RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 55 Issue 4: 416-421 (August 2021)

Initiation of Flowering and Bulbils in Shallot with Various Treatments in Tropical Regions

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10.18805/IJARe.A-621

ABSTRACT

Background: Shallots are one of the horticultural commodities with low production growth value in Indonesia. Shallots production in Indonesia is limited by the quality of planting material and the climate, especially temperature. The flowering of shallots is a significant obstacle to the regulation of tuber development and bulbils. This study aimed to examine the effect of treatment on the initiation of flowering and the formation of bulbils.

Methods: The method used was a complete randomized block design with six treatments: without treatment, vernalization at 5°C, Immersion GA3 100 ppm, Immersion IAA 100 ppm, Immersion NAA 100 ppm, injury to the flower stalk and three replications.

Result: The treatment of flowering initiation and the formation of bulbils had a significant effect on the flowering day, the number of flower stalks, the percentage of flowering plants, number weight of bulbils and weight of bulbils. GA3 and vernalization can speed up the appearance of flowers and increase the percentage of flowering plants. GA3 and the opening of the flower stalks can encourage the formation of bulbils with a higher weight and diameter of the tubers. The flowering day had a negative correlation with the number of bulbils formed. Bulbils formation requires a higher content of endogenous GA3. GA3 can initiate the flowering and formation of shallot bulbils.

Key words: GA3, Hormone, Low temperature, Stalk injury, Vernalization.

INTRODUCTION

Shallots are one of the horticultural commodities with a low production growth value in Indonesia, namely 5.11% (Statistical Yearbook of Indonesia, 2019). Shallot production in Indonesia is influenced by the quality of planting material. Based on the research results of (Yulyatin *et al.,* 2019) that 60% of Indonesian farmers use consumption tubers as planting material. Continuous use of consumption tubers can lead to systemic disease transmission through seed tubers, causing a decrease in quality and production (Golubkina *et al.,* 2019). One of the unpopular planting materials for shallots, namely bulbils. Based on Triharyanto *et al.* (2020) research, the planting material with bulbils shows the highest production, namely 17.1 tonnes ha^{-1}, so that bulbils are used as planting material to increase shallot production. Plants obtained from bulbils are more resistant to various diseases, including viral infections (Luis *et al.* 2015). Bulbils are usually treated as a byproduct of both shallot and garlic. Garlic bulbils can be used as planting material and contain vitamin C, total polyphenols, antioxidants and carotenoids (Kopec *et al.,* 2020).

Bulbils are tubers that are formed from other parts of the plant above the soil surface, which usually originate on the axillary stem and in certain circumstances, can be formed from flowers (Ewing *et al.,* 1992). Shallots can form bulbils by inhibiting the shoot apical meristem (Shalom *et al.,* 2015; Wu *et al.,* 2015). Bulbils are widely known in tuberous plants such as begonias, potatoes, and *Dioscorea* sp (Pokrzywinski *et al.,* 2020; Terui and Okagami, 1998). Bulbils are a vegetative part that can be used for the benefit of seedlings. The formation of bulbils and onion flowering is strongly influenced by climate, namely temperature, and photoperiod (Wiebe,1990; Medina-alonso *et al.,* 2020). The red bottom is difficult to flower and bulbils are formed in tropical areas such as Indonesia. This is because shallots are long-day plants that require irradiation of > 12 hours (Siswadi *et al.,* 2020) and the initiation of flowering requires low temperatures (Dianawati *et al.,* 2021). In sub-tropical countries, bulbils can naturally form. This is because bulbils can be formed at a length of 12 hours of radiation (Fragoso-jimenez *et al.,* 2021).

Induction of flowering and bulbils in shallots was carried out by vernalization treatment with a temperature between 10-15°C for a certain period (Fragoso-jimenez *et al.,* 2020; Ritz *et al.,* 2010). Vernalization can be categorized as vernalization of seeds (low temperature treatment of seeds) or vernalization of green plants (low temperature treatment of plants of certain ages) (Song *et al.,* 2012; Wu *et al.,* 2015).

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How to cite this article: Triharyanto, E., Pujiasmanto, B. and Handoyo, G.C. (2021). Initiation of Flowering and Bulbils in Shallot with Various Treatments in Tropical Regions. Indian Journal of Agricultural Research. 55(4): 416-421. DOI: 10.18805/IJARe.A-621.

