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Morphological and Flowering Characteristics of Shallot (Allium cepa Var. Aggregatum) in Response to Gibberellic Acid and Vernalization

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Morphological and Flowering Characteristics of Shallot (*Allium cepa* Var. Aggregatum) in Response to Gibberellic Acid and Vernalization

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Abstract

The research was conducted to determine the effect of gibberellic acid (GA_3) and vernalization on the morphological and flowering character of shallot. The first experiment was arranged in the field and organized in a completely randomized block design with two factors and three replications. The first factor was 5 varieties of shallots (Bauji, Bima Brebes, Super Philip, Tajuk, and Thailand). The second factor was the concentration of GA_3 (0, 50, 100, and 150 mg L^{-1}). The site was first cultivated and made into a mound plots of 100 cm x 120 cm each and 30 cm height. The bulbs were planted in a spacing of 20 cm x 20 cm. Plants were maintained for 65 days until harvesting. The second experiment was organized in completely randomized design with 3 replications. Five varieties of shallot were evaluated with and without vernalization treatment. Vernalization of shallot bulbs at 8 ^{o}C was carried in storage room for 6 weeks. A higher response of the number of leaves and bulbs at all GA_3 concentrations were observed on Super Phillip variety. A concentration of GA_3 at 100 mg L^{-1} increased the plant height up to 45.74 cm. Based on the vernalization treatment, 5 varieties of shallots were clustered into 3 groups, which were indicated that the morphological and flowering variations, especially in terms of the flowering competence. Vernalization was effective in increasing flowering of Ilokos variety. But,there was no increase in all flowering characters in the Sumenep variety which confirmed as a non-sensitive flowering variety.

Key words : Flower induction; Gibberellic acid; Shallot varieties; Vernalization

Introduction

Shallot (*Allium cepa* var. Aggregatum) is one of the economically important crop belong in the Liliaceae family. Shallot bulb contains important nutritive vegetable and medicinal (Mohammadi-Motlagh et al., 2011; Marlin et al., 2019). Usually, shallot is vegetatively propagated using its bulbs. Shallot cultivation requires specific edaphoclimatic conditions and agricultural management to grow, overcome bulb dormancy, induce flower development, reproduce bulbs, and true seeds (Tendaj and Mysiak, 2013; Farhadi and Salteh, 2018).

Shallot responses to agricultural management and environmental conditions differ among different variety. Selection of the elite variety is an essential for obtaining desired growth and quality of bulbs, and induces the flower formation. The varieties of shallots in Indonesia have the ability to produce flowers, except for the Sumenep variety (Marlin et al., 2018). Cultivation techniques for developing shallot flowering initiation have not been widely developed. Shallot growth and development can be induced by optimizing genetic ability and manipulating the growing environment.

Treatments to induce flowering and seed formation can be carried out using growth regulators such as gibberellic acid and vernalization treatments. Gibberellic acid and vernalization treatment play a role in the plant growth and the process of flowering initiation. Both treatments work by stimulating the formation of flowering genes such as the SOC1 gene (suppressor of overexpression of constant 1) and the LEAFY gene (Corbesier and Coupland, 2006). The LEAFY gene is the main gene that controls flowering in shallots and predicticably have been related to flowering pattern (Marlin et al., 2018).

Bio-regulators like gibberellic acid (GA_3) have been known to play a vital role in building of plants and involved in plant growth together with stem elongation (Rahman et al., 2006), and the transition from vegetative growth to flowering (Sumarni et al., 2013). The treatment at low temperatures (vernalization) can stimulate flower formation in shallot (Song et al., 2012). Vernalization is an important adaptation of plants to initiate flowering in response to prolonged exposure to low temperatures (Finnegan et al., 2001; Song et al., 2012). Elsiddig et al. (2015) showed that vernalization treatment at a temperature of 4-5 ^{o}C for 90 days was a major factor to induce flowering in Texas Grano cultivar onions. This studies were conducted to determine the effect of applying GA_3 and vernalization in stimulating plant growth and flower initiation of shallot (*Allium cepa* var. Aggregatum).

Materials and Methods

<u>The Effect of Gibberellic Acid on Shallot Growth and Yield</u>

The research was carried out in the field located in 700 m above sea level in the planting season 2019 and 2020. The experiment arranged in a completely randomized block design with 2 factors and 3 replications. The first factor was 5 varieties of shallots, namely Bauji, Bima Brebes, Super Philip, Tajuk, and Thailand. The second factor was the concentration of GA_3 which were 50, 100,150 mg L^{-1}, and without GA_3 as a control.

Shallot bulbs sized of 3-5 grams were used as planting materials. The experimental land was cultivated and made into a mound of plots measuring 100 cm x 120 cm each, and 30 cm height. The soil was mixed with manure (at 10 ton ha^{-1}) and the plots covered with silver black plastic mulch. Planting holes were made with a spacing of 20 cm x 20 cm. Inorganic fertilizers of Urea, SP-36, and KCl were given as basic fertilizers, at 250, 150, and 150 kg ha^{-1}, respectively. Shallot bulbs were cut off one third on the bulb top, and then soaked for one hour in the GA_3 solution with concentration as described previously. Then, the shallot bulbs were planted through the holes by immersing the bulb into the soil and covering with a thin layer of soil. Plant maintenances included watering and controlling pests with pesticides were done before harvesting at 65 days after planting. Harvesting was done in the morning or during sunny conditions by carefully pulling the shallot plants.

Observation were carried out on the growth and yield variables, which included : plant height, number of leaves, number of tillers, bulb diameter, number of bulbs, fresh weight of bulb, and dry weight of bulb. Data were statistically analyzed with ANOVA at 5% using SAS program version 9.1, and further tested by a *Least Significance Different* (LSD) test with a 95% confidence level.

<u>The Effect of Vernalization on Shallot Growth and Flower Initiation</u>

The experiment was carried out in a completely randomized design, with two factors. The first factor was 5 varieties of shallot as described previously and the second factor was vernalization treatment, which was with and without vernalization of the shallot bulbs. Vernalization was carried in storage room for 6 weeks at 8 ^{o}C. The shallot bulbs were planted in polybags with a diameter of 45 cm containing 10 kg of planting medium (which was mixed of soil, manure, and rice husk in ratio of = 2: 1: 1). Each polybag was planted with three shallot bulbs. Before planting, the shallot bulbs were immersed for 15 minutes in a fungicide solution containing *Benomyl* 2 g.L^{-1} for 15 minutes. Then, the shallot bulbs were soaked again for another 15 minutes in the PGPR (*plant growth promoting rhizobacteria*) solution at 5 g. L^{-1}. The plants were fertilized with NPK mixture fertilizer (15:15:15) at a 2.4 g per polybag, or similar to 600 kg. ha^{-1}. Plant maintenances were carried out similar to previous experiment. The shallot bulbs were harvested at 65 days after planting.

Morphological characters of the bulb weight were carried out by weighing the bulb before planting, while the characters of plant height, number of tillers, number of leaves were observed at 5 weeks after transplanting. Flowering characters observed as sprouting time, time to flowering, number of umbels, umbel diameter, length of umbel stalk, and time to umbel broke, were done when 75% of the plants shown those characteristics. The percentage of flowering plants was observed by counting the number of flowering plants divided by the number of plants for each treatment in each replication.

Data were analyzed statistically using ANOVA to determine the effect of vernalization on the morphological and flowering characters. Further analysis was carried out based on *Least Significance Different* (LSD) test with a 95% confidence level. The analysis using the SAS program version 9.1. A cluster analysis was conducted using an unweighted pair group method arithmetic with means (UPGMA). This analysis was conducted with the Cluster package from the R-software package (R version 3.2.2).

Results and Discussion

The Effect of GA_3 on 5 Varieties of Shallot

The results of the analysis of variance on growth and yield of shallots showed the interaction between GA_3 application and shallot varieties which observed significantly effects on the number of leaves, and number of bulbs. The further analysis with LSD test is presented in Table 1. A higher response of the number of leaves and bulbs at all GA_3 concentrations were observed on Super Phillip variety. Meanwhile, Bima Brebes had the highest number of leaves and bulbs at 100-150 mg L^{-1} GA_3. The plant growth regulators might be needed to increase shallot production, however GA_3 influenced growth by promoting elongation of stem and internodes of plant. Sravani et al. (2020) reported that the highest plant was obtained under the treatment of GA_3 at 25 mg. L^{-1}. This might be due to the increasing of cell wall extensibility by GA_3. Application of the exogenously GA_3 might have activated the endogenous hormonal activities which ultimately led to leaf elongation of plant.

Gibberellic acid (GA_3) is one of the main regulators of the growth and development of plants which stimulates not only the growth and promoting of cell division and elongation (Olszewski et al., 2002), but also plays a major role in diverse growth processes including seed development, organ elongation, senescence and control of flowering time (Yamaguchi 2008; Ouzounidou et al., 2011). The increase in the number of leaves per plant is mainly due to the enhancement of cell elongation and cell division. It enhances also the photosynthesis and respiration which catalyze the metabolism activities in plant. The results are conformed with the findings of earlier reports in onion (Hye et al., 2002; Tiwari et al., 2003; Patel et al., 2010;), and garlic (Singh et al., 2014; Govind et al., 2015).

Five varieties of shallot showed different growth and yield variables (Table 2). The variety of Tajuk showed the highest plant height (43.3 cm) and had the highest number of shoots per plant (8.1 shoots). Tajuk variety also showed higher yield compared to other varieties. It had the highest responses in the number of bulbs (9.41 bulbs/plant), bulb fresh weight (90.1 g/plant) and bulb net weight (75 g/plant).

The application of GA_3 singly had no significant effect on another growth and yield of shallot (Table 3). These results are similar to those reported in garlic, observed that the plant height or the stem length at 15 and 25 days after planting were not significantly different among various concentration of GA_3 (Rahman et al., 2006). However, the results showed that the plant heights were observed of 44.61-45.74 with the application of GA_3 at 0-100 mg L^{-1}, while the application of GA_3 at 150 mg L^{-1} was only 40.11 cm. Shaikh et al., (2002) reported that the application of GA_3 at 50 mg L^{-1} to large or medium bulbs produced a significantly higher seed yield per hectare, germination and vigour values on onion. Kucera et al. (2005) showed that the applications of GA_3 on plants increased the total plant height of onion and garlic by 35% and 25% of the control, respectively.

Helaly et al. (2016) reported that GA_3 application on *Allium cepa* did not significantly affect the plant fresh weight, but increased the number of leaf, plant height and could allow for higher plant density, therefore higher total yield. A vigorous onion and garlic growth and yield were promoted by GA_3 application (Kucera et al., 2005; Ouzounidou et al., 2011). GA_3 application stimulated and integrated the overall growth, development and reproduction of shallot.

The Effect of Vernalization on Growth and Flower Initiation of Shallot

The ANAVA showed that there was an interaction between vernalization treatment and the variety of shallot observed on time to sprout and the flowering characters of shallot. The interactions were able to increase the number of umbel, the diameter of umbel, the length of umbel stalk, and time to umbel broke in all varieties except Sumenep variety (Figure 1).

The flowering ability of shallots depends on the genetic variability and environmental conditions. The vernalization treatment can stimulate flowering and produce more seeds (Khokhar, 2014). The vernalization signal received by plants is permanent and persists in subsequent crop development (Song et al., 2012). Plant growth environment becomes exogenous factors which has a strong influence in determining the ability of flowering. Inflorence develops from the apical meristem under suitable conditions.

The interaction between variety and vernalization gave a significantly different effect on the flowering quantitative character of shallot. Observations on the quantative characters of flowering showed that the Ilokos variety was responsive to vernalization treatment. While the Sumenep variety was not sensitive to flowering. There was no increase in all flowering characters in the Sumenep variety. The interaction effect between variety and vernalization treatment was able to increase umbel diameter characters in the varieties of Bima Brebes, Ilokos and Tajuk (Fig. 1G). The reports by Mardiana (2016) and Kusumadewi et al. (2017) showed that vernalization was effective in increasing flowering of shallots.

The average of shallot flowering without vernalization was 20%, while with vernalization increased up to 39%. The Bentanis variety showed no difference in the percentage of flowering between those treated or not treated with vernalization. This indicated that the Bentanis variety is a sensitive variety to flowering, and able to produce flowers in all growing conditions. The result showed the importance of vernalization treatment to initiate flowering which might relate to the temperate origin of shallot. It was reported by Lee et al. (2013) and Marlin et al. (2018) that vernalization blocked flowering repressor and induced expression of genes responsible for the flowering (florigen). Vernalization could also promote the up-regulation of some key cytokinin signaling regulators which induced flowering (Wen et al., 2017). In contrast to the Sumenep variety that it was not able to increase the ability of flowering even though it was treated with vernalization.

The results showed that variety had a significant effect on the character of the initial bulb weight, time to flowering, umbel number, umbel diameter, length of umbel stalk, and the percentage of flowering (Figure 2). The LSD test results showed that the varieties of Bentanis, Bima Brebes and Tajuk had higher initial tuber weights (5.43-6.80 g) compared to the Ilokos and Sumenep variety (3.31-3.65 g).The variety of Bentanis, Bima Brebes and Tajuk were higher than those of Ilokos and Sumenep. It was suspected that with the larger size of bulbs, the varieties of Bentanis, Bima Brebes and Tajuk had more food reserves, which affected the growth of plant height. On the other hand, the Ilokos and Sumenep varieties had a greater number of leaves and tillers than the other three varieties.

The quantitative character of flowering was controlled by many genes and is also influenced by environmental factors. The percent flowering of shallot were strongly influenced by variety and vernalization, but no interaction was found. Bentanis variety has the same flowering percentage as Bima Brebes and Tajuk, which is around 32-52%, while the Ilokos variety has 22% flowering percentage, and the Sumenep variety has no flowering ability.

