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Physiological Responses of Indigenous Vegetable of Sintrong (*Crassocephalum crepidioides*) due to Exposure to High Temperature

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Abstract

Sintrong is an Indonesian indigenous vegetable with leaves used for vegetables, digestive disorders, and burns. Changes in the environment due to an increase in temperature affect the growth and quality of sitrong, and its existence in the nature is threatened. This study aims to obtain information about the effect of exposure to high temperatures on the physiological character and flavonoid content of indigenous sintrong vegetables and obtain accession of sintrong, which can be developed as a functional vegetable. The Nested randomized group design was applied with two factors, temperature differences as the main plot and accession as a second plot. Four replications were conducted for each accession in the Cikabayan experimental garden of IPB. The results showed that exposure to high temperatures up to 32 °C increased the speed of flowering age, which was 4.76% and 7.14% faster and showed a high wilting rate of 36.66%, but decreased leaf area index up to 30.30% and 42.42% at the conditions above ambient temperature exposure (control). Flavonoid content did not show any effect due to exposure to high temperatures. The flavonoid content reached 1695.38 and 1834.83 mg QE 100 g^{-1}. Bogor 1 accession showed the best performance so that the plants can be developed for functional vegetables. Based on the research findings, sintrong should be harvested earlier before flowering to obtain high leaf production and good-quality vegetables.

Keywords: anthocyanins; flavonoid; heat stress; stomata conductance; vegetables

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INTRODUCTION

Sintrong *(Crassocephalum crepidioides)* is one of the indigenous vegetables. The part of sintrong that is commonly used is the leaf. Adjatin et al. (2013) conducted the phytochemical screening of sintrong and reported that the leaves contain cathectic tannins, gallic tannins, coumarins, flavonoids and steroids. Sintrong leaves are used traditionally to treat digestive disorders and burns (Can et al., 2020). Sintrong has anti-bacterial (Owokotomo, 2018), antioxidant (Aniya et al., 2005), anti-inflammatory (Akinpelu et al., 2019), antitumor (Tomimori et al., 2012) and antidiabetic activity (Bahar et al., 2017).

Climate change is a threat to nutrition and food security (Tanimonure, 2021), especially food that is still harvested from the wild. Global climate change is indicated by an increase in the average temperature of the earth's surface that reached 0.87 °C in 2006 to 2015 (IPCC, 2021). The increase in average temperature at the end of the 21st century is relatively larger, between 2.4 and 3.5 °C (IPCC, 2022). The development of sintrong, which is an indigenous vegetable, is a challenge because some of them are not cultivated. Environmental changes due to

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increasing temperatures will affect the growth and quality of vegetables and will even threaten their existence in nature. Vegetables have different responses to changes in temperature, depending on each plant species (Bashandy and El-Shaieny, 2021). High temperatures affect all phases of plant growth and metabolism (Sailaja et al., 2014).

High temperature is a significant limiting factor in supporting the productivity and quality of rice harvests (Liu et al., 2013). Jaisyurahman et al. (2020) reported that high temperatures 32.72 °C after the generative phase in rice affected the character of the total number of tillers, the number of filled grains per panicle, the number of empty grains per panicle, the total number of grains per panicle, the rate of seed filling, the percentage of filled grain and the total grain weight per plant. Heat stress over a long period of time in several tomato cultivars resulted in a significant decrease in all reproductive traits, such as pollen viability, pollen count, mud fertility, fruit set and number of flowers per flowering (Xu et al., 2016). Alhaithloul et al. (2021) reported that the identification of total phenolics, flavonoids and the abundance of secondary metabolites in tomato seedlings was associated with thermal stress. This accumulation is a defense mechanism due to thermal stress.

The impact of exposure to high temperatures can affect the availability of nutritious food for the community, especially food available in nature, because its growth depends entirely on the environment (Giulia et al., 2020). Conditions of increasing temperature above the tolerance limit can threaten the food sustainability. This study examined the effect of temperatures above the ambient temperature on the physiological character of the sintrong. This study will provide information about the effect of temperature exposure on physiological and flavonoid characteristics, as well as the best accessions that are able to adapt to exposure to high temperature.

