Trichomes and Stomata Diversity in Soybean (*Glycine max* L. Merill) Lines

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Abstract. Modified epidermal tissues on the leaf surfaces, such as trichomes and stomata, are very useful for plants because it helps prevent pest attacks and support photosynthesis. The characteristics of a plant can be determined based on an observation conducted on trichomes and stomata to improve the genetic potentials of the plant. This research aimed to discern the diversity of trichomes and stomata in 10 soybean lines and determine the correlation between the characters. This experimental study employed a randomized complete block design with three replications. The results showed that there were significant differences in all leaf anatomical characters including the number of trichomes and stomata, as well as the length and the width of the trichomes and stomata. Some observed characters were also correlated. Positive correlations were found between the number of stomata and the width of the trichomes, the width of the lower stomata and the length of the trichomes, and the width of the upper stomata and the width of the trichomes. On the other hand, a negative correlation was observed between the length of the stomata and the number of trichomes.

Keywords: Diversity, soybean, trichomes, stomata

1. Introduction
Plant breeding is the process of improving the genetic potentials of a plant to yield crops with favorable traits such as being resistant to a specific disease or highly productive [1-2]. It may, for instance, begin with crossing a superior variety with a particular genotype in soybean which is resistant to CpMMV [3]. A study of diversity needs to be conducted to recognize the characters of the offspring that results from crosses. The study of diversity refers to a visual characterization of a plant to classify it based on its quantitative or qualitative traits [4-5]. One of the important facets of a diversity study is the observation of leaf anatomical characters.

Leaf constitutes the main organ of a plant which plays a direct pivotal role in photosynthesis and in determining the optimum photosynthetic capacity through various forms of adaptation mechanisms [6]. A leaf can also help control the production level of a plant [7]. The leaf surface is covered with...
epidermal tissues that protect the inside structure of the leaf. The epidermal tissues can be found on the upper (adaxial) or the lower surface (abaxial) of the leaf [8]. The leaf epidermis varies in shape, size, and structure. It also contains a considerable number of derivative cells, including stomata, trichomes, periderm cells, and bulliform cells [9].

Trichomes are modified epidermal tissues that can be found on the surface of a plant’s organ, such as leaf, flower, stem, and pod. Trichomes may be unicellular or multicellular, constituting the main trait of a plant that makes it resistant or tolerant to biotic or abiotic threats. Trichomes help reduce water loss and reflect excessive light. The density of the leaf trichomes can affect the larval feeding capacity; the tighter the trichomes, the more difficult for a larva to devour [10]. Trichomes come in the form of fine hair. This distinguished trait of trichomes can help protect the leaf, especially the soybean leaf, from pest attack [11]. Lupeol (triphenyl) content in soybean trichomes affects whitefly activity. High level of lupeol can result in decreasing the number of whiteflies that are trying to attack the plant. It can be concluded that lupeol content determines the level of soybean resistance to pests [12].

Besides trichomes, stomata also play a crucial role in the plant’s growth. A stoma is an opening between epidermis bordered by a pair of guard cells. The guard cells can change their form to adjust the stomata diameter or in other words to widen or narrow the stomatal opening [13]. In general, a light stimulus can trigger a stomatal opening, thus allowing the light to come in and contribute to photosynthesis [14]. Stomata serve some vital functions in transpiration, photosynthesis and plant’s innate immune system which helps prevent pathogenic infection [15-16]. The number of stomata found in the virus-infected plants will be less than that found in the healthy plants which normally range between thousands and a hundred thousand per cm² [17]. It is obvious then that virus or pest attack can decrease the number of plants’ stomata [18]. The distribution of stomata is also associated with the transpiration rate and intensity and is influenced by the condition of the environment where the plant grows. Stomata in soybean are distributed randomly. They function as an agent to protect the soybean plant from pathogens [19-20]. Trichomes and stomata observation is important to help plant breeders recognize the characteristics of high quality parental for a genetic assembly. This study, thus, was conducted to investigate the characteristics of trichomes and stomata found in the leaves of 10 soybean lines and further analyze the correlation between the observed variables.

2. Methods

2.1. Research Setting
This research was conducted from May to July 2017 at the Nuts and Tuber Crops Research Installation (INLITKABI) in Kepanjen, Malang (8°05'36.0"S 112°47'54.0"E) and at the Biology Laboratory of Universitas Negeri Malang.

