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Research on Epidermis and Epidermal Derivates of Bitter Mustard (Brassica juncea L.) and Petsai Mustard (Brassica pekinensia L.) Leaves in Batang Regency

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ABSTRACT

This research is an exploratory study conducted in Batang Regency. The research samples, namely bitter mustard (Brassica juncea L.) and petsai (Brassica pekinensia L.) leaves, were collected in December 2022. The selection of research samples is based on the level of production and availability. The data analysis method used in the research is descriptive quantitative analysis. The results showed that bitter mustard (Brassica juncea L.) and petsai mustard (Brassica pekinensia L.) have the same epidermal characteristics, namely irregularly shaped epidermal cells with notched cell wall edges and elongated polygonal cells with flat cell wall edges. The epidermal derivatives found are stomata and trichomes. The stomata of the study sample have anisocytic type, closed slit condition, but have different sizes (length and width), index and density. Trichomes have a simple needle-like hair type on the edge of the leaf, but have different sizes (length and width). The differences in the research samples can be observed quantitatively.

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Introduction

This research is an exploratory study conducted in Batang Regency. The research samples, namely bitter mustard (Brassica juncea L.) and petsai (Brassica pekinensia L.) leaves, were collected in December 2022. The selection of research samples is based on the level of production and availability. The data analysis method used in the research is descriptive quantitative analysis. The results showed that bitter mustard (Brassica juncea L.) and petsai mustard (Brassica pekinensia L.) have the same epidermal characteristics, namely irregularly shaped epidermal cells with notched cell wall edges and elongated polygonal cells with flat cell wall edges. The epidermal derivatives found are stomata and trichomes. The stomata of the study sample have anisocytic type, closed slit condition, but have different sizes (length and width), index and density. Trichomes have a simple needle-like hair type on the edge of the leaf, but have different sizes (length and width). The differences in the research samples

can be observed quantitatively.

Method

This research is an *ex post facto* Exploratory research was conducted in Batang Regency to determine the abundance of the Brassicaceae family and bitter mustard (Brassica juncea L.) and petsai mustard (Brassica pekinensia L.) were selected as research samples. Sampling was done by collecting leaves from both plants and then preparing them using the leaf clearing technique. The observation of research samples was done in Batang Regency and the microscopic observation was done in the Integrated Laboratory of Campus 4 UAD Yogyakarta.

Results and Discussion

The research samples came from Batang Regency, specifically in Kayuabang Hamlet, Gerlang Village, Blado District, Batang Regency has the characteristics of a highland area. The research samples were collected at a location that is a garden owned by local residents in December 2022. The location point of research sampling is measured using the Google Earth application can be seen in Table 1.

Table 1. Research Sampling Location Point

Research Samples	Location
Bitter Mustard (*Brassica juncea* L.)	7°09'20.3"S 109°49'46.4" E
Petsai Mustard (*Brassica pekinensia* L.)	7°09'21.1"S 109°49'47.7" E

Site environmental parameters such as light intensity, soil moisture, soil pH, air temperature, air humidity, and sampling site elevation were measured. The results of measuring the environmental parameters of the research sampling site at 10:20 am are shown in Table 2.

Table 2. Environmental Parameter Measurement Results

Enviromental Parameter	Measurement Results
Light intensity (lux)	1050
Soil moisture (%)	60
Soil pH	6
Air temperature (^{o}C)	19
Air humidity (%)	90
Site elevation (mdpl)	1750

The results of measuring the height of the site at the coordinates of the research sampling site show that the site is included in the highland area. According to Thana (2016: 59-61), the land surface with the height of >700 masl or highland area is very suitable for mustard growth with the air humidity of 80-90%, so the air humidity of the sampling location supports mustard growth. According to Lama & Kune (2016: 27-28), the air temperature suitable for mustard growth is between 19-21 oC with a fairly high light intensity of 70-100% because mustard needs sufficient sunlight for growth, but too high air temperature affects mustard growth. The results of soil moisture and soil pH measurements show that the soil conditions are in good condition for mustard growth according to Wahyuni & Sofyadi (2019: 42), which explains that optimal soil conditions for mustard growth have soil moisture of 50-70% and soil pH of 6-7. The results of the overall parameter measurements show that the research sample location is very good for mustard plant growth, so the research sample has the potential to grow optimally with the structures and tissues present in it.