MATERIALS AND METHOD

The field research was conducted at 6°33’01” S and 106°42’51” E Cikabayan experimental field, IPB University, using UV house. Analysis of flavonoid content was conducted at Post Harvest Laboratory of Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, from May to September 2022. High-temperature condition was obtained in a modification of the UV house building on the percentage of wall covering so as to get the temperature range based on the treatment. Temperature measurement was carried out using a thermo recorder. Thermo recorder can record temperature every 30 minutes. Average daily temperature was obtained from the average air temperature at 00:00 to 23:30 during treatment period. The maximum temperature was obtained from the highest daily average temperature and the minimum temperature was obtained from the lowest daily average temperature during the period. This study used a nested randomized block design with two factors of treatment, namely temperature difference as the main plot and accession as the second plot. The temperature difference consists of three levels, including the ambient temperature (T1), the daily average air temperature in the UV house with an average of 30±2 ℃ (T2), and the average daily air temperature in the UV house with an average of 32±2 ℃ (T3). Plant accessions consist of Bogor 1 (Bogor, lowland), Bogor 2 (Bogor, highland) and Cianjur 1 (Cianjur, lowland), based on the seeds. At each accession level, four replications were conducted to obtain 12 experimental units. One experimental unit was a plot of 1.5 x 1.5 m^2, with a population per plot of 25 plants with a spacing of 20 x 20 cm^2, so that the total plants were 900. High-temperature treatment was provided from transplanting stage until 45 days after planting (DAP). Plant maintenance includes watering, weeding, and fertilizing. Sintrong was harvested at 45 DAP.

Observation was conducted at flowering stage, at the age of full flowering of 50%. The wilting rate was observed by assessing the percentage of leaf fall. Leaf area index (LAI) was measured by the total leaf area on the area of land covered by the plant. The leaf area was measured with ImageJ. Relative growth rate (RGR) was measured at 30 and 40 DAP using the Hoffman and Poorter (2002) and pigment content was analyzed using Sims and Gamon (2004). The rate of photosynthesis, stomatal conductance, and transpiration was measured using the Licor-6400 XT portable photosynthesis system. Anatomical observations include the density of stomata and trichomes using an Olympus CX 23 led binocular microscope.

The preparation of flavonoid sample was conducted with a sample of dried leaves at a temperature of 60 °C for 24 hours. The sample was mashed and sifted, and then stored in a container with silica gel. A sample of 0.01 g was weighed for extraction analysis and powder moisture content was established by weighing. The samples of 1 to 2 g were then ventilated at 105 °C for 5 hours. Extraction was conducted by taking 2 g of simplicial powder and 70% ethanol solvent, homogenized using a vortex for 15 minutes, then sonicated with sonicator for 15 minutes and macerated for 24 hours in dark space, centrifuged for 15 minutes to produce filtrate. The filtrate was then mixed and evaporated until the extract volume was 10 ml (Istiqomah, 2020). The flavonoid content was analyzed by the $AlCl_3$ method (Pothitirat et al., 2009). Briefly, 5 ml of 2% aluminum trichloride in methanol was mixed with the same volume of sample (500 µg ml^{-1}). Absorption reading at 415 nm was done after 10 minutes against a blank sample without aluminium trichloride using UV–visible spectrophotometer Shimadzu Type UV-1280. The standard used for the analysis of flavonoids was quercetin with the standard curve at concentrations of 2, 5, 25, 50 and 75 mg l^{-1}. The total flavonoid content was counted as grams of quercetin equivalents (QE) 100 g^{-1} of the extract (Vongsak et al., 2013). Collected data were analyzed statistically according to one-way analysis of variance (ANOVA) technique. If the treatment gave statistically significant effect ($p < 0.05$), the different sources of variation were compared using Duncan's multiple range test (DMRT) using SAS 9.0.

RESULTS AND DISCUSSION

Temperatures above ambient have a significant effect on flowering age, wilting rate, LAI and RGR of sintrong plants (Table 1). The flowering ages of the sintrong at 30.49±0.25 and 32.23±0.28 °C were faster, 4.76% and 7.14% at above ambient temperature exposure conditions. The flowering ages at 28.89±0.40, 30.49±0.25, and 32.23±0.28 °C were respectively 42, 40 and 39 days. Flowering age was calculated when the flowers first appeared on the sintrong based on nine samples of plant each plot. The transition from the vegetative to the generative phase is an important event; the flowering age has an important role in plant reproduction and the ability to adapt to the environment (Guirao et al., 2019). Factors that affect flowering time include vernalization, photoperiodicity, autonomic and gibberellin pathway (Mouradov et al., 2002). The flowering signals appear on the leaves and then transmitted to the apical meristems in the shoots where they flower (Tsoy and Mushegian, 2022). High temperatures will trigger a flowering signal so that flowering will occur more quickly. The regulatory gene in flowering is *Flowering Locus C* (FLC). FLC will encode protein for flowering (Xu et al., 2016). In addition, high temperatures reduce the flowering period, which in turn reduce the accumulation of dry matter in *Tanacetum cinerariifolium* (Suraweera et al., 2020).

The exposure of sintrong to high temperature shows a wilting response with the characteristics of the leaves falling or drooping. The temperature treatment of 32.23±0.28 °C showed a high wilting rate of 36.66% (Table 2). This is presumably because exposure to high temperatures causes the plant to lose a lot of water due to a faster transpiration rate. Guo et al. (2022) reported that heat stress in tomato plants induces an increase in the rate of transpiration, which results in dehydration of plant organs so that the leaves fall down and wither.