2.2. Plant Materials
Materials used in this research were 10 soybean lines obtained from the Indonesian Legume and Tuber Crops Research Institute (ILETRI), Indonesian Agency for Agricultural Research and Development, namely Snb/1087-148-2-1 (G1), Snb/ 1087-147-2-2 (G2), Snb/ 1087-147-2-7 (G3), Snb/1087-148-2-10 (G4), Snb/1087-148-2-3 (G5), Snb/1087-210-1-1 (G6), Snb/1087-210-4-12 (G7), Sby/Pdm 651 (G8), Mlbr/MLG 0927-15 (G9), and MLGG 0896 (G10). The lines with Snb/1087 are derived from Sinabung and MLGG 1087 crossing. Sby/Pdm 651 is derived from Sibayak and Panderman crossing. Mlbr/MLG 0927-15 is derived from Malabar and MLGG 0927 crossing. MLGG 0896 is from Ponorogo, East Java.

2.3. Research Design
This experiment employed a randomized complete block design with three replications. Before planting the soybean seeds, the soil was weeded and cultivated. The seeds were grown on a land area of 2.24 m² and a planting spacing of 40 × 15 cm² with intensive watering, pests and diseases control, and fertilization. An observation on the leaf trichomes and stomata was conducted on day 47 after planting the soybean seeds, the soil was weeded and cultivated. The seeds were grown on a land area of 2.24 m² and a planting spacing of 40 × 15 cm² with intensive watering, pests and diseases control, and fertilization. An observation on the leaf trichomes and stomata was conducted on day 47 after planting the soybean seeds, the soil was weeded and cultivated. The seeds were grown on a land area of 2.24 m² and a planting spacing of 40 × 15 cm² with intensive watering, pests and diseases control, and fertilization. An observation on the leaf trichomes and stomata was conducted on day 47 after
sowing. The number of the leaf trichomes was observed from three fields of view and with a field area of $128\times102.4$ mm$^2$ at 258.5X magnification. The soybean leaf was placed under the dinolite microscope to get a clear view of the trichomes before they were counted. Meanwhile, the length and the width of the trichomes were measured by separating some trichomes that were still attached to the leaf and putting them under the microscope for observation (Figure 1).

**Figure 1.** Number of trichomes (left); the length and the width of the trichome (right)

A stomata section was created by dropping some limpid nail polish or gelatin on the leaf surface. The section was left dried for 2-3 minutes and sealed with adhesive tape. The tape was removed right before the observation was conducted from three different fields of view with an area of $128\times102.4$ mm$^2$. The number of the leaf stomata was observed at 258.5 X magnification while the length and the width of the stomata were observed at 2585.3 X magnification (Figure 2).

**Figure 2.** Number of stomata (Left); the length and width of the stomata (Right)

2.4. *Observation Parameters*

Variables observed included the number of trichomes found on the upper and lower surfaces of the leaf as well as the length and width of the trichomes. The observation was also performed to the number of stomata found on the upper and lower surfaces of the leaf as well as the length and the width of the stomata.
2.5. Data Analysis

The normality and homogeneity of the data were tested before performing the one-way ANOVA, t-test, and BNT test. Additionally, a correlation test was conducted to examine the relationships between research variables.

3. Results and Discussion

The result of the t-test showed that there was a significant difference in the number of trichomes, the length of the trichomes, and the number of stomata. No notable differences were reported for the width of the trichomes, and the length and width of the stomata. Table 1 suggested that the lower leaf surface contained more trichomes and stomata. The lower surface trichomes and stomata were found longer than those on the upper surface. However, both upper and lower leaf surfaces were covered by trichomes and stomata with the same width.

