Morphological and Anatomical Characteristics of leaves of Ten Soybean (Glycine max L. Merill) Lines

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Abstract. The characterization of the leaf morphology and anatomy can be useful to determine the superiority of the plant. The purpose of this study was to observe and analyze the morphological and anatomical leaves characteristics of the 10 soybean lines. The experiment employed a randomized block design with three replications. The results showed that the highest scores of the leaf length, leaf width, leaf thickness, petiole length, and petiole diameter could be observed in Brg/Myp-3 line. The largest leaf ratio was reported by Anj/MLGG 0511-29, and the lowest scores in the leaf length, leaf width, petiole length, petiole diameter, and leaf ratio were found in MLGG 0892 line. Grb/Lwt-12 have the highest number of trichomes, both on the upper and lower surfaces of the leaf, while Grb/Myp-65 had the smallest number. Anj/MLGG 0511-20 and Grb/Myp-14 had the longest trichomes, while Grb/Lwt 17 had the shortest trichomes. The highest number of stomata was recorded in Grb/Lwt-17. The results of the correlation test showed some significant relationships between the leaf characteristics: the length of the leaf and the width of the leaf and the petiole diameter; the length of the upper trichome and the length of the lower trichome, and the width of the upper trichome and the number of the lower stomata.

Keywords: Morphology, anatomy, soybean, leaves

1. Introduction
Soybeans are the third most important crop commodities after rice and maize. Soybeans provide essential plant-based protein which plays a crucial role in the improvement of public health and nutrition. The demand for soybeans is increasingly high as the human populations continue to grow and use soybeans as the main ingredient of various food products, such as tofu, tempeh, soy milk, and tauco i.e. preserved fermented soybeans [1]. Therefore, the Indonesian government has decided to import at least 2.5 million tons of soybeans every year to fulfill their needs. Sadly, the facts showed that local production could only yield 859 thousand tons of soybeans in 2016 and 675 thousand tons in 2017. Even though these numbers could improve to 2.2 million in 2018, as reported by the Bureau of Central Bureau of Statistics, they still cannot cover the shortages for local consumption [2].
Virus infection may result in decreasing the productivity of soybeans by 90 to 100%. Research on developing the superior quality of soybean that is resistant to CpMMV (Cowpea Mild Mottle Virus) has been conducted [3]. A conventional way such as crossing some soybean genetics has also been applied to create a new superior variety [4]. Soybeans are known to have a vast genetic diversity due to their high tendency to adjust and adapt to many different environments [5]. Therefore, to identify and analyze the physical characteristics of soybeans, a diversity study needs to be conducted. Aspects that can be observed include the morphological and anatomical traits of a leaf.

A leaf is a plant’s vegetative organ which serves an important function in soybean photosynthesis. Therefore, it is considered as a determinator factor of soybean production. The leaf area and the number of leaves growing from soybean can determine the productivity of the plant [6]. Leaf’s morphological character that can be used to inform the physical diversity of the plant is trichomes. Trichomes are derivatives of epidermal tissue that are spread throughout leaf surfaces. Soybean trichomes vary according to soybean’s type and variety [7]. Trichomes constitute an organ that is directly related to the attachment of the host. Soybean IAC-100 and IAC-80-596-2 with long and tight trichomes are exemplary pests-resistant lines [8]. Morphological characters possessed by plants are used as a genotypic defense system for pests and diseases [9]. Previous studies on leaf morphology have been carried out on healthy soybeans, and CpMMV attacked soybeans. Infected leaves are characterized by mosaic symptoms that turn leaves into pale, dry, brownish, and covered with lines or scratches [10]. In addition to morphological characters, anatomical characters are also important to determine the shape of cells and tissues in plant organs. The study of anatomical structure plays an important role in observing the effect of disease infection. Therefore, this study needs to be carried out to determine the morphological and anatomical characters of the 10 expected soybean lines and determine the correlation between the observed variables.

2. Methods

2.1. Research Setting

This research was carried out at two places, the Nuts and Tuber Crops Research Center in Jambegede, Kepanjen, Malang, East Java, Indonesia and the biology laboratory of Universitas Negeri Malang.

