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Effectiveness of Sucrose and Casein Hydrolysate Concentration Combinations on The In Vitro Growth *Dendrobium Sp*. Plantlets

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Abstract:
Background*: The growth medium is a critical factor influencing the quality of Dendrobium sp. plantlet development. The addition of sucrose as an energy source and casein hydrolysate as a growth supplement to the medium can enhance plantlet growth. This study aimed to evaluate the effects of sucrose and casein hydrolysate combinations on the growth of Dendrobium sp. plantlets cultivated in vitro*.
Materials and Methods*: The research was conducted in the in vitro Laboratory of Agrotechnology, Faculty of Agriculture, Halu Oleo University, from August to November 2020, using a Completely Randomized Design (CRD) with 12 treatments and 4 replications. Treatments consisted of various combinations of sucrose concentrations (20 g/L, 30 g/L, 40 g/L) and casein hydrolysate concentrations (50, 100, 150, 200 mg/L). Data were analyzed using Duncan's Multiple Range Test (DMRT).*)
Results*: The results showed that the combination of sucrose 30 g/L and casein hydrolysate (100–150 mg/L) yielded the highest plantlet performance, including height increment (2.57 cm and 2.42 cm), leaf number increment (6.17 and 6.13 leaves), and fresh weight increment (2.13 g and 2.10 g). Furthermore, the combination of sucrose 40 g/L and casein hydrolysate (50–150 mg/L) resulted in the highest root performance, with root number increment (6.43 roots) and root length increment (9.63 cm and 9.67 cm).*
Conclusion: *These findings demonstrate that specific sucrose and casein hydrolysate combinations can significantly optimize the in vitro growth of Dendrobium sp. plantlets.*
Key Word: *Planlet Growth; Dendrobium sp.; Sucrose; Casein Hydrolysate; In-vitro cultivation.*

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I. Introduction

Orchids (Dendrobium, sp.) hold significant commercial potential as ornamental plants. Despite their typically slow growth and variation across species, these orchids are highly sought after due to their beauty and adaptability to diverse environmental conditions (1). The increasing interest in orchids creates a substantial opportunity for large-scale and commercial orchid production. Orchid production, which reached 11.35 million in 2021, declined to 6.79 million in 2022 (2). This decline is attributed to a lack of high-quality seeds, inefficient cultivation practices, and inadequate post-harvest management. Traditionally, orchids are propagated using generative methods with seeds or vegetative methods by separating offshoots and clumps. However, these approaches have notable drawbacks, including prolonged germination periods, limited offshoot production (3) and low seed germination rates (4). Therefore, an efficient and rapid propagation method is essential to address these challenges.

One alternative propagation technique is in vitro culture through tissue culture methods. This approach allows propagation without requiring extensive space or donor plants, as propagation materials are readily available (5). Additional benefits include the ability to produce a large number of high-quality, uniform plants in a short time. Furthermore, in vitro propagation can disrupt the cycle of disease degeneration, making it a critical step in improving overall orchid production.

Successful in vitro propagation depends on several factors, such as the composition of the culture medium, the type of explants used, the addition of organic compounds, and the inclusion of sucrose in the basal medium (6,7). Sucrose plays a vital role in supporting vegetative growth, including the development of roots, leaves, and stems. This process is driven by the need for substantial carbohydrates during cell division to build cell walls composed of protoplasm and cellulose. Both cellulose and protoplasm are primarily formed from sugars, which act as substitutes for photosynthetic products in explants, serving as a carbon source (8,9). Additionally, sucrose contributes significantly by providing energy for respiration, stabilizing membranes, regulating osmotic pressure, and facilitating the formation of new plant cells (8).

