BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 13, Number 2, October 2024 | Pages: 459-465 | DOI: 10.14421/biomedich.2024.132.459-465 ISSN 2540-9328 (online)

The New Technology for In Vitro Culture with Induction of Nanobubbles ($NBsN_2$ and $NBsO_2$) in *Cattleya* sp.

Moch. Mustakim, Azis Mawardi, Lina Purnama Dewi, Nela Vede Rhofa Putri, Tintrim Rahayu, Gatra Ervi Jayanti*
Biology Department, Faculty of Mathematics and Natural Science, Universitas Islam Malang,
Jl. MT. Haryono No 193 Malang 65141, Tel. 0341 551932, Fax. 0341552249, Indonesia.

Corresponding author*
gatra.ervi@unisma.ac.id

Manuscript received: 12 June, 2024. Revision accepted: 30 September, 2024. Published: 22 October, 2024.

Abstract

The growth and development of orchids take a relatively long time, while the demand for high-quality orchids continues to increase significantly. Tissue culture is a technique of isolating plant parts in organs, tissues, and cells and then culturing these plants on artificial media in a sterile environment. Tissue culture techniques can reproduce plants in a relatively short time, with the same properties and quality, so efforts to optimize tissue culture with technology are the right solution, one of which is using nanogenerator technology that produces Nanobubbles (NBs). NBs are one of the nanotechnologies that are ≤100 nm in size with induced gas needed by plants. This study aims to determine the effectiveness of growth and development from the induction of $NBsN_2$ and $NBsO_2$ in *Cattleya* sp. planlet in vitro culture. This research method uses in vitro culture with a Randomized Group Design. The results of the MsO treatment showed higher weight than other treatments, with a total plant weight value of 0.3550 g and a total plant height value of 1.3983 cm. Murashige-Skoog + NBsNitrogen (MsN) treatment showed higher root length than other treatments with a total value of 1.2367 cm. In the treatment of Ms, MsO, OJ and NJ, a live percentage of 83-100% was observed. The statistical test results indicate that NBs positively affect *Cattleya* sp. plantlets. This effect includes increasing plant weight, height, and root length and promoting new shoots growth. Additionally, nitrogen supplements play a role in colour changes, indicating the plant's health and photosynthetic efficiency. In the treatment with Induction O_2, the initial colour changes from Strong Yellow Green (141 D) to Strong Yellowish Green (141 C), while in the treatment with Induction $NBsN_2$, the initial colour changes from Strong Yellow Green (141 D) to Deep Yellow Green (141 B).

Keywords: Nanobubbles; $NBsO_2$; $NBsN_2$; *Cattleya* sp.; Tissue Culture.

Abbreviations: NBs: Nanobubbles; O_2: Oxygen; N_2: Nitrogen; LAF: Laminar Air Flow; ATS: Air Treatment Sanitizer; PHC: Plant Healthy Check, Media with a thinwall container (Ms: Murashige-Skoog, MsO: Murashige-Skoog + nanobubbles Oxygen, MsN: Murashige-Skoog + nanobubbles nitrogen, O: Nanobubbles Oxygen, N: Nanobubbles nitrogen); Media with a jar bottle container (MsJ: Murashige-Skoog, MsOJ: Murashige-Skoog + nanobubbles Oxygen, MsNJ: Murashige-Skoog + nanobubbles nitrogen, OJ: Nanobubbles Oxygen, NJ: Nanobubbles nitrogen).

INTRODUCTION

The development of science and technology in all fields is a new challenge in developing a better system. Nanobubbles (NBs) are one of the latest technologies in the world of biology they are focused on improving their quality and quantity (Haryanto *et al.*, 2022). NBs are filled with gases needed by plants, such as Oxygen (O_2), Carbon dioxide (CO_2), Nitrogen (N_2) and others. NBs are gas cavities with a radius of approximately tens to hundreds of nanometers (nm) (Rahayu *et al.*, 2023). With a tiny size, plants can easily and quickly absorb a solution, nanobubbles can significantly increase plant height and root length in plants. NBs treatment stimulates the synthesis of the growth hormone gibberellin and increases the regulation of plant nutrient absorption genes, thereby increasing the absorption and utilization of nutrients by the roots (Wang *et al*., 2021). proven in the research of Ahmed *et al*. (2018), the use of NBs can accelerate seed germination time and seed growth; Rahayu (2023) also proves that the efficiency of using NBs in accelerating orchid growth is better with greater stem diameter growth than not using NBs. In addition, orchids have a relatively long growth period with market consumer demand that tends to increase. Orchid data globally reaches 25,000-30,000 species, while orchids in Indonesia consist of ± 5,000 species (Nisa *et al*., 2021). Orchid flowers have durability or freshness over a relatively long period and are a factor in the high selling value of orchids, one of the most popular orchid species is *Cattleya* sp. which is nicknamed the "Queen of Orchids" because it has large flowers (Yasmin, 2018). In a relatively long and complex production process, orchid breeding needs to consider several factors including air, water, light, food (nutrients) Growth Regulators are in a balanced state (Firmansyah et al., 2023). According to Latifah (2017), the demand for high-quality orchids in Indonesia continues to increase yearly. However, the volume of imports still surpasses the volume of exports. To meet this demand, one practical approach is to utilize tissue culture for mass orchid propagation, as it allows for faster and more manageable growth, thereby addressing the market needs.