The research samples, namely bitter mustard (Brassica juncea L.) and petsai mustard (Brassica pekinensia L.) in Batang Regency, were then observed for their morphology with the results in Table 3. The research samples, namely bitter

mustard (Brassica juncea L.) and petsai mustard (Brassica pekinensia L.) leaves from Batang Regency, were selected based on the availability or the highest amount of vegetable production in Batang Regency. The selection of the two samples was based on data from the website of the Central Bureau of Statistics (BPS) of Batang Regency in 2015. Research sampling uses certain conditions, according to Sinaga (2014: 6-8) states that sampling with certain planning or requirements aims to make the research sample have some uniformity of characteristics so that the data obtained is more accurate and consistent. The sample requirements used include the age and height of the plants in Table 3, then morphological observations are made and measurements of leaf length and width are made on the 4th leaf of the open bud.

Table 3. Morphological Observation Results of Research Samples

Aspects Observed	***Brassica juncea* L.**	***Brassica pekinensia* L.**
Sample age (minggu)	6	6
Sample height (cm)	29,5	29,7
Leaf length (cm)	26,6	20,3
Leaf width (cm)	13,4	13,6
Leaf count	15	15

The samples used are 6 weeks or about 1.5 months old, Anjarwati et al (2022: 278) states that optimal growth of mustard greens is between 1,5 until 2 months old plants. The results of measuring environmental parameters are consistent with the results of previous relevant research, so it can be hypothesized that the research samples used are in good condition. The research samples were taken from the 4th leaf of the opened leaf bud, and then the tip and the middle of the leaf blade were cut with a size of 1cm2. The cutting of the 4th leaf is done at the end and in the middle of the leaf blade so that the leaves used are not too young or vice versa. The leaf samples were prepared using the leaf clearing technique, then the leaf epidermal tissue and its derivatives were observed microscopically. The results of microscopic observations and measurements are presented in Table 4 and Table 5.

Table 4. Results of Microscopic Observations of Leaf Epidermis of Research Samples

Num	**Aspects Observed**	***Brassica juncea* L.**	***Brassica pekinensia* L.**
1.	Leaf tip epidermis shape		
	Adaxial surface	Irregular	Irregular
	Abaxial surface	Elongated polygonal	Elongated polygonal
2.	Epidermal cell wall edge ujung daun		
	Adaxial surface	Squiggly	Squiggly
	Abaxial surface	Flat	Flat

Num	Aspects Observed	*Brassica juncea* L.	*Brassica pekinensia* L.
3.	Shape of leaf mid-epidermis		
	Adaxial surface	Irregular	Irregular
	Abaxial surface	Irregular	Irregular
4.	Epidermal cell wall edge tengah daun		
	Adaxial surface	Squiggly	Squiggly
	Abaxial surface	Squiggly	Squiggly
5.	Leaf tip epidermal cell length (μm)		
	Adaxial surface	144,36±35,26	166,70±48,64
	Abaxial surface	156,18±33,87	190,40±41,18
6.	Leaf tip epidermal cell width (μm)		
	Adaxial surface	70,80±21,86	66,45±23,09
	Abaxial surface	80,55±36,79	68,60±15,25
7.	Leaf mid-epidermal cell length (μm)		
	Adaxial surface	150,39±34,61	121±20,72
	Abaxial surface	159,22±39,73	130,74±36,56
8.	Cell width of leaf mid-epidermis (μm)		
	Adaxial surface	54,43±9,12	45,47±9,24
	Abaxial surface	75,80±19,00	48,19±10,20

Observations were made on the adaxial and abaxial surfaces of the leaves at the tip and center of the leaf blade. The results of microscopic observations of the epidermis of bitter mustard (Brassica juncea L.) and petsai (Brassica pekinensia L.) leaves in Batang Regency at the tip of the leaf blade are shown in Figure 1.

