Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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OPEN ACCESS Asian Journal of Plant Sciences
ISSN 1682-3974
DOI: 10.3923/ajps.2024.238.243

Research Article
Morphophysiological Characteristics of Shallot on Application of Boron and Benzyl Amino Purin

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Abstract
Background and Objective: Boron is an important micro nutrient that functions for the growth and development of shallot plants. Benzyl amino purine (BAP) is a growth regulator which is classified as a synthetic cytokinin which is most active in influencing plant physiological processes. This research aims to identify the role of boron and BAP on the morphophysiological characteristics of shallots. **Materials and Methods:** The study was conducted at Screen House, Faculty of Agriculture, Universitas Sumatera Utara, from September to December, 2023. This research was conducted using a factorial randomized block design with 2 factors and 3 replications. The first factor was boron treatment at 0, 2, 4 and 6 kg/ha. The second was the application of benzyl amino purine at 0, 100 and 200 ppm. The observed variables include plant length, number of leaves, chlorophyll a, chlorophyll b and total of chlorophyll. **Results:** Boron treatment 6 kg/ha tends to increase number of leaves 6 WAP, while application of benzyl amino purin 200 ppm tends to increase plant length 2-4 WAP and number of leaves 3-4 WAP. There was a tendency that the application of 0-200 ppm BAP reduced the chlorophyll a, chlorophyll b and total chlorophyll content of leaves. The interaction between 6 kg/ha boric acid treatment and 200 ppm BAP increased plant height 2-4 WAP and number of leaves 2-4 WAP. **Conclusion:** The application of 0-200 ppm BAP reduced the chlorophyll a, chlorophyll b and total chlorophyll content of leaves.

Key words: Chlorophyll, growth, shallot, boron, benzyl amino purin

Citation: Hasanah, Y., D.S. Hanafiah, Nursa'adah, H. Hanum and A.M. Kusriarmin, 2024. Morphophysiological characteristics of shallot on application of boron and benzyl amino purin. Asian J. Plant Sci., 23: 238-243.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Shallots are a leading horticultural commodity and spice which is ranked second after chili plants which are always needed by the Indonesian people. Several studies state that shallots are classified as a strategic commodity which is one of the biggest contributors to inflation domestically, apart from rice, red chilies, chicken and beef[1-4]. Shallots act as a food flavoring, raw material for the food industry and biopharmaceuticals because they contain bioactive compounds such as saponins, flavonoids, essential oils, allicin, quercetin and alliin[5-9].

In general, shallot propagation materials use bulbs as seeds. The use of botanical seeds (true shallots seed = TSS) as seeds for propagating shallot plants is an alternative that needs to be developed, because it has the advantage of increasing plant productivity by up to 100% when compared to using bulbs, the need for fewer seeds (±3-6 kg/ha), while tubers require (±1-1.2 t/ha), more practical, TSS seeds can be stored for up to 1-2 years, compared to tubers (only 4 months), transportation costs are cheaper, stronger and healthier free of *Fusarium* sp., *Colletotrichum* sp., *Alternaria* sp. and viruses from the original plants that were attacked and larger tubers[10-13]. Shallots are cultivated in the highlands and lowlands. The main obstacles in the cultivation and production of TSS seeds in the lowlands are differences in environmental conditions (edaphic and climatic), the availability of superior varieties according to environmental conditions as well as inappropriate cultivation and post-harvest methods, low flower and seed set.

Temperature conditions in the lowlands (>25°C) can inhibit the initiation of shallot flowering. However, there are indications that capsule and seed formation is more suitable in the lowlands compared to the highlands. The research results of Putra *et al.*[14] reported that flowering rates and seed production in the highlands are greater than in the lowlands, conversely the quality of the seeds produced in the lowlands is better than in the highlands. Therefore, the highlands have great potential for developing shallot seed production.

The efforts to overcome this problem are the application of benzyl amino purine (BAP) and boron which can increase flowering, pollen viability and production and the quality of TSS seeds in the lowlands, because BAP which is classified as a cytokinin PGR is able to increase the process of cell division, increase the formation of chlorophyll and protein synthesis and stimulate flowering[15]. Apart from that, the micro nutrient boron can also be applied which plays a role in flower formation[16,17].