Tissue culture is defined as isolating plant parts (organs, tissues, cells or protoplasm) and then growing them on artificial media under sterile and controlled environmental conditions. The benefits of this system are that it can be used for plant propagation in a relatively short time, with the same properties and quality (Yachya *et al*., 2022). This technique has several advantages over traditional methods, because in addition to producing large numbers of plants quickly, this technique is also independent of the season (Putriana *et al*., 2019). NBs are one of the solutions in optimizing this technique, using N_2 and O_2 which can significantly effect plant growth and development as a fulfilment of nutrients, can also maintain market demand for quality orchids with the appropriate quantity.

This research aims to determine the growth effectiveness of the inducing of $NBsN_2$ and $NBsO_2$ on *Cattleya* sp. planlets in *vitro* culture. Therefore, optimization in the world of tissue culture needs to be continuously developed by accelerating plant growth and development effectiveness.

MATERIALS AND METHODS

Study area

This research was conducted at the Orchidology Laboratory and Nursery in Universitas Islam Malang, located at Jl. MT Haryono No. 193 Dinoyo Malang City. The research method uses *in vitro* culture applying a Randomized Group Design with $NBsO_2$ and $NBsO_2$ in liquid media tested as an addition to the media on the effect of growth and development on orchids *Cattleya* sp. In this study, there were two groups with five treatments each and repeated six times so that there were 72 experimental units. Thinwall group, there are media; Murashige-Skoog (Ms), Ms + $NBsO_2$ (MsO), Ms + $NBsN_2$ (MsN), $NBsO_2$ (O), $NBsN_2$ (MsN), while the Jar group, there are media; Murashige-Skoog (MsJ), Ms + $NBsO_2$ (MsOJ), Ms + $NBsN_2$ (MsNJ), $NBsO_2$ (OJ), $NBsN_2$ (NJ). Observation parameters consisted of plants weight (g), number of live plant (%), length of leaves and roots of plant (cm), plant colour, and contamination.

The tools used in this study were Laminar Air Flow (LAF), Air Treatment Sanitizer (ATS), "Nanogenerator (*Yixing Holly Technology Co*.., Ltd, China), large tweezers and small tweezers, sterile blade, scissors, *thinwall*, *beaker*, measuring cup, petri dish, pH meter (*Multifunction)*, micropipette, sterile tip, bunsen, lighter, magnetic stirrer (*Thermo Scientific*), analytical balance (Ohaus), alcohol sprayer, sterile wipes, tissues, markers, large plastic wrap and small plastic wrap, stove, plastic, and pipettes, Autoclave, Millimeter block, magnifying glass, *Green Pointer* and *Royal Horticultural Society (*RHS) *Color Chart*. Meanwhile, the materials used in this research are 7-month-old *Cattleya* sp orchids, NBsO solution$_2$, NBsN solution$_2$ 70% alcohol, 96% alcohol, sterile water, stock solution A: NH_4NO_3 (SmartLab A-2131), B: KNO_3 (SmartLab A-2131), C: $CaCl_2.H_2O$ (SmartLab A-2114), D: $MgSO_4._7H_2O$ (SmartLab A-2024) and KH_2PO_4 (SmartLab A-2024), E: $FeSO_4.\ _7H_2$ $O.Na_2$ $EDTA._2H_2O$ (SmartLab A-2059), F: H_3O_3 (SmartLab A-2013), $MnSO_4._4H_2O$ (SmartLab A-2100), and $ZnSO_4.\ _7H_2O$ (SmartLab A-2100), and G: Cl (SmartLab A-2038), Na_2 $MoO_4._2H_2O$ (Himedia PCT0117), $CuSO_4.\ _5H_2$ O (Himedia PCT0104), and $CoCl_2._6H_2$ O (Himedia PCT0117), vitamin: *nicotinic-acid* (Phygenera), *pyridoxine-HCl*, *Thiamine-HCl* (Caisson Lab), and glycine (Caisson G007), Methionine (Caisson Lab), Peptone (Himedia PCT0806), *Myo-oinositol* (Phygenea AD102), *Naphthaleneacetic acid (*NAA), *-Benzylaminopurine (*BAP), sugar, and distilled water.