Table 1. Effect of high temperature and accession on the flowering age of sintrong, LAI and RGR

Treatment	Flowering age (days)	Wilting rate (%)	LAI	RGR (g day^{-1})
Temperature (°C)				
T1 (28.89±0.40)	42±0.50a	9.6±6.31c	3.3±0.34a	0.28±0.03a
T2 (30.49±0.25)	40±0.27b	22.05±7.66b	2.3±0.30b	0.19±0.04b
T3 (32.23±0.28)	39±0.62c	36.66±8.17a	1.9±0.45c	0.15±0.02c
Accession				
Bogor 1	41±1.64	24.40±11.31	2.78±0.59a	0.24±0.08
Bogor 2	41±1.25	21.89±13.65	2.35±0.71b	0.20±0.07
Cianjur 1	41±1.37	22.10±13.54	2.48±0.62b	0.18±0.07

Note: Numbers followed by the same letter in the same column show no significant difference based on DMRT at level of 5%

Table 2. Effect of high temperature and accession on leaf pigment content

Treatment	Pigment content (mg g^{-1})				
	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids	Anthocyanins
Temperature (°C)					
T1 (28.89±0.40)	1.06±0.08b	0.37±0.03b	1.43±0.11b	0.37±0.02b	0.02±0.005b
T2 (30.49±0.25)	1.09±0.12b	0.39±0.05b	1.48±0.17b	0.38±0.04b	0.03±0.004b
T3 (32.23±0.28)	1.27±0.13a	0.47±0.05a	1.75±0.18a	0.43±0.03a	0.05±0.01a
Accession					
Bogor 1	1.17±0.11a	0.42±0.04a	1.60±0.15a	0.40±0.03a	0.03±0.01
Bogor 2	1.19±0.13a	0.38±0.06b	1.45±0.19b	0.37±0.04b	0.03±0.02
Cianjur 1	1.06±0.17b	0.42±0.06a	1.61±0.23a	0.41±0.05a	0.03±0.01

Note: Numbers followed by the same letter in the same column show no significant difference based on DMRT at level of 5%

LAI is the ratio of the total leaf area of the plant to the projected area covered. The ambient temperature treatments with 30.49±0.25 and 32.23±0.28 °C showed LAI of 3.3, 2.3, and 1.9, respectively. The percentage of decrease in LAI above the ambient temperature of 30.49±0.25 and 32.23±0.28 °C was 30.30% and 42.42%, respectively (Table 2). The LAI decreases and the temperature increases. High LAI values at ambient temperature indicate that young leaves on the shoots are able to absorb large amounts of radiation and high CO_2 assimilation rate and translocate to other plant parts in large quantities. High temperatures with an average of 35.3 °C reduced LAI in peas, and LAI value in Arka Chaitra variety peas aged 60 days after sowing was 0.88 (Verma, 2019). Exposure to high temperatures also decreased LAI faster due to the presence of wheat leaf senescence. Senescent induces a signal of seed maturity so that the plant will complete its life cycle faster (Chen et al., 2017). LAI is influenced by leaf distribution and density, which are related to plant spacing and population.

The closer the spacing, the higher the leaf density and the lower the exposure to sunlight reaching the lower leaf layers so LAI value increases (Du et al., 2022). The inhibition of leaf expansion will have an impact on decreasing the capacity of the leaves to absorb light, so that it will minimize the performance of the rate of photosynthesis in plants.

RGR observations were at the age of 30 (vegetative phase) and 40 DAP (maximum vegetative phase) with destructively weighing the dry weight of roots, stems and leaves at 80 °C for 2 x 24 hours. Exposure to high temperature decreased RGR compared to ambient temperature. The RGR value at 30.49±0.25 °C decreased by 32.14% while at 32.23±0.28 °C decreased by 46.42% (Table 2). This decrease is thought to be due to changes in photosynthate allocation (Hoffmann and Poorter, 2002). RGR observations were carried out at the age of 40 DAP, when the sintrong entered its maximum vegetative state, so that the accumulation of photosynthate was no longer focused on vegetative growth, which would reduce the RGR. RGR was also lower during the stress phase in heat-sensitive wheat genotypes (Abdelhakim et al., 2021).

Differences in accessions did not affect flowering age, wilting rate and RGR, but differences in accessions affected LAI. Accessions of Bogor 1, Bogor 2 and Cianjur 1 have the same flowering age of 41 days. Bogor 1 has the largest LAI value of 2.78, followed by Cianjur 1 and Bogor 2, which are not significantly different. This is presumably because Bogor 1 comes from the lowlands and is shaded, so it has a wider leaf area than other accession. The higher leaf area will increase the LAI value.

