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Original article

Protocorm Like Bodies (PLB) Growth of *Dendrobium* sp. Orchid with induction of oxygen nanobubbles ($NBsO_2$) by *In Vitro*

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Abstract

Nanobubbles (NBs) are a technology applicable in various sectors and have proven to be able to accelerate plant germination and growth and increase agricultural yields. The use of NBs in tissue culture still needs to be done. Tissue culture is a technology that can produce a large number of seeds in a short time especially for orchid plants. NBs in orchid tissue culture are expected to accelerate orchid growth. This research aims to identify the growth of Protocorm Like Bodies (PLB) of *Dendrobium* sp. orchids induced by $NBsO_2$, and to determine the effects of $NBsO_2$ on the growth of *Dendrobium* sp. orchids. The research method involves various steps such as sterilization, making pre-treatment media, making liquid media, sterilizing tools and materials, sterilizing nanogenerators, making $NBsO_2$ solutions, screening NBs, pre-treatment, induction of Nanobubbles, and measuring plants. The observation parameters include PLB weight (g), PLB height (cm), number of living PLB (%), PLB color, root emergence, root length, shoot emergence, number of new shoots, embryo emergence, and number of embryos. The results showed that the growth of *Dendrobium* sp. orchid PLB induced by $NBsO_2$ experienced a rapid and significant growth rate compared to treatments that were not induced by $NBsO_2$. The use of $NBsO_2$ affects the growth of *Dendrobium* sp. orchids by increasing the time of embryo emergence, number of embryos, plant weight, plant height, color, and percentage of life to increase the accelerated growth rate of *Dendrobium* sp. orchid PLB.

Keywords: *Dendrobium* sp., Growth, *In Vitro*, PLB, $NBsO_2$

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Introduction

In the last few years, Nanobubbles (NBs) have become an innovative and versatile technology in various fields including agriculture, aquaculture, food engineering, wastewater treatment, and the medical field. NBs are bubbles with a size of less than 100 nm (10^{-9}) and are different from other bubbles. NBs consist of gases, such as oxygen ($NBsO_2$) or nitrogen ($NBsN_2$), encapsulated in a liquid, commonly water, and are stabilized by a molecular layer at the gas-liquid interface. Due to their distinct properties, nanobubbles have attracted considerable interest across various scientific and industrial fields. Notably, their exceptional characteristics, including prolonged stability, enhanced gas dissolution, and improved gas interfacial diffusion, have been the subject of extensive research (Garcia et al., 2023). NBs have the advantages of high gas mass transfer efficiency and long shelf life. In particular, NBs combined with reactive oxygen have broad application prospects due to their low-cost, safe, and efficient characteristics (Zhou et al., 2022). In agriculture, accumulating evidence has shown that NBs enhance the growth process twofold compared to plain water. NBs are reported to be able to accelerate seed germination because the Reactive Oxygen Species (ROS) in NBs can produce endogenous ROS in seeds to promote seed germination (Oshita et al., 2023). The use of NBs in tissue culture is still rarely done and is an interesting topic in this research.

Plant tissue culture or *in vitro* culture is an effective and relatively economical plant propagation method. Plant tissue culture is based on the theory of cell "totipotency", that is, each cell can grow and redifferentiate to form structures and tissues to become a complete plant if given the appropriate conditions (Wijerathna-Yapa & Hiti-Bandaralage, 2023). This method has been widely used for plants that have high economic value and plants that are difficult to propagate conventionally because it is able to produce superior, pathogen-free plants, and can produce large numbers of plant seeds in a relatively fast time (Irsyadi, 2021).

Orchids are one of the plants that are widely bred in tissue culture. *Dendrobium* orchid is the most popular orchid and is favored by around 34% of the public. This orchid is mostly used as an ornamental plant for cut flowers. Another advantage of *Dendrobium* orchid is that it has a relatively long freshness period and high productivity which makes orchids have great economic prospects (Amalia et al., 2022). Orchids provide quite positive market prospects and increase the interest of plant breeders In producing new hybrid orchids. Orchid production in Indonesia in 2017 amounted to 20,045,577 stalks and will increase every year (Jupri et al., 2023).

The increasing demand for orchids means that the market needs have not been met. To fulfill the orchid trade in Indonesia, it still relies on imported seedlings (Erfa et al., 2020). Fluctuating orchid production caused by the slow growth of orchid plants makes the fulfillment of orchid plants cannot be achieved optimally. To meet the needs of orchids, it is necessary to increase produc-

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tion through improved cultivation technology (Hairuddin et al., 2018). So that plant propagation using tissue culture methods provides a great opportunity to produce plant seeds in large quantities and in a relatively short time. In addition, this technique is efficient for clonal propagation of plants, can reproduce explants with superior characters through the induction of somaclonal variations or genetic engineering techniques (Deswiniyanti & Lestari, 2022).

