Journal of Tropical Crop Science Vol. 4 No. 3, October 2017
www.j-tropical-crops.com

In Vitro Adventitious Shoot Proliferation of Three Basil Species (*Ocimum* sp. L.) by Addition of Naphthalene Acetic Acid (NAA) and Benzyl Amino Purine (BAP)

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Abstract

Basils are mainly used as food flavoring and source of aromatic oil, but the leaves have also been used for herbals and cosmetics. Basil is propagated by seeds but the germination is often low in the field. This study aims to investigate the effect of different concentrations of NAA (Naphthalene Acetic Acid) and BAP (Benzyl Amino Purine) to induce the adventitious shoot proliferation of three species of basil (*Ocimum* sp. L.) *in vitro*. The research was conducted at the Tissue Culture Laboratory II of the Department of Agronomy and Horticulture,Bogor Agricultural University (IPB), Indonesia, from November 2016 to October 2017. This research consisted of three separate experiments with one basil species for each experiment, Lemon Basil, “Greek Minette”, and “Thai Siam Queen”. The experiments were organized in a completely randomized block design with two factors: NAA concentrations (0, 0.5, and 1 mg.L^{-1}) and BAP (0, 1, 2, and 3 mg.L^{-1}) using Murashige and Skoog (MS) medium supplied with 25 ml.L^{-1} coconut water. The three basil species responded differently to NAA and BAP in the media. The optimum concentration of NAA and BAP to induce callus growth in Lemon Basil was 0.5 mg.L^{-1} and 3 mg.L^{-1}, respectively, with BAP alone showing the best effect on proliferation. In “Greek Minette”, however, the addition of NAA and BAP had no significant effect on the callus growth, proliferation, height, and number of leaf, node and root per explant. Media without growth regulators had the best results in increasing height, explant proliferation rate, number of nodes and shoots, and percentage of proliferating shoots of “Thai Siam Queen”.

Keywords: cytokinin, “Greek Minette”, Lemon Basil, “Thai Siam Queen”, synthetic auxin

Introduction

Basil (*Ocimum* sp.) is an annual herbaceous plant that has various benefits as it can be used as a vegetable, food flavoring and herbals (Setiari and Nurchayati, 2009). Plants belonging to the *Ocimum* genus can grow in the lowlands at an altitude of 1100 meters above sea level and contains essential oils, saponins, flavonoids and tannins (Shahabuddin, 2015) which can be used as a biological pesticides and to prevent a wide arrays of medical conditions including tumors (Sentana, 2010).

There are different species of basils, including Lemon Basil (*Ocinum sactum),* Basil *(Ocimum basilicum)*, and Sweet Basil *(Ocimum spp.)*. Basil is propagated by seeds, but the seeds have a low germination rate (34%) particularly during physiological maturity (Suwarno *et al,* 2014). Propagation *in vitro* can potentially increase a more rapid generation of explants of *Ocimum* sp. L. as compared to propagation by seeds (Lewinsohn et al ., 2000). As basil is normally consumed fresh it is important that basil leaves are free of pesticides and microorganisms. Restaurants in star hotels have started to include little basil potted plants as addition to the menu. Basil potted plants that are propagated *in vitro* is relatively free of microorganisms and pesticides. The advantage of tissue culture techniques is that it can multiply pesticide and microorganism-free explants with shorter growing cycle, not seasonally dependent, and do not require large space to grow (Sriyanti, 2000).

Propagation through tissue culture can be successful by considering several factors including explant genotype, type of base medium, with the type and concentration of growth regulator used (Kosmiatin et al., 2005). Solid media such as Murashige and Skoog (MS) contains high nitrate, ammonium, and potassium (Wattimena et al., 2011). Growth regulator can be added to the media to help stimulate growth and development of the explant.

Several growth regulators that have been used in tissue culture propagation include auxin, cytokinin, and gibberellin (Gunawan, 1992). Naphthalene Acetic Acid (NAA) is a synthetic auxin that can influence apical dominance, inhibition of axillary and adventitious shoots (Karlianda et al., 2013) and can induce callus growth in explants (Widyawati, 2010). Benzyl Amino Purine (BAP) is a type of cytokinin that stimulates cell division in tissue cultured explant and may stimulate the growth of shoots (Widyawati, 2010). Rahmi et al. (2010) reported that the application of 2.5 mg.L^{-1} BAP on MS medium can increase the proliferation and induced earlier shoot growth in orange. Coconut water is an organic compound containing Growth hormone regulation from auxin and cytokinin groups (Surachman, 2011). The use of coconut water in tissue culture medium is very important to control organogenesis and morphogenesis in shoot and root formation and the formation and development of callus (Lestari, 2011). A study by Karjadi and Burchory, (2008) showed that the combination of NAA, BAP, as well as the addition of coconut water to MS medium was able to induce the growth of meristem tissues in potato culture. Based on these reports addition of NAA and BAP to the basal MS media is expected to increase the proliferation of basil adventitious shoots.