The results showed that the vernalization treatment singly had a very significant effect on the character of the initial bulb weight and the percentage of flowering (Figure 3). The results showed that the plant height, the number of tillers, and the percentage of shallot flowering actually increased with the vernalization treatment. However, the vernalization treatment reduced the bulb initial weight.

Vernalization was an important adaptation of plants to initiate flowering in response to prolonged exposure to low temperatures (Finnegan et al., 2001; Song et al., 2012). The vernalization treatment had a stressful effect on plants which caused the plants to use more energy during their early growth period. The bulbs without vernalization treatment still store a lot of energy that can be used for optimal growth. Wu et al. (2016) stated that the vernalization in garlic inhibited the number of leaf, pseudostem diameter, and plant height. The vernalization of garlic bulbs at 4˚C (for 2 months) resulted in bolting, inflorescence formation and true seed production in 9 varieties whereas non-vernalized failed to result into bolting, i.e. no true seed production was determined.

Cluster analysis showed that 5 varieties of shallots were divided into 3 groups according to the similarity of morphological and flowering characters (Figure 4). The 3 patterns of flowering ability in shallot varieties, namely natural (sensitive flowering), medium sensitive, and non sensitive flowering ability. The natural flowering ability in shallot shown by the ability to flower naturally in shallot varieties with or without external stimulation. The medium sensitive variety of shallot will produce flowers in the presence of stimulation from external treatments, such as vernalization. Meanwhile, a non-sensitive variety was not able to produce flowers naturally even with external stimuli.

The clustering of morphological and quantitative flowering characters was visualized graphically with a matrix representation of the degree of dissimilarity between the 5 local varieties of shallots. The 5 varieties of shallot were grouped into 3 groups based on their flowering ability with similar morphological and quantitative flowering characters in which were given vernalization treatment and without vernalization treatment. The first group consisted of variety Bentanis (G1), Bima Brebes (G2), and Tajuk (G4). In the second group there was the Ilokos (G3) variety, and in the third group there was Sumenep (G5) variety. Each variety in the same group were similar based on morphological and flowering characters. Analysis of the 12 morphological and quantitative flowering characters of shallots further confirmed the different ability patterns of the tested shallot varieties. The difference in the grouping of the 5 shallot varieties indicates that there are morphological and flowering variations among the five varieties, especially in terms of their flowering competence.

The results showed that the Sumenep variety had a different flowering pattern with other varieties, both without vernalization and with vernalization treatment. The Sumenep variety had the highest dissimilarity value compared to other varieties. The large disimilarity value indicated that the Sumenep variety has the different morphological and flowering characters from others. The Sumenep variety is a non sensitive flowering variety, even with the induction treatment such as vernalization treatment. Sumenep varieties are generally difficult to produce flowers (Idhan et al., 2015), The ideal grouping of varieties is when all the varieties in a group have a dissimilarity value equal to zero, but with varieties from other groups the dissimilarity value is equal to one. Identification of the morphological diversity and flowering ability of shallots is very useful knowledge in the efforts of onion breeding and cultivation development programs.

Conclusions

The GA_3 might be important for increasing shallot production and influence growth processes by promoting shoot growth and bulb initiation. With the application of GA_3 at up to 100 mg L^{-1}, the height of plants reached from 44.61 to 45.74 cm. Tajuk variety showed better yield characters compared to other varieties. It had the highest responses in bulb number of 9.41 bulbs/plant, bulb fresh weight of 90.1 g/plant, and bulb net weight of 75 g per plant. Five varieties of shallots were clustered into 3 groups according to the similarity of morphological and flowering characters. Based on flowering characters, Bentanis variety is sensitive to vernalization and able to produce flowers in all growing conditions, Ilokos variety is responsive to vernalization, while Sumenep variety is not sensitive to vernalization. The environment conditions were the exogenous factors for plant growth which had a strong influence in determining the ability of flowering.

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Authors Contribution Statement

Marlin Marlin and Hartal Hartal designed and performed experiments. Marlin Marlin performed data analysis was in charge of the overall direction and planning, writing, and interpretation of the manuscript and interpretation of results. Atra Romeida and Reny Herawati participated in data collection and statistical analysis. Marulak Simarmata and other authors were involved in writing and review the article.

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Table 1. Interaction between GA_3 concentrations and 5 varieties of shallots on the number of leaves and bulbs of shallot

Variety	Concentration of GA_3 (mg L^{-1})	Number of leaves	Number of bulbs
Bauji	0	21.60e	5.60d
	50	27.63d	5.87d
	100	32.87c	7.03cd
	150	27.73d	6.15d
Bima Brebes	0	41.93bc	8.41bc
	50	39.46bc	8.67bc
	100	48.53ab	10.68abc
	150	32.13c	10.12abc
Super Philip	0	48.93ab	9.25bc
	50	52.93a	12.50ab
	100	56.60a	14.35a
	150	44.20b	11.21abc
Tajuk	0	40.06bc	10.55abc
	50	39.13bc	8.36bc
	100	33.80c	7.91c
	150	44.46b	10.83abc
Thailand	0	31.6c	8.05bc
	50	40.16b	9.12bc
	100	38.87bc	8.23bc
	150	36.66bc	8.87bc

The numbers followed by the same letter within each column are not significantly different based on the LSD test at 5% level.

Table 2. Morphological characters on 5 varieties of shallots

Variety	Plant height (cm)	Number of leaves	Number of shoots	Bulb diameter (cm)	Number of bulbs	Bulb fresh weight (g)	Bulb net weight (g)
Bauji	32.6c	24.1c	4.7ab	19.8a	5.60c	26.1c	24.4c
Bima Brebes	33.0c	40.51b	4.7b	18.5a	9.61b	41.6bc	40.3b
Super Philip	38.1b	50.66a	6.3ab	16.7a	11.83a	50.2b	33.2bc
Tajuk	45.3a	39.6b	8.1a	20.8a	9.41b	90.1a	75.0a
Thailand	38.4b	32.0bc	5.6ab	17.2a	8.0b	44.2bc	34.7bc

The numbers followed by the same letter within each column are not significantly different based on the LSD test at 5% level.

Table 3. Morphological response of shallots at different GA_3 concentrations

Concentration of GA_3 (mg L^{-1})	Plant height (cm)	Number of leaves	Number of shoots	Bulb diameter (cm)	Number of bulbs	Bulb fresh weight (g)	Bulb net weight (g)
0	45.74a	43.64a	8.00a	1.82a	9.41a	51.03a	38.39a
50	44.61a	43.84a	8.80a	1.71a	9.85a	50.02a	34.24a
100	41.89ab	46.31a	8.88a	1.81a	11.62a	52.06a	38.70a
150	40.11b	40.26a	8.22a	1.66a	10.28a	43.79a	31.53a

The numbers followed by the same letter within each column are not significantly different based on the LSD test at 5% level.

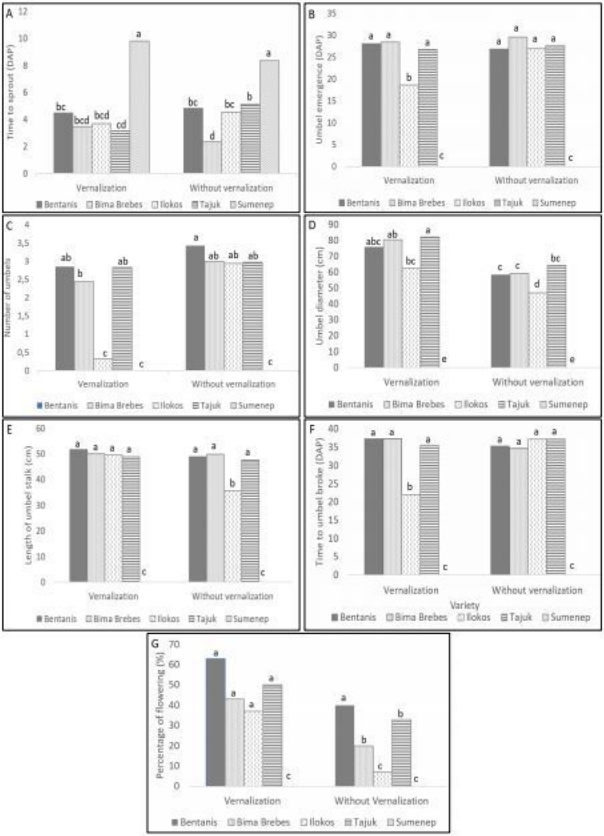

Figure 1. The interaction between shallot varieties and vernalization on time to sprout (A), day of umbel emergence (B), number of umbel(C), umbel diameter (D), length of umbel stalk (E), time to umbel broke (F), and percentage of flowering (G) of shallot. Same letters in each variable response indicated no significant differences based on LSD test at 5% level.

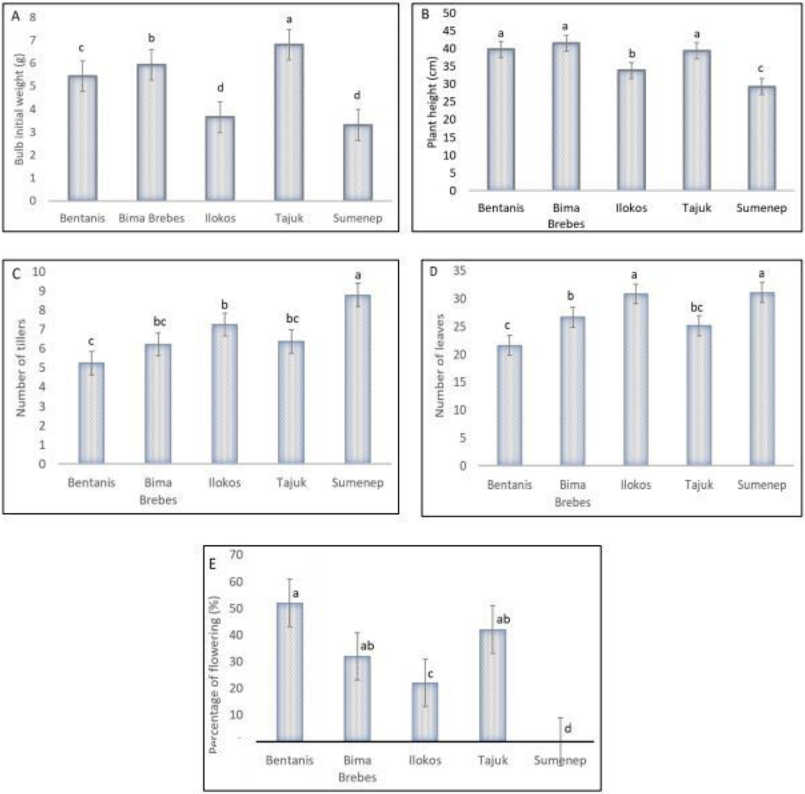

Figure 2. Effect of variety on bulb initial weight (A), plant height (B), number of tillers (C), number of leaves (D), and percent of flowering (E) of shallots. Same letters within each variable response indicated no significant differences by LSD test at 5% level.

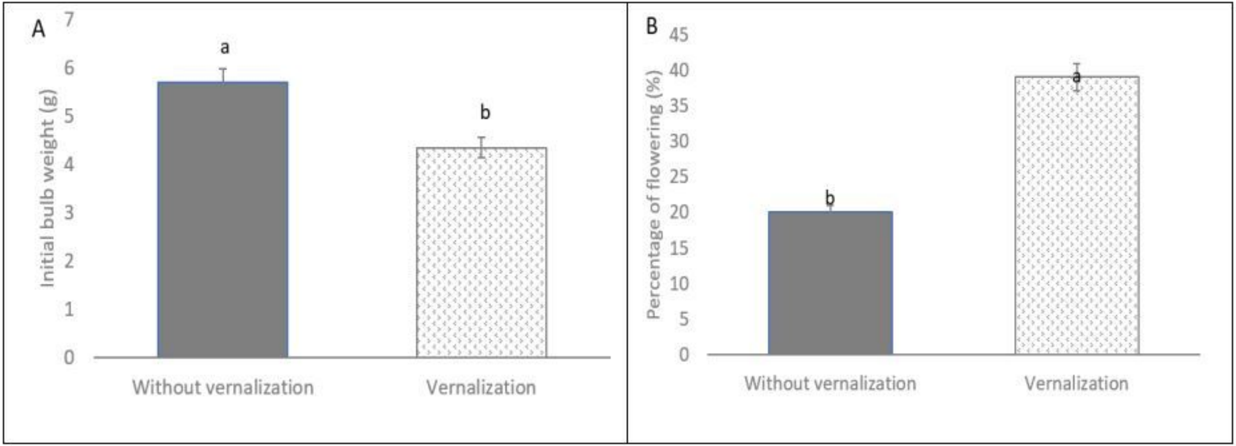

Figure 3. The effect of vernalization on bulb initial weight (A) and percent of flowering (B) of shallots. The numbers followed by the difference lowercase letter are significantly different based on the LSD test at 5% level. The numbers followed by the difference capital letter are significantly different based on the LSD test at 5% level.

