The Effect of Sucrose on Growth and Morphology of Rodent Tuber (*Typhonium flagelliforme*) Plantlets in Minimal Growth Preservation *in vitro*

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**Abstract.** Rodent tuber (*Typhonium flagelliforme*) is a herbal plant that has anticancer bioactive compounds. *In vitro*, conservation is attempted to preserve germplasm under aseptic conditions. Conservation techniques are used to store germplasm and minimize somaclonal variation. This study aims to identify sucrose’s effect on the minimum growth of rodent tuber *in vitro* culture and obtain the right sucrose concentration to store rodent tuber *in vitro* for extended shelf life. This study was used plantlets from Bogor and Pekalongan using ½MS medium with 40, 50, 60, and 70 g/L sucrose treatment. In the treatment of 70 g/L sucrose concentration on rodent tuber from Bogor inhibited the increase of the number of shoots, number of leaves, root length, number of roots was 0.50, 2.45, 1.43 cm, and 15.38, respectively. The growth of rodent tuber from Pekalongan on media with a sucrose concentration of 70 g/L resulted in the average number of shoots, number of leaves, root length, number of roots was 0.98, 1.47, 2.10 cm, and 13.74, respectively. Morphological changes in rodent tuber from Bogor and Pekalongan occurred in the number of shoots, the number of leaves, root length, and the number of roots with 70 g/L sucrose treatment.

1. Introduction
Rodent tuber (*Typhonium flagelliforme*) is a native Indonesian herbal plant origin from Java [1]. This plant contains secondary metabolites such as flavonoids, glycosides, alkaloids, steroids [2], phytol [3], and triterpenoids, which have cytotoxic activity against cancer cells [4]. Secondary metabolite compounds from rodent tuber plants have the potential to be anticancer and need to be cultivated. The bioactive compounds in rodent tuber can inhibit the proliferation of breast cancer [5], lung cancer [6], and leukemia [7].

The propagation of rodent tuber naturally occurs by vegetative with the tubers. Tissue culture techniques have been used to reproduce this plant. This method has advantages, including multiplying in large quantities, fast, and genetically like the parent. This technique was developed for
anticipating increased exploitation of plants from nature. Rodent tuber plant is limited in wild nature because it is susceptible to the environment[8]. Rodent tuber has been successfully propagated by tissue cultures such as rodent tuber from Bogor[9], Pekalongan[10], and Medan[11]. Gamma-ray irradiation has been carried out on rodent tuber from Bogor to increase genetic diversity in vitro culture [12].

In vitro conservation is a germplasm preservation technique under aseptic (sterile) conditions. In vitro conservation techniques can be used for storage in the growing state (short term), minimal growth (medium-term), and freezing/cryopreservation (long time)[13]. The preservation of in vitro culture has several advantages, such as saving rare (endangered) plants, storing vegetative plants, maintain plants disease-free, and requiring small space[14]. This technique is efficient in slow-growing plants and overcomes somaclonal variations in subcultured plants [15]. Minimal Growth Preservation is a technology in plant storage for a long time. Technology will be able to store rodent tuber germplasm in all conditions. The rodent tuber plants stored in vitro can be transferred to the field and processed into anticancer drugs and functional drinks.

Various methods are often used in in vitro conservation, such as reducing the macro and micro sodium chloride (NaCl) from 1/2 to 1/4 composition [16], lowering the temperature to 4-12°C [17], providing osmotic pressure by adding osmotic materials such as mannitol or sucrose, and growth inhibitors such as abscisic acid (ABA)[18], the utilization of retardants (paclorobutrazol and ancymidol)[19], and a lower atmospheric pressure of oxygen [20].

