The Effect of NAA Concentration and Different Parts of Stem on Growth of Patchouli (*Pogostemon cablin* Benth.)

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Abstract: The need for patchouli is increasing with the increase of population and the development of the cosmetics industry. The supply of healthy and of high production patchouli cutting is necessary to ensure the optimum of production. The purpose of this study was to determine the effect of NAA concentration and the right parts source of cuttings and the interaction between the two on the growth of patchouli. The study was conducted in the Experimental Field and Plant Physiology Laboratory of the Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh, in January to April 2019. The study used a factorial randomized block design with a 4x3 factorial pattern with three replications. NAA was applied using *Growtone*, a brand with an NAA concentration of 3%. Factors studied were *Growtone* concentration at 0, 4.0, 8.0, and 12.0 g L^{-1} water, and parts of stem source (shoots, middle, and base). The results showed that the best growth of patchouli cuttings was at *Growtone* concentration of 4.0 g L^{-1} water, while the best shoot length and leaf area was found in the treatment of *Growtone* concentration of 12.0 g L^{-1} water. Meanwhile, the best growth of patchouli cuttings was found in the stem taken from the shoot's part. There was no significant interaction between NAA concentration and the source of the different parts of stem on the growth of patchouli.

1 INTRODUCTION

Patchouli (*Pogostemon cablin* Benth.) is a highest ranked essential oil producing plants (Singh et al., 2015). It has a strategic potential in the world market where the oil is used as a scent binding agent in perfumes, cosmetics, medicines and aromatherapy (Swamy and Sinniah, 2016; Yang et al., 2013). Patchouli oil can also be used as insect repellent (Maia and Moore, 2011) and antiseptic (Haryudin and Maslahah 2011). Recently, no synthetic ingredients or substitutes have been found to match the benefits of patchouli oil. The largest quantity of patchouli oil is produced in Indonesia (Swamy and Sinniah, 2016).

Patchouli cultivation in Indonesia was originally developed in Aceh, North Sumatra, West Sumatra and Bengkulu (Haryudin and Maslahah 2011). Three superior quality of patchouli varieties (Tapak Tuan, Lhokseumawe, and Sidikalang varieties) have been resealed by the Indonesian Research Institute of Spices and Medical Plants, Bogor, Indonesia. Tapak Tuan varieties is superior for its production, Lhokseumawe varieties has high oil content, while Sidikalang varieties tolerant to bacterial wilt and nematode (Nuryani, 2006).

In recent years, according to Kementerian Pertanian Republik Indonesia (2019), Indonesian patchouli production is unstable and does not show any progress (2.207 tons in 2017 and 2.211 tons in 2019). The problem of unincreased production and quality of Indonesian patchouli is caused by many factors such as plant genetic quality, non-intensive cultivation, poor seedlings, limited seed sources, varied seedling, reduced planting area, decreased level of soil fertility, harvest and postharvest mechanism, and patchouli oil distillation that is far from perfect (Nuryani, 2006; Setiawan and Rosman, 2013).

The formation of adventitious roots of plant is controlled by genetic and environmental factors, among which phytohormone auxin plays a major role (Zhao et al., 2014). Exogenous auxin application (e.g., naphthalene acetic acid, NAA) can increase adventitious root formation in cuttings of most plant

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species (Damiano et al., 2008; Ragonezi et al., 2010. The growth of patchouli cuttings can be stimulated by the application of growth regulators containing auxin (exogenously) to stimulate root growth and can physiologically influence plant growth.

Currently, there are many growth regulators in the market, including *Growtone* with Naphthalene acetic acid (NAA) content of 0.3% and acetamide 1-naphthalene of 0.75%. NAA serves to stimulate root growth and reduce the risk of cuttings decay. Faizin (2016) found that the number of leaves, shoot length, number of roots, and root length of patchouli plant cuttings were best shown at *Growtone* concentration of 6.0 g L^{-1} water. This shows that *Growtone* concentration of 6.0 g L^{-1} water has been able to increase the growth of patchouli cuttings more than any other treatment. Handriyano (2007) states that the length of cuttings of 25 cm with a soaking time of 45 minutes at *Growtone* concentration of 0.8 g L^{-1} water, increase the growth of Jatropha cuttings where the root length, root volume and buds of Jatropha cuttings appear better than in other treatments.