The purpose of this research was to examine the effect of (NAA and BAP concentration on the standard MS media) in inducing the adventitious shoot proliferation of Basil *(Ocimum citratum),* ”Greek Minette” *(Ocimum basilicum),* and “Thai Siam Queen” *(Ocimum basilicum) in vitro.*

Materials and Methods

This research was conducted in the tissue Culture Laboratory II of the School of Agronomy and Horticulture at the Bogor Agricultural University in November 2016 to September 2017. The study consisted of three separate experiments using different basil species. The experiment consisted of 12 treatments, with three replications. Each experimental unit had nine explants so that there were 324 units of experimental observation. The species used in this study were Lemon Basil, which were obtained from seeds, and ”Greek Minette” and ”Thai Siam Queen” which were both obtained from plantlets derived from subculture of shoots that were previously maintained at Tissue Culture Laboratory II.

Basil seeds were soaked in 2 g.L^{-1} fungicide Dithane M-45 (a.i. Mancozeb) and 2 g.L $^{-1}$ bactericide Agrept (a.i. streptomycin sulphate) overnight. The seeds were then filtered using a sieve while pressed slowly to separate the seed from the mucus. The filtered seeds were then soaked in 30% Clorox for 30 minutes while shaking and soaked in GA_3 for two days prior to surface sterilized with 10% Chlorox for 10 minutes. Seeds were then grown in a MS0 + 4 mg.L^{-1}CaP medium. Germinated seeds were left to grown for six weeks and sub cultured on MS13K media (MS + 0.1 mg.L^{-1} IAA + 1.5 mg.L^{-1} 2IP + 4 mg.L^{-1} CaP + 100 ml.L^{-1} coconut water + 30 g.L^{-1} sugar). The treatment consist of MS + 4 mg.L^{-1} CaP + 25 ml.L^{-1} coconut water and added with BAP and NAA in accordance with the treatment (described below) and a control medium of MS + 4 mg.L^{-1} CaP + 25 ml.L^{-1} coconut water without addition of NAA and BAP. The media used 7 g.L^{-1} agar, 30 g.L^{-1} sugar, and pH was set to 6.0 before autoclaving.

This research was organized in a completely randomized factorial design with two factors, NAA and BAP concentrations, conducted on three basil species, Lemon Basil, ”Greek Minette”, and “Thai Siam Queen”. The concentrations of NAA were 0; 0.5 and 1 mg.L^{-1} and the BAP concentration consisted of four levels, 0, 1, 2, and 3 mg.L^{-1} and a control without growth regulators. Each culture used 25 ml of media. Shoot explants from 6-week-old plants were incubated in a culture room with a temperature of 22 ± 2^{o}C with a light intensity of ± 1340 lux and a photoperiod of 24 hours each day.

Scoring was conducted on (1) seedling germination percentage; (2) the percentage of callus on explants; (4) number of shoots in each explant, which is from the number of shoots growing; (5) plantlet height, measured from the base of the stem to the tip of the shoot; (6) the number of nodes on each explant by counting the segment marked with the appearance of the leaf from the bottom of the node to the tip of the shoot (excluding shoots); (7) the number of leaves per explant was calculated from each explant marked by leaves that fully opened; (8) number of roots per explant. Root number per explant was determined using scores where score 1 is for explants with <5 roots per explant; score 2 for explants with 6 to 10 roots; score 3 for explants with 11 to 15 roots; and score 4 for explants with 16 to 20 roots, and score 5 for explants with more than 20 roots. Scoring was conducted weekly for eight weeks after planting.

The data was analyzed using Microsoft Excel and SAS (Statistical Analysis System) version 9.0 with F-test at 5% level to determine the effects of treatments. Treatments that had significant effects were tested further using Duncan Multiple Range Test (DMRT) at 5% level.

