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# TRUE SHALLOT (*Allium cepa* var *ascalonicum*) SEED PRODUCTION DURING OFF SEASON

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**ABSTRACT**

Seed cultivation for true shallot is an alternative for the more common cultivation practice in which 30% of the harvested tubers are used for cultivation purposes. Seed production of this temperate tuber in the tropical region, however, is quite challenging due to its low flowers and seed formation. Several studies have shown that vernalization (cold induction) and application of Benzil Amino Purin (BAP) had improved the flowering and seed production of shallot. However, such studies were conducted during the best cultivation period for about 3 months and thus, limit the production period of seeds during the rainy season. This study was conducted to observe the effect of both methods outside cultivation periods on the flower and capsule numbers, fruit set, and weight of 100 seeds when compared with commonly practiced cultivation during the dry season. The onion bulbs vernalized at 10 $^{o}$C for 30 days were subjected to synthetic hormone (BAP) prior to planting. The shallot group treated with BAP had the lowest values for all observed parameters, i.e., 1,552.67 number of flowers; 312.11 number of capsules; 22.5% seed set; and 0.2244 g weight of 100 seeds, compared to those in the vernalization treated group, i.e., 1,592.44 number of flowers; 623 number of capsules; 30.5% seed set; 0.2261 g weight of 100 seeds and control group 6,774.67 number of flowers; 3,898.44 number of capsules; 57.06% seed set; 0.3304 g weight of 100 seeds. In conclusion, the commonly practiced cultivation of sowing bulbs directly without vernalization and plant growth regulator treatment is probably the better method to produce shallot seeds during the offseason, the rainy season.

**Keywords:** Benzil Amino Purin, True Shallot Seeds, Vernalization

## INTRODUCTION

Shallot (*Allium ascalonicum*) is one of the most important tubers in Indonesia, which is commonly used as food ingredients and traditional medicine. Market demand for the certain commodity has increased annually with 5.30% average consumption growth (Kementerian Pertanian 2015). The shallot farmers in Indonesia usually cultivate this commodity by its vegetative form. However, this method has several challenges, namely 1. short storage period of planting stock (Suwandi & Himan 1995); 2. variable quality (Balai Penelitian dan Pengembangan Pertanian 1995); 3. high susceptibility to disease spread (Wibowo *et al.* 2016); 4. high production cost (Gina & Rofik 2010); and 5. significant amount of unsold harvested tuber (Permadi & Putrasamedja 1991; Basuki 2009). These factors have prevented the true shallot total production to fulfill its market demand.

To meet its market demand, the improvement of national shallot production through the use of botanical seeds or true shallot seed (TSS) in shallot cultivation is necessary. Seeds have longer storage time, up to six times their vegetative form, and eliminate the need for large storage room (Basuki 2009) thereby also reducing the production cost (Permadi & Putrasamedja 1991; Basuki 2009).

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However, most of the local growers have not applied this method due to the very limited amount of available true shallot seeds (Rosliani 2013). Environmental factors, such as average temperature, photoperiod, average humidity, are believed to be the limiting factors for seed production in Indonesia (Fahrianty 2013; Wu *et al.* 2015). This biennial plant produces bulb as an overwintering stage of the life cycle and produces flowers in the spring after a period of winter or vernalization (Brewster 2008). Furthermore, true shallot also requires long photoperiod (> 12 hours) to ensure flowering and seed production (Kamenetsky & Rabinowich 2001). Vernalization of bulb before planting ensures early flowering of seed crop (Brewster 1994) and enables the seeds to produce a heavier yield (Jones & Mann 1963; Mollah *et al.* 2005; Ami *et al.* 2013) as the result of increasing gibberellin endogen and auxin production (Dinarti *et al.* 2011). Therefore, most true shallot seed producers in Indonesia apply low-temperature shock treatment to the mother bulb and establish the plantation in higher elevation.

Moreover, improving the production application of plant growth regulators (PGR) is one of the common practices. A small number of plant regulators can be applied to adjust plant physiology, such as plant growth and development (Yamaguchi & Kamiya 2000). Control of flowering and seed formation are among the most important practical aspects of seed production. Among all plant regulators, cytokinin plays an important role from seed germination to delaying the onset of senescence (Chatsudthipong & Muanprasat 2009). Furthermore, synthetic cytokinin is available, such as Benzil Amino Purin (BAP). Some studies showed the beneficial effect of BAP on flower numbers, flower size, flower longevity, and the number of seed produced (Youngkoo *et al.* 2006; Roslian *et al.* 2012; El-Kinany *et al.* 2019).

