ISBN : 978-602-14917-9-9

ENDOGENOUS HORMONES AT THE BEGINNING OF LEAF GROWTH AFTER VERNALIZATION OF GARLIC BULB (ALLIUM SATIVUM L.) IN INDONESIAN LOCAL VARIETIES

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Abstract. Flowering of garlic in tropical regions such as Indonesia has never been found, so that generational multiplication cannot be done yet. Induction of garlic flowering can be done by vernalizing the seed tubers to trigger flowering. This study aims to determine the hormone status of IAA, GA_3, at the time of initial growth of leaf tissue, after going through vernalization. Bulbs of Indonesian local varieties of Lumbu Kuning and Tawangmanu varieties were vernalized for 4 weeks, 8 weeks, and without vernalization treatment as a control. The contents of IAA and GA hormones in the leaf tissue of garlic plants aged 2 weeks were analyzed using method *high performance liquid chromatography* (HPLC). The results showed that the Lumbu Hijau variety with 4 weeks vernalization contained IAA of 1615,595 ng / g; $GA_{3\ is}$ 7.297 ng / g. At 8 weeks vernalization the IAA content is 2169,475 ng / g; GA_3 of 9,757 ng / g; In the Tawangmanu variety with 4 weeks vernalization containing IAA of 1459,168 ng / g; $GA_{3\ is}$ 6.617 µg / g. Whereas for 8 weeks vernalization of IAA content of 2007,195 ng / g; $GA_{3\ is}$ 8.315 ng / g.

1. Introduction

Garlic (*Allium sativum* L.) is cultivated in Indonesia for centuries asexually using seed tubers, with productivity that tends to decrease with an average yield of only 6 tons / ha from 20 tons / ha in the previous period. This is considered as a result of asexual mutations due to the lack of genetic diversity. Lately, it has been realized that asexual mutations will not occur if we can maintain genetic diversity through sexual propagation, by using the true seeds of garlic or better known as *true garlic seed* (TGS) [1].

Sexual garlic propagation through true seeds began to be developed since the discovery of 3 genotypic groups based on their ability to flower (*bolting*) of 613 genotypes. The first group was *garlic bolting* (300 genotypes), the second group was *garlic non-bolting* (205 genotypes), and the third group was *garlic bolting* (108 genotypes) [2]. Bolting or flowering is a problem that always occurs in the genus Allium [3]. The factors that most influence the flowering of the genus Allium, including the plants of garlic (*Allium sativum* L.), the main ones are low temperature, day length, light intensity, nutrients, hormones, and vitamins [4]. Onions experience bolting at temperatures between 10-15˚C [5] and onion plants in the tropical regions require treatment of cold temperatures or vernalization at 5˚C for 4 weeks and grown in an environment with air temperature below 18ºC [6].

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Vernalization usually aims to accelerate the release of flowers because cold temperatures can stimulate flower initiation (bolting) [7]. This condition can be obtained naturally from the area of origin, especially for subtropical plants. For tropical regions such as Indonesia, it is very difficult to obtain, except in high places. Therefore, artificial low temperature is needed which is known as vernalization technique.

The minimum vernalization period required for flowering differs from species to species, but usually lasts for several weeks [8]. Most species require temperatures between -1 to 10^{o}C effective for vernalization. Vernalization of garlic plants has a positive effect on flowering and garlic yield [9]. Furthermore, it was explained that the effect of vernalization can increase the activity of enzyme promoted peroxidase (POD) and superoxidase dismutase `

ISBN : 978-602-14917-9-9

(SOD) [10]. In India using the garlic vernalization technique at 4°C for 2 months can increase flowering, umbel formation, and seeds in some varieties tested [11].

It is known that flowering which involves changes in the differentiation patterns of apical buds and axilar from vegetative phase to generative phase to flower buds is the interaction of internal and external factors [12]. The effect of cold temperatures through vernalization techniques as external factors seems to affect flowering hormones in response to internal factors, especially gibberellin and auxin acids.

