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Research Article
Role of Cultivation Methods on Physiological Characteristics and Production of Shallot Varieties under Lowland Condition

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Abstract

Background and Objective: An understanding of the method of cultivating shallots from TSS will provide accuracy in selecting the right varieties of shallots from TSS cultivated in the lowlands as well as the appropriate cultivation technology to increase the productivity and quality of shallot yields. The study aims to identify the best cultivation method for the production and physiological characters of shallots from TSS in the lowlands. **Materials and Methods:** This study was conducted in the community land in Tanjung Sari Medan from June to September, 2021. This study used a factorial randomized block design. The first factor is the variety of shallots from the bulb (Lokananta and Sanren F1) and the second factor is cultivation methods consisting of seed supplier recommendation, modification of seed supplier recommendation, multiple production recommendation, modification of multiple productions. **Results:** Lokananta variety has higher production and chlorophyll content compared to Sanren F1. The cultivation method from a seed supplier is the best method for increasing the production of shallots from TSS. The interaction between the Lokananta variety and the cultivation method from the seed supplier increased the production of shallots from TSS. **Conclusion:** The use of the cultivation method of seed supplier recommendation and Lokananta variety increased the bulb dry weight per plot by 24.87% compared to multiple production recommendations. The use of Lokananta variety increased bulb weight per plot (1×1 m) by 50.42% compared to Sanren F-1.

Key words: Shallot, variety, lowlands condition, physiological characteristic, true shallot seed, fatty acids, paclobutrazol

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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 classified as the main commodity of horticulture and high-value spices, have very good prospects and act as food seasonings, food industry ingredients and biopharmaceutical sources because they contain bioactive compounds such as quercetin, saponins, flavonoids, essential oils, alliin and allicin[1-5]. Shallots contain carbohydrates, sugars, fatty acids, a source of vitamins B, C, potassium, phosphorus, protein and other minerals that humans need[6].

In general, shallot cultivation is done by using bulbs as seeds. Constraints in using bulbs as seeds are the cost of providing seed bulbs is quite high (±40% of the total production cost), the quality of the bulb is not guaranteed due to the presence of bulb-borne pathogens such as *Fusarium* sp., *Colletotrichum* sp., *Alternaria* sp. and viruses from the original plants that are attacked can reduce yield productivity[7]. One way to overcome this problem is the use of true shallot seed (TSS).

To increase the productivity and quality of shallots, the cultivation of shallots from TSS needs to be carried out because it has advantages such as bulb yields increasing up to two times (production of 26 t ha^{-1}), less seed volume (seed requirement ±7.5 kg ha^{-1} than tubers ±1.5 t ha^{-1}), cheaper transportation costs, easier storage, stronger and healthier. After all, they are free of viruses, larger tubers, longer seed shelf life (1-2 years) while planting material from tubers can only be stored for 4 months[8].

Until now, there are still limited studies on the method of cultivating shallots from TSS in the lowlands that will provide accuracy in selecting the right shallot varieties from TSS cultivated in the lowlands as well as the appropriate cultivation technology to increase the productivity and quality of shallot yields.

Previous studies have studied the role of sulfur and paclobutrazol on the productivity of shallots from TSS[9], the effect of plant growth regulator[10,11] shallot as natural plant growth regulator[12] the effect of ecoenzyme[13], agricultural management and environmental requirements for TSS production[14].

Based on this background, this study aims to identify the best cultivation method for production and physiological characters of shallots from TSS in the lowlands.

MATERIALS AND METHODS

Time and location: The research was conducted on the community land of Tanjungsari, Medan with an altitude of ±25 meters above sea level in July to October, 2021. The materials used in this study were shallots TSS Sanren F-1 and Lokananta varieties, NPK fertilizer, ZA fertilizer, paclobutrazol, KCl fertilizer, manure, SP-36, *Trichoderma harzianum*, paper and silver plastic black mulch.

The results of the soil analysis at the research site are presented in Table 1 which shows that it shows that the content of N (0.41%), P (0.30%), K (0.30%), C-organic (1.92%), pH (4.80).

Research design: The study used a factorial randomized block design with 2 factors and 3 replications. The first factor is the variety, namely V_1 = Lokananta and V_2 = Sanren F1. The second factor is the TSS cultivation method, namely C_1: Treatment from seed supplier recommendations, C_2: Modification of seed supplier recommendations with the addition of paclobutrazol, C_3: Double production modification with the addition of paclobutrazol, C_4: Double production recommendations, as presented in Table 2.