Submitted:15-02-2021 **Accepted:** 22-05-2021 **Online:** 22-07-2021

Vernalization can be carried out at temperatures ranging between 0 and 18°C, but optimal vernalization temperature at 5°C and temperatures above 18°C caused devernalization (Webb *et al.,* 2014). Induction of shallot flowering was successfully carried out by vernalization at 10°C for four weeks in the plains. However, based on the results of research by Siswadi *et al.* (2020) that shallots are still difficult to flower in the lowlands after being vernalized, the combination of vernalization and GA3 treatment has a significant effect on shallot flowering.

GA3 plays a role in encouraging flowering and increasing the fast elongation of flower stalks (bolting) (Liu *et al.,* 2019). The elongation of flower stalks in shallots is followed by the discharge of flowers and is often followed by the formation of bulbils. Initiation and growth of bulbils are controlled by multi-phytohormones (Atif *et al.,* 2020). Gibberellin (GA3) is a vital phytohormone produced by the diterpenoid pathway (Olszewski *et al.,* 2002). Several studies have shown that GA3 induces airborne tubers' formation in radishes (Nishijima *et al*., 2005) and garlic (Yamazaki *et al.,* 2015). The research results of (Triharyanto *et al.,* 2018) show that bulbils can be formed in Indonesia with gibberellin treatment. However, the results obtained for bulbils are still minimal, so a study is needed to increase bulbils' production. This study aims to examine the effect of seed treatment on the initiation of flowering and bulbils' formation.

MATERIALS AND METHODS

The research was conducted in Karanganyar Regency, Central Java, Indonesia, with an altitude of 92 m above sea level, temperatures ranging from 18-3°C and an average rainfall of 7231.4 mm per year. Endogenous gibberellin analysis was carried out at the Biology Laboratory, Faculty of Biology, Gadjah Mada University, Yogyakarta, Indonesia. The materials used are shallot seeds (*Allium cepa* L.) in the form of tubers (cloves) from the Bima Brebes variety as planting material, growth regulators, namely GA3 concentration of 100 ppm, IAA 100 ppm, NAA concentration of 100 ppm, organic vermicompost and inorganic fertilizers (Urea, KCl, SP-36 and ZA). Gibberellin content analysis using HPLC apparatus model Shimadzu system controller CBM 20 A, Solvent delivering unit LC 20AT, Column oven CTO 10 ASVP, Detector SPD 20-1 UV-Vis, Detector Column Shim-pack VP ODS µm a50 x 4.6 mm, Column temperature 25°C, Mobile phase Acetonitrile: H_3PO_4 0.1% (45: 55 v / v), Mobile phase Isocratic method, Flow rate 0.6 ml/min, Injection volume 50 µl Wavelength detector 260 nm, Rum timer 25 minutes.

The method used was a complete randomized block design consisting of one treatment factor for the initiation of flowering and the formation of bulbils consisting of 6 levels, namely: without treatment, Vernalization at 5°C, Immersion at 100 ppm GA3 concentration, Immersion at 100 ppm IAA concentration, Immersion at 100 ppm NAA concentration, injury to flower stalks and repeated three times so that there were 18 experimental units. The vernalization treatment was carried out by storing shallot seeds in the refrigerator at 5°C for one week. The GA3, IAA and NAA treatments were applied to the seeds according to the treatment level; they were soaked for 2 hours and then drained. The wound treatment of the flower stalks is done by breaking the part of the flower stalk starting to appear on the plant.

The analysis of gibberellin content was tested on bulbs and leaf shallots using the Nefed'eva and Mazey (2009) method using High-performance liquid chromatography. The sample material used is 10 g. The variables observed included the day on which the flowers appeared, the number of flower stalks planted, the percentage of flowering plants, the number of bulbils, the weight of bulbils, the diameter of bulbils. Data were analyzed by analysis of variance based on the F test of 5% level. If the treatment affects the measured variables, data analysis is continued with a comparison of the treatment means using the Duncan Multiple Range Test (DMRT) with a level of 5%.

RESULTS AND DISCUSSION

Initiation of shallot flowering

The results showed that the treatment of flowering initiation and the formation of bulbils had a significant effect on the day the flowers appeared and the number of flower stalks planted (Table 1). The fastest flower appearance day on GA3 treatment was 28 days after planting. GA3 plays a role in making and initiating onion flowering (Regnault *et al.,* 2014; Ranwala and Miller, 2008). The flowering pathway with gibberellin affects the flowering time in response to internal developmental status (Fornara *et al.,* 2010). Furthermore, GA3 regulates flowering by controlling the expression of flower regulating genes such as flowering locus T (FT), twin

Table 1: Effect of treatment on the initiation of flowering.

