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Application of Kinetin and naphthalene acetic acid (NAA) for the growth and development of Aglaonema tricolor explant

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ABSTRACT

Two experiments were conducted to assess the effect of kinetin and NAA on the shoot growth and rooting of tissue-cultured Aglaonema tricolor using leaf bud explant. Likewise, it was also aimed to assess the suitability of Aglaonema leaf bud as explant and to determine the amount of NAA and kinetin needed in shoot formation and proliferation of the said foliage ornamental crop. NAA applied singly or in combination with kinetin did not affect the percentage survival of the explants, percent shoot formation, number of shoots, length of shoots, days to shoot formation and percent callus formation. Rates used were 0, 1.0 and 1.5 ppm kinetin and 0.25, and 5 ppm NAA A comparative evaluation of NAA and Kinetin was also conducted. In the follow-up study, percent callus formation, shoot length, number, and length of roots were significantly higher which showed a better effect with the application of 5 ppm NAA than 1.5 ppm Kinetin + 2.5 ppm NAA. In the first study, the optimum amount and the effective ratio of NAA and kinetin to enhance growth may not have been the ones tested. It is also possible that the variety used was not responsive to the growth regulator. The result of this study would add information on the use of growth regulators in the micropropagation of foliage crops. Studies like this will promote the advancement of foliage crop propagation for commercialization.

Keywords: shoot differentiation, Aglaonema tricolor, kinetin, Naphthalene Acetic Acid, cytokinin

INTRODUCTION

The term Aglaonema is derived from the root word aglaos meaning bright and "*nema*" meaning thread, referring to the glistening stamens of the plant. Aglaonema is also known as "*La Suerte*" and collectively referred as "*Chinese evergreens*" (Success with houseplants, n.d.). It is considered as one of the outstanding native ornamental plants in the Philippines. The genus Aglaonema includes several species of foliage plants which belong to Family Araceae or Aroid family. Its related family members are Philodendrons, Diffenbachias, and Spathyphyllum. These plants are distinguished for their subtly patterned gray, cream, red, and green leaves.

These are mainly grown due to their attractive appearance created by the handsome foliage and the minimum care and maintenance required to survive. These plants preferred to be kept under shade and diffused light conditions. These plants are observed to have tremendous variability affected by time, season, climate, stage of growth and growing conditions. Variations are observable with the leaf color, veins, stalks, stripes to occurrence of spathe, anthesis, leaf shape, and other plant characteristics.

It is considered a potential ornamental crop whose economic importance and popularity among ornamental growers and collectors are on the rise. The plant can serve both as landscape and indoor plant because of its satisfactory decorative appearance. Preliminary evaluation on the use of indoor plants for air purification and revitalization, include aglaonemas as second on the list of top ten plants in removing formaldehyde, benzene, and carbon monoxide on the air (Siar, 2003). These are conventionally propagated using cuttings of shoot tips or stems having two to three nodes. But this is slow and costly. A year old aglaonema with eighteen nodes can produce a maximum of six to nine new plants and rooted nodal cuttings can be marketed after five to eight months (Siar, 2013; Mendoza, 1982; Faustino et al., 1999).

Sourcing of planting materials of recently known hybrids is difficult and expensive. This makes micropropagation necessary to make aglaonema plantlets available and affordable.

The tissue culture of Aglaonema "*Silver leaf'*", it was found out that shoot formation was fastest in MS medium supplemented with 0.1 ppm 2,4 dichlorophenoxy acetic acid (2,4-D) and 0.1 ppm benzyl adenine (BA) (Mendoza, 1982). On the other hand, indole acetic acid (IAA), indole butyric acid (IBA), naphthalene acetic acid (NAA) or 2,4-dichlorophenoxy acetic acid (2,4-D) are often added to the nutrient media for the tissue culture of many agricultural crops (Chen & Yeh, 2014). Any living tissue therefore, can give rise to proliferating

undifferentiated callus or a shoot and finally a plantlet when excised and placed in a suitable culture medium (Zhang & Zhou, 2004; Ahmad, n.d.).

MATERIALS AND METHODS

The experiments were conducted in the Tissue Culture Laboratory of the University of Southeastern Philippines, Apokon, Tagum City, Davao del Norte. Initial trials were done in the Tissue Culture Laboratory of the Davao Oriental State College of Science and Technology, Mati, Davao Oriental. The materials used are those needed in the tissue culture laboratory such as culture media, glass wares, and culture bottles, measuring device, experimental plant, autoclave, sugar, coconut water, and the growth regulators. Aglaonema tricolor was used. This is a popular ornamental plant among nursery owners and plant collectors. This variety is known to be slow growing but with attractive foliage.