Table 1: Results of soil analysis at the research site

Parameter	Results (%)	Analytical method
pH H_2O	4.80	H_2O (1:5)-electrometry
C organic	1.92	Walkley and black with spectrophotometer
N	0.41	Kjeldahl with spectrophotometer
P	0.30	Dry ashing-HNO_3 with spectrophotometer
K	0.06	HNO_3 with spectrophotometer
S	1.33	Turbidimeter

Source: Socfind's Laboratory (2021)

Table 2: Treatment of cultivation methods of shallot varieties in lowlands

		An organic fertilizer (WAT)						
Treatments	Paclobutrazol (ppm)	-7	7	14	2 1	28	42	Spacing (em)
C_1	0		ZA 150 kg ha^{-1}	NPK (16-16-16) 200 kg ha^{-1}		NPK (16-16-16) 250 kg ha^{-1}	NPK (16-16-16) 250 kg ha^{-1} KCl 187.5 kg ha^{-1}	10×10
C_2	15	NPK (16-16-16) 500 kg ha^{-1}			NPK (15-15-15) 150 kg ha^{-1} ZA 150 kg ha^{-1}		NPK (15-9-20) 150 kg ha^{-1}	10×15
C_3	15		ZA 150 kg ha^{-1}	NPK (16-16-16) 150 kg ha^{-1}		NPK (16-16-16) 200 kg ha^{-1}	NPK (16-16-16) 200 kg ha^{-1} KCl 150 kg ha^{-1}	10 ×15
C_4	0	NPK (16-16-16) 500 kg ha^{-1}			NPK (15-15-15) 200 kg ha^{-1} ZA 150 kg ha		NPK (15-9-20) 200 kg ha^{-1}	10×10

Procedures: Making beds is done with a size of 1×1.5 m, then given manure (1 kg/bed) when tilling the soil. Before sowing the seeds were given ZA fertilizer (100 kg ha^{-1}). Nurseries were carried out for 5 weeks (35 days after planting).

The land was prepared before the seedlings were transplanted by cultivating the soil with a tillage depth of 20 cm and making a plot of 100×100 cm. The distance between the plots is 30 cm, while the distance between blocks is 50 cm. The SP-36 250 kg ha^{-1} was used as basic fertilizer and *Trichoderma harzianum* (100 kg ha^{-1}) was applied a week before planting by dissolving it in water and then watering it on each plot. Next, the black silver mulch was installed which had been perforated according to the planting hole.

Seedlings are ready to transplant at 35 days after sowing, 1 seedling/planting hole is planted, the spacing is 10×10 cm and 10×15 cm with the criteria that the seeds must be sturdy, the colour of the seeds is fresh green and has 4-6 leaves. The application of fertilizer is carried out according to the dose and cultivation method tested.

In dry weather conditions, watering is carried out with an intensity of 2 times a day, in the morning and evening. However, in the rainy season, watering is sufficient only once in the morning or evening or as needed. Weeding is done by removing weeds so that plant roots are not disturbed once a week. Pest control is carried out manually, namely by taking and removing pests that exist in plants. Disease control was carried out by spraying fungicides with a concentration of 2 cc L^{-1} in the field.

Harvesting is done when the plant has met the harvest criteria with the characteristics of the leaves starting to turn yellow, the top part of the plant starting to fall, the tubers already look solid and appear partially above the ground and the skin colour is shiny. Harvesting is done in the morning with sunny conditions, by pulling the plants carefully so that the stems do not break and the tubers do not stay in the soil. After being removed, the bulbs are collected and cleaned of soil.

The procedures to determine the chlorophyll, stomata and cuticle thickness is shallot samples aged 7 WAT were brought to the laboratory for analysis. The chlorophyll content was determined by referring to the method described by Kobayashi *et al.*[15]. Weighing 0.1 g of fresh shallot leaf samples for each treatment, then extracted with 10 mL of 80% acetone solution and each extract was filtered using filter paper, then the absorbance value was measured using a UV Vis spectrophotometer at wavelengths of 645 and 663 nm. The formula for determining the chlorophyll content is as follows: Chlorophyll-a = {(12.7×A663)-2.69×A645)}/10, Chlorophyll-b = {922.9×A645)-(4.68×A663)}/10, Total chlorophyll = {98.02×A663)+(20.2×A645)}/10. The unit of chlorophyll is expressed in mg g^{-1} of leaf fresh weight.