Procedure

The stage begins with making Plant Healthy Check (PHC) media by preparing a 30% sugar solution by dissolving 300 mg of granulated sugar in 700 ml of distilled water and homogenizing it with a hot plate and magnetic stirrer; The resulting solution is then stored in bottles for the subsequent sterilization process. In this study, the media used were Ms stock solution as well as NAA and BAP solutions, measured with a micropipette and homogenized in a jar using a magnetic stirrer at speed 250 rpm. Once homogeneous, the media was cooked on the stove until completely dissolved, followed by pH measurements until it reached the pH value of 5.4. The aim of the stage of providing PHC media is to check the health of *Cattleya* sp. planlets. by placing it in a sterile container and jar, 400 µl of PHC medium was added. The thinwalls containers and jar bottles were then covered tightly with plastic wrap, the tops were placed in a shaker, and the thinwalls were placed on an inclined surface for three days. Sterilize tools and materials using an autoclave at 121ºC and 2 Psi for 15 minutes. Then sterilization of the nanogenerator was achieved with a H_2O_2 3% solution and sterile distilled water, the solution was fed into the nanogenerator through the machine's output and input channels, followed by the same treatment with sterile distilled water. $NBsN_2$ and $NBsO_2$ were made by inducing sterile air with N_2 and $NBsO_2$ gas using a nanogenerator machine for 15 minutes. NBs filtering is done with a Green Pointer to confirm the presence of NBs through the waves created by the green light. NB induction involves treating healthy orchid plantlets with media induced with $NBsN_2$ and $NBsO_2$, the initial step is to remove the PHC media, then add 400-600 µl of media into the plantlets using a micropipette. The thinwalls and the jar are covered with plastic wrap, the jar is placed on a shaker, and the thinwalls are placed at an angle. Providing media to planlet was done by placing orchid plantlets in a jar to be transferred to a shaker, while plantlets located on thin sloping walls received media daily for 5 minutes at 10 am and 2 pm. Final plantlet measurements included initial and final assessments of weight, plant height, root length, colour and contamination, which were carried out at LAF. Weight was measured using digital scales, root and leaf dimensions were measured using millimetre blocks and petri dishes, and the colour of orchid planlet was assessed using RHS paper.

Data analysis

The data in this study were analyzed through two approaches, namely quantitative analysis and qualitative analysis. Quantitative analysis involved processing observational data using the *Multivariate Analysis of Variance* (MANOVA) Method, followed by Bonferroni testing if there were significant differences. Meanwhile, qualitative analysis involved reading and analyzing the research results, which were then presented through tables, figures, and graphs.

RESULTS AND DISCUSSION

Screening Nanobubbles (NBs)

The screening results show that the bottle containing $NBsO_2$ has a straight line on the bottle containing $NBsO_2$ which indicates the presence of nano bubbles in the solution (Figure 1). In contrast to sterile water, there is no straight line on the sauce bottle, so it can be concluded that the $NBsO_2$ used in the study contains nanobubbles.

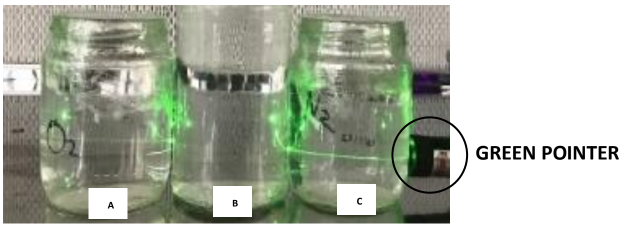

Figure 1. Screening of $NBsN_2$ and $NBsO_2$ using Green Pointer: (A) $NBsO_2$ (B) Aquades (C) $NBsN_2$

Plant weight and height of orchid *Cattleya* sp.

Induction of NBs Oxygen showed indicators of changes in the weight and height of orchids *Cattleya* sp. MsO treatment showed a higher average weight than other treatments with a total plant weight value of 0.3550 g and a total plant height value of 1.3983 cm. Bonferroni test showed no significant difference in plant weight in each treatment, while in plant height there was a significant difference in the MsO treatment against OJ, NJ, and Ms (Figure 2).

