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# Research Article
# Production and Physiological Characteristics Evaluation of Shallot (*Allium ascalonicum* L.) Lokananta Varieties via Sulphur and Paclobutrazol Application

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## Abstract

**Background and Objective:** Sulphur is very important to increase assimilate translocation to enlarge bulbs, forming essential amino acids. The presence of growth inhibitors is necessary to focus energy on the formation of shallot bulb. The aim of this research was to evaluate the role of sulphur and Paclobutrazol on the production and physiological characteristics of shallot from true shallot seed. **Materials and Methods:** The study was conducted in Medan, Sumatera Utara, from June-October, 2020. The experimental design used was a factorial randomized block design. The first factor was sulphur application (0, 75 and 150 kg ZA ha$^{-1}$) and the second factor was concentration of Paclobutrazol (0, 15 and 30 ppm) to observe the true shallot seeds variety. **Results:** The result of the research indicated that the highest of chlorophyll a, chlorophyll b, total chlorophyll, wet weight and dry weight of shallot bulbs were found in the application of sulphur 150 kg ZA ha$^{-1}$ and without application of Paclobutrazol. **Conclusion:** The highest of total chlorophyll, bulb dry weight wet weight of shallot bulbs, chlorophyll total, shallot bulb wet weight and shallot bulbs dry weight were found in the sulphur treatment 150 kg ZA. ha$^{-1}$ and without Paclobutrazol. The highest of chlorophyll total was found in Paclobutrazol 15-30 ppm application.

Key words: Chlorophyll, shallot, stomatal density, production, physiological characteristic

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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

Shallot (*Allium ascalonicum* L.) is a commodity horticultural plants that are widely used by the community as a mixture cooking spices after chili. Apart from being a mixture of cooking spices, shallot are also sold in processed forms such as powdered shallot, shallot extract, essential oils and fried shallots, even as medicinal ingredients to reduce hypercholesterolemia, anti diabetic, hypertension, arthritis, indigestion, allergies, asthma, osteoporosis neurodegenerative disorders, cataracts and leukimia[1-4]. The potential for development of shallot has very good prospects because shallot is a horticultural commodity that is widely consumed by the public, so that the potential for development is still wide open for both domestic and foreign needs. The main problem in increasing the productivity of shallot is that there is no guarantee of bulbs as quality planting materials throughout the growing season and the presence of bulb-borne pathogens can reduce the quality of yields, due to diseases caused by *Fusarium* sp., *Colletotrichum* sp. and *Alternaria* sp., as well as viruses that result in decreased plant productivity[5]. Therefore, it is necessary technology breakthrough by producing shallot seeds as planting materials, called True Shallot Seed (TSS)[6].

The main constraints in the cultivation of shallots from TSS are difficulties in senescence and long harvest life. The absence of aging in the shoots indicates that there is still assimilation accumulated in the leaves and translocated into the bulbs. A balance of stimulating and inhibiting hormones in plants produces bulbs. Therefore, the presence of retardants is needed to focus energy on the formation of bulbs. The use of Paclobutrazol as a retardant can increase the translocation of assimilates from leaves to tubers to increase tuber production[7]. Shallot also needs Sulphur (S) to increase the production and quality of shallot, because the role of S to increase assimilates translocation to enlarge bulbs, form the amino essential acids (thiamin, methionine and cysteine) and protein formation[8].

The previous studies have reported the response of shallot growth and production based on ammonium nitrate ratio on costal sandy soil[9], shallot residue compost and organic fertilizer[10] and green manure amendment for the management of Fusarium basal rot (*Fusarium oxysporum* f.sp. *cepae*) on shallot[11]. Until now, there are very limited studies examining the role of sulfur and Paclobutrazol on the agronomic and physiological facts of the shallot plant from TSS. According to the background, this study aimed to evaluate the role of Paclobutrazol and sulphur on the production and physiological characteristics of Lokananta variety of shallot from TSS.

## MATERIALS AND METHODS

**Time and location:** This research was conducted at Medan, Sumatera Utara. The soil used as planting media was analyzed in the Docfind Laboratory, Medan and the chlorophyll content and also stomatal density were analyzed at the Socfind Laboratory Medan of Plant Physiology and Tissue Culture, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara in June-October, 2020. The soil analysis result showed that a pH 5.0, C-organic 1.90%, N-total 0.2%, K-exchange (0.28 me 100 $g^{-1}$).