The use of NBs in tissue culture still needs to be done. Seeing the potential of NBs that have not been utilized optimally, the researchers intend to conduct research aimed at identifying the growth of PLB *Dendrobium* sp. orchids induced by $NBsO_2$ and to determine the effect of using $NBsO_2$ on the growth of *Dendrobium* sp. orchids. This research aims to identify the growth of Protocorm Like Bodies (PLB) of *Dendrobium* sp. orchids induced by $NBsO_2$, and to determine the effects of $NBsO_2$ on the growth of *Dendrobium* sp. orchids.

Methods

Study area

This research was conducted at the Orchidology Laboratory and Nursery, Faculty of Mathematics and Natural Sciences, Universitas Islam Malang. The method used was tissue culture using a thin film medium technique. The tools used were Laminar Air Flow (LAF), Air Treatment Sanitizer (ATS), nanogenerator (*Yixing Holly Technology Co.., Ltd, China*), Micropipette 100µl, Micropipette 200µl, Micropipette 1000µl, scalpel, sterile blade, spatula, tweezers, Scissors, Jam bottle, Beaker, Measuring cup, Petri dish, pH meter (*Multifunction*), Bunsen, Matches, Hotplate and Magnetic Stirrer (*Thermo Scientific*), Analytical Scales (*Ohaus*), Alcohol sprayer, Sterile tissue, Tissue, Markers, Large plastic wrap and small plastic wrap, stove, plastic, and Pipette. While the materials used are PLB of Orchid *Dendrobium* sp., $NBsO_2$, MS Media (Stock A: NH_4NO_3, Stock B: KNO_3, Stock C: $CaCl_2.H_2O$, Stock D: $MgSO_4.7H_2O$ and KH_2PO_4, Stock E: $FeSO_4.7H_2O.Na_2EDTA.2H_2O$, Stock F: H_3BO_3, $MnSO_4.4H_2O$, and $ZnSO_4.7H_2O$, Stock G: Kl, $Na_2MoO_4.2H_2O$, $CuSO_4.5H_2O$, dan $CoCl2.6H_2O$), Vitamins, Myo-Inositol, *Naphthaleneacetic acid (*NAA), *Benzyl Amino Purin* (BAP), H_2O_2 solution, 70% alcohol, 96% alcohol, spiritus burner, sterile distilled water, sugar, agar, and distilled water.

Procedures

The procedures in this research are:

- **Room Sterilization**

The room sterilization process is carried out using ATS. ATS is filled using hydrogen peroxide liquid, which is 1000 ml with a low and safe concentration. Then the ATS is turned on and releases steam. This sterilization process lasts for 30 minutes.

- **Preparation of Pre-Treatment Media**

In the process of making *pre-treatment* media, the first thing to do is to weigh 300 mg of sugar and then dissolve it with distilled water as much as 700 ml. Then the media is homogenized using a *hotplate and magnetic stirrer* and stored in a jar bottle. The sterilization process is carried out.

- **Preparation of Murashige and Skoog (MS) Growth Medium**

The process of making growth media was carried out by taking stock solutions A-G according to the dose using a micropipette, then dissolved with distilled water as much as 200 ml into a glass beaker. After that, myo-inositol, vitamins, and ZPT (NAA and BAP) were added using a micropipette and put into a glass beaker containing MS. The media was then added 200 mg of sugar and then homogenized using a magnetic stirrer at 120 rpm. After homogenization, the pH of the media was measured, the pH for the media is 5.4. If the pH of the media is appropriate, then the media is stored in jar bottles and sterilized.

- **Sterilization of Tools and Materials**

In the process of sterilizing tools and materials, namely using an *autoclave*. The tools to be sterilized must be washed first with detergent until clean, then the tool is wrapped using plastic wrap and then put into the autoclave for sterilization. The sterilization time is 15 minutes with a temperature of 121 °C.

- **Nanogenerator Sterilization**

Nanogenerator sterilization was carried out using 3% H_2O_2. H_2O_2 solution as much as 1000 ml is inserted into the generator through the output and input channels of the nanogenerator. Then the running process is carried out with a time of 15 minutes. After that, 1000 ml of sterile distilled water was taken and put into the machine through the output and input channels of the nanogenerator and a running process was carried out for 15 minutes to remove the remaining H_2O_2 in the nanogenerator.

- **Preparation of $NBsO_2$ Solution**

The process of making $NBsO_2$ solution is done by inducing O_2 gas through an oxygen hose into the nanogenerator machine. After that, sterile water was taken and put into the generator through the output and input hoses and then the nanogenerator running process was carried out for 15 minutes. After that, the solution was filtered using a bacterial filter to remove microorganisms that may be present in the NBs.

- **Screening NBs**

Screening of NBs was done using a laser pointer. The laser pointer was placed in front of a jar containing $NBsO_2$. The laser pointer was turned on and when the green light penetrated and a straight light formed on the jar, it proved that there were NBs in the solution.