The stomata density method of shallots was carried out by printing the stomata using transparent nail polish on the lower surface of the shallots, then dried and carefully removed. The impressed stomata were observed using a microscope with a magnification of 40×10). Stomatal density is determined using the formula[16]:

$$\text{Stomatal density} = \frac{\text{Number of stomata}}{\text{Width of the visual field}}$$

Cuticle thickness was observed by making a transverse leaf incision. The thickness of the cuticle layer observed was on the underside of the leaf (abaxial). The cuticle thickness unit used is the micrometre.

Data analysis: The data were analyzed using the analysis of variance, if there was a significant effect, further tests were carried out using Duncan's Multiple Distance Test at $\alpha = 5\%$.

RESULTS

Number of bulbs per plant: The treatment of varieties, cultivation methods and their interactions had no significant effect on the number of bulbs per plant (Table 3). Sanren F-1 variety tends to have more tubers per sample than Lokananta. The C_4 treatment produced the higher number of bulbs per plant, while the C_2 treatment produced the lowest number of bulbs per plant. The combination of C_4 treatment and Sanren F-1 variety had more leaves than the other treatment combinations.

Wet weight and dry weight of bulbs per plant: Varieties, cultivation methods and their interactions have a significant effect on the wet weight and dry weight of bulbs per plant. The Lokananta variety had a higher wet weight and dry weight of bulbs per plant than Sanren F-1. The C_1 cultivation method produced higher wet weight and dry weight of bulbs per plant than other cultivation methods. The interaction between Lokananta and the C_1 and C_3 cultivation methods resulted in the highest wet weight and dry weight of bulbs per plant (Table 3).

Dry weight of bulbs per plot: Varieties, cultivation methods and their interactions have a significant effect on bulbs dry weight per plot. The Lokananta variety produced a higher tuber dry weight of bulbs per plot than Sanren F-1. The C_1 cultivation method resulted in a higher dry weight of bulbs per plot than other cultivation methods. The interaction between Lokananta and the C_1 cultivation method resulted in the highest dry weight of bulbs per plot (Table 3).

Table 3: Bulbs production and harvest index of two shallot varieties from TSS with different cultivation methods

Variable observed	Variety	Cultivation method				Mean
		C_1	C_2	C_3	C_4	
Number of bulb/plant (bulb)	Lokananta (V_1)	1.70	1.57	1.83	1.87	1.74
	Sanren F-1 (V_2)	1.97	2.07	2.03	2.10	2.04
	Mean	1.83	1.81	1.93	1.98	
Wet weight of bulb/plant (g)	Lokananta (V_1)	22.98^{a}	14.97^{ab}	25.70^{a}	18.23^{b}	20.47^{a}
	Sanren F-1 (V_2)	15.67^{ab}	11.52^{c}	11.91^{bc}	16.02^{b}	13.78^{b}
	Mean	19.33^{a}	13.25^{b}	18.80^{a}	17.13^{a}	
Dry weight of bulb/plant (g)	Lokananta (V_1)	21.28^{a}	13.11^{ab}	23.48^{a}	16.45^{b}	18.58^{a}
	Sanren F-1 (V_2)	13.42^{b}	9.05^{d}	9.79^{bc}	13.68^{b}	11.49^{b}
	Mean	17.35^{a}	11.09^{b}	16.64^{a}	15.07^{a}	
Dry weight of bulb/plot (g)	Lokananta (V_1)	1882.77^{a}	817.80^{c}	1568.17^{b}	1414.53^{b}	1420.82^{a}
	Sanren F-1 (V_2)	964.20^{c}	503.90^{d}	397.87^{d}	950.10^{c}	704.01^{b}
	Mean	1423.48^{a}	660.85^{c}	983.02^{b}	1182.31^{ab}	
Harvest index	Lokananta (V_1)	0.70	0.61	0.67	0.70	0.67
	Sanren F-1 (V_2)	0.64	0.51	0.53	0.52	0.55
	Mean	0.67	0.56	0.60	0.61	

The numbers followed by the same letters and variable observed show no significant difference according to Duncan's Multiple Range Test at the level of $\alpha = 5\%$

Table 4: Chlorophyll content and cuticle thickness of two shallot varieties from TSS with the application of cultivation methods

