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Adaptive strategy of *Stevia rebaudiana* to environmental change in tropical climate based on anatomy and physiology characteristics

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Abstract. *Khuluq AD, Widaryanto E, Ariffin, Nihayati E. 2022. Adaptive strategy of* Stevia rebaudiana *to environmental change in tropical climate based on anatomy and physiology characteristics. Biodiversitas 23: 5710-5717.* High solar radiation and air temperature are the main influencing factors for plant growth and development in tropical climates. This study's objective was to understand stevia adaptation to microclimate changes at several altitudes in tropical climates. The study was conducted in a completely randomized design with altitude differences consisting of highlands, medium lands, and lowlands with ten replicates. The results showed that stevia was exposed to temperatures above 20ºC during growth in the highlands by 36.46%, and in the medium lands to lowlands by 92.85%-93.96%. Meanwhile, the temperature above 30ºC in the lowlands is 17.98% highest of the others. Stevia adapts to a high temperatures by increasing stomatal density, trichomes, and photosynthesis rate. Moreover, decreasing stomata (width, aperture, open percentage), leaf thickness, palisade length, xylem diameter, conductance, and transpiration rate. Stevia in the medium lands shows good adaptability with a high photosynthesis rate, percentage of open stomata 93.15%, leaf thickness 382.56 µm, and xylem diameter 30.97 µm, which is no different from the highlands. Thus, the medium lands have the potential as a new area of stevia development in a tropical climate, considering the increasingly competitive use of agricultural land in the highlands.

Keywords: Adaptation, altitude, *Stevia rebaudiana*

INTRODUCTION

Stevia (*Stevia rebaudiana* B.) is a natural sweetener that produces steviol glycosides, which are 200-300 times sweeter than sucrose. Steviol glycosides are non-calorie and non-carcinogenic, which is very good for diabetics. Stevia is a perennial plant originating from Paraguay (latitude 23ºS-24ºS, subtropics) with wide adaptability from the lowlands to the highlands and temperature tolerance between -6ºC to 43ºC (Moraes et al. 2013). Stevia is an obligate short-day plant with a critical point of 12-13 hours (Munz et al. 2018) and a sun-loving plant, which in its natural habitat grows in full sun conditions (Kumar et al. 2015; Gantait et al. 2018). The optimum temperature for stevia growth is 22-24ºC (Yadaf et al. 2011). Stevia can adapt well outside the origin area, such as in Japan, Australia, Brazil, Korea, Mexico, the United States, China, Indonesia, Tanzania, Thailand, India, Canada, and Russia (Libik-Konieczny et al. 2018). According to Sinta and Sumaryono (2019), a suitable growing environment for stevia in a tropical climate is a highland ranging from 700 to 1500 m a.s.l. with a temperature of 20-30ºC. On the other hand, the land available for stevia cultivation in the highlands is limited and the priority of its use is for vegetable commodities. Evaluation of stevia adaptation in the medium lands and lowlands is needed to find out how much influence it has on the growth of stevia plants. It can provide information related to efforts to assemble technology and varieties of stevia for development in the medium lands and lowlands.

Elevation gradients provide natural climatic variations that affect plant growth and development. Plants will adapt to related climatic conditions (Li and Bao 2014). In tropical climates, high solar radiation and air temperature are the main influencing factors for plant growth and development (Fahad et al. 2017), besides that plants are usually exposed to abiotic stresses, such as high temperatures and radiation, which are not suitable for growth and production of stevia plants (Ceunen and Geuns 2013). The increase in altitude causes an increase in air density, ultraviolet light intensity, and air humidity, while the air temperature decreases (Li and Jichao 2017; Istiawan and Kastono 2019). Temperature plays a role in controlling plant metabolic rate (Teixeira et al. 2013). Heat stress usually occurs when the temperature increases 10-15ºC from the ambient temperature (Tan et al. 2011; Ahmad et al. 2016). While Bita and Gerats (2013) explained that plants exposed to high temperatures at least 5ºC above the optimum temperature conditions for growth showed a response and cell metabolism to survive. Several studies have shown changes in leaf morphology and anatomical structure due to differences in longitude, latitude, and altitude gradients (Guo et al. 2017). Changes in the environment for growing at temperatures higher than the optimum growth conditions affect morphology, anatomy and physiology (Chen et al. 2014). High temperatures cause leaf aging, growth inhibition, and changes in leaf morphology (Fahad et al. 2017). High-temperature stress causes reduced cell size, stomata closure, increased stomatal density (Hasanuzzaman et al. 2013; Shahzad et al. 2015), an increasing number of trichomes, decreasing size of xylem vessels (Chen et al. 2014).

