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MULTIDISCIPLINARY APPROACH TO IN VITRO PROPAGATION OF *COELOGYNE PANDURATA LINDL*. FROM KALIMANTAN: SYNERGISTIC EFFECTS OF NAPHTHALENE ACETIC ACID AND YEAST EXTRACT WITH A MATHEMATICAL BIOLOGY PERSPECTIVE

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Abstract: This study investigates the synergistic effects of Naphthalene Acetic Acid (NAA) and yeast extract on the in vitro propagation of *Coelogyne pandurata* (black orchid) using a multidisciplinary approach that combines biological experimentation and statistical analysis. The research aimed to optimize vegetative growth, specifically shoot, leaf, and root development, by applying different concentrations of NAA (0, 1, 3, and 5 mg/L) and yeast extract (0, 0.5, 1, and 1.5 g/L). Data analysis involved Pearson correlation, Kruskal-Wallis test, and hierarchical cluster analysis. The Pearson correlation matrix revealed a strong positive relationship between shoot and leaf growth (r = 0.93), indicating that increased shoot formation was associated with leaf development. A weaker correlation was observed between shoot and root growth ($r = 0.39$), and a moderate correlation was found between leaf and root growth ($r = 0.55$). Statistical analysis using the Kruskal-Wallis test showed significant differences in root number ($p_{value} = 0.04$) among various NAA treatments, with the 1 mg/L NAA concentration resulting in the highest root

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number. Yeast extract concentration also significantly influenced shoot ($p_{value} = 0.03$) and root ($p_{value} = 0.03$) development, while leaf number remained unaffected (p = 0.07). Cluster analysis using Ward's method revealed distinct treatment groups based on growth patterns. Treatments with moderate yeast extract concentrations (1–1.5 g/L) clustered together, showing enhanced shoot and root growth, while high concentrations of NAA combined with yeast extract resulted in suppressed root development. These findings indicate that a balanced combination of NAA and yeast extract optimizes the vegetative growth of black orchids. This study contributes to the understanding of growth regulation in *Coelogyne pandurata* and offers insights for improving orchid propagation in vitro.

Keywords: coelogyne pandurate; naphthalene acetic acid; yeast extract; in vitro propagation, Pearson correlation; Kruskal-Wallis; cluster analysis.

2010 AMS Subject Classification: 92B05.

1. INTRODUCTION

Orchids are ornamental plants with high economic value due to their unique flower shapes and striking colors [1], [2]. The enchanting beauty of orchids has attracted widespread attention from domestic and international enthusiasts [3]. Orchids belong to one of the most prominent plant families with a global distribution[4]. Indonesia is home to about 20% of the total orchid species in the world, with an estimated number of species ranging from 5,000 to 6,000 [5], [6]. One notable species is the black orchid (*Coelogyne pandurata*), which is endemic to Kalimantan and serves as the mascot of East Kalimantan Province [7].

The black orchid is unique, especially its black labellum and its ability to emit a fragrant aroma when it blooms [1]. The flowers of the black orchid usually bloom between April and July, with a bloom duration of only 5–6 days [8]. Its beauty and uniqueness make it highly sought after by collectors and orchid enthusiasts. However, the biggest challenge in propagating the black orchid lies in its short blooming period and the difficulty of crossbreeding[9]. Conventional propagation methods have been hindered by the long time required to produce seedlings and the limited number of seedlings produced [10]. Additionally, the seeds of the black orchid cannot be used for propagation as they do not contain endosperm, thus requiring a mutualistic symbiosis with mycorrhizal fungi[1].

To overcome these challenges, in vitro propagation has become a promising solution [11], [12], [13], [14], [15]. This technique allows plants to develop into complete plants from isolated cells, tissues, or organs under sterile conditions. The success of in vitro culture is influenced by

several endogenous and exogenous factors, including plant growth regulators (PGRs). PGRs are plant hormones that can be sourced from synthetic or organic materials. One synthetic auxin commonly used in in vitro culture is Naphthalene Acetic Acid (NAA), due to its superior stability compared to other synthetic auxins like Indole Acetic Acid (IAA) [16], [17].

The importance of other components in the culture medium, such as vitamins, amino acids, and complex organic materials, cannot be overlooked [4], [18]. Yeast extract, an affordable organic material, significantly enhances plant growth [19], [20]. Using yeast extract as an additive in the culture medium can synergize with PGRs to improve the success of in vitro propagation. Previous studies have shown that combining PGRs and organic materials like yeast extract often results in better plant growth than using PGRs alone [21].

However, despite the numerous studies exploring the effects of combining PGRs and organic materials, no specific research has investigated the effects of NAA and yeast extract on the growth of *Coelogyne pandurata*. Therefore, this study aims to evaluate the combined impact of NAA and yeast extract in in vitro culture to enhance the vegetative growth of black orchids, particularly in the parameters of shoots, leaves, and roots, using the Murashige and Skoog (MS) medium, which has been proven effective for various plant species.

The use of mathematical biology and statistics in this research is crucial to provide a more systematic scientific approach in evaluating and understanding the interaction between NAA concentration and yeast extract on the vegetative growth of black orchids. Mathematical biology allows researchers to model complex biological phenomena and gain deeper insights into the dynamics of plant growth, especially in in vitro culture. Meanwhile, statistical techniques are used to analyze experimental data to identify the influence of the tested variables, such as NAA concentration and yeast extract, on plant growth parameters.

Pearson correlation is used to evaluate the linear relationship between the number of shoots, leaves, and roots in black orchids treated with various concentrations of NAA and yeast extract. This correlation is essential to identify how changes in one growth parameter are related to changes in other parameters. To analyze differences between the treatments, the Kruskal-Wallis test is used as an alternative to analysis of variance (ANOVA). The Kruskal-Wallis test helps identify if there are significant differences in the number of shoots, leaves, or roots among different treatment groups [22]. The results of this test provide an overview of the impact of NAA and yeast extract

treatments on black orchid growth. As a follow-up test, the Mann-Whitney test is used to compare the two treatment groups further [23], which shows significant differences after the Kruskal-Wallis test. This technique allows us to determine which treatment is more effective in promoting the plant growth of shoots, leaves, or roots.

Additionally, Ward's method uses cluster analysis to group treatments based on similarities in black orchid growth responses [24], [25]. This technique identifies treatments that result in similar growth patterns, whether in the number of shoots, leaves, or roots. This clustering will help determine the optimal combination of NAA and yeast extract that produces the best growth in black orchids.