- **Pre-treatment**

In the pre-treatment process carried out is to check the health of the PLB. PLB that will be used is taken using a spatula and weighed as much as 100-120 mg and then put into a sterile thinwall. After that, 400 µl of pre-treatment media was added using a micropipette. Thinwall is then closed and closed using plastic wrap and placed on a shelf and then waited for 3 days.

- **Induction of NBs**

Healthy orchid PLB were then induced using growth media. P1 is the treatment with MS media, P2 is the treatment with MS media plus $NBsO_2$, and P3 is the treatment with $NBsO_2$. The first step is to remove the

pre-treatment media by vacuuming using a micropipette. After that, orchid PLB was added by each treatment media as much as 500 µl using a micropipette. Then the thinwall was closed and sealed using plastic wrap and placed back on the shelf. This induction is done every week. At this stage, the treatment was repeated in three replicates.

- **Crop Measurement**

Plant measurements were taken at the beginning and end of the study. Plant measurements were made using millimeter blocks, the Royal Horticultural Society (RHS) Color Chart and digital scales. In addition, the emergence of roots, shoots and embryos was also observed during the treatment.

Data analysis

This study consisted of 3 different media treatments namely MS (P1), MS with the addition of $NBsO_2$ (P2) and $NBsO_2$ (P3) then replicated 8 times so that there were 24 experimental units. The observation parameters consisted of PLB weight (g), PLB height (cm), number of live PLB (%), PLB color, root emergence, root length, shoot emergence, number of new shoots, embryo emergence, and number of embryos. So that data analysis is done in two ways, namely qualitative data analysis and quantitative data analysis. Qualitative data analysis is done by reading and processing the study result and then presented in the form of tables, figures and graphs. Meanwhile, quantitative data analysis was carried out by processing the observation data using normality test, homogeneity test, and multivariate test. If it is known that there is a real or very real difference, it will be continued with a univariate comparison test and further tests using the games-howell test and the bonferroni test.

Results

Presence of NBs in the solution was confirmed through a screening process. This involved utilizing a laser pointer, where the appearance of a straight line served as evidence of NBs, a phenomenon explained by the Tyndall effect. (Han et al., 2023). The screening results demonstrate the presence of a straight line in the bottle containing $NBsO_2$, confirming the existence of nanobubbles in the solution (Figure 1). Conversely, no straight line was observed in the bottle with sterile water, supporting the conclusion that the $NBsO_2$ used in this study contains nanobubbles.

Figure 1. Screening of NBs, Note: Jar Bottle = NBs, Sauce Bottle = Sterile Water

The results of the study were obtained at the beginning of 0 days after planting and the end of the study after 30 days after planting through observation of a Binocular Stereo type Motic microscope at 1x magnification with the following results (Figure 2-4).

Figure 2. PLB of *Dendrobium* sp. orchid with MS media treatment; (a) 0 days after planting, (b) 30 days after planting

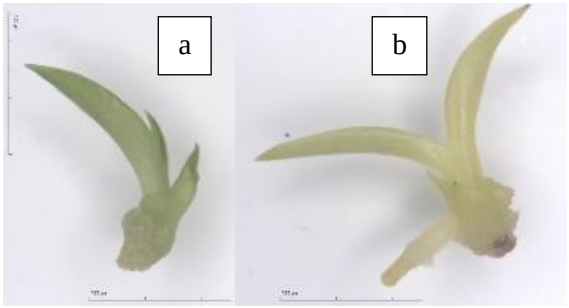

Figure 3. PLB of *Dendrobium* sp. orchid with MS media induced by $NBsO_2$; (a) 0 days after planting, (b) 30 days after planting

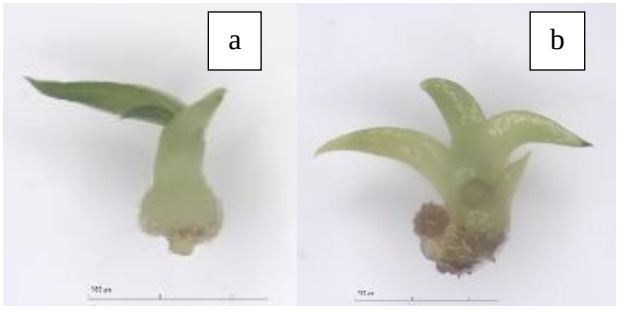

Figure 4. PLB of *Dendrobium* sp. orchid with $NBsO_2$ media; (a) 0 days after planting, (b) 30 days after planting

Orchid Embryos

NBs are bubbles less than 100 nm in size that have many advantages. The use of NBs for the growth of orchid PLBs has been done with the results listed below. Induction of NBs in Orchid PLB affects the faster emergence of new embryos. P3 has a fast embryo emergence time with a value of 13.88 compared to other treatments. The results of games howell test showed that in treatment P2 the average number of new embryos was high and not significantly different from treatment P1. However, both are significantly different from the P3 treatment (Figure 5).