High-temperature stress causes a decrease in net photosynthetic rate, stomatal conductivity (Tan et al. 2011; Pal et al. 2015), and senescence acceleration (Fahad et al. 2017). Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is very sensitive to increasing temperature (Bita dan Gerats 2013). An increase in temperature above 30ºC during the day causes a decrease in Rubisco activation, thereby reducing the net photosynthetic rate (Killi et al. 2017). An increase in temperature causes an increase in the evaporation rate and water loss, which results in dehydration. This is related to soil dryness, which affects the water potential in cells and tissues (Munz et al. 2018). Decreased water potential causes a decrease in stomata conductivity (Rodríguez-Gamir et al. 2019). Drought conditions can induce abscisic acid. Increasing abscisic acid causes a decrease in stomata conductance, thereby inducing stomata closure to reduce water loss from the transpiration process (Killi et al. 2017). Thus, a decrease in the transpiration rate and stomata conductance reduces the photosynthesis rate of Stevia.

The objective of this study was to understand the adaptation of stevia plants to changes in microclimate at several altitudes through observations of the characteristic of anatomy, physiology, and climatology so that it is expected to provide information on the suitability of growing stevia characteristics for wider-scale development in tropical climates.

MATERIALS AND METHODS

Study area

The experiment was carried out during the dry season (May to October 2021) at the three elevations. The research was conducted in Malang, East Java, which has a tropical climate, at a different altitude, i.e. location 1: Junggo (highland, 7°48'25''S 112°31'43''E; 1300 m a.s.l.), location 2: Karangploso (medium land, 7°54'27''S 112°37'22''E; 560 m a.s.l.), location 3: Jambegede (lowland, 8°10'29''S 112°33'35''E; 330 m a.s.l.) (Figure 1).

Plant material and crop management

Planting material using shoot cuttings from the Garut cultivar, which is a local adaptive cultivar of stevia. The selected shoot cutting had comparable sizes (4-5 pairs of leaves). The study was conducted in a completely randomized design with three altitude differences consisting of highlands, medium lands, and lowlands, which were repeated 10 times. Pots were arranged with a spacing of 30 cm x 30 cm. Soil used inceptisol with a dose of manure 70 g pot^{-1}. Fertilization was carried out with 2.08 g N pot^{-1}, 0.78 g P_2O_5 pot^{-1} and 2.34 g K_2O pot^{-1} and applied at 7 days after pruning (DAP). Irrigation was done every 2 days during plant growth.

Climate observation

Measurements were carried out in the dry season (May to October) on the variables of temperature and humidity. The measuring instrument used a thermohygrometer (Elitech RC-4HC, ElitechLog V6.0.3 software) data logger system, which is set with measurements every 30 minutes.

Anatomical study

Leaf thickness (µm), palisade length (µm), and xylem diameter (µm) was measured by leaf and stem sections from fresh, fully expanded sun leaves, collected at 30 DAP (Day After Planting) by systematic sampling and measured by light microscope (Olympus BX53-32F0, camera DP73, cellSens software). Stomatal density (stomata mm^{-2}) was measured from nail varnish impressions of the leaf abaxial. Stomatal pore length (µm) and width (µm) were measured on the same recorded digital images. Dimension of the stomata was used to calculate the equivalent area of stomata aperture (µm^2) by the following formula: ($\pi \times$ length $\times$ width)/4, according to Gratani et al. (2013). Open stomata (%) were calculated by comparing open and closed stomata. Trichome was measured by stereo microscope (Zeiss stemi 2000-C, camera AxioCam ERc 5s, Axiovision software).

