

# The effect of red onion bulb extract concentration as a growth regulator on the growth of *Cattleya* sp. orchid plantlets

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**Abstract.** *Cattleya* orchids are one of the most popular types of ornamental orchids due to their beautiful flowers and economic value. Tissue culture is an in vitro plant propagation technique that uses sterile artificial media to produce large quantities of uniform, disease-free seedlings. Red onion bulb extract contains plant growth hormones such as auxin, gibberellin, and vitamin B1, which play a crucial role in stimulating plant growth, particularly root formation and tissue mass increase. This study aims to investigate the effect of red onion bulb extract concentration as a plant growth regulator (PGR) on the in vitro growth of *Cattleya* orchid plantlets. The method used was a Randomized Block Design with treatments of red onion extract at concentrations of control, 50, 75, 100 g/L, along with a control using MS ½ medium, each repeated six times. The parameters observed included increases in plantlets height and fresh weight during culture. The results showed that red onion extract significantly influenced plantlets growth, with the 100 g/L concentration yielding the highest increase in fresh weight (0.123 g). These findings indicate the potential of red onion extract as an effective plant growth regulator for *Cattleya* orchid tissue culture.

Keywords: *Cattleya* orchid, tissue culture, red onion extract, plant growth regulator, plantlets growth.

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## 1. Introduction

*Cattleya* sp. orchids are one of the most famous and widely cultivated orchid genera in the world, mainly due to the beauty of their large, brightly colored flowers. This genus originates from tropical regions in the Americas, with the most common species found in Brazil, Colombia, and Venezuela. *Cattleya* sp. is known for its striking flower shape and fragrant aroma, making it a favorite among plant enthusiasts and in the world of floriculture. Additionally, this orchid has significant economic value, often used in floral arrangements for various special events. *Cattleya* sp. boasts a wide range of species and hybrids offering diverse colors and forms, from purple, red, to white, further enhancing its appeal [1]. Generative orchid propagation often faces physiological challenges such as low germination capacity and the lengthy time required for seeds to germinate. Orchid seeds have very little, or almost no, endosperm, resulting in an unavailability of food reserves during early seed germination, which delays germination [2]. This constraint is why orchid propagation is more often done vegetatively, namely through tissue culture techniques. Tissue culture is a technique for isolating plant parts, whether organs, tissues, cells, or protoplasm, and then culturing these plant parts in artificial media under sterile and controlled environmental conditions [3]. These plant parts can regenerate and form a complete plant [4].

Micropropagation is a laboratory culture method that enables the rapid multiplication of plant material using a segment called an explant. This explant can be any plant part, including an immature embryo, seed, leaf section, root, shoot, anther, pollen grain, ovule, meristem, or apex. The micropropagation process involves cultivating these explants to promote the growth of either undifferentiated or specialized cells [5]. Tissue culture is a way to grow plants by taking cells, small parts of a plant, or even whole parts like leaves or stems and placing them in a special mix of nutrients and hormones in a clean, controlled environment. This process is also called *in vitro* culture. The idea behind tissue culture comes from the cell theory, which was developed by Schawan and Scheleiden. This theory says that each plant cell has the ability to grow into a whole new plant, a property called totipotency. In tissue culture, small plant parts like meristems, leaves, or tubers are placed in a medium that has all the nutrients and hormones needed to help them grow. The benefits of tissue culture include keeping the same traits of the original plant, helping to grow more plants with the same qualities, making it easier to spread plants, creating plants that are free from viruses, storing genetic material for future use, and even making new plant varieties using genetic changes. The advantages of tissue culture include being able to grow seedlings any time of the year, producing a large number of seedlings quickly—like at least 10,000 in one year—making seedlings that are all the same and healthy, and making useful chemicals from plants without having to wait for them to grow to full size [6].

Red onion bulbs are one part of the plant that contains auxin, gibberellin, and vitamin B1 hormones. These hormones play an important role in plant growth, especially in root development. Auxin hormones stimulate root formation and elongate root cells, thereby increasing the plant's ability to absorb water easily and nutrients from the soil. Additionally, gibberellin accelerates the growth and development of plants, including stimulating cell division that supports stronger root growth. Meanwhile, vitamin B1 (thiamine) contributes to energy metabolism and helps improve root health, enabling plants to grow more optimally. The combination of these three components makes red onion bulbs a valuable source for supporting healthy and productive root growth in plants [7].