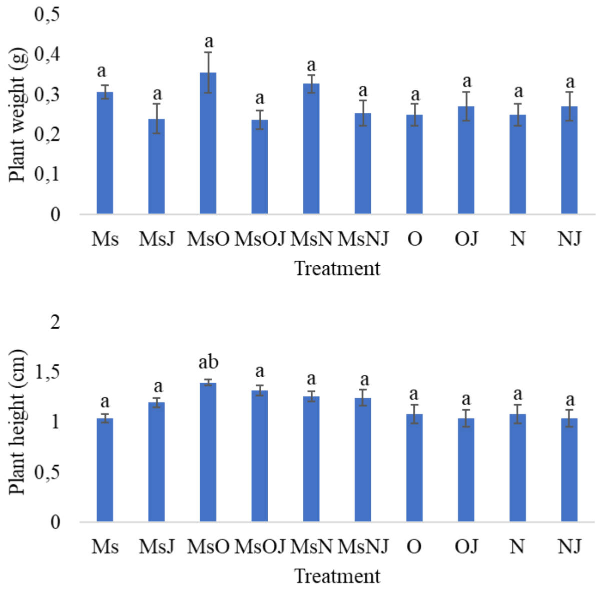

Figure 2. Average weight and height of *Cattleya* sp. planlets in the treatment groups of thinwall containers and Jar bottles

Root length analysis of orchid *Cattleya* sp.

$NBsN_2$ induction affects the root length of *Cattleya* sp. orchids as evidenced by the results of MsN treatment showing a higher average root length than other treatments with a total value of 1.2367 cm. However, the results of the Bonferroni test of root length parameters show that each treatment is not significantly different (Figure 3).

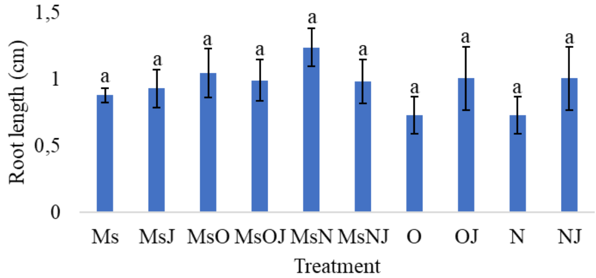

Figure 3. Average root length of *Cattleya* sp. planlets in *thinwall* container and jar bottle treatments.

Percentage alive and number of new shoots

The Ms, MsO, OJ and NJ treatments showed the highest average percentage of live planlets with a total value of 100%. However, the graph did not show any significant difference in the average percentage value between 83.33-100%, contamination of the planlets occurred in MsO 6th replication day 26. The test results also showed that the MsN treatment (Figure 4) showed a higher average number of new shoots than the other treatments with a total value of 3.67 cm.

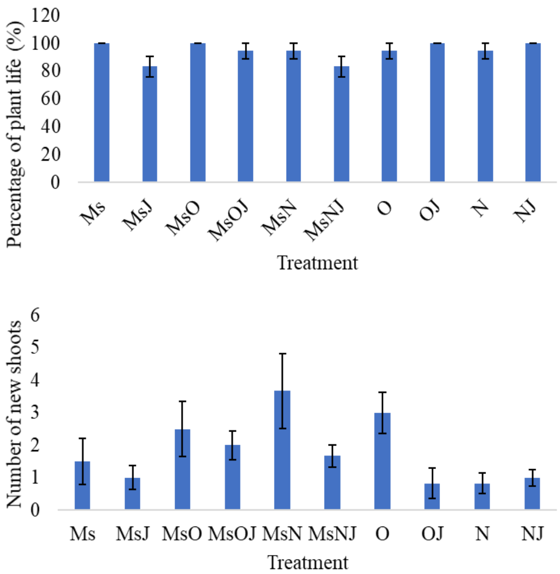

Figure 4. Average percentage of survival and number of new shoots of *Cattleya* sp. planlets in the treatment of thinwall container groups and jar bottles.

Plant color at RHS

NBs induction affect on plant colour based on observations using RHS, NBs have a role in increasing the color of greener plants. $NBsO_2$ induction was able to increase the initial color of *Strong Yellow Green* (141 D) to *Strong Yellowish Green* (141 C), $NBsN_2$ induction experienced colour changes from the initial Strong Yellow *Green (141* D) to *Deep Yellow Green (141* B), while the treatment without NBs induction did not experience colour changes in *Strong Yellow Green* (141 D) (Figure 5).