Flowering induction affected by gibberellins is a substitute role for long days and induces flowering in short-day plants [13]. This hormone increases the hydrolysis of starch and fructane to fructose and glucose. The hexose-hexose from the starch hydraxis is a source of energy, especially for cell formation and causes the potential of water to be low. This causes a decrease in water potential. Furthermore, water from outside the cell diffuses easily into the cell, so the cell can enlarge. Cell enlargement caused by GA_3 can reach 15 times greater than cells that are not treated with GA_3 [14]. Administration of GA_3 10 mg / l can induce flowering of olive plants [15]. Philodendron growth and flowering can increase with the provision of a concentration of GA_3 125 mg / l to 1,000 mg / l [16]. Auxin is a key endogenous hormone as a regulator in the constituent of cells in flower primordia, but in high concentrations it can inhibit flowering of plants. Inhibition of the formation of flowers as a result of ethylene production which is stimulated by the high production of auxin. Inhibition of flowering plants is one of the effects of the natural mechanism of ethylene. The role of auxin in addition to affecting flower development can also inhibit the fall of flowers and fruit, because auxin stimulates photosynthetic activity through increased stomata opening, CO phosphorylation and fixation. With increased photosynthesis activity will increase the supply of assimilates, so that the fruit will grow and develop properly [17]. Adequate auxin concentration will maintain the absentee zone not sensitive to ethylene. Zone friction absences against ethylene due to low auxin content, characterized by increased hydrolytic enzyme activity. Increased hydrolytic enzyme activity causes damage to the cell wall in the abscess zone and causes the separation of plant organs from the parent [17].

Induction of flowering of garlic in tropical regions such as Indonesia needs to be done for restoration of garlic fertility. Furthermore, it can be used for sexual hybridization and seed production, in order to avoid asexual mutations and higher yields. Therefore, tubing vernalization efforts for flowering initiation were tried using local Indonesian garlic genetic sources. The purpose of this study was to determine the initial response to the status of GAhormones$_3$ and IAAafter vernalization of garlic seed tubers in two varieties, namely Lumbu Hijau varieties and Tawangmanu varieties.

2. Method

The research was carried out in the Bromo Plateau with an altitude of 1350 m above sea level in May to September 2018. The experimental design used was a factorial randomized block design with two factors. The first factor is variety (A), consisting of (A1) green lime varieties and (A2) Tawangmanu varieties. The second factor (B) vernalization time, consists of (B0) without vernalization; (B1) vernalization up to 4 weeks; (B2) vernalization up to 8 weeks. Thus, there are six combinations of treatments with three repetitions. Totaly there are 18 experimental units. The experimental unit is a unit of polybag with a size of 5 kg.

Bulbs of green garlic seeds and Tawangmanu varieties used have a shelf life of 2 months. Naturally, garlic seeds can complete a dormancy period of around 9 months. Seedlings were then vernalized for 4 weeks and 8 weeks respectively, and without vernalization treatment as a control. Planting media consists of a mixture of top soil with organic matter in a ratio of 3: 1. The soil used is local soil which is given NPK fertilizer (16:16:16). Fertilizer is given twice by splashing evenly throughout the beds at 4 weeks and 6 weeks after planting. Seed bulbs, according to vernalization treatment, are planted in the media using 3 seed tubers for each polybag. Maintenance includes watering and weeding. The media is kept to keep it moist. As for preventing pests, yellow traps and selective insecticides are installed. While to prevent disease attacks selective fungicides are used once a week. Observations included the percentage of seed bulbs that grew at the age of 1 week, namely the number of seed tubers that germinated from the number of bulbs planted, the number of leaves, and the height

ISBN : 978-602-14917-9-9

of the plant at 2 weeks. The initial growth data were analyzed using analysis of variance, using the SPSS version 17. IAA and GA content analysis was$_3$ carried out using the HPLC method using the Shimadzu model HPLC tool, using a solvent delivering LC 20AT unit, and a SPD M20-A Photo Diode Array detector Detector, mobile phase acetonitrile in water (25:75 v / v). The results of the subsequent analysis are described descriptively.

