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Research Article
Physiological Characteristics of Shallot (*Allium ascalonicum*) Varieties in Highlands and Lowlands

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Abstract
Background and Objective: Variations in chlorophyll content, stomatal density and cuticle thickness in plants are determined by plant genetic factors and plant growth environment. The objective of the study is to evaluate the physiological characteristics of shallots in the lowlands and highlands. **Materials and Methods:** This study used a survey method in shallot cultivation sites in the highlands (>700 m asl) and lowlands (<700 m asl), on May-August, 2021, by taking samples of shallot varieties. Analysis of chlorophyll content, stomatal density and cuticle thickness was carried out at the laboratory. **Result:** The result showed that the highest chlorophyll a and total chlorophyll were found in shallot of Lokananta variety from Saran Padang 1 (highland). The highest stomatal density was found in the shallots of the Sanren F1 variety (Panei Simalungun). The thickest cuticle was found in the Lokananta variety in Dolok Sanggul 2 (highland). **Conclusion:** There are variations in chlorophyll, stomatal density and cuticle thickness in the highlands and lowlands. The stomatal density of shallot varieties at each location varied between 57.48-118.83 units stomata. mm^{-2}. The variation of cuticle thickness ranged from 5071.66-32428.07 µm.

Key words: Chlorophyll, shallot, stomatal density, cuticle thickness, lowland, highland, quercetin

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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 (*Allium ascalonicum*) is a leading spice and horticultural commodity that acts as a food flavouring ingredient, a mixture of food industry ingredients ornamental plants and functional foods because of their bioactive substances that can cure various diseases. Shallots contain carbohydrates, amino acids, minerals, enzymes, vitamins A, B and C, sulfur compounds and several bioactive compounds in shallots including quercetin, saponins, essential oils, allicin and allicin[1-4]. The efficacy of quercetin in shallots is for the prevention and treatment of coronary heart disease, cancer, obesity, diabetes, hypertension, digestive disorders, osteoporosis asthma, neurodegenerative disorders, hypercholesterolemia, allergies, arthritis, cataracts and leukaemia[5-12.]

In general, bulbs of shallot are consumed, while the leaves are not commonly consumed. In contrast to green onions, which are consumed by the leaves and become an important export commodity for several countries, such as the Netherlands and Egypt. It has been used for vegetables and spices around the world for centuries[13]. Green shallot in Indonesia is sold at high prices for limited consumption of Chinese cuisine and local dishes[14].

The main part of the leaf that plays a role in the process of photosynthesis is chlorophyll. Chlorophyll is a pigment that plays a role in the photosynthesis process of plants by absorbing and converting light energy into chemical energy. The three main functions of chlorophyll in the process of photosynthesis are to utilize solar energy, triggers CO_2 fixation to produce carbohydrates and provide energy for the ecosystem as a whole. Carbohydrates produced in photosynthesis is converted into protein, fat, nucleic acids and other organic molecules. Chlorophyll absorbs light in the form of electromagnetic radiation in the visible spectrum. Sunlight contains all colours of the visible spectrum from red to violet but not all long waves are well absorbed by chlorophyll. Chlorophyll can accommodate light absorbed by other pigments through photosynthesis, so chlorophyll is referred to as photosynthetic reaction centre pigment. Higher plants have two types of chlorophyll namely chlorophyll a ($C_{55}H_{72}O_5N_4Mg$) which is dark green and chlorophyll b ($C_{55}H_{70}O_6N_4Mg$) which is coloured light green. Chlorophyll a and chlorophyll b most strongly absorbs light in the red part (600- 700 nm) and absorbs the least light green (500-600 nm)[15].

Until now, research on shallots that have been reported mainly on shallots as medicinal plants and spices[16-17], the effect of fertilization and the use of plant growth regulator on shallots[18-20] but studies that examine the physiological characteristics of shallot varieties planted in the highlands and lowlands have not been widely reported.