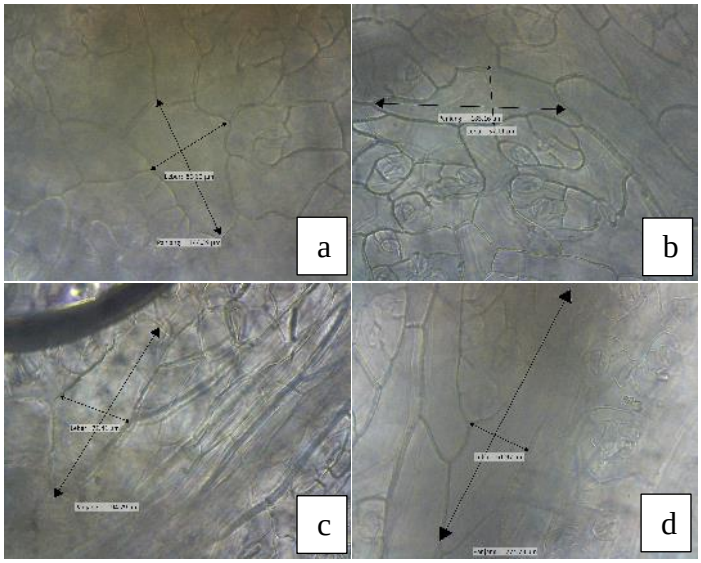

Figure 1. Characteristics of the leaf epidermis at the tip of the leaf blade of research samples at 400X magnification Description: a. Epidermis of the adaxial surface of bitter mustard leaves (Brassica juncea L.) b. Epidermis of the adaxial surface of petsai mustard leaves (Brassica pekinensia L.) c. Epidermis of the abaxial surface of bitter mustard leaves (Brassica juncea L.) d. Epidermis of the abaxial surface of mustard greens leaves (Brassica pekinensia L.)

Observations showed that the shape of the epidermal cells of the tip of the leaf blade on the adaxial surface of the two research samples was irregular with notched cell wall edges. Observations on the abaxial surface of the leaf blade tip of both research samples are elongated polygonal with flat cell wall edges. The results of the observations made on the research samples in the center of the leaf blade are shown in Figure 2.

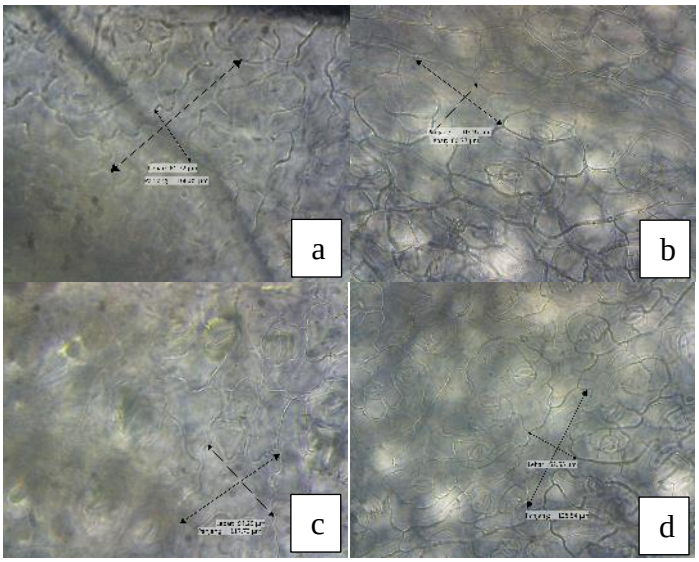

Figure 2. Characteristics of the leaf epidermis of the center of the leaf blade of research samples at 400X magnification Description: a. Epidermis of the adaxial surface of bitter mustard leaves (Brassica juncea L.) b. Epidermis of adaxial surface of Petsai mustard leaf (Brassica pekinensia L.) c. Epidermis of abaxial surface of bitter mustard leaf (Brassica juncea L.) d. Epidermis of abaxial surface of mustard leaf (Brassica pekinensia L.)