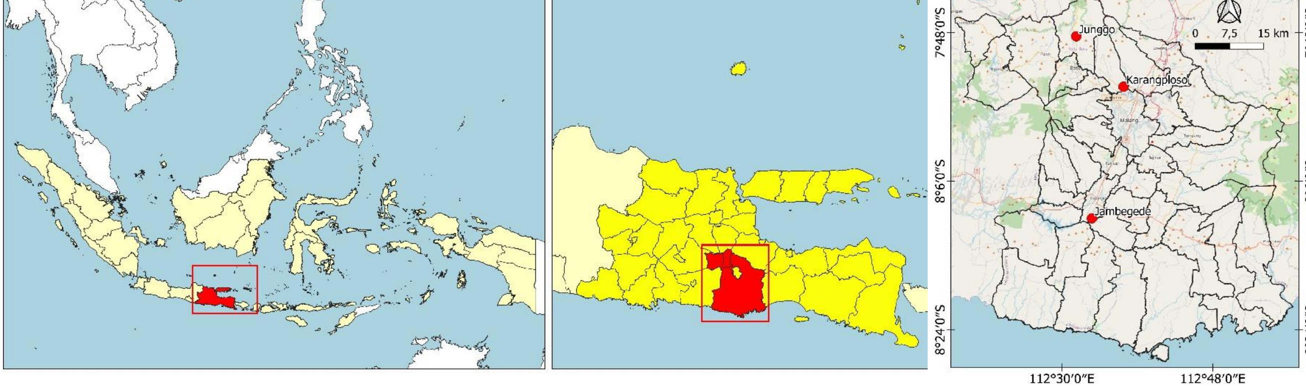

Figure 1. Location of field experiment at a different altitude, location 1: Junggo (highland), location 2: Karangploso (medium land), location 3: Jambegede (lowland)

Physiological study

Gas exchange measurement was taken with an open-system portable photosynthesis meter (LI-6400; LI-COR Inc., Lincoln) equipped with the standard leaf chamber (encloses 6 cm^2 of leaf area) and CO_2 injection system. The light intensity for measurement was set from 200 to 2000 μmol $m^{-2}s^{-1}$ (interval 300 μmol $m^{-2}s^{-1}$) provided by a red-blue light source. After enclosing the chamber that contained the leaf sample, 2 to 3 minutes were allowed for the photosynthetic rate to stabilize. Net photosynthetic rate (Pn, μmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (g_s, mol H_2O $m^{-2}s^{-1}$), transpiration rate (E, mmol H_2O $m^{-2}s^{-1}$) were measured in the morning, from 8.00 a.m. to 12.00 p.m. Observations were made at the age of 30 days after pruning.

Data analysis

SPSS software (version 26.0; IBM Corp.) was used to analyze the data, and LSD's test was used to compare treatments when the analysis of variance (ANOVA) showed a significant difference between means at $P \leq 0.05$; P-value less than 0.05 was regarded as statistically significant. The temperature was calculated as a percentage based on a certain range during plant growth.

RESULTS AND DISCUSSION

Microclimate descriptions at several altitudes

Microclimate was measured during the growth of the stevia plant. The difference in altitude affects microclimate conditions. Generally, increasing altitude causes decreasing in temperature and increasing in humidity (Table 1).

The maximum temperature in the highlands reached 29ºC and the maximum temperature in the lowlands was 34.1ºC with a difference of 0.7ºC compared to the medium lands. While the minimum temperature in the highlands reached 10.3ºC and an increase of 5.4ºC in the medium lands and 7.1ºC in the lowlands. The average temperature in the medium lands (24.5ºC) has approached the optimum temperature of stevia compared to the highlands and lowlands. The highest percentage of exposure to optimum temperature (22-24ºC) was found on medium land at 35.69%. In the lowlands, Stevia accepted the highest value at temperatures above 24ºC by 38.19% and temperatures above 30ºC by 17.98 %. The temperature in the highlands is lower than in the medium land and lowlands with a very large exposure below 20ºC, approximately 63.54% (Table 1).

The difference in altitude also has an effect on relative humidity (RH). Average humidity ranges from 74.7% - 87.9% at all altitudes. There was a tendency for an increase in RH along with an increase in altitude with an equation $y = 6.6x + 67.233$ ($R^2 = 0.95$). The increase in humidity was followed by decreasing in temperature. It means that the water vapor content in the highlands is quite a lot compared to the medium land and lowlands.

Anatomical characteristics

Stomata

Altitude differences affected stomatal characteristics. The decrease in altitude caused a decrease in stomata in the open percentage, length, and aperture. Stomatal density decreased with decreasing altitude. The increase in stomata density was seen in the lowlands by 258.97 units mm^{-2}. Stomata density in the highlands was significantly different ($P \leq 0.05$) from the stomata density in the medium lands and lowlands. Significant differences were also seen in the length and aperture of stomata in the highlands compared to the medium lands and lowlands, but the width of the stomata was not significantly different at all altitudes. The open percentage of stomata in the highlands and medium lands was not significantly different (Table 2) with a difference of 2.97%.

Trichome

Trichomes on the leaf and stem in the highlands were seen less than in the medium lands and lowlands with relatively the same length (Figure 2, Figure 3). The number of leaf trichomes at all altitudes ranged from 4.23 to 8.08 (scale 100 μm). The number of abaxial trichomes is more than the adaxial part. A significant difference was seen in the number of trichomes in the highlands and did not show significant difference in the number of trichomes in the medium lands and lowlands (Table 3). Qualitatively, the stem trichomes were thicker in the lowlands than in the highlands (Figure 3). As the altitude decreases, there is a tendency to increase the number of trichomes both on the stems and leaves of stevia plants.

