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VERNALIZATION AND BENZIL AMINO PURIN APPLICATION UNABLE TO ENHANCE TRUE SHALLOT SEED (TSS) PRODUCTION DURING OFF SEASON**

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ABSTRACT

The application of seed for true shallot cultivation is an alternative of the more common cultivation practice, in which 30% of harvested tubers used for cultivation purposes. The seed production of this temperate tuber, in the tropical region, is quite challenging due to low flowers and seed formation. Several studies showed that vernalization (cold induction) and application of Benzil Amino Purin (BAP) could be applied to improve flowering and seed production. However, such studies were conducted during the best cultivation period for about 3 months and thus, limit the production period of seeds. This study was conducted to observe the effect of both methods outside cultivation periods to flower and capsule numbers, fruit set, and weight of 100 seeds compared with common cultivation. In this study, bulbs of onion vernalized at 10^{o}C for 30 days then became subjected to synthetic hormone (BAP) prior planted while control group The results showed that BAP treated shallot group has the lowest values for all observed parameters (1552.67, 312.11, 22.5%, 0.2244 gram) compared to those vernalization treated group (1592.44, 623, 30.5%; 0.2261 gram) and control group (6774.67; 3898.44; 57.06%; 0.3304 gram). Based on this study, it could be concluded that common cultivation is a better method to produce true shallot seeds during the offseason.

Keywords: Benzil Amino Purin, True Shallot Seeds, Vernalisasi

INTRODUCTION

True shallot (*Allium ascalonicum*) is one of the most important tubers in Indonesia. Market demand for this commodity has increased annually with 5.30% average consumption growth (Kementrian Pertanian 2015). In Indonesia, true shallot farmers usually cultivate this commodity by its vegetative form. This method has several disadvantages, such as (1) short storage period (Suwandi & Himan 1995), (2) inconsistent quality (Balai Penelitian dan Pengembangan Pertanian 1995), (3) higher possibility of disease spreading (Permadi 1995), (4) higher production cost (Gina & Rofik 2010), and (5) significant amount of harvested tuber unable to be sold (Permadi &

Putrasamedja 1991, Basuki 2009) which prevented total true shallot production to fulfill market demand.

To meet the market demand, the improvement of the national shallot production through the use of botanical seeds or true shallot seed (TSS) in shallot cultivation is necessary. The seeds have longer storage time, up to six times of generative form, and nullified the need for large storage room (Basuki 2009) while it also reduces production cost (Permadi & Putrasamedja 1991, Basuki 2009).

However, most of the local true shallot growing areas have not applied this method due to the limited amount of seed availability (Rosliani 2013). Environmental factors, such as average temperature, photoperiod, average humidity, are believed to be the limiting factor 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 (known as 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 produces a heavier yield of seeds (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 high land.

In order to improve seed production, synthetic plant hormone-like Benzil Amino Purin (BAP) is also applied to mother bulb prior to planting. Some studies reported the positive effect of this substance on flower and seed production (Youngkoo *et al*. 2006, Roslian *et al*. 2012).

It is possible to produce true shallot seeds in Indonesia by the application of the vernalization of mother bulb and plantation in the highland, mostly in dry seasons. Present study focused on the possibility and limitation of the application of the vernalization technique and BAP for true shallot seed production during the rainy season. The result of this study may provide valuable information to increase the true shallot seed production through the development of the production system during the rainy season, a less optimum period for production.

MATERIALS AND METHODS

Study area and period

The study was conducted at the greenhouse of the East West Seed research station and School of Life Sciences and Technology, Institut Teknologi Bandung. True shallot was cultivated from October 2016 to April 2017 at the East West Seed research station, while the quality of seed produced was assessed at Institut Teknologi Bandung. The field experiment was conducted at rainy

season with the average temperature between 19 to 23^{o}C which is considered as off-season for true shallot seed production.

Variety

Variety Bima was used in the research program. It is released by Balai Penelitian Tanaman Sayuran. This variety is considered as the most widely

Vernalization of mother bulbs

Bulbs of onion 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. All bulbs were dipped into fungicide to prevent fungi attack before planted.

Land Preparation

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

Plant spacing

The planting distances between rows and between bulbs were 25 cm and 20 cm, respectively. Each plot contained four rows. Fifteen seed bulbs were sown in each row. a total of 180 bulbs were sown at 7 cm depth.

Application of fertilizer and cultivation practices

True shallot was fertilized with recommended doses of N:P:K 16:16:16 and dolomite. Watering, weeding, and fungicide applications was conducted once a week during shallot cultivation. During flowering period, flowers were covered with plastic cover to prevent it from the destruction by rainfall. Pollination was conducted by hand pollination.

Harvesting and processing

The duration of true shallot cultivation was 115-130 days. When the seeds inside the capsules become black and more than 25% black seeds were exposed on the umbel, each umbel was

cut with 5 cm of the flower stalk. Harvesting was conducted on day 116, 123, and 130. The umbels were 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 reduced to below 8%. The seeds of each harvest were processed separately and contained in a separate paper bag and preserved for further use (Mollah *et al.* 2015).