Based on this background, this study aimed to evaluate the physiological characteristics of shallots in the lowlands and highlands.

MATERIALS AND METHODS

Time and location: This study used a survey method in shallot cultivation sites in the highlands (>700 m asl) and lowlands (<700 m asl), on May-August, 2021. Based on the survey results, obtained 14 shallot planting locations consisting of 3 locations in the lowlands (Patumbak, Batang Kuis and Pantai Cermin) and 11 locations of shallot cultivation in the highlands (Saran Padang 1, Saran Padang 2, Saran Padang 3, Saran Padang 4, Saran Padang 5, Berastagi, Dolok Sanggul 1, Dolok Sanggul 2, Dolok Sanggul 3, Panei Simalungun, Barus Jahe).

Analysis of chlorophyll content, stomatal density and cuticle thickness was carried out at the Plant Physiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan.

Materials: The research materials used were shallot plants of various varieties in the highlands and lowlands, clear nail polish, acetone, glass preparations, deck glass, paper bags, name tags and filter paper. The equipment used consisted of a cuvette, spectrophotometer, microscope, microtome, sample vial, mortar and pestle.

Research design: The research was conducted in two stages, namely the first stage of descriptive exploratory research, while the second stage of analytical research. The first phase of the research used a survey method by collecting field data in the form of highland and lowland shallot plants. The second stage of the research was the analysis of the physiological characteristics of shallots from TSS in the highlands and lowlands. The samples obtained were grouped by location, then analyzed for chlorophyll content, stomatal density and cuticle thickness.

Procedures: The shallot plant samples obtained were then taken to the laboratory for analysis. The determination of chlorophyll content refers to the method described by Kobayashi *et al.*[21]. The method of determining the chlorophyll content was carried out by weighing 0.1 g of leaf fresh shallot samples from each location, then extraction with 10 mL 80% acetone solution. Next, each extract was filtered through filter paper and its absorbance was measured using a UV Vis spectrophotometer at wavelengths of 645 and 663 nm. Determination of chlorophyll content was carried out with the equation:

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

$$\text{Chlorophyll b} = \frac{(922.9 \times \text{A645}) - (4.68 \times \text{A663})}{10}$$

$$\text{Total chlorophyll} = \frac{(98.02 \times \text{A663}) + (20.2 \times \text{A645})}{10}$$

The unit of chlorophyll is expressed in mg g^{-1} of leaf fresh weight.

Leaf stomatal density in shallots was determined by the method of printing stomata using transparent nail polish on the surface of the lower leaves of the shallots, then dried and carefully removed. The impressed stoma was observed under a microscope to obtain a clear visuality with a magnification of 40×10). Determination of stomata density is calculated by the equation:

$$\text{Stomatal density} = \frac{\text{Number of stomata}}{\text{Wide field of view}}$$

Stomatal density is expressed based on the total number of stomata observed per area (unit.mm^{-2})[22]. Variables observed at the incision The transverse leaf is the thickness of the lower cuticle layer (abaxial) of the shallot leaf. The cuticle thickness unit used is μm.

Data analysis: Data were analyzed by analysis of variance and continued with DMRT 5% if there was a treatment that had a significant effect.

RESULTS AND DISCUSSION

Content of chlorophyll: Based on Table 1, it can be seen that there are variations in chlorophyll content in the highlands and lowlands. The highest chlorophyll a was found in shallots of Lokananta variety from Saran Padang 1 (highland), while the lowest chlorophyll a was found in shallots from Dolok Sanggul 3 (highland). The highest chlorophyll b was found in shallots of Sanren F1 variety from Panei Simalungun (highland), while the lowest chlorophyll b was found in plants shallots of the Sumenep variety from Saran Padang 5 (highland). The highest total chlorophyll was found in the Lokananta variety from Saran Padang 1 (highland), while the lowest total chlorophyll was found in the Lokananta variety from Dolok Sanggul 3 (highland).