The observation of epidermal cells in the center of the leaf blade shows the same results between the two research samples, both on the adaxial and abaxial surfaces. The results of the observations include the irregular shape of the epidermal cells with the edges of the cell walls being notched. The observations of the epidermal cells in the research samples as a whole have one thing in common, namely the arrangement of the cells is dense. According to Mulyani (2019: 133-134), the epidermal tissues have different shapes, sizes, and arrangements, but the arrangement of the cells is tight so that there is no space between the cells.

Measurements of the average length and width of epidermal cells show differences, but not so significant. The average measurement of the longest and widest epidermal cells in both research samples is on the abaxial surface of the leaf. These results are consistent with the research of Akbar & Begum (2020: 17-18) who compared the three species of Brassicacea family and showed the results of the longest and widest epidermal cell measurements on the abaxial surface of the leaves. Epidermal cell size is also related to organ function. The difference in epidermal cell size according to Salamah, et al (2022: 15-16) explained that epidermal cell size can be influenced by light intensity, the higher the light intensity received by the leaf surface, the smaller the epidermal cell size. The results of comparing the epidermal cell measurements of research samples from Batang Regency show that mustard greens (Brassica pekinensia L.) have larger epidermal cell size than the epidermal cells of bitter mustard leaves (Brassica juncea L.).

Table 5. Results of Microscopic Observations of Epidermal Derivatives of Leaf Samples of Research

Num	Aspects Observed	***Brassica juncea* L.**	***Brassica pekinensia* L.**
1.	Location of stomata	*Adaxial surface*, *Abaxial surface*	*Adaxial surface*, *Abaxial surface*
2.	Stomatal type		
	Adaxial surface	*Anisocytic*	*Anisocytic*
	Abaxial surface	*Anisocytic*	*Anisocytic*
3.	Leaf tip stomatal length (μm)		
	Adaxial surface	28,23±1,87	23,12±1,18
	Abaxial surface	27,84±3,87	23,97±1,85
4.	Leaf tip stomata width (μm)		
	Adaxial surface	19,71±1,35	17,94±0,89
	Abaxial surface	19,99±1,29	17,55±1,62
5.	Leaf center stomatal length (μm)		

Num	Aspects Observed	*Brassica juncea* L.	*Brassica pekinensia* L.
	Adaxial surface	37,20±3,1	29,95±2,79
	Abaxial surface	37,39±9,15	27,7±2,19
6.	Leaf center stomatal width (μm)		
	Adaxial surface	26,19±3,27	23,6±2,12
	Abaxial surface	23,05±5,14	22,82±2,05
7.	Leaf tip stomatal density		
	Adaxial surface	156,92±27,98	228,62±65,10
	Abaxial surface	219,15±48,70	304,38±42,18
8.	Leaf center stomatal density		
	Adaxial surface	98,75±20,13	288,16±72,96
	Abaxial surface	110,93±15,22	328,73±61,96
9.	Leaf tip stomatal index (%)		
	Adaxial surface	26,92±7,98	24,38±5,18
	Abaxial surface	39,15±8,70	28,62±15,10
10.	Leaf mid-stomata index (%)		
	Adaxial surface	18,75±3,22	18,16±3,96
	Abaxial surface	24,75±7,13	28,73±6,96
11.	Condition of the stomata gap at the tip of the leaf		
	Adaxial surface	Closed	Closed
	Abaxial surface	Closed	Closed
12.	Condition of the central stomata gap of the leaf		
	Adaxial surface	Closed	Closed
	Abaxial surface	Closed	Closed
13.	Trichome type		
	Leaf edges	simple hair resembling a needle	simple hair resembling a needle
14.	Trichome length (μm)		
	Leaf edges	534,64±78,2	717,02±82,1
15.	Trichome width (μm)		