The novelty of this study lies in the fact that no specific research has been conducted on the combination of NAA and yeast extract to optimize the growth of black orchids, particularly in terms of shoots, leaves, and roots. Overall, the use of mathematical biology and statistics in this research provides a more scientific and systematic approach to understanding the physiological mechanisms involved in the vegetative development of Coelogyne pandurata. The statistical techniques help identify patterns in the data and clarify the interactions between the growth regulators (PGRs) used in in vitro culture. With a deeper understanding of these interactions, this research is expected to identify the optimal combination of NAA and yeast extract that can enhance the vegetative growth of black orchids, thus contributing to practical applications in the black orchid propagation industry and ornamental plant cultivation in general.

2. PRELIMINARIES

2.1 STATISTICAL ANALYSIS

In this study, identifying the interrelationships between growth parameters was considered an important preliminary step before conducting group comparisons. Since the responses of *Coelogyne pandurata* to the various combinations of yeast extract and Naphthalene Acetic Acid (NAA) could potentially involve related physiological processes, correlation analysis was performed to explore the associations between shoot, leaf, and root development.

Pearson correlation was used to evaluate the degree of linear association between each pair of continuous growth variables. As a parametric test, it assumes that the variables are normally distributed and exhibit linearity [26]. The resulting correlation coefficient (r) ranges from -1 to 1,

where values closer to 1 or -1 indicate stronger positive or negative relationships, respectively, and values near 0 indicate little to no linear association. The hypotheses tested are:

H_0 : There is no significant linear correlation between the two variables ($r = 0$).

H_1 : There is a significant linear correlation between the two variables ($r \neq 0$).

The Pearson correlation coefficient is calculated using the formula:

$$r = \frac{\sum_{i=1}^{n}(X_i - \bar{X})\,(Y_i - \bar{Y})}{\sqrt{\sum_{i=1}^{n}(X_i - \bar{X})^2\,(Y_i - \bar{Y})^2}} \quad (1)$$

Where X_i and Y_i represent the values of the two variables, and $\bar{X}$ and $\bar{Y}$ are their respective means. A p_{value} is then used to assess the significance of the correlation; correlations with $p_{value} < 0.05$ are considered statistically significant. The resulting correlation matrix is often visualized in a heatmap to highlight the strength and direction of relationships between the growth parameters [27], [28].

This study employed nonparametric statistical methods to examine the effects of Naphthalene Acetic Acid (NAA) and yeast extract on the in vitro growth of black orchid (*Coelogyne pandurata*). Given the possibility that the data did not meet the assumptions required for parametric tests such as normality and homogeneity of variance, the Kruskal-Wallis test was chosen as the primary method of analysis. The Kruskal-Wallis test is a nonparametric, rank-based statistical method used to determine whether there are statistically significant differences among three or more independent groups on an ordinal or numerical (interval or ratio) dependent variable. It serves as an alternative to the one-way analysis of variance (ANOVA) when the assumptions of normality and homogeneity of variance are not met [22]. The hypotheses tested are:

H_0 : The independent variable has no significant effect on the dependent variable.

H_1 : The independent variable has a significant effect on the dependent variable.

The test statistic is compared to the chi-square distribution with $k - 1$ degrees of freedom, where k is the number of groups. The null hypothesis is rejected if the $p_{value} < \alpha$, indicating significant differences among the groups. The test statistic is computed using the following formula:

$$H = \frac{12}{N(N+1)}\sum_{i=1}^{k}\frac{R_i^2}{n_i} - 3(N+1) \quad (2)$$

where:

N : total number of observations,

k : number of groups,

R_i : sum of ranks in group i,

n_i : number of observations in group i.

When the Kruskal-Wallis test yields a statistically significant result, post-hoc analysis is required to determine which specific groups differ. For this purpose, the Mann-Whitney U test is commonly used to perform pairwise comparisons between independent groups. The Mann-Whitney U test is designed to assess whether two independent samples come from the same distribution. It does so by ranking all observations from both groups together, then analyzing the sum of ranks for each group. The hypotheses tested are:

H_0 : There is no significant difference between the two independent groups.

H_1 : There is a significant difference between the two independent groups.

The test statistic is compared to the Mann-Whitney U distribution for the corresponding sample sizes. The null hypothesis is rejected if the $p_{value} < \alpha$, indicating a significant difference between the two groups. The test statistic is computed using the following formula:

$$U_1 = (m_1.m_2) + \frac{m_1(m_1+1)}{2} - R_1 \quad (3)$$

$$U_2 = (m_1.m_2) + \frac{m_2(m_2+1)}{2} - R_2 \quad (4)$$

where:

m_1 : number of observations in group 1,

m_2 : number of observations in group 2,

R_1 : sum of ranks for group 1,

R_2 : sum of ranks for group 2.

The smaller value between U_1 and U_2 is used as the final test statistic for comparison or for determining the p_{value} [23], [29].

Following the identification of significant differences between treatments using Mann-Whitney U tests, hierarchical cluster analysis was applied to further categorize the treatments based on similarity in their growth responses. This analysis aimed to reveal underlying patterns in the data by grouping treatments that produced comparable shoot, leaf, or root development.

Ward's method was selected as the clustering approach due to its effectiveness in minimizing

within-cluster variance. It begins by treating each treatment as a separate cluster and successively merges them based on the smallest increase in total variance, ensuring more homogeneous groupings at each step. The dissimilarity between clusters is computed using the formula:

$$D_{ij} = \frac{n_i . n_j}{n_i + n_j} \left\| \bar{x}_i - \bar{x}_J \right\|^2 \quad (5)$$

Where n_i and n_j are the sizes of clusters i and j, and $\bar{x}_i$ and $\bar{x}_j$ are their centroids. The Euclidean distance between centroids $(\| . \|)$ serves as the basis for measuring dissimilarity [30].

The clustering results are illustrated in a dendrogram, which visually represents the hierarchical relationships among treatments [31], [32], [33]. Treatments that merge at lower heights in the dendrogram are considered more similar in their growth outcomes, allowing for a clearer biological interpretation of how specific combinations of yeast extract and NAA influence in vitro development in *Coelogyne pandurate.*

2.2 MATERIALS AND METHODS

The experimental material used in this study consisted of *Coelogyne pandurata* (black orchid) explants measuring approximately 1–1.5 cm in height, each bearing two leaves and no roots. The explants were obtained from the Tissue Culture Laboratory, Faculty of Mathematics and Natural Sciences, Mulawarman University.

The experiment was arranged using a factorial Completely Randomized Design (CRD), involving two factors: four concentrations of Naphthalene Acetic Acid (NAA) (0, 1, 3, and 5 mg/L) and four concentrations of yeast extract (0, 0.5, 1, and 1.5 g/L), resulting in 16 treatment combinations. Each treatment was replicated three times, producing a total of 48 experimental units.

