



OPTIMIZATION OF *ALOCASIA AMAZONICA* PROLIFERATION THROUGH *IN-VITRO* CULTURE TECHNIQUE

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ABSTRACT

Excised explants were *in-vitro* cultured on multiplication medium of Murashige and Skoog (MS). This study was carried away inside the tissue culture lab. Horticulture Research Institute, Agricultural Research Center. Giza, Egypt through the period from 2015 to 2017, to research some factors affecting *in-vitro* propagation of the indoor ornamental plant *Alocasia amazonica* using benzyladenine amino purine (BAP) and Kinetin at 0, 1, 2, 3, 4 ppm and their interaction. The obtained results indicated that BAP gave the greatest number of shoots, plus the lowest values for shoot length, shoot fresh weight, number regarding roots and total chlorophyll content. Meanwhile, kinetin achieved the highest values for shoot length, shoot fresh weight although it was not necessarily significant. The same was observed in number of leaves, number of roots and total chlorophyll content with no significant difference. MS medium free of hormones demonstrated the greatest number of leaves, number of roots and total chlorophyll content, and the lowest values of number of shoots and shoot length. Using cytokinin at 1 ppm gave the highest shoot length and number of leaves; and the second position for number of shoot and roots. As for 2 ppm of cytokinin application, it gave the greatest values of shoot length, number of leaves and shoot fresh weight, despite the last one was not significant. this concentration got also the other position for number of shoots, 3 ppm had the greatest number of shoots, and the lowest shoot length, number of roots and shoot fresh weight and 4 ppm occupied the second grade concerning number of shoots, and the lowest

grades for shoot length, shoot fresh weight, number of leaves, number of roots and total chlorophyll. Regarding the interaction between cytokinin type and concentration found that, the control treatment (Free MS) gave the highest number of leaves. Using BAP at 2 or 3 ppm attained the highest number of shoots. Using Kin at 1 or 2 ppm attained the highest shoots length. Also, Using Kin at 2 ppm attained the highest fresh weight. The application of Kin at 1 ppm was connected with the highest value of number of leaves. The development of roots showed great values on free medium of BAP and Kin as well as medium supplemented with Kin at 1 and 2 ppm. Whereas, root did not demonstrate any presence at higher concentrations of BAP of 2, 3 and 4 ppm. It is usually recommended to use the MS medium supplemented with BAP at 3 ppm which often gave the highest number of shoots. However, the highest values for shoot length, shoot fresh weight and number of roots were recorded on particularly on MS medium supplemented with Kin at 2 ppm.

Keywords: *Alocasia*, BAP, Kin, multiplication.

INTRODUCTION

Over 100 species were found of *Alocasia* known to technology and all are normally found in the region of Southeast Asian countries and neighboring island countries of the Pacific Sea. *Alocasia x amazonica* is a new hybrid and not a new species. It was developed in 1950's of the particular last century from Asiatic parents *Alocasia sanderiana* (Schott)G. Don) x *Alocasia longiloba*(Miq.). This is a hybrid fellow member of the family Araceae. This plant has in

no way existed within the rain woodland of the Amazon or perhaps South America. It provides never been observed normally in any virgin woodland in the world. **InterNet Site 1 (2020)**.

Alocasia species are very well-known ornamental plants among plant collectors and landscape gardeners due to their foliar charm, patterns of leaf variegation and texture, along with tolerance to limited sunlight. *Alocasia* species are conventionally propagated through seeds and corms. However, conventional methods of propagation are time consuming in addition to restrict the mass propagation of *Alocasia* species. **(Jo et al 2008)**.

In-vitro culture methods of propagation are the alternatives method to meet the growing demand of both the domestic and the international markets. Despite the increasing commercial demand for *Alocasia* plants, only one tissue culture report is available for *Alocasia macrorrhizos* **(Adelberg and Toler 2004)**, and there are few reports of in vitro propagation of *A. amazonica* and other species **(Jo et al 2008 & 2009 and Bhatt et al 2013)**.

Hence it is necessary to be able to develop efficient and economically viable micro-propagation protocols in order to ensure conservation and meet the commercial demand for *Alocasia* species. Regarding the use of various cytokinins at different level represent an important value for proliferation of in vitro propagated plants.