Table 1. Description of microclimate components at several altitudes in a tropical climate

Climate variable	Highland	Medium land	Lowland
Temperature maximum (ºC)	29.0	33.4	34.1
Temperature minimum (ºC)	10.3	15.7	17.4
Average temperature (ºC)	19.1	24.5	25.2
Humidity maximum (%)	100.0	99.9	99.9
Humidity minimum (%)	40.5	38.0	37.7
Average humidity (%)	87.9	78.7	74.7
Percentage of temperature exposure to stevia during cultivation			
Temperature < 20ºC (%)	63,54	7.15	6.04
Temperature 22ºC - 24ºC (%)	14.37	35.69	30.97
Temperature > 24ºC (%)	6.70	30.12	38.19
Temperature > 30ºC (%)	0.03	14.75	17.98

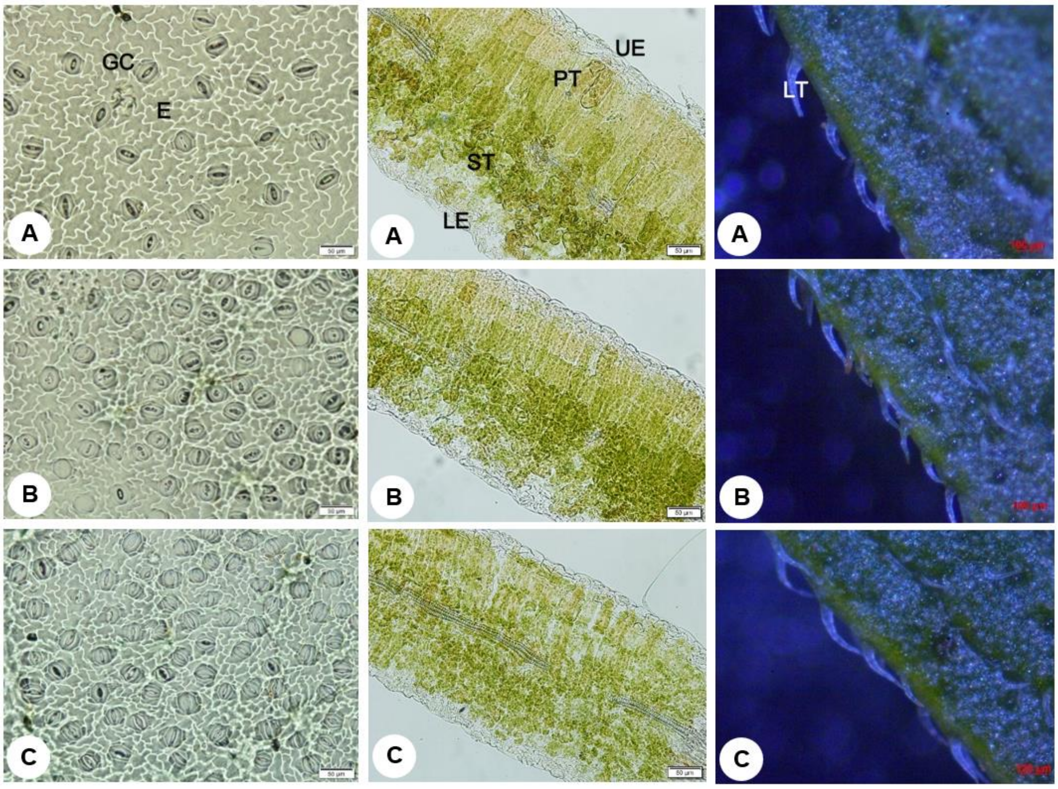

Figure 2. Stomata (left), leaf cross sections (middle), and leaf trichome (right) of *Stevia rebaudiana* at different altitudes. A: highland, B: medium land, C: lowland, GC: guard cell, E: epidermis, UE: upper epidermis, LE: lower epidermis, PT: palisade tissue, ST: sponge tissue, LT: leaf trichome