Variable observed	Variety	Cultivation method				Mean
		C_1	C_2	C_3	C_4	
Chlorophyll-a (mg g^{-1} of fresh weight)	Lokananta (V_1)	8.89^{ab}	8.31^{b}	7.86^{b}	10.12^{a}	8.79^{a}
	Sanren F-1 (V_2)	8.51^{b}	6.78^{c}	3.52^{c}	8.07^{b}	6.72^{b}
	Mean	8.7^{ab}	7.54^{b}	5.69^{c}	9.09^{a}	
Chlorophyll-b (mg g^{-1} of fresh weight)	Lokananta (V_1)	11.46^{b}	10.86^{b}	1.29^{d}	18.44^{a}	10.52^{a}
	Sanren F-1 (V_2)	4.24^{c}	0.52^{d}	2.53^{cd}	0.97^{d}	2.07^{b}
	Mean	7.85^{ab}	5.69^{b}	1.91^{c}	9.71^{a}	
Total of chlorophyll (mg g^{-1} of fresh weight)	Lokananta (V_1)	20.36^{b}	19.18^{b}	9.15^{d}	28.56^{a}	19.31^{a}
	Sanren F-1 (V_2)	12.75^{c}	7.30^{e}	6.05^{f}	9.04^{d}	8.79^{b}
	Mean	16.55^{b}	13.24^{c}	7.60^{d}	18.80^{a}	
Cuticle thickness (µm)	Lokananta (V_1)	5.23^{bc}	5.99^{bc}	6.78^{bc}	5.21^{c}	5.80^{b}
	Sanren F-1 (V_2)	11.55^{a}	4.86^{c}	8.60^{b}	7.05^{bc}	8.01^{a}
	Mean	8.39^{a}	5.42^{b}	7.69^{a}	6.13^{b}	

The numbers followed by the same letters and variable observed show no significant difference according to Duncan's Multiple Range Test at the level of $\alpha = 5\%$

Harvest index: Varieties, cultivation methods and their interactions have no significant effect on the harvest index. The Lokananta variety has a higher yield index than the Sanren F-1. The C_1 cultivation method produces a higher index than other cultivation methods. The interaction between Lokananta and the C_1 or C_4 cultivation method resulted in the highest harvest index (Table 3).

Chlorophyll content: Based on Table 4, it can be seen that varieties, cultivation methods and their interactions significantly affect the content of chlorophyll-a, chlorophyll-b and total chlorophyll. Lokananta variety has higher chlorophyll-a, chlorophyll-b and total chlorophyll content than Sanren F-1. The C_4 cultivation method produced the highest content of chlorophyll-a, chlorophyll-b and total chlorophyll, while the C_3 cultivation method produced the highest content of chlorophyll-a, chlorophyll-b and total chlorophyll.

In the Lokananta variety, the C_4 cultivation method produced higher chlorophyll-a, chlorophyll-b and total chlorophyll content, while the C_3 cultivation method produced the lowest chlorophyll-b and total chlorophyll content. In the Sanren F-1 variety, the C_1 cultivation method increased the chlorophyll-a, chlorophyll-b and total chlorophyll content, while the M_3 cultivation method produced the lowest chlorophyll-b and total chlorophyll content.

Cuticle thickness: Sanren F-1 variety had a significantly higher cuticle thickness than the Lokananta. The treatments C_1 and C_3 resulted in significantly higher cuticle thickness than C_2 and C_4. In Lokananta variety, all cultivation method treatments (C_1, C_2, C_3 and C_4) produced relatively the same cuticle thickness, while in Sanren F-1 variety, C_1 cultivation method treatment produced significantly higher cuticle thickness than other cultivation methods (Table 4).

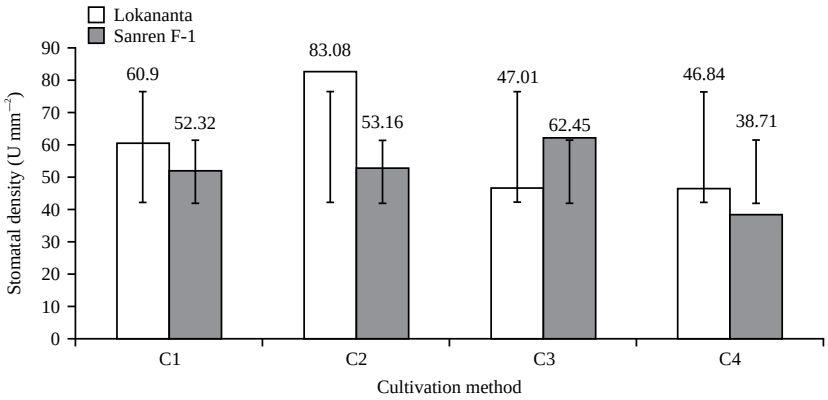

Fig. 1: Stomatal density of two varieties of shallot from TSS with different cultivation methods

Stomatal density: Based on Fig. 1, it can be seen that in the Lokananta variety, the C_1 cultivation method resulted in the highest stomatal density, while the C_4 treatment resulted in the lowest stomatal density. In the Sanren F-1 variety, the C_3 treatment resulted in the highest stomatal density, while the C_4 treatment resulted in the lowest stomatal density.

