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ARTICLE

Composition of media for *in vitro* slow growth storage (sgs) of Aglaonema

Composição de meios de crescimento lento para armazenamento *in vitro* (sgs) de Aglaonema

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Abstract: Aglaonema is one of the ornamental plant commodities often affected by falling prices in the ornamental plant market. This phenomenon requires a strategy for storing seeds of rare and exotic cultivars for the short and medium term. *In vitro* storage is one way to anticipate it. This study aims to obtain the suitable composition of *in vitro* growing media for storing Aglaonema plants by slow growth storage (SGS). This study used a completely randomized design with one factor, consisting of seven treatments, i.e., media 1/4 Murashige and Skoog (MS), 1/2 MS, and full MS, with the addition of 1.0 mg L^{-1} and 2.0 mg L^{-1} Benzyl aminopurine (BAP), and the addition of Indoleacetic Acid (IAA) 1.0 mg L^{-1} and 2.0 mg L^{-1}. The results showed that the composition of the planting medium on ¼ MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1} was the best for slow-growth storage of Aglaonema *in vitro*.
Keywords: *Aglaonema commutatum* 'Lady Valentine', benzyl aminopurine, *in vitro* storage, indoleacetic acid, Murashige and Skoog.

Resumo: Aglaonema é uma das commodities de plantas ornamentais frequentemente afetada pela queda dos preços no mercado de plantas ornamentais. Este fenômeno exige uma estratégia de armazenamento de sementes de cultivares raras e exóticas no curto e médio prazo. O armazenamento *in vitro* é uma forma de antecipar isso. Este estudo teve como objetivo obter a composição adequada de meios de cultivo *in vitro* para armazenamento de plantas de Aglaonema por armazenamento de crescimento lento (SGS). Este estudo utilizou um delineamento inteiramente casualizado com um fator, composto por **7** tratamentos, ou seja, meio 1/4 MS, 1/2 MS e MS completo, com adição de 1**,**0 mg L^{-1}, 2**,**0 mg L^{-1} de Benzil aminopurina (BAP) e a adição de ácido indolacético (IAA) 1,0 mg L^{-1}, 2**,**0 mg L^{-1}. Os resultados mostraram que a composição do meio de plantio em ¼ MS + BAP 1,0 mg L^{-1} + IAA 1,0 mg L^{-1} foi a melhor para armazenamento de crescimento lento de Aglaonema *in vitro*.
Palavras-chave: *Aglaonema*, benzilaminopurina, armazenamento *in vitro*, ácido indolilacético, Murashige e Skoog.

Introduction

Aglaonema is a type of leaf ornamental plant with a good selling value in the world of ornamental plant business. Some cultivars of the Aglaonema plant have even reached prices of up to millions of rupiahs in Indonesia. The price fluctuations in the Aglaonema ornamental plant trading need to be anticipated. By making collections of seeds and germplasm with *in vitro* storage. *In vitro* plant cultures can be stored for a long time, but they are still alive, their genetic authenticity is maintained, and if necessary, they can be used at any time. Efforts to store or conserve plant germplasm can be carried out on-site (in the field) or *in vitro* (in culture bottles). There are types of in vitro storage, namely short-term *in vitro* storage and long-term *in vitro* storage (Rajasekharan and Sahijram, 2015).

Initially, *in vitro* storage was carried out to preserve rare plant germplasm so they would not become extinct. Subsequent developments from the study of Kulak et al. (2022) stated that *in vitro* storage also has an essential meaning in biocultural preservation. *In vitro*, storage of plants for the short and medium term is now also being carried out on ornamental plants (Silva et al., 2018), in line with the development of the ornamentals plant industry. The slow-growth storage technique is an *in vitro* approach to conserving some vegetatively propagated species by controlling plantlet growth and development, saving storage space and labor, and reducing costs (Benelli et al., 2022). The *in vitro* plant storage method for the short to medium term is generally carried out using the slow growth or minimal growth storage method (Chauhan et al., 2019), while for the long term, it is carried out using the cryopreservation technique, namely the low-temperature treatment of *in vitro* culture (Silva et al., 2018). Slow-growth storage (medium-term storage) reduces the metabolic activity, i.e., the growth rate of *in vitro* cultures, by maintaining them on modified growth media or under altered culture conditions. Research on *in vitro* storage from various aspects has been presented by Chauhan et al. (2019). *In vitro* techniques to conserve plant biodiversity include micropropagation based on apical or axillary meristem shoots, somatic embryogenesis, cell culture technology, embryo rescue techniques, and *in vitro* low-temperature storage.