Weight of 100 seeds

One hundred seeds were selected at random from each harvest. The 100-seed weight was recorded on an electric balance and expressed in gram (g).

Seed germination

Germination test was carried out in a plastic tray according to the International Rules for Seed Testing (ISTA, 1996). A paper towel dipped in liquid fertilizer was used as a substrate for the germination test. The plastic tray was filled with a moist paper towel. Adequate moisture was maintained in the substrate. One hundred seeds were taken at random and placed in the tray. The number of normal seedlings, abnormal seedlings, dead seeds, and ungerminated seeds was counted for two weeks. Germination percentage was determined by the following formula.

Germination = [(Number of seedlings / Number of seeds tested)] x 100% (1)

Data Analysis

The normality of data was analyzed by One-Sample Kolmogorov-Smirnov. The differences among treatment on flowering initiation period, flower numbers, capsule numbers, fruit set, seed numbers, weight on 100 seeds, and seeding rates were analyzed by one-way ANOVA with a significant level of $P<0,05$. Tukey analysis was conducted as the post hoc test when ANOVA showed significant value. All analyses were conducted by SPSS 16.0.

RESULTS AND DISCUSSION

Flowering initiation

The time required to produce flowers in untreated shallot plants (control group) was significantly longer than those in other groups. While those in V+BAP and V groups were relatively similar (Fig. 1).

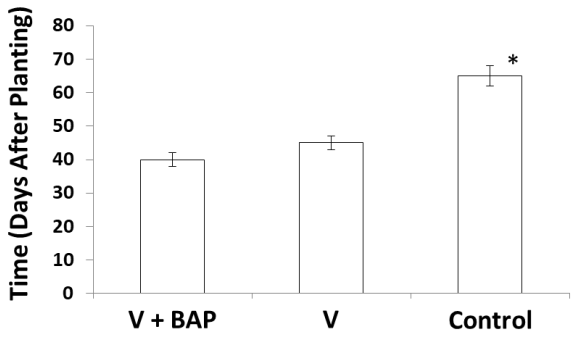

Figure 1 Time required for producing flowers among all treatments. V + BAP = Vernalization + Benzil Amino Purin (BAP) treatment, V = Only vernalization. (*) significant at $P < 0.05$

This result is in accordance with previous studies which revealed that vernalization treatment on shallot bulbs required shorter time to produce flowers (Satjadiputra 1990, Yan *et al.* 2003, Islam *et al*. 2010, Andres and Coupland 2012, Fahrianty 2013, Ream *et al*. 2013, Wu *et al.* 2015). The result showed the importance of vernalization treatment to initiate flowering which might relate to the temperate origin of true shallot. It is reported that vernalization blocked flowering repressor and induced 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 flowering (Wen *et al.* 2017). Application of BAP, a cytokinin synthetic, might induce gibberellin signaling that reduced flowering initiation time (Tarkowska 2012, Wong, *et al.* 2013).

Number of flowers, capsules, and seeds produced

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

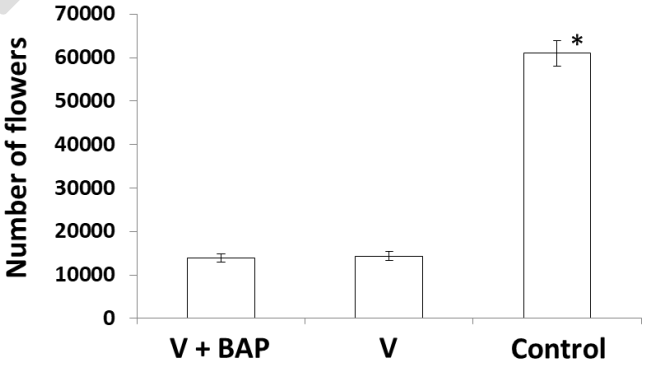

Figure 2 Number of true shallot flowers among treatments. V + BAP = Vernalization + Benzil Amino Purin (BAP) treatment, V = Only vernalization. (*) significant at $P < 0.05$

The previous study (Fahrianty, 2013) reported the positive effect of vernalization on flower initiation and the number of flowers produced by shallot. However, the study was conducted on dry season, the best season for true shallot seed production. The lower number of flowers produced by vernalization groups in this 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).

Lower numbers of flowers resulted in fewer number of capsules (Fig. 3A) and seeds (Fig. 3B) of vernalization treated shallot groups. Most of the seed losses were caused by flower abortion and infection by fungi on the flowers of vernalization groups.

