Flowering and yield of true shallot seed from bulb and different seedling age vernalized at low temperature

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Abstract. True Shallot Seed (TSS) is an alternative source of shallot (Allium cepa var. ascalonicum) seeds. The constraint of TSS production currently being developed is bulb seeds availability which is limited in a timely manner, amount and quality. The study aim was to observe flowering response from bulb-to-seed method compared to seedling (seed-to-seed method). The study was conducted in Margahayu Experimental Field, IVegRI, Lembang (1,250 m asl) from February to December 2017. The randomized block design with 4 replications was used. The treatments include bulb seeds that are vernalized at 10 °C for 4 weeks, vernalized TSS then 6 weeks sowing, vernalized seedlings aged 4, 5, 6, and 7 weeks at 10 °C for 3 weeks, and non-vernalized seedlings aged 6 weeks. The variety used is Trisula. Observations included vegetative and generative parameters. Data were analysed with ANOVA and DMRT test at the 5% level. The results showed that TSS plant material resulted in higher flowering and TSS production compare to that derived from bulb. Six-weeks vernalized seedling produce the highest florets number per umbel, seed weight per umbel and per plant. Results of the experiment indicate that TSS from seedling showed potential for further development.

1. Introduction
Shallot (Allium cepa var. ascalonicum) is one of the most important vegetable crops showing high economic value and market as it is consumed almost every day. Its propagation method is mainly from bulb in Indonesia while there is a potential to propagate using True Shallot Seed (TSS). The last method is believed could provide the requirement in a large amount continually year around since the ratio of propagation is higher (1:200-300) compared to that from bulb (1:10). The constraint facing the farmers using TSS is lack availability of that planting material from local varieties that well adapted in the field. The farmers will choose shallot that characterized by its shape, color, smells and storage capability [1]. The innovation of TSS including suitable and new high yielding varieties has introduced to the field since 2013 especially in production center of the commodity and new
development areas outside of Java [2-3]. The effort is hoped to meet the national requirement of shallot planting material.

The development of TSS production in Indonesia so far is using bulbs as planting material, called bulb to seed method [4-5]. Dormant period and limitation of storage period are those that can cause unavailability of bulb at suitable stadia and suitable amount at planting season. In the tropics like Indonesia, TSS is mostly produced in the highland [6] at temperature of 17 – 18 °C and mainly in the dry season [7-8] so that it can avoid from the rain and disease for flowering and seed development process. Hence, TSS should available at early dry season or end rainy season from April to Mei. While utilization of TSS or seedling as planting material for producing TSS (seed to seed method) so far has not developed yet. The comparative advantage of using TSS is that it does not have dormant period and longer storage period (> 1 year) at cold storage [9-10]. Seed to seed TSS production in the subtropics is using seedling with 5 – 9 leaves [11] and seedling age is significantly affected to bolting [12-14].

Bolting for shallot directly determines the production of seed. The trigger for bolting process is temperature [15] that affects the transition from vegetative to generative phase. It is the critical temperature for flowering and seed formation. [16] reported that seed treatment with low temperature (vernalization) during storage process is important for flower initiation. The temperature needed by onion bulb for bolting is about 9 – 12 °C [17] and 9 – 10 °C for shallot [18]. For onion seedling, the effect of vernalization on flowering is determined by seedling age and plant size having 10 – 12 leaves [17]. However, those criteria for vernalization of shallot in Indonesia is unknown. Environmental condition after planting also affect bolting [19]. High temperature during growing period could decrease inducted inflorescence [20] as it happens for shallot flowering that planting from bulb in lowland [21]. To achieve maximum flower and seed development, TSS should be produced in the highland [6, 22].

Based on that mention above, an experiment was conducted to observe response of flowering and seed production of shallot to bulb and seedling use at different stadia that vernalized at low temperature.