Exposure to high temperature had a significant effect on chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and anthocyanins (Table 2). Chlorophyll a and b increased with increasing temperature. The increases in chlorophyll a and b at a temperature of 32.23±0.28 °C were 16.53% and 21.27%, respectively. Chlorophyll a is in the reaction center of photosystems I and II, and in the antenna pigment, while chlorophyll b is found in the antenna pigment, only which is useful for capturing light (Handayani et al., 2013). Exposure to high temperatures caused the loss of pigment content in flag leaves, by reducing the chlorophyll a+b content in two wheat cultivars JM 22 and XM 26 by 1.031 and 0.778 mg ml^{-1} g FW^{-1} (Feng et al., 2014). The results of this study showed that chlorophyll a and b increased along with plants exposed to higher than ambient temperatures. This is presumably because the sintrong plant is tolerant to high-temperature exposure conditioned in this study. In addition, it is suspected that the sintrong plant is found in the nature so that it has a good level of adaptation to high temperatures as evidenced by the increase in chlorophyll. The low decrease in chlorophyll content, the ratio of chlorophyll a:b, and total chlorophyll indicate the tolerance to high temperatures (Almeselmani and Viswanathan, 2012).

Carotenoids are terpenoid compounds that become photosynthetic pigments with a color effect between red and yellow. Violaxanthin is a member of the carotenoids found in the chloroplast membrane, which causes a yellow color (Zaripheh and Erdman, 2002). High temperatures increase the carotenoid content in sintrong plants. The carotenoid content at 32.23±0.28 °C was significantly different with those at 28.89±0.40 and 30.49±0.25 °C. The increase in carotenoids was at the temperature of 32.23±0.28 °C when compared to the ambient temperature of 12.5%. However, in this study, the increase in carotenoids is a form of plant defense against photooxidation damage at high-temperature conditions (Rossi and Huang, 2022).

Anthocyanins belong to the class of flavonoid compounds that are included in the group of natural pigments in plants dissolved in water, which function to give color to flowers, fruits and vegetables (Maulid and Laily, 2015). The anthocyanin content at ambient temperature of 30.49±0.25 °C was not significantly different, but at 32.23±0.28 °C, it was significantly different. The increase in anthocyanins at a temperature of 32.23±0.28 °C was by 60%, compared to those at ambient temperatures. Anthocyanins function as specific light shields that absorb visible and UV radiation in the vacuole and prevent UV rays from penetrating into the tissue (Subira et al., 2021). High anthocyanin content can increase absorption and tolerance to UV radiation and increase its antioxidant capacity.

The difference in accessions showed a significant effect on chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. Chlorophyll a contained in Bogor 1 and Bogor 2 was higher than that in Cianjur 1. Leaf surface area streamlines the capture of light energy for photosynthesis (Dharmadewi, 2020). It is considered that Bogor 1 and Bogor 2 accessions are able to harvest higher light energy so that the chlorophyll a content is higher because of a wide leaf area. Chlorophyll b contained in Bogor 1 and Cianjur 1 accessions was higher than that in Bogor 2. Bogor 1 and Cianjur 1 accessions had higher chlorophyll b content, presumably because Bogor 1 and Cianjur 1 were accessioned from the lowlands. In the lowlands, light exposure is more optimal than in the highlands. Bogor 2 has low chlorophyll b, which reduces the total chlorophyll. This is so because Bogor 2 was from highland. The total chlorophyll Bogor 1 and Cianjur 1 accessions were higher than Bogor 2. High total chlorophyll will encourage plants to carry out photosynthesis more optimally.

Exposure to high temperature has a significant effect on the rate of photosynthesis, stomata conductance and transpiration (Table 3). Photosynthesis rate at temperature 32.2±0.28 °C was 2.52% higher when compared to the rate at ambient temperature 28.89±0.40 °C with a photosynthetic rate of 31.05 µmol CO_2 m^{-2} s^{-1}, while at a temperature of 30.49±0.25 °C, the photosynthesis rate increased 8.61%, reaching 27.16 µmol CO_2 m^{-2} s^{-1}. The rate of photosynthesis is sensitive to the changes in temperature, which will result in an imbalance of energy in the cell. High temperature affects the photochemical reactions in thylakoids and carbon metabolism in the stroma affects each

Table 3. Effect of high temperature and accession on photosynthesis rate, stomatal conductance and transpiration

Treatment	Photosynthesis rate (µmol CO_2 m^{-2} s^{-1})	Stomatal conductance (mmol H_2O m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})
Temperature (°C)			
T1 (28.89±0.40)	24.82±1.09c	1.19±0.36a	3.36±0.50b
T2 (30.49±0.25)	27.16±0.80b	0.73±0.13b	4.95±0.45a
T3 (32.23±0.28)	31.05±1.64a	0.69±0.13b	5.05±0.75a
Accession			
Bogor 1	28.21±3.00	0.82±0.29	4.58±0.60
Bogor 2	27.15±2.81	0.93±0.32	4.48±1.26
Cianjur 1	27.66±2.71	0.87±0.35	4.58±0.92

Note: Numbers followed by the same letter in the same column show no significant difference based on DMRT at level of 5%

other (Wang et al., 2018). The application of sulfur in the conditions with an increase in high temperature in canola showed a photosynthetic rate of 6.12 mol m^2 s^{-1} (Waraich et al., 2021). High temperature can damage photosynthetic apparatus, such as PS I, PS II, *cytochrome b6f (Cytb6f)* complex and rubisco inactivation (Hu et al., 2020). In this study, the opposite applies to high temperatures, in which the rate of photosynthesis increases. This is presumably due to the availability of sufficient water during the growth so that it can compensate for water loss due to increased transpiration.

