Inducing Somatic Embryogenesis Of Salak Sidempuan (Salacca Sumatrana Becc.) With The Addition Of Lysine

Elimasni , Haryati, Khairiyah Khairuddin

ABSTRACT: The research of inducing somatic embryogenesis from salak sidempuan (Salacca sumatrana Becc.) with adding of lysine had been conducted. This study aimed to determine the effect of lysine treatments on the growth and the development of somatic embryos of salak sidempuan (Salacca sumatrana Becc.). The samples collected directly at salak farmers at Padang Sidempuan, North Sumatra. MS Cultures Media were used complete with adding of amino acid lysine. The concentration of lysine were range from 0 mg/L; 10 mg/L; 20 mg/L; 30 mg/L; 40 mg/L and 50 mg/L. The results obtained were analyzed statistically by ANOVA analysis random design. The results showed the best survival rate found on 10 mg/L of lysine concentration., the best wet weight found on 40 mg/L of lysine concentration. This lysine concentration also had good ability to form somatic embryo of salak sidempuan. The histological analysis showed that somatic embryos formed at globular and heart–shape phase.

Keywords: Tissue culture, Lysine, Salacca sumatrana Becc., Somatic embryogenesis.

1. INTRODUCTION

As a tropical country, Indonesia is known to have many native fruits, such as snake fruits or Salak [23]. There are two types of snake fruits that have a relatively high economic value which are Salacca sumatrana Becc. and Salacca zalacca [23] and [1]. Salacca sumatrana Becc is found around Padang Sidempuan, North Sumatra. The cultivation of this snake fruits is mainly done by traditionally, so the yield of the fruits is consider low and taken such a long time [23]. To enhance the productivity as well as to supply good quality snake fruit seedling, appropriate alternative tissue culture techniques needs to be done. Somatic embryogenesis is in vitro technique that has most advantages to a species of high economic value [2], [15] and [20]. The success of the embryogenesic technique is influenced by several factors, namely 1) genotypes of plants, 2) the physiological condition of the plant [12] and [13], 3) the type and physical condition of the medium, 4) the growth regulators and 5) environmental culture [27]. Environmental factors is one of the major factor for media culture growth. The media is a mixture of water, compactor, sugar, plant growth regulators and amino acids [5] and [6]. Amino acids are generally added to increase callus induction, regeneration and growth of shoots [29]. The addition of amino acids in the media can increase the success of embryogenic callus formation [5] and [6]. The exact concentration of amino acids can influence culture positively. Some types of amino acids needed for culture such as alanine, arginine, asparaginine, cysteine, glutamine, glycine, leucine, isoleucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. Lysine can help hydrogen bonding formation as well as to change the speed of chemical reactions [29].

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2. MATERIALS AND METHODS

This research was conducted by using embryo Sidempuan Salak. Embryos of Sidempuan Salak were collected directly from the salak farmers at Padang Sidempuan, North Sumatera

2.1 Induction and Multiplication of Somatic Embryos

Embryogenic callus induced from the Padangsidempuan embryo explants. Sterilization of explants were done based on Zulkarnain [31], performed by washing the seeds Salak from the field, then soak the seeds by 1% of sodium hypochlorite and 4 drops of Tween 80 for 30 minutes. Seeds were rinsed with sterile distilled water for 5 minutes and then soaked again in a solution of 0.1% HgCl2 for 30 minutes and the last were rinsed into sterile distilled water three times for 5 minutes each The explants were isolated and cultured to MS medium (Murashige and Skoog, 1962). Lycine inducing were done by adding 2,4-D and Kinetin 1 mg / l and lysine (0, 10, 20, 30, 40, 50 mg / l). After 3 month, formed cultures were subcultured into new media. Then, the cultures were incubated at 25 ° C for two months.

2.2 Histological analysis

Histological analysis were conducted to observe embryogenetic phase of the new somatic embryos. The methods performs by using methods paraffin Johansen (1940) in [16], which is consists of (1) fixation, (2) dehydration, (3) infiltration, (4) planting, (5) cutting, (6) purification, (7) staining and (8) the observation in the microscope.

2.3 Data analysis

The data were analyzed by completely randomized design using ANOVA test at 5% level, then continued with Duncan test at 5% used SPSS version 22 if needed.

3. RESULTS AND DISCUSSION

The parameters needed to observe any growth that occurs were the proliferation of the type of culture, the culture and the wet weight of somatic embryo formation.