In this study, minimal growth preservation was carried out by reducing the concentration of compounds Murashige and Skoog (MS) media to ½MS in vitro. The decrease in concentration can inhibit plant growth from achieving minimal growth. The primary energy source in culture media is sucrose. The sucrose concentration was varied to identify the optimal concentration to produce minimal growth preservation. The high concentration of sucrose in culture media can affect the osmotic pressure and inhibit plant growth [24]. The minimum growth parameter desired is stunted plant growth without affecting plant genetics. Therefore, rodent tuber plants can be stored for a long time. According to Roostika[23] showed that sucrose treatment gave a better response to the development of purwaceng (Pimpinella pruatian) culture compared to mannitol treatment. Many in vitro plant conservation studies have been carried out such as Dewa leaves (Senecio divaricatus) with the use of paclorobutrazol and plant growth regulators [24], rodent tuber plants from Pekalongan using paclorobutrazol[25], curcuma (Curcuma xanthorrhiza) with paclorobutrazol [2], pineapples (Ananas comosus) with paclorobutrazol and ABA[26,27], mannitol and sucrose[23]. This study aims to identify sucrose’s effect on the minimum growth of rodent tuber in vitro culture and obtain the right sucrose concentration to preserve rodent tuber for extended shelf life.

2. Materials and Methods

2.1. Plant Materials
The plant material was used in vitro rodent tuber shoots from Pekalongan (Central Java) and Bogor (West Java) in Sianipar’s collection. [9,10]. Rodent tuber from Pekalongan was grown on Murashige & Skoog (MS) medium with benzyl amino purine (BAP) 0.5 mg/L and naphthaleneacetic acid (NAA) 0.5 mg/L treatment. Rodent tuber plants from Bogor were grown on MS media with BAP treatment of 0.5 mg/L and NAA 1 mg/L.

2.2. Plant Control Media and Minimal Growth
The control media (MS0) were used Murashige & Skoog (MS) media with the addition of NAA 0.5 mg/L and BAP 0.5 mg/L for rodent tuber from Pekalongan of NAA 1 mg/L and BAP 0.5 mg/L in rodent tuber from Bogor. The minimal growth media treatment was made from ½ MS medium with growth inhibitor and paclorobutrazol. Sucrose is given at several concentration levels at 40, 50, 60, and 70 g/L.
2.3. The propagation of Rodent Tuber on Control Media

In vitro culture of rodent tuber originated from plantlets at 10 weeks, then subcultured to optimal media and ½MS. Plant morphology is observed every week until the week 10th. The parameters were investigated: number of shoots, number of leaves, root length, and number of roots. The number of shoots was monitored in the number of new plants growing from the culture media. The number of leaves was observed the number of entirely free leaves. Root length was measured from the maximum root hair length produced from plantlet tubers on the culture media. The number of roots is the averages of all root in the rodent tuber plantlet.

2.4. In vitro Preservation of Rodent Tuber

In vitro culture of rodent tuber has been propagated from optimal media into minimal growth media. Subcultures were carried out as many as ten replications at different sucrose concentrations. The sucrose concentrations are 40 g/L, 50 g/L, 60 g/L, and 70 g/L. The parameters were examined: number of shoots, number of leaves, root length, and number of roots.

2.5. Statistical Analysis

This study was used as a Completely Randomized Design (CRD) with one factor. The CRD factor includes sucrose concentration are 40, 50, 60, and 70 g/L sucrose. Data number of shoots, number of leaves, root length, and number of roots in the last week of observation were analyzed using SPSS 19 software using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (MRT).

3. Results and Discussion

3.1. Number of Shoots

The effect of increasing sucrose concentration in tissue culture media on the number of rodent tubers shoots from Bogor and Pekalongan was shown in Figure 1. Bogor’s rodent tuber had the highest number of shoots in control and the least on media with a 70 g/L sucrose concentration. Rodent tuber from Pekalongan had the highest number of shoots in control media. Rodent tuber from Pekalongan had the highest number of shoots at week 5 to 8 in ½MS medium + 40 g/L sucrose. The increase in the number of shoots rodent tuber from Bogor and Pekalongan in control was influenced by plant growth regulator (PGR). Control media in rodent tuber from Bogor has PGR, which added by naphthalene acetic acid (NAA) of 1 mg/L and benzyl amino purine (BAP) of 0.5 mg/L [9]. The control media in rodent tuber from Pekalongan had a PGR concentration of 0.5 mg/L of NAA and 0.5 mg of BAP [10]. Control media was the optimal medium for the growth and development of rodent tuber in vitro.
Figure 1. The average increase in the number of shoots rodent tuber from Bogor and Pekalongan with various sucrose treatments on culture media.