All tools and materials were cleaned using detergent, rinsed thoroughly, and sterilized by soaking in 70% ethanol and autoclaving at 121°C and 1 atm for 30 minutes. The culture medium was based on Murashige and Skoog (MS) formulation, prepared by dissolving MS salts and sucrose in sterile distilled water. NAA and yeast extract were added according to the treatment plan. The medium's pH was adjusted to 5.6–5.8 using 1 N NaOH or HCl, then solidified with agar and sterilized by autoclaving. The yeast extract was prepared by dissolving commercial dry yeast (Fermipan) in sterile water, heating and homogenizing the solution at 70°C, and filtering it to obtain the supernatant used in the culture media.

Explant inoculation was conducted aseptically in a Laminar Air Flow Cabinet (LAFC) that had been sterilized using 70% ethanol and ultraviolet (UV) light. Explants were separated from clumps and residual media using a sterile scalpel and tweezers, then transferred into sterile culture bottles containing the respective treatment media. The culture bottles were sealed with heat-resistant plastic and wrapped with plastic film, then labeled with treatment codes and inoculation dates.

All cultures were incubated under controlled environmental conditions for a period of 12 weeks. Observations were made weekly, and the recorded growth parameters included the number of shoots, number of fully expanded leaves, and number of roots per explant. These measurements were then analyzed statistically to evaluate treatment effects.

2.3 RESEARCH STAGE WITH R SOFTWARE

To evaluate the in vitro growth response of *Coelogyne pandurata* under various combinations of yeast extract and NAA, a structured analysis workflow was conducted using R software. The process involved a combination of descriptive statistics, correlation analysis, nonparametric testing, and multivariate clustering. The flow of analysis is summarized in the Figure 1.

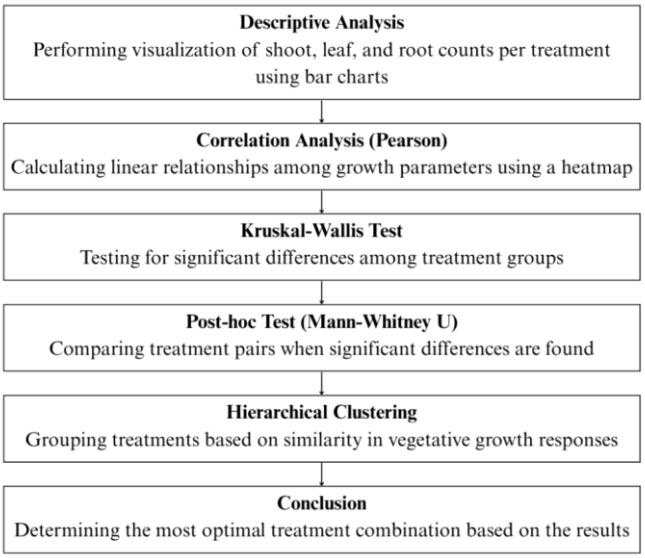


Figure 1. Research stage

Pseudocode: Pearson Correlation

```
# Step 1: Input paired data from the same observations
X = values of variable 1 (e.g., shoot count)
Y = values of variable 2 (e.g., leaf count)

# Step 2: Calculate mean of each variable
mean_X = average of X
mean_Y = average of Y

# Step 3: Compute the numerator of the correlation formula
numerator = sum of (Xᵢ - mean_X) * (Yᵢ - mean_Y)

# Step 4: Compute the denominator
denominator = square root of [sum of (Xᵢ - mean_X)^2 * sum of (Yᵢ - mean_Y)^2]

# Step 5: Calculate correlation coefficient (r)
r = numerator / denominator

# Step 6: Compute the t-statistic
t = r * sqrt((n - 2) / (1 - r^2))

# Step 7: Compute p-value using t-distribution
p_value = probability of t with (n - 2) degrees of freedom

# Step 8: Interpret result
If p_value < α:
   Conclude that there is a significant correlation between the two variables
Else:
    Conclude that there is no significant correlation

Output: Correlation coefficient (r), p-value
```

Pseudocode: Kruskall-Wallis Test

```
Input: Data consisting of observations from multiple groups

# Step 1: Combine all data from different groups into one dataset
combined_data = combine(data_group_1, data_group_2, ..., data_group_k)



# Step 2: Sort the combined data in ascending order and assign ranks
sorted_data = sort(combined_data)
ranks = assign_ranks(sorted_data)

# Step 3: Calculate the rank sum for each group
rank_sum_per_group = []
for each group in data_groups:
    group_rank_sum = sum_ranks(group)
    rank_sum_per_group.append(group_rank_sum)

# Step 4: Calculate the H statistic
N = total_number_of_observations  # Total number of data points across all
groups
H = (12 / (N * (N + 1))) * sum(rank_j^2 / n_j for each group) - 3 * (N + 1)

# Step 5: Determine degrees of freedom (df)
df = k - 1  # Where k is the number of groups

# Step 6: Compare H with Chi-Square distribution
critical_value = chi_square_critical_value(df)
p_value = 1 - chi_square_distribution(H, df)

# Step 7: Interpret the result
if H > critical_value:
    print("Null hypothesis rejected, there is a significant difference between
groups.")
else:
    print("No significant difference between groups.")

Output: H statistic, degrees of freedom, p-value
```

Pseudocode: Man-Whitney U Test

```
Input: Data consisting of observations from two independent groups (Group 1, Group 2)

# Step 1: Combine the two groups' data into one dataset
combined_data = combine(data_group_1, data_group_2)

# Step 2: Sort the combined data in ascending order and assign ranks
sorted_data = sort(combined_data)
ranks = assign_ranks(sorted_data)

# Step 3: Calculate the rank sum for each group
rank_sum_group_1 = sum_ranks(group_1)
rank_sum_group_2 = sum_ranks(group_2)

# Step 4: Calculate the U statistic for each group
n1 = number_of_observations_group_1
n2 = number_of_observations_group_2
U1 = rank_sum_group_1 - (n1 * (n1 + 1)) / 2
U2 = rank_sum_group_2 - (n2 * (n2 + 1)) / 2

# Step 5: Choose the smaller U statistic
U = min(U1, U2)

# Step 6: Determine the critical value from the U distribution table
critical_value = get_critical_value(n1, n2, alpha)

# Step 7: Compare U with the critical value
if U < critical_value:
   print("Null hypothesis rejected, there is a significant difference between the groups.")
else:
   print("No significant difference between the groups.")

Output: U statistic, p-value, result of hypothesis test
```