Arab et al (2014) mentioned that the longest span of new microshoots associated with the GxN15 (hybrid of almond x peach) rootstock was obtained within hormone-free MS medium. generally there is a significant connection between hormone level and even plantlet, in order that an rise in BAP level lead significantly in height lower. The existence of high amounts involving BAP in culture moderate triggered influence on shoot development.

Thus the target of this study was to find the proper cytokinin and concentration on proliferation of *Alocasia amazonica*.

MATERIALS AND METHODS

This kind of study was carried out inside the Tissue culture Lab, Horticulture Research Institute, Gardening Research Center, Giza, Egypt throughout the period from 2015 to 2017, to research the effect of cytokinin type and levels on *in-vitro* shoot growth of *Alocasia amazonica*. Inside vitro excised shoot explants of *Alocasia amazonica* have been obtained from the tissues culture facility in Zohryia

Garden. A factorial test was carried out making use of BAP and Kin as the first factor, from concentration of 0, 1, 2, 3 and 4 ppm as the second factor. The explants were inoculated onto MS medium. 1 bud explant was inoculated into each glass container.

Every treatment was replicated 3 times, with 6 containers in each replicate. These kinds of treatments were completely randomized. Jars of this research were kept in typically the incubation room at 26±2°C, under florescent lighting of 2000-2500 lux from 16/8 day \night varying for 8 weeks. Info of number of sets, shoot length, shoot fresh weight, number of root base and total chlorophyll content according to **Saric et al (1976)** were recorded. Information were statistically analyzed making use of analysis of variance while described by **Snedecor and Cochran (1989)** and means had been separated according to Duncan multiple comparison test with a probability level of 5% **(Duncan, 1955)** by SAS 95 computer program

RESULTS

Number of shoots

Cytokinin concentration, and type and their interaction showed significant effect on number of shoots **(Table 1)**. The effect of cytokinin concentration on number of shoots was significant. The highest number (8.33 shoots) was obtained when cytokinin were applied at 3 ppm. The lowest one (4.33 shoots) was a result of using no cytokinins at all. The effect of cytokinin type was significant. Applying BAP gave an increase in the number of shoots when compared to Kin (8.67 and 3.93 shoots, respectively).

Table 1. Effect of cytokinin type, concentration and their interaction on number of shoots.

Conc. (ppm)	Cytokinin type		Mean
	BAP	KIN	
0	4.33 cd	4.33 cd	4.33 B
1	7.33 b-d	4.00 cd	5.67 AB
2	9.00 a-c	5.00 b-d	7.00 AB
3	13.00 a	3.67 d	8.33 A
4	9.67 ab	2.67 d	6.17 AB
Mean	8.67 A ¹	3.93 B ¹	

Means with the same letter are not significantly different

As for the interaction, it affected number of shoots significantly. The highest record in this concern resulted when BAP was used at 3 ppm (13.00 shoots). However, using BAP at 2 and 4 ppm shared also in the highest rank (9.00 and 9.67 shoots, respectively). The lowest numbers were a result of using kinetin at 3 and 4 ppm (3.67 and 2.67 shoots, respectively).

Shoot length (cm)

The concentration and type of cytokinin and their interaction showed significant effect on shoot length (Table 2). As for the concentration, applying cytokinins at 1 or 2 ppm resulted in higher shoots length (6.33 and 6.00 cm, respectively) compared to concentrations of 0, 3 or 4 ppm (3.45, 3.10 and 2.58 cm, respectively).

Table 2. Effect of cytokinin type, concentration and their interaction on shoot length (cm).

Conc. (ppm)	Cytokinin BAP	Type KIN	Mean
0	3.45 bc	3.45 bc	3.45 B
1	3.17 bc	9.50 a	6.33 A
2	2.50 bc	9.50 a	6.00 A
3	2.13 c	4.07 b	3.10 B
4	2.00 c	3.17 bc	2.58 B
Mean	2.65 B ¹	5.94 A ¹	

Means with the same letter are not significantly different

Cytokinin type revealed significant effect on shoot length. The use of Kin gave higher shoot length than those induced by BAP (5.94 and 2.65 cm, respectively).

Regarding the effect of interaction, using Kin at 1 or 2 ppm gave the highest shoots length (9.50 cm for both combinations). The lowest shoot lengths were obtained when explants were treated by BAP at 3 or 4 ppm (2.13 and 2.00 cm, respectively).