Results and Discussion

Callus Formation

Callus is undifferentiated cells which is usually formed on one or all slices of explant and is caused by tissue injury and hormone explant response (Andaryani, 2010). Addition of 0.5 mg.L^{-1} of NAA had the best callus formation whereas medium without NAA gives the lowest callus formation. However, a higher concentration of NAA tends to decrease the number of Lemon Basil explants with callus. The addition of BAP of 1 mg.L^{-1} in Lemon Basil gave more shoots per explant than without BAP. Application of BAP at high concentrations decreased the formation of new shoots.

Similar to Lemon Basil, addition of NAA resulted in more callus formation in “Greek Minette” compared to without NAA, but differences were noted between NAA at 0.5 and 1 mg.L^{-1}. BAP had no significant effect on callus formation in “Greek Minette” (Table 1). In “Thai Siam Queen”, the addition of NAA and BAP had no significant effect on callus formation. It is possible that the concentrations of NAA and BAP used were not optimal for this species (Widyawati et al., 2010).

The callus was brown in color and had crumbs texture which may be due to the presence of high phenolic compounds in the explant, as reported by Purba et al. (2008). According to Andarwulan et al (2010) basil has a phenol content of 0.812 ± 0.119 mg GAE (Gallic Acid Equivalent). The brown and crumbed calli were not only found in the “Greek Minette” callus, but also in the two other basil species, Lemon Basil and “Thai Siam Queen”.

The Formation of Adventitious Shoots on Basil Explants

In this study some adventitious shoots developed from callus differentiation. According to Lawalata (2011), adventitious shoots can grow from callus induced in response to growth regulators. Adventitious shoots were developed on the explants of Lemon Basil, “Greek Minette”, and “Thai Siam Queen” starting two to three weeks after planting.

The interaction between NAA and BAP at week four, six, and eight had no significant effect on adventitious shoot formation on Lemon Basil explants (Table 2). NAA in various concentrations did not affect the number of Basil shoots. BAP significantly increased the number of shoots compared to the control (Table 3).

There was no significant interaction between NAA and BAP in affecting “Greek Minette” shoots per explant at week four, six and eight (Table 3). Media without NAA had the highest increase in shoot numbers whereas media with NAA at 0.5 mg.L^{-1} had the lowest and did not differ significantly with NAA at 1 mg.L^{-1}. BAP increased the number of adventitious shoots significantly on the “Greek Minette” explant at weeks six and eight (Table 3).

Table 1. Percentage of explant with callus of the three basil species in MS media supplemented with different concentrations of NAA and BAP

Treatment	Explant with callus (%)					
	Lemon Basil		“Greek Minette”		“Thai Siam Queen”	
	4 WAP	8 WAP	4 WAP	8 WAP	4 WAP	8 WAP
NAA (mg.L^{-1})						
0	1.85b	25.93	1.00b	2.78b	5.56	22.22
0.5	**20.36a**	47.22	**11.11a**	**36.11a**	11.11	52.78
1	10.19b	30.56	**19.44a**	**47.22**	19.44	55.56
NAA	**	ns	*	**	ns	ns
BAP (mg.L^{-1})						
0	2.15b	4.94b	1.00	8.64	11.11	37.04
1	**18.52a**	**51.85a**	7.41	24.69	9.88	51.85
2	4.93ab	40.74a	11.11	37.04	17.28	44.44
3	7.28ab	40.74a	22.22	44.44	9.88	40.74
BAP	*	**	ns	ns	ns	ns
NAA X BAP	**	ns	ns	ns	ns	ns
CV (%)[1)]	10.33	8.11	11.60	9.91	11.51	8.80

Note: * significantly different at 5%; ** significantly different at 1 %; ns= non-significantly different; [1)] the CV values were transformed (x + 0.5); the values followed by different letters in the same column are significantly different according to DMRT test at 5%.

The combination of NAA and BAP in “Thai Siam Queen” showed similar results to the Lemon Basil and “Greek Minette”, which was not significant during the course of the study (Table 2). Explants on media without NAA had the highest number of shoots per explant (Table 2). Media without the BAP also showed the best results in increasing the number of shoots whereas at high concentration (3 mg.L$^{-1)}$ produced the lowest number of shoots. Rahmi et al. (2010) suggested that shoot proliferation requires a high concentration of cytokinin, or auxin at very low concentrations. Shoot proliferation of the three basil species in this study can be seen in Figure 1.

Number of Leaves per Explant

The number of leaf is very important as the leaf is the main part of the basil plants for consumption (Rukmana and Yudirachman, 2016). Table 8 shows that NAA and BAP had no effects on “Greek Minette” and “Thai Siam Queen” explants, but had significant interaction in affecting the number of leaf in Lemon Basil. Explants on media with NAA at 0.5 mg.L^{-1} and BAP of 1 mg.L^{-1} had the lowest number of leaves.