Pseudocode: Ward's Hierarchical Clustering

```
Input: Data consisting of observations with features

# Step 1: Initialize each data point as its own cluster
clusters = initialize_clusters(data)

# Step 2: Compute the distance matrix (Euclidean distance between each pair of points)
distance_matrix = compute_distance_matrix(data)

# Step 3: Repeat until all clusters are merged
while len(clusters) > 1:
    # Step 3.1: Find the two closest clusters based on Ward's method
    cluster_pair = find_closest_clusters(distance_matrix)

    # Step 3.2: Merge the two closest clusters into a new cluster
    new_cluster = merge_clusters(cluster_pair)

    # Step 3.3: Update the distance matrix to reflect the new cluster
    update_distance_matrix(distance_matrix, new_cluster, cluster_pair)

    # Step 3.4: Update the list of clusters with the new merged cluster
    update_clusters(clusters, new_cluster, cluster_pair)

# Step 4: Construct the dendrogram based on the hierarchical clustering
dendrogram = construct_dendrogram(clusters)

# Step 5: Determine the number of clusters based on the dendrogram
clusters_cut = cut_dendrogram(dendrogram, number_of_clusters)

# Output: Final clusters
Output: clusters_cut
```

3. MAIN RESULTS

The effects of yeast extract and Naphthalene Acetic Acid (NAA) on the in vitro growth of black orchid (*Coelogyne pandurata*) were evaluated based on shoot, leaf, and root development across different treatment combinations. Data from three biological replicates per treatment were summarized and visualized using bar charts to highlight variations in growth responses. These visualizations provide an initial descriptive assessment, allowing examination of central tendencies, inter-replicate consistency, and the relative effectiveness of each treatment.

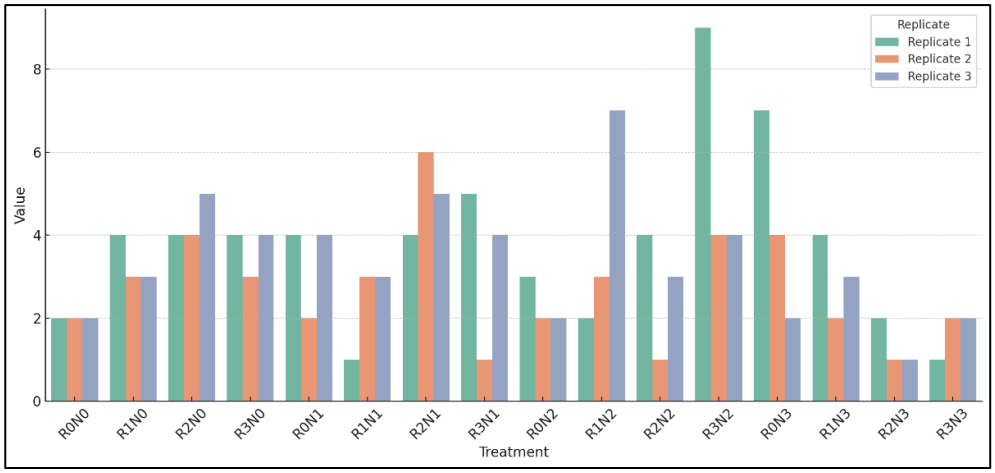


Figure 1. Shoot growth of replicate 1, 2, and 3 for each treatment

Based on Figure 1, there is a noticeable variation in shoot growth of black orchid (*Coelogyne pandurata*) across the different combinations of yeast extract and NAA treatments. The control treatment (R0N0), which received no yeast extract or NAA, showed the lowest and most uniform shoot number across all replicates, indicating minimal stimulation of shoot development. In contrast, the treatment with 3 g/L yeast extract and 2 ppm NAA (R3N2) resulted in the highest number of shoots, particularly in the first replicate, which reached a value of 9. This suggests that a high concentration of yeast extract combined with a moderate concentration of NAA may have the most significant effect in promoting shoot formation. Additionally, the R2N1 treatment also showed a relatively high and consistent number of shoots across replicates, supporting the idea that there may be a synergistic interaction between yeast extract and NAA. However, some treatments such as R1N2 and R2N2 showed noticeable variability among replicates, indicating that plant responses to treatment may not always be stable. Overall, these results suggest that the

application of yeast extract and NAA, whether individually or in combination, can enhance shoot growth, with the degree of effectiveness depending on the specific concentrations used.

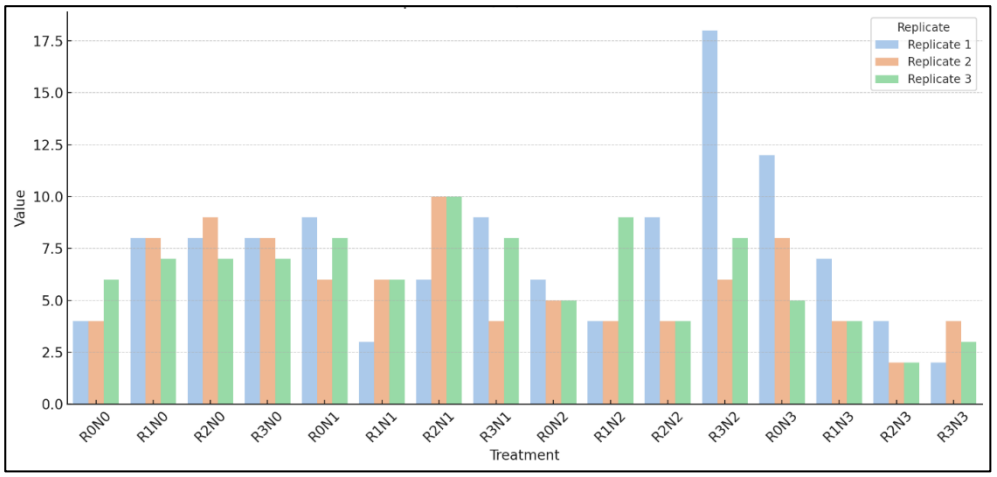


Figure 2. Leave growth of replicate 1, 2, and 3 for each treatment

Based on Figure 2, it can be observed that the number of leaves in black orchid (*Coelogyne pandurata*) varies noticeably across the different combinations of yeast extract and NAA treatments. The R3N2 treatment (3 g/L yeast extract and 2 ppm NAA) stands out as the treatment with the highest average number of leaves, particularly due to replicate one reaching a value of 18, far exceeding the other treatments. This suggests that the combination is highly effective in stimulating leaf formation. In addition, treatments such as R0N3 and R2N0 also showed relatively high and consistent leaf numbers across replicates, indicating the potential benefits of both single and combined applications of yeast extract and NAA. In some treatments, such as R2N3 and R3N3, the number of leaves remained consistently low across all replicates. This outcome suggests that high concentrations of both yeast extract and NAA do not always lead to favorable results and may even suppress leaf development. These findings highlight the importance of concentration balance, as the effectiveness of leaf growth appears to be strongly influenced by the specific combination and dosage of the growth regulators used.