In Indonesia, the true shallot seeds have been produced by applying either vernalization or BAP to the mother bulb and planting in the highland during the dry seasons (Rosliani *et al.* 2012). The present study, however, explored the possibility and limitation of the application of the vernalization technique and BAP on true shallot seeds during the rainy season, with the intent to improve its production by providing viable seeds all year round.

## MATERIALS AND METHODS

### Study Site

The study was conducted at the field greenhouse of PT East West Seed Indonesia Research Station in Lembang and School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia. Shallot for seed production was cultivated from October 2016 to April 2017 at the greenhouse, while the quality of seed produced was assessed at the institute. The field experiment was conducted during the rainy season at average temperature of 19 ºC to 23 ºC which is considered as off-season for true shallot seed production.

### Vernalization of the Bima Mother Bulbs

Bima variety, which is widely planted in center of shallot production and released by the Balai Penelitian Tanaman Sayuran was used as mother bulbs in this study. One hundred and twenty Bima mother bulbs were put in white cotton cloth bags and vernalized in a refrigerator at a calibrated temperature of 10 °C for 30 days. After bulbs vernalization, a total of 60 bulbs were subjected to synthetic hormone treatment by dipping them in the BAP solution, while another 60 bulbs were stored under controlled temperature (21±3 °C) and serve as the untreated control. To prevent the mother bulbs from fungi attack, all of the mother bulbs were dipped into fungicide solution before planting.

### Land Preparation

The land was thoroughly prepared by plowing and cross plowing followed by laddering. The subsequent operations were done with harrow, spade, hammer, and other tools. Weeds and stubbles were collected and removed from the field. Irrigation and drainage channels were made around the plots with the corners trimmed by the spade.

### Plant Spacing

The planting distances between rows and between bulbs were 25 cm and 20 cm, respectively. Each of the 3 plots contained four rows with 15 bulb seeds sown in each row, amounting to 180 bulbs sown at 7 cm depth.

### Application of Fertilizer and Cultivation Practices

The true shallot planting stock was fertilized with the recommended doses of N:P:K 16:16:16 and dolomite. Watering, weeding, and fungicide applications were conducted once a week during the cultivation period (115 - 130 days). During the flowering period, the plants were protected under plastic sheets, harbored above them to protect them from rainfall damage, while pollination was conducted by hand.

### Harvesting and Processing

The cultivation period lasted for 115 - 130 days. When the seeds inside the capsules turned black and more than 25% black seeds were exposed on the umbel, each umbel was cut at 5 cm of the flower stalk. Harvesting was conducted on days 116, 123, and 130 and the umbels were then sun-dried. Threshing was done by light beating and hand rubbing of the umbels. The seeds were cleaned and sun-dried up to 7 days until seed moisture was reduced to below 8%. Each of the harvested groups of seeds were processed separately and contained in separate paper bags and preserved for later use (Mollah *et al.* 2015).

### Seed Weight and Germination

One of the methods to determine the quality of seeds is by measuring the total weight and germination rate of 100 seeds. One hundred seeds were randomly selected from each harvest and weighed (in gram, g) on an electric balance, placed in a plastic tray, and allowed to germinate. The plastic tray was filled with a paper towel previously dipped in liquid fertilizer.

Germination test was carried out according to the International Rules for Seed Testing (ISTA 1996). The number of normal seedlings, abnormal seedlings, dead seeds, and ungerminated seeds were recorded for two weeks. Then the germination percentage was determined by the following formula.

$$\text{Germination} = [(\text{Number of seedlings}/\text{Number of seeds tested})] \times 100\% \quad (1)$$

### Data Analysis

The normality of data was analyzed by the One-Sample Kolmogorov-Smirnov method. The differences among treatments on flowering initiation period, flower numbers, capsule numbers, fruit set, seed numbers, weight of 100 seeds, and seeding rates were analyzed using one-way ANOVA with a significant level of $P < 0.05$. Tukey analysis was conducted as the post hoc test when ANOVA showed significant differences. All analyses were conducted using SPSS 16.0.

## RESULTS AND DISCUSSION

### Flowering Initiation

The time required to produce flowers in the untreated shallot plants (control group, with temperature of 21±3 °C) was significantly longer than those in the other groups, while those in the V+BAP and V groups were relatively similar (Fig. 1).

These results conformed with previous studies that vernalization treatment on shallot bulbs required a shorter time to produce flowers (Satjadiputra 1990; Yan *et al.* 2003; Islam *et al.* 2010; Andres & Coupland 2012; Fahrianty 2013; Ream *et al.* 2013; Wu *et al.* 2015). The results showed the importance of vernalization treatment to initiate the flowering, which might be related to the temperate origin of true shallot. Vernalization blocked the flowering repressor and induced the expression of genes responsible for the flowering (florigen) (Lee *et al.* 2013). Vernalization could also promote the up-regulation of some key cytokinin signaling regulators which induced the flowering (Wen *et al.* 2017). The application of BAP, a cytokinin synthetic, might have induced gibberellin signaling that reduced the flowering initiation time (Tarkowska 2012; Wong *et al.* 2013).

