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Optimization of Production Botanily Seeds (TSS) Shallot (*Alliun cepa* var. *ascalonicum*) Biru Lancor Variety through improvement of hand pollination in the lowland

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Abstract. The productivity of shallots is influenced by bulb quality, disease, and low vigor characteristics, leading to many ideas for developing true shallot seed (TSS). TSS planting material advantage is the use of less seed, it can be said maintenance would be easier, minimization of seed-borne diseases, and has a fairly long shelf life compared to seed tubers. It is hoped that improved pollination through modification of Hand Artificial Pollination can replace or increase the success of flowers into fruit and be followed by the formation of true seeds or True Shallot Seed (TSS) of the biru lancor variety of red onion in the lowland. The research aims to analyze the treatment of Hand Artificial Pollination in replacing insect pollination in the production of true seed (TSS) of the lancor blue variety in the Lowlands. The research method was to prepare the bulbs of the biru lancor variety shallots given vernalization treatment by storing in the refrigerator (showcase) at temperatures of 5^{o}C, 10^{o}C, and room temperature for 4 weeks. Furthermore, the tuber seeds after undergoing vernalization are planted. Research results show vernalization with a temperature of 10^{o}C has no significant effect compared to tubers without vernalization. Vernalization with 5^{o}C temperature can increase flowering and fruit formation (number of umbels per clump, number of flowers per umbel, number of fruit per umbel), viability, and pollen count, production of TSS seeds (number of seeds per umbel, seed weight per umbel, seed weight per clump).

1. Introduction

Shallot cultivation in Indonesia is carried out in an agro-climatic and efficient manner, as well as growth optimization, is mostly cultivated in the lowlands when compared to the highlands [1]. One of the varieties of shallot (Allium cepa var. ascalonicum) is biru lancor. Based on the Decree of the Minister of Agriculture Number: 2830/KPTS/SR.120/7/2009 origin of the lancor blue onion from the Cabean Hamlet, Pabean Village, Dringu District, Probolinggo Regency, East Java Province. Probolinggo as the center of shallots in East Java has an average yield of 8.9 tons/ha, while the potential yield can reach more than 15 tons/ha. Prolonged use of tubers as seeds at the farm level can lead to asexual mutations [2]. The government needs to organize the distribution of production centers, distribution of crop yields between regions, and monitor and evaluate shallot price policies. These policies aim to ensure the adequacy and smooth distribution of shallots [3].

The low productivity of shallots is influenced by the low quality of tubers available at the farm level, the presence of diseases and low vigor characteristics, which have led to many ideas for developing True Shallot Seed (TSS). The advantages of TSS planting material are the use of less seeds, it can be said that

it will be easier to maintain, minimize congenital diseases from seeds, and have a fairly long shelf life compared to seed tubers [4].

The vernalization method of shallot bulbs at a temperature of 4oC for 2 months can increase flowering, umbel formation and seeds in several varieties tested, although all the seeds produced have not been able to germinate. Based on the results of the research that has been done, this research will try to develop a method for flowering by giving the effect of vernalization temperatures of 5oC and 10oC for 4 weeks and combined with growth regulators (ZPT) using benzyl amino purines (BAP) and BAP which has been proven can give a positive response to induce flowering in the Bima and Trisula varieties of shallots, given that endogenous hormone conditions often still need to be improved again to encourage flowering through the application of PGR, to be able to further encourage the flowering process on shallots, especially the Biru lancor variety which has been proven adapted well in the lowland area as one of the shallot centers in East Java. Based on this, this research was conducted to study the effect of vernalization and the application of ZPT benzyl amino purine and BAP on the flowering of the Biru lancor variety of shallots.

2. Methods

Research on the vernalization of tuber seedlings was conducted at the Jember State Polytechnic Plant Laboratory. The results obtained will then be planted in the Lowlands, Leces District, Probolinggo Regency at an altitude of 89 meters above sea level from June 2021 to October 2021. Preparation The bulbs of the biru lancor variety of shallot seeds were subjected to vernalization treatment by storing in a refrigerator (showcase) at temperatures of 5ºC, 10ºC, and room temperature for 4 weeks. Furthermore, the tuber seeds after undergoing vernalization are planted. Planting was carried out in 2 months in polybags with a diameter of 22.5 x 40 cm.

The planting media used were topsoil, compost, and manure in a ratio (1:1:1) and placed under a transparent plastic house to avoid direct exposure to rainwater, while drip irrigation was used for irrigation, considering the need for water is very vital. Fertilization using NPK fertilizer (16:16:16) at a dose of 600 kg/ha and manure at a dose of 10 tons/ha and dolomite at a dose of 1.0 tons/ha. Meanwhile, to help pollinate, assisted by insect pollinators *Apis mellifera* and hand pollination. Insect pest control is carried out using insecticides with the active ingredient abamectin following the recommendations.