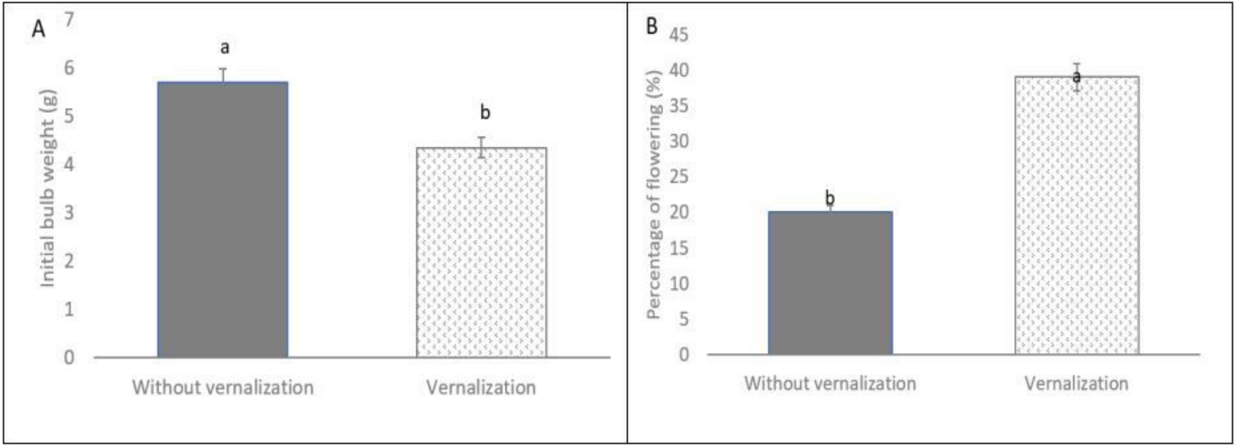

Figure 3. The effect of vernalization on bulb initial weight (A) and percent of flowering (B) of shallots. The numbers followed by the difference lowercase letter are significantly different based on the LSD test at 5% level. The numbers followed by the difference capital letter are significantly different based on the LSD test at 5% level.

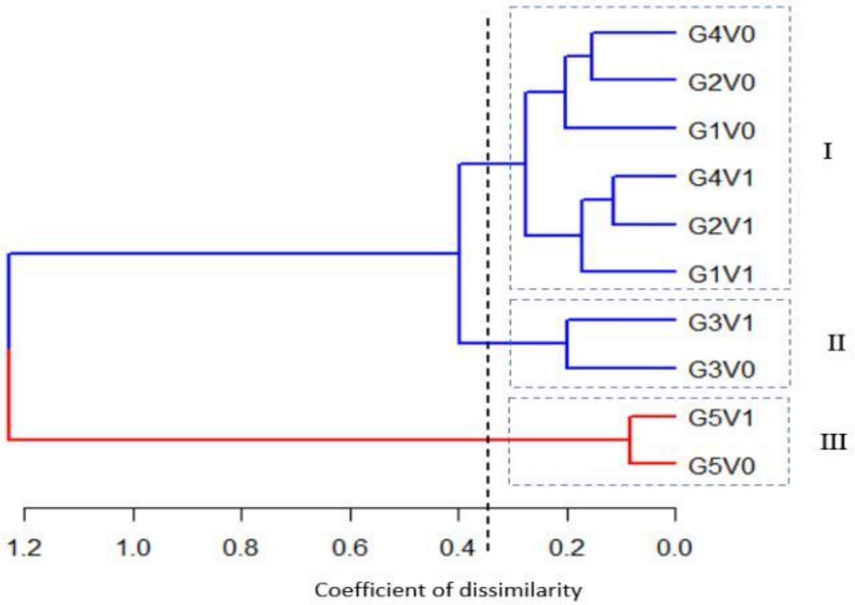

Figure 4. Hierarchical cluster of the dissimilarity matrix of vernalization treatments of 5 shallot varieties. Without vernalization (V0), and vernalization (V1). Variety of Bentanis (G1), Bima Brebes (G2), Ilokos (G3), Tajuk (G4), Sumenep (G5).

Consent of Patient

I give my consent for the material to appear in article, entitle ‘Morphological and Flowering Characteristics of Shallot (*Allium cepa* Var. Aggregatum*)* in Response to Gibberellic Acid and Vernalization’.
I confirm that I am legally entitled to give this text or other material consent. I understand the article may be published in a journal which is distributed worldwide. The article, including the Material, may be the subject of a press release, and may be linked to from social media and/or used in other promotional activities. Once published, the article will be placed on Emirate Journal of Food and Agriculture website and may also be available on other websites. The text of the article will be edited for style, grammar and consistency before publication.

April 24. 2021

Dr. Marlin
Department of Crop Science Faculty of Agriculture
University of Bengkulu, INDONESIA

Marlin UNIB <marlin@unib.ac.id>

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noreply@uaeu.ac.ae <noreply@uaeu.ac.ae> 23 April 2021 pukul 12.05
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Marlin UNIB <marlin@unib.ac.id>

Submission progress

Marlin <marlin@unib.ac.id> 26 April 2021 pukul 22.58
Kepada: "ejfa@uaeu.ac.ae" <ejfa@uaeu.ac.ae>

Dear Editors,

I have submitted article entitle : Morphological and Flowering Characteristics of Shallot (Allium cepa Var. Aggregatum) in Response to Gibberellic Acid and Vernalization, for consideration for publication in Emirate Journal of Food and Agriculture. I have submitted the complete article, but in the article status it is stated as an uncomplete submission. Is there an incomplete item uploaded? Please help so that the article can be processed and can be published in Emirate Journal of Food and Agriculture. Thank you for your help and cooperation.

Sincerelly yours.

Dr. Marlin

Department of Crop Production

Faculty of Agriculture, University of Bengkulu

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ejfa <ejfa@uaeu.ac.ae> 27 April 2021 pukul 15.21
Kepada: Marlin <marlin@unib.ac.id>

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Marlin UNIB <marlin@unib.ac.id>

Submission Confirmation

Emirates Journal of Food and Agriculture <noreply@ejmanager.com> 27 April 2021 pukul 22.26
Balas Ke: Emirates Journal of Food and Agriculture <ejfa@uaeu.ac.ae>
Kepada: marlin@unib.ac.id

Dear Marlin Marlin,

Your submission entitled **Morphological and Flowering Characteristics of Shallot (Allium cepa Var. Aggregatum) in Response to Gibberellic Acid and Vernalization** (Manuscript Number: EJFA-2021-04-206) has been received by **Emirates Journal of Food and Agriculture**.

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Thank you for submitting your work to our journal.

Best regards,

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Article Revision Letter for Authors - (EJFA-2021-04-206)

Emirates Journal of Food and Agriculture <noreply@ejmanager.com> 9 Juni 2021 pukul 15.44
Balas Ke: Emirates Journal of Food and Agriculture <ejfa@uaeu.ac.ae>
Kepada: marlin@unib.ac.id

Dear Marlin Marlin,

Your manuscript entitled \"Morphological and Flowering Characteristics of Shallot (Allium cepa Var. Aggregatum) in Response to Gibberellic Acid and Vernalization\" (Ms.Nr. EJFA-2021-04-206) was reviewed by editorial board members of the Emirates Journal of Food and Agriculture. As initial decision, your manuscript was found interesting but some revisions have to be made before it can reach a publishable value.
Please answer all the comments below point-by-point in an accompanying response letter to your revised submission.

You should send your revised manuscript via the online system of eJManager on http://my.ejmanager.com.

Sincerely yours,

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Impact Factor: 1.008
2019 Journal Citation Reports® (Clarivate Analytics, 2020)

COMMENTS for Authors:

=> Reviewer # 1

In the manuscript “Morphological and Flowering Characteristics of Shallot (Allium cepa Var. Aggregatum) in Response to Gibberellic Acid and Vernalization” the authors evaluated the effect of gibberellic acid (GA3) and vernalization on different morphological and flowering traits of shallot (Allium cepa var. Aggregatum).

The manuscript has elements of originality, especially through the studied biological material (varieties of shallot), the large number of analyzed characteristics and statistical analyzes, but unfortunately, it also has different shortcomings. Because of these, the manuscript must be thoroughly revised.

The abstract is not well enough structured to contain a short explanation of the motivation of the research (why the authors conducted the study, respectively the background of this investigation), what question(s) the authors aimed to answer (objectives). The presentation of how the authors performed the study (methods) is unclear and with not enough coherence for readers. The presentation of final founds (results: major data, relationships), and the interpretation and main consequences of the findings (conclusions) are not really relevant, or are insignificant or superfluous (\"Based on the vernalization treatment, 5 varieties of shallots were clustered into 3 groups, which were indicated that the morphological and flowering variations, especially in terms of the flowering competence. Vernalization was effective in increasing flowering of Ilokos variety. But,there was no increase in all flowering characters in the Sumenep variety which confirmed as a non-sensitive flowering variety.\").
In addition, the wording of the manuscript is very simplistic, starting with the Abstract (i.e., \"The research was conducted to determine the effect of gibberellic acid (GA3) and vernalization on the morphological and flowering character of shallot.\"; \"The first experiment was arranged in the field and organized ...\"; \"The first factor was 5 varieties of shallots...\". There are also various language inaccuracies, editing and typographical errors.

The above deficiencies extend generally, to the entire manuscript. Some of the major issues are listed below.

The Introduction must summarize in a more pertinent manner the relevant literature so that the reader will understand the necessity of this study. At the end of the Introduction, the authors must explain (but duly justified) the aim of the study.

The presentation of the Material and Method chapter is not adequate and does not provide the reader with a clear understanding of the methodology and workflow. Please organize your presentation so that readers will understand the logical flow of the experiment; adequate subheadings (but not overlapping with those of \'Results and Discussion\'!!) work well for this purpose.
E.g. ‘Description of the study site’ (where did the research take place???); ‘Biological material’; ‘Experimental OR sampling design’; ‘Experimental procedures OR Protocol for collecting data’; ‘Qualitative analysis AND/OR statistical procedures’ etc.

The results are consistent through the number of characteristics analyzed and statistical analyzes.

They referred to \"Morphological characters\", i.e. the number of leaves and bulbs of shallot (Table 1), plant height, number of leaves and shoots, bulb diameter, number of bulbs, bulb fresh weight and bulb net weight (Tables 2 and 3); the interaction between vernalization treatment and the variety of shallot observed on time to sprout and the flowering characters of shallot (Figure 1); the genotypes effect on the character of the initial bulb weight, time to flowering, umbel number, umbel diameter, length of umbel stalk, and the percentage of flowering (Figure 2).

Except these \"Morphological characters\", it would have been interesting also to analyze other traits, i.e. quality elements or chemical composition of the bulbs etc.

Cluster analysis may appear redundant in the context of the information of interest it provides. Probably, a regression between the five levels of Concentration of GA3 (mg L-1): 0, 50, 100, 150 and the analysed traits could be more relevant for the provided information than the cluster analysis.

In addition, the text and illustration should be reviewed:

- Improve all figures, as design, information; e.g. Figure 2 – it is mandatory to include for error bars the parameter used, e.g. SD or SEM, explanation etc.

- Avoiding mistakes, e.g.

row 176: “The ANAVA showed…” (instead ANOVA) etc., but please revise the whole manuscript in order to avoid other inadvertences and mistakes as technical or formal flaws.

#reviewer 2

-Introduction should be more focused on the previous works done on this topic

-Please clearly mention the objectives

-Detailed methodology is not needed, instead, provide standard method references

-Add photographs from the experiments

-Conclusion should be in a separate section

-Provide author contributions as separate section before reference

-References should be exactly as per the journal format, refer a recent article from the current issue in www.ejfa.me

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Revised Article Submission

Emirates Journal of Food and Agriculture <noreply@ejmanager.com> — 1 Juli 2021 pukul 23.00
Balas Ke: Emirates Journal of Food and Agriculture <ejfa@uaeu.ac.ae>
Kepada: marlin@unib.ac.id

Dear Marlin Marlin,

Your REVISED ARTICLE entitled **Morphological and Flowering Characteristics of Shallot (Allium cepa Var. Aggregatum) in Response to Gibberellic Acid and Vernalization** (Mns No:EJFA-2021-04-206) has been received by **Emirates Journal of Food and Agriculture**.

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Thank you for submitting your REVISED version of your article.

Best regards,

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JOURNAL REVISION LETTER

July. 1st. 2021

Editors
Emirates Journal of Food and Agriculture [E JFA]

Dear Editors,

It is with excitement that I resubmit to you a revised version of manuscript 137-1619535481, entitle: **Morphological and flowering characteristics of shallot (*Allium cepa* var. Aggregatum*)* in response to gibberellic acid and vernalization**" by Marlin Marlin, Hartal Hartal, Atra Romeida, Reny Herawati, and Marulak Simarmata, for the *Emirates Journal of Food and Agriculture [EJFA]*.
Thank you for giving me the opportunity to revise and resubmit this manuscript. I appreciate the time and detail provided by each reviewer and by you and have incorporated the suggested changes into the manuscript to the best of my ability. The manuscript has certainly benefited from these insightful revision suggestions. I look forward to working with you and the reviewers to move this manuscript closer to publication in the *Emirates Journal of Food and Agriculture [EJFA]*.

I have responded specifically to each suggestion below. To make the changes easier to identify where necessary, I have numbered them.

Reviewer's suggestions:
Most notably, your revised manuscript should:

1. The abstract is not well enough structured to contain a short explanation of the motivation of the research (why the authors conducted the study, respectively the background of this investigation), what question(s) the authors aimed to answer (objectives).

(Reviewer 1 and Reviewer 2)

- I have improved the abstract and added short explanation of the experiment background; see pp. 2.

2. The Introduction must summarize in a more pertinent manner the relevant literature so that the reader will understand the necessity of this study. At the end of the Introduction, the authors must explain (but duly justified) the aim of the study. (Reviewer 1 and Reviewer 2)

    - I have summarized the introduction with relevant literatures, see pp. 3-4. I have also added the explanation about the aimed of the study at the end of introduction, see pp. 4.

3. The presentation of the Material and Method chapter is not adequate and does not provide the reader with a clear understanding of the methodology and workflow. Please organize your presentation so that readers will understand the logical flow of the experiment; adequate subheadings (but not overlapping with those of \'Results and Discussion\'!!) work well for this purpose (Reviewer 1).
Detailed methodology is not needed, instead, provide standard method references (Reviewer 2).

    - I have organized the presentation of Material and Methods, and provided with workflow subheading, see pp. 4-7.

4. The results are consistent through the number of characteristics analyzed and statistical analyzes.
(Reviewer 1)

    - I attempted to frame the experiment findings that I highlighted in the discussion. on pp. 7-13.