**Materials:** The materials of this research were TSS, NPK fertilizer, KCl, ZA, Paclobutrazol, clear nail polish, acetone, fungicides and insecticides. The research used tools such as sprayer, polybags, scale, bucket, cuvette, microscope, camera and microscope.

**Research design:** The experimental design used a factorial randomized block design with 2 factors and 3 replications. As the first factor is sulphur application (0, 75 and 150 kg of ZA $ha^{-1}$). The second factors are Paclobutrazol application (0, 15 and 30 ppm).

**Procedures:** The research activity was started by making a nursery bed (100-120 cm). The sowing of the husks on the beds was carried out to a thickness of 15-20 cm, after which they are burned at a fire point of 1 m until they burn for 1 night. After sitting for 1 night, do enough watering and tilling the soil. Sowing seeds (0.5 g per plot), but first given fungicide treatment. The planted seeds were covered with fine soil/compost/husk. Seeds begin to grow 5-7 Days After Planting (DAS), NPK fertilizer (16:16:16) is given at a dose of 0.5 g $L^{-1}$ after 21 DAS by watering. A plastic shade was installed to prevent direct rain exposure.

Seedlings were ready for transplanting after 35-42 DAS. Seeds were planted as much as 1 seedling/planting-hole with a spacing of $10\times15$ cm. The seedlings should be sturdy, fresh green in color and have 4-6 leaves. Watering is done in the morning and evening. The sulphur application was carried out at 7 days after transplanting. The Paclobutrazol application was carried out at 20 and 35 days after transplanting according to the treatment. Fertilization was carried out 4 times, namely at 7 Days After Transplanting (DAT) in the form of organic fertilizer (mixed in the beds), 14, 28 and 42 DAT in the form of NPK (16:16:16) 750 g 200 $L^{-1}$ of water; 1 kg 200 $L^{-1}$ of water and KCl 750 g 200 $L^{-1}$ of water (by watering it), respectively.

Weed control was carried out at 14-21 DAT. Integrated pest control was carried out with pesticides according to the types of pests and diseases that attack. Harvest was done after 75% of the upper leaves have fallen at the age of 60-70 days after transplanting, by pulling the tubers carefully, tied and dried in the sun for 7 days according to weather conditions. The observed variables consisted of plant length and number of leaves.

**Chlorophyll content:** The content of chlorophyll a, chlorophyll b and total chlorophyll refer to the method described by Henry and Grimes[12]. Determination of chlorophyll content were carried out by taking 0.1 g of shallot leaf sample and then macerated with 10 mL of acetone using a mortar. Determination of chlorophyll a, chlorophyll b and total chlorophyll content using the following Eq:

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

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

$$\text{Chlorophyll total} = \frac{(22.9\times A649)+(20.2\times A645)}{10}$$

**Stomatal density:** The determination of stomatal density was conducted by applying clear nail polish with a size of 1×2 cm on the abaxial part of the shallot to give a surface impression. After drying, the sample was taken using a clear tape and then placed on a microscope slide was carried out at a magnification of 40×10. The stomatal density is expressed as unit $mm^{-2}$.

**Statistical analysis:** The data were analysed using a two way analysis of variance (ANOVA) using the SAS computer program version 12 and for the treatment that had the significant effect, it was followed by a mean different test using Duncan's Multiple Range Test (DMRT) p = 0.05.

## RESULTS AND DISCUSSION

The application of sulphur and the interaction between sulphur and Paclobutrazol had significantly affected the wet weight and dry weight of shallot bulbs per plot. Application of sulphur150 kg of ZA $ha^{-1}$ had a significant effect on increasing the wet weight and the dry weight of Lokananta varieties shallot bulbs/plot. The treatment sulphur (150 kg ZA $ha^{-1}$) produced the highest the wet weight and dry weight of shallot bulbs per plot (7 plants). The interaction between 0 ppm of Paclobutrazol and 150 kg ZA $ha^{-1}$ indicated the highest of the wet and dry of shallot bulbs (Table 1).