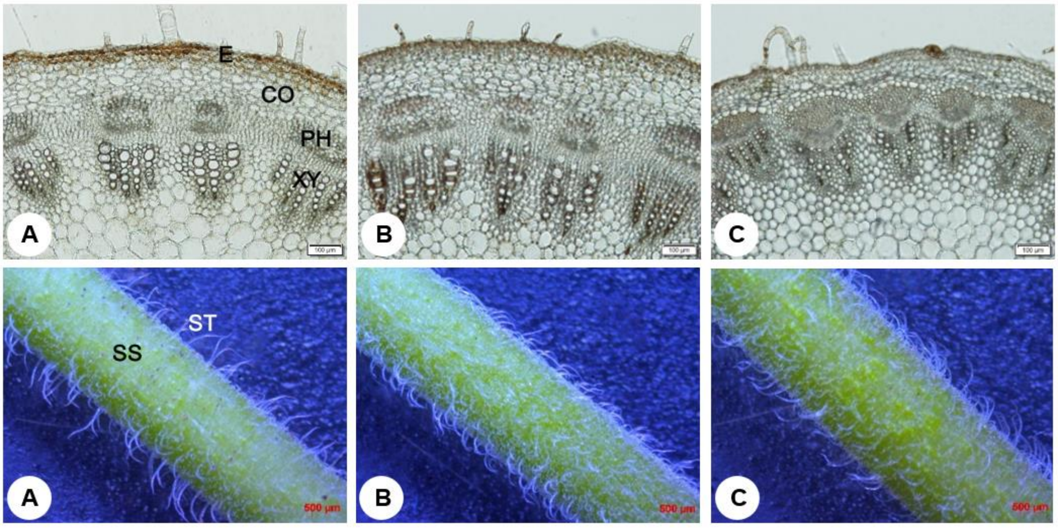

Figure 3. Stem cross-sections (top) and stem trichome (bottom) of *Stevia rebaudiana* at different altitude. A: highland, B: medium land, C: lowland, E: epidermis, CO: cortex, PH: phloem, XY: xylem, ST: stem trichomes, SS: stevia stem

Table 2. Characteristics of stevia stomata on environmental changes at several altitudes

Different altitude	Stomata density (unit/mm^2)	Open percentage (%)	Stomata dimension		
			Length (µm)	Width (µm)	Aperture (µm^2)
Highland	160.54 a	96,12 b	24.35 b	12.04 a	231.45 b
Medium land	245.76 b	93,15 b	21.27 a	11.31 a	190.04 a
Lowland	258.97 b	87,08 a	21.36 a	11.10 a	187.34 a

Note: Rows with different letters across treatments are significantly different at $P < 0.05$ according to the LSD test

Table 3. Characteristics of stevia leaves on environmental changes at several altitudes

Different altitude	Leaf thickness (µm)	Palisade length (µm)	Leaf trichome number		Xylem diameter (µm)
			Adaxial	Abaxial	
Highland	415.25 b	174.41 b	4.23 a	6.07 a	32.91 b
Medium land	382.56 ab	155.61 ab	6.21 b	8.03 b	30.97 ab
Lowland	376.32 a	144.88 a	6.69 b	8.68 b	29.14 a

Note: Rows with different letters across treatments are significantly different at P < 0.05 according to the LSD test

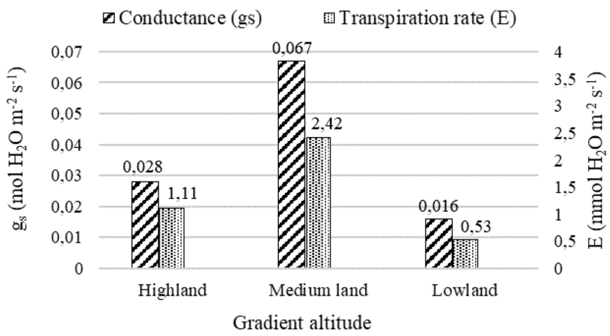

Figure 4. Conductance (g_s) and transpiration rate (E) of stevia with PPFD level at 1400 µmol m^{-2} s^{-1} at several altitudes

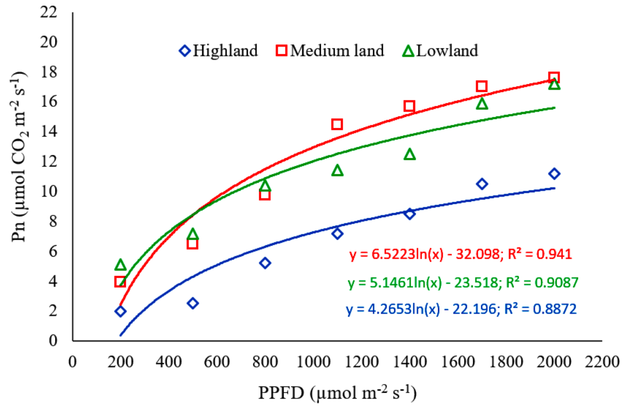

Figure 5. Photosynthesis rate in light intensity level ranges from 200 s/d 2000 (µmol m^{-2} s^{-1}) at several altitudes