3. Results and Discussion

The results of the analysis of the variance in the effect of varieties and varieties of vernalization on the initial growth of garlic are presented in Table 1. The results showed that there was no interaction between varieties and vernalization duration of growth, plant height, and number of leaves. From Table 1 it can be shown that in observing the growth power, both green lumbu varieties and Tawangmangu, both showed a growth power of zero, meaning that when observed two varieties had not grown, especially in the treatment without ververalization.

From Table 1 it can be seen that the use of green lime varieties and tawangmangu varieties does not provide significant differences in plant height and number of leaves, with an average plant height of 15.74 cm in Tawangmangu varieties and in varieties green spice of 18.31 cm. The average data was obtained from the average plant height from vernalization treatment 4 weeks and 8 weeks in the two varieties tested.

Likewise, differences in varieties also did not significantly affect the number of leaf parameters. In green lumbu varieties the average number of leaves was 1.95, while the average number of leaves in the tawangmangu variety was 2.15, which was obtained from the average number of leaves in the vernalization treatment 4 weeks and 8 weeks in both varieties.

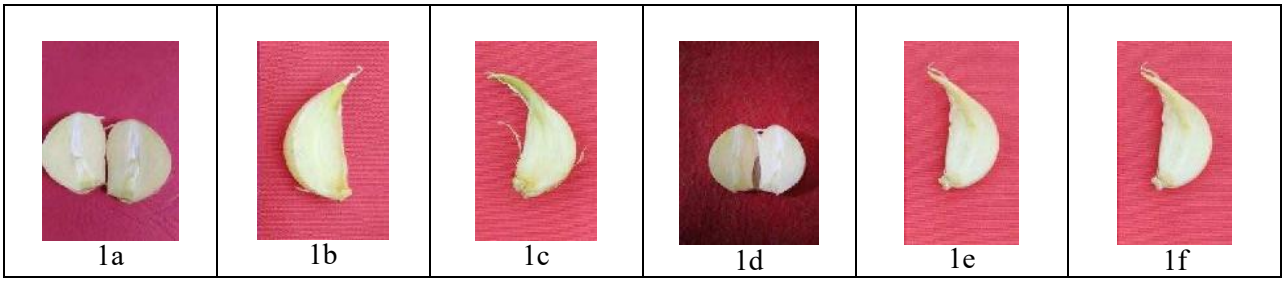
Table 1. Growth Power, Plant Height, and Number of Garlic Leaves After Vernalization

Treatments	Parameters		
	Growing Power (%)	Plant Height (cm)	Number of Leaves
Varieties			
Green Lumbu	0 a	18.31 ± 1.50 a	1.95 ± 0.51 a
Tawangmangu	0 a	15.74 ± 1.54 a	2.15 ± 0.14 a
Duration of vernalization			
Without vernalization	0 a	0 a	0 a
Vernalization 4 weeks	100 b	17.79 ± 2.21 b	2.15 ± 0.40 b.
Vernalization 8 weeks	100 b	16.27 ± 3.30 b	1.95 ± 0.51 b

Another interesting thing is that in observing the growth of plants at 2 weeks, there is data zero in the treatment without vernalization of the growth power, which means that at the age of plant weeks both Lumbu Hijau varieties and Tawangmangu varieties have not shown any growth at all. The data on the average height of plants and the average number of leaves due to vernalization time factors were obtained in the treatment of vernalization duration 4 and 8 weeks. Based on Table 1, it can be seen that the treatment of vernalization duration of 4 weeks averages plant height of 17.79 cm, while the average plant height at 8 weeks vernalization is 16.27 cm. While the average number of leaves at 4 weeks vernalization was 2.15, while at 8 weeks vernalization was 1.95, although the length of vernalization did not show a significant difference in plant height or leaf number.

