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

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12

13 **Abstract**

14

15 The research was conducted to determine the effect of gibberellic acid (GA₃) and
16 vernalization on the morphological and flowering character of shallot. The first
17 experiment was arranged in the field and organized in a completely randomized block
18 design with two factors and three replications. The first factor was 5 varieties of shallots
19 (Bauji, Bima Brebes, Super Philip, Tajuk, and Thailand). The second factor was the
20 concentration of GA₃ (0, 50, 100, and 150 mg L⁻¹). The site was first cultivated and made
21 into a mound plots of 100 cm x 120 cm each and 30 cm height. The bulbs were planted
22 in a spacing of 20 cm x 20 cm. Plants were maintained for 65 days until harvesting. The
23 second experiment was organized in completely randomized design with 3 replications.
24 Five varieties of shallot were evaluated with and without vernalization treatment.
25 Vernalization of shallot bulbs at 8 °C was carried in storage room for 6 weeks. A higher
26 response of the number of leaves and bulbs at all GA₃ concentrations were observed on
27 Super Phillip variety. A concentration of GA₃ at 100 mg L⁻¹ increased the plant height
28 up to 45.74 cm. Based on the vernalization treatment, 5 varieties of shallots were
29 clustered into 3 groups, which were indicated that the morphological and flowering
30 variations, especially in terms of the flowering competence. Vernalization was effective
31 in increasing flowering of Ilokos variety. But, there was no increase in all flowering
32 characters in the Sumenep variety which confirmed as a non-sensitive flowering variety.

33

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

35

36 **Introduction**

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

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

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

59

60 Bio-regulators like gibberellic acid (GA₃) have been known to play a vital role in
61 building of plants and involved in plant growth together with stem elongation (Rahman
62 et al., 2006), and the transition from vegetative growth to flowering (Sumarni et al., 2013).
63 The treatment at low temperatures (vernalization) can stimulate flower formation in
64 shallot (Song et al., 2012). Vernalization is an important adaptation of plants to initiate
65 flowering in response to prolonged exposure to low temperatures (Finnegan et al., 2001;
66 Song et al., 2012). Elsiddig et al. (2015) showed that vernalization treatment at a
67 temperature of 4-5 °C for 90 days was a major factor to induce flowering in Texas Grano
68 cultivar onions. This studies were conducted to determine the effect of applying GA₃ and
69 vernalization in stimulating plant growth and flower initiation of shallot (*Allium cepa* var.
70 *Aggregatum*).

71

72 **Materials and Methods**

73 **The Effect of Gibberellic Acid on Shallot Growth and Yield**

74 The research was carried out in the field located in 700 m above sea level in the
75 planting season 2019 and 2020. The experiment arranged in a completely randomized
76 block design with 2 factors and 3 replications. The first factor was 5 varieties of shallots,
77 namely Bauji, Bima Brebes, Super Philip, Tajuk, and Thailand. The second factor was
78 the concentration of GA₃ which were 50, 100,150 mg L⁻¹, and without GA₃ as a control.

79 Shallot bulbs sized of 3-5 grams were used as planting materials. The experimental
80 land was cultivated and made into a mound of plots measuring 100 cm x 120 cm each,
81 and 30 cm height. The soil was mixed with manure (at 10 ton ha⁻¹) and the plots covered
82 with silver black plastic mulch. Planting holes were made with a spacing of 20 cm x 20
83 cm. Inorganic fertilizers of Urea, SP-36, and KCl were given as basic fertilizers, at 250,

84 150, and 150 kg ha⁻¹, respectively. Shallot bulbs were cut off one third on the bulb top,
85 and then soaked for one hour in the GA₃ solution with concentration as described
86 previously. Then, the shallot bulbs were planted through the holes by immersing the bulb
87 into the soil and covering with a thin layer of soil. Plant maintenances included watering
88 and controlling pests with pesticides were done before harvesting at 65 days after
89 planting. Harvesting was done in the morning or during sunny conditions by carefully
90 pulling the shallot plants.

91 Observation were carried out on the growth and yield variables, which included :
92 plant height, number of leaves, number of tillers, bulb diameter, number of bulbs, fresh
93 weight of bulb, and dry weight of bulb. Data were statistically analyzed with ANOVA at
94 5% using SAS program version 9.1, and further tested by a *Least Significance Different*
95 (LSD) test with a 95% confidence level.

96

97 **The Effect of Vernalization on Shallot Growth and Flower Initiation**

98 The experiment was carried out in a completely randomized design, with two
99 factors. The first factor was 5 varieties of shallot as described previously and the second
100 factor was vernalization treatment, which was with and without vernalization of the
101 shallot bulbs. Vernalization was carried in storage room for 6 weeks at 8 °C. The shallot
102 bulbs were planted in polybags with a diameter of 45 cm containing 10 kg of planting
103 medium (which was mixed of soil, manure, and rice husk in ratio of = 2: 1: 1). Each
104 polybag was planted with three shallot bulbs. Before planting, the shallot bulbs were
105 immersed for 15 minutes in a fungicide solution containing *Benomyl* 2 g.L⁻¹ for 15
106 minutes. Then, the shallot bulbs were soaked again for another 15 minutes in the PGPR
107 (*plant growth promoting rhizobacteria*) solution at 5 g. L⁻¹. The plants were fertilized

108 with NPK mixture fertilizer (15:15:15) at a 2.4 g per polybag, or similar to 600 kg. ha⁻¹.
109 Plant maintenances were carried out similar to previous experiment. The shallot bulbs
110 were harvested at 65 days after planting.

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

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

124

125 **Results and Discussion**

126 **The Effect of GA₃ on 5 Varieties of Shallot**

127 The results of the analysis of variance on growth and yield of shallots showed the
128 interaction between GA₃ application and shallot varieties which observed significantly
129 effects on the number of leaves, and number of bulbs. The further analysis with LSD test
130 is presented in Table 1. A higher response of the number of leaves and bulbs at all GA₃
131 concentrations were observed on Super Phillip variety. Meanwhile, Bima Brebes had the

132 highest number of leaves and bulbs at 100-150 mg L⁻¹ GA₃. The plant growth regulators
133 might be needed to increase shallot production, however GA₃ influenced growth by
134 promoting elongation of stem and internodes of plant. Sravani et al. (2020) reported that
135 the highest plant was obtained under the treatment of GA₃ at 25 mg. L⁻¹. This might be
136 due to the increasing of cell wall extensibility by GA₃. Application of the exogenously
137 GA₃ might have activated the endogenous hormonal activities which ultimately led to leaf
138 elongation of plant.