5. Improve all figures, as design, information (Reviewer 1 and Reviewer 2)

- A figure has been improved; see figure 1-4.

6. Avoiding mistakes, e.g. row 176: “The ANAVA showed…” (instead ANOVA) etc., but please revise the whole manuscript in order to avoid other inadvertences and mistakes as technical or formal flaws (Reviewer 1)
   - I have attempted to change those typing mistakes, on pp. 9. I have also revised the typing mistakes in whole manuscript.

7. Conclusion should be in a separate section (Reviewer 2)
   - I have provided the conclusion in separated section, on pp. 13.

8. Provide author contributions as separate section before reference (Reviewer 2).
   - I have provided the author contributions as separate section before reference, on pp. 14.

9. References should be exactly as per the journal format, refer a recent article from the current issue in www.ejfa.me (Reviewer 2).
   - I have attempted to change the references as EJFA current issue format, see pp. 14-19.

Sincerely yours,

Dr, Marlin
Department of Crop Production
Faculty of Agriculture, University of Bengkulu
Indonesia

Emir. J. Food Agric

Morphological and Flowering Characteristics of Shallot (Allium cepa Var. Aggregatum) in Response to Gibberellic Acid and Vernalization

Journal Name :	Emirates Journal of Food and Agriculture
Manuscript ID :	EJFA-2021-04-206
Manuscript Type :	Regular Article
Submission Date :	27-Apr-2021

Authors :	Marlin Marlin|Hartal Hartal|Atra Romeida|Reny Herawati|Marulak Simarmata|

Morphological and Flowering Characteristics of Shallot (*Allium cepa* Var. Aggregatum) in Response to Gibberellic Acid and Vernalization

Marlin Marlin[1*], Hartal Hartal[2], Atra Romeida[1], Reny Herawati[1], Marulak Simarmata[1]

[1]*Department of Crop Production, Faculty of Agriculture, University of Bengkulu, Indonesia*

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Abstract

Shallot plants have variations in morphological and flowering characters. Flowering ability can be induced by the treatment of gibberellic acid (GA_3) and exposing to cold temperature (vernalization). The objectives of the research were to determine the effect of GA_3 and vernalization on the morphological and flowering characters of 5 shallot varieties. Field study was organized in a completely randomized block design with three replications to evaluate the responses of 5 varieties of shallots (Bauji, Bima Brebes, Super Philip, Tajuk, and Thailand) to GA_3 with the concentrations ranged from zero to 150 mg L^{-1}. Plants were maintained for 65 days until harvesting. The second study was organized in a completely randomized design with 3 replications to evaluate the responses of the five varieties of shallot to vernalization. The vernalization of shallot bulbs were done at 8 ^{o}C for 6 weeks. The results indicated that a Super Phillip variety showed the highest response to GA_3 observed in the number of leaves and bulbs. At 100 mg L^{-1} of GA_3 increased the plant height up to 45.74 cm. The results from the second study showed that vernalization was effective to increase flowering only on the Ilokos variety. But the other varieties were not sensitive to vernalization. Based on morphological and flowering characters, 5 varieties of shallots were clustered into 3 groups, namely: sensitive flowering included Bentanis, Bma Brebes and Tajuk variety, medium sensitive flowering included Ilokos variety, and non-sensitive flowering included Sumenep variety.

Key words: Flower induction; Gibberellic acid; Shallot varieties; Vernalization

Introduction

Shallot (*Allium cepa* var. Aggregatum) is one of the economically important crop belong in the Liliaceae family. Shallot bulb contains important nutritive vegetable and medicinal (Mohammadi-Motlagh et al., 2011; Marlin et al., 2019). Shallot cultivation requires specific edaphoclimatic conditions and agricultural management to grow, overcome bulb dormancy, induce flower development, reproduce bulbs, and true seeds (Tendaj and Mysiak, 2013; Farhadi and Salteh, 2018).

Shallot responses to agricultural management and environmental conditions differ among different variety. Selection of the elite variety is an essential for obtaining desired growth and quality of bulbs, and induces the flower formation. The varieties of shallot in Indonesia have the ability to produce flowers, except for the Sumenep variety (Marlin et al., 2018). Cultivation techniques for developing shallot flowering initiation have not been widely developed. Shallot growth and development can be induced by optimizing genetic ability and manipulating the growing environment.

Treatments to induce flowering and seed formation can be carried out using growth regulators such as gibberellic acid and vernalization treatments. Gibberellic acid and vernalization treatment play a role in the plant growth and the process of flowering initiation. Both treatments work by stimulating the formation of flowering genes such as the SOC1 gene (suppressor of overexpression of constant 1) and the LEAFY gene (Corbesier and Coupland, 2006). The LEAFY gene is the main gene that controls flowering in shallot and predicticably have been related to flowering pattern (Marlin et al., 2018).

Bio-regulators like gibberellic acid (GA_3) have been known to play a vital role in building of plants and involved in plant growth together with stem elongation (Rahman et al., 2006), and the transition from vegetative growth to flowering (Sumarni et al., 2013). The treatment at low temperatures (vernalization) can stimulate flower formation (Song et al., 2012). Vernalization is an important adaptation of plants to initiate flowering in response to prolonged exposure to low temperatures (Finnegan et al., 2001; Song et al., 2012). Elsiddig et al. (2015) showed that vernalization treatment at a temperature of 4-5 ^{o}C for 90 days was a major factor to induce flowering in Texas Grano cultivar onions. The use of gibberellins and vernalization treatments to stimulate the growth and flowering for shallot has not been described. This studies were conducted to determine the effect of applying GA_3 and vernalization in stimulating plant growth and flower initiation of shallot (*Allium cepa* var. Aggregatum).

Materials and Methods

<u>Experimental 1:</u>

Experimental Site and Layouts

Field experiment was carried out in the field located in 700 m above sea level in the planting season of 2019 and 2020. The experimental land was cultivated and made into a mound of plots measuring 100 cm x 120 cm each, and 30 cm height. The soil was mixed with manure at 10 tons ha^{-1} and the plots covered with silver black plastic. Planting spacing was 20 cm x 20 cm. Inorganic fertilizers of urea, super phosphat-36, and potassium chloride were given as basic fertilizers, at 250, 150, and 150 kg ha^{-1}, respectively.

The experiment with two factors was organized in a randomized complete block design with three replications. The first factor was 5 varieties of shallots, namely Bauji, Bima Brebes, Super Philip, Tajuk, and Thailand. The second factor was the concentration of GA_3 which were 50, 100,150 mg L^{-1}, and without GA_3 as a control.

Experimental Material

Five varieties of local shallot, namely Bauji, Bima Brebes, Super Philip, Tajuk, and Thailand were used as planting material. The average size of the bulbs was 3-5 grams. Shallot bulbs were cut off one third on the bulb top, and then soaked for one hour in the GA_3 solution with concentration as described previously. Then, the shallot bulbs were planted through the holes by immersing the bulb into the soil and covering with a thin layer of soil. Plant maintenances included watering and controlling pests with pesticides were done before harvesting at 65 days after planting. Harvesting was done in the morning or during sunny conditions by carefully pulling the shallot plants.

Data Collection and Analysis

Observation was carried out on the growth and yield variables, which included: plant height, number of leaves, number of tillers, bulb diameter, number of bulbs, fresh weight of bulb, and dry weight of bulb. Data were collected from selected plants in each unit plot. To avoid border effect with the highest precision, 10 plants were selected randomly from each plot. Data were statistically analyzed with ANOVA at 5% using SAS program version 9.1, and further tested by a *Least Significance Different* (LSD) test at a 95% confidence level.

<u>Experiment 2:</u>

Experimental Site and Layouts

The experiment was carried out in a completely randomized design, with two factors. The first factor was 5 varieties of shallot as described previously and the second factor was vernalization treatment, which was with and without vernalization of the shallot bulbs. Vernalization was carried in storage room for 6 weeks at 8 ^{o}C. The shallot bulbs were planted in polybags with a diameter of 45 cm containing 10 kg of planting medium (which was mixed of soil, manure, and rice husk in ratio of = 2: 1: 1). Each polybag was planted with three shallot bulbs. The plants were fertilized with NPK mixture fertilizer (15:15:15) at a 2.4 g per polybag, or similar to 600 kg. ha^{-1}. Plant maintenances were carried out similar to previous experiment. The shallot bulbs were harvested at 65 days after planting.

Experimental Material

Five varieties of local shallot as described previously were used as planting material. Before planting, the shallot bulbs were immersed for 15 minutes in a fungicide solution containing *Benomyl* 2 g.L^{-1} for 15 minutes. Then, the shallot bulbs were soaked again for another 15 minutes in the PGPR (*plant growth promoting rhizobacteria*) solution at 5 g. L^{-1}. The shallot bulbs were planted and maintenance as described previously.

Data Collection and Analysis

Morphological characters of the bulb weight were carried out by weighing the bulb before planting, while the characters of plant height, number of tillers, number of leaves were observed at 5 weeks after transplanting. Flowering characters observed as sprouting time, time to flowering, number of umbels, umbel diameter, length of umbel stalk, and time to umbel broke, were done when 75% of the plants shown those characteristics. The percentage of flowering plants was observed by counting the number of flowering plants divided by the number of plants for each treatment in each replication.

Data were analyzed statistically using ANOVA to determine the effect of vernalization on the morphological and flowering characters. Further analysis was carried out based on *Least Significance Different* (LSD) test with a 95% confidence level. The analysis using the SAS program version 9.1. A cluster analysis was conducted using an unweighted pair group method arithmetic with means (UPGMA). This analysis was conducted with the Cluster package from the R-software package (R version 3.2.2).

Results and Discussion

<u>The Effect of Gibberellic Acid on Shallot Growth and Yield</u>

The results of the analysis of variance on growth and yield of shallot showed the interaction between GA_3 application and shallot varieties which observed significantly effects on the number of leaves, and number of bulbs. The further analysis with LSD test is presented in Table 1. A higher response of the number of leaves and bulbs at all GA_3 concentrations were observed on Super Phillip variety. Meanwhile, Bima Brebes had the highest number of leaves and bulbs at 100-150 mg L^{-1} GA_3. The plant growth regulators might be needed to increase shallot production, however GA_3 influenced growth by promoting elongation of stem and internodes of plant. Sravani et al. (2020) reported that the highest plant was obtained under the treatment of GA_3 at 25 mg. L^{-1}. This might be due to the increasing of cell wall extensibility by GA_3. Application of the exogenously

GA_3 might have activated the endogenous hormonal activities which ultimately led to leaf elongation of plant.

Gibberellic acid (GA_3) is one of the main regulators of the growth and development of plants which stimulates not only the growth and promoting of cell division and elongation (Olszewski et al., 2002), but also plays a major role in diverse growth processes including seed development, organ elongation, senescence and control of flowering time (Yamaguchi 2008; Ouzounidou et al., 2011). The increase in the number of leaves per plant is mainly due to the enhancement of cell elongation and cell division. It enhances also the photosynthesis and respiration which catalyze the metabolism activities in plant. The results are conformed with the findings of earlier reports in onion (Hye et al., 2002; Tiwari et al., 2003; Patel et al., 2010;), and garlic (Singh et al., 2014; Govind et al., 2015).

Five varieties of shallot showed different growth and yield variables (Table 2). The variety of Tajuk showed the highest plant height (43.3 cm) and had the highest number of shoots per plant (8.1 shoots). Tajuk variety also showed higher yield compared to other varieties. It had the highest responses in the number of bulbs (9.41 bulbs/plant), bulb fresh weight (90.1 g/plant) and bulb net weight (75 g/plant).

The application of GA_3 singly had no significant effect on another growth and yield of shallot (Table 3). These results are similar to those reported in garlic, observed that the plant height or the stem length at 15 and 25 days after planting were not significantly different among various concentration of GA_3 (Rahman et al., 2006). However, the results showed that the plant heights were observed of 44.61-45.74 with the application of GA_3 at 0-100 mg L^{-1}, while the application of GA_3 at 150 mg L^{-1} was only 40.11 cm. Shaikh et al., (2002) reported that the application of GA_3 at 50 mg L^{-1} to large or medium bulbs produced a significantly higher seed yield per hectare, germination and vigour values on onion. Kucera et al. (2005) showed that the applications of GA_3 on plants increased the total plant height of onion and garlic by 35% and 25% of the control, respectively.

Helaly et al. (2016) reported that GA_3 application on *Allium cepa* did not significantly affect the plant fresh weight, but increased the number of leaves, plant height and could allow for higher plant density, therefore higher total yield. A vigorous onion and garlic growth and yield were promoted by GA_3 application (Kucera et al., 2005; Ouzounidou et al., 2011). GA_3 application stimulated and integrated the overall growth, development and reproduction of shallot.

The Effect of Vernalization on Growth and Flower Initiation of Shallot

The ANOVA showed that there was an interaction between vernalization treatment and the variety of shallot observed on time to sprout and the flowering characters of shallot. The interactions were able to increase the number of umbel, the diameter of umbel, the length of umbel stalk, and time to umbel broke in all varieties except Sumenep variety (Figure 1).

The flowering ability of shallot depends on the genetic variability and environmental conditions. The vernalization treatment can stimulate flowering and produce more seeds (Khokhar, 2014). The vernalization signal received by plants is permanent and persists in subsequent crop development (Song et al., 2012). Plant growth environment becomes exogenous factors which has a strong influence in determining the ability of flowering. Inflorence develops from the apical meristem under suitable conditions.

The interaction between variety and vernalization gave a significantly different effect on the flowering quantitative character of shallot. Observations on the quantative characters of flowering showed that the Ilokos variety was responsive to vernalization treatment. While the Sumenep variety was not sensitive to flowering. There was no increase in all flowering characters in the Sumenep variety. The interaction effect between variety and vernalization treatment was able to increase umbel diameter characters in the varieties of Bima Brebes, Ilokos and Tajuk (Fig. 1G). The reports by Mardiana (2016) and Kusumadewi et al. (2017) showed that vernalization was effective in increasing flowering of shallot.

