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THE ROLE OF AgNPs AND AB Mix IN THE GROWTH OF *Aglaonema* 'LADY VALENTINE' IN VITRO & EX VITRO

Peran AgNPs dan AB Mix dalam Pertumbuhan *Aglaonema* 'Lady Valentine' In Vitro & Ex Vitro

Damasa Ines Larrisa, Pangesti Nugrahani*, Sri Wiyatiningsih
Universitas Pembangunan Nasional "Veteran" Jawa Timur
*Email: pangesti_n@upnjatim.ac.id

ABSTRACT
Aglaonema 'Lady Valentine' is a popular ornamental plant with significant economic value. Conventional propagation methods have limitations, making tissue culture and acclimatization viable alternatives. However, low shoot multiplication and slow acclimatization remain major challenges. This study investigated the effects of silver nanoparticles (AgNPs) and AB Mix nutrient concentrations on in vitro and ex vitro plantlet growth using a two-factorial completely randomized design with four levels and three replications. The results showed varied growth responses, with the best performance observed in the 2 ppm AgNPs and 3 ml/L AB Mix treatment, which increased plantlet height, volume, and visual health. In ex vitro conditions, both 2 ppm and 3 ppm AgNPs combined with 3 ml/L AB Mix supported better plantlet adaptation, as seen in increased height, number of leaves and shoots, and seedling viability. Overall, the combination of 2 ppm AgNPs and 3 ml/L AB Mix proved most effective for enhancing multiplication and acclimatization.

Keywords: *Acclimatization, AB Mix, Aglaonema, AgNPs, Tissue culture*

ABSTRAK
Aglaonema 'Lady Valentine' merupakan tanaman hias yang tengah digemari masyarakat dan memiliki nilai ekonomi tinggi. Perbanyakan secara konvensional masih menemui berbagai kendala, sehingga diperlukan metode alternatif seperti kultur jaringan dan aklimatisasi. Namun, rendahnya multiplikasi tunas dan pertumbuhan lambat pada tahap aklimatisasi masih menjadi kendala dalam metode ini. Penelitian ini bertujuan menetahui pengaruh kombinasi konsentrasi nanopartikel perak (AgNPs) dan nutrisi AB Mix terhadap pertumbuhan plantlet secara in vitro dan ex vitro. Metode yang digunakan yaitu menggunakan RAL faktorial dua faktor (AgNPs dan AB Mix) dengan empat taraf dan tiga ulangan. Pemberian AgNPs dan AB Mix menunjukkan variasi respons pertumbuhan pada Aglonema var. Lady Valentine. Hasil penelitian pada tahap in vitro menunjukkan kombinasi konsentrasi 2 ppm AgNPs dan 3 ml/L AB Mix menghasilkan pertumbuhan terbaik, ditandai dengan peningkatan tinggi, volume, dan kondisi kultur planlet secara visual dan kuantitatif. Sama halnya dengan kombinasi perlakuan 2 ppm AgNPs dan 3 ml/L AB Mix dan 3 ppm AgNPs dan 3 ml/L AB Mix, berpotensi optimal dalam mendukung pertumbuhan dan adaptasi planlet Aglaonema pada kondisi ex vitro, ditinjau dari parameter tinggi, jumlah daun, jumlah tunas, dan viabilitas bibit. Dapat disimpulkan bahwa penggunaan AgNPs dan AB Mix pada konsentrasi optimal efektif dalam mendukung multiplikasi dan aklmatisasi tanaman.

Kata kunci: *Aklimatisasi, AB mix, Aglaonema, AgNPs, Kultur jaringan*

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INTRODUCTION

Aglaonema 'Lady Valentine' is one of the varieties of ornamental plants that is popular in the community because it has a unique beauty of color and leaf patterns and a high selling value. The high market demand for this plant has not been fully offset by the availability of superior seeds in large quantities. Conventional plant propagation, such as stem cuttings, is considered less efficient due to the limited number and quality of seedlings produced and susceptible to diseases. Tissue culture (*in vitro*) and acclimatization (*ex vitro*) techniques have been developed as a method of plant propagation that is able to produce seedlings in large and uniform numbers. However, these two methods also have limitations. Previous research has shown that the rate of bud multiplication in tissue culture is still low (Chen and Yeh, 2007), and plantlet growth at the acclimatization stage tends to be slow (Barakat and Gaber, 2018).