2. Material and Method

The experiment was conducted at Experimental Station of Indonesian Research Institute for Vegetable, Lembang – West Java (1.250 m above sea level) from June to December 2017. Weather data shows that the average temperature at the experimental site from July to December ranges from 18.4-19.87 °C, the maximum temperature ranges from 24.87 to 25.37 °C, and the minimum temperature ranges from 14.45 to 15.55 °C (Table 1). Meanwhile, the rainfall from July to September is very low even in August there is no rain. Starting from October, it enters the rainy season with an average rainfall of 370 mm per month but the rain starts to fall on the 3rd and 4th weeks. The treatments were arranged at a randomized block design with 4 replications, consist of shallot bulb vernalized for 4 weeks at 10 °C [5, 18], vernalized TSS and 6 weeks sowing, TSS seedling at 4, 5, 6, 7 weeks vernalized for 3 weeks at 10 °C, and 6 weeks old seedling without vernalization.

**Table 1.** Temperature and rainfall data from July to December 2017 at the IVegRI experimental site in Lembang

| Temperature data | July | August | September | October | Nopember | December |
|------------------|------|--------|-----------|---------|----------|----------|
| Temperature (°C) | 19.49| 19.87  | 19.30     | 18.40   | 18.90    | 18.50    |
| Maximum          | 25.29| 24.87  | 24.94     | 25.04   | 25.37    | 25.19    |
| Minimum          | 15.29| 15.23  | 14.45     | 15.55   | 15.37    | 15.19    |
| Rain Fall (mm/month) | 0.95 | 0      | 58        | 370     | 403      | 426      |
Trisula variety was used in this experiment where the bulb (2 months after harvest) and seedling (grown in a plate with media of soil, compost and husk charcoal with 1:1:1 composition and cover with plastic) were placed in cold storage for vernalization process. The bulb and seedling were then soaked in Benzylaminopurine 37.5 ppm for 1 hour and planted in polybag with 8 kg soil. Soil in the polybag was mix with horse and chicken dung, phosphorous, dolomite, insecticide and fungicide 1 week before planting. Additional fertilizer consists of: solution of NPK fertilizer (4 kg/plant) was applied 10 times every week started 10 days after planting; KCl fertilizer (1 gram/liter) was applied 5 times every 2 weeks; and Boron (20 mg/plant) was applied at 3, 5 and 7 weeks after planted. Seedling TSS in polybag was placed in the bed cover with black plastic mulch and all seedling was cover with transparent plastic to avoid from rain.

Seed harvest was done at 120 days after planting when the capsule was brown in colour and the seed was black in colour. Observations were made to number of tillers per plant, number of flowering umbel (inflorescence) per plant, percentage of flowering plant, umbel diameter size, number of florets per umbel, number of capsules per umbel, percentage of capsules formed, number and weight of seed per umbel, weight of seed per plant. Data were analyzed with ANOVA and DMRT test at the 5% level.

3. Results and Discussion
Results indicated that treatments of seedling and bulb significantly different for number tiller per plant, number of umbels per plant and diameter umbel; but it was not significantly different to percentage of flowering plants (Table 2). The average of flowering plants both from bulb and seed/seedling was from 44 to 56.9%. However, the plant from bulb produces more tiller than that from seed as there were buds can produce tiller. At favorable environment, each of tiller with its apical buds would proceed into flower primordial [23]. The average of tiller produced of plant from bulb was 8.33 while that from seedling was from 2.68 to 3.7. Several studies have reported that the capability of each seedling to produce sprouts is limited (up to 3) [9, 24]. It can also be presumed because of decreasing genetic character of the varieties breed between shallot and onion. Data in Table 2 also indicated that the older the seedling the lower the production of tiller, and it is similar to that found by Muhammad et al. [13]. Hence, results of this experiment showed that tiller production of TSS is influenced by seedling age.