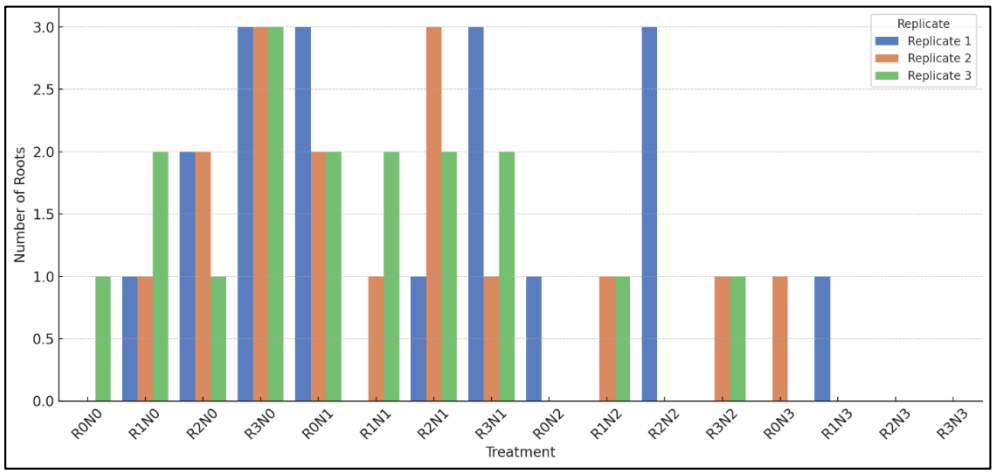


Figure 3. Root growth of replicate 1, 2, and 3 for each treatment

Based on Figure 3, root growth in black orchid (*Coelogyne pandurata*) varies across the different combinations of yeast extract and NAA treatments. The R3N0 treatment (3 g/L yeast extract without NAA) showed the highest and most consistent root number, with all three replicates producing three roots each. This indicates that yeast extract at high concentration, when applied without NAA, is highly effective in promoting root formation. Treatments such as R0N1 and R2N1 also resulted in relatively high root numbers, although with slightly less consistency than R3N0. This suggests that low concentrations of NAA can still support root development when combined with appropriate levels of yeast extract. In some treatments, such as R2N2, R3N2, R2N3, and R3N3, the number of roots remained consistently low or absent across most replicates. This outcome implies that high concentrations of NAA may inhibit root initiation rather than stimulate it. Overall, the results suggest that the use of yeast extract alone, particularly at higher concentrations, is more favorable for root formation, while the addition of NAA must be carefully optimized to avoid suppressing root growth.

The variation in shoot, leaf, and root numbers observed across treatments reflects the morphogenetic response of *Coelogyne pandurata* to different combinations of yeast extract and NAA, while also aligning with the species' inherent morphological potential. This pattern of vegetative development corresponds with key structural features such as pseudobulbs, oblong leaves, and a sympodial root system, which characterize the species and can be seen in Figure 4.

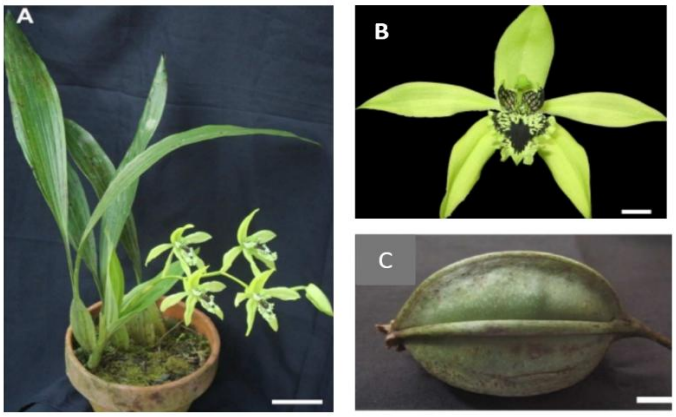


Figure 4. Morphological structure of *Coelogyne pandurata*., showing (A) whole plant, (B) flower, and (C) fruit.

Figure 4 presents the morphological characteristics of *Coelogyne pandurata*. This species develops an inflorescence that can grow upright or pendulous, bearing multiple flowers in a racemose arrangement (1-14 flowers per cluster). Sepals appear light green, lanceolate, and pointed, measuring 5-16 cm in length. The narrower petals share the same shape and color. A distinctive feature of this species is its black labellum, which is covered with trichomes. Pseudobulbs range from 2-10 cm in length and 1.5-8 cm in width, supporting oblong leaves that may extend up to 50 cm. Fruits are oblong, approximately 7 cm long and 2-3 cm wide.

Pearson correlation analysis was then used to examine the relationships among shoot, leaf, and root growth under different treatment combinations. The heatmap in Figure 5 visualizes the resulting correlation matrix, with color intensity indicating the strength and direction of the linear association between each pair of vegetative parameters.

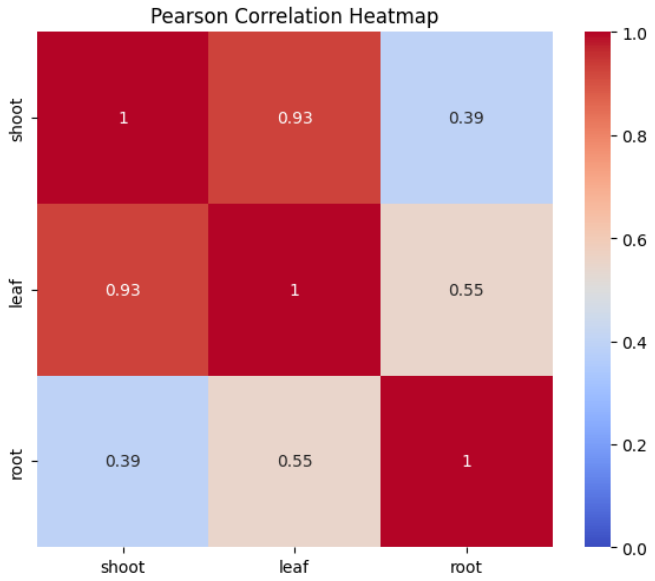


Figure 5. Pearson correlation heatmap

Based on Figure 5, the correlation between shoot and leaf numbers was 0.93, indicating a very strong and positive relationship. This suggests that shoot formation is often accompanied by intensive leaf development, reflecting a close association between the two organs during the in vitro morphogenetic process. The high correlation value implies a consistent pattern in which both variables tend to increase together.