Beyond sucrose as a primary energy source, casein hydrolysate is another critical component supporting explant growth. Casein hydrolysate is an organic nitrogen source rich in essential amino acids and peptides. This nitrogen is essential for protein and enzyme synthesis, which in turn supports cell growth, tissue division, and the development of shoots (10–12). Several studies provide relevant insights. Saleh et al. (13) demonstrated that applying 20 mg/L sucrose and 0.5 mg/L nicotinic acid in MS media produced the best results in root length, leaf count, and overall subculture growth of Dendrobium orchids. Similarly, Murgayanti, Putri, & Nuraini, (14) found that combining sucrose and glucose with cytokinins BAP and TDZ positively influenced shoot proliferation, development, and growth in C. zedoaria plants.

Other research highlights that adding 100 ppm glutamine amino acids to MS media stimulated shoot growth and development in sugarcane (15). Maysyaroh & Ermawati (16) also found that combining 100 ppm glutamine with 30 g/L sucrose resulted in optimal growth in terms of plantlet height and weight. Furthermore, studies by Kailola (17) and Istiningdyah, Tambing, & Bustami (18) revealed that 0.5 mg/L BAP and 150 mg/L casein hydrolysate significantly enhanced plant height, shoot numbers, and leaf count. Based on these findings, this research aims to investigate the effects of combining sucrose and casein hydrolysate on the in vitro growth of Dendrobium orchid plantlets.

II. Material And Methods

The research was conducted in the in vitro laboratory of the Agrotechnology Department, Faculty of Agriculture, Halu Oleo University. The study took place from August to November 2020. The materials used in this research included Dendrobium sp. orchid seedlings derived from germination cultures, which were part of the in vitro laboratory collection and one year old. Additional materials included MS medium without sucrose, casein hydrolysate, sucrose, and distilled water.

Study Design: The orchid seedlings, consisting of two leaves, were subcultured onto MS basal media according to the treatments. The study utilized a Completely Randomized Design (CRD) with 12 treatments and four replications. The treatments consisted of various concentration combinations: 20 g/L sucrose + casein hydrolysate (50, 100, 150, and 200 mg/L), 30 g/L sucrose + casein hydrolysate (50, 100, 150, and 200 mg/L), and 40 g/L sucrose + casein hydrolysate (50, 100, 150, and 200 mg/L). The results were analyzed using Duncan's Multiple Range Test (DMRT).

The culture bottles containing the plantlets were placed on culture racks under TL 36-watt lamps, providing a photoperiod of 12/12 hours of light and dark, with a room temperature maintained at 18°C. Observations were made weekly for 12 weeks after planting, covering parameters such as plantlet survival rate, increase in plantlet height, number of leaves, number and length of roots, and plantlet fresh weight. Data from the observations were analyzed using an F-test and further tested using DMRT at a 5% significance level

III. Result

Plantlet Survival, Plantlet Height Increase, And Leaf Number Growth

Increase in plant height is a commonly used parameter to assess plant growth. The elongation of a plantlet is attributed to the processes of meristematic cell division and cell elongation, which collectively lead to increased plant height (19,20). Based on the results of the DMRT test presented in Table 1, the treatment combinations of 30 g/L sucrose and 100–150 mg/L casein hydrolysate yielded significantly higher increases in plantlet height, reaching 2.57 cm and 2.42 cm, respectively, compared to other treatments. This is likely due to the synergistic effect of sucrose and casein hydrolysate in supporting the physiological and morphogenetic processes of plantlets.

Sucrose plays a vital role in vegetative plant growth by acting as an osmotic pressure regulator and enhancing the tissue's ability to absorb water from the medium into the plant. During cell division, a substantial amount of carbohydrates is required to build cell walls composed of protoplasm and cellulose ((13,21).

Casein hydrolysate functions as an organic nitrogen and amino acid source, supporting protein synthesis and enzyme metabolism. Nitrogen from casein hydrolysate enhances photosynthesis, ultimately producing higher amounts of photosynthates. This contributes to plantlet growth and accelerates the increase in plant height ((10,22,23). According to Maysyaroh and Ermawati (16), a combination of 100 ppm glutamine and 30 g/L sucrose yields optimal plantlet weight and plant height. However, treatments with 20 g/L sucrose and 50 mg/L casein hydrolysate, as well as 40 g/L sucrose and 200 mg/L casein hydrolysate, showed the lowest increases in plantlet height, at 1.53 cm and 1.55 cm, respectively. This may be attributed to the inadequacy of 20 g/L sucrose in providing an optimal carbon source for plantlet growth. Conversely, a sucrose concentration of 40 g/L might cause excessive osmotic pressure in the medium, inhibiting water and nutrient uptake by the plantlets.