The average of shallot flowering without vernalization was 20%, while with vernalization increased up to 39%. The Bentanis variety showed no difference in the percentage of flowering between those treated or not treated with vernalization. This indicated that the Bentanis variety is a sensitive variety to flowering, and able to produce flowers in all growing conditions. The result showed the importance of vernalization treatment to initiate flowering which might relate to the temperate origin of shallot. It had been reported by Lee et al. (2013) and Marlin et al. (2018) that vernalization blocked flowering repressor and induced expression of genes responsible for the flowering (florigen). Vernalization could also promote the up-regulation of some key cytokinin signaling regulators which induced flowering (Wen et al., 2017). In contrast to the Sumenep variety that it was not able to increase the ability of flowering even though it was treated with vernalization.

The results showed that variety had a significant effect on the character of the initial bulb weight, time to flowering, umbel number, umbel diameter, length of umbel stalk, and the percentage of flowering (Figure 2). The LSD test results showed that the varieties of Bentanis, Bima Brebes and Tajuk had higher initial tuber weights (5.43-6.80 g) compared to the Ilokos and Sumenep variety (3.31-3.65 g).The variety of Bentanis, Bima Brebes and Tajuk were higher than those of Ilokos and Sumenep. It was suspected that with the larger size of bulbs, the varieties of Bentanis, Bima Brebes and Tajuk had more food reserves, which affected the growth of plant height. On the other hand, the Ilokos and Sumenep varieties had a greater number of leaves and tillers than the other three varieties.

The quantitative character of flowering was controlled by many genes and is also influenced by environmental factors. The percent flowering of shallot was strongly influenced by variety and vernalization, but no interaction was found. Bentanis variety has the same flowering percentage as Bima Brebes and Tajuk, which is around 32-52%, while the Ilokos variety has 22% flowering percentage, and the Sumenep variety has no flowering ability.

The results showed that the vernalization treatment singly had a very significant effect on the character of the initial bulb weight and the percentage of flowering (Figure 3). The results showed that the plant height, the number of tillers, and the percentage of shallot flowering actually increased with the vernalization treatment. However, the vernalization treatment reduced the bulb initial weight.

Vernalization was an important adaptation of plants to initiate flowering in response to prolonged exposure to low temperatures (Finnegan et al., 2001; Song et al., 2012). The vernalization treatment had a stressful effect on plants which caused the plants to use more energy during their early growth period. The bulbs without vernalization treatment still store a lot of energy that can be used for optimal growth. Wu et al. (2016) stated that the vernalization in garlic inhibited the number of leaf, pseudostem diameter, and plant height. The vernalization of garlic bulbs at 4˚C (for 2 months) resulted in bolting, inflorescence formation and true seed production in 9 varieties whereas non-vernalized failed to result into bolting, i.e. no true seed production was determined.

Cluster analysis showed that 5 varieties of shallot were divided into 3 groups according to the similarity of morphological and flowering characters (Figure 4). The 3 patterns of flowering ability in shallot varieties, namely natural (sensitive flowering), medium sensitive, and non sensitive flowering ability. The natural flowering ability in shallot shown by the ability to flower naturally in shallot varieties with or without external stimulation. The medium sensitive variety of shallot will produce flowers in the presence of stimulation from external treatments, such as vernalization. Meanwhile, a non-sensitive variety was not able to produce flowers naturally even with external stimuli.

The clustering of morphological and quantitative flowering characters was visualized graphically with a matrix representation of the degree of dissimilarity between the 5 local variety of shallot. The 5 varieties of shallot were grouped into 3 groups based on their flowering ability with similar morphological and quantitative flowering characters in which were given vernalization treatment and without vernalization treatment. The first group consisted of variety Bentanis (G1), Bima Brebes (G2), and Tajuk (G4). In the second group there was the Ilokos (G3) variety, and in the third group there was Sumenep (G5) variety. Each variety in the same group were similar based on morphological and flowering characters. Analysis of the 12 morphological and quantitative flowering characters of shallot further confirmed the different ability patterns of the tested shallot variety. The difference in the grouping of the 5 shallot varieties indicates that there are morphological and flowering variations among the five varieties, especially in terms of their flowering competence.

The results showed that the Sumenep variety had a different flowering pattern with other varieties, both without vernalization and with vernalization treatment. The Sumenep variety had the highest dissimilarity value compared to other varieties. The large disimilarity value indicated that the Sumenep variety has the different morphological and flowering characters from others. The Sumenep variety is a non sensitive flowering variety, even with the induction treatment such as vernalization treatment. Sumenep varieties are generally difficult to produce flowers (Idhan et al., 2015), The ideal grouping of varieties is when all the varieties in a group have a dissimilarity value equal to zero, but with varieties from other groups the dissimilarity value is equal to one. Identification of the morphological diversity and flowering ability of shallot is very useful knowledge in the efforts of onion breeding and cultivation development programs.

Conclusions

The GA_3 can increase the yield of shallot by promoting shoot growth and bulb initiation. With the application of GA_3 up to 100 mg L^{-1}, the height of plants reached from 44.61 to 45.74 cm. Tajuk variety showed better yield characters compared to other varieties which was observed in bulb number, bulb fresh weight, and bulb net weight of 9.41 bulbs/plant, 90.1 gram/plant, and 75 gram/plant, respectively. Five varieties of shallot were clustered into 3 groups according to the similarity of morphological and flowering characters, namely very responsive included Bentanis, Bima Brebes, and Tajuk; medium responsive included Ilokos; and non-responsive included Sumenep.

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Authors Contribution Statement

Marlin Marlin and Hartal Hartal designed and performed experiments. Marlin Marlin performed data analysis was in charge of the overall direction and planning, writing, and interpretation of the manuscript and interpretation of results. Atra Romeida and Reny Herawati participated in data collection and statistical analysis. Marulak Simarmata and other authors were involved in writing and review the article.

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Table 1. Interaction between GA_3 concentrations and 5 varieties of shallots on the number of leaves and bulbs of shallot

Variety	Concentration of GA_3 (mg L^{-1})	Number of leaves	Number of bulbs
Bauji	0	21.60 ± 0.14e	5.60 ± 0.15d
	50	27.63 ± 0.09d	5.87 ± 0.09d
	100	32.87 ± 0.08c	7.03 ± 0.00cd
	150	27.73 ± 0.00d	6.15 ± 0.08d
Bima Brebes	0	41.93 ± 0.06bc	8.41 ± 0.03bc
	50	39.46 ± 0.00bc	8.67 ± 0.09bc
	100	48.53 ± 0.20ab	10.68 ± 0.06abc
	150	32.13 ± 1.00c	10.12 ± 0.06abc
Super Philip	0	48.93 ± 0.01ab	9.25 ± 0.03bc
	50	52.93 ± 0.07a	12.50 ± 0.03ab
	100	56.60 ± 0.03a	14.35 ± 0.08a
	150	44.20 ± 0.08b	11.21 ± 0.05abc
Tajuk	0	40.06 ± 0.00bc	10.55 ± 0.03abc
	50	39.13 ± 0.08bc	8.36 ± 0.05bc
	100	33.80 ± 0.01c	7.91 ± 0.03c
	150	44.46 ± 0.06b	10.83 ± 0.00abc
Thailand	0	31.60 ± 0.01c	8.05 ± 0.02bc
	50	40.16 ± 0.05b	9.12 ± 0.08bc
	100	38.87 ± 0.08bc	8.23 ± 0.08bc
	150	36.66 ± 0.00bc	8.87 ± 0.04bc

The numbers followed by the same letter within each column are not significantly different based on the LSD test at 5% level. Values are means with standard deviation of triplicate determinations.

Table 2. Morphological characters on 5 varieties of shallots

Variety	Plant height (cm)	Number of leaves	Number of shoots	Bulb diameter (cm)	Number of bulbs	Bulb fresh weight (g)	Bulb net weight (g)
Bauji	32.6 ± 0.08c	24.1 ± 0.05c	4.7 ± 0.10ab	19.8 ± 0.01a	5.6 ± 0.02c	26.1 ± 0.08c	24.4 ± 0.05c
Bima Brebes	33.0 ± 0.03c	40.5 ± 0.08b	4.7 ± 0.08b	18.5 ± 0.08a	9.6 ± 0.03b	41.6 ± 0.08bc	40.3 ± 0.08b
Super Philip	38.1 ± 0.08b	50.6 ± 0.00a	6.3 ± 0.00ab	16.7 ± 0.00a	11.8 ± 0.00a	50.2 ± 0.00b	33.2 ± 0.08bc
Tajuk	45.3 ± 0.01a	39.6 ± 0.08b	8.1 ± 0.07a	20.8 ± 0.05a	9.4 ± 0.00b	90.1 ± 0.02a	75.0 ± 0.00 a
Thailand	38.4 ± 0.08b	32.0 ± 0.04bc	5.6 ± 0.06ab	17.2 ± 0.00a	8.0 ± 0.08b	44.2 ± 0.08bc	34.7 ± 0.04bc

The numbers followed by the same letter within each column are not significantly different based on the LSD test at 5% level. Values are means with standard deviation of triplicate determinations.

Table 3. Morphological response of shallots at different GA_3 concentrations

Concentration of GA_3 (mg L^{-1})	Plant height (cm)	Number of leaves	Number of shoots	Bulb diameter (cm)	Number of bulbs	Bulb fresh weight (g)	Bulb net weight (g)
0	45.7 ± 0.00a	43.6 ± 0.08a	8.0 ± 0.12a	1.8 ± 0.10a	9.4 ± 0.00a	51.0 ± 0.00a	38.4 ± 0.03a
50	44.6 ± 0.08a	43.8 ± 0.06a	8.8 ± 0.08a	1.7 ± 0,08.a	9.9 ± 0.08a	50.0 ± 0.03a	34.2 ± 0.08a
100	41.9 ± 0.06ab	46.3 ± 0.00a	8.9 ± 0.08a	1.8 ± 0.06a	11.6 ± 0.10a	52.1 ± 0.03a	38.7 ± 0.00a
150	40.1 ± 0.05b	40.3 ± 0.08a	8.2 ± 0.05a	1.7 ± 0.10a	10.3 ± 0.10a	43.8 ± 0.00a	31.5 ± 0.00a

The numbers followed by the same letter within each column are not significantly different based on the LSD test at 5% level. Values are means with standard deviation of triplicate determinations.

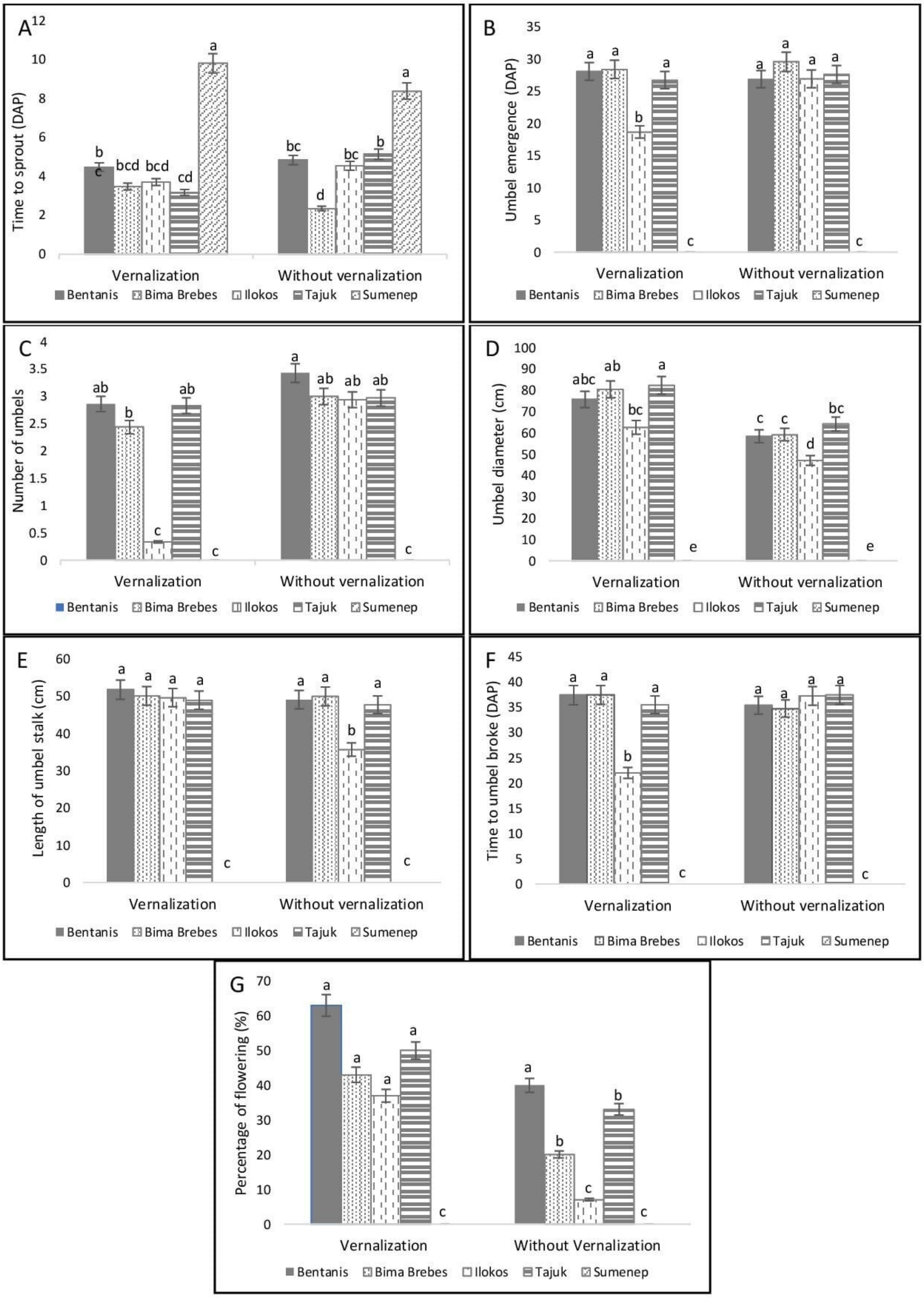

Figure 1. The interaction between shallot varieties and vernalization on time to sprout (A), day of umbel emergence (B), number of umbel(C), umbel diameter (D), length of umbel stalk (E), time to umbel broke (F), and percentage of flowering (G) of shallot. Values are means with standard deviation of triplicate determinations. Means with different lowercase letter are significantly different at on LSD test at 5% level. Values are means with standard deviation of triplicate determinations.