139 Gibberellic acid (GA₃) is one of the main regulators of the growth and development
140 of plants which stimulates not only the growth and promoting of cell division and
141 elongation (Olszewski et al., 2002), but also plays a major role in diverse growth
142 processes including seed development, organ elongation, senescence and control of
143 flowering time (Yamaguchi 2008; Ouzounidou et al., 2011). The increase in the number
144 of leaves per plant is mainly due to the enhancement of cell elongation and cell division.
145 It enhances also the photosynthesis and respiration which catalyze the metabolism
146 activities in plant. The results are conformed with the findings of earlier reports in onion
147 (Hye et al., 2002; Tiwari et al., 2003; Patel et al., 2010;), and garlic (Singh et al., 2014;
148 Govind et al., 2015).

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

154 The application of GA₃ singly had no significant effect on another growth and yield
155 of shallot (Table 3). These results are similar to those reported in garlic, observed that

156 the plant height or the stem length at 15 and 25 days after planting were not significantly
157 different among various concentration of GA₃ (Rahman et al., 2006). However, the results
158 showed that the plant heights were observed of 44.61-45.74 with the application of GA₃
159 at 0-100 mg L⁻¹, while the application of GA₃ at 150 mg L⁻¹ was only 40.11 cm. Shaikh
160 et al., (2002) reported that the application of GA₃ at 50 mg L⁻¹ to large or medium bulbs
161 produced a significantly higher seed yield per hectare, germination and vigour values on
162 onion. Kucera et al. (2005) showed that the applications of GA₃ on plants increased the
163 total plant height of onion and garlic by 35% and 25% of the control, respectively.

164 Helaly et al. (2016) reported that GA₃ application on *Allium cepa* did not
165 significantly affect the plant fresh weight, but increased the number of leaf, plant height
166 and could allow for higher plant density, therefore higher total yield. A vigorous onion
167 and garlic growth and yield were promoted by GA₃ application (Kucera et al., 2005;
168 Ouzounidou et al., 2011). GA₃ application stimulated and integrated the overall growth,
169 development and reproduction of shallot.

170

171 **The Effect of Vernalization on Growth and Flower Initiation of Shallot**

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

177 The flowering ability of shallots depends on the genetic variability and
178 environmental conditions. The vernalization treatment can stimulate flowering and
179 produce more seeds (Khokhar, 2014). The vernalization signal received by plants is

180 permanent and persists in subsequent crop development (Song et al., 2012). Plant growth
181 environment becomes exogenous factors which has a strong influence in determining the
182 ability of flowering. Inflorescence develops from the apical meristem under suitable
183 conditions.

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

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

203 that it was not able to increase the ability of flowering even though it was treated with
204 vernalization.

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

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

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

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

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

243 The clustering of morphological and quantitative flowering characters was
244 visualized graphically with a matrix representation of the degree of dissimilarity between
245 the 5 local varieties of shallots. The 5 varieties of shallot were grouped into 3 groups
246 based on their flowering ability with similar morphological and quantitative flowering
247 characters in which were given vernalization treatment and without vernalization
248 treatment. The first group consisted of variety Bentanis (G1), Bima Brebes (G2), and
249 Tajuk (G4). In the second group there was the Ilokos (G3) variety, and in the third group

250 there was Sumenep (G5) variety. Each variety in the same group were similar based on
251 morphological and flowering characters. Analysis of the 12 morphological and
252 quantitative flowering characters of shallots further confirmed the different ability
253 patterns of the tested shallot varieties. The difference in the grouping of the 5 shallot
254 varieties indicates that there are morphological and flowering variations among the five
255 varieties, especially in terms of their flowering competence.

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

267

268 **Conclusions**

269 The GA₃ might be important for increasing shallot production and influence growth
270 processes by promoting shoot growth and bulb initiation. With the application of GA₃ at
271 up to 100 mg L⁻¹, the height of plants reached from 44.61 to 45.74 cm. Tajuk variety
272 showed better yield characters compared to other varieties. It had the highest responses
273 in bulb number of 9.41 bulbs/plant, bulb fresh weight of 90.1 g/plant, and bulb net weight

274 of 75 g per plant. Five varieties of shallots were clustered into 3 groups according to the
275 similarity of morphological and flowering characters. Based on flowering characters,
276 Bentanis variety is sensitive to vernalization and able to produce flowers in all growing
277 conditions, Ilokos variety is responsive to vernalization, while Sumenep variety is not
278 sensitive to vernalization. The environment conditions were the exogenous factors for
279 plant growth which had a strong influence in determining the ability of flowering.

280

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285

286 **Authors Contribution Statement**

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

292

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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	21.60e	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 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	45.74a	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.

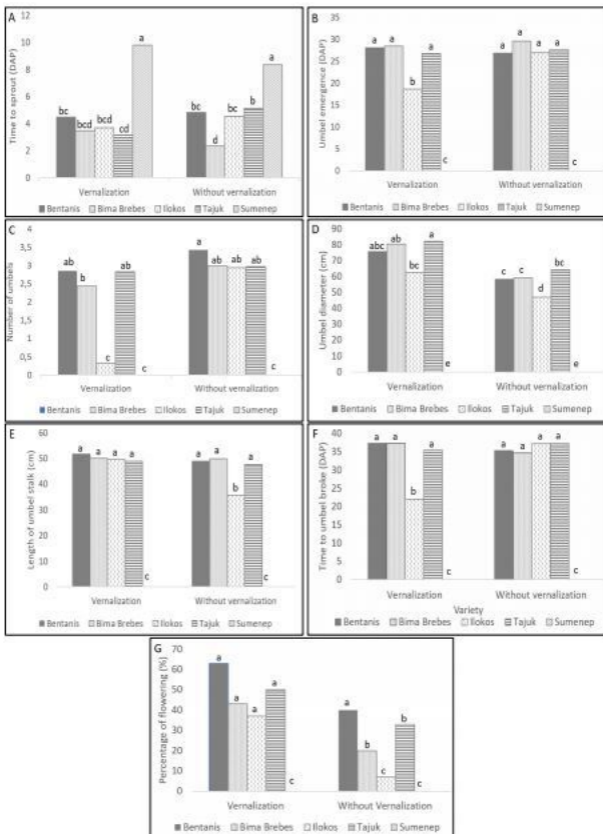


Figure 1. The interaction between shallot varieties and vernalization on time to sprout (A), day of umbel emergence (B), number of umbel(C), umbel diameter (D), length of umbel stalk (E), time to umbel broke (F), and percentage of flowering (G) of shallot. Same letters in each variable response indicated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