Similarly, at casein hydrolysate concentrations of 50 and 200 mg/L, the lowest plantlet height increases were observed. This may be due to the high nitrogen content in casein hydrolysate. This finding aligns with Widiastoety and Nurmalinda (10), who stated that adding casein hydrolysate to culture media could enhance the growth of Vanda orchid plantlets. However, increasing the concentration of casein hydrolysate up to 200 mg/L

did not yield better results compared to 100 mg/L. This finding contrasts with the research of Istiningdyah et al. (18), which reported that treatments using 0.50 mg/L BAP and 150 mg/L casein hydrolysate had a more positive effect on plant height, shoot numbers, and leaf numbers in Cucumis melo L. (melon) cultivated in vitro.

Table 1. Average Percentage of Plantlet Survival, Plantlet Height Increase, and Leaf Number Growth with Various Combinations of Sucrose and Casein Hydrolysate Concentrations at 12 Weeks After Planting (WAP)

Treatment	Plantlet Survival Percentage (%)	Plantlet Height Increase (cm)	Leaf Number Growth
S1 = Sucrose 20 g/L+Casein Hydrolysate 50 mg/L	87.66	1.53 ^{e}	3.92 ^{e}
S2 = Sucrose 20 g/L+Casein Hydrolysate 100 mg/L	90.00	1.70 ^{de}	3.98 ^{e}
S3 = Sucrose 20 g/L+Casein Hydrolysate 150 mg/L	94.33	1.82 ^{cd}	4.80 ^{cd}
S4 = Sucrose 20 g/L+Casein Hydrolysate 200 mg/L	90.00	1.85^{cd}	4.47 ^{de}
S5 = Sucrose 30 g/L+Casein Hydrolysate 50 mg/L	97.33	2.30 ^{b}	5.30 ^{bc}
S6 = Sucrose 30 g/L+Casein Hydrolysate 100 mg/L	96.67	2.57 ^{a}	6.17 ^{a}
S7 = Sucrose 30 g/L+ Casein Hydrolysate 150 mg/L	98.33	2.42^{ab}	6.13 ^{a}
S8 = Sucrose 30 g/L+Casein Hydrolysate 200 mg/L	96.66	1.95 ^{c}	5.87 ^{ab}
S9 = Sucrose 40 g/L+ Casein Hydrolysate 50 mg/L	98.33	1,97 ^{c}	5.93 ^{ab}
S10= Sucrose 40 g/L+Casein Hydrolysate 100 mg/L	95.00	1.85 ^{cd}	5.37 ^{bc}
S11= Sucrose 40 g/L+Casein Hydrolysate 150 mg/L	94.31	1.78 ^{cd}	5.13 ^{c}
S 12= Sucrose 40 g/L+Casein Hydrolysate 200 mg/L	91.67	1.55 ^{e}	4.43 ^{de}
Average of Plantlet Survival Percentage	94.19		

Note: Numbers followed by the same letter within the same column indicate no significant difference according to DMRT at the 5% significance level.

Observation results indicated that the survival percentage of Dendrobium sp. orchid plantlets 12 weeks after planting (WAP) (Table 1) reached a relatively high range of 87.66% to 98.33%, with an average survival rate of 94.19%. This high survival rate can be attributed to the use of explants derived from previous in vitro cultures, which were in sterile conditions. As stated by Yatim (2016), the success of tissue culture techniques is influenced by several factors, including the type of explants, size, age, physiological phase of the tissues used, and aseptic environmental conditions.