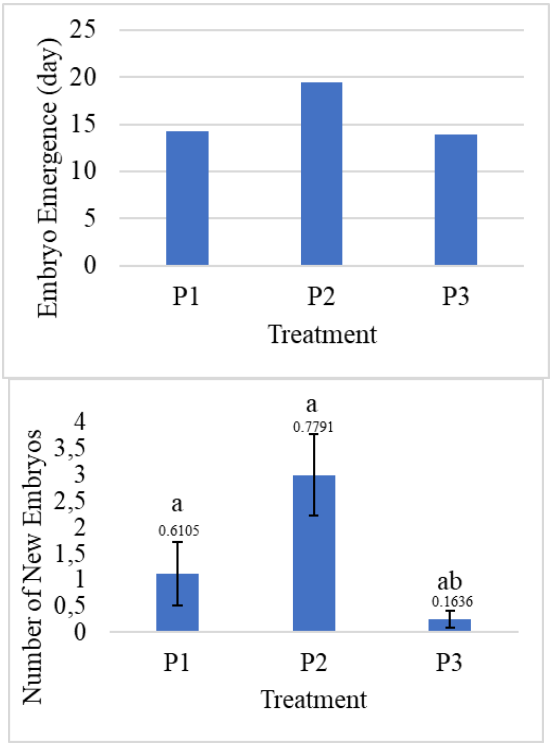

Figure 5. Embryo Emergence and Number of New Embryos *Dendrobium* sp. Orchid

New Shoots of PLB

P1 treatment has a faster bud emergence time compared to P2 and P3 with a value of 18.75. Based on Bonferroni test, the highest number of buds was found in treatment P1 with an average of 11.5 buds and no significant effect on the number of buds in P2 with an average of 11.38 buds. Both treatments were significantly different from the P3 treatment (Figure 6).

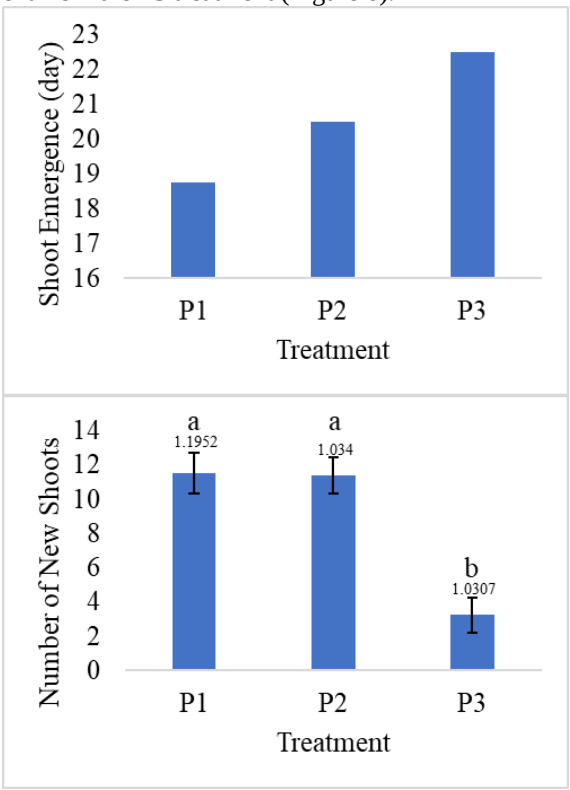

Figure 1. Time to Shoot Emergence and Number New Shoots of *Dendrobium* sp. Orchid

PLB color

NBs induction also contributed to the increase in PLB color to a greener color observed using the RHS Colour Chart. PLB induced by NBs (P3) which was originally *Strong Yellow Green* B: Green Group 143B changed to a greener color with Strong Yellow Green A: Green Group 143A (Figure 7). Induction of NBs in the media was also able to change the color which was originally Strong Yellow Green B: Green Group 143B changed to a greener color with Strong Yellow Green A: Green Group 143A (Figure 8). In contrast to PLB that was not induced by NBs (P3) which showed no change in color and was still *Strong Yellow Green B*: Green Group 143B (Figure 9).

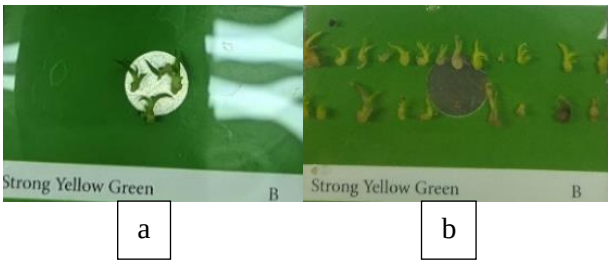

Figure 7. Color changes in PLB of *Dendrobium* sp. orchid with MS media treatment; (a) Before treatment, (b) After treatment