In “Greek Minette” media without NAA had more leaves per explant compared to the media with NAA whereas NAA at 0.5 mg.L^{-1} and BAP of 1 mg.L-1 had the lowest number of leaf per explant.

The increase in NAA concentration in “Thai Siam Queen” did not increase the number of leaves per explant possibly because NAA has roles in inducing callus formation, suspension culture, and and root formation through increased cell division within the cambium tissue (Fitriani, 2008). Explants with BAP at 1 mg.L^{-1} had the highest number of leaf per explant even though it was not significantly different from media without BAP. However, the highest concentration of BAP at 3 mg.L^{-1} resulted in the lowest number of leaf in “Thai Siam Queen” (Table 3).

Table 2. Shoot proliferation of three basil species in MS media supplemented with different concentrations of NAA and BAP

Treatment	Number of shoots per explant								
	Lemon Basil			“Greek Minette”			“Thai Siam Queen”		
	4 WAP	6 WAP	8 WAP	4 WAP	6 WAP	8 WAP	4 WAP	6 WAP	8 WAP
NAA (mg.L^{-1})									
0	1.6	3.2	5.2	**3.8a**	**4.8a**	**5.6a**	**4.7a**	**5.4a**	**6.6a**
0.5	1.7	3.4	5.4	2.4b	3.4b	3.7b	**3.3b**	**5.4b**	**5.6b**
1	1.7	3.3	5.2	2.4b	3.6b	3.9b	3.6b	4.7ab	6.5ab
NAA	ns	ns	ns	**	*	**	*	*	*
BAP (mg.L^{-1})									
0	**0.9b**	3.6	5.0b	2.5	**4.9**	**5.5a**	**4.4a**	**9.7a**	**9.2a**
1	**2.1a**	3.5	**6.2a**	3.4	4.4	4.9ab	4.3a	6.1ab	7.4ab
2	1.8a	3.1	5.6ab	2.7	3.3	**3.5b**	4.0ab	5.2bc	6.0bc
3	1.7a	3.0	**4.5b**	2.9	3.2	3.6b	**2.9b**	**3.8c**	**4.5c**
BAP	**	ns	*	ns	*	*	*	**	**
NAA X BAP	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%) [1)]	29.3	21.0	21.0	36.8	30.6	30.9	31.0	33.7	33.0

Note: * significantly different at 5%; ** significantly different at 1 %; ns= non-significantly different; [1)] the CV values were transformed (x + 0.5); the values followed by different letters in the same column are significantly difference according to DMRT test at 5%.

Figure 1. Shoot proliferation of three basil species; left: Lemon Basil, middle: “Greek Minette”, right: “Thai Siam Queen”.

Table 3. Number of leaf per explant of the three basil species in MS media supplemented with different concentrations of NAA and BAP

Treatment	Number of leaf per explant								
	Lemon Basil			“Greek Minette”			“Thai Siam Queen”		
	4 WAP	6 WAP	8 WAP	4 WAP	6 WAP	8 WAP	4 WAP	6 WAP	8 WAP
NAA (mg.L^{-1})									
0	7.5	18.3	**26.7a**	**5.2a**	**19.2a**	**23.6a**	**19.0a**	25.7	28.3
0.5	7.9	18.3	23.7ab	10.5b	13.3b	14.2b	14.7b	18.6	21.2
1	7.6	19.8	**22.4b**	10.4b	13.3b	15.0b	15.5b	20.3	24.5
NAA	ns	ns	*	**	**	**	**	ns	ns
BAP (mg.L^{-1})									
0	**9.7a**	18.8a	**20.7b**	12.1	**17.4a**	20.2	17.6a	**27.5a**	30.9
1	7.3b	**21.1a**	**26.3a**	14.6	**17.9a**	19.9	**18.5a**	22.4b	26.5
2	7.2b	19.0a	**26.5a**	9.9	13.6b	15.4	16.8a	20.1bc	22.2
3	6.4b	**15.8b**	23.5ab	11.6	12.9b	14.9	**12.7b**	**16.2c**	19.2
BAP	**	**	**	ns	**	ns	**	*	ns
NAA X BAP	ns	**	ns	ns	ns	ns	ns	ns	ns
CV (%) [1)]	1 9.2	15.2	15.3	22.7	25.3	28.3	18.9	21.5	24.2

Note: * significantly different at 5%; ** significantly different at 1 %; ns= non-significantly different; [1)] the CV values were transformed (x + 0.5); the values followed by different letters in the same column are significantly different according to DMRT test at 5%.

