

The analysis of endogenous auxin of shallot and its effect on the germination and the growth of organically cultivated melon (*Cucumis Melo*)

M B Yunindanova^{1*}, MTh S Budiastuti¹ and D Purnomo¹

¹Sebelas Maret University, Jl. Ir. Sutami 36A, Kentingan, Surakarta, Indonesia.

*Email:mercybientri_fp@staff.uns.ac.id

Abstract. Nowadays, the demand for organic melon increases along with the enhancement of people awareness of the role of organic products in human health. However, there is limited source of organic hormone to support organic cultivation. Therefore, this experiment aimed to identify endogenous hormone of shallot and to investigate the effect of the hormone on germination and the growth of melon (*Cucumis Melo*). The results showed that 45 days and 80 days after harvest the shallot contained 3 types endogenous auxin hormones (Indole-3-Acetic Acid, 2,4 Dichlorophenoxyacetic acid and α -Naphthalene Acetic Acid) and Cytokines (6-Benzyl Amino Purine). Overall, 45 days after harvest the shallot contained more hormones than it did 80 days after harvest (DAH). It contained 0.73-0.75 ppm Indole-3-acetic acid. Additionally, 0.85 ppm 6-Benzyl Amino Purine was detected in the shallot 45 DAH or 121% more than that found in the shallot 80 DAH. However, 2,4-Dichlorophenoxy acetic acid and α -Naphthalene acetic acid were found only in the shallot 45 DAH. Extract of the shallot 45 DAH was applied on melon seeds by soaking them for 24 hours with different extraction methods (juicer and blender) and at several concentrations (75%, 50%, 25% and 12.5%). The shallot extract resulting from the juicer was effective to increase the germination rate, the fresh weight and the dry weight of melon plant. Meanwhile, there was not any significant difference among the different concentrations. It might be related to low concentration of the shallot extract. This experiment showed that the shallot extract was potential as organic hormone source.

1. Introduction

Melon (*Cucumis melo*) is an important horticultural commodity. Nowadays, the demand for the melon is higher because the fruit has a fresh and unique taste. In addition to conventional cultivation, it is now also cultivated using organic systems. The organically grown melon could give harvesting result that is not significantly different from the harvesting result of the plant grown using conventional fertilizers [1]. Compared to conventional farming conditions, muskmelons grown in organic farming conditions gave the same result, including total soluble solids (TSS) and soluble sugar contents [2]. The demand for the organic melon increased because it was considered as more superior than the conventional product in terms of health and environmental safety [3].

There is a constraint in the organic cultivation, which is the scarcity of organic hormones. It takes place because there is limited source of organic hormone to support organic cultivation. Growth regulators or growth hormones are organic substances in addition to nutrients, synthesized in plants, causing alteration in their cellular metabolism [4]. The use of plant growth regulators, for example synthetic hormones, has rapidly progressed in the recent years [5,6]. There are several plants that can be the sources of the plant growth hormone such as shallot. However, the plant growth hormone has



not been experimented in melon. The shallot has been used several times as the source of the hormones in growing horticultural crops and had a positive effect. The application of shallot extract could increase the growth of long [7]. Auxin and gibberellin were detected in shallot and could enhance the growth of seeds [8].

The analysis of secondary metabolism in shallot still focuses on pharmacology aspects, while there has not been any analysis of hormone levels. Some studies suggested that shallot bulbs contained quercetin, isorhamnetin, glycosides [8], phenol compounds and antioxidant compounds [9]. Therefore, this study aimed to determine the levels of endogenous auxin contained in shallot and its effect on the germination and the growth of organic melon.

2. MATERIAL AND METHODS

This study was conducted in Green House Jumantono, the Faculty of Agriculture, SebelasMaret University from March 2015 to June 2015. The experiment used shallot (*Allium ascalonicum*Hort.) of Bima variety and taken 45 days and 80 days after harvest (DAH), while the melon used in the study was F1 Hybrid-Action 434. It used 2 factors, namely extraction methods (juicer and blender) and concentrations (75%, 50%, 25% and 12.5%). It was arranged in completely randomized design (CRD) with three repetitions. The shallot extract was applied by soaking melon seeds for 24 hours with shallot 45 DAH. The melons were cultivated organically using alfisol soil, compost fertilizers, liquid organic fertilizers and PetroganikTM fertilizer.

The analysis of the auxin and the BAP of the shallots 45 DAH and 80 DAH was made using HPLC. The auxin hormones consisted of Indole-3-acetic acid (IAA), 2,4-Dicholophenoxy acetic acid (2,4 D) and a-Naphthalene acitic acid (NAA). Additionally, 6-Benzyl amino purine (BAP) was also observed in form of cytokines. Observations were carried out during the process of germination and plant growth. The germination speed was measured by calculating the seeds germinated on the fourth day. Meanwhile, the germination rate was counted on the seventh day after treatments.

