Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

IN VITRO PROPAGATION OF KULO VARIETY CHRYSANTHEMUM NODULE EXPLANTS IN MURASHIGE AND SKOOG MEDIA WITH NAPHTALENE ASETIC ACID AND BENZILAMAMINO PURINE

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ABSTRACT

The purpose of this study was to obtain new buds of kulo chrysanthemum by in vitro propagation technique or in vitro multiplication. It's also aimed to discover the influence of NAA and BAP in *kulo chrysanthemum* bud multiplication. This study used completely randomized design which was arranged by factorial with treatments which consisted of 0 ppm ; 0,5 ppm ; 1 ppm of NAA which were combined with 1 ppm ; 2 ppm ; 3 ppm of BAP. The explants used were *kulo chrysanthemum* nodules. Observed variables consisted of: budding time, callusing time, number of buds, height of buds, number of leaves, umber of roots, length of roots, percentage of grown cultures, percentage of unresponsive cultures, percentage of contaminated cultures. Data was analyzed using analysis of variance and continued by 5 % LSD test. The result was that there were interactions between NAA and BAP in their influence on number of buds, height of buds, number of roots, root length. NAA and BAP individually influenced the number of leaves.

KEYWORDS : Kulo chrysanthemum, in vitro, NAA, BAP, nodule explants, bud multiplication.

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INTRODUCTION

Chrysanthemum is a decorative plant with beautiful flowers. Other than as a decoration for gardens or as an attractive potted plant, *chrysanthemum* can also be used for traditional medication, insecticide producing plant and can also be used as a cut flower which can compete with other cut flowers. In Indonesia *chrysanthemum* is a rather popular cut flower and is the highest rank among non-orchid cut flowers. This is because *chrysanthemum* has fragrant scent, varying shapes and sizes and colors, thus having a unique appeal[1]. Demands for cut flower, especially *chrysanthemum* in major cities such as: Jakarta, Bandung, Malang, Manado, and Denpasar increase by an average of 10% per year[2]. In 1993 *chrysanthemum* export was 198,3 ton valued at US $ 243,7 thousand to Japan, Hongkong, Malaysia, and Singapore, while in the same year *Chrysanthemum* import was 3,8 ton, valued at US $ 22,1 thousand from Netherlands and Malaysia, so there was a surplus of US $ 221,6 thousand. In 1999 *chrysanthemum* needs in DKI was 12.220.800 stalks. According to Directorate General of Horticulture, *chrysanthemum* production kept increasing since 2005 to 2010, i.e. in 2005 it was 47.465.794 stalks and in 2010 it was 120.485.701 stalks. This is presented in table 1. The target of Directorate General of Horticulture to 2014 was reaching 352.956.738 stalks production (Table 2). The *chrysanthemum* production consists of various types of *chrysanthemum* and several types of *chrysanthemum* in Indonesia which are developed by various breeding techniques which can be seen in the following figure.

Table 1
NATIONAL CHRYSANTHEMUM PRODUCTION 2005 – 2010

Chrysanthemum Production	YEAR					
	2005 (Stalk)	2006 (Stalk)	2007 (Stalk)	2008 (Stalk)	2009 (Stalk)	2010 (Stalk)
National Chrysanthemum	47.465. 794	63.716. 256	66.979. 260	101.777.126	107.847.072	120.485.701
Increase		16.250.462	3.263.004	34.797. 866	6.069. 946	12.638. 629
Percentage of Increase		34,2%	5,1%	52%	6%	11,7%
Source: Strategic Planning of Directorate General of Horticulture 2010*) Temporary Number						

Table 2
Chrysanthemum Production Target 2010 – 2014 Directorate General of Horticulture

Production	YEAR				
	2010 (Stalk)	2011 (Stalk)	2012 (Stalk)	2013 (Stalk)	2014 (Stalk)
National Production	248.080.490	270.770.892	295.661.436	322.888.796	352.956.738
Target Increase (%)		22.690.402	24.890.544	27.227.360	30.67.942
		9,1 %	9,2 %	9,2 %	9,3 %
Source: Strategic Planning of Directorate General of Horticulture 2010 - 2014					

Figure 1. High Yielding Variety of Chrysanthemum Comodity released by Decorative Plants Research Center 2010 (image source: Balithi Segunung)