Parameters Observations were carried out by observing the growth of shallot plants and the number of flowers, namely: a) Average Plant Height in Vernalization Treatment, b) Number of BAP Leaves, c) Number of BAP tillers, d) Flowering Time of Vernalization and BAP Interaction, e) Flowering Percentage of Plants, f) Number of Flowers per Umbel, g) Flowering Time on Vernalization Interaction and BAP Seed Weight Per Umbel, h) Seed Weight Per Plant on Vernalization Treatment and BAP Interaction.

3. Result and Discussion

The results showed that the vernalization and benzyl amino purine treatments affected plant height, number of leaves, number of tillers, and number of flowers of the biru lancor variety of shallots in the lowlands. Vernalization technology allows for the acceleration of the sprouting process which is much faster than without vernalization so that through this technology it is hoped that it will be able to provide sustainable onion seeds [5]. Benzyl amino purine or (BAP) triggers flowering, as well as cell regeneration. Benzyl amino purine or (BAP) is a cytokinin group of plant growth hormones that naturally promotes cell division, cell division, tissue differentiation, dormancy, flowering phase, and fertilization [6].

The table above shows that the vernalization treatment did not have a significant effect on the height of shallot plants. There was no difference in plant height because plant growth was determined by genetic factors rather than vernalization treatment. This is by the opinion of experts that vernalization, in general, is more directed to generative growth than growth [7]. Benzyl amino purine treatment effects 28 days after planting. Benzyl Amino Purines are included in the cytokinin group which plays a role in

shoot growth. Benzyl Amino Purine is a type of cytokinin that is resistant to degradation [8]. The relationship between vernalization and BAP has a significant effect at 42 days after planting.

Table 1. Average Plant Height on Vernalization Treatment

Vernalization Treatment	Plant Height					
	7 DAP	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP
V_o	9,81a	20,16a	26,53a	32,15a	33,16a	31,16a
V_1	7,63b	17,14b	23,25a	29,93b	33,12a	32,23a

Note: The numbers followed by the same letter in the same column show no significant difference in the 5% BNT test. V0 = Non-Vernalization, V1 = Vernalization.

Table 2. The average number of BAP leaves

Vernalization Treatment	Number of Leaves					
	7 DAP	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP
B_o	11a	20b	26b	32b	35b	49c
B_1	13a	24a	31a	37ab	43a	69ab
B_2	12a	20b	30a	39a	33b	75a
B_3	11a	20b	25b	32b	38ab	61b
BNT	5%	5%	5%	5%	5%	5%

Note: The numbers followed by the same letter in the same column show no significant difference in the 5% BNT test. B0 = 0 ppm; B1 = 50 ppm; B2 = 100 ppm; and B3 = 150 ppm= 0 ppm; B1 = 50 ppm; B2 = 100 ppm; dan B3 = 150 ppm

Table 3. The average number of BAP tillers

Vernalization Treatment	Number of Tubers					
	7 DAP	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP
B_o	0a	0a	0a	24b	25b	28a
B_1	0a	0a	0a	26a	27a	31a
B_2	0a	0a	0a	25ab	27a	31a
B_3	0a	0a	0a	20c	20c	23b
BNT	5%	5%	5%	5%	5%	5%

Note: The numbers followed by the same letter in the same column show no significant difference in the 5% BNT test. B0 = 0 ppm; B1 = 50 ppm; B2 = 100 ppm; and B3 = 150 ppm= 0 ppm; B1 = 50 ppm; B2 = 100 ppm; dan B3 = 150 ppm

Vernalization and BAP treatments had no significant effect on the number of leaves parameters, while vernalization and BAP treatments affected 42 days after planting. The vernalization treatment on day 42 can have a very significant effect because the vernalization treatment can stimulate plant growth, especially plant elongation growth through an acceleration of dormancy so that plants can grow faster normally. This growth acceleration was due to an increase in cell division activity and endogenous gibberellins and auxin content. The number of leaves will greatly determine the surface area to receive

sunlight for the photosynthesis process. In this process the carbohydrates produced will be distributed throughout the plant organs for growth [9][10].

The vernalization treatment with BAP showed no significant effect on the number of shallot tillers. A significant effect occurred on the interaction of vernalization treatment with BAP at 42 days after planting. The number of shallot tillers correlated with the size of the bulbs caused by cell enlargement which was more dominant than cell division. The increase in the wet weight of the tubers was influenced by the amount of water absorption and the accumulation of photosynthetic products in the leaves to be translocated for tuber formation. So, the difference in water content will affect the wet weight of the tubers produced [11].