Vascular bundle

The cross-section of stems differs in the size of the vascular bundle at all altitudes. In the lowlands, the epidermis and cortex are arranged thinner and the cell size is smaller than in the highlands and medium lands. Likewise, the size of the vascular bundle (xylem) in the lowlands is smaller than the others (Figure 3). Xylem diameter showed a significant difference in the highlands and lowlands ($P \leq 0.05$). The largest xylem diameter was found in the highlands at 32.91 µm and it was not significantly different from the xylem diameter in the medium lands (Table 3). The increase in the diameter of the xylem tissue was not only in the stem but also in the petiole (data not shown).

Physiological characteristics

Stomata conductance and transpiration rate

Differences in altitude had an effect on stomata conductance and transpiration rate. Conductance was measured at a PPFD level of 1400 mol $m^{-2}s^{-1}$. The highest conductance was found in the medium lands (0.067 mol H_2O $m^{-2}s^{-1}$) and followed by the highlands and the lowlands. Increased stomata conductance induces stomata opening thereby increasing gas exchange and water transpiration. The transpiration rate increased with increasing stomata conductance. The highest transpiration rate of 2.42 mmol H_2O $m^{-2}s^{-1}$ was found in the medium lands and the lowest in the lowlands was 0.53 mmol H_2O m^{-2} s^{-1} (Figure 4). The value of conductance and transpiration rate doesn't show a tendency to always decrease with decreasing altitude.

Photosynthetic rate

The net photosynthetic rate (Pn) curves made under different light intensities generally show a trend of increasing Pn with increasing light intensity (Figure 5). The Pn of stevia in the highlands is measured as the lowest compared to the medium lands and lowlands. The Pn curve in the highlands is noted to increase slowly with increasing light intensity and tends to be constant after PPFD levels of 2000 mol $m^{-2}s^{-1}$. At low light intensity (PPFD levels 200-400 mol $m^{-2}s^{-1}$), the Pn of stevia in the lowlands is highest and then tends to decrease compared to Pn in the medium lands. Meanwhile, the photosynthesis rate in the medium lands was recorded as the highest and tends to increase with increasing light intensity by forming a logarithmic curve (R^2=0.94). At PPFD levels of 1400 mol $m^{-2}s^{-1}$, the highest CO_2 assimilation was recorded in medium lands with a difference of 3.2 mol CO_2 $m^{-2}s^{-1}$ compared to lowlands and 7.17 mol CO_2 $m^{-2}s^{-1}$ compared to highlands.

Discussion

In recent years, the increasing demand for stevia in the world has led to an increase in the development of stevia production in several parts of the world, both in sub-tropical climate areas as the center of origin for stevia to tropical climate areas, such as Brazil, India, Thailand, Malaysia, and Indonesia. It was reported that people with diabetes in 2030 are expected to increase to 366 million people in the world and 21.3 million people in Indonesia (Wild et al. 2004; Sharma et al. 2016). The stevia plant has the potential to meet the ever-increasing demand for sweeteners for diabetics. Stevia can adapt to tropical climates, although its growth is not as good as in sub-tropical climates. Temperatures in tropical climates are relatively higher than in sub-tropics because they receive more solar radiation with the radiation energy reaching the surface on average of 700 Wm^{-2}. However, tropical climate conditions are quite heterogeneous depending on rainfall (season), distance from the equator, and altitude (Galvin 2016). The highlands temperature is relatively lower than the lowlands. The average temperature is quite related to the average humidity with a high correlation of -0.98. The characteristics of climate in the highlands have high humidity and low temperature. The high-intensity radiation that reaches the surface is mostly absorbed by water vapor and heat energy is stored in the form of latent heat. Besides that, solar radiation encounters a process of reflection, absorption, and transmission by matter in the atmosphere, such as water vapor (clouds), suspended particles (dust, smoke), pollutant gases (CO, NO_2, SO_2), and ozone (Galvin 2016; Ariffin et al. 2020). So that the ambient air temperature in the highlands was low.