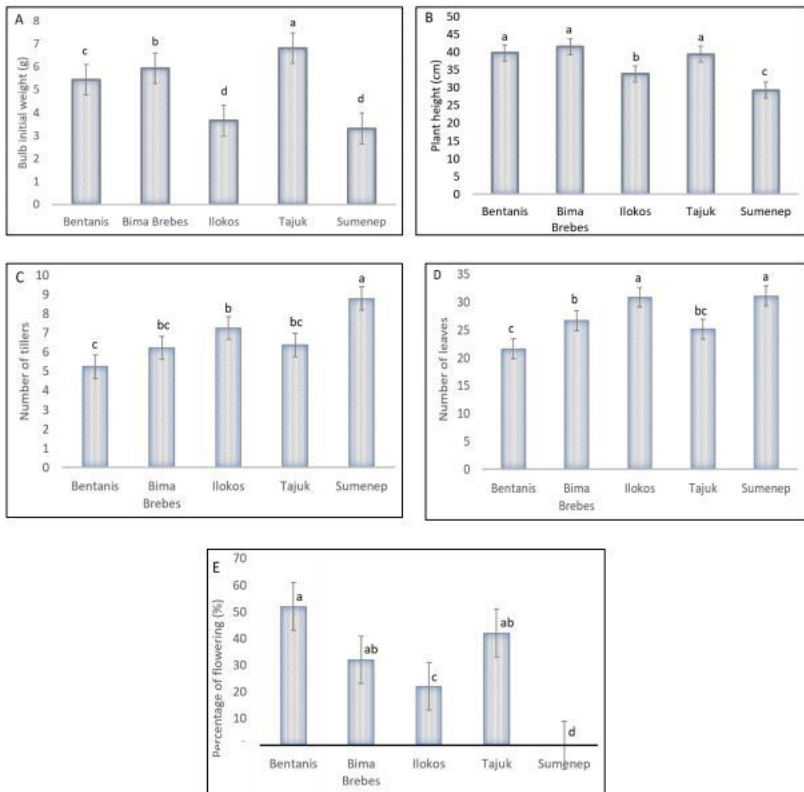


Figure 2. Effect of variety on bulb initial weight (A), plant height (B), number of tillers (C), number of leaves (D), and percent of flowering (E) of shallots. Same letters within each variable response indicated no significant differences by LSD test at 5% level.

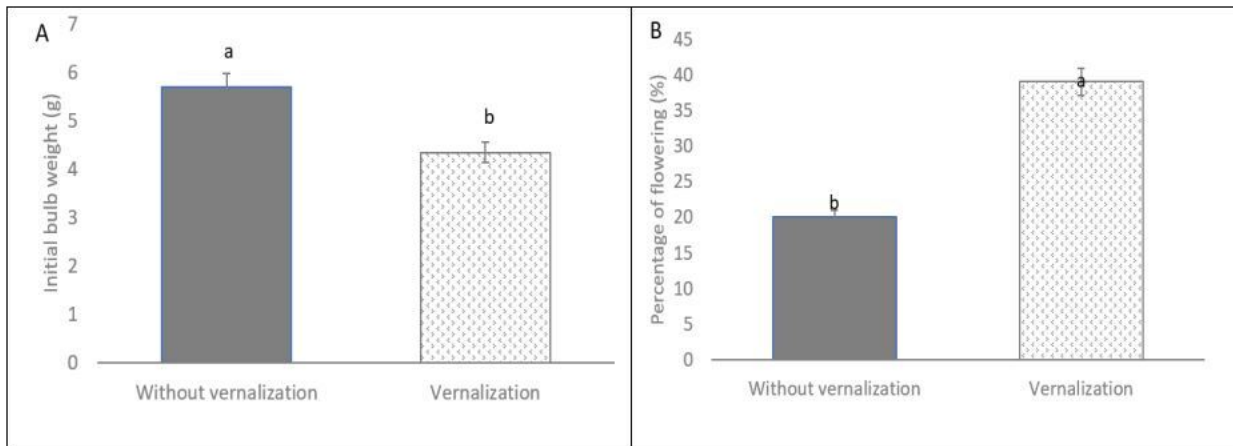


Figure 3. The effect of vernalization on bulb initial weight (A) and percent of flowering (B) of shallots. The numbers followed by the difference lowercase letter are significantly different based on the LSD test at 5% level. The numbers followed by the difference capital letter are significantly different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

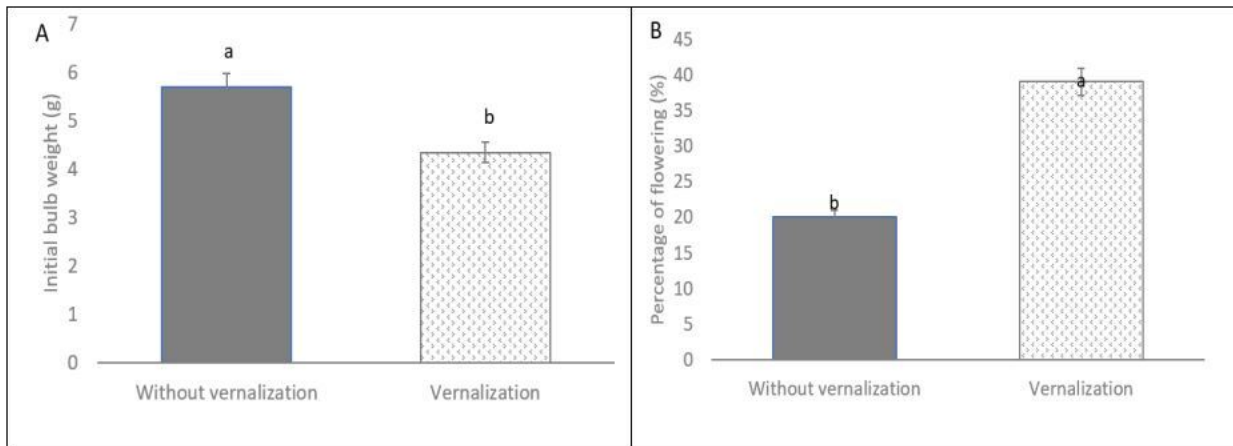


Figure 3. The effect of vernalization on bulb initial weight (A) and percent of flowering (B) of shallots. The numbers followed by the difference lowercase letter are significantly different based on the LSD test at 5% level. The numbers followed by the difference capital letter are significantly different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

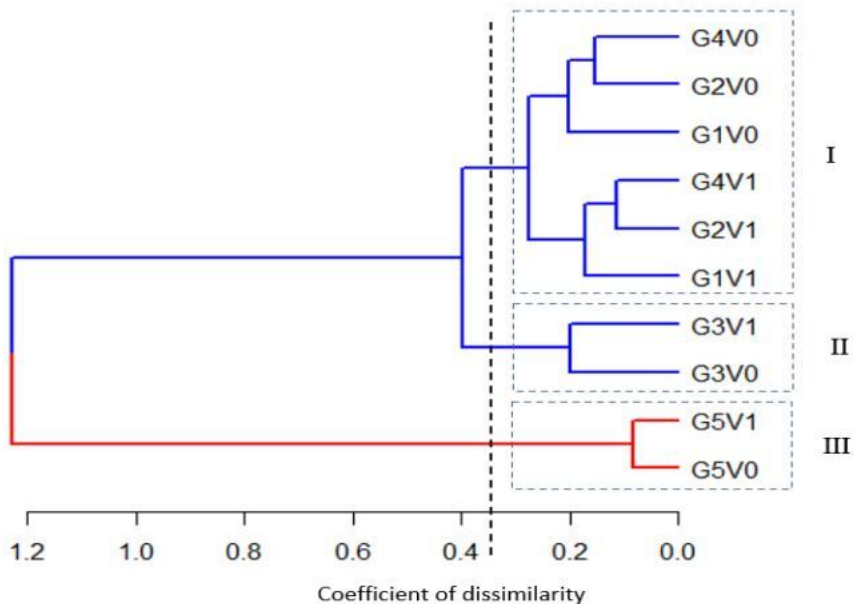


Figure 4. Hierarchical cluster of the dissimilarity matrix of vernalization treatments of 5 shallot varieties. Without vernalization (V0), and vernalization (V1). Variety of Bentanis (G1), Bima Brebes (G2), Ilokos (G3), Tajuk (G4), Sumenep (G5).