Treatments	Days when flowers appear (DAP)	Number of flower stalks per plant	Percentage of flowering plants
Without treatment	36.00 d	4.67 a	58.67 a
Vernalization at 5°C	30.33 ab	11.00 b	76.33 b
Immersion at GA3 100 ppm	28.00 a	13.67 b	80.67 b
Immersion at 100 ppm IAA	32.00 bc	10.00 b	71.33 ab
Immersion at 100 ppm NAA	32.00 bc	13.00 b	66.67 ab

Note: DAP: Days after planting. Numbers followed by the same letters in the same column showed no significant differences based on the DMRT level of 5%.

sister of FT (TSF), squamosa promoter binding proteinlike (SPL), suppressor of overexpression of (SOC1), and LEAFY (LFY) (Galvao *et al.,* 2012). However, the day of flower appearance on GA3 treatment with vernalization treatment was not significantly different. This is because the vernalization treatment (5°C) has a role in stimulating bolts (Guevara-figueroa *et al.,* 2018). Based on the research results by (Wu *et al*., 2016), the vernalization treatment significantly increases the rate of bolting. Low temperature is a major factor in spurring growth, flowering and tuber formation (Mojtahedi *et al.,* 2013). Vernalization activates sugar and protein metabolism, triggers the buildup of small molecule compounds, and increases reproduction (Li *et al.* 2010). The combination of cold temperature vernalization with GA3 accelerates the bolting time, stem elongation, and flowering time (Shi-wei *et al.,* 2019). GA3 has been effectively used as a substitute for vernalization to stimulate the breakdown of dormancy and encourage flowering (Oh *et al.* 2018). Low temperature treatment (vernalization) in plant organs can increase endogenous gibberellin activity, cell division and increase auxin (Dinarti *et al.,* 2011; Nazeer *et al.,* 2020). The day of interest on the GA3 treatment was significantly different from the IAA and NAA applications (Table 1). These results indicate that the IAA and NAA applications show the appearance of flowers is more elongate, namely 30 days after planting. Apart from gibberellin, IAA and NAA also have essential effects on flowering (Kurtar and Ayan, 2005).

GA3 application also shows the highest number of flower stalks planted, namely 13.67 stalks. This is because most of the flowering gene expression is influenced by GA3 treatment (Guan *et al*., 2019). These results were supported by the GA3 content of tubers and leaves in flowering plants (Table 4). Endogenous GA3 in the leaves and tubers of flowering plants showed lower yields than endogenous GA3 in non-flowering plants. Based on the results of the research of (Triharyanto *et al.* 2020) that endogenous GA3 in flowering plants is higher than in non-flowering plants. Endogenous GA3 increased after exogenous GA3 application and decreased at 44 days after flowering (Zhang *et al.* 2016). GA3 is an environmental signal to induce stem elongation and flowering (Winterhagen *et al.* 2020). The application of exogenous GA3 also stimulates the synthesis of endogenous GA3 and indole-3-acetic acid (IAA) but reduces levels of abscisic acid (ABA) (Cole *et al.,* 2016). Endogenous GA3 and indole three acetic acids, which increasingly affect the development of flowers, the number and identity of flower organs. Auxins can stimulate division, enlargement, cell differentiation and protoplasmic flow in plants' vegetative growth, including root organs (Cheng and Zhao, 2007).

The results showed that the treatment had a significant effect on the percentage of flowering plants (Table 1). GA3 treatment showed the highest percentage of flowering plants (Table 1). This study's results are consistent with (Chang and Huang, 2018) that exogenous GA3 increases the percentage of flowering plants. This is because GA3 promotes the transition from vegetative growth to reproductive growth (King *et al.,* 2008). However, the percentage of flowering plants in GA3 treatment was not significantly different from the vernalization treatment, flower stalk wound treatment, and NAA. Auxins play an essential role in stimulating plant vegetative growth (Nikmah and Slamet, 2017).