Num	Aspects Observed	*Brassica juncea* L.	*Brassica pekinensia* L.
	Leaf edges	106,30±34,6	128,90±30,5

Epidermal derivatives are differentiations of the epidermis that differ in shape, structure, and function. Microscopic observations on both samples showed the presence of epidermal derivatives, namely stomata and trichomes. Stomata are gaps bounded by a pair of covering cells, while epidermal derivatives are found on both surfaces of the leaf, namely the adaxial and abaxial surfaces (Anu, et al. 2017: 71). Observations were made to determine the presence of epidermal derivatives at the tip and center of the leaf blade. The stomata found at the tip of the leaf blade of the research sample can be seen in Figure 3.

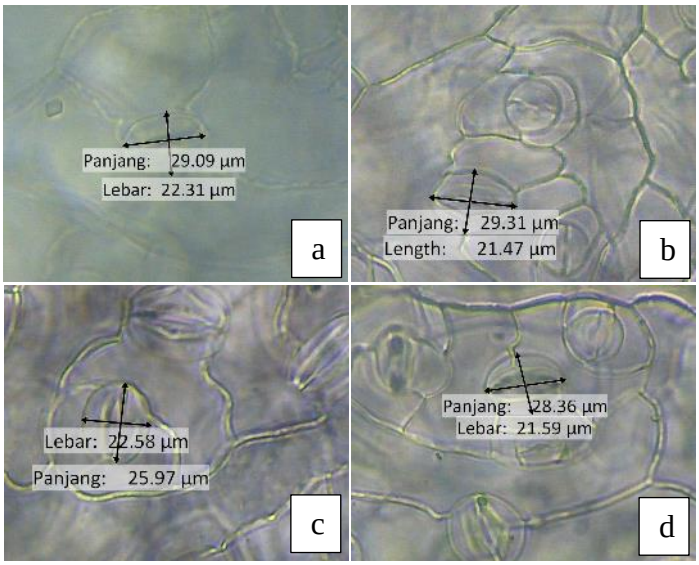

Figure 3. Stomata Characteristics of the Tip of the Leaf Blade Samples Examined at 400X Magnification Description: a. Stomata of the adaxial surface of bitter mustard leaves (Brassica juncea L.) b. Stomata of the adaxial surface of petsai mustard leaves (Brassica pekinensia L.) c. Stomata of the abaxial surface of bitter mustard leaves (Brassica juncea L.) d. Stomata of the abaxial surface of petsai mustard leaves (Brassica pekinensia L.)

The results of observations of the two research samples at the tip of the leaf blade showed that the two research samples had anisocytic type stomata. According to Akbar & Begum (2020: 23-24), the stomata of Brassica genus are surrounded by three additional cells or epidermal cells with one cell size smaller than the other cells, so it is called anisocytic type. Stomata can be found in both research samples covering the leaf surface including adaxial and abaxial surfaces, so including amphistomatic leaves. Mulyani (2019: 118) states that leaves with stomata on both surfaces are called amphistomatic leaves. The same results were also found when observing the center of the leaf blade of the two research samples.