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



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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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Emirates Journal of Food and Agriculture

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

Emirates Journal of Food and Agriculture9 Juni 2021 pukul
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<noreply@ejmanager.com>

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,

Editor
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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 finds (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

1 Juli 2021 pukul
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Balas Ke: Emirates Journal of Food and Agriculture <ejfa@uaeu.ac.ae>

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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.

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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

For your questions please send message to ejfa@uaeu.ac.ae

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

3 **Marlin Marlin**^{1*}, Hartal Hartal², Atra Romeida¹, Reny Herawati¹, Marulak Simarmata¹

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12

13 **Abstract**

14

15 Shallot plants have variations in morphological and flowering characters. Flowering
16 ability can be induced by the treatment of gibberellic acid (GA₃) and exposing to cold
17 temperature (vernalization). The objectives of the research were to determine the effect
18 of GA₃ and vernalization on the morphological and flowering characters of 5 shallot
19 varieties. Field study was organized in a completely randomized block design with three
20 replications to evaluate the responses of 5 varieties of shallots (Bauji, Bima Brebes,
21 Super Philip, Tajuk, and Thailand) to GA₃ with the concentrations ranged from zero to
22 150 mg L⁻¹. Plants were maintained for 65 days until harvesting. The second study was
23 organized in a completely randomized design with 3 replications to evaluate the
24 responses of the five varieties of shallot to vernalization. The vernalization of shallot
25 bulbs were done at 8 °C for 6 weeks. The results indicated that a Super Phillip variety
26 showed the highest response to GA₃ observed in the number of leaves and bulbs. At
27 100 mg L⁻¹ of GA₃ increased the plant height up to 45.74 cm. The results from the
28 second study showed that vernalization was effective to increase flowering only on the
29 Ilokos variety. But the other varieties were not sensitive to vernalization. Based on
30 morphological and flowering characters, 5 varieties of shallots were clustered into 3
31 groups, namely: sensitive flowering included Bentanis, Bma Brebes and Tajuk variety,
32 medium sensitive flowering included Ilokos variety, and non-sensitive flowering
33 included Sumenep variety.

34

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

36

37 **Introduction**

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

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

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

59

60 Bio-regulators like gibberellic acid (GA₃) have been known to play a vital role in
61 building of plants and involved in plant growth together with stem elongation (Rahman
62 et al., 2006), and the transition from vegetative growth to flowering (Sumarni et al., 2013).
63 The treatment at low temperatures (vernalization) can stimulate flower formation (Song
64 et al., 2012). Vernalization is an important adaptation of plants to initiate flowering in
65 response to prolonged exposure to low temperatures (Finnegan et al., 2001; Song et al.,
66 2012). Elsiddig et al. (2015) showed that vernalization treatment at a temperature of 4-5
67 °C for 90 days was a major factor to induce flowering in Texas Grano cultivar onions.
68 The use of gibberellins and vernalization treatments to stimulate the growth and flowering
69 for shallot has not been described. This studies were conducted to determine the effect of
70 applying GA₃ and vernalization in stimulating plant growth and flower initiation of
71 shallot (*Allium cepa* var. *Aggregatum*).

72

73 **Materials and Methods**

74 **Experimental 1:**

75 **Experimental Site and Layouts**

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

83 The experiment with two factors was organized in a randomized complete block
84 design with three replications. The first factor was 5 varieties of shallots, namely Bauji,
85 Bima Brebes, Super Philip, Tajuk, and Thailand. The second factor was the concentration
86 of GA₃ which were 50, 100,150 mg L⁻¹, and without GA₃ as a control.

87

88 **Experimental Material**

89 Five varieties of local shallot, namely Bauji, Bima Brebes, Super Philip, Tajuk,
90 and Thailand were used as planting material. The average size of the bulbs was 3-5 grams.
91 Shallot bulbs were cut off one third on the bulb top, and then soaked for one hour in the
92 GA₃ solution with concentration as described previously. Then, the shallot bulbs were
93 planted through the holes by immersing the bulb into the soil and covering with a thin
94 layer of soil. Plant maintenances included watering and controlling pests with pesticides
95 were done before harvesting at 65 days after planting. Harvesting was done in the morning
96 or during sunny conditions by carefully pulling the shallot plants.

97

98 **Data Collection and Analysis**

99 Observation was carried out on the growth and yield variables, which included:
100 plant height, number of leaves, number of tillers, bulb diameter, number of bulbs, fresh
101 weight of bulb, and dry weight of bulb. Data were collected from selected plants in each
102 unit plot. To avoid border effect with the highest precision, 10 plants were selected
103 randomly from each plot. Data were statistically analyzed with ANOVA at 5% using SAS
104 program version 9.1, and further tested by a *Least Significance Different* (LSD) test at a
105 95% confidence level.

106

107 **Experiment 2:**

108 **Experimental Site and Layouts**

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

119

120 **Experimental Material**

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

127

128 **Data Collection and Analysis**

129 Morphological characters of the bulb weight were carried out by weighing the bulb
130 before planting, while the characters of plant height, number of tillers, number of leaves

131 were observed at 5 weeks after transplanting. Flowering characters observed as sprouting
132 time, time to flowering, number of umbels, umbel diameter, length of umbel stalk, and
133 time to umbel broke, were done when 75% of the plants shown those characteristics. The
134 percentage of flowering plants was observed by counting the number of flowering plants
135 divided by the number of plants for each treatment in each replication.