Table 4. Average Time of Interest in Vernalization and BAP Interaction

Vernalization Treatment x BAP	Flower time
	63 DAP
V_0B_0	0c
V_0B_1	0c
V_0B_2	0c
V_0B_3	0c
V_1B_0	29a
V_1B_1	25b
V_1B_2	22b
V_1B_3	24b
DMRT	5%

Note: The numbers followed by the same letter in the same column show that they are not significantly different in the multiple-distance test (DMRT) 5%. V0B0 = Non Vernalization + 0 ppm BAP; V0B1 = Non Vernalization + 50 ppm BAP; V0B2 = Non Vernalization + 100 ppm BAP; V0B3 = Non Vernalization + 150 ppm BAP; V1B0 = Vernalization + 0 ppm BAP; V1B1 = Vernalization + 50 ppm BAP; V1B2 = Vernalization + 100 ppm BAP; V1B3 = Vernalization + 150 ppm BAP

Based on the results of the F test (Table 4), it can be seen that the interaction between the vernalization factor and the BAP factor has a very significant different effect on the parameter of the percentage of flowering plants. While the single factor vernalization gave no significant effect on the parameters of the percentage of flowering plants, as well as the BAP gave an insignificant different effect on the parameters of the percentage of flowering plants.

The flowering process of plants occurs through four stages, namely induction, flower initiation, flower differentiation, maturation of flower parts, and anthesis, initiation of flowering is this stage where morphological changes occur to form generative buds and transition from vegetative shoots to generative buds that can be detected from changes in shape. as well as bud size, as well as subsequent processes that begin to form generative organs. Flowering response and yield of shallot seeds increased with a combination of vernalization treatment (10^{o}C) for 4 weeks on seed tubers. BAP was found to stimulate flowering and increase production [12]. The increase in production is due to the presence of cytokinin in the plant which stimulates an increase in the size of the reproductive meristem so that it can produce more flowers [13].

Amount of Interest Per Umbel Based on the results of the F test (Table 4), the interaction between the vernalization factor and the BAP factor has a very significant effect on the parameter of the amount

of interest per umbel. While the vernalization factor gave an insignificant different effect on the parameter of the amount of interest per umbel, as well as the BAP gave an insignificant different effect on the parameter of the amount of interest per umbel.

Table 5. Average Parameter of Flowering Plant Percentage

Treatment	Percentage of flowering plants
	63 DAP
V_0B_0	0c
V_0B_1	0c
V_0B_2	0c
V_0B_3	0c
V_1B_0	58ab
V_1B_1	71a
V_1B_2	67ab
V_1B_3	50b
DMRT	5%

Note: The numbers followed by the same letter in the same column show that they are not significantly different in the multiple-distance test (DMRT) 5%. V0B0 = Non Vernalization + 0 ppm BAP; V0B1 = Non Vernalization + 50 ppm BAP; V0B2 = Non Vernalization + 100 ppm BAP; V0B3 = Non Vernalization + 150 ppm BAP; V1B0 = Vernalization + 0 ppm BAP; V1B1 = Vernalization + 50 ppm BAP; V1B2 = Vernalization + 100 ppm BAP; V1B3 = Vernalization + 150 ppm BAP

Non-vernalization treatment resulted in a longer flower emergence time and BAP did not stimulate more tillers to produce umbel shoots. BAP is more involved in increasing the size of differentiated meristems producing the number of flowers per umbel rather than stimulating stumps of tillers. BAP application has no effect on flower emergence time because 0 and 50 ppm flowers appear first because low concentrations of BAP accelerate flower growth, the flowering period is not affected by BAP application, possibly because vernalized plant conditions can reduce the effect of BAP to the acceleration of flowering plants.

Cytokinins have an important role in meristem morphogenesis, thus allowing the development of larger flower meristems. However, the application of BAP at various concentrations did not affect the increase in the number of umbels per clump. This means that BAP does not stimulate more tillers to produce umbel shoots. It is possible that BAP plays a more important role in increasing the size of the differentiated meristems resulting in the number of flowers per umbel rather than stimulating swarming tillers. This happens because PGR is known to promote plant growth and development at low concentrations but at high concentrations, it can inhibit plant growth and development. This study showed that 50 ppm BAP was the optimum concentration to promote shallot flowering [14].

Number of Capsules Per Umbel Based on the results of the F test (Table 4) shows that the interaction between the vernalization factor and the BAP factor has a very significant different effect on the parameter of the number of capsules per umbel. While the single factor of vernalization gave an insignificant different effect on the parameter of the number of capsules per umbel, as well as the single BAP gave an insignificant different effect on the parameter of the number of capsules per umbel.