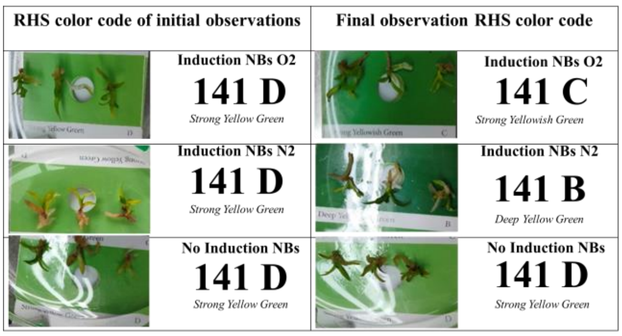

Figure 5. Changes in color of planlets treated without NBs induction and NBs induction using RHS parameters.

Discussion

Nanotechnology with NBs products is one of the latest technologies applied to plants as a breakthrough in improving the quality of plants, one of which is the orchid itself. NBs have a rapid response that can affect the size of the orchid diameter because NBs can provide a transportation mechanism for gas delivery to membranes or cells thereby affecting trans membrane proteins or membrane structure, hydrophobic gases will push into the membrane and thus will change the curvature of membrane which will have an effect on trans membrane proteins or global effects on membrane structure (Rahayu *et al.*, 2023). Screening of NBs was performed to confirm the presence of NBs in solution. Screening is done using a laser pointer and if there is a straight line it indicates the presence of NBs caused by the Tyndall effect (Han *et al*., 2023). The observation results show in Figure 1 that there is a *Tyndall* effect on the induction of NBs in both O_2 and N_2 with a straight line on the laser beam from the *green pointer* while in Figure 1 B, which is the treatment without induction of NBs (Aquades) there is no straight line of the *green pointer* beam.

The test results show that $NBsO_2$ influences the weight and height of *Cattleya* sp. orchid plants. According to Rahayu *et al.*, (2023) stated that the diameter of the stem of the largest orchid plant in the $NBsO_2$ treatment, therefore the additional weight is caused by the application of $NBsO_2$ in orchid tissue culture. The use of O_2 gas induced through the nanogenerator functions in absorbing nutrients that are useful for the formation of new tissues and plants getting bigger (Krisna, 2017). $NBsO_2$ has a tiny bubble size and low buoyancy so it can quickly enter plant cells including mitochondria. Oxygen that enters will be easily absorbed by plants and used for the respiration process. The process of respiration in plants will produce energy for various activities such as energy metabolism (carbon fixation in photosynthesis organisms), signal transduction (transduction of plant hormone signals), and environmental adaptation. $NBsO_2$ can increase the respiration process to produce much greater energy and biological processes in plants take place quickly which causes faster growth of orchid plants (Figure 6) so that plants have good and fast energy transfer in the growth process and make plants have a faster weight and height from the use of $NBsO_2$ media.

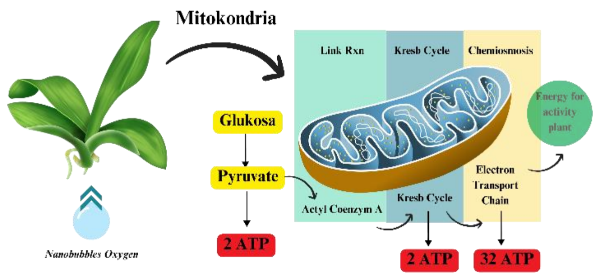

Figure 6. NBsO2 mechanism in *Cattleya* sp. planlets.

Based on the research of Wang *et al*. (2021) showed that the effect of NBs on plant growth and nutrient absorption was evaluated in the laboratory. Laboratory experiments showed that NBs significantly increased plant height and root length in rice seedlings, with NBs treatment stimulating the synthesis of growth hormone gibberellin and upregulating plant nutrient absorption genes, namely *OsBT, PiT-1 and SKOR*, resulting in increased absorption and utilization of nutrients by roots.

The highest increase in root length was shown in the treatment with $NBsN_2$ induction, with an average value of 1.2367 cm. Roots are a vital in the plant's response to water shortage. One of the essential that must be evaluated is root morphology, because roots can absorb water by maximizing the root system. Plants with large roots will be able to absorb more water than plants with smaller roots (Ai & Torey., 2013). Increased absorption and utilization of nutrients by plant roots. The number of roots in the use of tissue culture techniques indicates that orchid plants are able to absorb nutrients in the media well. A larger number of roots also indicates that the reach of the plant is wider and the plant absorbs more nutrients and nutrients, so that the spread of nutrients from the media to the plant can be done smoothly (Rahma, et al., 2018).