Based on statistical analysis (Duncan's Multiple Range Test (DMRT)), the addition of sucrose concentration into the media had a significant effect on the number of shoots rodent tubers from Bogor (Table 1). In contrast, the rodent tuber from Pekalongan was not significantly different. The average number of shoots rodents tuber from Bogor was mostly found in control, with an average of 4.67. The sucrose concentration of 30 g/L in the control was the optimal concentration in producing rodent tuber's shoots. Culture media, with a higher concentration of sucrose, had a smaller number of shoots. The increasing sucrose in the culture media affects the osmotic pressure, thereby inhibiting plantlet formation. The cytokinin hormone BAP in the controlled media affects plant growth and development. The BAP compound functions to encourage the formation of main shoots and side shoots in vitro culture media. A higher BAP concentration increased the stimulation of rodent tuber shoots [28]. The cytokinin hormone BAP in the controlled media affects plant growth and development. The cytokinins in culture media (exogenous), also had endogenous cytokinins in plants [29]. Endogenous cytokinins functioned as regulators in shoot formation in the other four media treatments, except in control media. The least average number of shoots was obtained on ½ MS medium + 70 g/L sucrose with the number of rodent tuber shoots from Bogor of 0.5 and Pekalongan of 0.98.

Table 1. The average comparison of the number of shoots rodent tuber from Bogor and Pekalongan

| Treatments                  | Number of Shoots | Bogor | Pekalongan |
|-----------------------------|------------------|-------|------------|
| MS + 30 g/L sucrose (Control) | 4.67<sup>b</sup> | 1.34<sup>a</sup> |
| ½ MS + 40 g/L sucrose       | 1.67<sup>a</sup> | 1.61<sup>a</sup> |
| ½ MS + 50 g/L sucrose       | 1.33<sup>a</sup> | 0.98<sup>a</sup> |
| ½ MS + 60 g/L sucrose       | 2.00<sup>a</sup> | 1.13<sup>a</sup> |
| ½ MS + 70 g/L sucrose       | 0.50<sup>a</sup> | 0.98<sup>a</sup> |

Noted: Numbers followed by the same letters in the same column are not significantly different based on honestly significant difference (HSD) test at 0.5 level.

The differences growth of rodent tuber from Bogor on various media with sucrose treatment was shown in Figure 2. The lowest sucrose concentration, 30 g/L (control), had the most optimal plant development. Meanwhile, minimal growth for in vitro conservation with the least number of shoots was seen at ½ MS + 70 g/L sucrose. Media with a sucrose concentration of 70 g/L is a suitable medium to produce the least number of shoots in rodent tuber from Bogor and Pekalongan.
Figure 2. The growth of rodent tuber from Bogor at the 10th week. (A) Control, (B) ½MS + 40 g/L sucrose, (C) ½MS + 50 g/L sucrose, (D) ½MS + 60 g/L sucrose, (E) ½MS + 70 g/L sucrose.

3.2. Number of Leaves

There was an increase in the number of leaves rodent tuber from Bogor from the first week to the 10th week, as seen in Figure 3. The fastest growth in the number of leaves occurred in control media and medium ½MS + 40 g/L sucrose. At week 9th, there was a decrease in the number of rodent tuber leaves in culture media with a 40 g/L sucrose concentration. This was due to the inhibition of plant growth, so there was no other number of leaves. The highest average number of leaves rodent tuber from Bogor occurred on the 10th day of the control. Rodent tuber from Pekalongan, in the first week to 7th week, there was an increase in the number of leaves, but after that, there was a decrease in the number of leaves, except in the control media. Inhibition of leaf formation at week 8 to 10 is due to inhibition of nutrient absorption by roots, compounds in the media have been used when plant growth in the previous week and endogenous hormones in plants have decreased.