The correlation between shoot and root numbers was 0.39, representing a weak to moderate relationship. This indicates that although root number may increase with shoot formation, the association is not particularly strong and may be influenced by additional factors such as media composition or hormone concentration. This pattern suggests that root development may follow a different or more complex regulatory pathway compared to shoot and leaf formation. The correlation between leaf and root numbers was 0.55, showing a moderate positive relationship. This may indicate that leaf growth supports root development, potentially through photosynthate allocation or hormonal signaling, although it is not the primary determinant of root number.

While correlation analysis offers an overview of the relationships among vegetative growth variables, it does not confirm whether the observed differences in shoot, leaf, and root numbers between treatment groups are statistically significant. Therefore, the Kruskal-Wallis test was applied using R software. The results indicated a significant difference in the root number variable

among the various NAA treatments applied to *Coelogyne pandurata* in vitro (p_{value}= 0.04). Among the three vegetative growth variables observed, only root number showed a significant response to the treatments, whereas shoot and leaf numbers did not differ significantly (p_{value}= 0.24 and p_{value}= 0.13, respectively).

To further examine the differences in root number, a Mann-Whitney test was conducted. The results are presented in Figure 6.

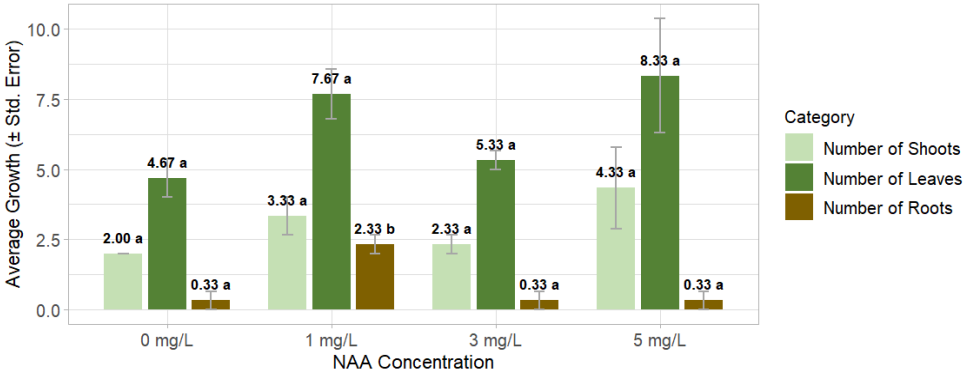


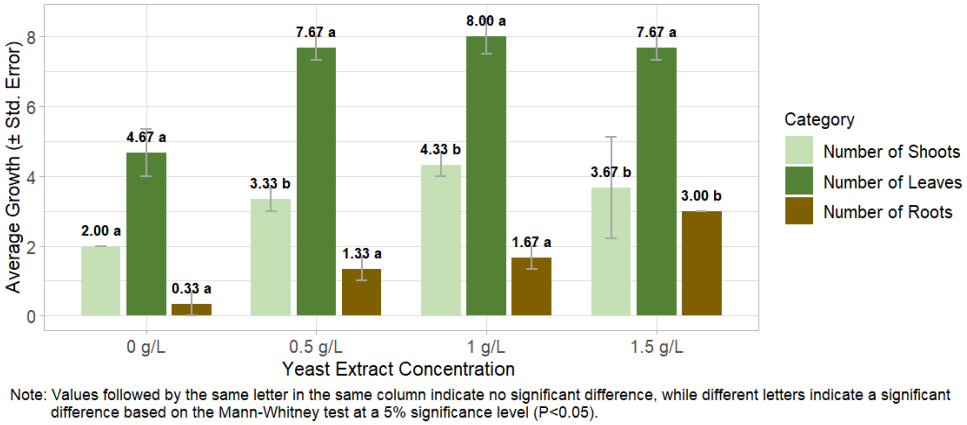
Figure 6. The effect of NAA concentration on the average number of shoots, leaves, and roots

Based on Figure 6, the number of roots produced by *Coelogyne pandurata* varied depending on the NAA concentration. The treatment with 1 mg/L NAA is grouped separately, as indicated by the letter ‘b’. In contrast, the control group (0 mg/L) and the treatments with 2, 3, 4, and 5 mg/L NAA share the same grouping letter, ‘a’. This pattern indicates that only the 1 mg/L treatment resulted in a significantly higher root number, while the remaining concentrations did not differ from one another. The distinct grouping of the 1 mg/L treatment suggests that this concentration was the most effective for root induction under in vitro conditions. The lack of difference among the other treatments implies that increasing the NAA concentration beyond 1 mg/L did not lead to further enhancement of root formation.

The Kruskal-Wallis test was performed using R software to evaluate the effect of yeast extract concentrations on the vegetative growth variables of *Coelogyne pandurata* . The results revealed significant differences in both shoot number (p_{value}= 0.03) and root number (p_{value}= 0.03) among the treatments, while leaf number did not differ significantly (p_{value}= 0.07). These

findings indicate that shoot and root development were affected by the yeast extract concentrations, whereas leaf production remained relatively unchanged across treatments.

To explore the observed differences further, a Mann-Whitney test was conducted. The results are presented in Figure 7.



Note: Values followed by the same letter in the same column indicate no significant difference, while different letters indicate a significant difference based on the Mann-Whitney test at a 5% significance level (P<0.05).

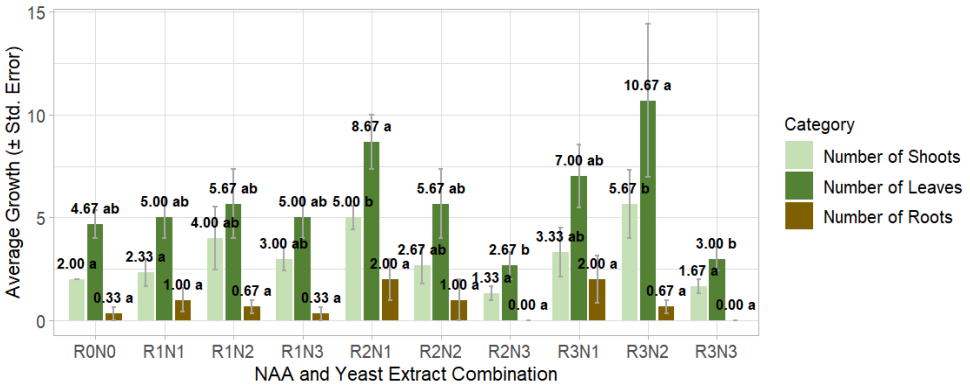
Figure 7. The effect of yeast extract on the average number of shoots, leaves, and roots

Based on Figure 7, the shoot number varied depending on the concentration of yeast extract. Treatments with 1 and 1.5 g/L were grouped together under the letter ‘b’, indicating higher shoot numbers compared to the control (0 g/L), which was grouped under ‘a’. The 0.5 g/L treatment showed an intermediate response and was not significantly different from either group. This pattern suggests that the application of 1 to 1.5 g/L yeast extract promotes shoot formation under in vitro conditions. A similar trend was observed in root number. The highest root formation occurred at 1.5 g/L, which was assigned a distinct grouping letter ‘b’, while the control, 0.5, and 1 g/L treatments were grouped under ‘a’. This indicates that only 1.5 g/L of yeast extract significantly enhanced root development. For leaf number, although numerical differences were observed, all treatments shared the same grouping letter, reflecting no significant differences. Overall, these results indicate that yeast extract has a concentration-dependent effect on vegetative growth, particularly on shoot and root formation, with 1–1.5 g/L being the most favorable range for *Coelogyne pandurata* cultured in vitro.