In vitro storage using the Slow Growth Storage technique is carried out in several ways, including the use of growth retardant and osmotic compounds (Dewi et al., 2020; Syahid, 2020), encapsulation (Hassanen, 2021), storage in dark conditions and control of the light spectrum (Rodrigues et al., 2022), modification of the growing media by reducing sucrose levels and concentration of primary media (Budiarto et al., 2020), a combination of diluting media with growth inhibitory substances (Syahid, 2021; Syahid and Parlindung, 2023) and the use of a specific light spectrum (Rodrigues et al., 2022). Modifying the media composition by reducing the content of sugars, minerals, growth regulators, or osmotic agents such as sorbitol and mannitol can inhibit cell division and significantly limit callus formation and shoot development. Reducing macronutrients by diluting MS media is one way to slow down the growth of *in vitro* culture (Syahid, 2021). Technically, *in vitro* plant storage methods extend the time between subcultures, lower the risk of germplasm loss through handling errors, such as contamination problems, and reduce the risk of genetic instability due to reduced subcultures (Benelli et al., 2022). Research on *in vitro* micropropagation to stimulate explant growth and increase seedling production continues to be carried out, but on the other hand, *in vitro* storage also needs to be developed. For this reason, it is necessary to research the *in vitro* storage of ornamental plants in the context of preserving germplasm, storage of sterile cultures, and collections of exotic and rare ornamental plants.

SGS has been developed in various plants. However, no publications have been reported regarding the SGS technique for Aglaonema. This method is important for storing Aglaonema germplasm, especially for expensive, rare and unique types of Aglaonema. This study aims to obtain the correct composition of slow growth growing media for *in vitro* storage of Aglaonema plants.

Materials and Methods

The research was conducted from May to November 2022. The sterile culture explants of Aglaonema var. Lady Valentine were used as plant materials. The medium was the basic medium of Murashige and Skoog (MS),

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with the addition of 30 g L^{-1}sugar and 7 g L^{-1} agar solid. Plant Growth Regulatory (PGR) added to the growing media according to treatment are Benzyl Amino Purine (BAP) and Indole Acetic Acid (IAA). This study used a completely randomized design with one factor. Media composition treatment factors, as listed in Table 1.

Table 1. Media composition

No.	Treatment code	Media composition
1	P0	MS without PGR
2	P1	MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}
3	P2	MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}
4	P3	½ MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}
5	P4	¼ MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}
6	P5	½ MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}
7	P6	¼ MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}

Each experiment had 5 repetitions, with a total of 35 experimental units. One experimental unit was three culture bottles consisting of three explants. The explants used were shoots of Aglaonema plant culture measuring ± 0.3 cm. According to the treatment, the explants were inoculated in bottles jars containing 30 mL of medium. Observations were made one week after planting on the growing explants: time of shoots appearing (days), time of roots appearing (days), number of shoots, number of leaves, number of internodes, and number of roots. The initial growth observations were made between 7 and 56 days after planting, starting from the explant's growth initiation, which typically occurs between 1 to 7 weeks after planting. At that time, it is likely that the explant will grow quickly. Subsequent observations at the age of 91 days after planting determine the length of the plant and the minimum growth chart. Data analysis was carried out using ANOVA, followed by a 5% Tukey's test.

Results and Discussion

The variance analysis showed differences in the effect of MS media concentrations with the addition of BAP and IAA on the time of emergence of shoots and roots of Aglaonema explants. Table 2 presents the average time of emergence of Aglaonema shoots and roots. Treatment of MS medium concentration with the addition of BAP and IAA did not affect the time of emergence of new Aglaonema leaves. Table 2 shows that the average time for the fastest shoot growth to appear was in treatment P0 (MS), which was 12.56 days, significantly different from treatment P4 (1/4 MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}). The longest time for new shoots to appear was in treatment P4 (1/4 MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}), which was 24.89 days. This phenomenon is different from the time new roots appear. The longest time for roots to appear was in treatment P0 (21.89 days), while the time for new roots to appear was the fastest, occurring in treatment P1 (MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}), for 4 .94 days (Table 2).