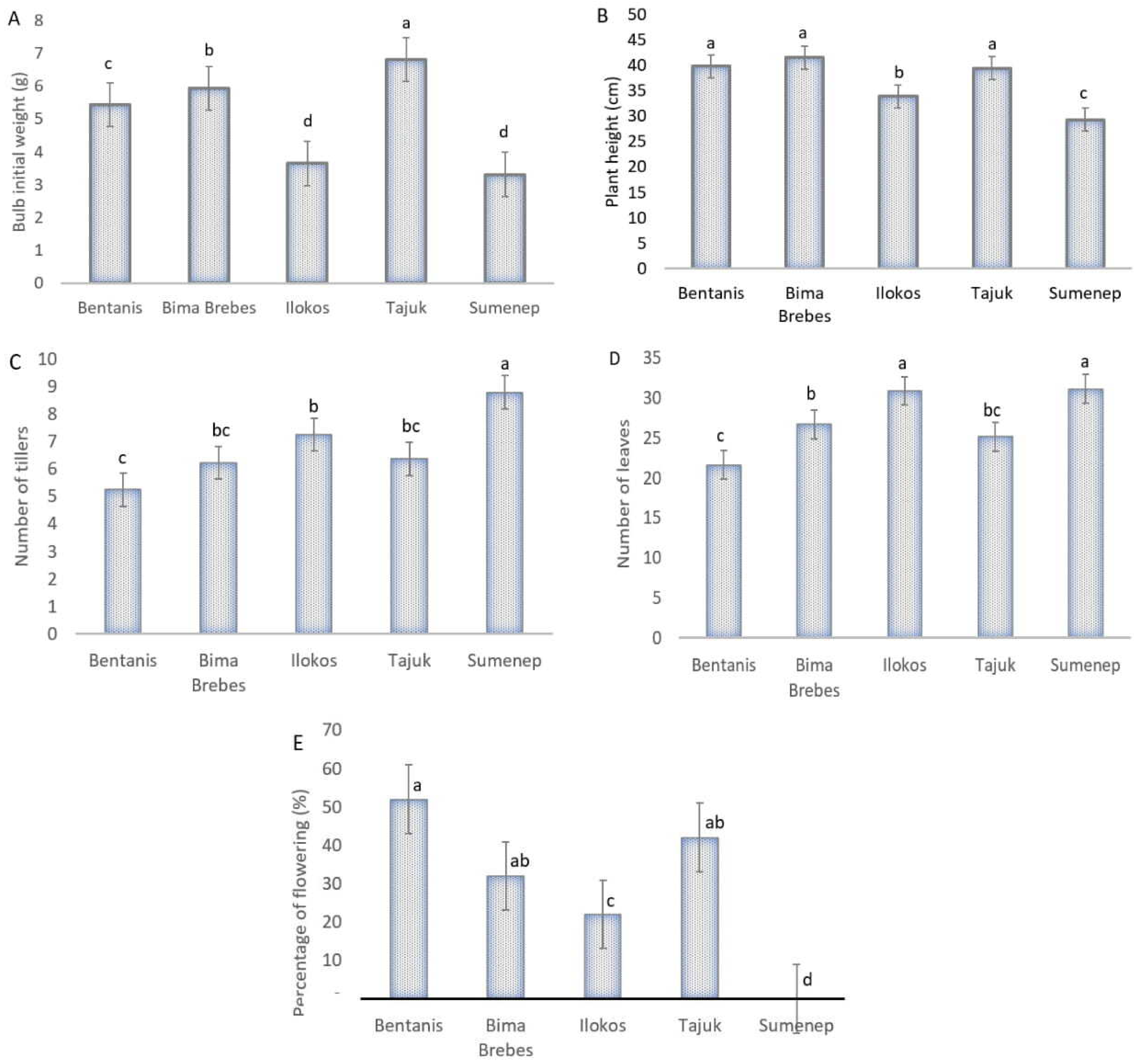

Figure 2. Effect of variety on bulb initial weight (A), plant height (B), number of tillers (C), number of leaves (D), and percent of flowering (E) of shallots. Same letters within each variable response indicated no significant differences by LSD test at 5% level. Values are means with standard deviation of triplicate determinations.

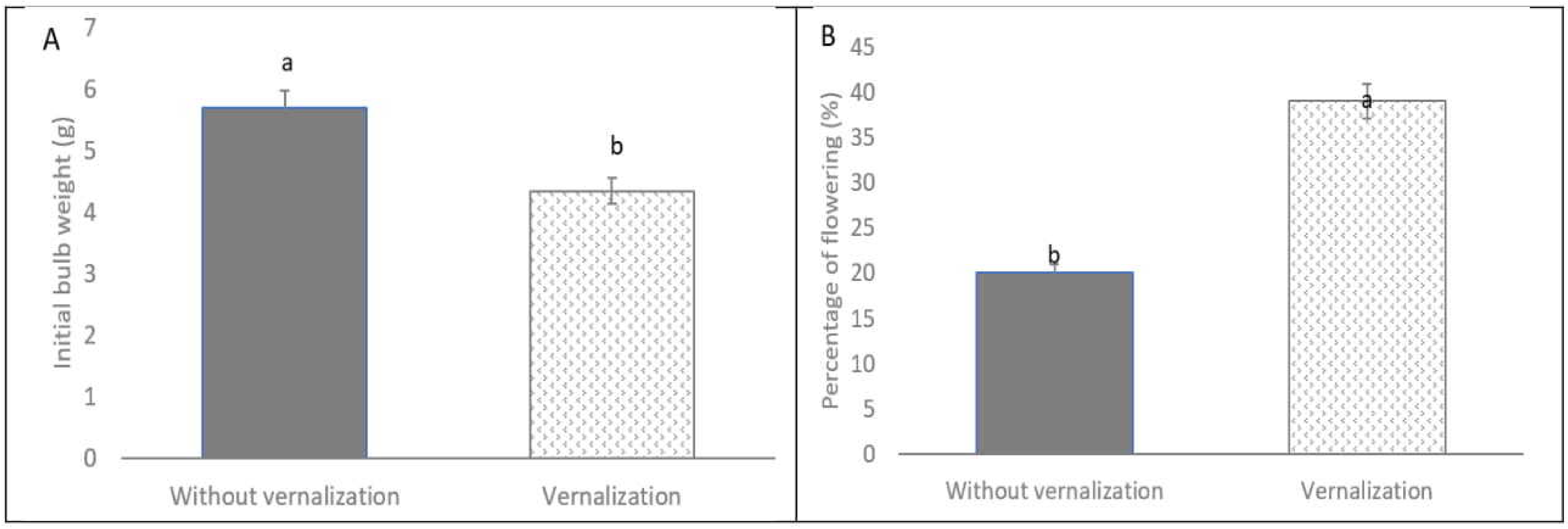

Figure 3. The effect of vernalization on bulb initial weight (A) and percent of flowering (B) of shallots. The numbers followed by the difference lowercase letter are significantly different based on the LSD test at 5% level. The numbers followed by the difference capital letter are significantly different based on the LSD test at 5% level. Values are means with standard deviation of triplicate determinations.

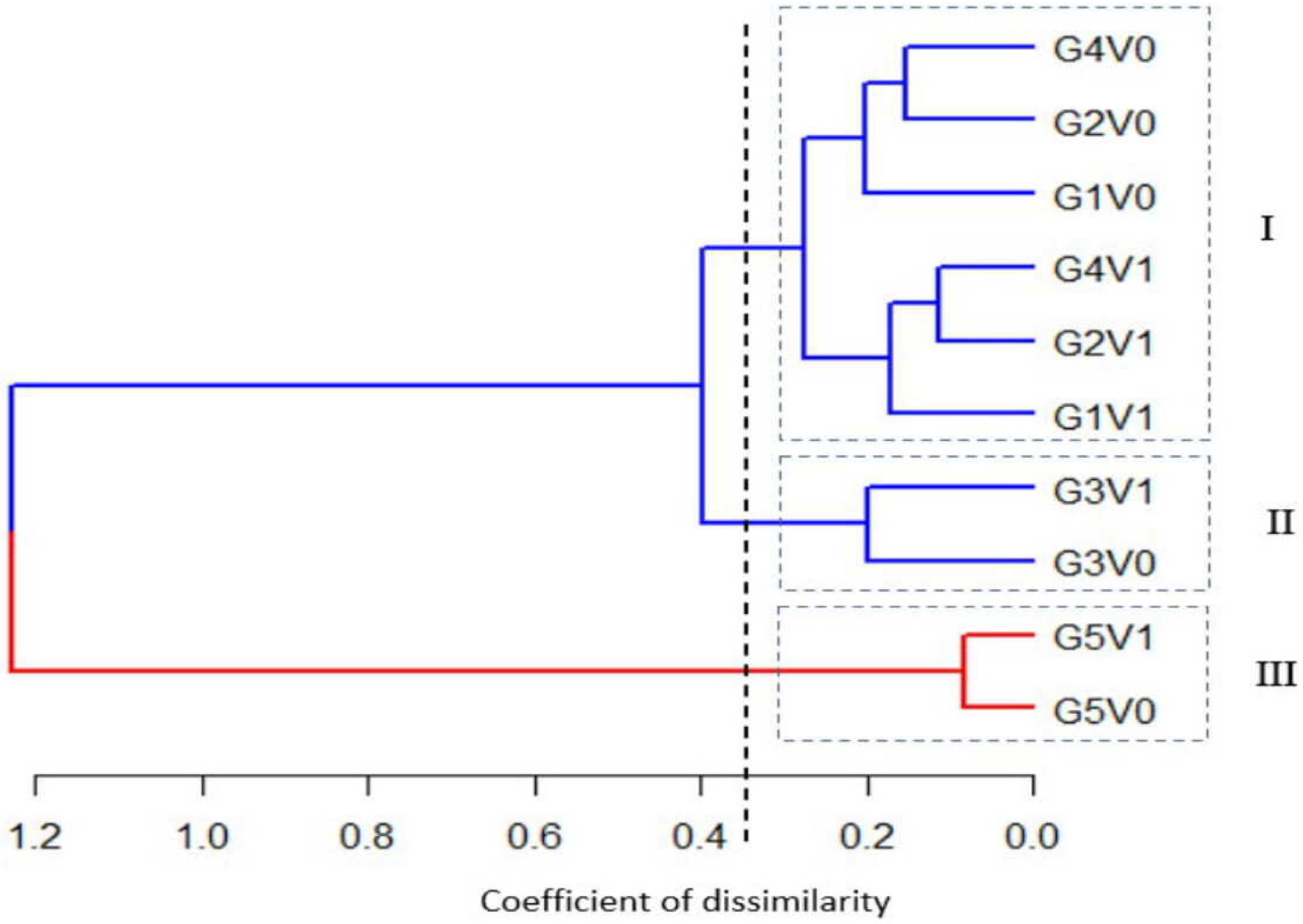

Figure 4. Hierarchical cluster of the dissimilarity matrix of vernalization treatments of 5 shallot varieties. Without vernalization (V0), and vernalization (V1). Variety of Bentanis (G1), Bima Brebes (G2), Ilokos (G3), Tajuk (G4), Sumenep (G5).

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RESEARCH ARTICLE

Morphological and flowering characteristics of shallot (*allium cepa* var. Aggregatum) in response to gibberellic acid and vernalization

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ABSTRACT

Shallot plants have variations in morphological and flowering characters. Flowering ability can be induced by the treatment of gibberellic acid (GA_3) and exposing to cold temperature (vernalization). The objectives of the research were to determine the effect of GA_3 and vernalization on the morphological and flowering characters of 5 shallot varieties. Field study was organized in a completely randomized block design with three replications to evaluate the responses of 5 varieties of shallots (Bauji, Bima Brebes, Super Philip, Tajuk, and Thailand) to GA_3 with the concentrations ranged from zero to 150 mg L^{-1}. Plants were maintained for 65 days until harvesting. The second study was organized in a completely randomized design with 3 replications to evaluate the responses of the five varieties of shallot to vernalization. The vernalization of shallot bulbs were done at 8 °C for 6 weeks. The results indicated that a Super Phillip variety showed the highest response to GA_3 observed in the number of leaves and bulbs. At 100 mg L^{-1} of GA_3 increased the plant height up to 45.74 cm. The results from the second study showed that vernalization was effective to increase flowering only on the Ilokos variety. But the other varieties were not sensitive to vernalization. Based on morphological and flowering characters, 5 varieties of shallots were clustered into 3 groups, namely: sensitive flowering included Bentanis, Bma Brebes and Tajuk variety, medium sensitive flowering included Ilokos variety, and non-sensitive flowering included Sumenep variety.