Propagation by cuttings is one of vegetative propagation technique that is widely used in horticultural crops such as ornamental plants (Oinam *et al*., 2011) and forestry plants (Nakhooda *et al*., 2016) or for the propagation of elite genotypes on a large scale. As patchouli plants are rarely produce seeds, it is mostly propagated using stem cutting or *in vitro* multiplication (Swamy et al., 2010; Saravanan et al., 2015). The success of vegetative propagation mainly depends on the efficient selection of stem cuttings. Rathnayake et al. (2015) found in *Pogostemon heyneanus*, two nodal hardwood cuttings performed better in rooting parameters when compared to semi-hardwood and softwood cuttings.

Cuttings must be available in good conditions, because it is likely that cuttings will decay after planting. For the best result, cuttings are suggested to be prepared in nurseries before planting them directly in the field (Nuryani et al., 2007). Differences in stem cuttings affect plant growth, while cuttings for patchouli plants can be used at the shoots, the middle and the base of the stem. Melati et al., (2006) found that almost all growth parameters (plant height, number of branches, number of leaves) observed showed that the growth of leafy cuttings of patchouli was better than non-leaf cuttings. Conversely, Iskandar (2014) found that the suitable planting material was base cuttings compared to other parts of stem. The results of his study showed that the plant height, number of leaves, and the number of shoots were higher.

The length of time for a stem to produce root was a problem faced by patchouli farmers in patchouli planting (Pandji and Sofyan, 1986). The capacity to form adventitious roots in stem cuttings varies between cuttings and within plant species or even genotypes (De Klerk et al., 1999). Stuepp et al. (2014) suggested that age, size, juvenility and maturity levels of vegetative cuttings play a key role in the establishment of better rooting. The cuttings are taken from woody stems and parts of plants that are not too old, and the cuttings chosen for seedlings must be free from pests and diseases.

The use of suitable patchouli cuttings along with a combination of IBA concentration provide a high percentage of cuttings life, initially buds emerge, number of shoots, root length, root volume, biomass dry weight and root dry weights, exceeding the base cuttings and middle cuttings of patchouli (Purba et al., 2017).

Based on the description above, this study used several concentrations of *Growtone* as a source of NAA and different parts of stem cutting to determine the highest growth of patchouli plant. This study aims to determine the effect of *Growtone* concentration and different parts of stem as the best part of stem cutting and the interaction between the two factors on the growth of patchouli plant.

2 MATERIALS AND METHODS

2.1 Experimental Site

The research was carried out at the Experimental Field and Plant Physiology Laboratory of the Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh, which was carried out in January to April 2019.

2.2 Tools and Materials

The tools used in this study were analytical scales, 100 ml measuring cups, calipers, Tapak Tuan varieties patchouli cuttings from different parts of stem (shoots, middle stem and base), *Growtone* of 72 g, polybags with a size of 25 cm x 30 cm as much as 108 sheet, and Urea fertilizer as much as 216 g. Paranet was used as a shading material.

2.3 Research Implementation

Planting media used were soil and manure with a ratio of 3:1 (based on volume). Patchouli cuttings from different plant parts with a length of 25 cm were soaked in *Growtone* with a concentration according to treatment for 24 hours. Patchouli cuttings control treatment soaked with water without *Growtone*. Each patchouli cutting was planted in each polybag with a depth of 5 cm. Maintenance of patchouli plant includes watering, fertilizing with Urea of 2 g per polybag at one week after planting (DAP), weeding and losing the soil was carried out at 25, 45 and 55 DAP. Plant revocation was done at the age of 75 DAP.

2.4 Experimental Design

This study used Randomized Block Design with a 4x3 factorial pattern with 3 replications. There were 2 factors studied, the first factor was *Growtone* concentration consists of 4 levels (0, 4.0, 8.0 and 12.0 g L^{-1} water). The second factor was cuttings from different parts of stem (shoots, middle and base parts of stem). Each experiment unit consists of three polybags. Data were analyzed with analysis of variance (ANOVA), and analysis of differences in mean values using Tukey Test at 5% significant level.

2.5 Observation Parameters

Observations were made on the number of shoots, shoot length, shoot diameter, number of leaves at 15, 30, 45, 60 and 75 DAP. Measurement of leaf length, leaf width, leaf area, fresh and dry weight of biomass (using an oven for 3x24 hours with a temperature of 60ºC to a constant weight), number of roots, root length and root volume of patchouli plant were performed at 75 DAP.