Table 2. The average time of emergence of shoots, roots and leaves at 27 days after planting

Media composition	average time of emergence		
	shoots	roots	leaves
P0: MS without PGR	12.56 a	21.89 c	1.89
P1: MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}	13.56 a	4.94 a	-
P2: MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}	16.44 a	13.50 bc	-
P3: ½ MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}	13.67 a	17.83 c	-
P4: ¼ MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}	24.89 b	13.33 b	-
P5: ½ MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}	12.78 a	18.33 c	-
P6: ¼ MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}	13.11 a	14.67 bc	-
Tukey's 5%	3.91	4.39	

Data in the column followed by the same letters were not significantly different according to the Tukey's 5%.

Treatment of MS media concentration with the addition of BAP and IAA had no significant effect on the emergence time of Aglaonema leaves. Table 1 shows that only the P0 (MS) treatment increased the number of Aglaonema leaves with an emergence time of 1.89 days at 27 days after planting (DAP). Other treatments showed leaf emergence 27 days after planting. P1 treatment with full MS media provided enough nutrition for the emergence of leaves. Diluting MS medium concentration to 50% (½ MS) or 25% (¼ MS) does not support leaf growth. This information is also in line with Syahid's research (2021) on *Hypericum perforatum* L. plants, that the use of ¼ MS + 0.1 mg L^{-1} BAP could minimize plant growth (number of shoots, long shoots, and number of leaves) during in vitro storage.

The analysis of variance showed that the treatment given had a significant effect on the length of plantlets, the number of shoots, and the number of roots of Aglaonema plantlets. Table 2 shows the average results of plantlet length, number of shoots, and number of Aglaonema roots at 91 days after planting. The results of plantlet length observations in Table 3 show that treatment P4 (1/4 MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}) produced the lowest plantlet length (2.17 cm), while the control treatment (full MS without PGR) showed pretty good growth with a plantlet length of 4.86 cm.

The addition of BAP and IAA growth regulators significantly affected the increase in the number of Aglaonema shoots at 91 days after observation. Table 3 presents the average number of Aglaonema shoots. At 91 days after planting, observations showed that the highest average number of shoots was in treatment P5 (1/4 MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}) with a yield of 3.00 shoots. Treatment P1 (Full MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}) showed the lowest number of shoots at 1.44 but was not significantly different from treatment P4 (1/4 MS + BAP mg L^{-1} + IAA 1.0 mg L^{-1}) as much as 1.89 pieces.

Table 3. The mean length of plantlets, number of shoots, and number of roots of Aglaonema at 91 days after planting

Media Composition	Plantlet length (cm)	Number of Shoots	Number of Roots
P0: MS without PGR	4.86 b	1.61 a	1.89 b
P1: MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}	2.42 a	1.44 a	0.78 ab
P2: MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}	2.83 a	2.33 ab	0.56 ab
P3: ½ MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}	3.56 ab	1.78 a	1.89 b
P4: ¼ MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}	2.17 a	1.89 ab	0.11 a
P5: ½ MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}	3.44 ab	3.00 b	1.11 ab
P6: ¼ MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}	3.94 ab	2.33 ab	0.89 ab
Tukey's 5%	1.72	1.15	1.37

Data in the column followed by the same letters were not significantly different according to the Tukey's 5%.

Treatment of MS medium concentration and the addition of PGR also significantly affected the number of Aglaonema roots (Table 3). The highest average number of roots was found in treatment P0 (full MS without PGR) and P3 (1/2 MS + BAP 1.0 mg L^{-1}+ IAA 1.0 mg L^{-1}) with a yield of 1.89 roots. The P4 treatment (1/4 MS + BAP 1.0 mg L^{-1}+ IAA 1.0 mg L^{-1}) on the number of roots gave the lowest yield of 0.11. Reducing nutrients in the MS planting medium by reducing the concentration or dilution to 25% (1/4 MS) causes the plantlets to grow less optimally, according to Budiarto et al. (2020) modification of the planting medium by reducing the sucrose level and the concentration of the basic media. Reducing the concentration of basic media with PGR combinations is one of the slow-growth storage techniques (Syahid and Parlindung, 2023). The number of roots in the P0 treatment (full MS without PGR) showed promising results (1.89 strands). This phenomenon also occurs in Aldeen and Mona's research (2021) on *Aglaonema commutatum* cultures. It identifies that the explants have sufficient endogenous hormones to induce roots.