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

142

143 **Results and Discussion**

144 **The Effect of Gibberellic Acid on Shallot Growth and Yield**

145 The results of the analysis of variance on growth and yield of shallot showed the
146 interaction between GA₃ application and shallot varieties which observed significantly
147 effects on the number of leaves, and number of bulbs. The further analysis with LSD test
148 is presented in Table 1. A higher response of the number of leaves and bulbs at all GA₃
149 concentrations were observed on Super Phillip variety. Meanwhile, Bima Brebes had the
150 highest number of leaves and bulbs at 100-150 mg L⁻¹ GA₃. The plant growth regulators
151 might be needed to increase shallot production, however GA₃ influenced growth by
152 promoting elongation of stem and internodes of plant. Sravani et al. (2020) reported that
153 the highest plant was obtained under the treatment of GA₃ at 25 mg. L⁻¹. This might be
154 due to the increasing of cell wall extensibility by GA₃. Application of the exogenously

155 GA₃ might have activated the endogenous hormonal activities which ultimately led to leaf
156 elongation of plant.

157 Gibberellic acid (GA₃) is one of the main regulators of the growth and development
158 of plants which stimulates not only the growth and promoting of cell division and
159 elongation (Olszewski et al., 2002), but also plays a major role in diverse growth
160 processes including seed development, organ elongation, senescence and control of
161 flowering time (Yamaguchi 2008; Ouzounidou et al., 2011). The increase in the number
162 of leaves per plant is mainly due to the enhancement of cell elongation and cell division.
163 It enhances also the photosynthesis and respiration which catalyze the metabolism
164 activities in plant. The results are conformed with the findings of earlier reports in onion
165 (Hye et al., 2002; Tiwari et al., 2003; Patel et al., 2010;), and garlic (Singh et al., 2014;
166 Govind et al., 2015).

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

172 The application of GA₃ singly had no significant effect on another growth and yield
173 of shallot (Table 3). These results are similar to those reported in garlic, observed that
174 the plant height or the stem length at 15 and 25 days after planting were not significantly
175 different among various concentration of GA₃ (Rahman et al., 2006). However, the results
176 showed that the plant heights were observed of 44.61-45.74 with the application of GA₃
177 at 0-100 mg L⁻¹, while the application of GA₃ at 150 mg L⁻¹ was only 40.11 cm. Shaikh
178 et al., (2002) reported that the application of GA₃ at 50 mg L⁻¹ to large or medium bulbs

179 produced a significantly higher seed yield per hectare, germination and vigour values on
180 onion. Kucera et al. (2005) showed that the applications of GA₃ on plants increased the
181 total plant height of onion and garlic by 35% and 25% of the control, respectively.

182 Helaly et al. (2016) reported that GA₃ application on *Allium cepa* did not
183 significantly affect the plant fresh weight, but increased the number of leaves, plant height
184 and could allow for higher plant density, therefore higher total yield. A vigorous onion
185 and garlic growth and yield were promoted by GA₃ application (Kucera et al., 2005;
186 Ouzounidou et al., 2011). GA₃ application stimulated and integrated the overall growth,
187 development and reproduction of shallot.

188

189 **The Effect of Vernalization on Growth and Flower Initiation of Shallot**

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

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

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

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

223 The results showed that variety had a significant effect on the character of the initial
224 bulb weight, time to flowering, umbel number, umbel diameter, length of umbel stalk,
225 and the percentage of flowering (Figure 2). The LSD test results showed that the varieties

226 of Bentanis, Bima Brebes and Tajuk had higher initial tuber weights (5.43-6.80 g)
227 compared to the Ilokos and Sumenep variety (3.31-3.65 g). The variety of Bentanis, Bima
228 Brebes and Tajuk were higher than those of Ilokos and Sumenep. It was suspected that
229 with the larger size of bulbs, the varieties of Bentanis, Bima Brebes and Tajuk had more
230 food reserves, which affected the growth of plant height. On the other hand, the Ilokos
231 and Sumenep varieties had a greater number of leaves and tillers than the other three
232 varieties.

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

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

244 Vernalization was an important adaptation of plants to initiate flowering in response
245 to prolonged exposure to low temperatures (Finnegan et al., 2001; Song et al., 2012). The
246 vernalization treatment had a stressful effect on plants which caused the plants to use
247 more energy during their early growth period. The bulbs without vernalization treatment
248 still store a lot of energy that can be used for optimal growth. Wu et al. (2016) stated that
249 the vernalization in garlic inhibited the number of leaf, pseudostem diameter, and plant

250 height. The vernalization of garlic bulbs at 4°C (for 2 months) resulted in bolting,
251 inflorescence formation and true seed production in 9 varieties whereas non-vernalized
252 failed to result into bolting, i.e. no true seed production was determined.

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

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

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

285

286 **Conclusions**

287 The GA₃ can increase the yield of shallot by promoting shoot growth and bulb
288 initiation. With the application of GA₃ up to 100 mg L⁻¹, the height of plants reached
289 from 44.61 to 45.74 cm. Tajuk variety showed better yield characters compared to other
290 varieties which was observed in bulb number, bulb fresh weight, and bulb net weight of
291 9.41 bulbs/plant, 90.1 gram/plant, and 75 gram/plant, respectively. Five varieties of
292 shallot were clustered into 3 groups according to the similarity of morphological and
293 flowering characters, namely very responsive included Bentanis, Bima Brebes, and
294 Tajuk; medium responsive included Ilokos; and non-responsive included Sumenep.

295

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300

301 **Authors Contribution Statement**

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

307

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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 ± 0.14e	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 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 ± 0.00a	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.