3 RESULT AND DISCUSSION

3.1 Effect of *Growtone* Concentration on Growth of Patchouli Cuttings

The results of analysis of variance showed that *Growtone* concentration had a very significant effect on all observed growth variables. The average growth of patchouli cuttings due to the treatment of *Growtone* concentration were as indicated in Table 1. The results showed that the highest number of shoots at 15, 30 and 75 DAP were obtained at *Growtone* concentration of 4.0 g L^{-1} water and the highest number of shoots at 45 and 60 DAP at *Growtone* concentrations of 8.0 g L^{-1} water. The largest shoot diameter at 15 and 30 DAP were obtained in control treatment, while at 45 and 60 DAP at *Growtone* concentrations of 8.0 g L^{-1} water, and at 75 DAP was the largest at *Growtone* concentrations of 12.0 g L^{-1} water. The highest shoot lengths at 15, 30, 45 and 60 DAP were found at *Growtone* concentrations of 12.0 g L^{-1} water, while at 75 DAP was at *Growtone* concentrations of 8.0 g L^{-1} water. The highest number of leaves at ages 15, 30, 45 and 60 DAP were found at *Growtone* concentration of 8.0 g L^{-1} water but not significantly different from *Growtone* concentrations of 4.0 g L^{-1} water. The highest number of leaves at 75 DAP was found at *Growtone* concentration of 4.0 g L^{-1} of water, and was not significantly different from control. The highest leaf length and leaf width were found at *Growtone* concentration of 8.0 g L^{-1} water, while the largest leaf area was found at *Growtone* concentration of 12.0 g L^{-1} water. Fresh and dry biomass weights, the highest number of roots and root volume were found at *Growtone* concentration of 4.0 g L^{-1} water, while the highest root length was found at *Growtone* concentration of 8.0 g L^{-1} water.

The best *Growtone* treatment was found at a concentration of 4.0 g L^{-1} water. This fact indicated that the use of *Growtone* concentration of 4.0 g L^{-1} water had been able to provide a better growth of patchouli cuttings. Faizin's (2016) found that *Growtone* concentrations of 6.0 g L^{-1} water which was applied to patchouli plants showed the best results compared to other treatments. This fact indicated that *Growtone* concentrations of 6.0 g L^{-1} water was optimal enough to stimulate the formation of new patchouli plant roots, cell division, formation and growth of patchouli plant cuttings. This also indicated that the lower the use of *Growtone* concentration, the better the growth of patchouli cuttings. According to Zhao (2010) and Heddy (2006) auxin as a plant growth regulator (PGR) play its role in plant growth and development by affecting membrane proteins, so protein synthesis and nucleic acid can be faster and auxin influence the formation of new roots, cell division and the formation of new shoots. The best shoot length and leaf area parameters were found in the treatment of *Growtone* concentration of 12.0 g L^{-1} water. The increasing concentration of *Growtone* containing auxin (naphthalene acetic acid) play a role in stimulating growth, thus *Growtone* at the base of plant cuttings increased the speed of growth of patchouli shoots and enlarge the leaf area. Purba et al. (2017) suggested that auxin hormone in cuttings was active enough to divide the plant plus exogenous PGR to provide optimal auxin conditions in the growth and development of patchouli cuttings. The formation of adventitious roots is the main condition for its success in propagation.

Table 1. Average growth of patchouli cuttings due to different *Growtone* concentrations.