Number And Length Of Roots, Fresh Weight Of Plantlets

Increase in Root Number and Length

Root formation is a key indicator of success in plantlet development during the in vitro micropropagation process. The average increase in root number, root length, and plantlet fresh weight was observed under various combinations of sucrose and casein hydrolysate concentrations after 12 weeks of planting (MST), as shown in Table 2. This presents the average increase in root number, root length (cm), and fresh weight (g) under different sucrose and casein hydrolysate treatments after 12 weeks of planting. Each treatment shows unique results, and the DMRT analysis at a 5% significance level highlights significant variations among treatments.

Table 2. Average Increase in Number and Length of Roots, Fresh Weight of Plantlets with Various Combinations of Sucrose and Casein Hydrolysate Concentrations at 12 Weeks After Planting

Treatment	Increase in Root Number	Increase in Root Length (cm)	Increase in Fresh Weight (g)
S1 = Sucrose 20 g/L+Casein Hydrolysate 50 mg/L	3.63^{d}	4.00 ^{f}	0.90 ^{cd}
S2 = Sucrose 20 g/L+ Casein Hydrolysate 100 mg/L	3.90 ^{d}	5.50 ^{ef}	0.96 ^{d}
S3 = Sucrose 20 g/L+ Casein Hydrolysate 150 mg/L	3.86 ^{d}	5.57 ^{ef}	1.80 ^{b}
S4 = Sucrose 20 g/L+Casein Hydrolysate 200 mg/L	3.73 ^{d}	7.00 ^{cde}	1.88 ^{ab}
S5 = Sucrose 30 g/L+Casein Hydrolysate 50 mg/L	5.87 ^{b}	5.83 ^{de}	1.87 ^{ab}
S6 = Sucrose 30 g/L+Casein Hydrolysate 100 mg/L	5.83 ^{b}	6.00 ^{cde}	2.13 ^{a}
S7 = Sucrose 30 g/L+Casein Hydrolysate 150 mg/L	5.93 ^{b}	7.10 ^{cde}	2.10 ^{a}
S8 = Sucrose 30 g/L+Casein Hydrolysate 200 mg/L	5.97 ^{b}	7.43 ^{bcd}	1.73 ^{b}
S9 = Sucrose 40 g/L+Casein Hydrolysate 50 mg/L	6.03 ^{b}	9.63 ^{a}	1.66 ^{b}
S10= Sucrose 40 g/L+Casein Hydrolysate 100 mg/L	6.10 ^{b}	9.67 ^{a}	1.66 ^{b}
S11= Sucrose 40 g/L+Casein Hydrolysate 150 mg/L	6.43 ^{a}	9.13 ^{ab}	1.07 ^{c}
S12= Sucrose 40 g/L+Casein Hydrolysate 200 mg/L	5.43 ^{c}	7.67 ^{b}	0.93 ^{cd}

Note : The notation "numbers followed by the same letter in the same column are not significantly different according to DMRT at a 5% significance level" is a statistical convention used in experimental research to interpret treatment comparisons

IV. Discussion

Plantlet Survival, Plantlet Height Increase, And Leaf Number Growth

Plantlet Survival Percentage

The highest plantlet survival percentage was observed in two treatments: Murashige and Skoog (MS) medium supplemented with sucrose 30 g/L and casein hydrolysate 150 mg/L, and the treatment with sucrose 40 g/L and casein hydrolysate 50 mg/L. Both treatments demonstrated a survival rate of 98.33%. This result indicates that these media combinations created optimal conditions for plantlet growth and survival in vitro. It can be inferred that these two treatments likely provided a balanced nutritional environment that supported optimal plantlet development, despite their differing compositions. According to Lutfiani et al. (24), the high growth percentage of explants is associated with the nutrient content in the media used.