Therefore, to increase the production and quality of shallot seeds, it is necessary to apply boron and BAP. The objective of this research was to identify the role of boron and BAP on the morphological characteristics of shallot under the lowlands condition.

MATERIALS AND METHODS

Time and location: This research was carried out at the USU Faculty of Agriculture's Screen House on October-December 2023. Initial soil analysis and boron uptake were carried out at the Integrated Laboratory, Faculty of Agriculture, USU Medan. Analysis of chlorophyll content was carried out at the Plant Physiology Laboratory, Faculty of Mathematics and Natural Science, Universitas Sumatera Utara. Analysis of quercetin content was carried out at the Research Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, Indonesia.

Materials and tools: The materials used are Bima Brebes variety shallots, manure, top soil, polybags, paper bags, bricks, boron, benzyl amino purine (BAP), insecticide and *Trichoderma harzianum*. The tools used are hoes, scales, tape measures, research nameplates, flush buckets, stakes, spectrophotometer Multiskan GO USA. Microscope Carl Zeiss Prime Star Built with AxioCam ERc5S, software ZEN lite 2012 USA, analytical balances Precisa type HGS (from Switzerland), ovens and several tools used for laboratory analysis.

Research design: The focus of research activities was evaluating the production and quality of shallot seeds through the application of boron and BAP in the lowlands. The research used a factorial randomized block design conducted in the lowlands. The first factor is boron with 4 levels consisting of 0, 2, 4 and 6 kg/ha, while the second factor is BAP with concentrations of 0, 100 and 200 ppm. There were 12 treatment combinations with 3 replications, so there were 36 experimental units. Each experimental unit consists of 5 plants, so 180 plants are needed. Planting material comes from seed tubers (5-7 g/tuber) of the Bima Brebes variety. The BAP treatment was watered three times, namely at the ages of 1, 3 and 5 weeks after planting (WAP) as much as 100 mL per polybag, while boron fertilization was carried out three times at the ages of 3, 5 and 7 WAP in the same way as the BAP application.

Before the shallot bulbs are planted, a vernalization process is carried out first for 2 weeks by storing the shallot bulbs in the refrigerator at a temperature of 2-8°C for 2 weeks. This slowed down the start of research. Shallot bulbs are planted in polybags (8 kg of top soil+manure). The NPK fertilizer dose used was 600 kg/ha, given according to the results of previous research Hasanah *et al.*[18]. To prevent and control stem rot disease caused by the fungus *Fusarium* *oxysporum*, the biological agent *Trichoderma harzianum* is used by sprinkling it on the plants. Each plant was watered with 250 mL of solution containing *Trichoderma harzianum*.

Research observations were made on growth components (plant length, number of leaves). The physiological analysis carried out included analysis of chlorophyll a, chlorophyll b and total chlorophyll.

Chlorophyll content: The chlorophyll analysis was carried out based on a method by Henry and Grime[19]. The chlorophyll content was determined by collecting leaves samples (0.1 g), then macerated with 10 mL of acetone using a mortar. The formula for determining chlorophyll content uses the formula as follows:

$$\text{Chlorophyll a} = \frac{(12.7 \times \text{A663}) - (2.69 \times \text{A645})}{10}$$

$$\text{Chlorophyll b} = \frac{(22.9 \times \text{A645}) - (4.68 \times \text{A663})}{10}$$

Content of chlorophyll: Determination of chlorophyll levels using the method described by Hendry and Grime[19]. The method used is to take 0.1 g of leaf sample which is macerated using 10 mL acetone. Chlorophyll levels are determined by the formula:

$$\text{Chlorophyll a} = \frac{(12.7 \times \text{A663}) - (2.69 \times \text{A645})}{10}$$

$$\text{Chlorophyll b} = \frac{(22.9 \times \text{A645}) - (4.68 \times \text{A663})}{10}$$

Statistical analysis: The data was subjected to two way Analysis of Variance (ANOVA) procedures, the SAS version 12 computer program and comparison of means were tested for significance using Duncan's Multiple Range Test (DMRT) $p = 0.05$.

