Effect of Sucrose on in vitro Bud Multiplication of Torch Ginger (Etlingera elatior)

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Abstract. Torch Ginger (Etlingera elatior (Jack) R. M. Smith) is plant material that can be used as a medicine, food, cosmetics, and bio-pesticides. The existence of torch ginger in nature is becoming more limited due to the utilization of health care services mainly as a medicine continues to increase. In conventionally, plant propagation of torch ginger is derived from a rhizome, but seedlings produced tend to have the level of low proliferation and susceptible to infections of soil pathogens. This research aimed to know the influence of sucrose and BAP concentration on bud growth of torch ginger in in vitro. The research was conducted in the Tissue Culture Laboratory Universitas Islam Negeri Sunan Gunung Djati Bandung. Research method used was a descriptiv design with experiment treatments i.e. sucrose (20 g/L, 40 g/L, and 60 g/L) and BAP (0 mg/L and 1 mg/L). The result showed that the use of sucrose 40 g/L + BAP 1 mg/L gave the best response to the shoot growth on 6 WAI and capable to produce green shoots on 8 WAI.

1. Introduction

Torch ginger (Etlingera elatior (Jack) R.M. Smith) is a native Indonesian herbal plant from the family Zingiberaceae which is widely used by people, as medicinal material, food, ornamental plants, cosmetic material, also as bio-pesticides. At this time, the use of torch ginger especially as medicinal material continues to increase. Like most other medicinal plants, torch ginger is also harvested directly from nature, so its existence is getting limited. This condition encourages the need of cultivation activity as an effort to preserve torch ginger so that its existence is maintained.

Conventionally, torch ginger propagation material comes from the rhizome, which is the part of the plant that is in the soil. Seedlings that are propagated through this method tend to have low proliferation rates and are very susceptible to pathogenic infections such as rhizome rots caused by Phytophthora species and leaf spot due to Colletotrichum species [1]. Such propagation techniques can lead to accumulation of pathogens in the seedlings, especially viruses that will be inherited through generations. This causes the supply of healthy torch ginger seedlings to be limited.

Effort that can be done to obtain disease-free torch ginger in large number and uniform plants with a relatively shorter time is by conducting plant propagation in in vitro [2]. The appropriate use of culture media and growth regulating substances become important factors as an effort to obtain optimum result in in vitro propagation.

In the use of medium, carbon sources is a very essential component because carbon functions as a source of energy needed by cells for growth [3]. Sucrose as an organic component consisting of glucose and fructose must be present in the culture media as an energy source for non-photosynthetic explants, so that they can perform growth [4]. In the culture medium, the addition of carbon sources becomes very important, because plants that are cultured in a bottle cannot obtain energy from the photosynthesis process as do plants that are conventionally cultivated. In order to meet the nutritional and energy needs,
it is necessary to provide carbon sources that can be directly used by plants in their growth. Sucrose is an important and abundant compound in plants. Sucrose as a carbohydrate, serves as a source of energy for explant growth. Besides carbon sources, torch ginger propagation also requires plant growth regulators (PGR). According to PGR which plays a role in shoot growth, especially is cytokinin. The purpose of adding cytokinin is to stimulate shoot growth, encourage metabolism and cell division, also reduce apical dominance to encourage lateral shoot initiation. BAP (Benzyl Amino Purine) is one of the groups of synthetic cytokinin which is most often used in plant propagation in in vitro culture, because it has a high enough effectiveness for shoot propagation, easily obtained and relatively cheaper.

2. Materials and Methods
A descriptive method was used in this research, which is the description of the growth response of torch ginger explants to the concentrations of sucrose and BAP given. Treatment design consisted of two factors, the first factor was the concentration of sucrose (s1 = 20 g/L, s2 = 40 g/L, s3 = 60 g/L), the second factor was the concentration of BAP (b0 = without BAP and b1 = 1 mg/L).

3. Results and Discussion
The results showed that explants began to show a growth response at 2 week after initiation (WAI). The response did not show the formation of shoot directly, but preceded by tissue swelling. The addition of sucrose and BAP on the culture medium greatly affect the growth pattern experienced by torch ginger explants. Of the total grow explants, the time required to demonstrate the first growth response was 2 weeks. Shoot formation is the main objective of this study. Shoots are a prospective new plants that grows from plant parts. Growth of shoots is very important in tissue culture, because shoots act as materials in the next propagation.

![Figure 1. Growth of shoot on medium with 40 mg/L Sucrose and 1mg/L BAP](image)

Based on the observation results, culture medium which was capable on growing the best shoot was s2b1 (40 mg/L Sucrose and 1 mg/L BAP). In this medium, explant was able to grow a bud at 6 WAI. The formation of buds began with the occurrence of tissue swelling in explants. Swelling was seen starting at 2 MSI, where the explants experienced an increase in size. This swelling pattern mainly occurred vertically and continued to occur up to 5 WAI. Entering 6 WAI, explants showed different conditions, the exterior was yellowish white with a stronger structure (Figure 1d). This was thought to be a previously swollen tissue had developed into a bud. The same condition was still seen in 7 WAI observations, a change occurred only in the slightly bud size increase. At 8 WAI, a significant change had occurred, where the previously formed buds changed color to green became a bigger shoot (Figure 1e).
Green color change that occurred in these shoot indicated the presence of chlorophyll in the tissue. As explained that the more green color produced, the higher the chlorophyll content. Chlorophyll is a green pigment located in the chloroplasts, which is the place where photosynthesis occurs in plants. Characteristics of green shoot indicated that shoot growth occurs well. Sucrose and BAP concentrations used had shown optimal shoot growth.

Treatment of S2B1, namely 40 g/L sucrose and 1 mg/L BAP was the best combination treatment that could produce optimal growth of torch ginger shoots. The use of 40 g/L sucrose had been able to meet the needs of carbon as an energy source for explants in its growth. BAP of 1 mg/L was a suitable concentration for regeneration of torch ginger explant tissue in shoot formation in vitro culture. With a proper combination of sucrose and BAP concentrations, so the best shoot growth of torch ginger explant can be generated.

In tissue culture, plants could not conduct photosynthesis processes as plants that are cultivated on open field, so they need additional carbohydrates as the energy source. [5] explained that the differences in growth regulators concentration in tissue culture cannot control the development of plants without the addition of carbohydrates. These carbohydrates have a fundamental role in the process of shoot proliferation and affect shoot growth and survival. The presence of sucrose in culture media in this study greatly influenced shoot growth. The use of sucrose with optimal concentration will ensure the availability of energy sources for the cell to grow [3]. If the carbon source is sufficient, the cells will form quickly, so that the explant is able to grow shoots.

Addition of cytokinin is also very important in the regeneration process of torch ginger shoots. Cytokinin is used to stimulate cell division, cell enlargement and shoot formation. [6] determined that the addition of cytokinin into culture medium is expected to overcome the problem of the low rate of cell division in plant shoots. In this study, the use of BAP was combined with sucrose.

4. Conclusion
The best concentration of sucrose and BAP for the growth of torch ginger shoot in vitro was in the combination of 40 mg/L sucrose and 1mg/L BAP which had been able to produce healthy and green shoot.

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