To overcome these limitations, various studies have been carried out to optimize the Aglaonema micropropagation process. Various studies have been conducted to optimize the micropropagation of Aglaonema, including regulation of the type and composition of plant growth regulators (ZPT) (Fang *et al*., 2013), as well as modifications of planting media and culture nutrients in vitro (El-Gedawey and Hussein, 2022), and ex vitro (Toscano *et al*., 2019). One approach that is starting to gain attention is the use of AB Mix hydroponic nutrients as an alternative to MS media.

The use of AB Mix hydroponic nutrients as a substitute for MS media is reported to be able to provide positive results on the growth of explants in vitro (Nhut et al., 2022). Based on the results of research by Pratiwi et al (2023), the application of AB Mix nutrition to in vitro culture media is able to significantly increase the growth of explants, so that it has the potential to be a more economical and practical alternative to conventional media such as MS. In addition, the use of nanoparticle technology, one of which is silver nanoparticles (AgNPs), has also begun to be used in micropropagation because it is antimicrobial and its ability to stimulate plant growth (Dang et al., 2018; Vasyukova et al., 2021).

The use of a combination of AB Mix and AgNPs nutrients is expected to be able to replace MS media in *in vitro* culture while acting as a substitute for conventional fertilizers at the *ex vitro acclimatization stage*. Therefore, this study has the value of up-to-date with the right regulation of the composition of the concentration of AgNPs and AB Mix nutrients, it is hoped that it can overcome the problem of slow growth of seedlings at both stages.

MATERIALS AND METHODS

Place and time of the research

The research will be carried out at the Biotechnology laboratory of the Faculty of Agriculture, National Development University "Veteran" East Java from September 2023 to November 2023.

Ingredients

This study uses Aglaonema sp var plant explants. 'Lady Valentine' from previous culture results obtained from the Biotrop-Seameo Biotechnology Laboratory, Bogor. Ingredients used in in vitro propagation include agar media, sucrose sugar, alcohol, sterile aqueducts, betadine, spirtus, amoxicillin, silver nanoparticles (AgNPs), and AB Mix nutrients. Meanwhile, the ex vitro material consists of a mixed planting medium of soil, compost, and fern (1:1:1), plastic pots with a diameter of 10 cm, AgNPs Bi Cons 9000 fertilizer, AB Mix Nutrition, aquades, aluminum foil, fungicide solution, bactericide. Tools used at the in vitro stage include laminar air flow (LAF), autoclave, Scanning Electron Microscope (SEM), magnetic hot stirrer, culture bottles, analytical scales, beaker glass, erlenmeyer, pH meter, and culture racks. Tools for the ex vitro stage include polybags, sprayers, paranets, plant shears, plastic covers, as well as measuring height and number of leaves.

Metode

The research method used a factorial Complete Random Design (RAL) with two treatment factors, namely the concentration of silver nanoparticles (AgNPs) and the

concentration of AB Mix nutrients, which were applied both at the in vitro stage for the observation of bud multiplication and at the ex vitro stage to assess plantlet growth after acclimatization. The first factor is the concentration of AgNPs which consists of three levels, namely 1 ppm (A1), 2 ppm (A2), and 3 ppm (A3). The second factor is the concentration of AB Mix nutrients which also consists of three levels, namely 1 ml/L (B1), 2 ml/L (B2), and 3 ml/L (B3). The combination of the two factors resulted in nine treatment combinations that were each repeated three times, resulting in 27 experimental units. Growth parameters were observed periodically, including the appearance of shoots, plantlet height, plantlet volume, culture conditions (in vitro), as well as plant height, number of leaves, number of shoots, and percentage of seedlings growing (in the ex vitro stage). The observation data were analyzed to evaluate the influence of each treatment and its interaction on the growth of Aglaonema sp var plants. 'Lady Valentine'.