Table 1. Mean, range, and the result of the t-test on the research variables

| Variable                        | Average | Range      | Sig (t-test) |
|---------------------------------|---------|------------|--------------|
| Number of trichomes             | Upper   | 49.066     | 41-56        | 0.000        |
|                                 | Lower   | 142.299    | 45-215       |              |
| The length of the trichomes (mm)| Upper   | 0.902      | 0.866-0.955  | 0.005        |
|                                 | Lower   | 0.994      | 0.879-1.127  |              |
| The width of the trichomes (mm) | Upper   | 0.027      | 0.024-0.031  | 0.726        |
|                                 | Lower   | 0.027      | 0.022-0.029  |              |
| Number of stomata               | upper   | 27.399     | 23-37        | 0.001        |
|                                 | lower   | 41.535     | 32-52        |              |
| The length of the stomata (mm)  | upper   | 0.023      | 0.023-0.024  | 0.122        |
|                                 | lower   | 0.024      | 0.023-0.025  |              |
| The width of the stomata (mm)   | upper   | 0.013      | 0.012-0.013  | 0.687        |
|                                 | lower   | 0.013      | 0.012-0.015  |              |

3.1. Number of trichomes

In soybean, trichomes can be found in the leaf and the pod. Trichomes that can be found on the leaf surface can affect the intensity of light which penetrates the leaf. An increase in the number of trichomes results in decreasing the amount of light that can be permeated [21]. The fine hair that covers the leaf surface can reflect the sun rays [22]. Eventually, to avoid the deficiency of light, a particular plant reduces the number of trichomes that can grow on the leaf surface. The results of the data analysis presented in Figure 3 indicated a big difference in the number of trichomes growing on the upper leaf surface of various soybean lines. Soybean MLGG 0896 (G10) reported the least trichomes with an average score of 41. This figure was not significantly different from the number of trichomes indicated in G1, G3, G4, and G7. Meanwhile, Soybean Snb/1087-210-4-12 (G7) shared many similarities with other lines but was dissimilar from G5. Soybean G6 was also observed to have similar properties with other lines, except with G10. These findings are in contrast with those found that no difference was found in the number of trichomes covering the upper leaf surface of soybeans [21].

The number of trichomes can vary from one soybean line to another. Figure 3 suggested a significant difference in the number of trichomes found on the lower leaf surface of soybean lines. Snb/1087-148-2-10 (G4) was observed to possess the most trichomes (with an average score of 215), and MLGG 0896 (G10) reported the least trichomes. Snb/1087-147-2-7 (G3) shared similarities with G6 but was significantly different from G1, G9, and G10. The diversity of the number of trichomes found in plants can be influenced by several factors. One of the adaptation forms that a plant can do to solve the light deficiency issue is by reducing the number of trichomes [22]. Therefore, trichomes may not be found on the leaf surface of some plants. If there is any, the number of trichomes must be small. In soybean, trichomes can be found more on the lower leaf surface than the upper leaf surface because
trichomes on the soybean leaf serve to defend the plant against specific pests which occasionally attack the lower surface of the leaf [10, 17]. This finding is in line with those indicated that an increase in the number of trichomes on leaves could lead to a decrease in the intensity of the armyworm’s (S. Litura) attack to soybean [23].

![Figure 3](image-url)  
**Figure 3.** The number of the upper surface trichomes (left); the number of the lower surface trichomes (right). Letters a-e show the significance of the differences.

### 3.2. **The length of the trichomes**

Soybean trichomes serve a mechanical defense function to protect the plant from a particular pest attack. The length of trichomes can affect the feeding power of larvae. Longer trichomes are more protective than the shorter ones [24]. The results of the current study shown in Figure 4 indicated that Snb/1087-210-4-12 (G7) possessed the longest trichomes with an average length of 0.955 mm, which created a great distinction between this soybean line with other lines, except with G1, G3, and G5. Snb/1087-147-2-2 (G2) and G10 shared a great number of similarities but had many different features compared to G1, G3, G5, G7, and G9. The potassium content in a plant can determine the length of trichomes [25]. Likewise, it had been shown that the application of a particular dosage of potassium on some soybean genotype indicated an interaction between UM 2-4 and K3 that resulted in longer trichomes [26].