3.1 Early Growth in culture (Survival Rate) HST

Initial culture growth was calculated and observed manually, starting from the day after planting (DAP) until the 90th day. The fastest growing time cultures found on 10 mg / L lysine concentration, whereas the longest time growth was at the 50 mg / L (Table 1).

Table 1. *Salak culture growth time at different lysine concentrations*

Lysine	Repeat							Amount	Average
treatment	**1**	**2**	**3**	**4**	**5**	**6**	**7**		
0 mg/L	10	-	8	20	14	20	20	92	15.3
10 mg/L	8	-	9	8	9	8	20	62	10.3
20 mg/L	14	12	10	14	12	14	15	91	13
30 mg/L	12	15	9	12	8	10	10	76	10.85
40 mg/L	10	17	8	8	12	-	20	75	12.5
50 mg/L	12	20	14	12	20	-	20	98	15.6
Total	66	64	58	74	75	52	105	494	77.5
Average	13,2	16	9,67	12,3	12,5	13	17,5	82,3	13

At the beginning of the 2nd week of culture, some treatments are already showed noticeable growth. However, analysis of variance result gave no differences of growth time on salak cultures. Therefore, the variation of lysine concentration given did not gave significant affect to Salak Sidempuan growing cultures. Observed cultures were only experienced swelling without producing callus, as shown in (Figure 1).

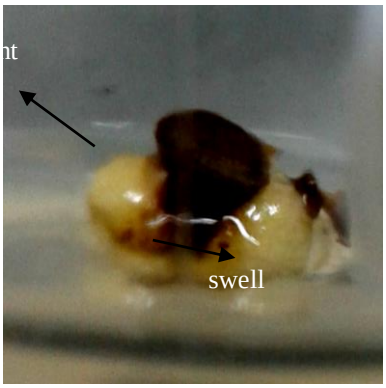

Figure 1. *Salak culture in the 2nd week after planting*

Early stage of culture growth, the embryo only make a contact to the media. It takes time before the media infiltrate into the embryo. According to [26] and [30], at the beginning, the culture will form on the wounding part of explants or explanst edges. Callus primarily are meristematic tissue for wound closure and it is also plant response to the occurrence of lesions occurred on the tissue or the cell Subcultures were conducted in order to yield a lot of callus. According to [9], subcultures were needed to maintain the life and continuous multiplication. By doing subcultures, callus can be multiplied because the new media means keeping the callus remained in exponential phase [4], [5] and [16].

3.2 Type of Culture Proliferation

Type of Salak proliferation in culture had been observed visually. It was showed that the callus tend to get friable and nodular callus that developed into embryogenic callus (Figure 2).

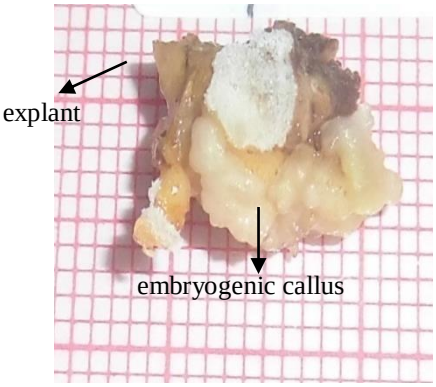

Figure 2. *Embryogenic callus of subculture results at week 8th*

Only 11 callus were obtained as figure 4.2, the others 20 only had swelling form (Table 2). In this case the shoots and roots were also found in the culture medium, but it only had 3 of buds and 4 of the roots.

Table 2. *Type of cultur Proliferation*

	Tipe of Proliferation			
Lysine	Swelling	Root	Bud	callus
L0	2	-	2	2
L1	2	1	1	2
L2	5	1	-	1
L3	5	-	-	2
L4	2	1	-	3
L5	4	1	-	1
Total	20	4	3	11

Shoot and root sprouts that appear are derived from embryos. [19], [11] and [10] explained that the explants derived from the seeds have the potential to germinate in the media. The process of the formation of organs of plants such as roots and shoots as well as complete plants from tissue obtained in this study were unxpected. According

[30], [3] and [7], rooted callus is not expected because it is harder to regenerate into plants. On the other hand, growth of shoots and roots in culture can showed a good thing. From the results obtained, there was 4 of lysine contained media had root grew in it which were L1, L2, L4 and L5 treatments. Meanwhile, 3 of the treatments had shoots grew. The L0 treatment had a high number of shoot growth followed by L1 treatment. Shoots and roots that grow in the culture medium are shown in Figure 3.