The Kruskal-Wallis test was carried out using R software to assess the effect of combined NAA and yeast extract treatments on the vegetative growth variables of *Coelogyne pandurata* cultured in vitro. The results showed no significant differences in shoot number (p_{value} = 0.54),

leaf number (p_{value}= 0.40), or root number (p_{value}= 0.27) across the treatment groups. These findings suggest that, overall, the combination of NAA and yeast extract did not produce distinct differences in vegetative growth when assessed collectively.

Although the Kruskal-Wallis test did not indicate significant differences across treatments, a Mann-Whitney test was still conducted to explore possible pairwise variations in shoot, leaf, and root numbers. The results of the analysis are presented in Table 4.3.



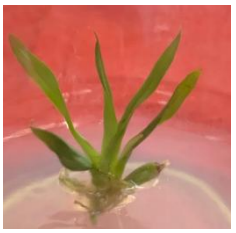
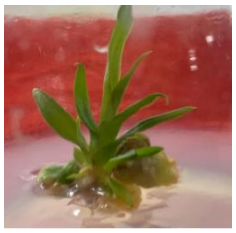
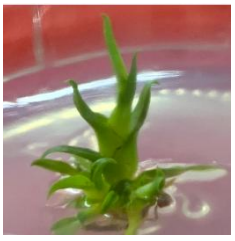
Note: Values followed by the same letter in the same column indicate no significant difference, while different letters indicate a significant difference based on the Mann-Whitney test at a 5% significance level (P<0.05).

Figure 8. The effect of NAA and Yeast extract combination on the average number of shoots, leaves, and roots

Based on Figure 8, shoot number varied depending on the combination of NAA and yeast extract. The highest average was observed in the R3N2 treatment (5.67), which was assigned the letter ‘b’. Treatments such as R2N3 and R3N3 were grouped under the letter ‘a’, indicating lower shoot production. Other combinations, including R2N1, R1N2, and R3N1, received intermediate groupings that reflect moderate responses. A similar trend was observed in leaf number. The R3N2 treatment recorded the highest average (10.67) and was grouped under the letter ‘a’, whereas R2N3 and R3N3 were grouped under the letter ‘b’, suggesting a lower leaf count. These groupings, although not supported by the overall Kruskal-Wallis result, indicate that certain combinations may influence vegetative development. For root number, most treatments were grouped under the same letter, suggesting a relatively uniform response across all combinations of NAA and yeast extract. This observation is consistent with the non-significant result reported earlier.

A summary of the average number of shoots, leaves, and roots in each treatment combination of NAA and yeast extract is presented in Table 2.

Table 2. The Effect of NAA and Yeast Extract Combination

NAA	Yeast Extract			
	R0 (0 g/L)	R1 (0.5 g/L)	R2 (1 g/L)	R3 (1.5 g/L)
N0 (0 mg/L)				
N1 (1 mg/L)				
N2 (3 mg/L)				
N3 (5 mg/L)				

Based on the results presented in Table 2, the application of NAA at 5 mg/L led to the highest average number of shoots (4.33) and leaves (8.33), suggesting its effectiveness in promoting aerial organ development. In contrast, NAA at 1 mg/L was associated with the highest root number (2.33), indicating that lower concentrations may be more favorable for root induction. Similarly, yeast extract at a concentration of 1 g/L enhanced shoot and leaf production, with average values of 4.33 and 8.00, respectively. For root development, the highest average (3.00) was observed at 1.5 g/L yeast extract, showing its role in stimulating below-ground organ formation. Among all treatment combinations, R3N2 (1.5 g/L yeast extract and 3 mg/L NAA) produced the highest average shoot and leaf numbers, reaching 5.67 and 10.67, respectively. For root number, the most favorable

combination was R3N1 (1.5 g/L yeast extract and 1 mg/L NAA), which yielded an average of 2.00 roots. These results highlight that the effectiveness of each regulator depends not only on its concentration but also on its combination with other growth-promoting agents.

To provide further insight into the overall growth response patterns under different treatment combinations, hierarchical clustering analysis using Ward's method was performed. This analysis was conducted to visualize the similarity in growth responses based on shoot, leaf, and root number across treatments and to strengthen the interpretation derived from the previous statistical tests.

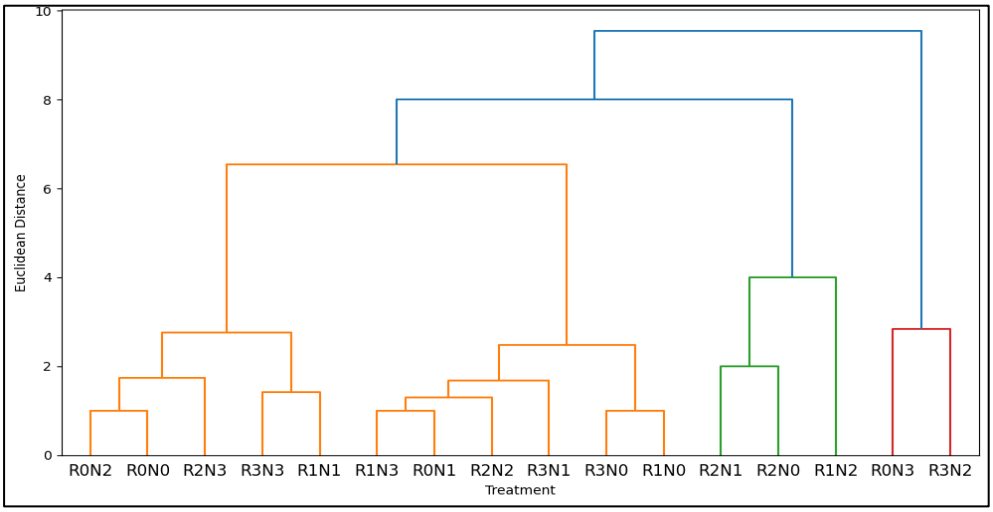


Figure 9. Dendrogram of shoot number clustering in *Coelogyne pandurata* under different NAA and yeast extract treatments (Ward's method)

The dendrogram reveals that certain treatment combinations produced similar responses in shoot number. Treatments such as R0N2, R0N0, and R2N3 were grouped within the same cluster, indicating low shoot formation, commonly associated with the absence of yeast extract or the application of high NAA concentrations. In contrast, treatments like R2N0, R2N1, and R3N0 formed a distinct cluster, suggesting that yeast extract at concentrations of 1–1.5 g/L effectively promoted shoot development. Notably, treatments R0N3 and R3N2 appeared at the outermost branches of the dendrogram, reflecting growth patterns that diverged significantly from other treatments. This may be attributed to negative interactions between excessive levels of NAA and yeast extract. Overall, the clustering pattern indicates that moderate concentrations of yeast extract, either alone or combined with low-to-moderate NAA, tend to result in higher and more consistent shoot formation compared to extreme combinations.