Both tables show that *chrysanthemum* is very important so it should be developed to obtain high yield. The flower production is determined by its maintenance, especially in good cultivation. *Chrysanthemum* cultivation highly determines the success of flower formation. If the cultivation isn't proper, the plant won't grow as expected. However, if the maintenance is proper, the result will be good and beautiful. The use of quality seeds will increase production with beautiful flowers. *Chrysanthemum* is also determined by environment, such as climate and soil. With increasing demand for *chrysanthemum*, there should be a better *chrysanthemum* cultivation system, especially in multiplication and seed quality, because conventional multiplication takes longer time and the plant quality produced isn't very good. One of the alternatives to get a large number of plants and in a short time is in vitro multiplication by tissue culture technique. Tissue culture technique is a technique in which an isolated part of a plant, whether cell, protoplasm, tissue or plant organ, is cultured in a suitable environment in aseptic condition, so it can grow and develop into a new plant[3]. The success of this culture technique is determined by several factors such as plant genotype, growth media, growth regulator and other environments 4-5 . Growth regulator is organic compounds in small amounts which can control plant growth and development 6. Sample callus tissue or plant organ piece which is grown in a media and given cytokinin will stimulate the formation of new buds. Growth regulators may be synthetic or fitohormon 7. One of the growth regulators used for bud formation from *chrysanthemum* tip is Benzilaminopurin (BAP). This growth regulator is more effective that cytokinin growth regulators 8. Several studies show that it's more successful in stimulating the formation of *chrysanthemum* buds. The study of Sompotan and Tilaar, 2009 shows that with culture, several *chrysanthemum* explants such as tips, stems, leaves and nodules grown in MS media with 1 to 5 ppm BAP addition produce new buds. However, this hadn't been conducted on local *chrysanthemum* varieties, so bud induction and *chrysanthemum* tip explants would be studied. The type or variety used was local variety from Tomohon, kulo variety. This variety is favored in Tomohon because it has large flower size and is white. Hence, it's called kulo variety, which means white. Tomohon Government tries to develop it as an icon of this region and considers in valuable in giving profit by planting the *chrysanthemum* 9. However there is a problem in seed shortage, which is the beginning of the plant production process. Therefore, to accelerate seed provision tissue culture technique above, also known as in vitro multiplication technique, can be used as a solution. Several studies have been conducted on in vitro multiplication on *chrysanthemum*, but on varieties different from kulo variety, so there is a possibility that the response is different to growth regulator such as auxin and cytokinin. So nodule explants of this kulo variety were studied by growing it in

media which is given NAA and BAP. The purposes of this study were to discover the influence of NAA and BAP on bud multiplication.

RESEARCH METHODS

This study was conducted in Agricultural Biotechnology laboratory of Faculty of Agriculture of Unsrat and lasted 4 months. The design used was factorial pattern completely randomized design. Treatments consisted of factor A with 0 ppm; 0,5 ppm and 1 ppm concentration levels of NAA and factor B with 1 ppm; 2 ppm ; 3 ppm of concentration levels BAP. Each treatment was repeated 5 times. Observed variables were: bud height, number of buds, number of leaves, bud wet weight, number of roots, root length. Statistical analysis used was variance analysis, followed by LSD Test.

RESULTS AND DISCUSSION

In this study the explants used was kulo chrysanthemum bud nodule which was cultured in the study with combined 0 : 0,5 ; 1 ppm NAA and 1 : 2 : 3 ppm BAP treatments. The observation was conducted on several chrysanthemum traits in the culture, such as bud weight, number of buds, bud height, number of roots, number of leaves and root length. They will be discussed in accordance with traits observed in the culture.

1. Number of Buds

The highest number of buds was in 0 ppm of NAA and 1 ppm of BAP combination which was 8,2 buds and the treatment was different from other treatments. This result was nearly the same as the study of 10 which is broccoli hypocotyls planting in cultures with 0,107 uM and 17,76 uM BAP treatments which produces around 6,4 new buds. Adventitious bud regeneration can be done for in vitro plant multiplication as produced in Brassica olerasea sub sp.Green Marvel. 11. 12 states that in hypocotyls explants culture with 1 mgl^{-1} BA 0; 0,1 and 0,2 mgl^{-1} of IBA treatment is a very good combination because it produces 3,5 to 7,4 buds. While 13 with stoloniferous nodule explants of H. Cordata produce 11,00 to 19,40 buds in MS medium which is given 2,22 to 17,74 uM of BAP. The study of Martin, 2004 uses stem internodule explants of *Andrographis paniculata* (Burm.F.) in which explants forms buds at 0,44 uM BAP. This study showed that the higher the BAP concentration, the lower the number of the buds. If 0,5 ppm of NAA was administered with 1 : 2 : 3 ppm of BAP on growth media, the number of the buds decreased. So did the number of buds in media administered with 1 ppm of NAA combined with 1 ; 2 ; 3 ppm BAP. So, high BAP concentration didn't support increase of number of buds, but inhibited it instead. In fact if combined with NAA as auxin it was more inhibiting. Administration of 1 ppm of BAP was enough to stimulate bud increase. The study was similar to the study of 14 on the influence of growth regulator on adventitious bud induction of explants in MS media of all treatments, especially BAP. The result was the higher the BAP concentration, the lower the result of adventitious bud induction. 15 show that bud induction decreases at 4,44 uM of BA and 2,68 uM of BAP combination with number of bud only reaching 3,6.