RESULTS AND DISCUSSION

Plant length: Based on Table 1, it can be seen that the boron treatment, BAP and the interaction between the two had no significant effect on plant height at 2-4 WAP. Boric acid treatment of 2 kg/ha tends to increase plant height by 2 WAP compared to other treatments. This is thought to be because boron has only been applied at 1 WAP so it has not had an effect on plant growth, because it takes time for the decolonization process of boron fertilizer to become cations that can be absorbed by plant roots.

There was a tendency to increase plant height from 2-4 WAP with increasing BAP application. This is thought to be because BAP is a growth regulator which most active in influencing plant physiological processes such as cell division and enlargement[20]. The interaction between 6 kg/ha boric acid treatment and 200 ppm BAP increased plant height 2-4 WAP.

Number of leaves: Based on Table 2, it can be seen that the boron treatment, BAP and the interaction between the two had no significant effect on the number of leaves 2-4 WAP. The 2 kg/ha boric acid treatment tended to increase the number of leaves at 2 WAP compared to other treatments. This was thought to be because boron has only been applied at 1 WAP so it has not had an effect on plant growth, because it takes time for the decolonization process of boron fertilizer to become cations that can be absorbed by plant roots.

There was a tendency to increase the number of leaves at 3 WAP with increasing BAP administration. This was thought to be because BAP is a growth regulator which is classified as a synthetic cytokinin with a molecular weight of 225.26 and the molecular formula $C_{12}H_{11}N_5$. The BAP is an adenine derivative substituted at position 6 which is most active in influencing plant physiological processes such as cell division and enlargement, shoot differentiation and modification of apical dominance[20]. The interaction between 6 kg/ha boric acid treatment and 200 ppm BAP increased plant height 2-4 WAP.

Actually, boron is an essential mineral element that regulates several important physiological processes including cell division and elongation, carbohydrate metabolism, assimilate translocation and cell division via RNA, so that it can form cell walls more quickly which leads to better growth processes[21]. However, in this study the role of boron in plant vegetative growth as indicated by an increase in the number of leaves was not seen significantly.

Chlorophyll a, chlorophyll b and total of chlorophyll: Based on Table 3, it can be seen that boron treatment, BAP and the interaction between the two have no significant effect on chlorophyll a, chlorophyll b and total chlorophyll. Chlorophyll is a pigment that plays an important role in photosynthetic activity and plant growth. Leaf chlorophyll levels can be used as an indicator of plant health[22].

The 4 kg/ha boric acid treatment tended to increase chlorophyll a, chlorophyll b and total chlorophyll compared to other treatments. This shows the role of boron in the

Table 1: Plant length 2-4 WAP of shallot on application of boron and BAP

		BAP (ppm)			
		A_0 (0)	A_1 (100)	A_2 (200)	
WAP	Boron (kg/ha)	(cm)			Mean
2	B_0 (0)	26.49	24.83	25.67	25.66
	B_1 (2)	25.78	26.20	25.93	25.97
	B_2 (4)	23.48	24.59	25.87	24.65
	B_3 (6)	24.99	25.15	26.51	25.55
	Mean	25.19	25.19	25.99	
3	B_0 (0)	29.79	27.58	28.29	28.55
	B_1 (2)	28.08	28.88	28.38	28.45
	B_2 (4)	26.55	27.25	28.25	27.35
	B_3 (6)	27.58	27.95	29.16	28.23
	Mean	28.00	27.91	28.52	
4	B0 (0)	32.38	30.53	31.14	31.35
	B1 (2)	30.48	31.00	31.36	30.95
	B2 (4)	27.42	28.99	31.02	29.14
	B3 (6)	28.68	29.93	32.40	30.34
	Mean	29.74	30.11	31.48	