There was no significant difference on number of flowering umbels per plant between plant from bulb compared to that from 5 – 6 weeks vernalized seedling and 6 weeks without vernalized seedling, but it was significantly different compared to vernalized TSS, 4 weeks and 7 weeks vernalized seedling (Table 2). The number of tillers from bulb that produce flowering umbel was 3.5 tiller from 8.33 tiller per plant, meaning that only 40% of tiller produce flowering umbel. On the contrary, the number of tillers produces flowering umbel from 5 – 6 weeks old vernalized seedling and that from 6 weeks old seedling without vernalization were 2.15 – 2.50 per plant from 2.68 – 3.30 tiller per plant (65 – 93%). There was indication that treatments from seed showed no difference between that vernalized and without vernalization. Flowering response to seedling vernalization is affected by age and size of the plant [17]. The critical physiological age of onion was seedling with 10 – 12 leaves [17] and 6 leaves for shallot [25] while it was 3 – 4 leaves of seedling used for this research (data not presented). Too small seedling of onion could not respond to vernalization application [11] and it seems was happen in this experiment due to less physiological age of seedling.

In line with diameter of umbel (Table 2), there was also a higher number of florets per umbel of plant from seedling compared to that of bulb (Table 3). Six weeks of vernalized seedlings has the highest umbel diameter (4.35 cm) and number of florets per umbel (280.5), while that from bulb has the lowest umbel diameter and number of florets per umbel at 3.14 cm and 153.9 respectively. It shows that there is potential to develop TSS production from seedling (seed to seed). This also shows that the older the seedling the higher umbel diameter and number of florets per umbel as response of seedling to vernalization [11]. Small seedlings need longer adaptation period in the field so that it affects required time for generative organ formation. The treatments of 6 weeks vernalized seedling
and that without vernalization seems did not differ significantly to umbel diameter and number florets as it also happens to the other flowering variable, showing that seedling vernalization at 10 °C is not enough to influence shallot flowering. This is different with that found from previous research that indicated the vernalization of bulb of shallot and onion at 9 – 10 °C significantly affect flowering initiation [18-19].

**Table 2.** Effect of bulb and different seedling age vernalized at low temperature to flowering of shallot var. Trisula

| Treatments                          | Number of tillers per plant | Number of flowering umbel per plant | Percentage of flowering plant (%) | Umbel diameter size (cm) |
|-------------------------------------|-----------------------------|------------------------------------|----------------------------------|--------------------------|
| A Bulb vernalized                   | 8.3±0.61ab                  | 3.4±0.73a                          | 44.1 a                           | 3.14±0.057b              |
| B Vernalized TSS + 6 weeks sowing   | 3.5±0.14b                   | 2.0±0.65b                          | 44.1 a                           | 3.63±0.388ab             |
| C 4 weeks vernalized seedling       | 3.1±0.38b abc               | 2.1±0.33b                          | 56.9 a                           | 3.51±0.208ab             |
| D 5 weeks vernalized seedling       | 2.8±0.49a                   | 2.5±0.21ab                         | 53.1 a                           | 3.67±0.155ab             |
| E 6 weeks vernalized seedling       | 2.7±0.55c                   | 2.2±0.58ab                         | 52.0 a                           | 4.35±0.126a              |
| F 7 weeks vernalized seedling       | 3.1±0.20bc                  | 2.1±0.22b                          | 42.8 a                           | 4.26±0.147a              |
| G 6 weeks seedling without vernalization | 3.3±0.26bc                 | 2.2±0.38ab                         | 53.8 a                           | 3.97±0.209ab             |

**Table 3.** Effect of bulb and different seedling age vernalized at low temperature to flowering and capsule of shallot var. Trisula

| Treatments                          | Number of florets per umbel | Number of capsules per umbel | Percentage of capsules formed (%) |
|-------------------------------------|-----------------------------|------------------------------|----------------------------------|
| A Bulb vernalized                   | 153.9±1.45c                 | 72.8±4.10b                   | 46.87a                           |
| B Vernalized TSS + 6 weeks sowing   | 198.5±9.58abc               | 85.5±5.72b                   | 42.67a                           |
| C 4 weeks vernalized seedling       | 186.4±5.80abc               | 86.8±3.73ab                  | 46.21a                           |
| D 5 weeks vernalized seedling       | 219.9±1.25abc               | 103.9±3.26ab                 | 47.02a                           |
| E 6 weeks vernalized seedling       | 280.5±1.90a                 | 125.8±8.51a                  | 44.60a                           |
| F 7 weeks vernalized seedling       | 274.7±1.56ab                | 126.3±7.24a                  | 45.70a                           |
| G 6 weeks seedling without vernalization | 268.9±1.37b                 | 123.6±4.15a                  | 45.72a                           |