Height of Explants

Explants on media with NAA at 1 mg.L^{-1} were the tallest whereas explants without or with low NAA (0.5 mg.L^{-1}) were the shortest (Table 4). NAA and BAP did not interact in affecting height of “Greek Minette” explants. The medium without NAA had the tallest explants as compared to the medium with the addition of NAA. However, there was a significant effect of BAP on the height of “Greek Minette”; explants on the media with the addition of 3 mg.L^{-1} of BAP were the shortest.

The tallest explant of “Greek Minette” and “Thai Siam Queen” were those grown on media without growth regulators, and increasing concentrations of NAA or BAP decreased explant height (Table 4). Media without growth regulators also had the highest callus formation in all basil species (Table 1).

Number of Nodes per Explant

The number of node is very important for proliferation *in vitro*. An increase in the number of node could potentially increase the number of shoot with leaves, which are the final products desired in basil (Rukmana and Yudirachman, 2016). NAA and BAP had no effect in the number of nodes in “Greek Minette” and “Thai Siam Queen” but had significant effects on Lemon Basil (Table 5). Medium without NAA and BAP had the highest number of nodes whereas BAP at 2 or 3 mg.L^{-1} without NAA had the lowest. Adding NAA to the media had no significant effect in the formation of basil node at weeks 4 and 6. The highest number of node per explants was from the explants grown on media without BAP.

NAA did not interact with BAP in affecting number of nodes “Greek Minette” whereas NAA significantly reduced node formation. “Greek Minette” explants in media without growth regulator, or with BAP at a low concentration had more nodes per explant (Table 5).

Similar to “Greek Minette”, NAA did not interact with BAP in affecting the number of nodes per explant in “Thai Siam Queen” (Table 6). NAA had no effects, whereas media without BAP generated the highest number of nodes (Table 5).

Table 5. Effect of NAA and BAP on number of node of the three basil species in MS media supplemented with different concentrations of NAA and BAP

Number of Roots per Explant

Root formation is important for acclimatization which is the next step to get the clean basil explants and ready grow on. On Lemon Basil NAA at 1 mg.L^{-1} or BAP at 1 mg.L^{-1} resulted in the highest number of root per explant.

Media without growth regulators produced more roots than the media with the addition of BAP, and high concentration of BAP decreased the number of roots

Table 4. Effect of NAA and BAP on explant height of the three basil species in MS media supplemented with different concentrations of NAA and BAP

Treatment	Explant height								
	Lemon Basil			"Greek Minette"			"Thai Siam Queen"		
	4 WAP	6 WAP	8 WAP	4 WAP	6 WAP	8 WAP	4 WAP	6 WAP	8 WAP
NAA (mg.L^{-1})									
0	14.10	30.54b	58.09	18.70	**25.22a**	**27.91a**	13.40	**20.44a**	21.17
0.5	5.15	33.33b	58.93	14.66	**18.09b**	**19.42b**	10.92	15.20b	20.57
1	6.73	**43.58a**	66.89	17.06	21.65ab	22.01b	11.74	17.65ab	22.69
NAA	ns	**	*	ns	*	*	ns	*	ns
BAP (mg.L^{-1})									
0	**32.95a**	**73.48a**	**79.57a**	**19.49a**	**26.54a**	**29.04a**	**14.98a**	**23.00a**	**30.70a**
1	11.16b	32.57b	67.64b	18.74ab	23.38ab	24.93ab	12.11b	19.52ab	24.42ab
2	8.69b	19.97c	57.59b	14.50b	19.06b	20.36bc	11.48bc	16.20bc	20.90bc
3	8.50b	17.26c	40.42c	**14.49b**	**17.63b**	**18.12c**	**9.51c**	**12.45c**	**15.73c**
BAP	**	*	**	*	*	**	**	**	**
NAA X BAP	ns	ns	ns	ns	ns	Ns	*	*	ns
CV (%) [1)]	21.44	15.20	19.19	25.30	27.63	28.38	19.78	24.79	33.76

Note: * significantly different at 5%; ** significantly different at 1 %; ns= non-significantly different; [1)] the CV values were transformed (x + 0.5); the values followed by different letters in the same column are significantly difference according to DMRT test at 5%.