Chlorophyll is a component of major chloroplasts and chlorophyll content is relatively positively correlated with the rate of photosynthesis. Chlorophyll is synthesized in the leaves and plays a role in catching the sun's rays different for each species. Chlorophyll synthesis is influenced by various factors such as light, sugar or carbohydrates, water, temperature, genetic factors, nutrients such as N, O, Mg, Fe, Mn, Cu, Zn and S[23].

Stomatal density: Stomata density is an important character that affects gas exchange. Stomata density of shallot varieties at each location varied between (57.48-118.83 units stomata. mm^{-2}) (Fig. 1). The highest stomatal density was

Table 1: Chlorophyll content of shallot varieties in highlands and lowlands

Location	Altitude (m asl)	Variety	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Chlorophyll total (mg g^{-1})
Berastagi	1400	Lokananta	15.32^{c}	3.76^{fg}	19.08^{cd}
Pantai Cermin	17	Maserati	15.84^{c}	10.47^{c}	26.31^{bc}
Batang Kuis	5.5	Lokananta	13.89^{c}	8.80^{d}	22.70^{bc}
Dolok Sanggul 1	1414	Lokananta	9.57^{d}	3.95^{fg}	13.53^{def}
Dolok Sanggul 2	1412	Lokananta	5.00^{d}	2.68^{gh}	7.68^{f}
Dolok Sanggul 3	1414	Lokananta	4.79^{d}	4.57^{ef}	9.36^{ef}
Barus Jahe	1347	Lokananta	21.54^{b}	7.52^{d}	29.06^{b}
Panei Simalungun	968	Sanren F-1	28.40^{ab}	13.95^{a}	42.35^{a}
Patumbak	75	Maserati	15.85^{c}	5.89^{e}	21.75^{bcd}
Saran Padang 1	1114	Lokananta	30.21^{a}	12.61^{b}	42.82^{a}
Saran Padang 2	1114	Solok	17.12^{c}	2.33^{h}	19.45^{cd}
Saran Padang 3	1114	Maja	21.60^{b}	2.13^{h}	23.74^{bc}
Saran Padang 4	1114	Bima Brebes	15.15^{c}	2.31^{h}	17.46^{cde}
Saran Padang 5	1114	Sumenep	16.74^{c}	1.54^{h}	18.28^{cde}

Numbers followed by a different superscripted letter on the same observation time and column showed significant differences based on Duncan's multiple range test at $\alpha = 5\%$

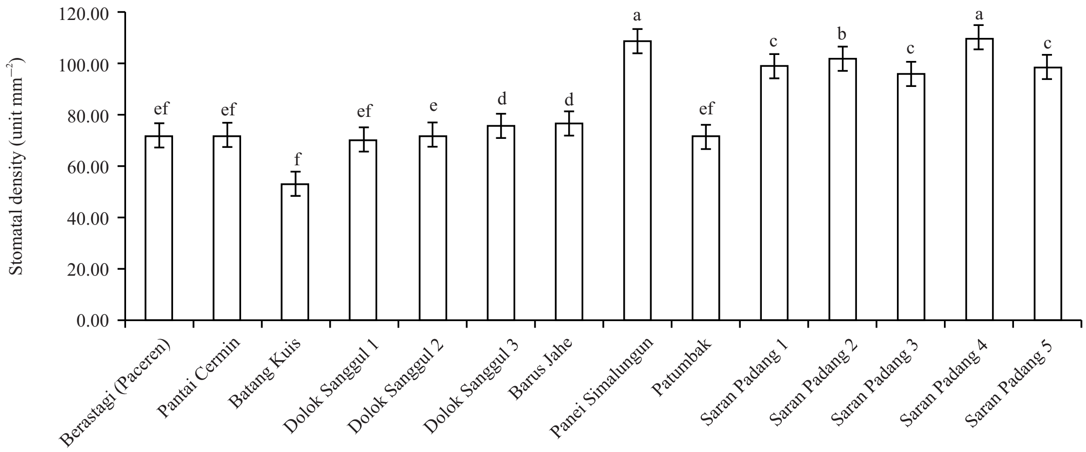

Fig. 1: Stomatal density of shallot in highlands and lowlands

Same letters above the bar chart indicate not significantly different according to Duncan's multiple range test at level = 5%