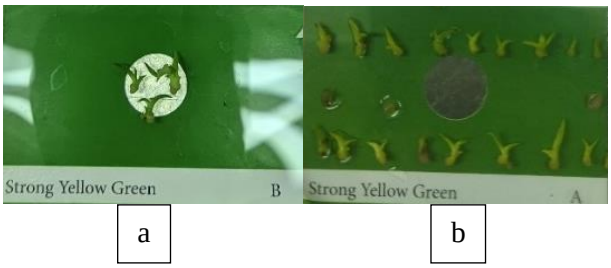

Figure 8. Color changes in PLB of *Dendrobium* sp. orchid with MS media induced by $NBsO_2$; (a) Before treatment, (b) After treatment

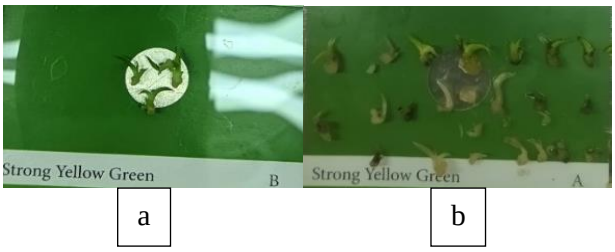

Figure 9. Color canger in PLB of *Dendrobium* sp. orchid with $NBsO_2$ media; (a) Before treatment, (b) After treatment

PLB roots

The P2 and P3 treatments showed faster root emergence time compared to the P1 treatment. Figure 4 shows that the induction of NBs in PLBs can accelerate the emergence of roots compared to PLBs that are not induced by NBs (P1). Howell's test showed that P1 and P2 were not significantly different on root length. However, both were significantly different from P3 (Figure 10). Based on the graph, NBs also play a role for induction on and have the same effect as media that are not induced with NBs.

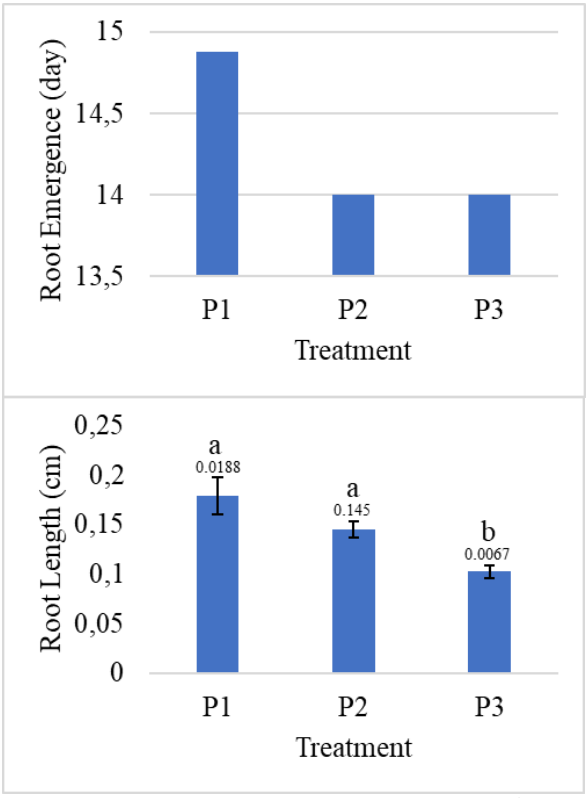

Figure 10. Root Emergence Time and Root Length PLB of *Dendrobium* sp. Orchid

Percentage alive

Bonferroni test results showed that in the P2 treatment the number of living protocorms reached 88% and was not significantly different from the P1 treatment at 83%. However, both were significantly different from P3 which had a live percentage of 20% (Figure 11).

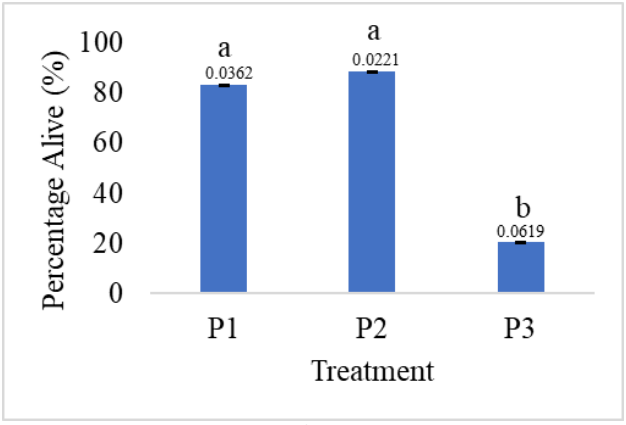

Figure 11. Percentage alive PLB of *Dendrobium* sp. Orchid

Discussion

The results of the multivariate analysis stated that there was an effect of treatment on the observed variables so that to determine the effect of treatment continued with the univariate test analysis. Analysis using the univariate test showed that the significance value in all variables was smaller than 0.05 except for the variables of plant weight and plant height. Based on this, there is a significant effect of treatment on the percentage of life, root length, number of new shoots, and number of new embryos.