Stomatal conductance describes the supply of CO_2 from the atmosphere to the intercellular space of the cell. Stomatal conductance at 30.49±0.25 and 32.23±0.28 °C was not significantly different. Stomatal conductance at temperatures above ambient 30.49±0.25 and 32.23±0.28 °C was 0.73 and 0.69 µmol CO_2 m^{-2} s^{-1}. Stomatal conductance decreases with increasing temperature and this is presumably because at high temperatures, the stomata will close to prevent water loss due to high transpiration (Anggraini et al., 2016). The high temperature inside the leaves results in a decrease in stomatal conductance in olives and this decrease in stomatal conductance aims to minimize the risk of xylem embolism. Similar to the study on *Cyamopsis tetragonoloba* with a high temperature of 42 °C, the stomatal conductance value was reduced to 45 mmol m^{-2} s^{-1}, lower than the control of 49 µmol CO_2 m^{-2} s^{-1} (Alshameri et al., 2019). At high temperatures, low water availability and high evapotranspiration favor a decrease in stomatal conductance because a higher transpiration rate results in lower xylem vessel pressure (Haworth et al., 2018).

The transpiration process starts from the absorption of groundwater by plant roots. The water was transported through the stems to the leaves and released as water vapor into the atmosphere. Transpiration increases with increasing temperature. Transpiration rate of sintrong plants exposed above ambient temperatures of 30.49±0.25 and 32.23±0.28 °C increased, compared to the ambient temperature with an increase of 32.12% and 33.46%. The water absorbed by plant roots from the soil is not fully used to produce dry matter, because most of the total water absorbed by the roots will be lost through the transpiration process. The main driving force of the transpiration process is the water potential gradient between the space in the stomata and the atmospheric air (Hidayati and Anas, 2016).

Exposure to high temperature had a significant effect on stomatal and trichome density; differences in accessions did not show a significant effect on stomatal and trichome density (Table 4; Figure 1 and 2). Stomata density increased as it increased above ambient temperature at 30.49±0.25 and 32.23±0.28 °C, with a stomata density of 127 mm^{-2}. The structure of stomata functions for the exchange of gases O_2, CO_2, and water vapor, which are highly important in the processes of photosynthesis, respiration and transpiration. The level of stomatal density is classified as low (< 300 mm^{-2}), medium (300 to 500 mm^{-2}), and high (> 500 mm^{-2}) (Marantika et al., 2021). From this category, the sintrong in all treatments belonged to the low-density category (density < 300 mm^{-2}). Stomata density is related to stomata size; if the density is high, the stomata usually has a small size, or vice versa. High density and number of stomata are the processes of plant adaptation to environmental conditions (Carrera et al., 2021). Trichomes are derived from the epidermis in the form of hair. At high temperatures, the trichomes increase because they function to prevent excessive evaporation of the leaves. This condition is mainly

Table 4. Effect of high temperature and accession on stomatal and trichome density

Treatment (°C)	Density (mm^{-2})	
	Stomata	Trichome
Temperature		
T1 (28.89±0.40)	82±12b	3±0.39c
T2 (30.49±0.25)	127±32a	4±0.54b
T3 (32.23±0.28)	127±30a	5±0.62a
Accession		
Bogor 1	114±37	4±1.24
Bogor 2	107±26	4±0.97
Cianjur 1	114±35	4±0.95

Note: Numbers followed by the same letter in the same column show no significant difference based on DMRT at level of 5%