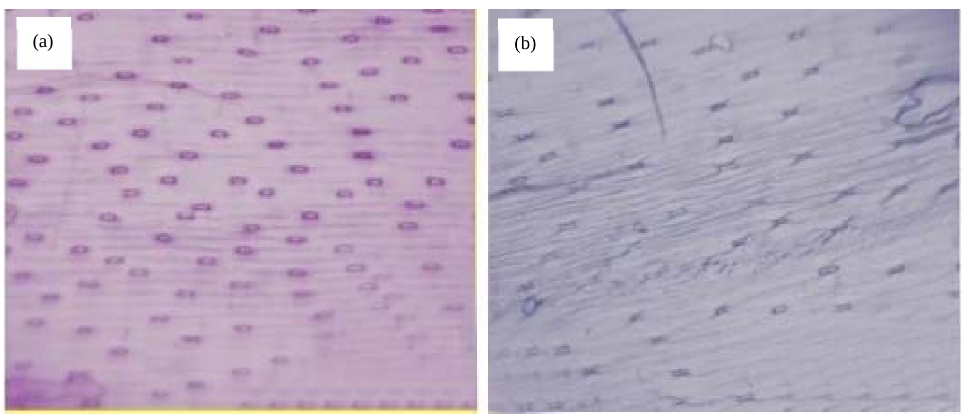

Fig. 2(a-b): Stomatal density in shallot, (a) the highest of stomatal density from Panei Simalungun, (b) the lowest of stomatal density from Batang Kuis

A microscopic observation of stomatal density with a magnification of 40×10

found in the shallots of the Sanren F1 variety (Panei Simalungun) and the Lokananta variety (Saran Padang 4) which had a higher stomata density compared to other locations, while the Lokananta shallots in the Batang Kuis location had the lowest stomata density. The performance of stomatal density can be seen in Fig. 2(a-b).

The difference in stomatal density of each variety is highly dependent on genetic factors. Variations in stomata density in varieties affect the ability of gas transportation. The value of stomatal density is influenced by the larger the size of the stomata, the smaller the stomata. The size of the stomata, the greater the density value. Leaves exposed to sunlight the sun at high light intensity has higher stomatal density compared to shaded leaves. Density low stomata when compared to a high number of epidermal cells, will result in a low stomatal index. On the other hand, high stomatal density when compared to the number of epidermal cells low will produce high stomata index[24,25].

Cuticle thickness: Based on Table 2, it can be seen that there are variations in the thickness of the cuticle on the underside of leeks in the lowlands and highlands. The variation of cuticle thickness ranged from 5071.66-32428.07 µm. The thickest cuticle was found in the Lokananta variety in Dolok Sanggul 2 (highland) but the thinnest cuticle was also found in the Lokananta variety in Berastagi (highland). The performance of cuticle thickness can be seen in Fig. 3(a-b).

The difference in cuticle thickness on shallots at different locations indicates the presence of adaptation to the growing environment of the plant. Previous research has reported that the cuticle has a function as a protective tissue of leaf cells against the penetration of sunlight and the entry of CO_2 into