ISBN : 978-602-14917-9-9

The results of research on the initial growth of plants seem to be strongly influenced by vernalization treatment. In Figure 1 showed that the tubers that were not given vernalization treatment showed no initial growth of shoots in the tubers (Figure 1a). Whereas in Figure 1b and 1c, respectively, with vernalization treatment 4 and 8 weeks, it has been shown that there was an initial shoot growth in the tuber. Likewise, Tawangmangu varieties that were not given vernalization treatment had not shown the initial growth of shoot shoots (Figure 1d). On the contrary, in Figure 1e and Figure 1f, it is clear that there is a clear shoot shoot growth in the seed tubers after 4 and 8 weeks vernalization treatment.



Remarks:
1a, 1b, 1c: Bulbs of green onion varieties (1a control, 1b vernalization 4 weeks, 1c vernalization 8 weeks)
1d, 1e, 1f: Bulbs of garlic tawangmangu variety (1a control , 1b vernalization 4 weeks, 1c vernalization 8 weeks)

Figure 1. Growth of Garlic Seed Bulbs

Content of IAA and GAHormone$_3$ in Early Growth of Leaf Tissue

Results of analysis reading of IAA and GA3 in the leaf tissue of garlic plants green lumbu variety at 2 weeks with vernalization of seed tubers for 4 and 8 weeks using the HPLC method shown in Figure 2.

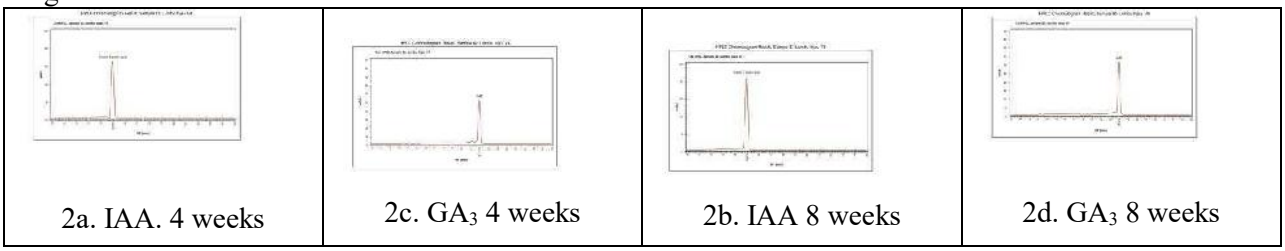
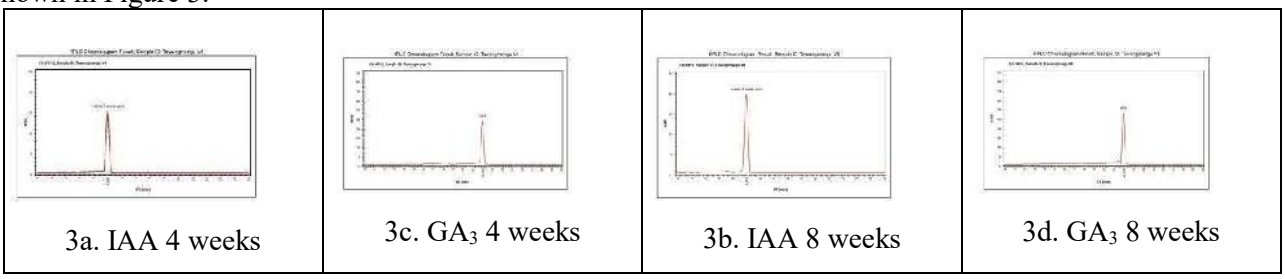


Figure 2. IAA and GA3 Hormones After Internalization of Green Lumbu

ISBN : 978-602-14917-9-9

Analysis results of IAA and GA3 hormone contents in garlic leaves tissue of 2 weeks old tawangmangu varieties with vernalization of seed tubers for 4 and 8 weeks using HPLC method shown in Figure 3.



3a. IAA 4 weeks | 3c. GA_3 4 weeks | 3b. IAA 8 weeks | 3d. GA_3 8 weeks

Figure 3. IAA and GA3 Hormones After Internalization of Tawangmangu

Based on the results of the reading of the HPLC analysis then compiled in summary Table 2.