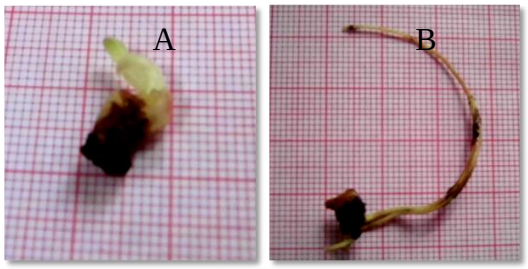

Figure 3. *A. shoot and B. Root at media cultures*

Table 3. *Wet Weight average of salak callus in media lysine*

Lysine concentration	repeat							Total	Average
	1	2	3	4	5	6	7		
0 mg/L	0.20	-	0.12	0.14	0.17	0.11	0.14	0.88	0.14
10 mg/L	0.30	-	0.28	0.14	0.47	0.22	0.29	1.77	0.28
20 mg/L	0.14	0.15	0.19	0.11	0.14	0.18	0.11	1.02	0.15
30 mg/L	0.22	0.26	0.30	0.14	0.15	0.21	0.20	1.48	0.21
40 mg/L	0,21	0.43	0.22	0.46	0.12	-	0.10	1.54	0.25
50 mg/L	0.12	0.11	0.14	0.46	0.10	-	0.21	1.14	0.19
Total	1.19	0.95	1.25	1.45	1.15	0.72	1.05	7.76	1.22
Average	0.19	0.23	0.20	0.24	0.19	0.18	0.175	1.29	0.20

The Table 3 explains that, treatment with lysine concentration of 10 mg / L provides the highest yield of callus with the average value of fresh weight callus was of 0.28 g. Treatment with a lysine concentration of 0 mg / L provided the lowest yield with the average value of The fresh weight callus was 0.14 g. The size of the fresh weight of culture was very diverse because of the size of the initial subcultures were different. According to [14[, the weight differences of cultures showed the differences in the growth process. The growth process is affected by a source of explants and media composition such as growth regulators.

3.4 Embryo somatic of Salak culture

Based on the characteristics of all treatments, 8 culture found embryogenic callus and only 3 cultures had non-embryogenic callus The highest embryonic callus found on lysine 40 mg / L, and the lowest number of embryonic callus found on concentrations of 10, 20 and 50 mg / L (Table 4.).

Table 4. *Embryogenic callus of Salak*

Lysine consentrations	Embryogenic Callus number
0 mg/L	1
10 mg/L	1
20 mg/L	1
30 mg/L	2
40 mg/L	3
50 mg/L	1

3.3 Fresh weight of cultures

.ANOVA analysis of the fresh weight of cultures showed no differences.. The variations concentration of lysine given into media did not significantly affect the fresh weight of cultures Salak Sidempuan. The average wet weight of Salak culture with variations of concentration of lysine can be seen in Table 3.

Eventhough Salak explants were growth on the similar media, but not all of the explants give similar response.. This is presumably caused by factors physiology of the explants themselves [8], [17], [22], [24] and [28]. According to [12] and [25], a specific tissue or organ parts plants of similar genotype may also have differ embriogenetic capabilities. Embryogenic callus obtained are characterized by the size of cells, dense cytoplasm, large nucleus and small vacuoles [18] and [25]. Histological analysis of embryogenetic salak showed that development phase of somatic embryos obtained from the culture of salak covers the phase of globular and heart-shaped phases (Figure 4).

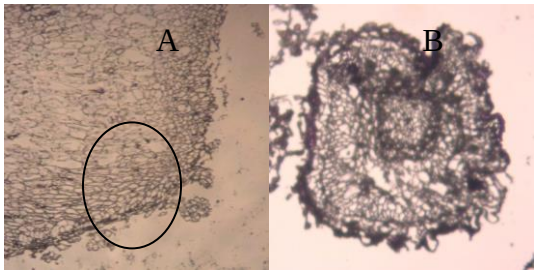

Figure 4. *Microscopic histology analysis of Embryonic salak. A. Globular phase and B. Heart shape phase.*

The number of somatic embryos obtained in this study were only 2 phase which are Globular embryo phase and heart-shape phase. Both phases progressed into the next

phase, which were formed cotyledon and germ. According [25] and [21], the number of heart shape somatic embryosof sago formed during the culture period is quite low. Plants monocots in general is rare to has embryo-torpedo shape.

4. CONCLUSION

Embryogenic callus growth from Salak Sidempuan had low frequency. The combination of basic medium MS + 2,4-D and Kinetin 1 mg / L + Lysine 40 mg / L gave the best response of embryogenic callus. The results of histological features of somatic embryogenic callus cultures Salak obtained was heart-shape phase and phase globular phase

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