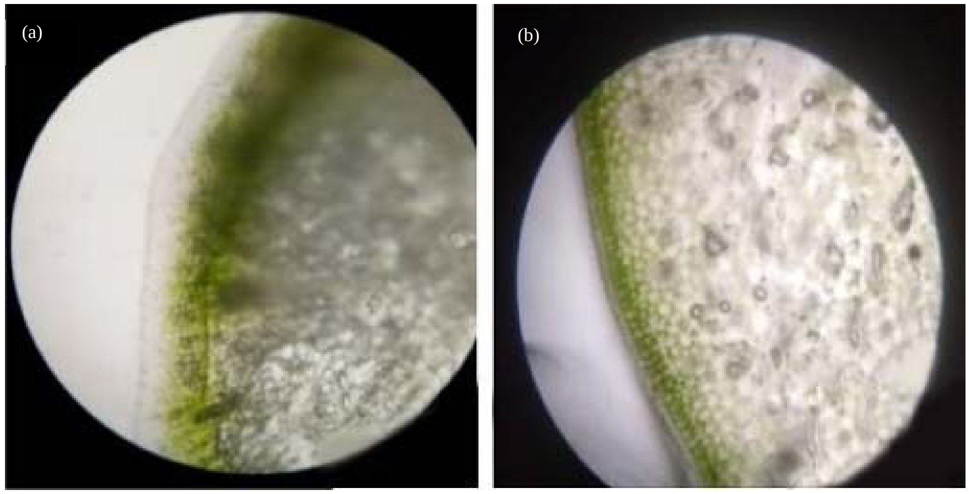

Fig. 3(a-b): Cuticle thickness in shallot, (a) the highest of cuticle thickness from Dolok Sanggul 2, (b) the lowest of cuticle thickness from Berastagi
A microscopic observation of cuticle thickness with a magnification of 40×10

Table 2: Cuticle thickness of shallot varieties in highlands and lowlands

Location	Altitude (m asl)	Variety	Cuticle thickness (μm)
BBerastagi	1400	Lokananta	11.36^{i}
PantaiCermin	17	Maserati	28.96^{b}
BatangKuis	5.5	Lokananta	20.73^{g}
DolokSanggul 1	1414	Lokananta	41.34^{e}
DolokSanggul 2	1412	Lokananta	109.27^{a}
DolokSanggul 3	1414	Lokananta	35.39^{gh}
BarusJahe	1256	Lokananta	31.10^{gh}
PaneiSimalungun	968	Sanren F-1	17.40^{hi}
Patumbak	75	Maserati	21.84^{hi}
Saran Padang 1	1114	Lokananta	29.58^{ef}
Saran Padang 2	1114	Solok	17.33^{e}
Saran Padang 3	1114	Maja	17.61^{c}
Saran Padang 4	1114	BimaBrebes	35.55^{e}
Saran Padang 5	1114	Sumenep	20.72^{d}

Numbers followed by a different superscripted letter on the same observation time and column showed significant differences based on Duncan's multiple range test at $\alpha = 5\%$

the leaf cells. Structural mechanism environmental changes related to anatomical characters include the presence of wax, cuticle thickness, density and size of stomata, cell nucleus and trichomes. The cuticle has a protective function plants from pests and diseases, reduce the rate of water transpiration and reflect sunlight. Very slippery cuticles can reduce attachment and spore development on the leaf surface so that the plant is protected from disease[26].

Based on the research results, it can be seen that the observed physiological characters (chlorophyll content, stomatal density and cuticle thickness) are influenced by genetic and environmental factors. This is in line with Casson's[27] and Tanaka *et al.*[28] research. The results of this study are expected to help researchers and farmers in determining the varieties to be cultivated in the highlands and lowlands. The selection of the right variety for specific locations will increase the productivity of shallots.

CONCLUSION

There are variations in chlorophyll, stomatal density and cuticle thickness at each observation location in the highlands and lowlands. The stomatal density of shallot varieties at each location varied between (57.48-118.83 units stomata. mm^{-2}). The variation of cuticle thickness ranged from 5071.66-32428.07 μm.