The optimum temperature for stevia growth was between 22-24ºC (Yadaf et al. 2011). The medium lands present suitable conditions for stevia by providing optimum growth temperature conditions during growth of 35.69%. Stevia in the highlands obtained the optimum temperature conditions for growth at only 14.37% and exposure to temperatures above 29ºC was very small, namely 0.03% (Table 1). Plants that are exposed to high temperatures at least 5ºC above the optimum growth temperature represent a response and cell metabolism to survive (Bita and Gerats 2013). It is claimed that the highlands are suitable for growing stevia in tropical climates. However our findings suggest that the exposure to optimum temperature in a tropical climate is greater in the medium lands than in the highlands.

Stevia adapts to changing microclimates at different altitudes to grow well and complete its life cycle. It can be seen that the anatomical characteristics are diverse at different altitudes. In this study, the enhancement of ambient temperature caused increasing in stomatal density, a decrease in the open percentage, and the stomata aperture. The decrease in a stomatal aperture in high-temperature conditions may reduce stomata size. This corresponds to Shahzad et al. (2015) and Shen et al. (2017) that high-temperature stress causes reduced cell size, stomata closure, and increased stomatal density. This is the response of stevia at ambient high-temperature conditions to reduce water loss in plants. High temperatures were often associated with reduced water availability and leaf water potential (Chen et al. 2014). It caused increasing in stomata sensitivity to increase abscisic acid transport to leaves (Medina and Gilbert 2015). Increasing abscisic acid causes a decrease in stomata conductance, thereby inducing stomata closure to reduce water loss from the transpiration process (Killi et al. 2017). Similar responses are also reported in *Solanum lycopersicum* (Zhang et al. 2014) and Rhododendron (Shen et al. 2017). The stomatal density of *Herpetospermum pedunculosum* decreased with increasing altitude (Zhao et al. 2019).

Differences in altitude affect the characteristics of leaf tissue. Stevia leaves in the highlands tend to be thicker than in the lowlands due to the larger cell size of leaf tissue. Increasing the cell size of stevia leaves was a form of plant adaptation to low-temperature conditions. Stevia can survive in the temperature range of -6ºC to 43ºC (Moraes et al. 2013; Munz et al. 2018). The higher altitude effect the increase in air density and UV-light intensity (Li and Jichao 2017). Leaf thickening was an adaptation mechanism of stevia plants to low-temperature conditions and high-intensity ultraviolet irradiation in the highlands (Liu et al. 2020). Leaf thickness increases under cold temperatures (Stewart et al. 2016; Hajihashemi et al. 2018). The palisade and spongy thickenings play a major function in reducing tissue damage caused by abundant solar radiation and maintaining high levels of photosynthesis. This suggests that leaf thickening contributes to stevia adaptation with low temperatures and high-intensity UV irradiation in the highlands.

In addition, the microclimate conditions of lowlands in the tropical are relatively unfavorable for the growth of stevia, especially the threat of high temperatures and drought. Stevia in the lowlands was exposed to temperatures above the optimum temperature (above 30ºC) of 17.98% during growth in the field, while in the highlands, it was only 0.03%. It is suspected that this condition causes temperature stress on stevia. This reason is in line with Bhusal et al. (2018) that wheat encounter high-temperature stress when grown at temperatures of 25-32ºC for 1 to 2 weeks, where the optimum temperature of wheat was 20-25ºC. So stevia in the lowlands has the potential to be exposed to high-temperature stress by showing a lower leaf thickness compared with the highlands. The thickness of a vertical section became thinner in the stressed leaves. Cells in the upper epidermis thinned, the length of cells in palisade tissue became shorter, and the ratio of palisade tissue to spongy tissue decreased (Zhang et al. 2014). *Brassica campestris* L. growing in high-temperature conditions decreased the thickness of the palisade mesophyll, and upper and lower epidermis (Yuan et al. 2017). Besides that, the potential of heat stress occurs quite large in the lowlands as indicated by higher electrolyte leakage compared to medium lands and highlands (data not shown).

The decrease in the cross-sectional area of the vascular tissue may be a response of stevia to high-temperature conditions and water deficit. An increase in environmental temperature causes an increase in the transpiration rate so that a decrease in the xylem diameter can reduce plant water loss. Chen et al. (2014) reported that high-temperature stress on high-temperature-sensitive plants of *Raphanus sativus* showed a decrease in the size of vessel elements in the petiole, while intolerant plants it tended to have no significant effect. This is reinforced by Shen et al. (2017) who reported a decrease in the thickness of the main vein in *Rhododendron* plants under conditions of high-temperature stress. The increase in altitude causes an increase in the main vein of the leaf, which has an important function in providing water to the leaves. The large main vein creates a large water transport capacity for plants. Increasing the thickness of the main vein is a plant strategy for increasing water transport at low-temperature conditions in the highlands (Wang et al. 2016; Liu et al. 2020). Water transport is mainly related to the structure of the xylem vessels in plant stems. The increase in xylem vessels in response to cold stress increase water transport from roots to leaves to prevent reductions in water potential (Hajihashemi et al. 2018).