2.2. Materials

Materials used in this research were obtained from Indonesian Legume and Tuber Crops Research Institute, Indonesian Agency for Agricultural Research and Development at Malang. They were ten soybean lines, i.e. Anj/MLGG 0511-20 (G1), Anj/MLGG 0511-29 (G2), Brg/MLGG 0511-29 (G3), Brg/Myp-14 (G4), Brg/Myp-3 (G5), Grb/Lwt-12 (G6), Grb/Lwt-17 (G7), Grb/Lwt-22 (G8), Grb/Myp-65(G9), and MLGG 0892 (G10). The plant materials differed in genetics background. Anj/MLGG 0511 was derived from Anjasmoro and MLGG 0511 crossing, Brg/MLGG 0511 was derived from Burangrang and MLGG 0511 crossing, Brg/Myp was derived from Burangrang and Menyapa crossing, Grb/Lwt was derived from Grobogan and Lawit crossing, Grb/Myp was derived from Grobogan and Menyapa crossing, and MLGG 0892 was a germplasm from Pasuruan East Java.

2.3. Research Design

This experiment employed a randomized group design with 3 replications and 10 treatments or 30 experimental units in total. The soybean seeds were planted in an area of 2.24 m² with a planting spacing of 40 cm × 15 cm. The plants were well-maintained and regularly controlled to protect them from weed, pests, and diseases. Observed variables included the leaf morphological and anatomical characteristics. The observations were conducted on day 47 after sowing.

Leaf anatomical characteristics were determined by observing the leaf samples prepared. A stomata section was created by dropping 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. The soybean leaf was placed under the DinoLite microscope to observe the number of the leaf trichomes.
Meanwhile, the length and the width of the trichome were measured by separating some trichomes that were still attached to the leaf and putting them under the microscope for observation. The number of leaf stomata and trichomes was observed at 258.5 X magnification (Figure 1).

![Figure 1. The length and width of the stoma (A); The length and the width of the trichome (B)](image)

2.4. **Observed Parameters**

An observation was conducted to the morphological characteristics of each of the soybeans’ leaves including the length of the leaf (mm), the width of the leaf (mm), the length of the leaf petiole (mm), the diameter of the petiole (mm), and the leaf ratio (width/length index). All the parameters were measured using a pair of calipers, except for the thickness of the leaf (mm) which was measured using a micrometer screw gauge. Meanwhile, the leaf anatomical characteristics that were going to be observed consisted of the number of trichomes, the length and the width of the trichomes, the number of stomata, and the length and the width of the stomata. All those characters were observed on the upper and lower surfaces of the leaves and then measured using a microscope which was connected to the DinoCapture 2.0 Microscope software.

2.5. **Data Analysis**

The research data were analyzed using ANOVA, followed by the Duncan test. A correlation test was accordingly performed to analyze the relationships between the research variables.

3. **Results and Discussion**

3.1. **Leaf Morphology**

There was a significant difference in the length of the leaf and the petiole of the ten soybean varieties at a significance level of 1% and in the width of the leaf at a significance level of 5%. Other morphological characteristics such as the thickness of the leaf, the diameter of the petiole, and the leaf ratio of the ten soybean lines did not differ significantly (Table 1).

The average length of the soybean leaves ranged from 73.22 to 112.76 mm. The longest leaf was observed in G5 (Brg/Myp-3) while the shortest was found in G10 (MLGG 0892). The statistics also showed various lengths of the soybean leaves as follows: G7 96.67 mm, G4 95.42 mm, G3 88.23 mm, G1 87.52 mm, G9 95.42 mm, G6 82.53 mm, G8 77.46 mm, and G2 76.94 mm (Figure 2).
Table 1. The results of the ANOVA analysis of the leaf morphological characteristics of the ten soybean lines

| Characteristics               | Lines Mean Square | Error of Mean Square |
|-------------------------------|-------------------|----------------------|
| Length (mm)                   | 392.055**         | 3.503                |
| Width (mm)                    | 141.259*          | 22.500               |
| Thickness (mm)                | 0.005             | 0.001                |
| The petiole length (mm)       | 1029.327**        | 83.733               |
| The petiole diameter (mm)     | 0.197             | 0.456                |
| Ratio (mm)                    | 0.004             | 0.003                |

*a a significance level of 5%; **a significance level of 1%

Figure 2. The average length of each of the ten soybeans’ leaves. Letters a – f shows the significance of the differences.