DISCUSSION

Lokananta variety produced a higher tuber dry weight per plot and harvest index than Sanren F-1. This proves that the Lokananta variety can adapt well in the lowlands. The interaction between Lokananta and the C_1 cultivation method resulted in the highest dry weight of tubers per plot (Table 3), proving that in the selection of shallot cultivation methods from TSS in the lowlands, the C_1 method could be used with Lokananta as the selected variety.

The increase in bulb dry weight/plot in the C_1 treatment is due to the relatively high NPK and ZA treatments (containing 21% sulfur) during the plant growth phase. Nitrogen (N), phosphorus (P), potassium (K) and sulfur (S) are four macro essentials that strongly supports the growth and production of shallots. Nutrient N is very useful for overall plant growth to be faster and increase crop yields. Nutrient P functions as a store and channel energy for all plant metabolic activities. The positive impact is that it stimulates root growth, stimulates tissue development, formation of fruit ripening tubers, increases disease resistance. Nutrient K is an enzyme activator that participates in plant metabolic processes. It also helps the process of absorption of water and nutrients in the soil. Nutrient K also helps distribute assimilated products from leaves to all plant tissues[17-21]. In addition, nutrient S plays a role in increasing the quality and quantity of onion bulbs produced. The distinctive aroma emitted from the tubers is also closely related to the content of sulfur[22,23].

Chlorophyll is the major pigment found in the thylakoid membranes of chloroplasts. The green pigment in leaves plays a role in absorbing light in phase I photosynthesis, namely the photolysis reaction. This pigment plays a role in the photosynthesis process of plants by absorbing and converting light energy into chemical energy. Chlorophyll-a, chlorophyll-b and total chlorophyll of shallots were significantly influenced by variety, cultivation methods and the interaction between these two factors. The Lokananta variety contains higher chlorophyll-a, chlorophyll-b and total chlorophyll than Sanren F-1. The M_4 cultivation method increased the content of chlorophyll-a, chlorophyll-b and total chlorophyll because the application of 400 kg ha^{-1} of NPK increased the formation of chlorophyll. The close relationship between the content of chlorophyll and nitrogen has been proven by many researchers[24-26]. This matter because N is a structural element of chlorophyll and protein molecules, so it affects the formation of chloroplasts and the accumulation of chlorophyll in them. Optimal nitrogen application can increase plant growth, increase protein synthesis and chlorophyll formation which causes the leaf colour to become greener[27].

Stomata is a natural hole that acts as a tool for evaporation, a tool for CO_2 exchange in physiological processes related to production. Stomatal density, defined as the number of stomata per unit leaf area, was theoretically shown to be correlated with stomatal conductance. Changes in stomatal density affect gas exchange[28,29]. In this study, the highest stomatal density was found in the Lokananta variety with C_2 treatment given 15 ppm paclobutrazol. This reinforces that the application of paclobutrazol increased stomatal density. In line with Waqas *et al.*[30] and Rodrigues *et al.*[31] statements that paclobutrazol application significantly increased stomatal density on both leaf surfaces.

The cuticle is a waxy layer found on the surface of stems and leaves that can prevent dryness in land plants. The cuticle layer is located on the outermost part of the epidermal cells, especially on plant parts that grow above the ground such as stems and leaves[32,33]. In this study, the difference in cuticle thickness with the variety and cultivation method proved that the cuticle thickness was influenced by genetic and environmental factors (treatment). The thickest cuticle was found in the Sanren F-1 variety which was treated with C_1 cultivation. The high administration of NPK in C_1 treatment is thought to increase the thickness of the cuticle.

CONCLUSION

Lokananta variety has higher production, chlorophyll-a, chlorophyll-b and total chlorophyll contents compared to Sanren F-1. The C_1 cultivation method is the best method for increasing the production and physiological characteristics of shallots. The best treatment interactions that increased production in this study were the C_1 cultivation method and the Lokananta variety.

SIGNIFICANCE STATEMENT

This study has discovered findings that there are differences in the interaction of shallot varieties and method of cultivation on the production and physiological characteristics of shallot varieties in lowlands. The research will assist researchers and farmers to use the best method for shallot cultivation and choose the best variety. Based on the research the new theory has been obtained that the use of the cultivation method of C_1 and Lokananta variety increased the bulb dry weight by 24.87% compared to the C_4 cultivation method. The use of Lokananta variety increased bulb weight per plot (1×1 m) by 50.42% compared to Sanren F-1.

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