Keywords: Flower induction; Gibberellic acid; Shallot varieties; Vernalization

INTRODUCTION

Shallot (*Allium cepa* var. Aggregatum) is one of the economically important crop belong in the Liliaceae family. Shallot bulb contains important nutritive vegetable and medicinal (Mohammadi-Motlagh et al., 2011; Marlin et al., 2019). Shallot cultivation requires specific edaphoclimatic conditions and agricultural management to grow, overcome bulb dormancy, induce flower development, reproduce bulbs, and true seeds (Tendaj and Mysiak, 2013; Farhadi and Salteh, 2018).

Shallot responses to agricultural management and environmental conditions differ among different variety. Selection of the elite variety is an essential for obtaining desired growth and quality of bulbs, and induces the flower formation. The varieties of shallot in Indonesia have the ability to produce flowers, except for the Sumenep variety (Marlin et al., 2018). Cultivation techniques for developing shallot flowering initiation have not been widely developed. Shallot growth and development can be induced by optimizing genetic ability and manipulating the growing environment.

Treatments to induce flowering and seed formation can be carried out using growth regulators such as gibberellic acid and vernalization treatments. Gibberellic acid and vernalization treatment play a role in the plant growth and the process of flowering initiation. Both treatments work by stimulating the formation of flowering genes such as the SOC1 gene (suppressor of overexpression of constant 1) and the LEAFY gene (Corbesier and Coupland, 2006). The LEAFY gene is the main gene that controls flowering in shallot and predictably have been related to flowering pattern (Marlin et al., 2018).

Bio-regulators like gibberellic acid (GA_3) have been known to play a vital role in building of plants and involved in

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plant growth together with stem elongation (Rahman et al*.,* 2006), and the transition from vegetative growth to flowering (Sumarni et al*.,* 2013).

The treatment at low temperatures (vernalization) can stimulate flower formation (Song et al*.,* 2012). Vernalization is an important adaptation of plants to initiate flowering in response to prolonged exposure to low temperatures (Finnegan et al*.,* 2001; Song et al., 2012). Elsiddig et al. (2015) showed that vernalization treatment at a temperature of 4-5 °C for 90 days was a major factor to induce flowering in Texas Grano cultivar onions. The use of gibberellins and vernalization treatments to stimulate the growth and flowering for shallot has not been described. This studies were conducted to determine the effect of applying GA_3 and vernalization in stimulating plant growth and flower initiation of shallot (*Allium cepa* var. Aggregatum).

MATERIALS AND METHODS

Experimental 1

Experimental site and layouts

Field experiment was carried out in the field located in 700 m above sea level in the planting season of 2019 and 2020. The experimental land was cultivated and made into a mound of plots measuring 100 cm x 120 cm each, and 30 cm height. The soil was mixed with manure at 10 tons ha^{-1} and the plots covered with silver black plastic. Planting spacing was 20 cm x 20 cm. Inorganic fertilizers of urea, super phosphat-36, and potassium chloride were given as basic fertilizers, at 250, 150, and 150 kg ha^{-1}, respectively.

The experiment with two factors was organized in a randomized complete block design with three replications. The first factor was 5 varieties of shallots, namely Bauji, Bima Brebes, Super Philip, Tajuk, and Thailand. The second factor was the concentration of GA_3 which were 50, 100,150 mg L^{-1}, and without GA_3 as a control.

Experimental material

Five varieties of local shallot, namely Bauji, Bima Brebes, Super Philip, Tajuk, and Thailand were used as planting material. The average size of the bulbs was 3-5 grams. Shallot bulbs were cut off one third on the bulb top, and then soaked for one hour in the GA_3 solution with concentration as described previously. Then, the shallot bulbs were planted through the holes by immersing the bulb into the soil and covering with a thin layer of soil. Plant maintenances included watering and controlling pests with pesticides were done before harvesting at 65 days after planting. Harvesting was done in the morning or during sunny conditions by carefully pulling the shallot plants.

Data collection and analysis

Observation was carried out on the growth and yield variables, which included: plant height, number of leaves, number of tillers, bulb diameter, number of bulbs, fresh weight of bulb, and dry weight of bulb. Data were collected from selected plants in each unit plot. To avoid border effect with the highest precision, 10 plants were selected randomly from each plot. Data were statistically analyzed with ANOVA at 5% using SAS program version 9.1, and further tested by a *Least Significance Different* (LSD) test at a 95% confidence level.

Experiment 2

Experimental site and layouts

The experiment was carried out in a completely randomized design, with two factors. The first factor was 5 varieties of shallot as described previously and the second factor was vernalization treatment, which was with and without vernalization of the shallot bulbs. Vernalization was carried in storage room for 6 weeks at 8 °C. The shallot bulbs were planted in polybags with a diameter of 45 cm containing 10 kg of planting medium (which was mixed of soil, manure, and rice husk in ratio of = 2: 1: 1). Each polybag was planted with three shallot bulbs. The plants were fertilized with NPK mixture fertilizer (15:15:15) at a 2.4 g per polybag, or similar to 600 kg. ha^{-1}. Plant maintenances were carried out similar to previous experiment. The shallot bulbs were harvested at 65 days after planting.

Experimental material

Five varieties of local shallot as described previously were used as planting material. Before planting, the shallot bulbs were immersed for 15 minutes in a fungicide solution containing *Benomyl* 2 $g.L^{-1}$ for 15 minutes. Then, the shallot bulbs were soaked again for another 15 minutes in the PGPR (*plant growth promoting rhizobacteria*) solution at 5 g. L^{-1}. The shallot bulbs were planted and maintenance as described previously.

Data collection and analysis

Morphological characters of the bulb weight were carried out by weighing the bulb before planting, while the characters of plant height, number of tillers, number of leaves were observed at 5 weeks after transplanting. Flowering characters observed as sprouting time, time to flowering, number of umbels, umbel diameter, length of umbel stalk, and time to umbel broke, were done when 75% of the plants shown those characteristics. The percentage of flowering plants was observed by counting the number of flowering plants divided by the number of plants for each treatment in each replication.

Data were analyzed statistically using ANOVA to determine the effect of vernalization on the morphological and flowering characters. Further analysis was carried out based on *Least Significance Different* (LSD) test with a 95% confidence level. The analysis using the SAS program version 9.1. A cluster analysis was conducted using an unweighted pair group method arithmetic with means (UPGMA). This analysis was conducted with the Cluster package from the R-software package (R version 3.2.2).

RESULTS AND DISCUSSION

The effect of gibberellic acid on shallot growth and yield

The results of the analysis of variance on growth and yield of shallot showed the interaction between GA_3 application and shallot varieties which observed significantly effects on the number of leaves, and number of bulbs. The further analysis with LSD test is presented in Table 1. A higher response of the number of leaves and bulbs at all GA_3 concentrations were observed on Super Phillip variety. Meanwhile, Bima Brebes had the highest number of leaves and bulbs at 100-150 mg L^{-1} GA_3. The plant growth regulators might be needed to increase shallot production, however GA_3 influenced growth by promoting elongation of stem and internodes of plant. Sravani et al. (2020) reported that the highest plant was obtained under the treatment of GA_3 at 25 mg. L^{-1}. This might be due to the increasing of cell wall extensibility by GA_3. Application of the exogenously GA_3 might have activated the endogenous hormonal activities which ultimately led to leaf elongation of plant.

Table 1: Interaction between GA3 concentrations and 5 varieties of shallots on the number of leaves and bulbs of shallot

Variety	Concentration of GA_3 (mg L^{-1})	Number of leaves	Number of bulbs
Bauji	0	21.60 ± 0.14^{e}	5.60 ± 0.15^{d}
	50	27.63 ± 0.09^{d}	5.87 ± 0.09^{d}
	100	32.87 ± 0.08^{c}	7.03 ± 0.00^{cd}
	150	27.73 ± 0.00^{d}	6.15 ± 0.08^{d}
Bima Brebes	0	41.93 ± 0.06^{bc}	8.41 ± 0.03^{bc}
	50	39.46 ± 0.00^{bc}	8.67 ± 0.09^{bc}
	100	48.53 ± 0.20^{ab}	10.68 ± 0.06^{abc}
	150	32.13 ± 1.00^{c}	10.12 ± 0.06^{abc}
Super Philip	0	48.93 ± 0.01^{ab}	9.25 ± 0.03^{bc}
	50	52.93 ± 0.07^{a}	12.50 ± 0.03^{ab}
	100	56.60 ± 0.03^{a}	14.35 ± 0.08^{a}
	150	44.20 ± 0.08^{b}	11.21 ± 0.05^{abc}
Tajuk	0	40.06 ± 0.00^{bc}	10.55 ± 0.03^{abc}
	50	39.13 ± 0.08^{bc}	8.36 ± 0.05^{bc}
	100	33.80 ± 0.01^{c}	7.91 ± 0.03^{c}
	150	44.46 ± 0.06^{b}	10.83 ± 0.00^{abc}
Thailand	0	31.60 ± 0.01^{c}	8.05 ± 0.02^{bc}
	50	40.16 ± 0.05^{b}	9.12 ± 0.08^{bc}
	100	38.87 ± 0.08b^{c}	8.23 ± 0.08^{bc}
	150	36.66 ± 0.00b^{c}	8.87 ± 0.04^{bc}

The numbers followed by the same letter within each column are not significantly different based on the LSD test at 5% level. Values are means with standard deviation of triplicate determinations

Gibberellic acid (GA_3) is one of the main regulators of the growth and development of plants which stimulates not only the growth and promoting of cell division and elongation (Olszewski et al., 2002), but also plays a major role in diverse growth processes including seed development, organ elongation, senescence and control of flowering time (Yamaguchi 2008; Ouzounidou et al., 2011). The increase in the number of leaves per plant is mainly due to the enhancement of cell elongation and cell division. It enhances also the photosynthesis and respiration which catalyze the metabolism activities in plant. The results are conformed with the findings of earlier reports in onion (Hye et al., 2002; Tiwari et al., 2003; Patel et al., 2010;), and garlic (Singh et al., 2014; Govind et al., 2015).

Five varieties of shallot showed different growth and yield variables (Table 2). The variety of Tajuk showed the highest plant height (43.3 cm) and had the highest number of shoots per plant (8.1 shoots). Tajuk variety also showed higher yield compared to other varieties. It had the highest responses in the number of bulbs (9.41 bulbs/plant), bulb fresh weight (90.1 g/plant) and bulb net weight (75 g/plant).

The application of GA_3 singly had no significant effect on another growth and yield of shallot (Table 3). These results are similar to those reported in garlic, observed that the plant height or the stem length at 15 and 25 days after planting were not significantly different among various concentration of GA_3 (Rahman et al., 2006). However, the results showed that the plant heights were observed of 44.61-45.74 with the application of GA_3 at 0-100 mg L^{-1}, while the application of GA_3 at 150 mg L^{-1} was only 40.11 cm. Shaikh et al., (2002) reported that the application of GA_3 at 50 mg L^{-1} to large or medium bulbs produced a significantly higher seed yield per hectare, germination and vigour values on onion. Kucera et al. (2005) showed that the applications of GA_3 on plants increased the total plant height of onion and garlic by 35% and 25% of the control, respectively.

Helaly et al. (2016) reported that GA_3 application on *Allium cepa* did not significantly affect the plant fresh weight, but increased the number of leaves, plant height and could allow for higher plant density, therefore higher total yield. A vigorous onion and garlic growth and yield were promoted by GA_3 application (Kucera et al., 2005; Ouzounidou et al., 2011). GA_3 application stimulated and integrated the overall growth, development and reproduction of shallot.

Table 2: Morphological characters on 5 varieties of shallots

Variety	Plant height (cm)	Number of leaves	Number of shoots	Bulb diameter (cm)	Number of bulbs	Bulb fresh weight (g)	Bulb net weight (g)
Bauji	32.6 ± 0.08^{c}	24.1 ± 0.05^{c}	4.7 ± 0.10^{ab}	19.8 ± 0.01^{a}	5.6 ± 0.02^{c}	26.1 ± 0.08^{c}	24.4 ± 0.05^{c}
Bima Brebes	33.0 ± 0.03^{c}	40.5 ± 0.08^{b}	4.7 ± 0.08^{b}	18.5 ± 0.08^{a}	9.6 ± 0.03^{b}	41.6 ± 0.08^{bc}	40.3 ± 0.08^{b}
Super Philip	38.1 ± 0.08^{b}	50.6 ± 0.00^{a}	6.3 ± 0.00^{ab}	16.7 ± 0.00^{a}	11.8 ± 0.00^{a}	50.2 ± 0.00^{b}	33.2 ± 0.08^{bc}
Tajuk	45.3 ± 0.01^{a}	39.6 ± 0.08^{b}	8.1 ± 0.07^{a}	20.8 ± 0.05^{a}	9.4 ± 0.00^{b}	90.1 ± 0.02^{a}	75.0 ± 0.00 ^{a}
Thailand	38.4 ± 0.08^{b}	32.0 ± 0.04^{bc}	5.6 ± 0.06^{ab}	17.2 ± 0.00^{a}	8.0 ± 0.08^{b}	44.2 ± 0.08^{bc}	34.7 ± 0.04^{bc}

The numbers followed by the same letter within each column are not significantly different based on the LSD test at 5% level. Values are means with standard deviation of triplicate determinations

Table 3: Morphological response of shallots at different GA3 concentrations

Concentration of GA_3 (mg L^{-1})	Plant height (cm)	Number of leaves	Number of shoots	Bulb diameter (cm)	Number of bulbs	Bulb fresh weight (g)	Bulb net weight (g)
0	45.7 ± 0.00^{a}	43.6 ± 0.08^{a}	8.0 ± 0.12^{a}	1.8 ± 0.10^{a}	9.4 ± 0.00^{a}	51.0 ± 0.00^{a}	38.4 ± 0.03^{a}
50	44.6 ± 0.08^{a}	43.8 ± 0.06^{a}	8.8 ± 0.08^{a}	1.7 ± 0,08.^{a}	9.9 ± 0.08^{a}	50.0 ± 0.03^{a}	34.2 ± 0.08^{a}
100	41.9 ± 0.06^{ab}	46.3 ± 0.00^{a}	8.9 ± 0.08^{a}	1.8 ± 0.06^{a}	11.6 ± 0.10^{a}	52.1 ± 0.03^{a}	38.7 ± 0.00^{a}
150	40.1 ± 0.05^{b}	40.3 ± 0.08^{a}	8.2 ± 0.05^{a}	1.7 ± 0.10^{a}	10.3 ± 0.10^{a}	43.8 ± 0.00^{a}	31.5 ± 0.00^{a}

The numbers followed by the same letter within each column are not significantly different based on the LSD test at 5% level. Values are means with standard deviation of triplicate determinations

The Effect of Vernalization on Growth and Flower Initiation of Shallot

The ANOVA showed that there was an interaction between vernalization treatment and the variety of shallot observed on time to sprout and the flowering characters of shallot. The interactions were able to increase the number of umbel, the diameter of umbel, the length of umbel stalk, and time to umbel broke in all varieties except Sumenep variety (Fig. 1).