Fig. 1. Effect of cytokinin concentration on number of shoots kinetin 1 ml / L

Shoot fresh weight (g/culture)

Effect of cytokinin concentration, type and their interaction on shoot fresh weight are presented in Table 3. The effect of cytokinin concentration did not show significant impact on shoot fresh weight. However, it could be detected that applying cytokinins at 2 ppm produced the heaviest fresh shoots (9.87 g/cluster), while cytokinins at 4 ppm induced the lightest ones (4.40 g/cluster).

Regarding the effect of cytokinin type did not gave significant effect on shoot fresh weight. Despite this finding, it was noticed that using Kin produced heavier fresh shoots when compared to BAP (6.71 and 6.41 g/cluster, respectively).

As for the interaction, Kin application gave significant increase in fresh shoots weight at 2 ppm (11.56 g/cluster). Applying Kin at 3 or 4 ppm gave the lightest fresh shoots (3.43 g/cluster for both combinations).

Table 3. Effect of cytokinin type, concentration and their interaction on shoot fresh weight (g/cluster)

Conc. (ppm)	Cytokinin	type	Mean
	BAP	KIN	
0	5.48 ab	5.48 ab	5.48 A
1	5.91 ab	9.66 ab	7.79 A
2	8.17 ab	11.56 a	9.87 A
3	7.09 ab	3.43 b	5.26 A
4	5.37 ab	3.43 b	4.40 A
Mean	6.41 A ¹	6.71 A ¹	

Means with the same letter are not significantly different



Fig. 2. Effect of cytokinin concentration on shoot fresh weight (Kinetin 2 ml/l)

Number of leaves

Effect of cytokinin concentration, type and their interaction on number of leaves are presented in **Table 4**.

The effect of cytokinin concentration revealed significant effect on number of leaves. The control treatment or cytokinins at 1 ppm gave higher number of leaves (17.83 and 17.17 leaves, respectively). On the other hand, the highest concentration of cytokinins at 4 ppm resulted in the lowest number of leaves (6.17 leaves).

Table 4. Effect of cytokinin type, concentration and their interaction on number of leaves

Conc. (ppm)	Cytokinin type		Mean
	BAP	KIN	
0	17.83 a	17.83 a	17.83 A
1	14.00 ab	20.33 a	17.17 A
2	12.33 a-c	14.83 ab	13.58 AB
3	7.33 bc	9.33 bc	8.33 BC
4	5.00 c	7.33 bc	6.17 C
Mean	11.30 A ¹	13.93 A ¹	

Means with the same letter are not significantly different

The effect of cytokinin type did not show significant effect on number of leaves. However, it could be noticed that using Kin induced more leaves when compared with BAP (13.93 and 11.30 leaves, respectively).

Concerning the interaction, it showed significant effect on number of leaves. The greatest number of leaves was obtained when explants were treated

with Kin was at 1 ppm followed by non-supplemented medium with cytokinin (20.33 and 17.83 leaves, respectively). Treatments of BAP at 1 or 2 ppm, and Kin at 2 ppm shared also in the highest position (14.00, 12.33 and 14.83 leaves, respectively). Whereas the lowest number of leaves were developed when BAP was used at 4 ppm (5.00 leaves).

Number of roots

Effect of cytokinin concentration, type and their interaction on number of roots is demonstrated in **Table 5**. Cytokinin concentration affected number of roots significantly. The greatest number of root was obtained when no cytokinins were used at all (14.67 roots). Values obtained at 1 ppm concentration occupied the second position (10.58 roots), while applying cytokinins at 3 or 4 ppm resulted in the lowest records (6.33 and 3.83 roots, respectively).

The effect of cytokinin type was significant. The use of Kin significantly increase number of roots when compared to BAP (12.57 and 4.40 roots, respectively).

Concerning for the interaction between cytokinin concentration and type, it showed significant effect on number of roots. The highest number of roots resulted when no cytokinins were applied at all (14.67 roots), followed by Kin at 1, 2 and 3 ppm (13.83, 14.00 and 12.67 roots, respectively). Whereas no roots were observed when BAP was used at 2, 3 or 4 ppm.