Table 1

Influence of NAA and BAP Interaction on the Number of Kulo Chrysanthemum Buds

Treatment NAA/ BAP (ppm)	Number of Buds	Notation
NAA 0 BAP 1	8,2	d
NAA 0 BAP 2	5,6	c
NAA 0 BAP 3	2.8	b
NAA 0,5 BAP 1	2,4	b
NAA 0,5 BAP 2	1,8	a
NAA 0,5 BAP 3	2,2	b
NAA 1 BAP 1	1,8	a
NAA 1 BAP 2	2,2	b
NAA 1 BAP 3	1,4	a
LSD 5 %	1,6	

2. Bud weight

Analysis of variance result showed that there was no interaction between NAA and BAP treatments. The analysis result showed that NAA didn't have significant influence on bud weight. However, there was a strongly significant influence of BAP on the weight of buds multiplied by plant tissue culture. This is shown in table 2.

Table 2
Influence of BAP on Chrysanthemum Bud Weight

Treatments BAP/ppm	Average wet weight bud /g	notation
1	0,42	a
2	0,62	b
3	0,22	a
5 % LSD	0,36	

The result of analysis of variance showed that only BAP influenced bud weight. It meant that bud weight was only determined by BAP while the combination of NAA and BAP as well as NAA itself didn't have significant influence on bud weight. Bud formation in bud nodule culture from induction stage was strongly influenced by BAP as one of the cytokinins. This BAP cytokinins could stimulate the formation of new buds in the culture. The large amount of formed buds made bud weight bigger and this was strongly influenced by BAP. The highest average of bud weight was in 2 ppm treatment which was 0,62 g. At 3 ppm of BAP concentration, bud weight was lower than other treatments, 1 and 2 ppm of BAP. This result showed that higher BAP concentration decreased new bud multiplication. Therefore the best concentration for bud weight was 2 ppm of BAP. That study was similar to the study of 14 which is about the influence of growth regulator on adventitious bud induction of explants in MS media of all treatments, especially BAP. The result is the higher the BAP concentration, the lower the adventitious bud induction result.

3. Bud height

The result of analysis of variance showed there was interaction between NAA and BAP in their influence on bud weight. So both factors, NAA and BAP, supported each other in increasing bud height. This is presented in table 3.

Table 3
The Influence of NAA and BAP Interaction On Bud Height

Treatments NAA/BAP(ppm)	Average Bud Height(Cm)	Notation
NAA 0 BAP 1	2,44	d
NAA 0 BAP 2	2,42	d
NAA 0 BAP 3	1,02	b
NAA0,5 BAP 1	3,46	e
NAA0,5 BAP 2	3,1	e
NAA 0,5 BAP 3	1,64	c
NAA 1 BAP 1	5,0	f
NAA 1 BAP 2	1,3	b
NAA 1 BAP 3	0,48	a
LSD 5 %	0,43	

The result of analysis of variance showed that F count was bigger than F Table of NAA and BAP combination. Both Growth Regulators supported the increase of bud height, especially NAA which influenced cell division and elongation. The result in this table showed that NAA had very significant on bud height. Auxins such as NAA stimulate cell division and cell elongation, so the role of NAA was very big in NAA and BAP interaction on bud height. 1 ppm of BAP concentration combined with all NAA concentrations in the treatments

support bud height. The best combination was 1 ppm of NAA and 1 ppm of BAP with 5 cm bud height. 16 in the study on in vitro broccoli produces the tallest bud in a treatment without NAA and BAP, but 1 ppm of NAA and 5 ppm of BAP concentration can increase bud height. It shows that NAA and BAP balance can stimulate the increase of bud height in the culture. The higher the BAP concentration combined with NAA, bud height growth is more inhibited. The study has the same response as the study of 15 in Terminalia arjuna Roxb. That the higher the BAP combined with NAA, the lower the bud height. In it the plant nodule explants grown in MS media administered with 2,22 uM of BAP combined with 0,05 uM, 0,53 uM and 2,68 uM of NAA produces bud length/height of 1,5 cm ; 1,6 cm and 0,5 cm, respectively.

4. Number of Leaves

The result of analysis of variance showed that there was no interaction between NAA and BAP in their influence on the number of leaves, however, NAA and BAP separately had significant influence on the number of leaves. NAA as well BAP played a role in morphogenesis and cell division and cell elongation for leaf enlargement. So NAA and BAP had significant influence in leaf formation and enlargement so that the number of leaves could increase. According to 6 cytokinin plays a role in cell division and cell elongation, even in leaf morphogenesis .