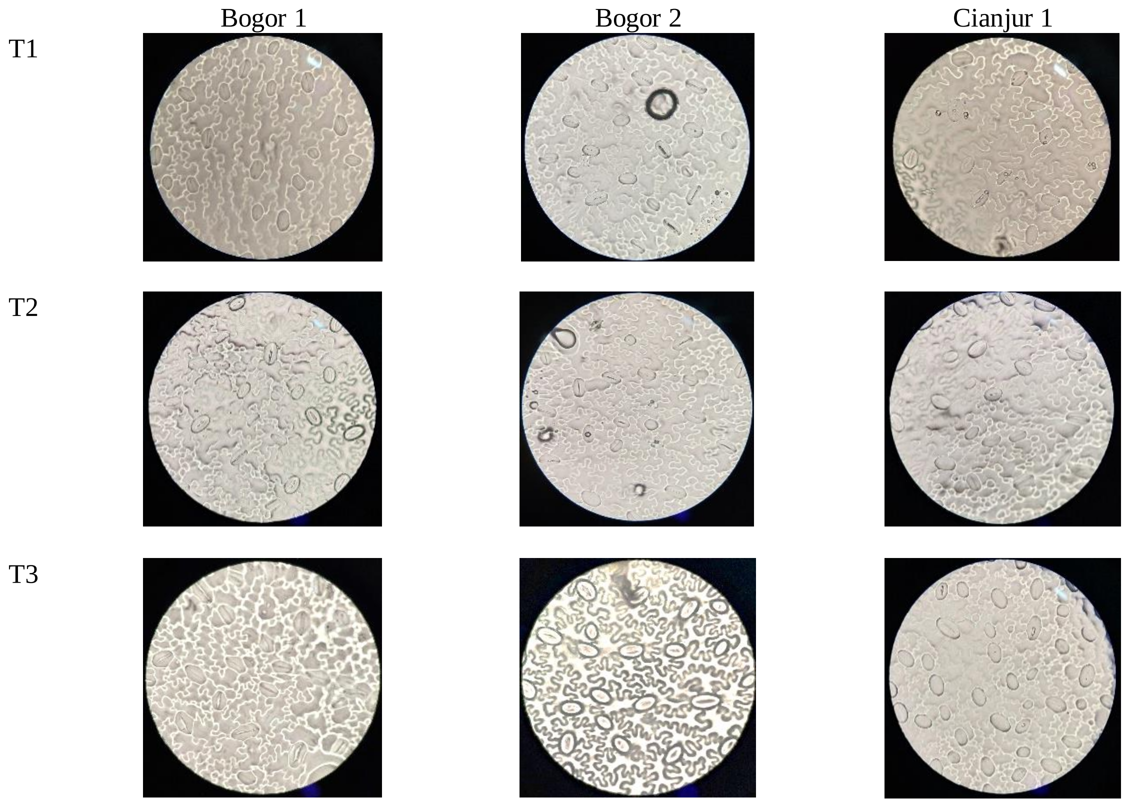

Figure 1. Stomata density of abaxial leave (T1 = 28.89±0.40 °C, T2 = 30.49±0.25 °C, T3 = 32.23±0.28 °C)