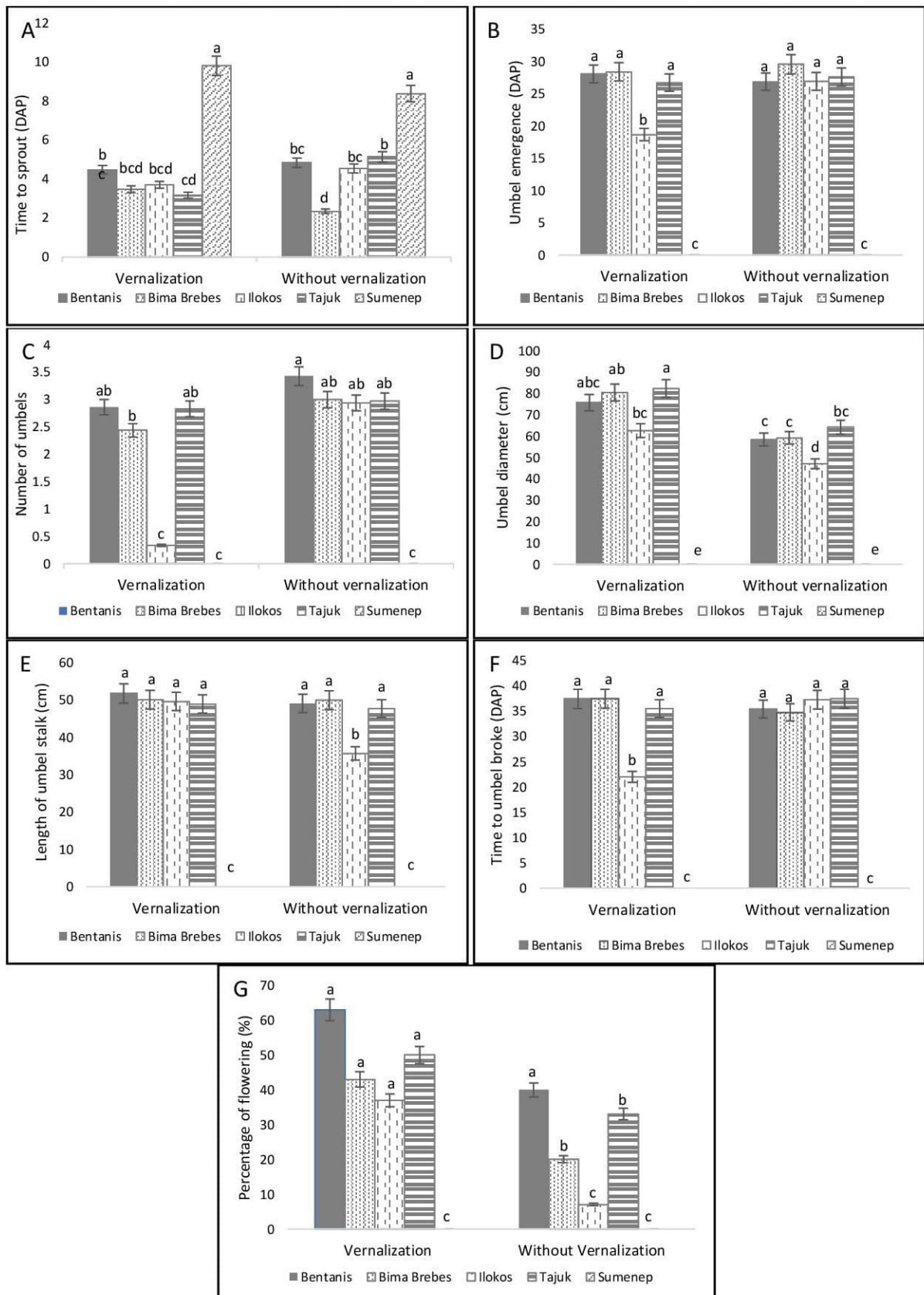


Figure 1. The interaction between shallot varieties and vernalization on time to sprout (A), day of umbel emergence (B), number of umbel(C), umbel diameter (D), length of umbel stalk (E), time to umbel broke (F), and percentage of flowering (G) of shallot. Values are means with standard deviation of 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.

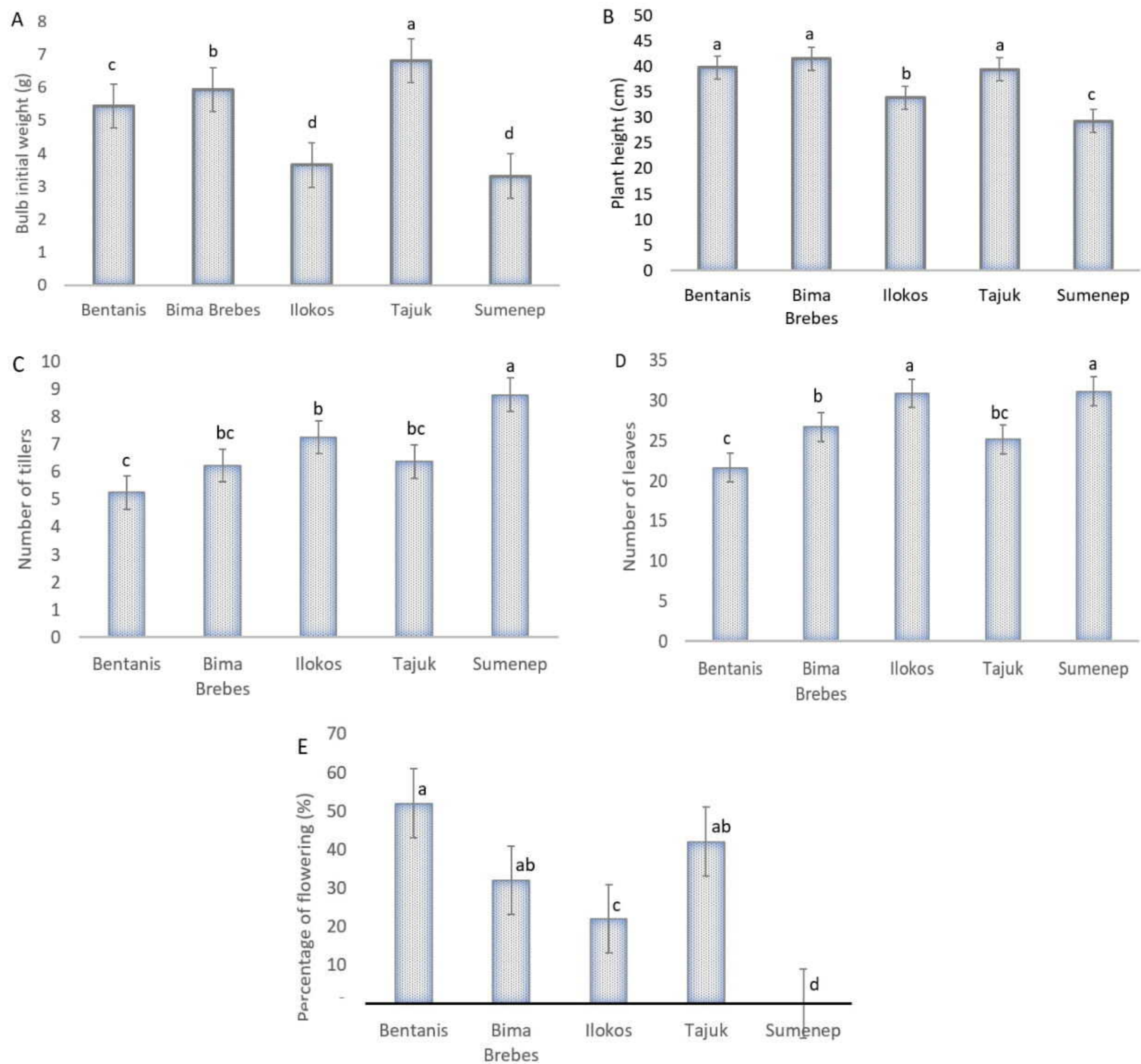


Figure 2. Effect of variety on bulb initial weight (A), plant height (B), number of tillers (C), number of leaves (D), and percent of flowering (E) of shallots. Same letters within each variable response indicated no significant differences by LSD test at 5% level. Values are means with standard deviation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