Table 5. Effect of cytokinin type, concentration and their interaction on number of roots

Conc. (ppm)	Cytokinin type		Mean
	BAP	KIN	
0	14.67 a	14.67 a	14.67 A
1	7.33 c	13.83 a	10.58 B
2	0.00 d	14.00 a	7.00 BC
3	0.00 d	12.67 ab	6.33 C
4	0.00 d	7.67 bc	3.83 C
Mean	4.40 B ¹	12.57 A ¹	

Means with the same letter are not significantly different

Total chlorophyll

Effect of cytokinin concentration, type and their interaction on total chlorophyll content are presented in **Table 6**. The highest total chlorophyll content resulted when MS medium was free of cytokinins (7.86 mg/g F.W.), while the lowest value resulted when cytokinin level was 4 ppm (3.25 mg/g F.W.).

Regarding the effect of cytokinins type, the use of Kin gave higher content of total chlorophyll when compared to application of BAP (6.59 and 3.99 mg/g F.W., respectively).

As for the interaction, the addition of Kin at 1 ppm produced the highest total chlorophyll content (8.07 mg/g F.W.), while BAP at 4 ppm induced the lowest content (1.85 mg/g F.W.)

Table 6. Effect of cytokinin type, concentration and their interaction on total chlorophyll content (mg/g F.W.)

Conc. (ppm)	Cytokinin BAP	type KIN	Mean
0	7.86	7.86	7.86
1	5.60	8.07	6.84
2	2.59	6.27	4.43
3	2.06	6.08	4.07
4	1.85	4.65	3.25
Mean	3.99	6.59	

DISCUSSION

The findings are in compliance using what a whole lot of authors have noted. of these coincidences **Thao et al (2003)** mentioned that MS medium supplemented with 5 mg/L {BA} gave the best take regeneration of the. xamazonica in addition to A. cuculata.

Muhammad et al (2006) investigated the typically the effect of BAP in addition to kinetin up to 8. 0 mg/L, on take proliferation of banana comprehensive resume. Basrai. they found of which the most of shoots/explant was achieved on MS medium containing 4. zero mg/L BAP. **Ružić and Vujović (2008)** observed that will the highest shoot range and length of shoot obtained on media with 0. 5-1. 0 ppm BA. on the particular contrary, poor multiplication seemed to be achieved on media using kinetin.

Maritano et al (2010) in comparison the effect of BA at 0-1 ppm about *Evolvulus glomeratus* and *E. arizonicus (convolvulaceae)*. They discovered that this hightest number in addition to shoot length of *Evolvulus glomeratus* and *E. arizonicus (convolvulaceae)* were obtained with 1 ppm BA. **Radmann et al (2011)** said that when different BAP concentrations were tested about 'Flordaguard' *Prunus* rootstocks, typically the maximum shoot number for every explant was obtained making use of with 4. 0 mg/L BAP. **Ismail et al (2012)** low concentration associated with BAP (0.1 - 0.5 mg/L) were sufficient with regard to shoot induction from crucial segments

of *Acacia auriculiformis*. **Bhatt et al (2013)** declared that MS moderate supplemented with 2. zero mg/L BA was maximum for the shoot growth of 5 *Alocasia* varieties. **Ling et al (2013)** found that kinetin with 1 mg/L was far better than kinetin at 3-7 mg/L in inducing typically the greatest number of shoots of *Labisia pumila var. alata*, (Fam. *Primulaceae*).

Arab et al (2014) stated the best shoot number associated with the GxN15 (cross of almond x peach)rootstock was found in MS medium supplemented with 1 mg/L BAP. Ashraf et al. (2014)cultured young take buds of *Chlorophytum borivilianum* on MS medium that contains BAP at (0, 2, 4, 6 ppm) in addition to kinetin(0, 2, 3. 8, 5. 7 ppm). They will declared that BAP with 2-6 ppm was considerably effective on shoot propagation, while kin at 2-2. 7 ppm was considerably effective on shoot elongation, compared to the management. **Karatas et al (2014)** observed that maximum take regeneration frequency from explant of *Ceratophyllum demersum* has been obtained at 0. 05 mg/L BA. **Tolera et al (2014)** observed that will MS media fortified using 2 and 3 mg/L BAP were found to have the best number of shoot per explant, shoot size and number of foliage per shoot of sugarcane variety N14 and B41-227, respectively.

Ferdous et al (2015) pointed out that maximum number of shoots along with the longest shoots associated with banana cultivars Amirtasagar and Sabri were induced simply by 0. 5 mg/L BAP. **Sujin et al (2016)** used different concentration (0-6 mg/L) of BAP and kinetin for multiplication of shoots of banana (*Musas sp.*) cv. Chenthuluvan. They found that typically the highest number and span of shoots were noticed in 3 mg/L involving BAP, compared to kinetin exact same concentration.