Explant preparation

Mother plants of A. tricolor were conditioned in clean dry room two weeks prior to surface sterilization. Plants were maintained with regular watering and foliar fertilization. Watering was done on the base of the stem of the said plant near the soil surface. Buds were obtained from the mother plant by cutting the upper portion of the stem. Leaf sheaths were removed and stems were washed with detergent and tap water several times. The stems were finally rinsed with sterile distilled water. Stem tissues were soaked in 50% sodium hypochlorite for 30 min. and rinsed with sterile distilled water five times. These were soaked again in sodium hypochlorite for another 20 min. (double sterilization). Two drops of sticker were added to the soaking solution. Tissues of nodal cuttings were rinsed again with sterile distilled water after soaking two times. Clean nodal cuttings were placed in sterile petri dish inside the inoculation chamber and swabbed with 95% ethyl alcohol. Undeveloped scale leaves were removed and buds were excised prior to implanting into the culture room. Leaf buds were excised from stem tissues and scooped individually. The surfaced sterilized tissues were washed with distilled water three times and the bleached portions exposed to sodium hypochlorite were removed. The remaining tissues were transferred to another petri dish for sectioning. Using sterile forceps and scalpel, the stem tissues were cut to separate each bud. These were washed again with sterile distilled water before the buds were placed in sterile culture bottles. The cultures were maintained at 26^0C for 10 hrs daily. All operations were done inside the sterile chamber.

Media preparation for shoot initiation

Modified MS medium was prepared and utilized (Pierik, 1987; Torres, 1989; Murashige & Skoog, 1962). This was added with 15% coconut water, 3% refined sugar plus growth regulators such as kinetin, NAA and 5ppm benzyl adenine.

The media was gelled with 7% agar dissolved by heating prior to addition to the basal medium. The pH of the medium was adjusted to 5.7 using 0.1 Normal HCL. Twenty-five ml of the medium mixture was dispensed into the culture bottles while still hot. Culture bottles were covered with metal caps and sterilized in the autoclave for 20 min at 15 1b pressure.

Samples were arranged using Completely Randomized Design (CRD) in the culture room inside the Tissue culture laboratory. When data were statistically significant, comparison between treatment means was made using Duncan's Multiple Range Test (DMRT) and T-test for comparative evaluation in the succeeding study. Eight bottle samples were used per replicate per treatment with one explant per bottle in the first study. Twenty samples each was used in the second study. There were ten treatments replicated three times in the first experiment and two treatments in the follow-up study.

RESULTS AND DISCUSSIONS

Growth parameters affected by NAA and Kinetin

In all parameters, the application of NAA and Kinetin did not affect the growth of A. tricolor explants as shown in Table 1. This could be attributed to the season, climate, type of plant, or variety tested. This plant possessed so much variability in its morphological characteristics. Different plants and different varieties could have varied responses to the application of any growth regulator.

Table 1. Different parameters on shoot growth and developmental. tricolor applied with different amounts and combinations of Kinetin and NAA 60 days after culture (transformed data).

Kinetin + NAA (ppm)	Percent Survival^{NS}	Percent shoot Initiation^{NS}	Number of shoots^{NS}	Length of shoots^{NS}	Days to Shoot Formation^{NS}	Percent Callus Formation^{NS}
1. 0 + 0 (Untreated)	41.66	0.71	0.71	0.71	0.71	0.71
2. 0 + 2.5	33.33	7.94	0.87	0.87	5.96	4.62
3. 0 + 5.0	62.50	7.67	2.02	1.91	5.79	2.97
4. 1.0 + 0	41.66	4.14	0.87	0.87	5.96	0.71
5. 1.0 + 2.5	41.66	3.6	0.87	0.87	7.52	0.71
6. 1.0 + 5.0	20.83	0.71	0.71	0.71	0.71	0.71
7. 1.5 + 0	37.50	5.05	0.87	0.87	6.74	0.71
8. 1.5 + 2.5	54.17	8.20	2.02	1.78	6.36	0.71
9. 1.5 + 5.0	33.33	6.81	1.58	1.55	6.36	0.71
10. 0.1 2,4-D + 0.1 Ki	45.83	0.71	0.71	0.71	0.71	7.11

This result contradicted previous studies on the effectiveness of Kinetin and NAA in shoot growth and development of some foliage plants such as Aglaonema "White tip", Commutatum and A. simplex (Chen & Yeh, 2014; Zhang & Zhou, 2004; Ahmad et al., n.d.). The use of 1 ppm 2,4-D + 0.1 ppm Kinetin did not

also promote root growth and callus formation. This result contradicted again the basic principle and findings of some studies on the use of 2,4-D to enhance callus formation and root growth in tissue cultured plants (Pierik, 1987; Torres, 1989; Debergh & Zimmerman, 1991; Mendoza, 1982). Percent shoot formation, number of shoots, length of shoots, days to shoot formation, and percent callus formation were likewise not affected by the application of kinetin and NAA IAA and kinetin were found out to have interacting effects in the differentiation and enhance growth of tobacco (Vasi & Trevor, 1994). Cytokinins (kinetin included) are often used to stimulate growth development. Commonly used are Kinetin, Benzyl Adenine Purine (BAP) and PBA (Promptep, 1981; Lorico, 1985). In anthurium, shoot formation was induced with the addition of IBA and kinetin (del Rosario, 1988). In a study on the in vitro propagation of Dracaena, another foliage crop, shoot growth, and proliferation was likewise achieved in liquid basal medium with 2 to 5 mg kinetin (Lorico, 1985). The same result was also obtained having high frequency shoot formation of foliage crop using MS medium with NAA levels ranging from 0.1 to 0.2 mg/L (Hussein, 2004). But another study on Ficus benjamina L. "Variegata"applied with various combinations of auxins and cytokinin resulted to no plantlet formation (Aurigue, 1986). In my study, when no shoots were formed, they were either dessicated, contaminated, or stayed as is (no growth).