Table 5. Effect of NAA and BAP on number of node of the three basil species in MS media supplemented with different concentrations of NAA and BAP

Treatment	Number of node per explant								
	Lemon Basil			"Greek Minette"			"Thai Siam Queen"		
	4 WAP	6 WAP	8 WAP	4 WAP	6 WAP	8 WAP	4 WAP	6 WAP	8 WAP
NAA (mg.L^{-1})									
0	0.7	1.6	3.5b	**1.9a**	**3.0a**	**3.8a**	2.7	**3 3.9a**	4.8
0.5	0.7	1.8	4.3ab	0.9b	1.5b	1.7b	2.0	3.0b	3.2
1	0.5	2.2	4.4a	0.9b	1.4b	1.6b	2.0	2.4ab	3.8
NAA	ns	ns	*	**	**	**	ns	*	ns
BAP (mg.L^{-1})									
0	**1.4a**	**3.0a**	3.0c	1.4	**2.6a**	**3.2a**	**3.3a**	**4.8a**	**5.6a**
1	0.5b	**2.7a**	**5.9a**	1.6	2.5a	2.9ab	2.1b	3.3b	4.2ab
2	0.3b	1.0b	4.4b	1.0	1.5b	2.0bc	2.0b	2.6bc	3.7b
3	0.3b	0.8b	2.6c	1.0	1.3b	1.4c	1.5b	1.8c	2.1c
BAP	**	**	**	ns	**	**	**	ns	ns
NAA X BAP	ns	*	**	ns	ns	ns	ns	6.1	6.5
CV (%) [1)]	7.7	6.3	21.4	7.8	6.7	6.7	6.4	ns	ns

Note: * significantly different at 5%; ** significantly different at 1 %; ns= non-significantly different; [1)] the values of CV were transformed (x + 0.5); the values followed by different letters in the same column are significantly difference according to DMRT test at 5%.

Table 7. Effect of NAA and BAP on root formation of the three basil species in MS media supplemented with different concentrations of NAA and BAP

Treatment	Number of roots per explant					
	Lemon Basil		"Greek Minette"		"Thai Siam Queen"	
	4 WAP	8 WAP	4 WAP	8 WAP	4 WAP	8 WAP
NAA (mg.L^{-1})						
0	**1.19a**	1.50	1.17	**1.80a**	1.33	3.03
0.5	1.00b	1.47	1.08	1.30ab	1.11	3.06
1	**1.00b**	1.61	1.00	**1.02b**	1.36	2.91
NAA	**	ns	ns	*	ns	ns
BAP (mg.L^{-1})						
0	**1.26a**	1.33b	1.18	1.55	**2.00a**	**3.96a**
1	1.00b	**1.77a**	1.14	1.74	1.07b	3.19ab
2	1.00b	1.74a	1.00	1.22	**1.00b**	2.81ab
3	1.00b	**1.25b**	1.00	1.00	**1.00b**	**2.04b**
BAP	**	*	ns	ns	**	*
NAA X BAP	**	**	ns	ns	ns	ns
CV (%) [1)]	5.21	27.38	22.93	6.99	21.46	6.66t

Note: * significantly different at 5%; ** significantly different at 1%; ns= non significantly different; [1)] the values of CV were transformed (x + 0.5); values followed by different letters in the same column are significantly different according to DMRT test at 5%.

in "Thai Siam Queen" (Table 7).
Addition of growth regulators to "Greek Minette" and "Thai Siam Queen" culture did not improve root initiation. It is possible that because the media in this study used coconut water that contains auxin (Surachman, 2011) so additional auxin did not increase root formation (Fitriani, 2008). NAA (auxin) combined with high concentrations of cytokinin inhibited root formation (Dode et al., 2003).

Acknowledgement

The authors thank Dr Krisantini for providing the "Greek Minette" and "Thai Siam Queen" seeds, and for her comments and suggestions for this manuscript.

Conclusion

Three species of basil responded differently to NAA and BAP. In "Greek Minette" BAP induced earlier shoot formation and shoot proliferation, increased the number of leaves, shoot height and number of nodes; NAA at 2 mg.L^{-1} and BAP at 2 mg.L^{-1} resulted in the highest number of proliferating explants. Media without growth regulators provide the best results in increasing height, explant proliferation rate, number of shoots, number of nodes and percentage of proliferating shoots of "Thai Siam Queen". In Lemon Basil 1 mg.L^{-1} BAP or 2 mg.L^{-1} NAA with 2 mg.L^{-1} BAP resulted in the proliferation of explants and the highest total number of shoots. Media with 2 mg.L^{-1} NAA with 2 mg.L^{-1} BAP had the highest number of nodes.

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