Sucrose concentrations of 30 g/L and 40 g/L were adequate to meet the energy requirements of the plantlets. Despite the difference in concentration, both levels appeared to have reached an optimal point to fulfill the energy needs of the plantlets without causing toxic effects due to excess sucrose. Research cited by Fardani (2005, in Saleh et al., (13) states that carbohydrates, particularly sucrose, serve as an energy source for plants in culture, replacing the energy that cannot be obtained through photosynthesis. Meanwhile, casein hydrolysate concentrations of 50 mg/L and 150 mg/L are suspected to contribute optimally to the survival of plantlets, even at varying concentrations. The addition of casein hydrolysate as a nitrogen source provides the advantage of faster absorption by plants. Casein hydrolysate contains amino acids that act as a source of organic nitrogen, stimulating the growth and development of plant tissues (10,18).

Plantlet Height Increase

Plantlet height growth is a commonly used parameter to measure plant growth. The increase in plantlet height occurs due to meristematic cell division and cell elongation processes, which collectively result in height increments (19,20,25). Based on the results of the DMRT test presented in Table 1, the treatment combination of sucrose at 30 g/L and casein hydrolysate at 100–150 mg/L showed a more significant plantlet height increase, reaching 2.57 cm and 2.42 cm, respectively, compared to other treatments. This is presumably due to the synergy between sucrose and casein hydrolysate in supporting the physiological and morphogenesis processes of the plantlets.

Sucrose plays a crucial role in vegetative plant growth, functioning as an osmotic pressure regulator and influencing the ability of tissues to absorb water from the medium into the plant. During the cell division process, a large amount of carbohydrates is required to construct cell walls composed of protoplasm and cellulose (13,21). Casein hydrolysate serves as a source of organic nitrogen and amino acids, supporting protein synthesis and enzyme metabolism. Consequently, the nitrogen in casein hydrolysate can enhance photosynthesis processes, ultimately producing a higher amount of photosynthates. This contributes to plantlet growth and accelerates height increase (10,22,23,25). According to Maysyaroh and Ermawati (16), the combination of glutamine at 100 ppm and sucrose at 30 g/L resulted in optimal plantlet weight and height.

However, treatments with sucrose at 20 g/L and casein hydrolysate at 50 mg/L, as well as sucrose at 40 g/L and casein hydrolysate at 200 mg/L, exhibited the lowest plantlet height increases, at 1.53 cm and 1.55 cm, respectively. The low sucrose concentration of 20 g/L is thought to be insufficient to provide an optimal carbon source for plantlet growth. In contrast, sucrose at 40 g/L may cause excessive osmotic pressure in the medium, inhibiting water and nutrient absorption by the plantlets.

Similarly, casein hydrolysate concentrations of 50 and 200 mg/L resulted in the lowest plantlet height increases. This is suspected to be due to the high nitrogen (N) content in casein hydrolysate. This observation aligns with the findings of Widiastoety and Nurmalinda (10), who noted that the addition of casein hydrolysate in culture media could improve the growth of Vanda orchid plantlets. However, increasing the casein hydrolysate concentration to 200 mg/L did not yield better results compared to 100 mg/L.This contrasts with the findings of Istiningdyah et al. (18), which indicated that treatments with 0.50 mg/L BAP and 150 mg/L casein hydrolysate had a more positive effect on plant height, shoot count, and leaf number in melon (Cucumis melo L.) under in vitro conditions.

Leaf Number Growth

Leaves are vital organs for plants, playing a crucial role in their survival. As the center of photosynthesis, leaves transform inorganic compounds into organic compounds with the aid of sunlight. Based on the DMRT test results presented in Table 1, the combination of sucrose at 30 g/L and casein hydrolysate at 100 mg/L produced the highest number of leaves at 12 MST (weeks after planting), reaching 6.17 leaves. This result was nearly identical to the combination of sucrose at 30 g/L and casein hydrolysate at 150 mg/L, which resulted in 6.13 leaves. These findings suggest that the more leaves an explant produces, the better its growth. The abundance of leaves is vital for plant development, as it correlates closely with the plant's ability to perform photosynthesis and other metabolic activities (19,20).