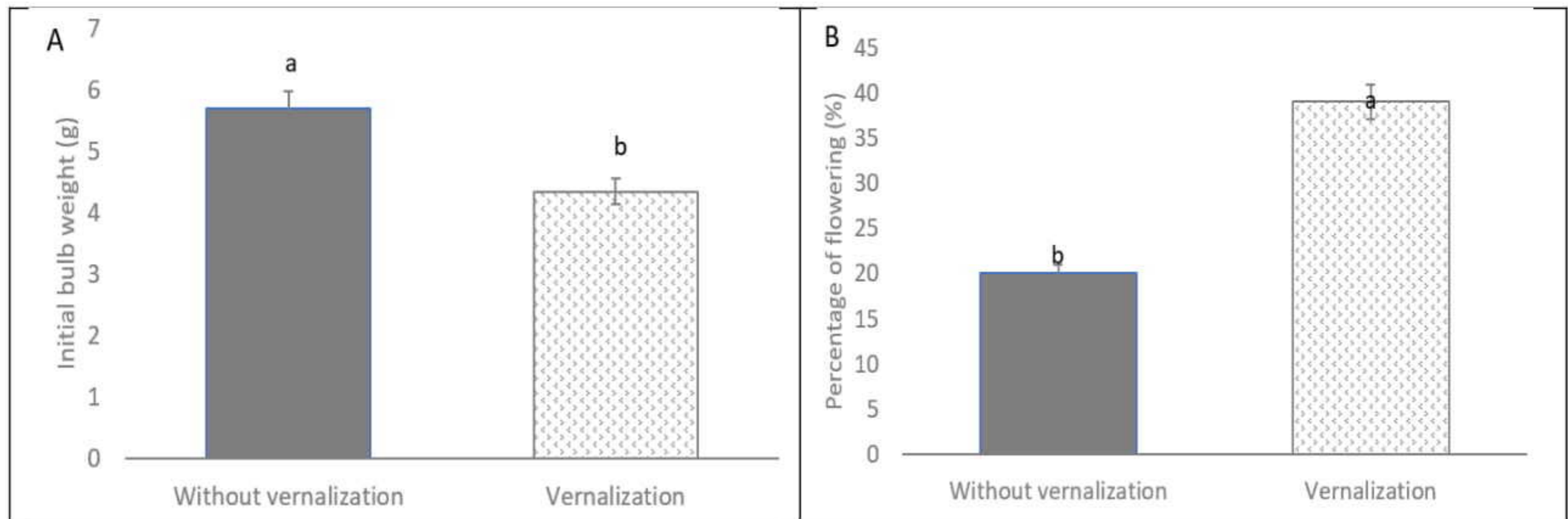


Figure 3. The effect of vernalization on bulb initial weight (A) and percent of flowering (B) of shallots. The numbers followed by the difference lowercase letter are significantly different based on the LSD test at 5% level. The numbers followed by the difference capital letter are significantly different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

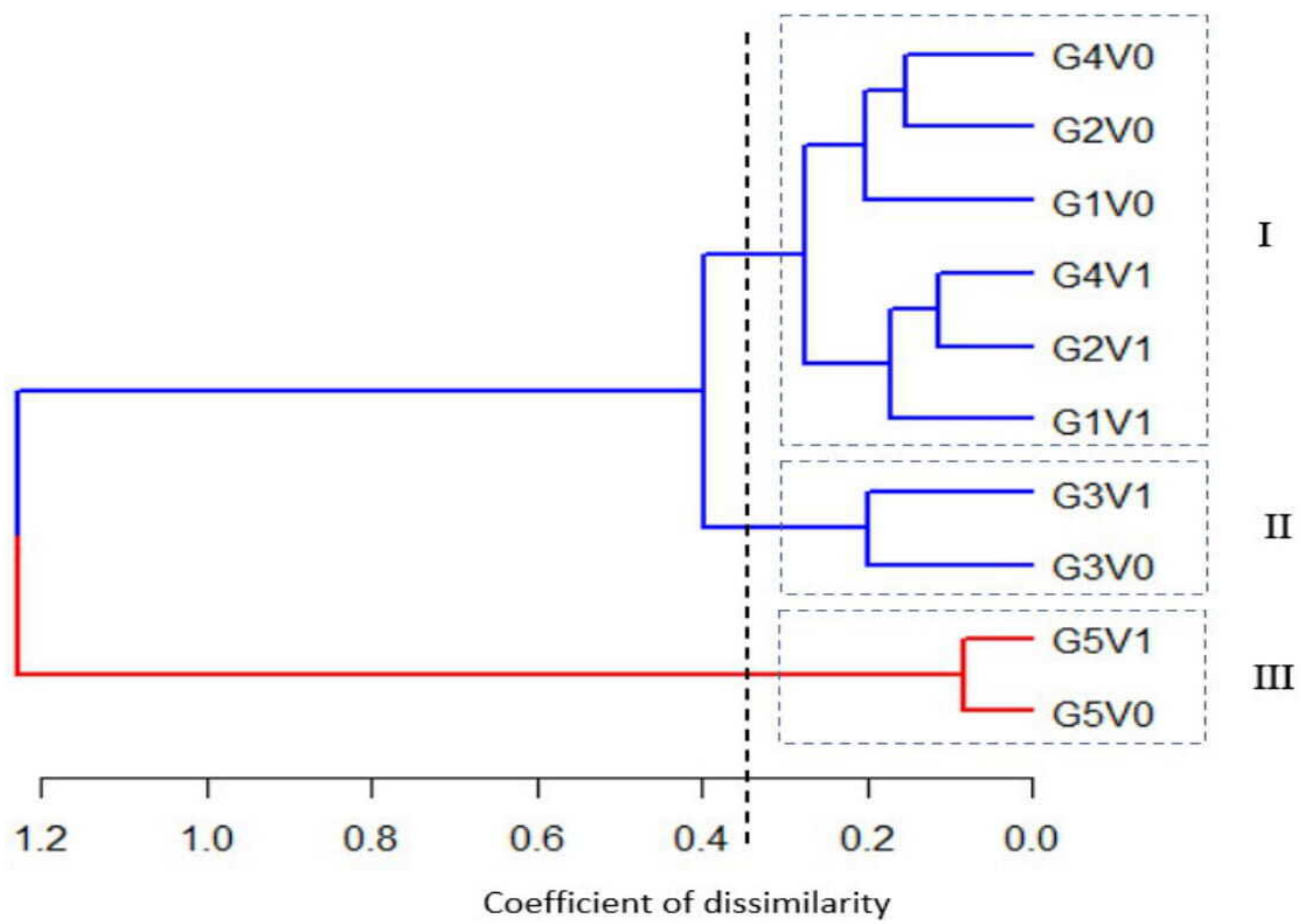


Figure 4. Hierarchical cluster of the dissimilarity matrix of vernalization treatments of 5 shallot varieties. Without vernalization (V0), and vernalization (V1). Variety of Bentanis (G1), Bima Brebes (G2), Ilokos (G3), Tajuk (G4), Sumenep (G5).



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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 (GA₃) and exposing to cold temperature (vernalization). The objectives of the research were to determine the effect of GA₃ 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 Phillip, Tajuk, and Thailand) to GA₃ 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 GA₃ observed in the number of leaves and bulbs. At 100 mg L⁻¹ of GA₃ 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 (GA₃) 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 GA₃ 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 GA₃ which were 50, 100, 150 mg L⁻¹, and without GA₃ 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 GA₃ 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 GA₃ 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 GA₃ concentrations were observed on Super Phillip variety. Meanwhile, Bima Brebes had the highest number of leaves and bulbs at 100-150 mg L⁻¹ GA₃. The plant growth regulators might be needed to increase shallot production, however GA₃ 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 GA₃ at 25 mg L⁻¹. This might be due to the increasing of cell wall extensibility by GA₃. Application of the exogenously GA₃

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

Gibberellic acid (GA₃) 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).