Data in Table 3 indicate that number of capsules per umbel from seed treatments (seed to seed method) was higher than that from bulb (bulb to seed method). It shows that the older the seedling the higher capsule production. It also indicates that when the plant produced high number of florets, the opportunity of seed reproduction will increase especially showed at treatments E, F and G at 6 and 7 weeks-old seedlings. Number of capsules at bulb to seed method was 72.8 which was significantly different from treatments E, F and G with average at 123.6 – 126.3 capsules per umbel (70% higher than that of bulb to seed method). Increasing number of florets and number of capsules per umbel at seed to seed method, however did not increase percentage of capsule. There was no significant difference in proportion of capsule forming between bulbs to seed method compared to all treatments of seed to seed method. Rosliani et al. [4] also found the same result on shallot var. Bima Brebes, showed that constantly develop of florets proportion to form the capsule. This seems that it is controlled by the mother plant to produce viable seed and it generally happened at annual crops like...
sweet cherry [26] or Prunus mahaleb [27]. Results of this experiment indicate that the average of capsule production at all treatments was in a range of 42.67 to 47.02%.

Data in Table 4 indicate that treatments of 6 and 7-weeks vernalized/without vernalized seedlings produce the highest seed weight per umbel (1.46-1.49 g). Those treatments also showed the highest number of seed per umbel, while the lowest was found at treatment of bulb vernalized because of low number of florets and number of capsules per umbel (Table 2 and 3). However, there was no significant difference in seed weight per plant between treatment of vernalized bulb and 6-weeks vernalized seedling. This 6-weeks vernalized seedling showed the highest seed weight per plant while the lowest was vernalized TSS with 6 weeks sowing. These results indicate that there is a potential to increase flowering and production of TSS based on seed production per plant (3.08 g) obtained from 6-weeks vernalized treatment. It is required to evaluate method of seedling vernalization including temperature and storage period as well as setting of crop moving from low temperature room to field environment to accelerate seedling adaptation.

Table 4. Effect of bulb and different seedling age vernalized at low temperature to yield of TSS var. Trisula.

| Treatments                      | Seed per Umbel | Weight of seed per plant (g) |
|--------------------------------|----------------|-------------------------------|
|                                | Weight (g)     | Number                        |                             |
| A Bulb vernalized              | 0.87±0.047d    | 219.7±12.6b                   | 2.70±0.60ab                 |
| B Vernalized TSS + 6 weeks sowing | 1.00±0.073c   | 258.5±17.4ab                  | 1.88±0.47b                  |
| C 4 weeks vernalized seedling  | 1.03±0.041c    | 261.8±11.7ab                  | 1.93±0.25ab                 |
| D 5 weeks vernalized seedling  | 1.24±0.038b    | 314.3±10.2ab                  | 2.87±0.24ab                 |
| E 6 weeks vernalized seedling  | 1.49±0.113a    | 379.5±25.3a                   | 3.08±0.86a                  |
| F 7 weeks vernalized seedling  | 1.49±0.080a    | 380.3±21.6a                   | 2.67±0.27ab                 |
| G 6 weeks seedling without vernalization | 1.46±0.034a | 372.7±11.9a                   | 2.66±0.13ab                 |

4. Conclusion
TSS plant material of shallot (seed to seed method) resulted in higher flowering and TSS production compare to that derived from bulb (bulb to seed method). Six-weeks vernalized seedling produce the highest number of florets per umbel, weight of seed per umbel and weight of seed per plant. Results of the experiment indicate that TSS from seedling (seed to seed method) showed potential for further development.

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