Figures 1 and 2 show the results of observations of the growth of shoots and roots of Aglaonema plantlets at the age of 7 - 91 days after planting. Meanwhile, Fig. 3 shows the results of visual observations of plantlet growth at observations 7 and 91 days after planting. Data on shoot growth in Fig. 1 and root growth in Fig. 2 show slow growth; even leaf growth is almost non-existent.

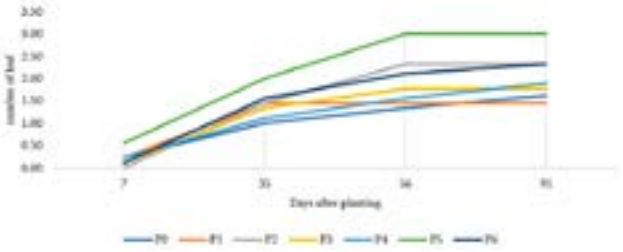

Fig. 1. Graph of shoot growth on 7 to 91 days after planting

Figure 1 shows that the number of Aglaonema shoots increased weekly from 7-91 days after planting observations. The increase in the number of shoots only ranged from one to three per explant planted. The lowest number of shoots in this study (Table 2 and Fig. 3) occurs in treatment P0 (full MS without PGR), P1 (MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}), and P4 (1/4 MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}). This data is in line with the research results presented by Syahid (2021) that the use of a composition of ¼ MS + 0.1 mg L^{-1} BA can suppress culture growth (number of shoots, shoot length, and number of leaves) without showing necrotic symptoms for up to three months of the storage period. Furthermore, Syahid and Parlindung (2023) stated that ¼ MS + 0.1 mg L^{-1} BAP was the best treatment for reducing growth during 12 weeks of storage.

According to the initial study on the safe storage of *Sorbus redliana* for slow growth, in vitro shoot cultures of the species can be kept for 52 weeks at 4 ºC in the dark on MS media supplemented with 3% sucrose, 2.8 µM of BAR, 0.6 µM GA3, and 1.48 µM IBA (Mendler-Drienyovszki and Magyar-Tábori, 2023).

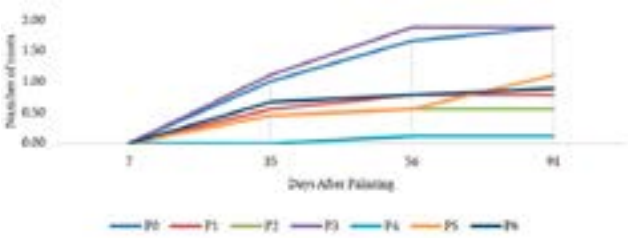

Fig. 2. Graph of root growth at 7 to 91 days after planting

Aglaonema plantlet roots generally increased from 7 to 91 days after planting (Fig. 2). The P4 treatment (1/4 MS + BAP 1.0 mg L^{-1} + IAA mg L^{-1}) showed the lowest root growth. The number of roots treated with P4 (1/4 MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}) at 91 days after planting was only 0.11 (Table 2 and Fig. 2). MS media enriched with PGR cytokinin and auxin in the right combination will encourage shoot initiation or multiplication of Aglaonema explants (Barakat and Gaber, 2018; Kaviani et al., 2019). On the other hand, the combination of diluting primary media with growth-inhibiting substances (Syahid, 2021; Syahid and Parlindung, 2023). In this study, treatment of reducing the concentration of MS media with the addition of BAP and IAA resulted in slow growth of Aglaonema culture.