The flowering ability of shallot depends on the genetic variability and environmental conditions. The vernalization treatment can stimulate flowering and produce more seeds (Khokhar, 2014). The vernalization signal received by plants is permanent and persists in subsequent crop development (Song et al., 2012). Plant growth environment becomes exogenous factors which has a strong influence in determining the ability of flowering. Inflorence develops from the apical meristem under suitable conditions.

The interaction between variety and vernalization gave a significantly different effect on the flowering quantitative character of shallot. Observations on the quantative characters of flowering showed that the Ilokos variety was responsive to vernalization treatment. While the Sumenep variety was not sensitive to flowering. There was no increase in all flowering characters in the Sumenep variety. The interaction effect between variety and vernalization treatment was able to increase umbel diameter characters in the varieties of Bima Brebes, Ilokos and Tajuk (Fig. 1G). The reports by Mardiana (2016) and Kusumadewi et al. (2017) showed that vernalization was effective in increasing flowering of shallot.

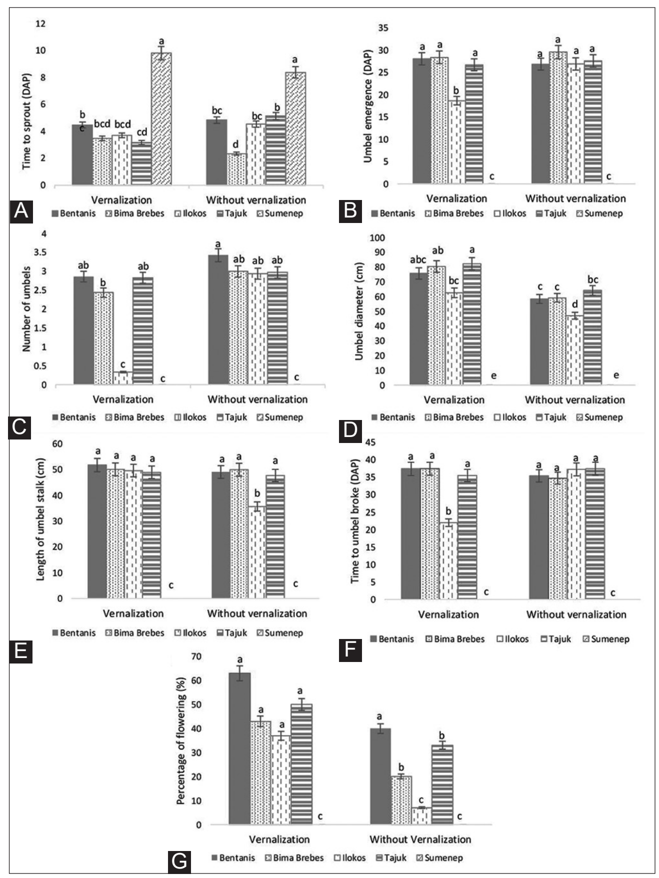

Fig 1. The interaction between shallot varieties and vernalization on time to sprout (A), day of umbel emergence (B), number of umbel (C), umbel diameter (D), length of umbel stalk (E), time to umbel broke (F), and percentage of flowering (G) of shallot. Values are means with standard deviation of triplicate determinations. Means with different lowercase letter are significantly different at on LSD test at 5% level. Values are means with standard deviation of triplicate determinations

The average of shallot flowering without vernalization was 20%, while with vernalization increased up to 39%. The Bentanis variety showed no difference in the percentage of flowering between those treated or not treated with vernalization. This indicated that the Bentanis variety is a sensitive variety to flowering, and able to produce flowers in all growing conditions. The result showed the importance of vernalization treatment to initiate flowering which might relate to the temperate origin of shallot. It had been reported by Lee et al. (2013) and Marlin et al. (2018) that vernalization blocked flowering repressor and induced expression of genes responsible for the flowering (florigen). Vernalization could also promote the up-regulation of some key cytokinin signaling regulators which induced flowering (Wen et al., 2017). In contrast to the Sumenep variety that it was not able to increase the ability of flowering even though it was treated with vernalization.

The results showed that variety had a significant effect on the character of the initial bulb weight, time to flowering, umbel number, umbel diameter, length of umbel stalk, and the percentage of flowering (Fig. 2). The LSD test results showed that the varieties of Bentanis, Bima Brebes and Tajuk had higher initial tuber weights (5.43-6.80 g) compared to the Ilokos and Sumenep variety (3.31-3.65 g). The variety of Bentanis, Bima Brebes and Tajuk were higher than those of Ilokos and Sumenep. It was suspected that with the larger size of bulbs, the varieties of Bentanis, Bima Brebes and Tajuk had more food reserves, which affected the growth of plant height. On the other hand, the Ilokos and Sumenep varieties had a greater number of leaves and tillers than the other three varieties.

The quantitative character of flowering was controlled by many genes and is also influenced by environmental factors.

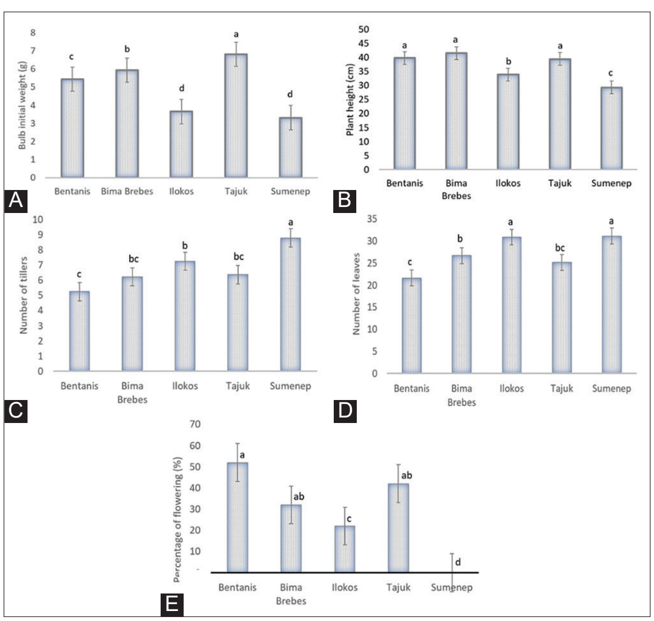

Fig 2. Efrfect of variety on bulb initial weight (A), plant height (B), number of tillers (C), number of leaves (D), and percent of flowering (E) of shallots. Same letters within each variable response indicated no significant differences by LSD test at 5% level. Values are means with standard deviation of triplicate determinations.

The percent flowering of shallot was strongly influenced by variety and vernalization, but no interaction was found. Bentanis variety has the same flowering percentage as Bima Brebes and Tajuk, which is around 32-52%, while the Ilokos variety has 22% flowering percentage, and the Sumenep variety has no flowering ability.

The results showed that the vernalization treatment singly had a very significant effect on the character of the initial bulb weight and the percentage of flowering (Fig. 3). The results showed that the plant height, the number of tillers, and the percentage of shallot flowering actually increased with the vernalization treatment. However, the vernalization treatment reduced the bulb initial weight.

Vernalization was an important adaptation of plants to initiate flowering in response to prolonged exposure to low temperatures (Finnegan et al., 2001; Song et al., 2012). The vernalization treatment had a stressful effect on plants which caused the plants to use more energy during their early growth period. The bulbs without vernalization treatment still store a lot of energy that can be used for optimal growth. Wu et al. (2016) stated that the vernalization in garlic inhibited the number of leaf, pseudostem diameter, and plant height. The vernalization of garlic bulbs at 4°C (for 2 months) resulted in bolting, inflorescence formation and true seed production in 9 varieties whereas non-vernalized failed to result into bolting, i.e. no true seed production was determined.

Cluster analysis showed that 5 varieties of shallot were divided into 3 groups according to the similarity of morphological and flowering characters (Fig. 4). The 3 patterns of flowering ability in shallot varieties, namely natural (sensitive flowering), medium sensitive, and non sensitive flowering ability. The natural flowering ability in shallot shown by the ability to flower naturally in shallot varieties with or without external stimulation. The medium sensitive variety of shallot will produce flowers in the presence of stimulation from external treatments, such as

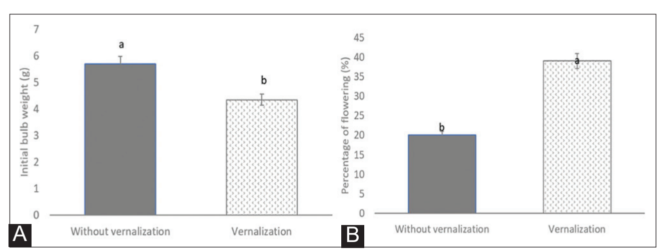

Fig 3. The effect of vernalixzation on bulb initial weight (A) and percent of flowering (B) of shallots. The numbers followed by the difference lowercase letter are significantly different based on the LSD test at 5% level. The numbers followed by the difference capital letter are significantly different based on the LSD test at 5% level. Values aremeans with standard deviation of triplicate determinations

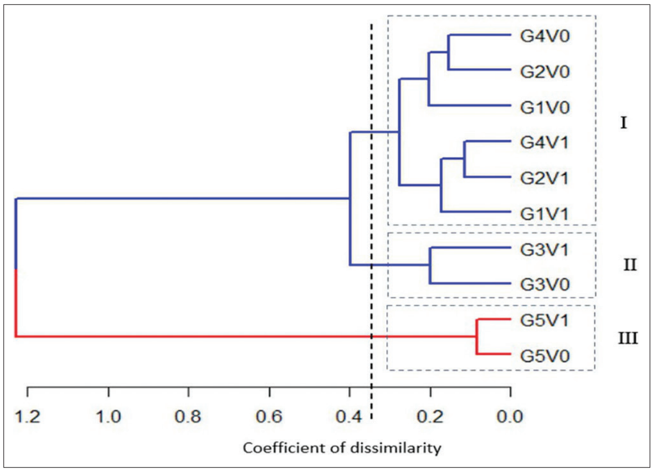

Fig 4. Herarchical cluster of the dissimilarity matrix of vernalization treatments of 5 shallot varieties. Without vernalization (v0), and vernalization (v1). Variety of Benatanis (G1), Bima Brebes (G2), Ilokos (G3), Tajuk (G4), Sumenep (G5)

vernalization. Meanwhile, a non-sensitive variety was not able to produce flowers naturally even with external stimuli.

The clustering of morphological and quantitative flowering characters was visualized graphically with a matrix representation of the degree of dissimilarity between the 5 local variety of shallot. The 5 varieties of shallot were grouped into 3 groups based on their flowering ability with similar morphological and quantitative flowering characters in which were given vernalization treatment and without vernalization treatment. The first group consisted of variety Bentanis (G1), Bima Brebes (G2), and Tajuk (G4). In the second group there was the Ilokos (G3) variety, and in the third group there was Sumenep (G5) variety. Each variety in the same group were similar based on morphological and flowering characters. Analysis of the 12 morphological and quantitative flowering characters of shallot further confirmed the different ability patterns of the tested shallot variety. The difference in the grouping of the 5 shallot varieties indicates that there are morphological and flowering variations among the five varieties, especially in terms of their flowering competence.

The results showed that the Sumenep variety had a different flowering pattern with other varieties, both without vernalization and with vernalization treatment. The Sumenep variety had the highest dissimilarity value compared to other varieties. The large disimilarity value indicated that the Sumenep variety has the different morphological and flowering characters from others. The Sumenep variety is a non sensitive flowering variety, even with the induction treatment such as vernalization treatment. Sumenep varieties are generally difficult to produce flowers (Idhan et al*.,* 2015), The ideal grouping of varieties is when all the varieties in a group have a dissimilarity value equal to zero, but with varieties from other groups the dissimilarity value is equal to one. Identification of the morphological diversity and flowering ability of shallot is very useful knowledge in the efforts of onion breeding and cultivation development programs.

CONCLUSIONS

The GA_3 can increase the yield of shallot by promoting shoot growth and bulb initiation. With the application of GA_3 up to 100 mg L^{-1}, the height of plants reached from 44.61 to 45.74 cm. Tajuk variety showed better yield characters compared to other varieties which was observed in bulb number, bulb fresh weight, and bulb net weight of 9.41 bulbs/plant, 90.1 gram/plant, and 75 gram/plant, respectively. Five varieties of shallot were clustered into 3 groups according to the similarity of morphological and flowering characters, namely very responsive included Bentanis, Bima Brebes, and Tajuk; medium responsive included Ilokos; and non-responsive included Sumenep.

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Authors contribution statement

Marlin Marlin and Hartal Hartal designed and performed experiments. Marlin Marlin performed data analysis was in charge of the overall direction and planning, writing, and interpretation of the manuscript and interpretation of results. Atra Romeida and Reny Herawati participated in data collection and statistical analysis. Marulak Simarmata and other authors were involved in writing and review the article.

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