Additionally, the combination of sucrose at 30 g/L with casein hydrolysate (100–150 mg/L) yielded the best results in terms of leaf count, indicating a synergy between the energy provided by sucrose and the supplemental nutrients from casein hydrolysate. Casein hydrolysate concentrations of 100–150 mg/L proved more effective in increasing the number of leaves compared to lower concentrations, such as 50 mg/L. This finding aligns with the study by Istiningdyah et al. (18), which reported that a treatment of 0.50 mg/L BAP combined with 150 mg/L casein hydrolysate positively impacted plant height, shoot count, and leaf count.

The combination of sucrose at 20 g/L and casein hydrolysate at 50 mg/L resulted in the lowest leaf count, at 3.92 leaves. This suggests that the nutrients in the medium may not be sufficient to meet the plantlet's needs, thus hindering leaf development. Nitrogen in casein hydrolysate plays a critical role as a macroelement in forming amino acids, chlorophyll, and other compounds. High chlorophyll levels enhance photosynthesis, which in turn increases photosynthate production, potentially boosting leaf growth in terms of length, width, and count (10,22).The research by Saklani et al. (26) indicated that 100 mg/L of casein hydrolysate in in vitro media significantly improved the shoot count of Elaeocarpus sphaericus, with an average of 11.68 shoots. Similarly, Gao et al. (27) demonstrated that 200 mg/L of casein hydrolysate in Amorpha fruticosa cultures increased shoot counts to an average of 8.77 shoots per subculture. These findings highlight the importance of optimizing sucrose and casein hydrolysate concentrations to support optimal leaf and overall plantlet development.

Number And Length Of Roots, Fresh Weight Of Plantlets

Increase in Root Number

The combination of sucrose at 40 g/L and casein hydrolysate at 150 mg/L yielded the best results, with an average root count of 6.43 roots in in vitro culture. This demonstrates a positive correlation between higher sucrose concentrations and optimal doses of casein hydrolysate in promoting root formation. Sucrose at 40 g/L is hypothesized to serve as an adequate carbon and energy source, supporting cellular metabolism and meristematic root tissue division. Concurrently, casein hydrolysate at 150 mg/L supplies essential amino acids and growth factors, accelerating root development. Rittirat et al (28) highlighted sucrose at 40 g/L as the most effective treatment for enhancing root number and length. Similarly, Zahara et al. (29), as cited in Karimah et al. (30), found that 40 g/L sucrose in the in vitro culture of Dendrobium orchids initiated the highest root count at 7.75 roots. Casein hydrolysate contributes significantly by providing essential amino acids that support protein synthesis, thereby stimulating tissue growth and development (10).

In contrast, combinations of sucrose at 20 g/L with casein hydrolysate at concentrations between 50–200 mg/L resulted in significantly lower root counts, ranging from 3.63 to 3.90 roots. The reduced root numbers in these treatments suggest that casein hydrolysate alone cannot replace sucrose as the primary energy source. Aruan et al. (31) also noted that using casein hydrolysate at concentrations of 50 and 200 mg/L combined with sucrose at 20 g/L produced fewer roots in strawberry plants.

Increase in Root Length (cm)

Table 2 further illustrates that treatments combining sucrose at 40 g/L with casein hydrolysate at 50, 100, and 150 mg/L produced the highest root lengths, measuring 9.63 cm, 9.67 cm, and 9.13 cm, respectively. These results indicate that sucrose at 40 g/L provides sufficient energy, working synergistically with casein hydrolysate to maximize root elongation. Samudera et al. (9) emphasized that the rooting phase requires abundant energy to support cell enlargement and elongation processes. Ruan (32), as cited in Karimah (30), also explained that sucrose serves as a vital carbon source regulating the cell cycle, crucial for cell division and root cell formation.

Additionally, Rahmawidowati et al. (33) reported optimal root elongation of 1.77 cm in Dendrobium orchids using sucrose at 40 g/L. On the other hand, the combination of sucrose at 20 g/L and casein hydrolysate at 50 mg/L resulted in the lowest root elongation, measuring only 4.00 cm. This suggests that the sucrose and casein hydrolysate levels in these treatments were insufficient to meet the metabolic demands necessary for optimal root growth. Saleh et al. (13) also noted that a combination of sucrose at 20 g/L and nicotinic acid at 0.5 mg/L produced an average root count of 8.20 but a root length of only 1.37 cm. This finding underscores the critical role of adequate sucrose and casein hydrolysate concentrations in supporting both root number and elongation during in vitro plantlet development.