Table 2. Hormone Content (ng / g) in Garlic Hormone

Varieties	Vernalization	Content (ng / g)	
		IAA	GA_3
Green	4 weeks	spices1615,475	7,297
	8 weeks	2169,475	9,757
Tawangmangu	4 weeks	1459,168	6,617
	8 weeks	2007,195	8,315

Data on hormone content in 2-week-old leaf tissue at the beginning of garlic plant growth both on Lumbu Hijuavarieties green and Tawangmangu varieties are more dominated by IAA than GA3. In green lime varieties IAA content is 1615,475 ng / g at 44 weeks vernalization. At 8 weeks vernalization there was an increase in IAA content to 2169,475 ng / g. Likewise, tawangmangu varieties with 4 weeks vernalization of IAA content were 1459,168 ng / g and increased to 2007,195 ng / g at 8 weeks vernalization. So that both the green lumbu varieties and tawangmangu varieties contain IAA will increase along with the vernalization period up to 8 weeks. If we look closely the data on IAA content in green lime varieties is generally higher than the content of IAA in tawangmangu plant varieties.

The GA3 content generally shows that the content of GA3 green lime varieties is also higher than the content of GA3 Tawangmangu varieties. With 4 weeks vernalization, the GA3 content was 7.297ng / g, increasing to 9.757ng / g with 8 weeks vernalization. Whereas in the 4 weeks vernalization withTtawangmangu variety, the GA3 content was 6.617ng / g and increased to 8.315ng / g by giving 8 weeks vernalization.

The high content of IAA compared to GA3 at the beginning of growth can be understood that at the beginning of the first organ growth that develops is root tissue. In the root tissue IAA hormone will be produced and translocated to the leaf tissue. In addition, the data obtained also showed that increasing vernalization up to 8 weeks also seemed to be accompanied by an increase in the content of IAA and GA3 in leaf tissue.

Garlic (*Allium sativum* L) which is cultivated in Indonesia has several local Indonesian varieties, such as umbu Hijau varieties, Lumbu Kuningbs, Lumbu, Tawangmangu, Sembalun, Sanur, Jatibarang, Bagor Layur, Kayu, etc. Of all these varieties it is rarely found the appearance of flowers naturally. Nonetheless, it is not uncommon in the garlic planting area to always find one, two or more garlic that are able to bolting (flowering), even though from the formed flowers rarely develop into perfect blooms, let alone produce seeds. Based on this, it seems that it is possible that some Indonesian local varieties are included in the category

ISBN : 978-602-14917-9-9

of bolting or semi-bolting [18]. Flowering can be done by giving vernalization treatment at 5ºC for 4 weeks - 8 weeks [8], [9], [10], [11]. Even so, the initial response to the growth of post-vernalization seedlings has not been widely known. It was found that flowering of garlic can occur if followed by changes in IAA hormone from 0.17 to 0.21 µg / g then decreases sharply to 0.06 µg / g in garlic leaf tissue that undergoes flowering [19]. The opposite was also followed by a significant increase in GA3 content from 5.5 µg / g to 9.5 µg / g. Therefore, with vernalization technology to encourage flowering is important to be done to measure the content of IAA and GA3 hormones at the beginning of growth. The results of this study are the initial stages of garlic flowering research by giving vernalization treatment and combined with ZPT treatment to encourage garlic flowering in tropical regions such as Indonesia

4. Conclusion

The results showed that the Lumbu Hijau variety with 4 weeks vernalization contained IAA of 1615, 595 ng / g; GA_3 of 7.297 ng / g. At 8 weeks vernalization the IAA content is 2169,475 ng / g; GA_3 of 9,757 ng / g; In the Tawangmangu variety with 4 weeks vernalization containing IAA of 1459,168 ng / g; $GA_{3\ is}$ 6.617 µg / g. Whereas for 8 weeks vernalization of IAA content of 2007,195 ng / g; GA_3 of 8.315 ng / g.

5. Acknowledgment

The author would like to thank the Central Directorate of Research and Community Service Directorate General of Research and Development Strengthening Ministry of Research, Technology and Higher Education In accordance with the Funding Agreement for the Implementation of Community Service Programs

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