The number of trichomes tends to increase with decreasing altitude. This is thought to be an adaptation of stevia in reducing the air flow rate on the leaf surface so that the movement of water from the leaves to the air runs slowly. So that the increase in trichomes can reduce the transpiration rate of plants. Trichomes can decrease the temperature by increasing the leaf surface boundary layer, which protects plants from water losses. The thickness of the boundary layer depends on the surface roughness. The thicker the boundary layer results in a decrease in air movement on the leaf surface, so the transpiration rate decreases (Ariffin et al. 2020). Trichomes can play an important role in protecting plants from damage through the physical structure. Trichomes can reduce the penetration of solar radiation and increase radiation reflection to reduce the effects of heat and damage by UV radiation (Zhang et al. 2020). The increase in the number of trichomes was a response of stevia to high-temperature environmental conditions that reduced excess water loss in plants.

Loss of water in plants due to the process of transpiration. In the lowlands, the transpiration rate was lower than in the highlands. There may be a mechanism of water-using efficiency by stevia in response to soil moisture deficit conditions. Water conservation in plants through decreases in stomatal conductance or a limitation of transpiration under high evaporative gradients (Sinclair 2012). Stomata play a critical role in regulating water loss. An increase in evaporation rate reduces guard cell turgor more than epidermal turgor, and aperture declines (Urban et al. 2017). Besides that, the increasing number of trichomes is known to play an efficient role in reducing water loss by decreasing the rate of transpiration (Ning et al. 2016). However, it is interesting to see the transpiration rate in the medium lands with the highest value compared to the others. This is presumably due to soil moisture being relatively maintained in the medium lands compared to the lowlands. The large size of the xylem and stomata aperture in the medium lands (Table 2; Table 3) causes the transpiration rate to be greater than the evaporation rate, so it is very beneficial for plants in the leaf cooling process to avoid damage to photosynthetic apparatus due to high temperature. In addition, an increase in stomata aperture increases the rate of CO_2 uptake in plants. So the high availability of water and CO_2 in leave can increase the photosynthesis rate of plants.

The photosynthesis process in plants is influenced by environmental factors, such as light intensity and temperature. Stevia in the lowlands and medium lands have a higher photosynthetic rate than those in the highlands. It is suspected that the temperature in the medium lands and lowlands is quite supportive in the photosynthesis process where exposure to temperatures above 20ºC was 92.85%-93,96%. Yamori et al. (2014) reported the optimum temperature of the photosynthesis process is generally between 20ºC and 30ºC. According to the above, the thermal optimum for photosynthesis causes photosynthetic rates to decrease, but the respiration rate continues to increase. Meanwhile, stevia in the highlands has a low photosynthesis rate. It is suspected that low temperatures below 20ºC dominate the stevia growth by 63.54% (Table 1), so the photosynthesis process takes place under optimum temperature conditions. Besides that, a high stomata conductance and transpiration rate in the medium lands make the availability of H_2O and CO_2 always maintained in the leaves to support a high photosynthesis rate.

Based on the anatomical and physiological characteristics of stevia, the plant in medium lands and highlands shows good adaptability. Accordingly, the potential for the production of stevia leaves will likely be obtained in large quantities. This is in accordance with Pal et al. (2015), which reported a dry leaf yield range of 0.79 to 1.91 ton ha^{-1} in highlands and medium lands, while in lowlands range of 0.39 to 0.83 ton ha^{-1}. Thus, the medium lands have the potential as a stevia development area in a tropical climate, considering the increasingly competitive use of agricultural land in the highlands. Water use efficiency technology is an important concern for the successful development of stevia in medium lands.

In conclusion, Differences in altitude generate differences in temperature and humidity. Stevia was exposed to temperatures above 20ºC during growth in the highlands by 36.46%, while in the medium lands to lowlands by 92.85%-93.96%. Changes in temperature and humidity affect anatomical and physiological characteristics. *Stevia rebaudiana* adapts to high temperature and low humidity environments by increasing stomatal density, trichomes, and photosynthesis rate. In addition, it reduces stomata width, stomata aperture, open percentage of stomata, leaf thickness, palisade length, xylem diameter, conductance, and transpiration rate. The response of stevia in the highlands and medium lands environment indicates good adaptability.

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