Figure 3. The average increase in the number of leaves rodent tuber from Bogor and Pekalongan with various sucrose treatments on culture media.

The statistical analysis results showed that the addition of sucrose concentration into the culture media had a significant effect on the number of leaves rodent tuber from Bogor and Pekalongan (Table 2). Treatment of sucrose concentration in plant media was significantly different from the number of leaves rodent tuber from Bogor. The highest number of leaves was identified on ½MS medium + 50 g/L sucrose with an average of 2.96, followed by control at 3.83, medium ½MS + 60 g/L sucrose by 2.76, medium ½MS + 40 g/L sucrose by 3.6 and the lowest on ½ MS medium + 70 g/L sucrose by 2.4. The number of rodent tuber leaves in Pekalongan medium ½MS + 40 g/L, 50 g/L, and 60 g/L sucrose was not significantly different, while the control medium and ½ MS + 70 g/L sucrose was significantly different from other treatments. The highest average number of leaves was seen on ½MS
+ 40 g/L media with an average of 2.83 and the least on ½MS + 70 g/L media with an average of 1.47. These data indicate that sucrose's increasing concentration has decreased the number of leaves in rodent tuber from Bogor and Pekalongan.

Sucrose in rodent tuber was a source of carbon and energy in plant growth and development. Sucrose in culture media could function as an osmotic regulator. Sucrose concentrations that were very high in the culture media would inhibit nutrient movement into the plant. Low nutrient absorption would inhibit the growth of plants in culture media [30]. Each plant has the optimal concentration for growth and development[31]. Rodent tuber from Bogor and Pekalongan had optimal growth at a sucrose concentration of 30 g/L with growth hormones such as naphthaleneacetic acid (NAA) and benzyl amino purine (BAP). Leaves formation in rodent tuber from Bogor and Pekalongan was the most optimal in control media (Table 2).

### Table 2. The average of comparison of the number of rodent tuber leaves from Bogor and Pekalongan

| Treatments                | Number of Leaves | Bogor | Pekalongan |
|---------------------------|------------------|-------|------------|
| MS + 30 g/L sucrose (Control) | 3.83c           | 3.54b |
| ½ MS + 40 g/L sucrose     | 3.60bc           | 2.83ab|
| ½ MS + 50 g/L sucrose     | 2.96abc          | 2.25ab|
| ½ MS + 60 g/L sucrose     | 2.76ab           | 2.34ab|
| ½ MS + 70 g/L sucrose     | 2.45a            | 1.47a |

Note: Numbers followed by the same letters in the same column are not significantly different based on honestly significant difference (HSD) test at 0.5 level.

Based on Figure 4, the rodent tuber plant from Bogor on the control media had the highest number of leaves. The same thing happened to rodent taro from Pekalongan. Minimal growth with the least number of leaves found in media with a sucrose concentration of 70 g/L. A sucrose concentration of 70 g/L in culture media was a suitable treatment for in vitro plant conservation.

**Figure 4.** The leaves of rodent tuber from Bogor at the 10th week. (A) MS + 30 g/L sucrose (control), (B) ½ MS + 40 g/L sucrose, (C) ½ MS + 50 g/L sucrose, (D) ½ MS + 60 g/L sucrose, (E) ½ MS + 70 g/L sucrose.

### 3.3. Length and Number of Roots

The root length of rodent tuber plants in vitro culture media with various sucrose concentrations was shown in Figure 5. The statistical analysis results showed that sucrose concentration into the media had a significant effect on the root length of rodent tuber from Bogor and Pekalongan. The root length of rodent tuber from Bogor in the control media was significantly different from the other treatments. The longest root lengths were found in ½MS medium + 40 g/L sucrose of 3.85 cm, ½MS medium + 50 g/L sucrose of 3.10 cm, control medium of 2.95 cm, ½MS medium + 60 g/L sucrose of 2.32 cm and the shortest in ½MS medium + 70 g/L sucrose of 1.43 cm. The rodent tuber Pekalongan has the longest roots in the control medium, 3.70 cm, and the shortest roots in the medium.
½MS + 70 g/L sucrose at 2.10 cm (Table 3). Media ½MS + 40 g/L sucrose was the optimal medium for ordering rodent tuber plants’ roots from Bogor and Pekalongan.