The combined effects of 2,4-D and Kinetin (0.1ppm + 1.0 ppm) on callus induction was evident but not statistically significant which confirmed the work of other scientists such as in aglaonema "Silver leaf" which resulted to better shoot and callus formation when buds were supplemented with 0.1ppm 2,4-D and 0.1ppm kinetin (Mendoza, 1982; Zhang & Zhou, 2004; Lorico, 1985). Callus formation in this treatment was 50%. Furthermore, the use of 2,4-D was observed to cause massive root growth comparative to a mangrove plant without shoots.

Comparative assessment on the effect of NAA and kinetin on shoot growth proliferation of A. tricolor

Five ppm NAA applied to aglaonema explants increased the shoot length, the number of roots and length of roots than those applied with 1.5 ppm kinetin combined with 2.5 ppm NAA (Table 2).

Table 2. Growth parameters of aglaonema explant applied with two combinations of NAA + Kinetin

Rates of Kinetin + NAA (ppm)	% Callus Formation*	% Shoot formationNS	Number of shootsNS	Shoot Length*(cm)	Number of Roots*	Length of roots*
0 + 5	26	52	2.0	2.91	9.14	4.12
1.5 + 2.5	10	56	2.17	1.85	5.14	2.44

*- Significant at 5 %, NS- Not significant

Percent callus formation

Application of five ppm NAA resulted to significantly higher callus formation than the combination of Kinetin and NAA (1.5 + 2.5 ppm). This supported the findings of workers who reported that optimization of growth regulators resulted to callus production of different species of plants (Diertert et al., 1982; Murata & Orton, 1987; Das, 1991; Yang et al., 1991).

Percentage shoot formation and number of shoots

These parameters were not affected by the application of kinetin and NAA at different rates and combinations when applied to aglaonema explants. This could be due to the explants used, the cultivar or plant species and the season when this was conducted. This result contradicted findings of another study where no or low levels of NAA with BAP promoted shoot formation of Brassica oleraceas (Ahman & Spoor, 1992).

Length of shoots (cm)

Length of shoots when measured 45 days after implantation was significantly longer when the culture medium was added with 5 ppm NAA without kinetin than Kinetin and NAA (Table 2). There was faster growth of shoots when explant was applied with 5 ppm NAA than 1.5 ppm Kinetin + 2.5 ppm NAA. Multiple shoot induction and consequently increased in shoot length was observed with MS medium supplemented with BA or various concentrations of Auxinx (either IAA or NAA) in vitro studies of *A. commutatum* Schott. Likewise, in vitro propagation of 3 species of aglaonema, it was concluded that culture media supplemented with cytokinin was crucial for shoot multiplication of aglaonema (Hussein, 2004). In another study (2011), Thioduzuron (TDZ) was found more effective than BAP, Zeatin. and kinetin on shoot proliferation (Chen & Yeh, 2007). This was further confirmed in a study on shoot multiplication of Aglaonema "White tip" which was applied with TDZ and observed to proliferate very fast in five weeks _period.

Number of roots

The number of roots is significantly increased with the use of NAA and Kinetin at 5ppm + 0 ppm respectively. NAA is effective for root formation in this study. The application of 2.5 ppm NAA + 1.5 ppm Kinetin produced fewer roots. While this is not a rooting set-up, it was observed roots were developed within 30 days of culture. This confirmed the statements of earlier researchers that low rates of auxin enhanced root formation (Pierik, 1987). But there is an optimum balance of concentration between these two growth regulators for shoot initiation and proliferation as well as rooting of explants.

Length of roots (cm)

Application of 5 ppm NAA + 0 ppm Kinetin produced significantly longer roots compared with 2.5 ppm NAA + 1.5 ppm Kinetin. This confirmed the efficacy of

NAA in root formation and elongation as growth regulator on the amount it was tested. The study revealed that 5ppm NAA is more effective in shoot and root growth and development of A. tricolor than kinetin. The result of these studies was not conclusive, it is possible that there are substantial cultivar differences in response to concentrations of NAA and kinetin.

Concentrations of growth regulators are critical and specific to explants type. Each species required particular hormone concentration for optimum growth.

Further study is recommended particularly on the sterilization technique and the application of other growth regulators at different rates which may provide consistent and conclusive results.

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