The density of trichomes in soybean is controlled by a single recessive gene [27]. The leaf trichomes have more prominent roles than other leaf morphological characters such as the leaf thickness and the leaf area in determining the soybean resistance to pests [24]. Trichomes in soybean also shown as a defense function [28]. The results of the analysis depicted in Figure 4 showed that there was a significant difference in the length of trichomes found on the lower surface of the soybean leaf. The longest trichomes were observed in Snb/1087-210-4-12 (G7) (1.127 mm), which resembled the length of trichomes in G3, G8, and G10. Meanwhile, the shortest trichomes were reported by Snb/1087-210-1-1 (G6), which also had some resemblance with the length of the trichomes in G1, G2, G4, G5, and G9. This finding is in line with the results of the study that the length of trichomes covering the lower surface of the soybean may differ in some soybean genes [29].
3.3. The width of the trichomes

The width and the length of a leaf trichomes can bring a different impact on the ability of the leaf to create a barrier between pests that may infect the leaf with the leaf, leaving the eggs no chance to stick on the leaf to inject the virus or poison [30]. The results of the analysis presented in Figure 5 indicated that the widest trichomes were observed in Sby/Pdm 651 (G8), while the narrowest trichomes were reported by MLGG 0896. Snb/1087-148-2-1 (G1) had shared many similarities with G4 and a little resemblance with other lines, except with G3 and G8. Meanwhile, G9, G10, and G8 held a significant difference. Research about some soybean lines has been proven that the application of gibberellin could affect the width of the upper surface trichomes which at the same time explained the significant difference found in the width of the trichomes in some soybean lines observed in the current study [31].

The position and the condition of trichomes in a plant depend on several factors, including the plant’s genetics, the environmental state, and the availability of the nutrients needed by the plant [32]. Nitrogen is one of the elements that play a significant role in leaf growth. A wider leaf has more chances to be covered by a lot of trichomes [33]. The application of a big dosage of nitrogen on a leaf may result in widening the leaf trichomes [29]. The results of the observations displayed in Figure 5 indicated that the most significant difference was found in MLGG 0896 (G10). On the other hand, G1-G9 did not show any significant difference. The average widths of the lower surface trichomes in some soybean lines were reported as follows: G2 (0.029 mm); G4 (0.029 mm); G8 (0.029 mm); G7 (0.028 mm); G1 (0.027 mm); G3 (0.027 mm); G5 (0.027 mm); G9 (0.026 mm); G6 (0.026 mm); and G10 (0.022 mm). To the contrary, [34] did not find any significant difference between UM lines and some soybean varieties, such as Gumitir and Wilis.
3.4. Number of stomata

A great number of stomata help improve transpiration which functions to keep the stabilization of the leaf’s temperature, maintain the cell’s turgidity, and accelerate the rate of nutrients transportation through xylem [35]. Figure 6 presented the results of the analysis that indicated a significant difference in the number of stomata found in each of the soybean lines observed in this study. The greatest number of stomata was observed in Snb/ 1087-148-2-10 (G4). This figure was reported to be significantly different from that observed in all soybean lines, except in G3 and G8. The index and the number of leaf stomata depend on the type of the plant and the location where the plant grows [36].

Stomata distribution with the intensity and the rate of leaf transpiration; the more gaps found in a certain area, the faster the transpiration rate will be [37]. However, when the distance between two stomata is too close, it is possible that one’s evaporation will inhibit the others due to the edges on the guard cells that allow water molecules to turn when passing through the stomatal gap. Besides serving a function in plant’s transpiration, stomata also play an important role in the photosynthesis. Stomata will control the in-and-out water and CO$_2$ circulation during the process [38]. If there are only a few stomata found on a leaf, the number of the leaf’s epidermal cells will increase; therefore, it can be concluded that the number of stomata and the number of epidermal cells have a negative correlation [39].

Plants need to decrease the transpiration rate to avoid drought to adapt to the terrestrial environmental conditions [40-42]. In general, the lower leaf surface is covered with more stomata than the upper leaf surface [13, 43]. The results of the present study presented in Figure 6 showed that the number of stomata in Snb/1087-210-1-1(G6) differed significantly from the number of stomata found in other soybean lines, except in G4. Snb/1087-148-2-1 (G1) shared some similarities with G2, G3, and G8, but not with other soybean lines. The density of the abaxial (lower) surface stomata is much higher than that of the adaxial (upper) surface stomata. The sun’s rays cannot directly penetrate through the epidermal layers on the abaxial leaf surface so that only a few stomata will be damaged by the light. This finding has proven that the light intensity can also determine the number of stomata found on a leaf. Besides, as the epidermis on the lower leaf surface is coated by a very thin cuticle layer, the transpiration process through stomata can be more effective [44]. An increase in the number of stomata can also be affected by the level of potassium absorbed by the plant to help stomata prevent water loss during transpiration and to increase the efficiency of the photosynthesis process [45-47].
3.5. The length of the stomata