Parameters		Concentration of *Growtone* (g L^{-1} water)				
		Control	4.0	8.0	12.0	Tukey (5%)
Number of shoot	15 DAP	1.19 a	1.41 b	1.07 a	1.19 a	0.22
	30 DAP	2.07 a	2.33 ab	2.26 ab	2.48 b	0.38
	45 DAP	4.93 a	5.41 a	6.22 b	5.41 a	0.47
	60 DAP	7.11 a	7.48 a	8.26 b	7.30 a	0.68
	75 DAP	6.44 a	9.52 c	8.85 c	7.93 b	0.78
Shoot’s diameter (mm)	15 DAP	0.55 b	0.47 ab	0.53 ab	0.43 a	0.11
	30 DAP	1.00 ab	0.96 a	1.06 b	0.95 a	0.16
	45 DAP	1.67 a	1.60 a	1.82 b	1.87 b	0.14
	60 DAP	2.58 a	2.49 a	2.71 ab	2.74 b	0.14
	75 DAP	3.09 a	3.16 a	3.10 a	3.59 b	0.21
Shoot’s length (cm)	15 DAP	3.03 a	3.51 b	4.20 c	5.43 d	0.42
	30 DAP	6.03 a	6.67 a	7.47 b	9.61 c	0.76
	45 DAP	9.18 b	7.76 a	8.99 ab	11.30 c	1.39
	60 DAP	10.68 ab	10.11 a	11.35 b	13.28 c	0.82
	75 DAP	19.75 b	18.49 a	21.50 c	21.35 c	1.14
Number of leaves	15 DAP	0.44 a	0.67 b	0.70 b	0.67 b	0.14
	30 DAP	0.85 a	1.93 c	2.04 c	0.67 b	0.14
	45 DAP	6.19 a	9.26 bc	10.11 c	8.41 b	1.25
	60 DAP	13.63 a	19.59 c	20.19 c	16.81 b	1.85
	75 DAP	39.41 b	47.48 b	46.44 b	26.11 a	11.23
Length of leaves (cm)		8.09 a	9.33 b	9.77 c	10.17 c	0.42
Width of leaves (cm)		6.58 a	7.37 b	7.82 c	7.79 c	0.29
Area of leaves (cm^2)		38.74 a	55,85 b	65.18 c	71.87 d	4.33
Fresh weight of biomass (g)		43.51 a	62.21 b	55.38 b	67.35 b	9.58
Dry weight of biomass (g)		8.90 a	14.51 c	10.76 b	12.09 b	1.76
Number of roots		22.58 a	25.67 b	23.56 a	24.67 ab	3.96
Length of roots (cm)		44.56 a	43.44 a	49.78 b	46.44 a	4.12
Volume of roots (ml)		17.11 b	20.67 c	12.78 a	14.67 ab	2.93

Notes: Numbers followed by the same letter in the same line are not significantly different at the 0.05 Tukey test level. DAP= Day After Planting.

Pacucar et al. (2014) argued that adventitious root growth is stimulated by interactions between phytohormones and external growth regulators. The stimulation of adventitious root formation is also shown to be positively influenced by ethylene, which may be through modulation of auxin transport (Druege et al., 2014; Wei et al., 2019), thus the production of ethylene induced by indole acetic acid can be a factor involved in the stimulation of adventitious root formation (Pan et al., 2002). Several hormones such as auxins, cytokines, and ethylene have long been known to regulate adventitious root formation (De Klerk et al., 1999). Adventitious roots can develop either from pericyclic cells or from various types of cells and tissues, which depend on the plant species and environmental stimuli involved (Druege et al., 2016). The synthesis of auxin-induced ethylene can play a role in the adventitious root initiation and is associated with increased cellulose activity (Kemmerer and Tucker, 1994).

The growth of patchouli cuttings in the control treatment was very low compared to other treatments. Control treatment that was not given *Growtone* was not able to stimulate the speed of cell division in the formation of plant organs such as roots, stems and leaves. Pasetriyani research (2014) stated that the control treatment or *Growtone* concentration of 0 mg/plant shows the lowest growth.

3.2 Effect of Different Parts of Stem Cutting on Growth of Patchouli

The results of the analysis of variance showed that cutting from different parts of stem had a very significant effect on the number of shoots at 30, 45, 60 and 75 DAP and the number of leaves at 15, 30, 45 and 60 DAP. The average growth of patchouli plants due to the treatment of cuttings from different part of stem is shown in Table 2. The table shows that the number of shoots at 30, 45, 60, 75 DAP and the number of leaves at 15, 30, 45 and 60 DAP were mostly found in shoot cuttings, and significantly different from other cuttings treatment. The number of shoots at 15 DAP, shoot diameter at 15, 30, 45, 60 and 75 DAP, the length of shoots at 15 and 75 DAP, the number of leaves at 75 DAP significantly different from the treatment of the middle and base stem cuttings.

The shoot lengths at 30, 45 and 60 DAP tend to be longer in the treatment of middle stem cuttings, although statistically not significantly different from the treatment of shoot and the base cuttings. Biomass fresh and dry weight tend to be higher in base stem cuttings, although was not significantly different from the treatment of shoot and middle stem cuttings.

The results showed that shoot's stem cutting with leaves provide the highest growth compared to the middle and base stem cuttings. At the shoot cuttings of patchouli, several leaves were present compared to middle and base stem cuttings, where in the presence of leaves also get the number of roots (Garbuio et al., 2007). In patchouli, stem cuttings with leaves are preferred for vegetative propagation because of the higher rooting and shooting capacity (Swamy and Sinniah, 2016). Shoot cutting contains a lot of carbohydrates and auxin to trigger the formation of shoots and leaves.