Every plant has hormones that stimulate growth and development, but there are hormones present in every plant in small amounts that need to be supplemented to accelerate growth and development and maximize results. The concentration of plant growth regulators (PGR) required by plants can influence the speed of height and weight

gain in plants. Based on the above, research is needed on the growth of orchid plantlets treated with plant growth regulators (PGR) derived from red onion bulb extract. The objective of this study is to determine the concentration of red onion bulb extract and its effect on the growth of *Cattleya* orchid plantlets. Based on this, it is hoped that the best growth treatments can be obtained, so that red onion bulb extract can be used as an alternative PGR to synthetic PGR.

## **2. Research Method**

### **2.1 Time and Place of Research**

This research was conducted from December 2024 to January 2025. The research location was the Orchidology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Islam Malang.

### **2.2 Research Design**

The research was conducted experimentally using a Randomized Block Design (RBD). There were three treatments of control, 50, 75, 100 g/L, along with a control using MS ½ medium as a comparison. Therefore, there were three treatments and one control group, which were repeated six times. The research layout is shown in Table 1, with the codes used being K=Control, P=Treatments, and U=Repetition. The parameters observed in this study were the increase in plant height and wet weight of the plantlets.

The materials used in this study were MS stock medium, red onion bulbs, agar powder, sucrose, 70% alcohol, 96% alcohol, NaOH, HCl, distilled water, water, and *Cattleya* sp. orchid plantlets.

The equipment used in this study included a Laminar Air Flow (LAF), autoclave, petri dishes, culture bottles, measuring cups, analytical balances, beaker glasses, hot plates, magnetic stirrers, Erlenmeyer flasks, mortars, scalpels, tweezers, spatulas, pH meter, dropper pipette, Bunsen burner, tissue, plastic wrap, PP plastic, tape, rubber bands, spray bottle, millimeter blocks, bolpoint, label paper, and camera.

### **2.3 Sterilization of Tools and Materials**

The tools used, namely culture bottles, tweezers, petri dishes, and measuring cups, are washed with water and detergent until clean, then dried. The tools are then wrapped in white paper/HVS and sterilized in an autoclave for  $\pm 20$  minutes at a temperature of 121 °C. The sterilized equipment is left in its wrapped condition and stored in a clean area or on a culture rack, ready for use.

### **2.4 Making Red Onion Extract**

The production of red onion extract can begin by preparing red onion bulbs according to the treatments level for use. The red onion bulbs are washed and cleaned thoroughly, then left to dry. The red onions are weighed according to the treatments level for use, then placed in a mortar and ground. Once the red onion bulbs are finely ground, they are ready to be added to the MS medium for use.

### **2.5 Preparation of Murashige and Skoog (MS) Medium**

The preparation of MS medium can be started by adding 50 ml of distilled water to a culture bottle, then adding each stock solution containing macro and micro nutrients, vitamins, and plant growth regulators (PGR) derived from organic materials (onion extract) according to the treatments level for use. Then, add 20 g/L of sugar and 100 ml of distilled water, then homogenize on a hot plate and magnetic stirrer. The homogenized medium is then added with 10 g/L of agar powder, then boiled for about 1 minute and the pH is measured using a pH meter. The optimal pH of the medium is between 5.8 and 6.0. If the

pH is below 5.8, NaOH solution can be added; if the pH is above 6.0, HCl is added to lower the pH of the medium. The medium with measured pH is poured into culture bottles at 25 ml per bottle, then sealed with PP plastic and secured with a rubber band. The medium is then sterilized in an autoclave at 121 °C for approximately 20 minutes. The sterilized medium is placed in an incubation chamber for 3 days to check for contamination.

### 2.6 Subculture of *Cattleya* sp. Orchid plantlets

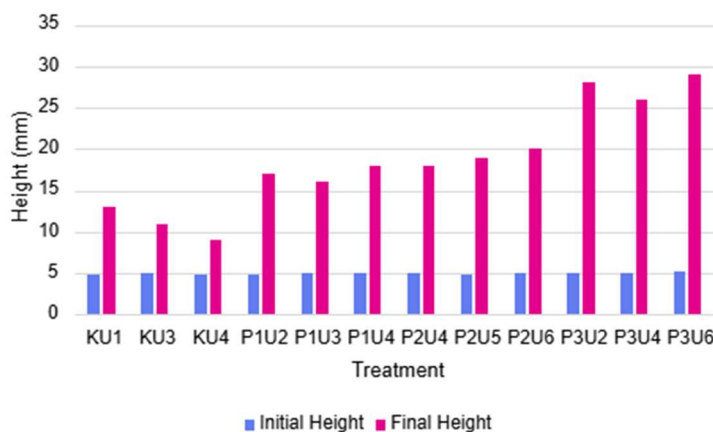
The subculture of *Cattleya* orchids can be started by cleaning the Laminar Air Flow (LAF) using 96% alcohol. All tools and materials must be sterilized before use in the LAF. *Cattleya* orchid plantlets ready for subculture are removed from the culture bottle and transferred to a culture bottle containing the medium specified for the treatments. They are then exposed to a Bunsen burner to maintain sterility, removed using forceps, and placed on a petri dish. The plantlets are then weighed using an analytical balance and measured on a millimeter block. The plantlets are then planted into culture bottles according to the treatments, with 5 plantlets in each bottle. The culture bottles are sealed with tape and plastic wrap to prevent contamination during the treatments.