$NBsO_2$ has a very small size and low buoyancy so that it can easily enter into plant cells including mitochondria. The oxygen absorbed by plants is efficiently utilized in the respiration process, which is vital for generating energy required for various plant functions. These functions include energy metabolism (e.g., carbon fixation in photosynthetic organisms), signal transduction (such as the mediation of plant hormone signals), and adaptation to environmental changes. The addition of NBsO2 enhances the respiration process, resulting in greater energy production and the acceleration of biological activities. As a result, the growth of orchid plants occurs at a faster rate (Figure 12). NBs have a very small size that easily penetrates plant tissue quickly and has low buoyancy. NBs have the ability to enhance the activity of the enzymes ent-kaurene synthase (KS) and ent-kaurene oxidase (KO), which play a crucial role in the biosynthesis of the growth hormone gibberellin within plastids. The enzyme ent-kaurene is first converted into 7-α-hydroxykaurenoic acid, which is subsequently processed into A12-aldehyde in the endoplasmic reticulum. This A12-aldehyde is then transformed into gibberellin (Wang et al., 2021). Gibberellin induces the synthesis of amylase and protease enzymes through de novo synthesis, enabling the breakdown of starch into glucose and thereby accelerating embryo development (Figure 13). The conversion of starch to glucose provides energy necessary for cell differentiation, resulting in the formation of the plumule, which initiates leaves and stems, and the radicle, which initiates root development. The use of NBs further accelerates plant and embryo growth by promoting the rapid emergence of plumules and radicles. Moreover, gibberellin stimulates the production of enzymes that soften cell walls, particularly proteolytic enzymes, which release tryptophan—a precursor of auxin. This increase in auxin levels further enhances plant growth (Igielski & Kępczyńska, 2017; Wulandari et al., 2014).

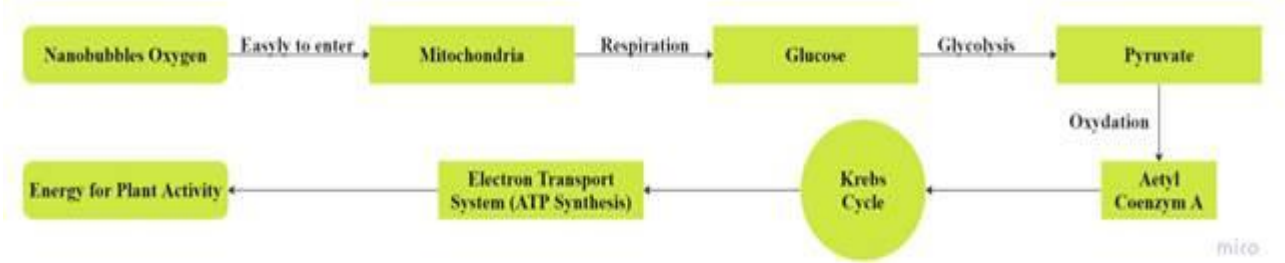

Figure 12. Mechanism of NBs on Plant Respiration

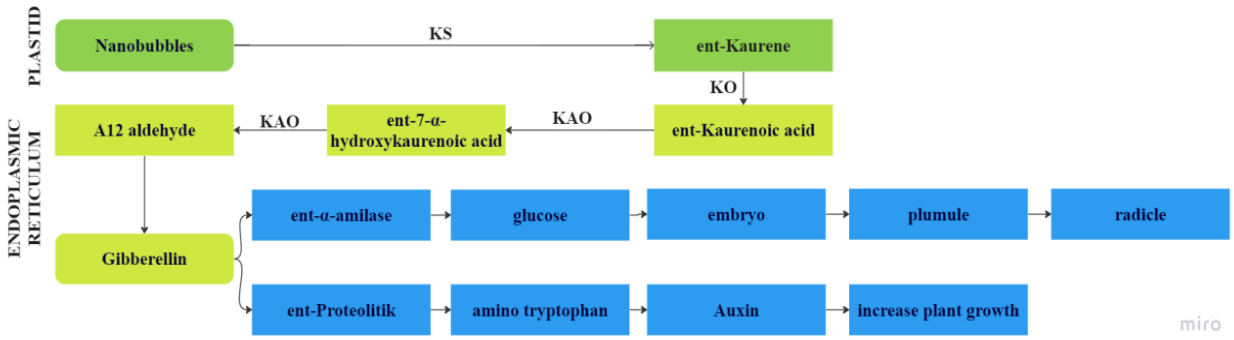

Figure 12. Mechanism of NBs on Gibberellin Synthesis and Growth Modified by Igielski & Kępczyńska, 2017 and Wulandari et al., 2014, Description: green color = occurs in plastid, yellow color = occurs in endoplasmic reticulum, blue color = effect of gibberelin

The color of the leaves serves as a marker of their quality; high-quality leaves are characterized by their green color due to their high chlorophyll content, which can increase photosynthetic efficiency. Orchid plants cannot survive in an overly wet environment, although they can acclimatize to humidity conditions. In thin liquid media, the planlet has no substrate to sit on, so nutrients are absorbed evenly throughout the planlet, rather than just through the plant's roots. If the amount of liquid medium absorbed throughout the plant increases, it is likely that the performance of the plant cell system will decrease. The higher water content outside the cell than inside the cell indicates that water can enter the cell with high intensity through the cell membrane, which may cause loss of cell content in the explant. The addition of O_2 NBs that have a very small size can store nutrients well and encourage optimal plant growth (Purnamasari et al., 2024).