Fresh Weight of Plantlets.

The fresh weight of plantlets serves as an important physiological indicator in plant tissue culture research, frequently employed to assess the efficacy of specific culture media formulations. Based on the data in Table 2, the highest mean fresh weight of Dendrobium sp. plantlets was recorded in the treatment combining 30 g/L sucrose with 100 mg/L casein hydrolysate, achieving a fresh weight of 2.13 g. This result was statistically comparable to the treatment involving 30 g/L sucrose and 150 mg/L casein hydrolysate, which yielded a fresh weight of 2.10 g. These findings suggest that these particular combinations of sucrose and casein hydrolysate

created an optimal culture environment, promoting enhanced cellular metabolic activity. This favourable condition significantly supported the growth of Dendrobium plantlets, leading to a greater fresh weight compared to other treatment groups. Sucrose is pivotal in providing the primary source of energy necessary for cellular metabolism and biomass production. Meanwhile, casein hydrolysate contributes essential amino acids that facilitate tissue development and cell differentiation in plants effectively (34,35).

The results of this study are consistent with the findings of Maysyaroh and Ermawati (16), which demonstrated that a combination of 100 ppm glutamine and 30 g/L sucrose resulted in the highest plantlet fresh weight. Furthermore, as Sitompul and Guritno (36) explained in Aruan et al. (31), the absorption of higher levels of amino acids by plants correlates with an increase in photosynthetic activity. This, in turn, positively influences fresh plant weight, as observed in strawberry plants, where the expansion of leaf area significantly contributes to an increase in fruit fresh weight.

In general, the fresh weight of plants is closely related to leaf area. Enhanced photosynthetic activity leads to broader leaf surfaces, allowing for improved sunlight absorption. Consequently, this optimises other metabolic processes that support plant growth. Treatments involving 20 g/L sucrose and 50 mg/L casein hydrolysate, as well as 40 g/L sucrose and 200 mg/L casein hydrolysate, exhibited lower fresh weights of 0.90 g and 0.93 g, respectively. These outcomes likely stem from insufficient concentrations of sucrose and casein hydrolysate in the media, limiting plant growth due to inadequate energy supply and essential nutrients. Conversely, excessively high levels of sucrose and casein hydrolysate may induce metabolic stress, particularly in nutrient uptake, resulting in the accumulation of potentially toxic nitrogen compounds. This condition can further impede plant growth (17). Additionally, research by Siregar, Chan, and Lim (37)highlighted that the inclusion of casein hydrolysate at a concentration of 5% (w/v) in the suspension culture medium of Eurycoma longifolia Jack led to reductions in fresh weight, dry weight, and total alkaloid content. This was in contrast to media formulations either devoid of casein hydrolysate or containing lower concentrations of 0.1–2% (w/v).

V. Conclusion

Based on the research findings, it can be concluded that the combination of sucrose and casein hydrolysate concentrations had a significant and optimal impact on various growth parameters of plantlets. These include survival rate, plantlet height, number of leaves, fresh weight, root number, and root length. The treatment combining 30 g/L sucrose with 100–150 mg/L casein hydrolysate demonstrated the most notable results in plantlet growth, producing the highest increases in plantlet height (2.57 cm and 2.42 cm), number of leaves (6.17 and 6.13 leaves), and fresh weight of plantlets (2.13 g and 2.10 g). Similarly, the combination of 40 g/L sucrose with 50–150 mg/L casein hydrolysate exhibited the best outcomes for root growth, including the highest increase in root count (6.43 roots) and root length (9.63 cm and 9.67 cm). These findings highlight the optimal nutrient composition necessary to enhance the physiological and morphological development of plantlets under in vitro conditions.

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