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 ± 0.08 ^b	8.23 ± 0.08 ^{bc}
	150	36.66 ± 0.00 ^b	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

The application of GA₃ 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 GA₃ (Rahman et al., 2006). However, the results showed that the plant heights were observed of 44.61-45.74 with the application of GA₃ at 0-100 mg L⁻¹, while the application of GA₃ at 150 mg L⁻¹ was only 40.11 cm. Shaikh et al., (2002) reported that the application of GA₃ 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 GA₃ 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 GA₃ 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 GA₃ application (Kucera et al., 2005; Ouzounidou et al., 2011). GA₃ 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. Inflorescence 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 quantitative 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

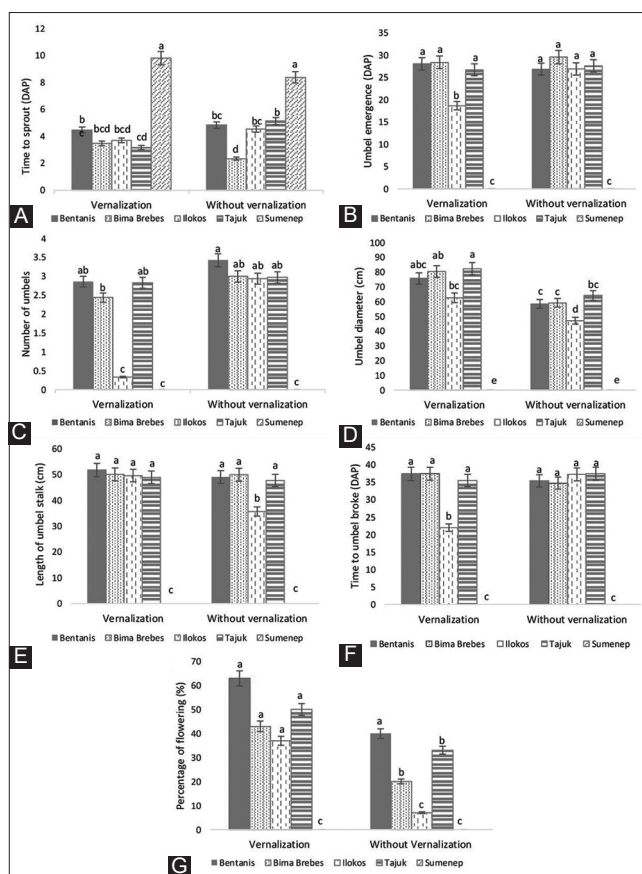


Fig 1. The interaction between shallot varieties and vernalization on time to sprout (A), day of umbel emergence (B), number of umbel (C), umbel diameter (D), length of umbel stalk (E), time to umbel broke (F), and percentage of flowering (G) of shallot. Values are means with standard deviation of triplicate 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

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.

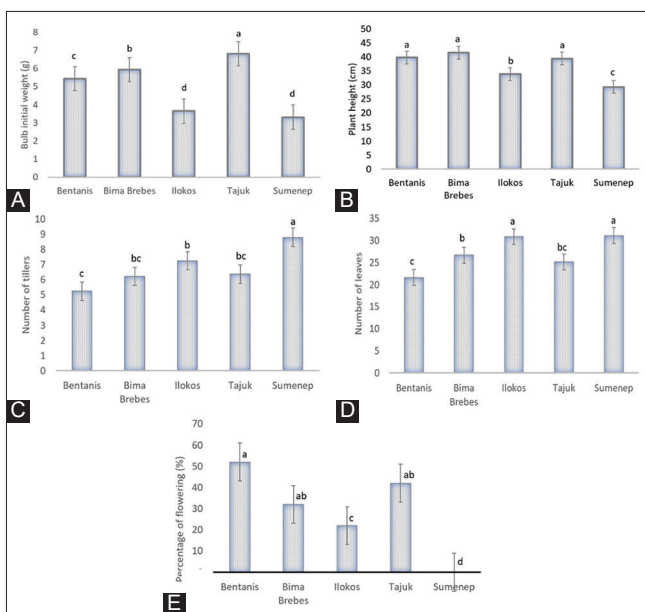


Fig 2. Effect of variety on bulb initial weight (A), plant height (B), number of tillers (C), number of leaves (D), and percent of flowering (E) of shallots. Same letters within each variable response indicated no significant differences by LSD test at 5% level. Values are means with standard deviation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

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

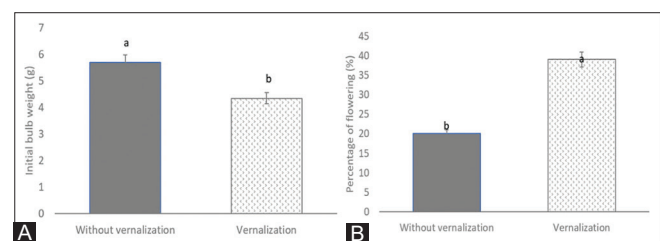


Fig 3. The effect of vernalization on bulb initial weight (A) and percent of flowering (B) of shallots. The numbers followed by the difference lowercase letter are significantly different based on the LSD test at 5% level. The numbers followed by the difference capital letter are significantly different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

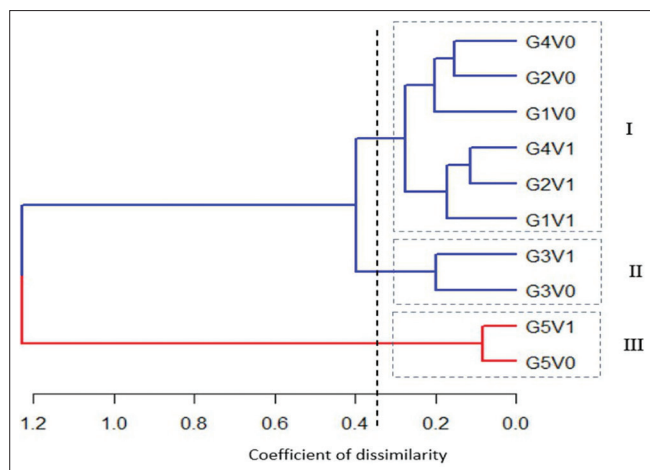


Fig 4. Hierarchical cluster of the dissimilarity matrix of vernalization treatments of 5 shallot varieties. Without vernalization (v0), and vernalization (v1). Variety of Benitanis (G1), Bima Brebes (G2), Ilokos (G3), Tajuk (G4), Sumenep (G5)

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 dissimilarity 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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