### Number of Flowers, Capsules, and Seeds Produced

The control groups produced significantly more flowers than the other groups. On the other hand, the number of flowers produced by both V+BAP and V groups were relatively similar (Fig. 2).

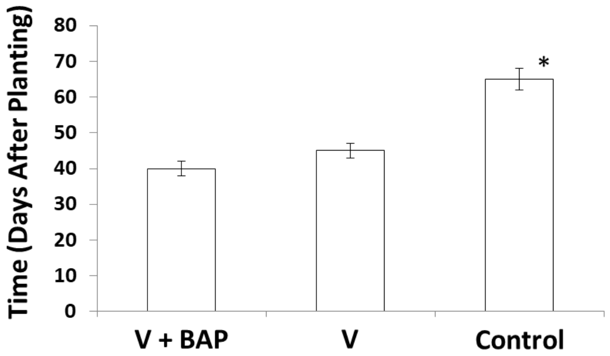


Figure 1 Time required for producing flowers among all treatments
Notes: V + BAP = Vernalization + Benzil Amino Purin (BAP) treatment; V = Only vernalization, Control = no treatment (with temperature of 21±3°C); * = significant at P < 0.05.

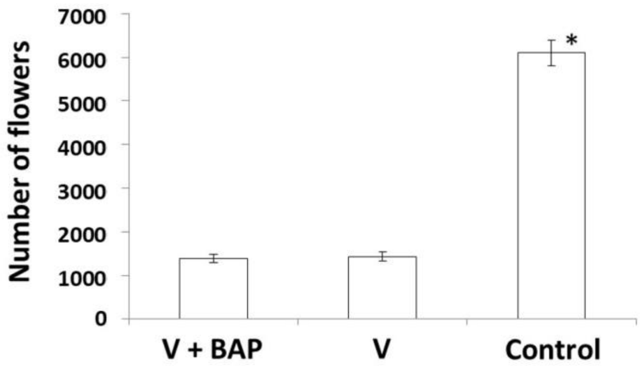


Figure 2 Number of true shallot flowers among treatments.
Notes: V + BAP = Vernalization + Benzil Amino Purin (BAP) treatment; V = Only vernalization; Control = no treatment (with temperature of 21±3°C); * = significant at P < 0.05.

Vernalization positively affected flower initiation and the number of flowers produced by shallot (Fahrianty 2013). However, the information was based on the study conducted in dry season, the best season for true shallot seed production. The lower number of flowers produced by vernalization groups in the current study could be related to insufficient photoperiod. The true shallot is a long day plant that required 12 hours light period (Currah & Proctor 1990). Shorter and epileptic light conditions during the rainy season may negate the positive effect of vernalization on flower production due to less optimal photoperiod (Dennish & Peacock 2009; Wu *et al.* 2015).

The lower number of flowers resulted in a fewer number of capsules (Fig. 3A) and seeds (Fig. 3B) of the vernalized shallot groups. Most of the seed losses were caused by flower abortion and infection by fungi on the flowers of vernalized groups.

The results indicated that plants of the vernalized groups had lower resistance to diseases probably due to the high humidity. Vernalization reduced the vegetative period which benefited the seed production because when plants were growing in optimum condition, most of the plant energy was fully used in seed production. However, under sub-optimal conditions, the plants have to overcome environmental stress and allocated less energy to seed production. On the other hand, the longer growing period of the control group could increase seed yield (Farghali 1995), probably due to more available energy for seed production.

## Seeds Quality

The weight of 100-seeds of the control groups was significantly higher than those of other groups, while the V+BAP group produced slightly heavier seeds than the V group (Fig. 4).