The use of $NBsN_2$ has great power over the growth and development of orchids, the results show that $NBsN_2$ has the potential to maintain the consistency of orchids in the percentage of life and the number of new shoots that are more than non-$NBsN_2$. According to Patti et al. (2013), N_2 functions as a synthetic material for chlorophyll, proteins, and amino acids. N_2 in the vegetative phase functions to increases vegetative growth, which includes increasing the number of tillers, grains per hill, and plant size. The growth regulators auxin and cytokinin in the media influence the emergence of buds. The primary role of auxin is to stimulate roots in stem and leaf cuttings and increase root branches, the formation of roots begins with the metabolism of nutrient reserves in the form of carbohydrates which produce energy which in turn encourages cell division and forms new cells in the tissue (Cytokinin functions to accelerate cell division resulting in cell enlargement, while auxin spurs cell elongation which will then differentiate and elongate plant tissue) (Kusuma *et al*., 2023). NBs can stimulate the enzymes ent-Kaurene *synthase* (KS) and ent-kaurene *oxidase* (KO) as the synthesis of growth the hormone gibberellin that occurs in plastids. The enzyme kaurene is converted to 7-alpha-hydroxykaurenoic *acid and* then converted to A12 *Aldehyde* in the endoplasmic reticulum. A12 aldehyde is gibberellin (Wang *et al*., 2021). Gibberellin stimulates the formation of amylase and protease enzymes through de-nevo synthesis which plays a role in the breakdown of amylum into glucose thereby accelerating embryo emergence. Breaking down amylum into glucose produces energy that is used for the cell differentiation process so that a plumula is formed, which is the beginning of leaves and stems and a radicle which is the beginning of roots. In addition, gibberellin will spur the formation of enzymes that soften the cell wall, especially proteolytic enzymes that release amino tryptophan (auxin precursor/former) so that auxin levels increase. (Igielski & Kępczyńska, 2017; Wulandari *et al*., 2014). Based on the research results, the pattern/mechanism of NBs in increasing Orchid growth was obtained (Figure 7).

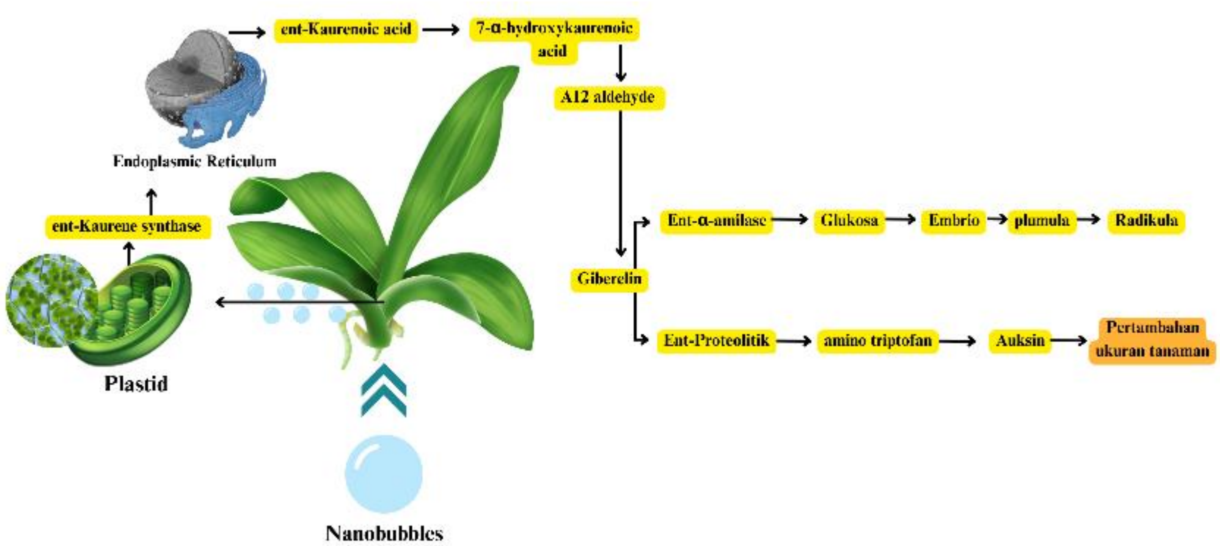

Figure 7. Mechanism of NBs in enhancing Orchid growth.