The diversity of sucrose concentrations in the growing medium significantly affected the number of rodent tuber roots from Bogor and Pekalongan (Table 3). Rodent tuber from Bogor has the most roots in media ½MS + 40 g/L sucrose with an average amount by 33.22, followed by media ½MS + 50 g/L sucrose by 31.94, ½MS + 60 g/L sucrose by 23.84, ½MS + 70 g/L sucrose by 15.38 and control by 14.50. The rodent tuber from Pekalongan was obtained as the highest number of roots in the growing medium ½MS + 40 g/L sucrose by 44.51 and the least on ½MS medium + 70 g/L sucrose at 17.4. The number of roots was obtained on ½ MS medium + 40 g/L sucrose, has a higher amount in other media. The utilization of sugar in vitro conservation has been widely used, such as comparing sorbitol, mannitol, and glucose [7-9]. The optimal concentration of sugar in the length and number of roots influenced by the effect of the combination of auxin hormones (NAA) and cytokines (BAP) on the control media also accelerated plant development roots. The hormones NAA and BAP in tissue culture media will increase plant roots and shoots [32].

Table 3. The average of length and number of rodent tuber roots from Bogor and Pekalongan

| Treatments                  | Root length | Number of roots |
|-----------------------------|-------------|-----------------|
|                             | Bogor       | Pekalongan      | Bogor       | Pekalongan      |
| MS + 30 g/L sucrose (Control) | 2.95b       | 3.70b           | 14.50a      | 17.68a          |
| ½ MS + 40 g/L sucrose       | 3.85c       | 3.33b           | 33.22b      | 44.51b          |
| ½ MS + 50 g/L sucrose       | 3.10bhc     | 3.20bc          | 31.94b      | 18.24a          |
| ½ MS + 60 g/L sucrose       | 2.32b       | 2.92ab          | 23.84ab     | 14.09a          |
| ½ MS + 70 g/L sucrose       | 1.43a       | 2.10a           | 15.38a      | 13.74a          |

Note: Numbers followed by the same letters in the same column are not significantly different based on honestly significant difference (HSD) test at 0.5 level.

Based on Figure 5, it can be seen that the root length and number of roots were at least in the medium with a sucrose concentration of 70 g/L. The minimum root length and number of roots indicate an inhibition in the absorption of nutrients from the culture medium to the plant. Inhibited nutrients into rodent tuber plants cause the growth of small plants. Media with a sucrose concentration of 70 g/L is the best medium for minimum growth in rodent tuber plants.

Figure 5. The length and number of roots of rodent tuber from Bogor at the 10th week. (A) Control (MS + 30 g/L sucrose), (B) ½ MS + 40 g/L sucrose, (C) ½ MS + 50 g/L sucrose, (D) ½ MS + 60 g/L sucrose, (E) ½ MS + 70 g/L sucrose.

4. Conclusion
The differences of sucrose concentrations of 30, 40, 50, 60, 70 g/L in vitro culture media have resulted in further growth in rodent tuber plants from Bogor and Pekalongan. The addition of sucrose concentration into the planting medium had a significant effect on the number of shoots, the number of leaves, the length of the roots, and the number of rodent tuber roots, except for the number of rodent tuber shoots from Pekalongan. Sucrose concentrations higher than 30 g/L (control) inhibited the growth and development of rodent tuber plants in vitro culture. The growth of rodent tuber on media with a sucrose concentration of 70 g/L has a minimal increase compared to other treatments. The sucrose concentration of 70 g/L is the best medium for rodent tuber preservation germplasm in vitro.

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