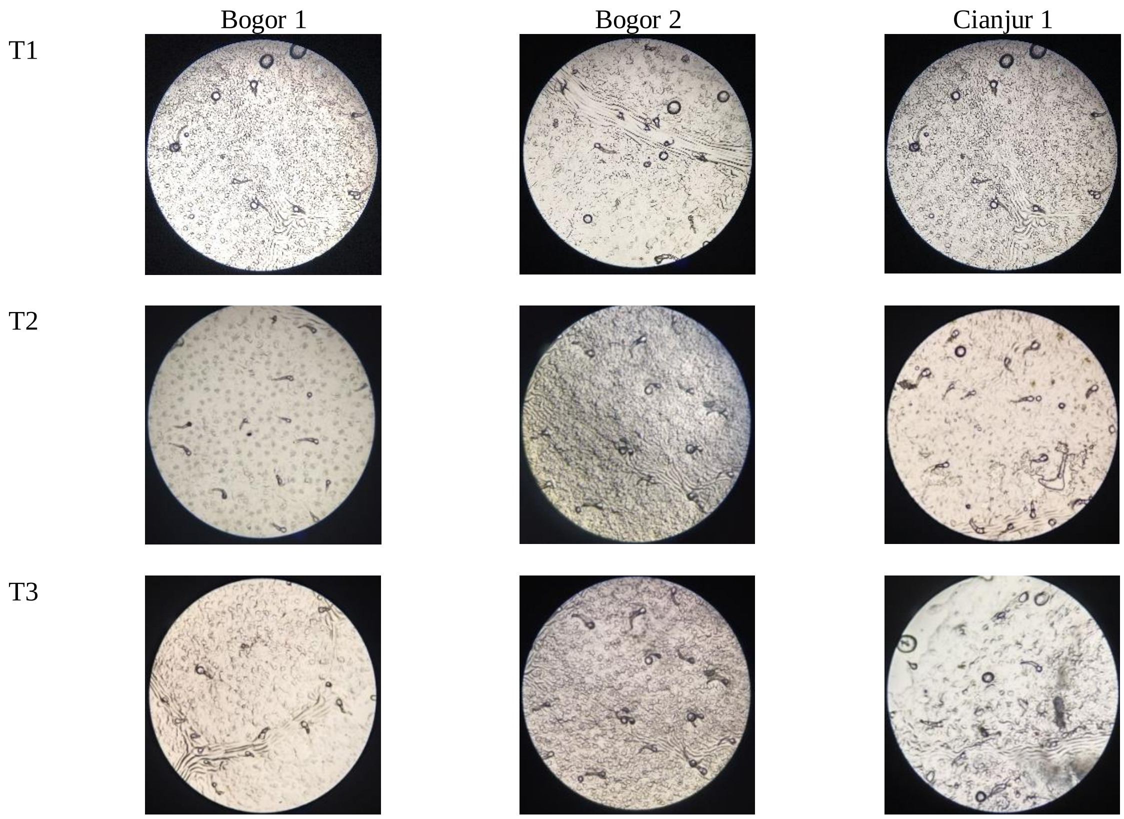

Figure 2. Trichome density of abaxial leaves (T1 = 28.89±0.40 °C, T2 = 30.49±0.25 °C, T3 = 32.23±0.28 °C)

due to the non-linear response of photosynthesis rate to temperature in *Metrosideros polymorpha*. Increasing leaf temperature affects trichomes, which can increase the activity of photosynthetic enzymes; leaf trichomes can increase the rate of photosynthesis (Amada et al., 2020).

The flavonoid content is expressed in mg 100 g^{-1} dry matter. Curve standard quercetin showed linear equation with R^2 value = 0.9985 (Figure 4). In this study, exposure to high temperatures up to 32 °C and accession had no effect on the flavonoid content (Figure 3). Flavonoids are part of the phenolic group, which have antioxidant activity and are found in many plants. The content of flavonoids at a temperature higher than the ambient temperature was 30.49±0.25 and 32.23±0.28 °C in a row by 1695.38 and 1834.83 mg 100 g^{-1} of dry matter. High temperatures result in an increase in free radicals in the form of reactive oxygen species (ROS), which is reactive in plant tissues and can trigger cell damage. Plants that are tolerant to ROS will adapt to such condition by producing antioxidant compounds (Slimen et al., 2014). In this study, flavonoids did not show any effect due to exposure to high temperatures, presumably because the groundwater status was always maintained at field capacity. In addition, it is suspected that the sintrong plant has a good tolerance for high temperatures. These results are supported by research by Obaid et al. (2016) that the flavonoid 3-monooxygenase and flavonoid 3-dioxygenase enzymes in the flavonoid biosynthetic pathway are inactive

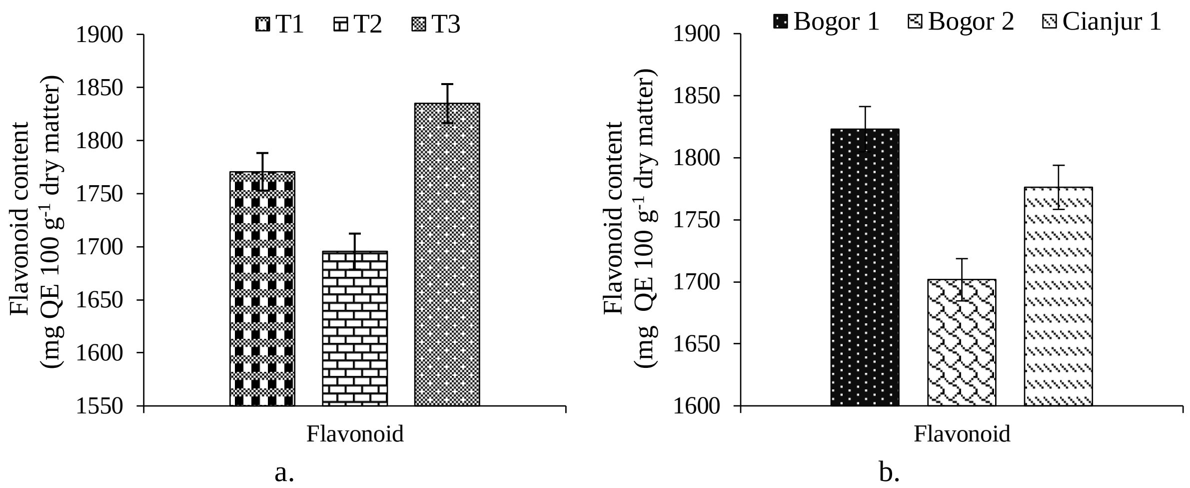

Figure 3. Content of flavonoid, (a) effect of high-temperature T1 = 28.89±0.40 °C, T2 = 30.49±0.25 °C, and T3 = 32.23±0.28 °C; (b) effect of accessions. Bar = standard deviation; n = 4