On the other hand, the role of $NBsN_2$ in research is visually proven to improve the quality of planlets. N_2 is a synthetic material for chlorophyll, proteins, and amino acids (Patti *et al*., 2013). The increase in the RHS code of leaf color shows the effect on absorption, which is more easily absorbed due to the induced solution of nano-sized N_2, which is able to play its role in chlorophyll synthesis.

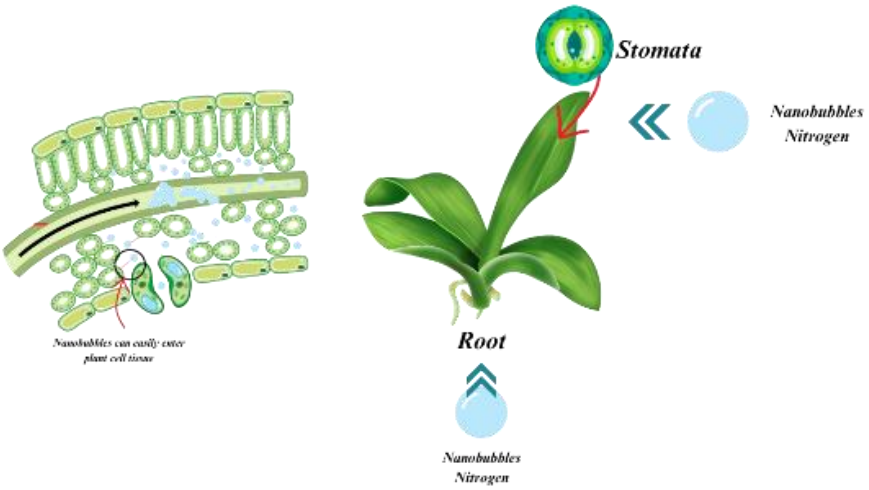

Figure 8. Mechanism of entry of $NBsN_2$ into *Cattleya* sp. planlets.

The increase in the RHS code of leaf color shows the effect on easier absorption due to the nano-sized N_2 induced solution that can play its role in chlorophyll synthesis (Figure 8). In the homogeneity test on plant weight, plant height, and root length to determine further tests, the results obtained a significance value of more than 0.05 (homogeneous data), which means the sig. >0.05, it can be concluded that the data variance is homogeneous, so the further test used is Bonferroni. At the further test stage using the Bonferroni test with multivariate tests, there are four statistical tests, namely *Pillai's Trace, Wilk's Lambda, Hotelling Trace,* and *Roy's Largest Root*. These four tests are based on eigenvalues. The multivariate test results showed Sig. <0.05, which stated that there was an effect of treatment on the observed variables. The analysis was continued with the univariate test to determine the effect of treatment. Analysis using the univariate test showed a significance value <0.05, indicating a significant effect of treatment on plant weight, plant height, and root length. The results of the statistical test state that NBs technology affects the induction of *Cattleya* sp. planlets by increasing plant weight, plant height, root length, giving rise to new shoots, and improving the quality of colour in plants.

CONCLUSIONS

Based on the results of the research that has been done, it can be concluded that the induction of the combination of Ms and $NBsO_2$ on *Cattleya* sp. planlet provides acceleration of weight and height, while the combination of Ms and $NBsN_2$ has an effect on root length, and for the highest presentation of life in the treatment of Ms, MsO, O, and N. The results of the statistical test state that NBs technology influences the induction of *Cattleya* sp. planlets by increasing plant weight, plant height, root length, giving rise to new shoots, and improving the quality of colour in plants.

Acknowledgements: Thanks to The Ministry of Education, Culture, Research, and Technology (Indonesian: Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi, abbreviated Kemendikbudristek) and Universitas Islam Malang and thanks also to the Orchidology Laboratory and Nursery in Universitas Islam Malang.

Authors' Contributions: Author one prepares the manuscript and designs the research (manuscript), author two analyzes the data, and authors three and four conduct the experiments, while the last author and associate professor directs the research and experimental design.

Competing Interests: The Authors declare that there are no conflicts of interest.

Funding: Thanks to the Ministry of Education, Culture, Research and Technology (Indonesian: Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi, abbreviated Kemendikbudristek) and Universitas Islam Malang for providing grants / research grants in PKM Riset Eksakta activities in 2023 with the title "The latest technology for In vitro Culture with Induction of Nanobubbles (NBs) N_2 and O_2 on *Cattleya* sp. Planlets".