NBs can provide a transport mechanism for gas delivery to membranes or cells and thus affect transmembrane proteins or membrane structureNanobubbles (NBs) function as carriers for gas transport to cellular membranes. The hydrophobic properties of gases drive their integration into the membrane, resulting in alterations to its curvature. These changes can affect the activity of transmembrane proteins or lead to more extensive modifications in the overall membrane structure (Rahayu et al 2023). Oxygen in the form of nanobubbles will capture substances in the liquid and float to the surface. Nanobubbles are able to penetrate small cavities so that they can wrap and make them lift up (Paradhiba et al., 2021). Based on research conducted by Puspa et al (2024), it is stated that the use of $NBSO_2$ has a higher effect on the root length of *Phalaenopsis* orchids than using leaf fertilizers. The use of $NBsN_2$ in the study also gave a better effect on the number of leaves, leaf length, wet weight of plants, and the number of roots.

The nutrient content contained in NBs helps facilitate the photosynthesis process by increasing the efficiency of nutrient utilization by plants. NBs have a relatively high stability in water, which is caused by the change in the size of gas bubbles from micro-scale to nano-scale through the process of gas diffusion from inside the bubble to the surrounding liquid. Then the bubble size will shrink to nano size. The presence of electrolyte ions around the bubble is able to reduce the surrounding gas pressure, so that the gas diffusion process from the bubble into the liquid becomes more restrained (Syafitri et al., 2024).

The use of $NBsO_2$ in orchid plants can accelerate plant growth. This is in line with research conducted by (Rahayu et al., 2023) which states that the use of NBs can accelerate the growth of *Dendrobium Imelda Marina masagung x Bumi Menangis* orchids by enlarging orchid stems, increasing plant height, growing new shoots and roots and can affect the color of leaves which become greener. The small size of NBs can cause the absorption of gas carried to be absorbed more quickly, and one of the effects is to induce hormones and genes. The use of NBs can be absorbed effectively and directly into the plant system by the roots and helps in increasing the availability of nutrients by plants (Puspa et al., 2024).

Syafitri et al (2024) in their research stated that the application of $NBsN_2$ had a significant effect on the growth of *Phalaenopsis* orchids at the acclimatization stage and had an effect on the addition of the number of leaves, number of roots, leaf length, root length, plant height, wet weight and dry weight of plants. NBs tend to be stable in water, this event is due to the change in the size of bubbles from micro to nano size by gas diffusion from inside the bubble to the liquid around the bubble, so that the size of the bubble shrinks to nano size. Electrolyte ions around the bubble will suppress the gas around the bubble so that the gas diffusion process from the bubble to the liquid will be restrained. NBs consumed by plants have a role in various metabolic reactions, namely maintaining plant anatomical functions. The application of NBs also has an influence on the growth of root length, number of roots and leaves, plant height, and wet and dry weight in Dendrobium sp. orchid plants (Istifadah et al., 2024).

Conclusion

PLB growth of *Dendrobium* sp. orchids induced by $NBsO_2$ experienced a rapid and significant growth rate compared to treatments that were not induced by $NBsO_2$. The use of $NBsO_2$ has an effect on the growth of *Dendrobium* sp. orchids by increasing the time of embryo emergence, the number of embryos, plant weight, plant

height, color, and percentage of life so as to increase the accelerated growth rate of PLB *Dendrobium* sp. orchids.

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References

Amalia, A. C., Mubarok, S., & Nuraini, A. 2022. Respons anggrek *Dendrobium* terhadap perbedaan naungan dan aplikasi zat pengatur tumbuh. *Kultivasi*, *21*(2):127–134.

Deswiniyanti, N. W., & Lestari, N. K. D. 2022. Pendampingan Petani Anggrek Dalam Mengembangkan Metode Kultur Jaringan dan Aklimatisasi Bibit Anggrek Di Desa Petiga Tabanan Bali. *Jurnal Widya Laksana*, *11*(2):252–260.

Erfa, L., Maulida, D., Sesanti, R. N., & Yuriansyah, Y. 2020. Keberhasilan Aklimatisasi dan Pembesaran Bibit Kompot Anggrek Bulan (*Phalaenopsis*) Pada Beberapa Kombinasi Media Tanam. *Jurnal Penelitian Pertanian Terapan*, *19*(2):122.

Garcia, S. M.V., Khan, M. S., Haidar, Z. S., & Acevedo Cox, J. P. 2023. NanoBubble-Mediated Oxygenation: Elucidating the Underlying Molecular Mechanisms in Hypoxia and Mitochondrial-Related Pathologies. *Nanomaterials*, *13*(23).