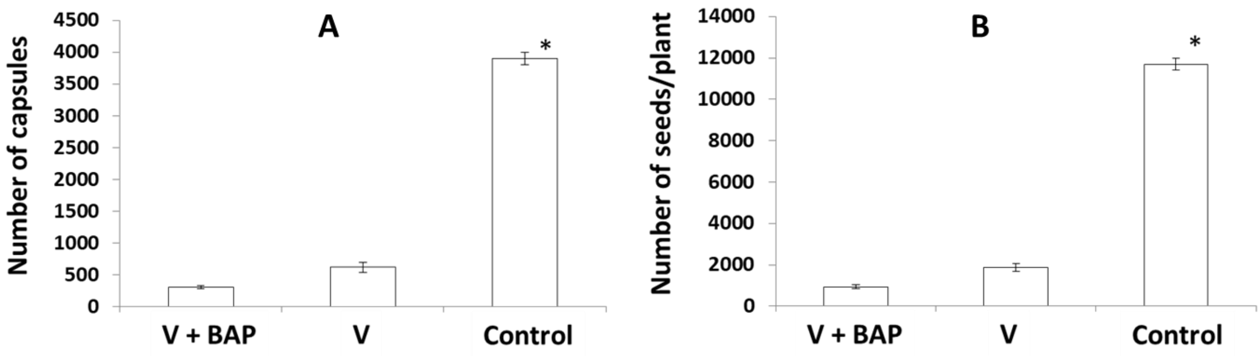


Figure 3 (A) Number of true shallot capsules and (B) Seeds per plant among treatments
Notes: V + BAP = Vernalization + Benzil Amino Purin (BAP) treatment; V = Only vernalization, Control = no treatment (with temperature of 21±3 °C); * = significant at $P < 0.05$.

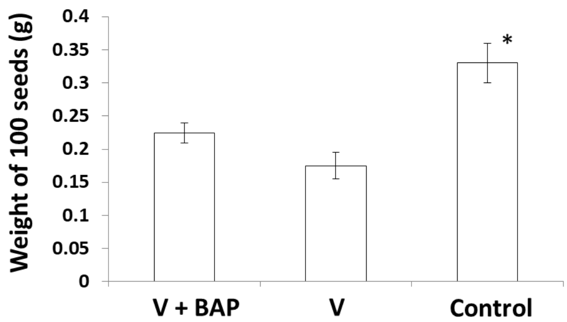


Figure 4 Weight of 100-seeds among treatments
Notes: V + BAP = Vernalization + Benzil Amino Purin (BAP) treatment; V = Only vernalization; Control = no treatment (with temperature of 21±3°C); * = significant at $P < 0.05$.

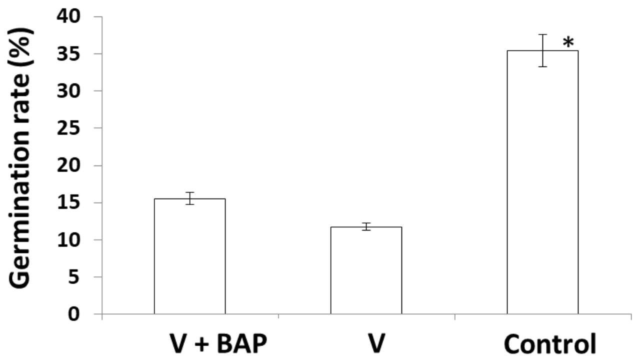


Figure 5 Germination rate of seeds produced among treatments
Notes: V + BAP = Vernalization + Benzil Amino Purin (BAP) treatment; V = Only vernalization; Control = no treatment (with temperature of 21±3°C); (*) = significant at $P < 0.05$.

The seed weights of the vernalization groups were in contrast with those in previous studies that indicated the positive effect of vernalization on seed weight (Mollah *et al.* 2005; Ami *et al.* 2013). The high humidity of the rainy season might have caused significant damage to the seed resulting in its reduced weight (Ku *et al.* 2008). The addition of BAP after vernalization has improved the seed weight as it could have induced cell growth and tissue differentiation (Rosliani 2013). Based on this current study, vernalization probably induced only flower production, while the seed quality depends on different mechanisms such as the effect of vegetative propagation, flower numbers and the availability of the pollinator (Krontal *et al.* 2000). Therefore, further study is suggested to test this hypothesis.

Germination rate of seeds produced by the control group was significantly higher than those of other groups, followed by V+BAP group and V group (Fig. 5). Germination rate of seed highly depends on seed weight which explained the low germination rate of the vernalization groups (Gamiely *et al.* 1990; Mollah *et al.* 2005). Germination rate in this study was also much lower than those produced in optimal season which indicated the importance of planting date (Mollah *et al.* 2005; El-Helaly & Karam 2012).

## CONCLUSION

Vernalization is required to induce the flowering of true shallot. However, the planting date plays an important role in the volume of seed production and quality. During the rainy season, the offseason, the commonly practiced cultivation technique is still the recommended method for the optimum production of true shallot seeds. Reducing the detrimental effect of the rainy season to the true shallot plant induced with vernalization, is also suggested, such as maintaining seed production inside a closed system with a controlled environment, which is obviously a key factor in the sustainable production of true shallot seed in Indonesia and other areas with similar climate.

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