RESULTS AND DISCUSSION

Growth of Aglonema var Lady Valentine Plantlets in Vitro

A combination of silver nanoparticle concentrations (AgNPs) and AB Mix showed a varied effect on the growth of Aglaonema var plantlets. Lady Valentine during the in vitro culture phase. Based on the observation results (Table 1.) the parameters of plantlet height, root volume, number of leaves, and culture conditions showed descriptively significant differences between treatments.

Table 1. Average Effect of the combination of AgNPs and AB Mix concentrations on the growth of *Aglaonema* 'Lady Valentine' plantlets in *vitro*

Group Treatment	Observation parameters			
	Buds Appear (Day)	Plantlet Height (cm)	Volume Planlet ($cm^{3)}$	Culture Conditions (score)
A1B1	4.66	4.33	0.16	2.33
A1B2	0.00	0.00	0.00	1.00
A1B3	9.00	1.66	0.03	2.33
A2B1	16.33	1.33	0.85	3.00
A2B2	7.00	3.00	0.20	2.33
A2B3	9.33	5.00	0.52	3.66
A3B1	7.00	0.66	0.02	2.00
A3B2	0.00	0.00	0.00	1.00
A3B3	4.66	4.00	0.32	2.33
BNJ 5%	TN	TN	TN	TN

Remarks: The average number followed by the same letter in the same treatment showed no significant difference in the 5% BNJ test; tn = no real effect

Based on Table 1. above, it can be seen that the combination treatment of A2B3 (AgNPs 2 ppm and AB Mix 3 ml/L) showed the most optimal results in almost all observation parameters. In this treatment, the shoots appeared the fastest on day 3, with a plantlet height of 5.00 cm, a plantlet volume of 0.52 cm^3, and the highest culture condition score of 3.66. In contrast, some combinations such as A1B2 and A3B2 showed no bud emergence, with a high value and plantlet volume of 0 and a low culture condition score (1.00). The treatment of A2B1 and A3B3 also gave fairly good results, with the appearance of buds on the 7th day and the volume of the plantlets being 0.85 and 0.32 cm^3, respectively. These results suggest that the combination of 2 ppm AgNPs with 3 ml/L AB Mix (A2B3) is the best treatment to increase bud multiplication and growth of Aglaonema var plantlets. 'Lady Valentine' in vitro compared to other treatments. This indicates that the combination has an effect on physiological processes and cell growth in an aseptic environment. AgNPs at low concentrations are known to stimulate cell division and enlargement through their antimicrobial effects and

activity as growth elictors. However, at high concentrations (3 ppm), plantlets show symptoms of phytotoxicity in the form of leaf tip necrosis and growth inhibition, suggesting that overdoses can disrupt the metabolic balance of plant cells (Khan *et al*., 2023).

In addition to AgNPs, AB Mix's nutrients also affect the growth and multiplication of agglonema explants. Nutrients are absorbed through diffusion and active transport, then utilized to accelerate meristematic cell division in roots and shoots. Pratiwi *et al*. (2023) reports that AB Mix accelerates growth, lengthens roots, and increases the number of shoots. Aisyah *et al*. (2021) also mentioned that the optimal concentration of AB Mix stimulates the multiplication of shoots in a short period of time.

Although it provides positive results, the growth of explants with AB Mix tends to be not as optimal as MS media because its nutritional content is not as complete and is not specifically formulated for tissue culture (George *et al*., 2020). MS media has a complete balance of macro, micro, vitamin, and growth regulating substances, while AB Mix only supplies basic nutrients (Pratiwi *et al*., 2023; Aisyah *et al*., 2021). One of the drawbacks of AB Mix is the absence of myoinositol, an important compound in MS media that plays a role in cell division, membrane stability, and differentiation response. Myoinositol also functions as an antioxidant that supports the viability of explants (Rout & Mohapatra, 2021; Ahmed *et al*., 2020).

Therefore, although AB Mix can be an alternative medium, the absence of essential components such as myoinositol limits its effectiveness in supporting optimal growth of explants.

Growth of Aglonema var Lady Valentine Acclimatization

At the acclimatization stage (*ex vitro*) it also shows a varied influence on the growth of Aglaonema var plantlets. Lady Valentine due to a combination of AgNPs concentration and AB Mix Nutrition (Table 2).