The highest petiole was reported by G5 (Brg/Myp-3) with a size of 63.88 mm, and the shortest petiole was observed in G10 (MLGG 0892) with a size of 35.93 mm. In average, the lengths of the leaves’ petiole were: G1 49.62 mm, G2 47.25 mm, G3 49.50 mm, G4 51.86 mm, G6 49.50 mm, G7 51.03 mm, G8 45.91 mm, and G9 46.69 mm (Figure 3). The width of each of the soybeans’ leaves spanned from 0.17 mm to 0.31 mm.

The thickest leaf was observed in G5 (0.31 mm) and the thinnest leaf was found in G7 (0.17 mm) and G9 (0.17 mm), followed by other lines: G1 0.24 mm, G2 0.26 mm, G3 0.19 mm, G4 0.22 mm, G6 0.21 mm, G8 0.22 mm, and G10 0.22 mm (Figure 4). The average thickness of each of the soybeans’ leaves spanned from 0.17 mm to 0.31 mm.

The longest petiole was reported by G5 138.29 mm and the shortest petiole was observed in G10 74.50 mm. The average lengths of the petioles of each of the varieties were presented in Figure 5 (G1 123.81 mm, G2 117.42 mm, G3 135.10 mm, G4 129.04 mm, G6 137.69 mm, G7 127.77 mm, G8 115.73 mm, and G9 120.53 mm).
A correlation test was performed to investigate the relationships between the research variables. The results indicated that there was a significant correlation between the length of the leaf and the width of the leaf (r = 0.900**) and the diameter of the petiole (r = 0.801**). This finding suggested that an increase in the length of the soybean leaf led to an increase in the width of the leaf. The width of the leaf also established a significant relationship with the length of the petiole (r = 0.828**) and the diameter of the petiole (r = 0.791**). Significant correlations were also found between the thickness of the leaf and the leaf ratio (r = 0.833**) and between the length of the petiole and the diameter of the petiole (r = 0.658*) at a significance level of 5% (Table 2).
**Figure 5.** The average length of each of the ten soybeans’ petioles. Letter a-d shows the significance of the differences.

**Table 2.** The results of the correlation test on the research variables

|                           | The Width of the leaf | The thickness of the leaf | The length of the petiole | The diameter of the petiole | The leaf ratio |
|---------------------------|-----------------------|---------------------------|---------------------------|-----------------------------|---------------|
| The length of the leaf    | 0.900**               | 0.343                     | 0.621                     | 0.801**                     | 0.391         |
| The width of the leaf     | 0.483                 |                           | 0.828**                   | 0.791**                     | 0.419         |
| The thickness of the leaf |                       | 0.079                     | 0.137                     | 0.833**                     |               |
| The length of the petiole |                       |                           |                           | 0.658*                      | -0.042        |
| The diameter of the petiole|                       |                           |                           |                             | 0.072         |

**A significance level of 0.01 (2-tailed).**  
**A significance level of 0.05 (2-tailed).**

The length and width of the leaf can be used to measure the index of the leaf area [7]. A wide leaf area indicates high chlorophyll levels and a high photosynthesis rate. According, the photosynthesis process highly depends on the height of a plant and the number of the leaves [8]. The length of the soybean petiole fell between 74.50 and 138.29 mm. The report that the length of a soybean petiole may be affected by the recessive complementary genes that are associated with recessive homozygotes [9].
3.2. Leaf Anatomy