The minimum percentage of leaves in cuttings of patchouli occurred as the consequence of low carbohydrate availability, as well as low reserve tissue, and higher ABA content (Kojima *et al*., 1993). Carbohydrates have been considered as one of the key factors that contribute to adventitious root formation (Shang et al., 2019). Faizin (2016) used shoot, middle and base stem cuttings and found that the best result was found on shoot treatment compared to other treatments. Shoot cuttings has been optimal enough to stimulate the speed of root formation, the emergence of early shoots and more leaf formation.

Table 2. The average growth of patchouli cuttings due to different parts of stem cuttings.

Parameters		Stem cutting			
		Shoot	Middle	Base	Tukey 5%
Number of shoots	15 DAP	1.56	0.97	0.97	-
	30 DAP	3.42 b	1.72 a	1.72 a	0.50
	45 DAP	8.42 b	3.78 a	4.28 a	0.63
	60 DAP	11.78 b	4.97 a	5.86 a	0.91
	75 DAP	13.78 b	5.67 a	5.11 a	1.05
Shoot's diameter (mm)	15 DAP	0.65	0.42	0.42	-
	30 DAP	1.22	0.89	0.87	-
	45 DAP	1.84	1.68	1.70	-
	60 DAP	2.70	2.61	2.59	-
	75 DAP	3.45	3.10	3.15	-
Shoot's length (cm)	15 DAP	4.18	3.93	3.97	-
	30 DAP	7.11	7.77	7.46	-
	45 DAP	8.15	10.87	8.90	-
	60 DAP	11.20	11.69	11.18	-
	75 DAP	21.13	19.62	20.07	-
Number of leaves	15 DAP	1.86 b	0.14 a	0.11 a	0.18
	30 DAP	3.67 b	0.78 a	0.44 a	0.55
	45 DAP	15.06 b	5.64 a	4.78 a	1.66
	60 DAP	28.58 b	12.72 a	11.36 a	2.47
	75 DAP	42.47	39.00	38.00	-
Length of leaves (cm)		9.70	8.69	9.63	-
Width of leaves (cm)		7.73	7.46	7.58	-
Area of leaves (cm^2)		60.68	52.92	60.13	-
Fresh weight of biomass (g)		57.32	48.75	65.27	-
Dry weight of biomass (g)		11.87	9.88	12.95	-
Number of roots		25.42	23.25	23.67	-
Length of roots (cm)		51.67	40.50	46.00	-
Volume of roots (ml)		19.17	11.92	17.83	-

Notes: Numbers followed by the same letter in the same line are not significantly different at the 0.05 Tukey test level.
DAP= Day After Planting.

Abidin (1990) argued that shoot cuttings contain a lot of auxin when compared to other parts, as endogenous auxin from a plant is produced from meristem tissue and causes apical dominance so that the formation of roots is faster and stimulates the emergence of shoots. Heddy (2006) argued that the role of carbohydrates to form roots and shoots is very large. The growth of good shoots and roots will lead to good leaf formation and increases the photosynthetic process, thus more carbohydrates are produced. Purba et al. (2017) found that the use of shoot cuttings in the provision of PGR IBA with a concentration of 100 ppm increased the growth of patchouli cuttings in all variables, such as the percentage of live cuttings, age of buds, number of shoots, root length, root volume, and root dry weight.

3.3 Effect of Interaction of *Growtone* Concentration and Difference Parts of Stem on the Growth from Patchouli

The results showed that there was no significant interaction between the concentration of *Growtone* and the stem cuttings on all patchouli growth variables. The growth of different patchouli stem cuttings due to differences in the concentration of *Growtone* applied was not affected by different parts of stem cuttings, and the treatment of stem cuttings were not affected by differences in concentration of *Growtone*.

4 CONCLUSION

The results showed that *Growtone* concentration had a very significant effect on all growth variables. The best growth of patchouli cuttings was found at *Growtone* concentration of 4 g L^{-1} water, while the highest shoot length, shoot diameter at 75 DAP and the leaf area was found in the treatment of *Growtone* concentration of 12 g L^{-1} water. Different stem cuttings have a very significant effect on the number of shoots at 30, 45, 60 and 75 DAP and the number of leaves at 15, 30, 45, and 60 DAP. The best growth of patchouli cuttings was found in the shoot cutting. There was no significant interaction between *Growtone* concentration and the different part of stem cutting on all patchouli growth variables that were observed.

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