Table 6. The average parameter of the amount of interest per umbel

Vernalization Treatment x BAP	Percentage of flowering plants
	63 DAP
V_0B_0	0c
V_0B_1	1c
V_0B_2	2c
V_0B_3	3c
V_1B_0	107ab
V_1B_1	96ab
V_1B_2	90b
V_1B_3	112a
DMRT	5%

Note: The numbers followed by the same letter in the same column show that they are not significantly different in the multiple-distance test (DMRT) 5%. V0B0 = Non Vernalization + 0 ppm BAP; V0B1 = Non Vernalization + 50 ppm BAP; V0B2 = Non Vernalization + 100 ppm BAP; V0B3 = Non Vernalization + 150 ppm BAP; V1B0 = Vernalization + 0 ppm BAP; V1B1 = Vernalization + 50 ppm BAP; V1B2 = Vernalization + 100 ppm BAP; V1B3 = Vernalization + 150 ppm BAP

Table 7. Average Flowering Time on Vernalization and BAP interaction

Vernalization Treatment x BAP	flower time
	63 DAP
V_0B_0	0c
V_0B_1	0c
V_0B_2	0c
V_0B_3	0c
V_1B_0	31a
V_1B_1	23b
V_1B_2	22b
V_1B_3	29a
DMRT	5%

Note: The numbers followed by the same letter in the same column show that they are not significantly different in the multiple-distance test (DMRT) 5%. V0B0 = Non Vernalization + 0 ppm BAP; V0B1 = Non Vernalization + 50 ppm BAP; V0B2 = Non Vernalization + 100 ppm BAP; V0B3 = Non Vernalization + 150 ppm BAP; V1B0 = Vernalization + 0 ppm BAP; V1B1 = Vernalization + 50 ppm BAP; V1B2 = Vernalization + 100 ppm BAP; V1B3 = Vernalization + 150 ppm BAP

Table 8. Average Seed Weight Per Umbel and Seed Weight Per Plant in V x B Interaction Treatment

Vernalization Treatment x BAP	Botanical Seed Production (TSS)	
	Seed Weight Per Umbel (g)	Seed Weight Per Plant (g)
V_0B_0	0 b	0 b
V_0B_1	0,17 ab	0,17 b
V_0B_2	0,18 ab	0,18 b
V_0B_3	0 b	0 b
V_1B_0	0,39 ab	0,39 ab
V_1B_1	0,64 a	0,73 a
V_1B_2	0,42 ab	0,42 ab
V_1B_3	0,43 ab	0,52 ab

Note: The numbers followed by the same letter in the same column show that they are not significantly different in the multiple-distance test (DMRT) 5%. V0B0 = Non Vernalization + 0 ppm BAP; V0B1 = Non Vernalization + 50 ppm BAP; V0B2 = Non Vernalization + 100 ppm BAP; V0B3 = Non Vernalization + 150 ppm BAP; V1B0 = Vernalization + 0 ppm BAP; V1B1 = Vernalization + 50 ppm BAP; V1B2 = Vernalization + 100 ppm BAP; V1B3 = Vernalization + 150 ppm BAP

The results of the 5% DMRT test analysis showed that there was an interaction between vernalization treatment and BAP which significantly affected the parameters of seed weight per umbel and seed weight per plant. The interaction treatment between vernalization and BAP (V1B1), increased seed weight per umbel and seed weight per plant which was heavier than the control treatment. The vernalization treatment with BAP could produce an average seed weight per umbel of 0.64 grams and an average seed weight of 0.73 grams per plant, but the weight of 100 grains was low, there was no significant interaction between the vernalization treatment and the BAP treatment. single treatment also did not affect the weight parameter of 100 grains.

The comparison of the weight of shallot seeds was more clearly seen in the V1B1 treatment which gave the effect of increasing the weight of shallot seeds on the parameters of seed weight per umbel and seed weight per plant by producing a difference of 0.09 grams. The interaction treatment between vernalization and BAP at a concentration of 50 ppm could induce maximum shallot flowering, especially in the Biru Lancor variety because it produced the highest seed weight compared to the control and other treatments. The best concentration for yields giving the highest value for seed weight per clump was 200 ppm BAP. So that in the application of BAP the concentration is lower in the seed weight of the Biru Lancor variety of shallots.

4. Conclusion

The interaction between vernalization and Benzyl amino purine (BAP) gave a significantly different effect on plant height parameters and flower parameters in shallots. single factor vernalization and BAP gave no significant effect on all parameters.

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