Table 3. The results of the ANOVA analysis of the leaf anatomical characteristics of the ten soybean lines

| Characteristics                          | Lines Mean Square | Error of Mean Square |
|------------------------------------------|-------------------|----------------------|
| The number of the upper surface trichomes| 1900.385**       | 17.407               |
| The number of the lower surface trichomes| 1783.633**       | 5.544                |
| The length of the upper surface trichome | 0.031*           | 0.011                |
| The length of the lower surface trichome | 0.027            | 0.015                |
| The width of the upper surface trichome  | 2.963×10⁻⁵       | 2.185×10⁻⁵           |
| The width of the lower surface trichome  | 2.370×10⁻⁵       | 2.926×10⁻⁵           |
| The number of the upper surface stomata  | 195.407*         | 29.930               |
| The number of the lower surface stomata  | 418.078**        | 0.322                |
| The length of the upper surface stoma    | 0.007            | 0.007                |
| The length of the lower surface stoma    | 0.007            | 0.006                |
| The width of the upper surface stoma     | 0.02             | 0.003                |
| The width of the lower surface stoma     | 0.02             | 0.002                |

*a significance level of 5%; **a significance level of 1%

The results of the analysis indicated some significant differences in the leaf anatomical aspects of the ten soybean varieties. The number of the upper trichomes, the number of the lower trichomes, and the number of the lower stomata were significantly different among the ten soybean lines. On the other hand, the length of the lower trichome, the width of the upper trichome, the width of the lower trichome, the length of the upper stoma, the width of the lower stoma, and the width of the lower stoma were reported similar in the ten soybean varieties. The average score for the number of the upper trichomes was 48.8 with a standard deviation of 26.

Figure 6 showed that the highest number of upper trichomes was found in G6 111 and the lowest was observed in G9 26. Meanwhile, the highest number of lower trichomes was reported by G6 (113 trichomes), and the lowest was observed in G2, G9 (39 trichomes). Trichomes can provide both benefits and harms for insects. The density, the length, the texture, the growth direction, and the structure of a trichome can protect the leaf from insects who intend to lay eggs or to feed on it [10].
Table 4. The average score, range, standard deviation, and results of the t-test on the ten soybean lines

| Characteristics                          | Average Score | Standard Deviation | Range             | T-test |
|------------------------------------------|---------------|--------------------|-------------------|--------|
| Number of the upper surface trichomes   | 49            | 26.022             | 13.00 – 124.00    | 0.000  |
| Number of the lower surface trichomes   | 70            | 25.204             | 29.00 – 126.00    |        |
| The length of the upper surface trichome| 0.992         | 0.136              | 0.72 – 1.26       | 0.236  |
| The length of the lower surface trichome| 0.968         | 0.135              | 0.74 – 1.24       |        |
| The width of the upper surface trichome | 0.027         | 0.005              | 0.02 – 0.03       | 0.211  |
| The width of the lower surface trichome | 0.025         | 0.005              | 0.02 – 0.03       |        |
| Number of the upper surface stomata     | 40            | 10.867             | 24.00 – 66.00     | 0.789  |
| Number of the lower surface stomata     | 40            | 14.129             | 22.00 – 78.00     |        |
| The length of the upper surface stoma   | 0.062         | 0.081              | 0.01 – 0.26       | 0.465  |
| The length of the lower surface stoma   | 0.060         | 0.079              | 0.01 – 0.26       |        |
| The width of the upper surface stoma    | 0.035         | 0.047              | 0.01 – 0.16       | 0.501  |
| The width of the lower surface stoma    | 0.034         | 0.046              | 0.01 – 0.13       |        |

Figure 6. The number of the upper trichomes (left) and the number of the lower trichomes (right). Letter a-h shows the significance of the differences.
Figure 7. The average length of the upper trichome. Letter a-c shows the significance of the differences.

Figure 7 indicated that the longest trichome (1.113 mm) was observed in G1, G4 and the lowest trichome was found in G7 with a size of 0.853 mm.

Figure 8. The average number of the upper stomata (left) and the average number of the lower stomata (right). Letter a – f shows the significance of the differences.