Table 2: Number of leaves 2-4 WAP of shallot on application of boron and BAP

		BAP (ppm)			
		A_0 (0)	A_1 (100)	A_2 (200)	
WAP	Boron (kg/ha)	(cm)			Mean
2	B_0 (0)	11.92	10.75	10.83	11.17
	B_1 (2)	11.58	11.08	11.08	11.25
	B_2 (4)	10.75	9.83	10.25	10.28
	B_3 (6)	10.58	11.17	11.83	11.19
	Mean	11.21	10.71	11.00	
3	B_0 (0)	13.83	13.08	13.17	13.36
	B_1 (2)	13.50	13.17	13.17	13.28
	B_2 (4)	12.50	11.92	12.50	12.31
	B_3 (6)	12.50	13.42	14.00	13.31
	Mean	13.08	12.90	13.21	
4	B0 (0)	14.83	15.08	15.12	14.87
	B1 (2)	15.00	14.83	15.00	14.94
	B2 (4)	14.75	14.42	13.67	14.28
	B3 (6)	14.92	15.08	15.67	15.22
	Mean	14.88	14.85	15.01	

Table 3: Chlorophyll a, chlorophyll b and total of chlorophyll of shallot on application of boron and BAP

		BAP (ppm)			
		A_0 (0)	A_1 (100)	A_2 (200)	
Chlorophyll	Boron (kg/ha)	Fresh weight (mg/g)			Mean
a	B_0 (0)	1.63	1.62	1.68	1.65
	B_1 (2)	1.78	1.48	1.33	1.53
	B_2 (4)	1.79	1.58	1.72	1.70
	B_3 (6)	1.49	1.75	1.68	1.64
	Mean	1.67	1.61	1.60	
b	B_0 (0)	2.75	2.84	2.75	2.78
	B_1 (2)	2.85	2.70	2.47	2.67
	B_2 (4)	2.93	2.79	2.84	2.85
	B_3 (6)	2.59	2.76	2.54	2.63
	Mean	2.78	2.77	2.65	
Total	B_0 (0)	4.38	4.46	4.43	4.42
	B_1 (2)	4.63	4.18	3.79	4.20
	B_2 (4)	4.72	4.37	4.56	4.55
	B_3 (6)	4.07	4.51	4.21	4.26
	Mean	4.45	4.38	4.25	

formation of chlorophyll a. Hegazi *et al.*[23] and Seth and Aery[24] stated that the addition of boron can also increase the chlorophyll content so that it will affect photosynthesis and produce optimum assimilate.

There was a tendency that the application of 0-200 ppm BAP reduced the chlorophyll a, chlorophyll b and total chlorophyll content of leaves. This is not in line with the previous research that BAP application significantly increased the expression of all genes coding for chlorophyll biosynthesis[25]. According to Lotfi *et al.*[26] blue light is relevant for chlorophyll biosynthesis, chloroplast development and stomata opening. Similar results were obtained by other authors with different plant species[27,28].

CONCLUSION

Based on the research, it can be concluded that, there was a tendency that the application of 0–200 ppm BAP reduced the chlorophyll a, chlorophyll b and total chlorophyll content of leaves. The interaction between 6 kg/ha boric acid treatment and 200 ppm BAP increased plant height 2-4 WAP and number of leaves 2-4 WAP. The application of 0-200 ppm BAP reduced the chlorophyll a, chlorophyll b and total chlorophyll content of leaves. Hence, the vegetative phase did not show a significant role of boron and BAP for plant growth.

SIGNIFICANCE STATEMENT

Plants need boron and benzyl amino purin for better growth and to stimulate flower formation in the generative phase, therefore the objective of this research was to identify the role of boron and BAP on the morphological characteristics of shallot under the lowlands condition. The vegetative phase the role of boron until 6 kg/ha tends to increase the number of leaves 6 WAP, while the application of benzyl amino purin 0-200 ppm tends to increase the number of leaves, plant length and decrease the content of chlorophyll. This research can help researchers and farmers regarding the best time to apply boron and BAP so that it has a significant impact on crop production.

ACKNOWLEDGMENT

The authors are grateful to the Universitas Sumatera Utara (USU) Research Institute for funding this research through TALENTA-Applied Research in 2023 with contract No: 13388/UN5.1.R/PPM/2023, 28 August, 2023.

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