SIGNIFICANT STATEMENT

This research has discovered findings that there are variations of total chlorophyll (7.68-42.82 mg g^{-1}), stomatal density (57.48-118.83 units stomata. mm^{-2}) and cuticle thickness (5071.66-32428.07 µm) in shallot varieties cultivated in the highlands and lowlands. These variations are influenced by genetic and environmental factors. The research will assist researchers to determine the variety to be cultivated in the highlands or lowlands. Based on research, it can be determined that the highest total chlorophyll was found in the Lokananta variety from Saran Padang 1 (highlands). The highest stomatal density was found in the Sanren F1 variety from Panei Simalungun (highlands) and the Lokananta variety from Saran Padang 4 (highlands). The thickest cuticle was found in the Lokananta variety in Dolok Sanggul 2 (highlands).

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REFERENCES

1. Lu, X., J. Wang, H.M. Al-Qadiri, C.F. Ross, J.R. Powers, J. Tang and B.A. Rasco, 2011. Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii*) using infrared spectroscopy. Food Chem., 129: 637-644.
2. Shahrajabian, M.H., W. Sun and Q. Cheng, 2020. Chinese onion, and shallot, originated in Asia, medicinal plants for healthy daily recipes. Notulae Sci. Biol., 12: 197-207.
3. Thuy, N.M., N.T.M. Tuyen, N.P. Cuong, L.T.N. Huyen and N.P. Phuong *et al*., 2020. Identification and extraction method of quercetin from flesh and skin of shallot (*Allium ascalonicum*) cultivated in Soc Trang province, Vietnam. Food Res., 4: 358-365.
4. Mohammadi-Motlagh, H.R., A. Mostafaie and K. Mansouri, 2011. Anticancer and anti-inflammatory activities of shallot (*Allium ascalonicum*) extract. Arch. Med. Sci., 1: 38-44.
5. Ferioli, F. and L.F. D'Antuono, 2016. Evaluation of phenolics and cysteine sulfoxides in local onion and shallot germplasm from Italy and Ukraine. Genet. Resour. Crop Evol., 63: 601-614.
6. Choi, I.S., E.J. Cho, J.H. Moon and H.J. Bae, 2015. Onion skin waste as a valorization resource for the by-products quercetin and biosugar. Food Chem., 188: 537-542.
7. Arpornchayanon, W., S. Klinprung, S. Chansakaow, N. Hanprasertpong, S. Chaiyasate, M. Tokuda and H. Tamura, 2019. Antiallergic activities of shallot (*Allium ascalonicum* L.) and its therapeutic effects in allergic rhinitis. Asian Pac. J. Allergy Immunol., 10.12932/ap-300319-0529.
8. Dibal, N.I., S.H. Garba and T.W. Jacks, 2018. Role of quercetin in the prevention and treatment of diseases: Mini review. Braz. J. Biol. Sci., 5: 647-656.
9. Li, Y., J. Yao, C. Han, J. Yang and M.T. Chaudhry *et al*., 2016. Quercetin, inflammation and immunity. Nutrients, Vol. 8, No. 3. 10.3390/nu8030167.
10. Mlcek, J., T. Jurikova, S. Skrovankova and J. Sochor, 2016. Quercetin and its anti-allergic immune response. Molecules, Vol. 21. 10.3390/molecules21050623.
11. Golubkina, N., S. Zamana, T. Seredin, P. Poluboyarinov and S. Sokolov *et al*., 2019. Effect of selenium biofortification and beneficial microorganism inoculation on yield, quality and antioxidant properties of shallot bulbs. Plants, Vol. 8. 10.3390/plants8040102.
12. Pobłocka-Olech, L., D. Głód, M.E. Żebrowska, M. Sznitowska and M. Krauze-Baranowska, 2016. TLC determination of flavonoids from different cultivars of *Allium cepa* and *Allium ascalonicum.* Acta Pharmaceutica, 66: 543-554.
13. El-Hamd A.S.A.A., M.T. EL-Abd, A.A.M. Mohamed and M.G.Z. EL-Din, 2016. Effect of some agricultural treatments on productivity and quality of green onion for export (*Allium cepa* L).Middle East J., 5: 37-44.
14. Putri, F., S.A. Aziz, N. Andarwulan, M. Melati and S. Suwarto, 2021. Leaf pigment, phenolic content, and production of green shallot of five different shallot varieties. Planta Tropika: J. Agro Sci., 9: 48-57.
15. Khaleghi, E., K. Arzani, N. Moallemi, M. Barzegar, 2012. Evaluation of chlorophyll content and chlorophyll fluorescence parameters and relationships between chlorophyll a, b and chlorophyll content index under water stress in *Olea europaea* cv. Dezful. World Acad. Sci. Eng. Technol. Int. J. Agric. Biosyst. Eng., 6: 636-639.
16. Sun, W., M.H. Shahrajabian and Q. Cheng, 2019. The insight and survey on medicinal properties and nutritive components of shallot. J. Med. Plants Res., 13: 452-457.
17. Amiri, Z., M.R. Asgharipour, D.E. Campbell, K. Azizi, E. Kakolvand and E.H. Moghadam, 2021. Conservation agriculture, a selective model based on emergy analysis for sustainable production of shallot as a medicinal-industrial plant. J. Cleaner Prod., Vol. 292. 10.1016/j.jclepro.2021.126000.
18. Elizani, P. and E. Sulistyaningsih, 2019. The correlation and regression analysis of the growth and physiological parameters: How paclobutrazol increases bulb yield on three cultivars of true shallot seed. Caraka Tani J. Sustain. Agric., 34: 128-139.