## 3. Results and Discussion

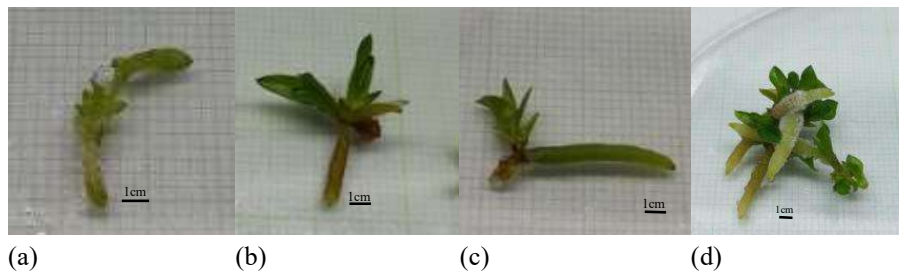
### 3.1 Increase in Plantlets Height

Based on the research conducted, the addition of red onion extract as a plant growth regulator had a significant effect on the height increase of *Cattleya* orchid plantlets. Of the three treatments with 1 control, namely MS 1/2, the average height increase of plantlets was 6,6 mm, the 50 g/L red onion extract treatments resulted in an average increase in plant height of 12 mm, the 75 g/L red onion extract treatments resulted in an average increase in plant height of 14 mm, and the 100 g/L red onion extract treatments resulted in a higher average increase in height of 22,6 mm.

The results in Figure 1 show that the 100 g/L red onion extract treatments had a better effect on the plant height increase parameter, with an average of 22,6 mm. Meanwhile, the 50 g/L red onion extract treatments showed a lower average plant height increase of 6,6 mm.



**Figure 1.** Initial height and final height after treatments using shallot bulb extract with controls, concentrations of 50, 75, and 100 g/L



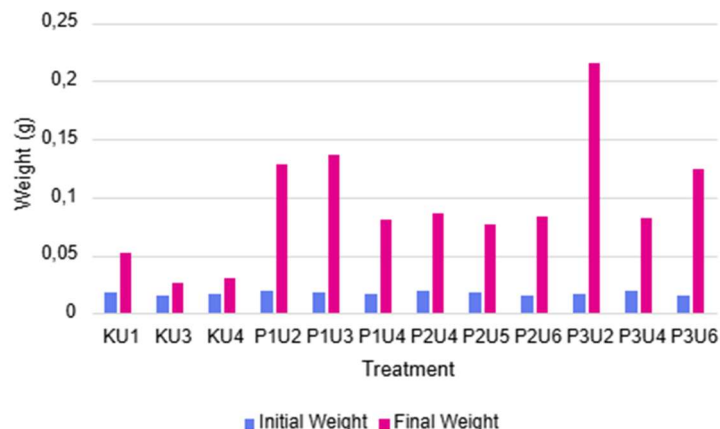
**Figure 2.** Final height and weight of *Cattleya* orchids after 1 month of treatments. (a) control MS 1/2, (b) 50 g/L, (c) 75 g/L, and (d) 100g/L after treatments.