Table 1: Wet weight and dry weight of shallot bulbs per plot with sulfur and paclobutrazol treatment

| Sulphur application (kg ZA $ha^{-1}$) | Paclobutrazol application (ppm) $P_0$ (0) | $P_1$ (15) | $P_2$ (30) | Mean |
|---|---|---|---|---|
| **Wet weight of shallot per plot (g)** | | | | |
| $S_0$ (0) | 100.47$^{e}$ | 136.37$^{cd}$ | 133.83$^{cd}$ | 123.56$^{b}$ |
| $S_1$ (75) | 170.73$^{b}$ | 163.70$^{bc}$ | 136.43$^{cd}$ | 156.96$^{a}$ |
| $S_2$ (150) | 205.83$^{a}$ | 127.87$^{d}$ | 165.07$^{bc}$ | 166.26$^{a}$ |
| Mean | 159.01 | 142.64 | 145.11 | |
| **Dry weight of shallot per plot (g)** | | | | |
| $S_0$ (0) | 80.67$^{e}$ | 104.33$^{cde}$ | 114.00$^{cd}$ | 99.67$^{b}$ |
| $S_1$ (75) | 145.00$^{b}$ | 140.00$^{bc}$ | 122.67$^{bcd}$ | 135.89$^{a}$ |
| $S_2$ (150) | 174.33$^{a}$ | 103.00$^{de}$ | 139.00$^{bc}$ | 138.78$^{a}$ |
| Mean | 133.33 | 115.78 | 125.22 | |

Numbers followed by the same letter on the same variable observed indicate no significant difference based on Duncan's Multiple Range Test at α = 5%. $P_0$: Paclobutrazol application 0 ppm, $P_1$: Paclobutrazol application 15 ppm, $P_2$: Paclobutrazol application 30 ppm

In this study, the ZA fertilizer used contained 24% sulphur, so that the application of 150 kg ZA $ha^{-1}$ was equivalent to 36 kg S $ha^{-1}$. This is closer to the research result by Mishu *et al.*[13] that the application of 40 kg S $ha^{-1}$ produced the highest of fresh and dries weight of shallot bulbs. Babalesh war *et al.*[14] and Hore *et al.*[15] stated that the foliar application of 60 kg S $ha^{-1}$ increased the crop growth and productivity in garlic. Asefa *et al.*[16] stated that the application of 21 kg S $ha^{-1}$ increased the shallot yield. Based on the state of Souza[17], the highest yield of shallot was achieved at application of 45 kg S $ha^{-1}$.

**Chlorophyll content observation:** Based on Table 2, it can be seen that the interaction treatment between sulphur and Paclobutrazol has a significant effect on the chlorophyll a and total chlorophyll contents. The highest chlorophyll a and total chlorophyll contents were found in the interaction of sulphur treatment of 150 kg ZA $ha^{-1}$ and without application of Paclobutrazol. This shows the role of sulphur in photosynthesis so that the chlorophyll content increases with the addition of sulphur.

Several researchers previously reported that S plays a very important role in photosynthesis and photorespiration, affects the growth phase of plants, plays a role in photosynthesis and the electron transport system as well as in coenzymes and prosthetic groups such as ferredoxin, which are important for nitrogen assimilation[18].

Table 2: Chlorophyll content of shallot with application of sulphur and paclobutrazol treatment

| Sulphur application (kg ZA ha$^{-1}$) | Paclobutrazol application (ppm) P$_0$ (0) | P$_1$ (15) | P$_2$ (30) | Mean |
|---|---|---|---|---|
| | ---------- mg g$^{-1}$ ---------- | | | |
| **Chlorophyll-a** | | | | |
| $S_0$ (0) | $0.07^{cd}$ | $0.09^{abcd}$ | $0.07^{cd}$ | 0.08 |
| $S_1$ (75) | $0.05^{d}$ | $0.10^{abc}$ | $0.11^{ab}$ | 0.09 |
| $S_2$ (150) | $0.12^{a}$ | $0.08^{bcd}$ | $0.08^{bcd}$ | 0.09 |
| Mean | 0.09 | 0.09 | 0.09 | |
| **Chlorophyll-b** | | | | |
| $S_0$ (0) | 0.06 | 0.07 | 0.08 | 0.08 |
| $S_1$ (75) | 0.08 | 0.07 | 0.08 | 0.07 |
| $S_2$ (150) | 0.07 | 0.07 | 0.07 | 0.08 |
| Mean | 0.06 | 0.08 | 0.06 | |
| **Total of chlorophyll** | | | | |
| $S_0$ (0) | $0.13^{d}$ | $0.16^{bcd}$ | $0.13^{d}$ | 0.14 |
| $S_1$ (75) | $0.09^{e}$ | $0.17^{abc}$ | $0.19^{ab}$ | 0.15 |
| $S_2$ (150) | $0.20^{a}$ | $0.14^{cd}$ | $0.16^{bcd}$ | 0.17 |
| Mean | 0.14 | 0.16 | 0.16 | |