19. Siburian, E. and L.A.M. Siregar, 2019. Various natural ingredients as plant growth regulator to increasing viability true seed shallot of onion. J. Pertanian Tropik, 6: 80-87.
20. Marlin, M., H. Hartal, A. Romeida, R. Herawati and M. Simarmata, 2021. Morphological and flowering characteristics of shallot (*Allium cepa* Var. *Aggregatum*) in response to gibberellic acid and vernalization. Emir. J. Food Agric., 33: 388-394.
21. Kobayashi, K., S. Fujii, D. Sasaki, S. Baba, H. Ohta, T. Masuda and H. Wada, 2014. Transcriptional regulation of thylakoid galactolipid biosynthesis coordinated with chlorophyll biosynthesis during the development of chloroplasts in arabidopsis. Front. Plant Sci., Vol. 5. 10.3389/fpls.2014.00272.
22. Zhang, L., H. Niu, S. Wang, X. Zhu, C. Luo, Y. Li and X. Zhao, 2012. Gene or environment? species-specific control of stomatal density and length. Ecol. Evol., 2: 1065-1070.
23. Croft, H., J.M. Chen, X. Luo, P. Bartlett, B. Chen and R.M. Staebler, 2017. Leaf chlorophyll content as a proxy for leaf photosynthetic capacity. Global Change Biol., 23: 3513-3524.
24. Hong, T., H. Lin and D. He, 2018. Characteristics and correlations of leaf stomata in different *Aleurites montana* provenances. PLoS ONE, Vol. 13. 10.1371/journal.pone.0208899.
25. Bertolino, L.T., R.S. Caine and J.E. Gray, 2019. Impact of stomatal density and morphology on water-use efficiency in a changing world. Front. Plant Sci., Vol. 10. 10.3389/fpls.2019.00225.
26. Wang, X., L. Kong, P. Zhi and C. Chang, 2020. Update on cuticular wax biosynthesis and its roles in plant disease resistance. Int. J. Mol. Sci., Vol. 21. 10.3390/ijms21155514.
27. Casson, S.A. and A.M. Hetherington, 2010. Environmental regulation of stomatal development. Curr. Opin. Plant Biol., 13: 90-95.
28. Tanaka, Y., S.S. Sugano, T. Shimada and I. Hara Nishimura, 2013. Enhancement of leaf photosynthetic capacity through increased stomatal density in arabidopsis. New Phytol., 198: 757-764.