The results of the observations suggested that the highest number of the upper stomata was observed in G7 (56 stomata), and the lowest number was found in G1 (32 stomata). Meanwhile, the highest number of lower stomata was reported by G7 (68 stomata), and the lowest number of lower stomata was observed in (32 stomata) (Figure 8).

The longest upper (0.167 mm) and lower stomata (0.173 mm) were found in the same lines (G7) while the shortest upper stoma was observed in G10 (0.020 mm). The widest upper (0.077 mm) and lower stomata (0.080 mm) were also reported by the same variety (G7), while the narrowest stoma (0.010 mm) could be found in four lines (G2, G3, G5, and G10). Adie et al. points out that plants from desert climates normally have smaller stomata than plants that receive enough water [11]. Drought and observation periods can also affect the width/length ratio of a stoma. The stoma index is one of the leaf anatomical characteristics that can be influenced by the environment [12].
The results of the correlation test showed that there was a significant correlation between the number of the upper trichomes and the lower trichomes \((r = 0.783^{**})\). A positive correlation was also found between the length of the upper trichome and the length of the lower trichome \((r = -0.699^{*})\). These findings were depicted in Table 5.

**Table 5. The results of the correlation test on the anatomical characteristics of the soybean leaves**

|       | NUT | LLT | PTB | WUT | NUS | NLS | LUS | LLS | WUS | WLS |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| NUT   | 0.783** | 0.269 | 0.282 | 0.100 | -0.019 | -0.238 | 0.265 | -0.064 | 0.072 | -0.104 |
| NLT   | 0.144 | 0.123 | 0.006 | -0.362 | -0.377 | 0.189 | 0.030 | 0.081 | -0.056 | -0.018 |
| LUT   | 0.995** | 0.764* | 0.115 | -0.557 | -0.699* | -0.622 | -0.604 | -0.299 | -0.350 |
| LLT   | 0.784** | 0.155 | -0.562 | -0.719* | -0.642* | -0.629 | -0.352 | -0.404 |
| WUT   | -0.102 | -0.392 | -0.682* | -0.351 | -0.389 | -0.186 | -0.214 |
| WLT   | 0.044 | -0.272 | -0.382 | -0.368 | -0.456 | -0.525 |
| NUS   | 0.468 | 0.647* | 0.654* | 0.562 | 0.513 |
| NLS   | 0.733* | 0.804** | 0.595 * | 0.636* |
| LUS   | 0.982** | 0.860** | 0.874** |
| LLS   | 0.872** | 0.882** |
| WUS   | 0.993** |

**"Significant at the 0.01 level (2-tailed). * Significant at the 0.05 level (2-tailed).**

Notes: NUT = the number of the upper trichomes, NLT = the number of the lower trichomes, LUT = the length of the upper trichomes, LLT = the length of the lower trichomes, WUT = the width of the upper trichomes, WLT = the width of the lower trichomes, NUS = the number of the upper stomata, NLS = the number of the lower stomata, LUS = the length of the upper stomata, LLS = the length of the lower stomata, WUS = the width of the upper stomata, WLS = the width of the lower stomata

Based on the results of the research it can be assumed that an increase in the number of the leaf trichomes may result in improving the density of the trichomes. The trichomes density is strongly associated with the number of trichomes found on the upper and lower leaf surfaces. The density of the leaf trichomes affect the damage caused by *Spodoptera litura*. If the number of trichomes is high, then the intensity of the armyworm attack is predictably low [13]. Plants that are covered with less dense stomata and trichomes will have thinner palisade parenchyma [14]. Stomata pores, index, and density, as well as the size of the epidermal cells which affect leaf growth, also depend on the sun’s rays.

4. Conclusion
Based on the results of the soybean leaves characterization, it can be concluded that there was a significant difference in the length and width of the leaves as well as in the length of the leaves petioles. Other significant differences were also found in the soybean leaves anatomical characteristics, including the number of trichomes and stomata. However, the length and width of the stomata were the same. Besides, a positive correlation was also found between the length of the trichomes on the upper and lower surfaces, and the width of the upper trichomes and the number of lower stomata. On the other hand, the length of the lower trichomes was negatively correlated with the length of the stomata.

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