The size of stomata can determine the number of stomata found on a leaf. If the size is small, the number will grow bigger, and vice versa [48]. Figure 7 indicated that G1 had the smallest stomata while the longest diameter of stomata was reported by G5. Snb/1087-148-2-1 (G1) was found to share some similarities with other soybean lines, except with G3 and G5. Snb/1087-148-2-3 (G5) differed significantly from other lines, except for G2, G3, G4, G7, G8, and G10. In contrast, found only one soybean line reported a different size while the others were similar [29]. However, the findings of this study are in line with the great number of similarities in soybean varieties, such as in Gumitir and Wilis [49].
position of the stomata, and the nitrogen content. Similarly, the application of a certain dosage of nitrogen on a soybean line can result in increasing the size of the stomata [29].

### 3.6. The width of the stomata

The width of stomata depends on the condition of the cells guarding the stomata. The guard cells control the diameter of the stomata by widening or narrowing the stomatal opening [13, 51]. The level of potassium absorbance can also affect the size of the stomatal opening [52-53]. The results of the analysis presented in Figure 8 indicated that the size of the G1 stomata did not differ greatly from those of other soybean lines, except that of G3. Moreover, G4 and G10 reported significantly different sizes of stomata from G3 which was observed to have the widest stomatal opening. Similar findings also found diversity in the width of stomata covering some soybean lines [26]. The length and the width of the stomatal opening and the length of the guard cells determine the effectiveness of the stomatal opening activity.

The width of stomata is associated with the opening of the stomatal pore [54]. Figure 8 showed a significant difference in the width of the stomata found on the lower surface of the soybeans leaf. MLGG 0896 (G10) shared plenty of similarities with G1, G2, G4, and G6 and a little resemblance to G3, G5, G8, and G9. Snb/1087-148-2-1 (G1) and G7 differed greatly in terms of the size of the stomata; G1 possessed the smallest stomata while G7 possessed the biggest stomata. The cells in stomata can shrink from responding to a dry situation [55]. The size of the stomata will adapt to this changing to prevent water loss through transpiration and to increase CO₂ assimilation.

![Figure 8](image.png)

**Figure 8.** The width of the upper surface stomata (left), the width of the lower surface stomata (right). Letters a-d show the significance of the differences

A correlation test was performed to investigate the relationships between the variables involved in the analysis. Table 2 indicated that the width of the upper surface trichomes established a positive correlation with the number of lower surface stomata. This finding suggests that many stomata can be found on the lower surface of a leaf that is covered with wide trichomes, and vice versa. The length of the upper surface trichomes and the width of the lower surface stomata were also correlated. If the trichomes found on the upper surface of the leaf is longer, then it is more likely to happen that the stomatal opening on the lower leaf surface will be wider. Unlike these four characters, a negative relationship was established between the number of the upper surface trichomes and the length of the lower surface stomata. This finding indicates that if one of the parameters increases then the others will decrease. In other words, if the number of trichomes on the upper leaf surface experiences a surge, the length of the stomata on the lower leaf surface becomes shorter.
Table 2. The results of the correlation test

|     | WUT   | NUT   | LUT   | WLT   |
|-----|-------|-------|-------|-------|
| NLS | 0.633* | 0.086 | -0.233| 0.415 |
| LLS | -0.700*| 0.121 |       | -0.269|
| WLS |       | 0.678*|       | 0.224 |
| WUS |       |       |       | 0.645*|

*. A significance level of 0.05 (2-tailed).

Note: NLS = the number of the lower surface stomata; LLS = the length of the lower surface stomata; WLS = the number of the upper surface stomata; WUS = the width of the upper surface trichomes; WUT = the width of the upper surface trichomes; LUT = the length of the lower surface trichomes; WLT = the width of the lower trichomes.

4. Conclusion

The results of the leaf anatomical characterisation suggest that a diversity of trichomes and stomata can be found in ten soybean lines. A negative correlation was reported between the length of the stomata on the lower leaf surface and the number of the upper surface trichomes, but the other three variables were observed to have positive correlations.

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