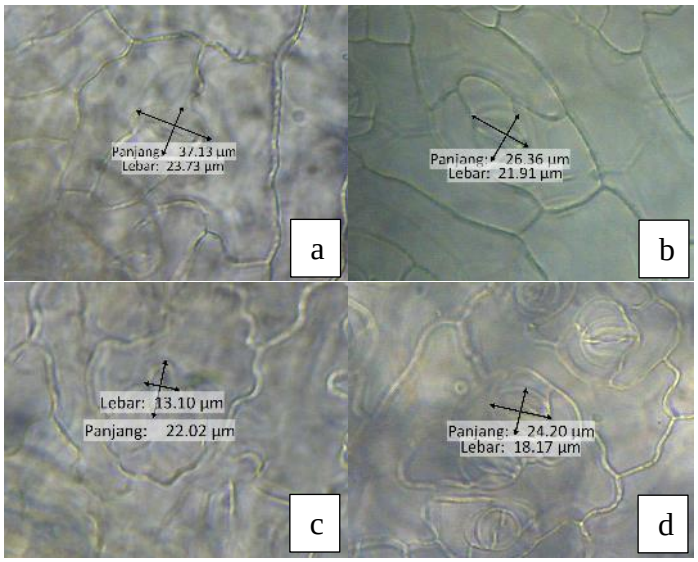

Figure 4. Stomata Characteristics of the Center of the Leaf Blade Samples Research with 400X Magnification Description: a. Stomata of the adaxial surface of bitter mustard leaves (Brassica juncea L.) b. Stomata of the adaxial surface of petsai mustard leaves (Brassica pekinensia L.) c. Stomata of the abaxial surface of bitter mustard leaves (Brassica juncea L.) d. Stomata of the abaxial surface of petsai mustard leaves (Brassica pekinensia L.)

The stomatal gap can open and close because it is related to the function of stomata in the transpiration process. Setiawati & Syamsi (2019: 148- 149) explain that stomatal gaps can open and close based on internal influences, such as biological time related to transpiration and the process of CO2 uptake during photosynthesis, and external influences, such as light intensity and other environmental conditions. The results of observations of the research sample showed that the stomatal gaps found were all closed. Based on external factors, according to Mutaqin, et al. (2016, 16-18), stomatal gaps are closed due to high light intensity and temperature and excessive water evaporation. This condition triggers stomatal closure to reduce excessive water loss.

The number of stomata is closely related to the intensity of transpiration in leaf organs and CO2 uptake. The greater the number of stomata or their density, the faster and higher the evaporation that can occur. The measurement results show that both samples, both at the tip and in the middle of the leaf blade, have a higher density on the abaxial surface. The results of observations and measurements of stomatal density with a field of view of 0.06 mm2 show that there are more stomata on the abaxial surface. The results of observations and measurements of stomatal density are in agreement with the results of research conducted by Salamah, et al. (2022: 16-17), which states that more stomata are found on the abaxial surface.

Tambaru, et al. (2019: 38-39) explain that if the number of stomata is more, the density value will be higher and vice versa. The results of measuring the stomatal density of the research samples show a quantitative difference between the stomatal density of bitter mustard leaves (Brassica juncea L.), which is lower than the stomata of the leaves of mustard petsai (Brassica pekinensia L.), this is because the number of stomata of mustard petsai (Brassica pekinensia L.) has a greater

number than the number of stomata of bitter mustard leaves (Brassica juncea L.). The results also show that stomatal size can affect stomatal density, stomata of bitter mustard leaves (Brassica juncea L.) have a larger size than stomata of petsai mustard leaves (Brassica pekinensia L.), so that in a given field of view more stomata will be found in research samples with smaller stomatal cell sizes.

The measurement of stomatal index is carried out to determine the level of stomatal density, more specifically, it can be said that the measurement of stomatal index is a representation of the value of stomatal density. The results showed that the stomatal index on the abaxial surface of the leaf, either at the tip or in the middle of the leaf blade, was higher than on the adaxial surface. These results are appropriate because the number of stomata is found more or the stomatal density is higher on the abaxial surface of the leaf. The research sample used is in an area with highland characteristics with relatively low light intensity, which may cause the stomatal index value of the adaxial surface to be lower than the abaxial surface. Tambaru, et al. (2019: 35-37) explain that the stomatal index can also be affected by external factors, namely environmental conditions such as light intensity.

Another epidermal derivative found on the leaves of bitter mustard (Brassica Juncea L.) and petsai mustard (Brassica Pekinensia L.) from Batang Regency are trichomes. Based on Salamah, et al (2022: 17), besides stomata, epidermal cells can also be modified into trichomes with different shapes and sizes. Trichomes are present on almost all surfaces of plant organs characterized by when touching the surface of plants that have trichomes, it will feel rough, itchy, sticky and have a pungent odor. Based on observations, both research samples have the same trichome shape. The results of the trichome observations of the research samples are shown in Figure 5.