3. RESULTS AND DISCUSSION

The period after the harvest greatly determined the endogenous hormones of shallot. The auxin concentration gradient was indicative of plant organ location and growth reactions to environment [10]. There were several factors contributing to the arrangement of auxin allocation in plant tissue. They were auxin biosynthesis, storage as inactive precursors, polar transport from sites of synthesis, and the degradation of auxin[11, 12, 13].

3.1. Hormone Levels

Table 1. The hormone content of the shallot

		Shallot (DAH)	
No.	Compounds	45	80
		ppm	
1.	Indole-3-acetic acid (IAA)	0.75	0.73
2.	2,4-Dicholophenoxy acetic acid (2,4 D)	2.92	ttd
3.	a-Naphthalene acitic acid (NAA)	0.77	ttd
4.	6-Benzyl amino purine (BAP)	0.84	0.38

Table 2. Hormone levels according to the concentration of the application

Extract Sources	Compound	Concentration (ppm)			
		12.5%	25%	50%	75%
Shallot 45 DAH	Indole-3-acetic acid (IAA)	0.094	0.188	0.375	0.563
	2,4-Dichlorophenoxy acetic acid (2,4 D)	0.365	0.730	1.460	2.190
	a-Naphthalene acetic acid (NAA)	0.096	0.193	0.385	0.578
	6-Benzyl amino purine (BAP)	0.105	0.210	0.420	0.630

Table 1 showed that the shallot 45 DAH had higher value than the shallot 80 DAH. It indicated that the longer was the storage period, the lower the hormone levels were. Both shallot extracts contained small amount of hormones or less than 5 ppm. IAA as the primary auxin in plant [14] was the lowest level of auxin as compared to 2.4 D and NAA. On the contrary, the highest level of auxin was in the form of 2.4 D, which was three times higher than IAA and NAA. The shallot 80 DAH still contained IAA, while 2.4 D and NAA were not detected. It indicated that IAA could last longer than other auxin forms. Additionally, although harvest periods differed greatly, IAA levels decreased only 0.02 ppm. 6-Benzyl amino purine (BAP) in the form of Cytokines was detected in both shallot types. The shallot 80 DAH had the BAP level that was almost 50% lower than that of the shallot 45 DAH.

3.2. The Effect of Extraction Methods

The shallot extracted using the juicer resulted in better germination and plant growth. It caused higher germination speed and germination rate and heavier plant fresh weight and plant dry weight (Table 3). On the contrary, the shallot extracted using the blender caused lower germination rate and germination speed as compared to control.

In contrast to the germination rate, the application of the extracts resulting from both the juicer and blender could enhance plant growth. However, the extract resulting from the blender resulted in the plant fresh weight that was 89.54% higher than control, while the extract resulting from the blender only caused 48.15% increase. Moreover, the extract resulting from the juicer boosted the plant dry weight more than twice as heavier as control. This result was consistent with the study conducted by [15] suggesting that shallot was known to contain growth hormone such as auxin and gibberelin, which could enhance the growth of seeds. Plant growth and development were stimulated by environmental or intrinsic cues, such as hormones [16, 17].

It showed that the extract of the shallot resulting from the juicer was more effective for the germination and the growth of melon. The extract was more concentrated and purer. On the contrary, there was less extract resulting from the blender and mixed with the dregs.

Table 3. The effect of extraction types to plant germination and growth.

Application	Germination Speed (%)	Germination Rate (%)	Plant Fresh Weight	Plant Dry Weight
Control	88,67ab	88,67ab	160,02 ^a	13,58a
Juicer	90,83 b	93,33 b	303,30 ^b	28,02 ^b
Blender	75,83a	84,17 a	237,07 ^{ab}	26,53 ^b

Note: The numbers followed by different letter in the same column are significantly different based on DMRT 5%.

3.3. The effect of shallot concentration

Concentration treatments exhibited the same impact to the germination and melon growth. There were no significant different among concentration 12.5 % to concentration 75%. This was likely due to the hormone levels contained in the extract of onion was very small (Table 1). Thus, although it was 75%, the hormone levels were below 5 ppm (Table 2) and it still has not affected the growth of melon.

Table 4. The effect of concentration to the germination and plant growth.

Concentration	Germination Speed	Germination Rate	Leaf Area	Root length	Plant Fresh Weight	Plant Dry Weight
%	%	%	cm ²	cm	g	g
75	85,00	88,33	105,32	26,95	281,75	27,02
50	83,33	91,67	111,10	21,68	260,14	24,23
25	86,67	95,00	114,57	26,31	278,11	28,85
12.5	78,33	80,00	127,71	27,43	260,74	28,99

This result was the same demonstrated by [18] which mentioned that application plant growth regulation in form of auxin and cytokines with various concentrations (0, 20 ppm, 50 ppm and 0, 20 ppm, 50 ppm, and 75 ppm) did not influence the development of basal leaf cutting. The use of hormones was done by soaking the cutting for 30 minutes. The ineffectiveness of the treatment (auxin and cytokines) is due to the concentration was technically inaccurate. This is in accordance with opinion [19] which states that auxin and cytokines are active at various concentrations and if the concentration and the time of application are appropriate, it will be useful and can play a role in stimulating the growth of cuttings.