Table 2. Average Effect of the combination of AgNPs concentration and AB Mix on ex *vitro* growth of Aglaonema *'Lady Valentine' plantlets*

Group Treatment	Observation parameters			
	Plant Height (cm)	Number of Leaves (strands)	Number of Buds	Percentage of Seedlings Growing (%)
P1Q1	8.33	4.00	0.66	97
P1Q2	8.16	3.66	0.66	97
P1Q3	6.83	3.00	0.66	97
P2Q1	8.00	2.66	0.66	98
P2Q2	7.50	5.00	1.00	95
P2Q3	8.00	2.66	0.33	98
P3Q1	6.16	1.66	1.33	95
P3Q2	7.83	3.33	1.33	96
P3Q3	7.33	3.66	1.66	96
BNJ 5%	TN	TN	TN	

Remarks: The average number followed by the same letter in the same treatment showed no significant difference in the 5% BNJ test; tn = no real effect

Based on Table 2. above, P1Q2 treatment produces the best plant growth at *the ex vitro stage*. This treatment showed that in the 7th week the highest plant height value was 8.16 cm, the number of leaves was 3.66 leaves, and the percentage of seedlings grew by 97%. In addition, the combination of P3Q3 also gave quite good results in week 7 with a plant height of 7.33 cm, the number of leaves of 3.66 leaves, the number of shoots of 1.66, and the percentage of seedlings growing 96%.

In contrast, the P3Q1 treatment showed the lowest growth in week 7 with a plant height of only 6.16 cm, a leaf count of 1.66 leaves, and a bud count of 1.33,

although the percentage of seedlings growing remained high (95%). In general, all treatments resulted in a seedling growth percentage above 95%, indicating a high acclimatization success rate.

These results indicate that a combination of certain treatments, particularly P1Q2 and P3Q3, has the potential to be optimal in supporting the growth and adaptation of Aglaonema plantlets under ex vitro conditions, judging from the parameters of height, number of leaves, number of shoots, and viability of seedlings.

The results of *in vitro* and *ex vitro* studies show that high concentrations of silver nanoparticles (AgNPs) tend to inhibit the growth of *Aglaonema* var plantlets. 'Lady Valentine' in the *in vitro* stage, but has a positive effect in the *ex vitro* stage (acclimatization). At the in vitro stage, the highly controlled and enclosed environment makes the plantlet highly sensitive to stress, including oxidative stress induced by high concentrations of AgNPs.

Some studies suggest that high doses of AgNPs can trigger an increase in reactive oxygen species (ROS) that damage cell membranes and disrupt plant metabolism, as reported in studies of callus fennel (*Pimpinella anisum* L.), where high concentrations of AgNPs decreased the activity of antioxidant enzymes such as SOD and caused toxicity (Fatemi *et al*., 2024). On the other hand, at the acclimatization stage, the plantlets have developed a more stable physiological system and are able to adapt to the outside environment. In this condition, AgNPs act as growth stimulants by increasing the activity of antioxidant enzymes, accelerating root and bud formation, and providing protection against pathogens thanks to their antimicrobial properties (Kim *et al*., 2018). Research on rice seedlings has also shown that the application of AgNPs can increase root length and chlorophyll content without causing physiological damage (Kim *et al*., 2018). Thus, these differences in responses suggest that the effectiveness of AgNPs is contextual, dependent on concentration, environmental conditions, and plant growth stages.

CONCLUSION

This study showed that the combination of silver nanoparticle concentration (AgNPs) and AB Mix nutrients had a significant effect on the growth of Aglaonema sp. var. 'Lady Valentine' at both in vitro and ex vitro levels. In the in vitro stage, high concentrations of AgNPs decrease the response of bud multiplication and plantet growth, possibly as a result of oxidative stress from the accumulation of AgNPs. The best treatment is A2B3 (2 ppm AgNPs and 3 ml/L AB Mix) which results in optimal bud and plantlet growth. In contrast, in the ex vitro stage, higher concentrations of AgNPs actually increase the plant's height, number of leaves, shoots, and percentage of seedlings growing, with the combination of P1Q2 and P3Q3 giving the best results. These results confirm that the effectiveness of AgNPs depends on the growth stage, so the concentration of AgNPs and nutrients of the AB Mix must be adjusted for optimal micropropagation results.

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