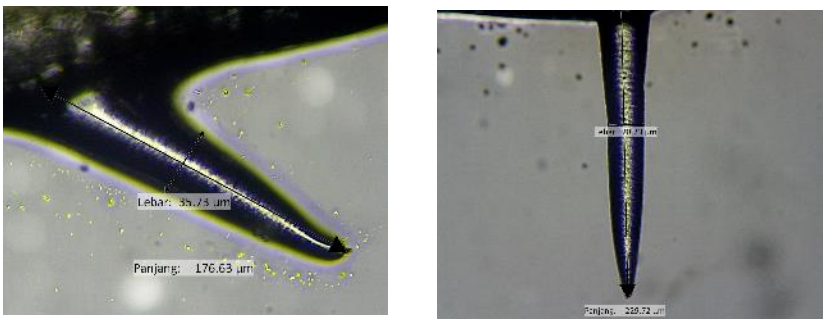

Figure 5. Characteristics of leaf margin trichomes of research samples at 100X magnification
Description: (left) Trichomes of bitter mustard leaves (Brassica juncea L.); (right) Trichomes of petsai mustard leaves (Brassica pekinensia L.)

The picture above shows that the trichomes found on the leaves of bitter mustard (Brassica juncea L.) and petsai (Brassica pekinensia L.) are simple hairs resembling needles (Akbar & Begum, 2020: 24-25). According to Dewi, et al. (2015: 209-218), trichomes with simple hairs resembling needles can be seen at 40x or 100x magnification and are usually clearly visible at the leaf margins.

Trichomes are epidermal derivatives that function in plant defense against insects, in addition to the study of trichomes including diversity and structure can be used in the identification of genera, species, subspecies and varieties of the different families studied (Dewi et al. 2015: 209-218). The results of the observation of trichomes in the research samples have the same shape, indicating the similarity of trichomes in a genus Brassica. The trichomes found in the research samples were located on the edge of the leaves, which is related to the function of trichomes as a leaf

defense against insects. Another function of trichomes on the edge of the leaf according to Dewi, et al. (2015: 209-218) is distinguished by their type, non-glandular trichomes function as a barrier to the entry of pathogens through stomata, while glandular trichomes function to release secondary metabolites. The results of the observations show the similarity of the shape of the trichomes, namely non-glandular trichomes with simple hair shapes resembling leaves. The existence of leaf trichomes found only on the leaf margin based on the function of trichomes indicates the need for further research on the adaxial and abaxial surfaces of the leaves. The results showed that the leaf trichomes of petsai mustard (Brassica pekinensia L.) had a longer size than the leaf trichomes of bitter mustard (Brassica juncea L.).

Conclusion

The epidermal characteristics of bitter mustard (Brassica juncea L.) and petsai (Brassica pekinensia L.) leaves from Batang Regency have the same epidermal cell shape, namely on the adaxial surface of the leaf tip, the adaxial and abaxial surfaces of the middle of the leaf are irregularly shaped with notched cell wall edges, on the abaxial surface of the leaf tip is elongated polygonal with flat cell wall edges. Derivatives of leaf epidermis found in both research samples are stomata of anisocytic type on both leaf surfaces and simple hair-like trichomes resembling needles on leaf edges.

Comparison of the characteristics of the size of the epidermal cells of petsai mustard leaves (Brassica pekinensia L.) is longer than the epidermal cells of bitter mustard leaves (Brassica juncea L.) at the tip of the leaf blade, but the opposite situation in the middle of the leaf blade. Derivatives of leaf epidermis in the form of stomata in bitter mustard leaves (Brassica juncea L.) have a larger size than the size of stomata in leaves of mustard greens (Brassica pekinensia L.), so that the stomatal density and stomatal index of bitter mustard leaves (Brassica juncea L.) are lower than the stomatal density and stomatal index of mustard greens (Brassica pekinensia L.). Trichomes of the same shape have a difference in size, namely trichomes on petsai mustard leaves (Brassica pekinensia L.) are longer than trichomes on bitter mustard leaves (Brassica juncea L.).

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