REFERENCES

Ahmed, A.K.A., Shi, X., Hua, L., Manzueta, L., Qing, W., Marhaba, T., & Zhang, W. (2018). Influences of Air, Oxygen, Nitrogen, and Carbon Dioxide Nanobubbles on Seed Germination and Plant Growth. *Journal of Agricultural and Food Chemistry, 66* (20), 5117-5124. Doi: https://doi.org/10.1021/acs.jafc.8b00333

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. Doi: https://doi.org/10.3390/nano12020237

Krisna, B., Putra, E.T.S., Rogomulyo, R., & Kastono, D. (2017). Effect of oxygen enrichment on root growth and yield of curly lettuce (*Lactuca sativa* L.) in floating raft hydroponics. *Journal of Vegetalika*, *6*(4),14-17. Doi: https://doi.org/10.22146/VEG.30900

Kusuma, R., Samsurianto, S., Azizah, W., & Yuliatin, E. (2023). Growth Response of *Protocorm Like Bodies* (PLB) of Sugarcane Orchid to Modification of *Murashige and Skoog* (MS) Media in *vitro*. *Paspalum: Scientific Journal of Agriculture*, *11*(1), 42-50. Doi: https://doi.org/10.35138/paspalum.v11i1.538

Latifah, R., Suhermiatin, T., & Ermawati, N. (2017). Optimization of *Cattleya* Plantlet Growth Through a Combination of *Murashige-Skoog* Media Strength and Organic Materials. *Agriprima: Journal of Applied Agricultural Sciences*, *1*(1), 59-62. Doi: https://doi.org/10.25047/agriprima.v1i1.20

Nisa, N.A., Rahayu, T., Jayanti, G.E. (2021). The Utility of BAP and Coconut Water in VW Medium on the Organogenesis of *Dendrobium* sp. *Metamorfosa: Journal of Biological Sciences*, *8*(2), 298-303. Doi: https://doi.org/10.24843/metamorfosa.2021.v08.i02.p14

Patti, P.S., Kaya, E., & Silahooy, C. (2013). Analysis of soil nitrogen status in relation to N uptake by paddy rice plants in Waimital village, Kairatu District, West Seram Regency. *Journal of Agrologia*, *2*(1), 51-58. Doi: https://dx.doi.org/10.30598/a.v2i1.278

Rahayu, T., Jayanti, G. E., & Hayati, A. (2023). Induction of Nanobubbles (NBs) for growth of *Dendrobium Imelda Marina Masagung x Bumi Menangis* orchids. *Journal of Metamorphosa*, *10*(1), 126-132. Doi: https://doi.org/10.24843/metamorfosa.2023.v10.i01.p13

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*(1), 1-9. Doi: https://doi.org/10.1016/j.scitotenv.2021.149627

Wulandari, D. C., Rahayu, Y. S., & Ratnasari, E. (2014). Effect of Gibberellin Hormone on Parthenocarpy Fruit Formation in Cucumber Plants of Mercy Variety. *LenteraBio*, *3*(1), 27-32. Doi:

Yachya, A., Sopandi, T., Slamet, P., Binawati, D.K., Ngadiani., Sukarjati., Ajiningrum, P.S., Andriani, V. (2022). Plants in East Java SMS Biology Mgmp Teachers. *Journal of Penamas Adi Buana*, *6*(1), 31-36. Doi: https://doi.org/10.36456/penamas.vol6.no01.a5498

Yasmin, Z. F. (2018). Nursery (tissue culture to enlargement) of *Phalaenopsis* Orchid at Hasanudin Orchids, East Java Nursery. Agrohorti Bulletin Journal, *6*(3), 430-439. Doi: https://doi.org/10.29244/agrob.v6i3.21113

Firmansyah, M. I., Rahayu, & Jayati, G. E., Agisimanto, D. (2023). Comparative Study of *Naphtalene Acetic Acid* (NAA) Concentration Variations on the Growth of *Dendrobium* sp. Orchid on White and Black Moss Media. *Journal of Metamorphosa*, *10*(1), 292-301. Doi: https://doi.org/10.24843/metamorfosa.2023.v10.i02.p13

Ai, N.S., & Torey, P. (2013). Root morphological characters as water-deficit indicators in plants. *Journal of Bioslogos*, *3(1)*, 32-39. Doi: https://doi.org/10.35799/jbl.3.1.2013.3466

Rahma, S., Rahayu, T., & Hayati, A. (2018). Study of Organic Material Addition in VW Planting Media on Organogenesis of Dendrobium Orchid in Vitro. *E-Journal of Known Nature 1(1)*, 93 - 103. Doi: https://doi.org/10.33474/j.sa.v1i1.1392