Other thing that may cause the ineffectiveness of hormone application is the possibility of antagonist interaction between hormones given with the hormones contained in the cuttings [18]. This was consistent with [20, 21] who noted that auxin and cytokines can experience several types of interactions that are antagonistic and synergistic. Auxins and cytokinins act synergistically to regulate cell division and antagonistically to control buds formation and lateral roots, suggesting multiple mechanisms of interaction [7]

However, the use of low doses of auxins also effective in several studies with different application techniques. The use of auxin in small dosage (a 0.5 mg L⁻¹ dose of auxin) was recommended for the enhancement of vegetative growth of linseed (*Linum usitatissimum* L.) and a combined dose of auxin (1.0 mg L⁻¹) and gibberellin (200 mg L⁻¹) is recommended for the enhancement of seed yield through three times sprayer on the apical tip of stem. Low doses of auxin are effective in growth promotion [22]. Application of 500g/L shallot extract by soaking for 12 hours, successfully increased number of leaf, green color intensity and shoot fresh weight of *Piper retrofractum* [23].

4. CONCLUSION

Shallot after harvested contained 3 types endogenous auxin hormones (IAA, 2,4 D, NAA) and cytokines (BAP). Shallot extract from juicer was effective to increase the germination speed, the rate of germination, plant fresh weight and plant dry weight. On the other hand, there was no significant different among various concentrations. Probably, this phenomenon was due to low concentration of shallot extract. This experiment revealed that shallot extract had potential to be used as organic hormone source. The use of shallot extract will likely be more effective as exogenous hormone if using pure extract.

References

- [1] Lopodota O, Leogrande R, Fiore A, Debiase G and Montemurro F 2013 *J. Plant Nutr.* **36** 415–428.
- [2] Song S, Lehne P, Le J, Ge T and Huang D 2009 *J. Plant Nutr.* **33** (1) 130–141.

- [3] Anifori M 2010 *Food Scip***45**
- [4] Rastogi A, Siddiqui A, Mirshra BK, Srivastava M, Pandey R, Misra P, Sighn M, and Shukla S 2013 *CBAB***13** 136–143.
- [5] Cai T, Xu H, Peng D, Yin Y, Yang W and Ni Y 2014 *Field Crop Res.* **155**172–183.
- [6] Gray, WM 2004 *PLoS Biol.* **2**(9)e311.
- [7] Coenen C and Lomax TL 1997 *Trend in Plant Sci.* **23**51–356.
- [8] Fattorusso E, Lorizzi M, Lanzotti V and Taglialatela-Scafati O 2002 *J. Agr. Food Chem.*, **50**(20)5686–5690.
- [9] Lu X, Wang J, Al-Qadiri HM, Ross CF, Powers JR, Tang J and Rasco BA 2011 *Food Chem.* **129**(2)637–644.
- [10] Hayashi K, Nakamura S, Fukunaga S, Nishimura T, Jenness MK, Murphy AS, Motose H, Nozaki H, Furutani M and Aoyama T 2014 *Auxin Transport Sites Are Visualized in Plant Using Fluorescent Auxin Analogs* Proc. National Academy of Sciences **111**(31) 11557–11562.
- [11] Hayashi K 2012 *Plant Cell Physiol.* **53**(6) 965–975.
- [12] Sauer M, Robert S, Kleine-Vehn J 2013 *J. Exp Bot* **64**(9) 2565–2577.
- [13] Petrásek J and Friml J 2009 *Develop.* **136**(16)2675–2688.
- [14] Kende H and Zeevaart JAD 1997 *The Plant Cell* **9**(1) 197–121.
- [15] Marfirani M 2014 *Pengaruh Pemberian berbagai Konsentrasi Filtrate Umbi Bawang Merah dan Rootone-F terhadap Pertumbuhan Stek Melati “RatoEbu”* Lentera Bio. **3**(1) 73–76.
- [16] Malamy JE 2005 *Plant Cell Environ.* **28**(1)67–77.
- [17] Wolters H and Jurgens G 2009 *Nat Rev Genet.* **10**(5)305–17.
- [18] Naibaho H. (2012). *Pengembangan Teknologi Perbanyakan Bibit Nenas Smooth Cayenne secara in vivo melalui Aplikasi Auksindan Sitokinin*. Thesis. (Bogor: Institut Pertanian Bogor) 82p.
- [19] Kusumo S 1990 *Zat Pengatur Tumbuh Tanaman* (Bogor: CV Yasaguna) p 73.
- [20] Lee DJ 2002 *Plant Sci.* **162**345–353.
- [21] Jones B, Gunnerås SA, Petersson SV, Tarkowski P, Graham N, May S, Dolezal K, Sandberg G and Ljung K 2010 *Plant Cell* **22**2956–2969.
- [22] Vwioko ED and Longe MU 2009 *Biosci. Res. Com.* **21**263–271.
- [23] Siswanto U, Sekta ND and Romeida A 2010 *J. Ind. Med. Plant* **3**(2)128–132.