Initiate the formation of bulbils

The results showed that the treatment of the initiation of flowering and bulbils' formation had a significant effect on the number of bulbils formed, weight and diameter of bulbils (Table 2). Seed treatment by immersing GA3 100 ppm showed the highest number of bulbils, namely 16 tubers. These results are supported by endogenous gibberellins' content in leaves and tubers, which are formed from plants that can form bulbils (Table 4). The content of gibberellins in leaves and tubers which can form bulbils is relatively high, namely 55.609 and 103.33 µg/g wet weight. These results indicate that the formation of bulbils requires endogenous gibberellins. This is because GA3 plays an essential role in the induction and growth of lateral shoots in some plants (Ni *et al.,* 2015; Elfving *et al.,* 2011). Application of GA3 to garlic led to an increase in the number of axillary meristems, lateral shoots in the early stages, and formation of bulbils (Hong-jiu *et al.,* 2020). Several studies have shown that GA3 induces bulbils formation in Indonesian radishes (Nishijima, *et al.,* 2005) and garlic (Yamazaki *et al.,* 2015). In sub-tropical countries, bulbils can naturally form (Rayamajhi *et al.,* 2017). The research results of Triharyanto *et al*. (2018) showed that bulbils could be formed in Indonesia with gibberellin treatment.

Table 2: Effect of treatment on bulbils formation.

Treatments	Number of bulbils per planting	Weight of bulbils (g)	Diameter of bulbils (cm)
Without treatment	5.33 a	2.64 a	0.80 a
Vernalization at 5°C	3.67 a	2.22 a	1.06 b
Immersion at GA3 100 ppm	16.00 b	8.99 b	1.14 b
Immersion at 100 ppm IAA	6,67 a	3.58 a	1.08 b
Immersion at 100 ppm NAA	7.33 a	2.87 a	0.21 a
Injury to flower stalks	13.33 b	6.61 b	1.10 ab

Note: Numbers followed by the same letters in the same column showed no significant differences based on the DMRT level of 5%.

Table 3: Correlation between flowering and formation of bulbils.

	Flower day	Number of bulbils	Weight of bulbils
Flower day	1	-.489*	-.528*
Number of bulbils	-0.489*	1	.926**
Weight of bulbils	-.528	.926**	1

Note: ** Correlation is significant at the 0.01 level (2-tailed) and * Correlation is significant at the 0.05 level (2-tailed).

Table 4: Gibberellins content in leaves and tubers.

Sample name	Relative content of gibberellin (µg/g wet weight)
Leaves do not flower	51.059
The leaves are formed bulbils	55.609
Flowering leaves	46.520
Bulbs do not flower	94.953
Bulbils are formed	103.331
Flowering bulbs	87.499

The results showed that GA3 treatment produced bulbils with the highest weight and diameter (Table 2). This is because GA3 plays a role in sucrose phosphosynthase activity, one of the main enzymes involved in sucrose synthesis and promotes tissue proliferation, which leads to a better assimilation process (Nguyen *et al.,* 2019). The number of bulbils formed, weight and diameter of bulbils in GA3 treatment was not significantly different from the wound treatment of flower stalks. The number of bulbils in the flower stalk wound treatment was 13 bulbils. This is because bulbils can be formed from injured plant parts due to disturbed assimilate translocation (Jackson, 1999). However, an injury to the flower stalk can inhibit the formation of flowers due to disturbed translocation of nutrients (Xue *et al.,* 2019). Table 1 shows the long flower appearance days and the percentage of flowering plants in the low flower stalk wound treatment. The day the flower appeared was negatively correlated with the number of bulbils formed (Table 3). These results indicate that the faster the flowering plants, the lower the number of bulbils formed. This study's results are consistent with (Yoshie, 2014) that flowering is inhibited due to an increase in shoot growth.

CONCLUSION

GA3 and vernalization treatments can accelerate the appearance of flowers at 28 days after planting and 30 days after planting. GA3 treatment resulted in the highest percentage of flowering plants, namely 80%. The treatment of GA3 and the bulbils' opening encourages the formation of bulbils with a higher weight and tuber diameter. The day the flower appeared was negatively correlated with the number of bulbils formed. Bulbils formation requires a higher endogenous GA3 content. GA3 can initiate shallot flowering and bulbils.

ACKNOWLEDGEMENT

The authors wish to express sincere thankfulness to Universitas Sebelas Maret for financial support on the Community Partnership Program with Non-Tax State Revenue funds for the 2020 budget year with contract number: 452/UN27.21/PN/2020.

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