Hairuddin, R., Yamin, M., & Riadi, A. 2018. Respon Pertumbuhan Tanaman Anggrek (*Dendrobium* Sp.) Pada Beberapa Konsentrasi Air Cucian Ikan Bandeng Dan Air Cucian Beras Secara in Vivo. *Cokroaminoto Palopo University*. *6*(2):23–29.

Han, Z., Chen, H., He, C., Dodbiba, G., Otsuki, A., Wei, Y., & Fujita, T. 2023. Nanobubble size distribution measurement by interactive force apparatus under an electric field. *Scientific Reports*, *13*(1):1–12.

Istifadah, H. 2023. Respon Pertumbuhan Anggrek Dendrobium sp terhadap Pemberian Ekoenzim dan Nanobubbles (NBs) CO2. Jurnal Ilmiah Biosainstropis (Bioscience-Tropic). 10(1), 1–37.

Igielski, R., & Kępczyńska, E. 2017. Gene expression and metabolite profiling of gibberellin biosynthesis during induction of somatic embryogenesis in Medicago truncatula Gaertn. *PLoS ONE*, *12*(7):1–30.

Irsyadi, M. B. 2021. Factors That Effect of the Optimal Plantlet Growth from Tissue Culture on the Acclimatization Stage. *Proc. Internat. Conf. Sci. Engin*, *4*(2):100–104.

Jupri, A., Saadah, R., Sukiman, Sukenti, K., & Jannah, W. 2023. Preservation of Orchid Plants through Cultivation as Nature Tourism Objects in Mount Rinjani National Park, Lombok Island. *Jurnal Biologi Tropis*, *23*(1):402–411.

Oshita, S., Boerzhijin, S., Kameya, H., Yoshimura, M., & Sotome, I. 2023. Promotion Effects of Ultrafine Bubbles/Nanobubbles on Seed Germination. *Nanomaterials*. *13*(10):1-10.

Paradhiba, A. M., Febriyanti, F., Rahmadania, E., Yanisa, F., Adelina, U., & Mukti, R. C. 2021. Pemanfaatan Teknologi Nanobubble untuk Produksi Anguilla sp pada Era Society 5.0 Utilization of Nanobubble Technology for the Production of Anguilla sp in Era Society 5.0. 1(2):435–444.

Purnamasari, V., Rahayu, T., Jayanti, G. E., & Agisimanto, D. 2024. Pengaruh Jenis Media Tanam dan Penambahan Nanobubbles O 2 terhadap Pertumbuhan Planlet Anggrek *Dendrobium* burana Green × Ong Ang Ai Boon secara in Vitro The Effect of the Type of Planting Media and The Addition of O 2 Nanobubbles on the Growth of *Dendrobium* burana Green × Ong Ang Ai Boon Orchid Plantlets in Vitro. 25(1), 49–58.

Puspa, A., Rahayu, T., & Jayanti, G. E. 2024. Analysis of Nanobubbles (NBs) Technology and Foliar Fertilization on the Growth of Phalaenopsis sp. Orchid. *Berkala Sainstek*, *12*(2), 37.

Rahayu, T., Jayanti, G. E., & Hayati, A. 2023. Induksi Nanobubbles (NBs) untuk Pertumbuhan Anggrek *Dendrobium* Imelda Marina Masagung X Bumi Menangis. *Metamorfosa: Journal of Biological Sciences*, *10*(1), 118-126.

Syafitri, F. I., Rahayu, T., & Jayanti, G. E. 2024. Potential of Ecoenzymes and N2 Nanobubbles on the Growth of Phalaenopsis sp. Orchid at the Acclimatization Stage. *Jurnal ILMU DASAR*, 25(1), 41.

Wang, Y., Wang, S., Sun, J., Dai, H., Zhang, B., Xiang, W., Hu, Z., Li, P., Yang, J., & Zhang, W. 2021. Nanobubbles Promote Nutrient Utilization And Plant Growth In Rice By Upregulating Nutrient Uptake Genes And Stimulating Growth Hormone Production. Science of the Total Environment. 800 (54):149-163.

Wijerathna-Yapa, A., & Hiti-Bandaralage, J. 2023. Tissue Culture—A Sustainable Approach to Explore Plant Stresses. *Life*, *13*(3), 1–16.

Wulandari, D. C., Rahayu, Y. S., & Ratnasari, E. 2014. Pengaruh Pemberian Hormon Giberelin terhadap Pembentukan Buah secara Partenokarpi pada Tanaman Mentimun Varietas Mercy. *LenteraBio*, *3*(1):27–32.

Zhou, Y., Cao, D., & Zhang, X. 2022. Degradation Mechanism of Micro-Nanobubble Technology for Organic Pollutants in Aqueous Solutions. *Nanomaterials*, *12*(15):10-18