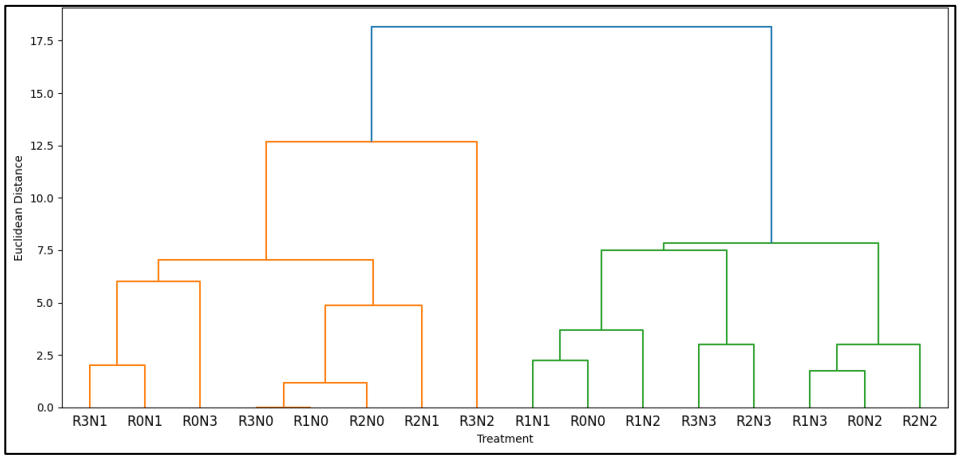


Figure 10. Dendrogram of leaf number clustering in *Coelogyne pandurata* under different NAA and yeast extract treatments (Ward's method)

The dendrogram for leaf number demonstrates that treatments such as R3N1, R0N1, and R0N3 are grouped closely, suggesting that these combinations produced similar leaf counts. These treatments are characterized by moderate concentrations of yeast extract and/or NAA. In contrast, treatments such as R2N2, R0N2, and R1N3 formed a separate cluster, generally involving higher NAA concentrations and lower yeast extract levels, which were associated with differing leaf growth patterns. Treatments like R2N1 and R2N0 were placed in distinct branches, indicating that yeast extract at 1 g/L may promote a unique leaf development response. This clustering implies that moderate doses of yeast extract, particularly when paired with low to medium NAA concentrations, tend to support more stable leaf formation, whereas extreme concentrations lead to inconsistent or reduced responses.

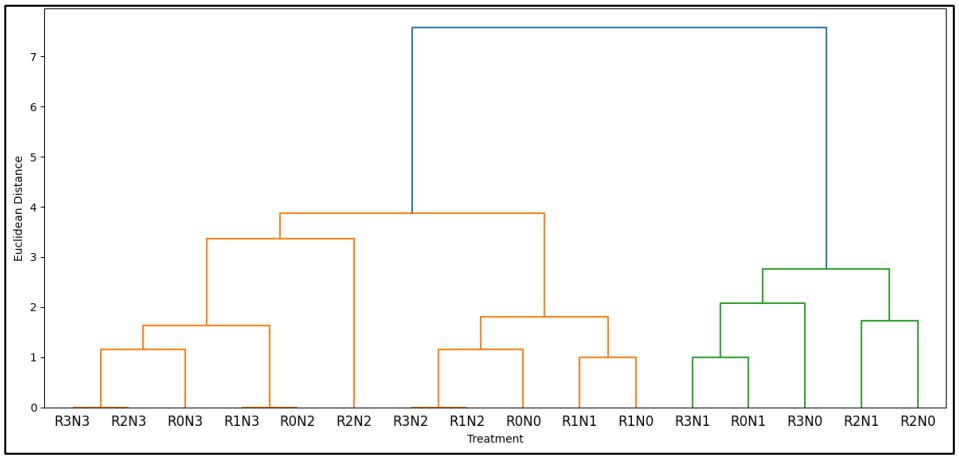


Figure 11. Dendrogram of root number clustering in *Coelogyne pandurata* under different NAA and yeast extract treatments (Ward's method)

The dendrogram for root number shows that treatments such as R3N3, R2N3, and R0N3 were grouped together, suggesting that high concentrations of NAA, particularly when combined with minimal or no yeast extract, resulted in similar root inhibition. A separate cluster consisting of R2N0, R2N1, and R3N0 highlights the role of moderate to high yeast extract concentrations in promoting more distinct root development responses. Treatments such as R1N2 and R1N0 were located on different branches from those involving high NAA, indicating that lower levels of both NAA and yeast extract may be more favorable for root formation. Collectively, these patterns suggest that moderate yeast extract levels, combined with low NAA concentrations, result in more uniform and enhanced root growth, while higher NAA levels may exert a suppressive effect.

4. CONCLUSIONS

This multidisciplinary study explored the synergistic effects of Naphthalene Acetic Acid (NAA) and yeast extract on the in vitro propagation of *Coelogyne pandurata* (black orchid) using a combination of biological and statistical approaches. The research highlights that the balance of NAA and yeast extract significantly enhances vegetative growth, particularly in shoots, leaves, and roots. By integrating mathematical biology and statistical techniques like Pearson correlation, Kruskal-Wallis test, and cluster analysis, the study provides a deeper understanding of the optimal conditions for propagating black orchids, offering valuable insights for the ornamental plant industry.

However, the study has some limitations. The findings are specific to *Coelogyne pandurata*, and their applicability to other orchid species or plants is uncertain. Experimental conditions may not fully reflect natural environments, limiting ecological relevance. Additionally, the molecular mechanisms behind the effects of NAA and yeast extract are not fully explored and require further research. The study's focus on in vitro conditions also restricts the applicability to field growth conditions, and the long-term effects on plant health and reproduction were not assessed, suggesting areas for future research.

CONFLICT OF INTERESTS

The author(s) declare that there is no conflict of interests.

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