In the study conducted, the addition of PGR in the form of shallot extract at the right concentration can provide optimal effects. In addition, the application of PGR at low concentrations also has an impact on the lack of stimulation in the development of orchid plantlets. Figure 2 shows that treatments with shallot bulb extract has a significant effect on increasing the height of *Cattleya* orchid plantlets compared to the control. The average increase in plant height in the MS 1/2 control was 11 mm, while in the treatments with onion extract concentrations of 50 g/L it was 17 mm, with a concentration of 75 g/L it was 19 mm, and with the highest concentration of 100 g/L it reached 27,6 mm. Thus, the higher the concentration of onion extract, the more significant the increase in plant height. The 100 g/L treatments provided the best results with the highest increase in plant height, indicating optimal growth stimulation thanks to the plant growth regulators in onion bulb extract. Conversely, the 50 g/L concentration, although still better than the control, showed a more minimal increase, indicating the need for a sufficient dose for growth stimulation. This increase in height is consistent with the effect of auxin hormones in red onion extract, which stimulate cell elongation and root formation, supporting better orchid plantlets growth. The references [8] stated that treatments using auxin hormones can stimulate root growth. The auxin hormones present in red onion extract function to influence cell elongation within plants by softening cell walls, enabling orchid plantlets to easily absorb water. In references [9] it was mentioned that auxin can boost the activity of enzymes that break down substances inside cells, which helps a lot in starting root growth. The use of growth regulators must be carefully considered, including concentration, timing of application, and the plant part used. High concentrations can inhibit seedling growth, while concentrations that are too low will not affect seedling growth. In references [10] it is explained that red onion extract contains hormones called auxin and cytokinin, which can help plants grow taller and encourage cell division in plant tissues. The plant growth regulators (PGR) added to the medium do not work alone but interact with each other, which can be seen through plant growth and development. The references [11] said that pepper cuttings treated with different concentrations, namely 0, 25, 50, 75, and 100, did not show much difference in results between the treatments. However, the concentration that showed the best results was 100%, which increased the survival rate, shoot length, and number of pepper leaves. This is because shallot bulbs contain three types of endogenous hormones, namely IAA, 2,4 D, and NAA. In references [12] it was mentioned that orchids are slow-growing plants, so they need proper nutrition to support their growth. One important nutrient for them is found in red onion skins. These skins have substances that help control growth, like auxin and gibberellin hormones. These hormones help plants grow and develop better. When more red onion extract was used, the plants showed better growth than when less extract was used. Therefore, it can be concluded that higher concentrations of red onion extract can efficiently promote the growth of *Cattleya* orchids.

### 3.2 Increase in Wet Weight of Plantlets

Based on the research data, the addition of red onion bulb extract affected on the increase in wet weight of *Cattleya* orchid plantlets. Of the three treatments with one control, namely MS 1/2, the average wet weight of the plantlets was 0.019 g. P1 gave an average wet weight of plantlets of 0.097 g, P2 gave an average wet weight of plantlets of 0.064 g, and P3 gave a higher average wet weight of 0.123 g.

Based on the figure 3, the addition of shallot extract affected the orchid plantlets weight gain parameter, with the average wet weight of orchid plantlets at P3 being 0.123 g, while the lowest average wet weight was at P2, which was 0.064 g.



**Figure 3.** Initial weight and final weight after treatments using shallot bulb extract with controls, concentrations of 50, 75, and 100 g/L

Growth regulators are substances that can promote growth when administered at the right concentration. However, if the concentration is too high or too low, it can also affect and inhibit the metabolic cycle within the plant. Red onion extract can stimulate root growth because red onions contain auxin and rhizokalin hormones. Additionally, they contain vitamin B1 (thiamine), which plays a role in shoot growth [13]. Red onion extract contains compounds similar to endogenous auxin it plays a role in promoting the elongation and development of root cells, thereby increasing the number and length of roots in plants.

The mechanism of onion bulb extract as a growth regulator in plants works due to the natural hormone content such as auxin and gibberellin in the extract. Auxin in onion bulb extract stimulates cell division and elongation, increases cell permeability, and promotes root formation. Gibberellin works in conjunction with auxin to stimulate the formation of vascular tissues such as xylem and phloem, as well as promoting cell division in the cambium, leading to an increase in stem diameter. These compounds optimize root and shoot growth by enhancing water and nutrient diffusion into cells, thereby accelerating plant development and growth [14]. In addition, red onion bulb extract contains the antibacterial compound allicin, which protects plants from microbial attacks and also acts as a plant growth regulator. Allicin, together with thiamine (vitamin B1), forms a chemical bond called allithiamine, which is easily absorbed by plant tissues, thereby improving metabolic efficiency. This smooth metabolic process triggers accelerated root and shoot formation by stimulating cell division and elongation, particularly in the meristematic regions. The antibacterial compound allicin in red onions plays an important role in plant tissue culture, not only as an agent that inhibits the growth of pathogenic microorganisms, but also as a plant growth regulator [15].

## 4. Conclusion

The treatments of red onion bulb extract concentration on the observed parameters showed an interaction. The most efficient concentration was 100 g/L for the parameters of height and wet weight of orchid plantlets.

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