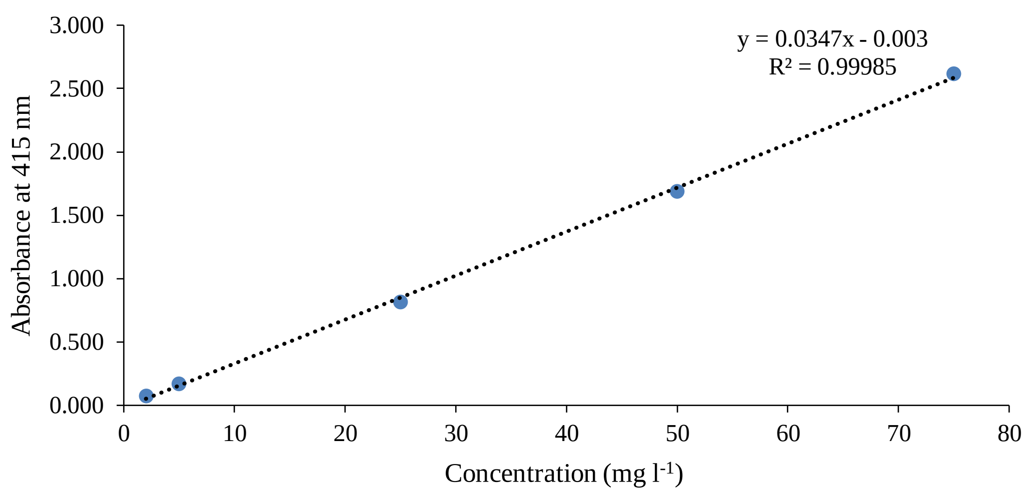

Figure 4. Curve standard of quercetin

Table 5. Pearson correlation test between parameters

Parameters	Flowering age	Wilting rate	LAI	RGR	Photosynthesis rate	Stomata conductance	Transpiration rate	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids	Anthocyanins	Stomata	Trichome	Flavonoid
Flowering age	1	-0.72	0.79	0.85	-0.73	-0.70	0.64	-0.40	-0.46	-0.42	-0.36	-0.62	-0.57	-0.78	0.03
Wilting rate		1	-0.64	-0.73	0.70	0.68	-0.44	0.60	0.61	0.60	0.57	0.55	0.37	0.61	-0.04
LAI			1	0.76	-0.64	-0.70	0.55	-0.34	-0.41	-0.36	-0.34	-0.59	-0.39	-0.64	-0.02
RGR				1	-0.80	-0.64	0.54	-0.44	-0.48	-0.46	-0.39	-0.57	-0.51	-0.79	-0.03
Photosynthesis rate					1	0.54	-0.46	0.58	0.64	0.60	0.53	0.57	0.39	0.78	0.19
Stomata conductance						1	-0.44	0.32	0.35	0.33	0.30	0.38	0.45	0.56	0.11
Transpiration rate							1	-0.24	-0.31	-0.26	-0.20	-0.46	-0.49	-0.61	-0.33
Chlorophyll a								1	0.55	0.51	0.51	0.49	0.06	0.56	-0.34
Chlorophyll b									1	0.98	0.91	0.69	0.19	0.61	-0.33
Total chlorophyll										1	0.94	0.61	0.19	0.58	-0.28
Carotenoids											1	0.48	0.16	0.47	0.005
Anthocyanins												1	0.25	0.58	0.25
Stomata													1	0.66	0.01
Trichome															0.01
Flavonoid															1

at 40 to 42 °C in young leaves of *Rhazya stricta*. Inactivity of the enzyme will reduce the total flavonoid content.

Based on Pearson's correlation among all parameters, several parameters were strongly correlated (r > 0.5), with positive and negative correlations (Table 5). The leaf area index was positively correlated with the relative growth rate and transpiration rate with the value of r > -0.5, but negatively correlated with the photosynthesis rate and trichome density. The increasing leaf area index will decrease the rate of photosynthesis. This is because leaves shade each other so that the lower leaves cannot conduct photosynthesis at optimal level (Santrum et al., 2021). The rate of photosynthesis was positively correlated with stomatal conductance, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, anthocyanins, and trichome density with the r value of > 0.5. The transpiration rate was negatively correlated with the density of trichomes with r value of > -0.5. This means that the higher the trichome density decreases the transpiration rate. Meanwhile, total chlorophyll was positively correlated with carotenoids, and anthocyanins with values of r > 0.5. This signifies that higher chlorophyll also contributes to higher anthocyanins and carotenoids. Stomata was positively correlated with trichomes with the r value of > 0.5. No correlation (r > 0.5) was found between flavonoids with all parameters.

CONCLUSIONS

Exposure to high temperatures between 30 and 32 °C increases the speed of flowering age, wilting rate, pigment content (chlorophyll a, chlorophyll b, total chlorophyll, anthocyanins and carotenoids), photosynthesis rate, transpiration rate, stomatal density, and trichomes, but decreases leaf area index, relative growth rate and stomatal conductance. The increasing temperature does not affect the flavonoid content in the leaves of sintrong. Therefore, sintrong should be harvested earlier before flowering to obtain high leaf production and good-quality vegetables. Bogor 1 accession has shown the best performance, so it can be developed for functional vegetables and needs further research to determine its nutritional content.

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