Fig. 3. Aglaonema plantlets at 91 days and 360 days after planting: P1:MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}, P2: MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}, P3: ½ MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}, P4: ¼MS + BAP 1.0 mg L^{-1} + IAA 1.0 mg L^{-1}, P5: ½ MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}, P6: ¼ MS + BAP 2.0 mg L^{-1} + IAA 2.0 mg L^{-1}, P0: MS

In vitro storage requires the stored germplasm material to remain alive and grow back normally when cultured on optimal media. In addition, no genetic changes occurred in the plant germplasm after storage. Therefore, explant materials used for storage should be differentiated tissues such as embryos, buds, plantlets, or shoot meristems compared to undifferentiated tissues such as calluses, cells, or protoplasts. Using a composition of ¼ MS + 0.1 mg L^{-1} BA can suppress culture growth (number of shoots, shoot length, and number of leaves) without showing necrotic symptoms for up to three months of storage (Syahid, 2021). This treatment can be used to minimize St. John's wort (*Hypericum perforatum* L.) for *in vitro* storage. By diluting the media to 1/4 or 1/2, it causes a reduction in macro elements, especially NH4+ and NO3-, so N as an essential element for growth decreases.

The performance of plants in culture was observed qualitatively at 91 days after planting, showing that the performance of plants for MS and ¼ MS media treatment supplemented with BAP and IAA 2 mg L^{-1}, respectively, showed plants with plant height and number of leaves smaller than with other treatments (Fig. 3). It is equal to the in vitro storage for Lilies, which could use ¼ MS + 7% sucrose treatment (Budiarto et al., 2020). Likewise, Syahid's research (2021) showed that using ¼ MS + 0.1 mg L^{-1} BAP could minimize plant growth (number of shoots, shoot length, and number of leaves) during in vitro storage of *Hypericum perforatum* L.

As shown by retardants (Dewi et al., 2020), slow growth is beneficial because it will prolong the subculture cycle. The subculture cycle will affect the storage time of in vitro germplasm. In addition, the less often a plant is a subculture, the lower the maintenance costs required (Benelli et al., 2022). Increasing the subculture period between 6 and 12 months will save costs, reduce the contamination rate, and prevent mutations in stored germplasm (Syahid, 2021). This condition indicates that low concentrations of nutrients induce suppression of leaf growth, and conversely, higher concentrations of MS nutrients induce the formation of taller leaves. Nutrients with low concentrations are one way to control minimal growth in vitro plant storage.

Various studies of Aglaonema tissue culture reported that slow growth occurred in control treatment without plant growth regulators, both from the auxin and cytokinin groups (Kaviani et al., 2019; Zahara and Win, 2020). Research by Hoda et al. (2022) showed that the Aglaonema micropropagation treatment on MS medium without plant growth regulators, as well as at the lowest concentration of BA addition (0.25 mg L^{-1}), resulted in slower explant growth compared to other treatments. Likewise, Barakat and Gaber's research (2018) showed that the growth of shoots and roots of the Aglaonema plant in the treatment without PGR was not better than in the treatment with PGR. Benelli et al. (2022) have stated that in vitro storage with the slow growth method can be applied by considering various factors, including the presence or absence of growth regulators.

In addition to concentration, the ratio of cytokinin and auxins added to the MS medium affects plantlet growth. Treatment with the addition of growth regulators, cytokinin, and Auxin at a certain concentration ratio will accelerate the growth of explants in vitro. In this study, adding BAP and IAA to MS media showed minimal growth at a 1:1 ratio with a concentration of 2.0 mg L^{-1} each. Sakr (2016) stated that growth was slow in the micropropagation of the *Dieffenbachia amoena* in MS media with the addition of 5.0 mg L^{-1} IBA and 5.0 mg L^{-1} IAA. Zahara and Win (2020) stated that using the micropropagation technique, a combination of plant growth regulators belonging to the auxin and cytokinin groups is required to propagate Aglaonema plants. The same comparison between Auxin and cytokinin class PGR can cause slow growth.

Conclusions

Adjusting the composition of the planting medium can do the slow growth method for *in vitro* storage of Aglaonema. Treatment of ¼ MS media with the addition of 1.0 mg L^{-1} BAP and 1.0 mg L^{-1} IAA gave the slowest growth results in the emergence of shoots and the lowest results in plantlet length, number of shoots, and number of roots.

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Author Contribution

PN: Conceptualization, writing original draft, funding acquisition. **HP:** Supervision. **SS:** Writing-review and editing

Conflict of interest

The authors declare that they have no potential conflict of interest in the submitted work.

Data Availability Statement

Data will be made available on request.

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