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تحسين إكثار نبات الألوكاسيا معملياً

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الموجز

للأفرع و4 جزء فى المليون: جاء فى المركز الثانى لصفة عدد الأفرع، والمرتببة الدنيا لصفات طول الأفرع، الوزن الرطب للأفرع، عدد الجذور، محتوى الكلوروفيل الكلى. وعند التفاعل بين نوع وتركيز السيتوكينينات وجد أن: معاملة الكنترول (بدون سيتوكينينات): نالت أعلى الدرجات لصفتي عدد الأوراق وعدد الجذور. 1 جزء فى المليون بنزايلى أدنينين: حصلت على أعلى عدد للأفرع وعدد الأوراق. 2 جزء فى المليون بنزايلى أدنينين: نالت أعلى عدد للأفرع وعدد الأوراق وأقل عدد للجذور. 3 جزء فى المليون بنزايلى أدنينين: أحرزت أعلى عدد للأفرع وأقل طول للأفرع وعدد الجذور. 4 جزء فى المليون بنزايلى أدنينين: نالت أعلى عدد للأفرع وعدد الجذور ومحتوى الكلوروفيل الكلى. 1 جزء فى المليون كينيتينين: نالت أعلى طول للأفرع، عدد الأوراق، عدد الجذور، ومحتوى الكلوروفيل الكلى. 2 جزء فى المليون كينيتينين: حصلت على أعلى الدرجات لصفات طول الأفرع، الوزن الرطب للأفرع، عدد الأوراق وعدد الجذور. 3 جزء فى المليون كينيتينين: أحرزت المركز الأعلى لصفة عدد الجذور، والمركز الثانى لصفة طول الأفرع، والمرتببة الدنيا لصفتي عدد الأفرع والوزن الرطب للأفرع. 4 جزء فى المليون كينيتينين: نالت المركز الثانى لصفة عدد الجذور، والدرجات الدنيا لصفتي عدد الأفرع والوزن الرطب للأفرع. وينصح باستعمال البنزايلى أدنينين بتركيز 3 جزء فى المليون للحصول على أكبر عدد من الأفرع، كم يمكن استعمال الكينيتينين بتركيز 2 جزء فى المليون للحصول على أعلى القيم لصفات طول الفرع، الوزن الرطب للأفرع، وعدد الجذور.

أجريت هذه الدراسة فى معمل زراعة الأنسجة، معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة، مصر فى الفترة من 2015 إلى 2017، لدراسة بعض العوامل المؤثرة على إكثار "ألوكاسيا أمازونيكاً" بواسطة زراعة الأنسجة. من نتائج البحث وجد أن: البنزايلى أدنينين: أعطى أفضل النتائج بالنسبة لصفة عدد الأفرع، وأقل النتائج بالنسبة لصفات التالفة طول الأفرع، الوزن الرطب للأفرع، عدد الجذور، محتوى الكلوروفيل الكلى بينما المعاملة بالكينيتينين: أعطت أفضل النتائج لصفات طول الأفرع، الوزن الرطب للأفرع (وإن يكن التأثير غير معنوى)، عدد الأوراق (وإن يكن التأثير غير معنوى)، عدد الجذور، محتوى الكلوروفيل الكلى.

أما بالنسبة لسيتوكينينات وجد أن: صفر جزء فى المليون (الكنترول): أعطى أفضل النتائج فيما يختص بصفات عدد الأوراق، عدد الجذور ومحتوى الكلوروفيل الكلى، والدرجات الأدنى لصفات عدد الأفرع وطول الأفرع والمعاملة بالسيتوكينيتين بتركيزات: 1 جزء فى المليون: نال أعلى درجة لصفة طول الفرع، عدد الأوراق، والمركز الثانى لصفتي عدد الأفرع والجذور. أما 2 جزء فى المليون: اعطى أفضل النتائج لصفتي طول الأفرع والوزن الرطب للأفرع (وإن كان التأثير غير معنوى للصفة الأخيرة)، عدد الأوراق، ونال هذا التركيز أيضاً المركز الثانى لصفة عدد الأفرع. كذلك 3 جزء فى المليون: أحرز الدرجة العليا لصفة عدد الأفرع، والمركز الأدنى لصفة طول الأفرع، عدد الجذور، الوزن الرطب