Table 4a
The Influence of NAA on Number Of Leaves

Treatment NAA/ppm	Average Number of Leaves	Notation
0	31,46	b
0,5	16,13	a
1	10,93	a
LSD 5 %	7,456	

Table 4 b
The Influence of BAP on Number of Leaves

Treatment BAP (ppm)	Average Number of Leaves	Notation
1	12	a
2	18	a
3	28	b
LSD 5 %	7,456	

The data of Table 4a showed increased NAA concentration would suppress the increase of the number of leaves. Conversely the data in table 4b showed that the higher the BAP concentration, the higher the increase of the number of leaves. This was because there were increased bud height and number of buds, so the number of leaves increased. So, BAP had an important role in leaf morphogenesis. The result of this study was the same as the result on broccoli culture by 16.

5. Number of Roots

The result of analysis of variance showed that there was significant interaction between NAA and BAP in their influence on the number of roots. So, both growth regulators supported each other in influencing root formation. NAA and BAP interacted to stimulate root formation. But in this case NAA as an auxin played a very important role in root formation so the role in stimulating root morphogenesis was bigger than BAP (Table 5). George E.F and P.D f (l984) state that auxin influenced root formation. Present data showed that NAA had an important role in root formation. So, it's similar to the statement of George and Sherrington that auxin plays a role in root formation.

Table 5
The Influence of NAA and BAP Interaction of the Number of Roots

Treatments NAA/BAP (ppm)	Average Number of Roots	Notation
NAA 0 BAP 1	0	a
NAA 0 BAP 2	0	a
NAA 0 BAP 3	0	a
NAA 0,5 BAP 1	1,6	a
NAA 0,5 BAP 2	9,8	b
NAA 0,5 BAP 3	10,4	b
NAA 1 BAP 1	12,8	c
NAA 1 BAP 2	8,0	b
NAA 1 BAP 3	7,8	b
LSD 5 %	2,24	

Root didn't form in MS Media administered with 1 ; 2 ; 3 ppm of BAP without NAA. The result showed that Benzilaminopurin (BAP) inhibited root formation. BAP as cytokinin only stimulated bud formation and nor for rooting. While when administered with 0,5 ppm of NAA combined 1 : 2 : 3 ppm of BAP roots started to grow. In the treatments there was root increase. 0,5 ppm concentration combined with 3 ppm of BAP had higher number of roots than when combined with 1 and 2 ppm of BAP. But the combinations of 0,5 ppm NAA and 2 ; 3 ppm BAP didn't have significant influence, so it was suspected that there was some inhibition in root formation which influenced the increase of the number of roots. The combination 1 ppm of NAA and 1 ppm of BAP showed the highest number of roots. The interaction between NAA and BAP was strongly significant in influencing the number of roots. While if there were 2 and 3 ppm of BAP additions combined with 1 ppm of NAA concentration, there was inhibition in root formation for the increase of the number of roots. So, the number of roots only increased at certain concentrations, such as 0,5 ppm of and 2 ppm of BAP combinations and 1 ppm of NAA and 1 ppm of BAP combination. If NAA and BAP concentrations were even, they would support each other to influence the number of roots.

6. Root Length

The result of analysis of variance showed that there was a strongly significant interaction between NAA and BAP on root length (Table 6). Both growth regulators stimulate root growth so roots became longer. Auxin stimulates cell division and elongation while cytokinin stimulates cell division 17.

Table 6
The Influence of NAA and BAP On Root Length.

Treatment NAA/BAP (ppm)	Average Root Length (cm)	Notation
NAA 0 BAP 1	0	a
NAA 0 BAP 2	0	a
NAA 0 BAP 3	0	a
NAA 0,5 BAP 1	0,74	b
NAA 0,5 BAP 2	1,46	d
NAA 0,5 BAP 3	1,06	c
NAA 1 BAP 1	5,48	f
NAA 1 BAP 2	2,78	e
NAA 1 BAP 3	0,9	b
LSD 5 %	3,2	

Data of table 6 showed that BAP without NAA inhibited root formation and elongation. While the combination of 0,5 ppm of NAA and 1 ; 2 ; 3 ppm of BAP and also the combination of 1 ppm of NAA and 1; 2; 3 ppm of BAP caused root formation and elongation. 1 ppm of NAA and 1 ppm of

BAP combination strongly stimulates root elongation and had different root length from other treatments. The data of the table above showed that high BAP concentration could inhibit root elongation.

CONCLUSION

There was interaction between NAA and BAP in their influence on number of buds, bud height, number of roots, root length. NAA and BAP separately influenced the number of leaves.

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