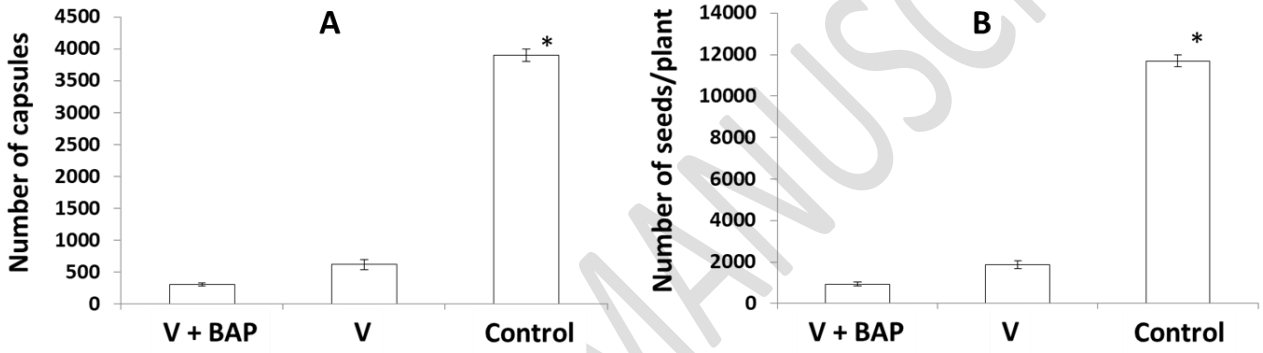

Figure 3 (A) Number of true shallot capsules and (B) Seeds per plant among treatments. V + BAP = Vernalization + Benzil Amino Purin (BAP) treatment, V = Only vernalization. (*) significant at $P < 0.05$.

The result might indicate plants of vernalization groups had lower resistance to diseases due to high humidity. Vernalization reduced vegetative period which benefited the seed production, when plant growth in optimum condition, as most energy produced by plant could be fully used to seed production. However, under sub-optimal conditions, the plant had to overcome environmental stress and allocated less energy to seed production. On the other hand, a longer growing period of the control group could increase seed yield as also reported by Farghali (1995), possibly through more energy available for seed production.

Seeds Quality

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

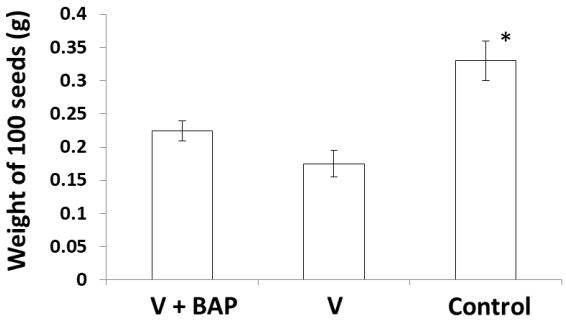

Figure 4 Weight of 100-seeds among treatments. V + BAP = Vernalization + Benzil Amino Purin (BAP) treatment, V = Only vernalization. (*) significant at $P < 0.05$.

The results of the seed weight of vernalization groups were on the the contrary with that found 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 cause significant damage to the seed that reduced its weight (Ku *et al*. 2008). The addition of BAP after vernalization improved the seed weight as it could induce cell growth and tissue differentiation (Rosliani 2013). Based on this study, it could be hypothesized that vernalization only induces flower 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 necessary to test this hypothesis.

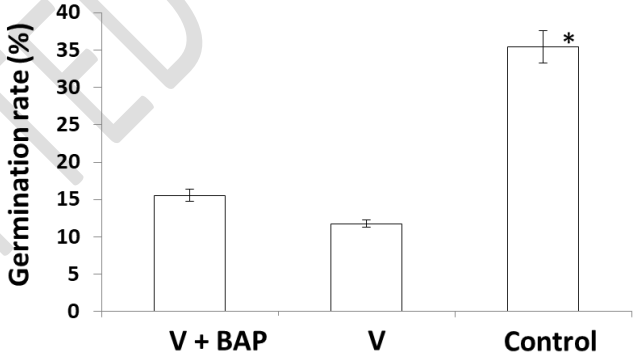

Figure 5 Germination rate of seeds produced among treatments. V + BAP = Vernalization + Benzil Amino Purin (BAP) treatment, V = Only vernalization. (*) significant at $P < 0.05$.

Germination rate of seeds produced by control groups were significantly higher than that of other groups, followed by V+BAP group and V group (Fig. 5). The germination rate of seed highly depends on seed weight which explained low germination rate of vernalization groups (Gamiely *et al.* 1990, Mollah *et al*. 2005). The germination rate of seed recorded in this study 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

This study showed that vernalization is required to induce flowering of true shallot. However, the planting date plays an important role in the production and quality of seeds. During the rainy season, common shallot cultivation is recommended to conduct in order to produce true shallot seeds. Reducing the detrimental effect of the rainy season to true shallot plant induced with vernalization, such as by maintaining the production inside the closed system with a controlled environment, may become the key factor to sustainable true shallot seed production in Indonesia and other areas with similar climate.

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