embryogenesis, but the competent or incompetent callus may be produced from the same explants. This indicates that identical cells can respond differently to the same stimuli and only certain cells can respond well. The responses is then translated into changes to cell reprogramming, that trigger competent cells into embryos while other incompetent cells may not change leading to asynchronous growth of callus [6]. Microscope image through histological analysis confirmed our findings that embryonic callus produced from medium were indeed somatic embryos. The stages of embryogenesis observed from Salak culture including globular, heart, torpedo and budding phase (Figure 4).

Globular somatic embryos are characterized by a spherical or round shape, with globular cells having diffused by their meristematic cells. Somatic globular phase embryo then develops into a heart phase that has a curve at the top like the heart. On the right and left sides there is a protrusion due to faster cell division in the area to form a torpedo stage embryo. Shoots are also part of embryonic
tissue. By looking into our results, it is clear that we have found the optimum cysteine concentration in MS medium to propagate Salak Sidempuan culture in laboratory.

4. Conclusions
The concentration of 10 mg.L\(^{-1}\) cysteine in MS medium gave the best result to Salak Sidempuan culture through all parameters including the time required for initial growth with average days of 10.14 and the best in proliferating cultures into callus and somatic embryos.

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