Numbers followed by the same letter on the same variable observed indicate no significant difference based on Duncan's Multiple Range Test at $\alpha = 5\%$. $P_0$: Paclobutrazol application 0 ppm, $P_1$: Paclobutrazol application 15 ppm, $P_2$: Paclobutrazol application 30 ppm

Table 3: Density of stomata of shallot from TSS with the application of sulphur and paclobutrazol

| Sulphur application (kg ZA ha$^{-1}$) | Paclobutrazol application (ppm) P$_0$ (0) | P$_1$ (15) | P$_2$ (30) | Mean |
|---|---|---|---|---|
| | ---------- Unit mm$^{-2}$ ---------- | | | |
| $S_0$ (0) | 82.46 | 87.35 | 90.15 | $86.65^{a}$ |
| $S_1$ (75) | 83.86 | 79.66 | 80.37 | $81.30^{b}$ |
| $S_2$ (150) | 73.38 | 82.46 | 83.86 | $79.90^{b}$ |
| Mean | 79.90 | 83.16 | 84.79 | |

$P_0$: Paclobutrazol application 0 ppm, $P_1$: Paclobutrazol application 15 ppm, $P_2$: Paclobutrazol application 30 ppm

In this study, the levels of chlorophyll a and total chlorophyll increased with increasing application of S fertilizer. This indicated the role of S in the formation of chlorophyll in leaves. This is in line with the previous researches which sulphur deficiency decreases the rate of photosynthesis through the effect of chlorophyll content, which decreases linearly with decreasing leaf sulphur and by decreasing the rate of photosynthesis per chlorophyll unit[19,20].

The application of Paclobutrazol 15-30 ppm had a tendency to increase the total chlorophyll. This is in line with previous research by Elizani and Sulistyaningsih[21] who reported that application of Paclobutrazol 15-30 mg L$^{-1}$ increased the chlorophyll content of TSS. This indicates that Paclobutrazol application can maintain greenish of leaves and plays a role in stimulating of cytokinins synthesis, whereas cytokinins serves to increase the chloroplast differentiation, biosynthesis of chlorophyll and prevent the chlorophyll degradation. Higher the content of chlorophyll and slower degradation of chlorophyll stimulates photosynthetic activity[22,23].

**Density of stomatal evaluation:** Based on Table 3, it can be seen that only the sulphur treatment has a significant effect on the density content of the stomata. The highest stomata density was found in the sulphur treatment of 0 kg ZA ha$^{-1}$. This shows that the application of sulphur reduces the density of stomata in plants. Stomata function for the transpiration process in plants. The main function of leaf stomata is the exchange of gases needed by plants for the photosynthesis process. Stomata or often known as leaf mouth are components of epidermal cells in leaves. Through stomata, $CO_2$, $H_2O$ and $O_2$ can go in and out to carry out the process of respiration and photosynthesis. However, these stomata are also one of the pathways for pollutants to enter plants[24]. The length and width of stomata greatly affect transpiration and absorption of air by plant. The water absorbed by plant is used for photosynthesis so that the rate of photosynthesis increases and plant growth and development also increases[25,26].

In this study, there was a tendency to increase the number of stomata with the increasing of Paclobutrazol application (0-30 ppm). This indicates the role of Paclobutrazol in increasing the density of shallot stomata.

## CONCLUSION

The application of sulphur 150 kg ZA ha$^{-1}$ and without Paclobutrazol increased the wet weight of shallot bulbs, the dry weight of shallot bulbs and chlorophyll total, compared without application of sulphur and Paclobutrazol. The application of Paclobutrazol 15-30 ppm increased the total of chlorophyll and density of stomata.

## SIGNIFICANCE STATEMENT

This research has discovered findings that there are differences in the interaction of sulphur and application of Paclobutrazol on the production and physiological characteristics. This research will assist researchers and farmers to use the dose of sulphur and application of Paclobutrazol on Lokananta variety of shallot from true shallot seed. The application of sulphur until 150 kg ZA kg ha$^{-1}$and without Paclobutrazol increased the wet weight and dry weight of shallot bulbs.

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