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Variability of *Catharanthus roseus* based on morphological and anatomical characters, and chlorophyll contents

SITI SAMIYARSIH♥, NETTYANI NAIPOSPOS♥♥, DIAN PALUPI♥♥♥
Faculty of Biology, Universitas Jenderal Soedirman. Jl. Dr. Suparno No. 63, Grendeng, Purwokerto, Banyumas 53122, Central Java, Indonesia. Tel.: +62-281-638794, Fax. +62-281-631700, ♥email: asih.fbio@gmail.com, ♥♥ nettyani.naipospos@gmail.com, ♥♥♥ dianpalupi08@gmail.com

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Abstract. *Samiyarsih S, Naipospos N, Palupi D. 2019. Variability of* Catharanthus roseus *based on morphological and anatomical characters, and chlorophyll contents. Biodiversitas 20: 2986-2993. Catharanthus roseus* (L.) is an important medicinal plant of the family Apocynaceae used to treat many of the diseases. *C. roseus* also possess good antioxidant potential**.** The purpose of this research was 1) to determine the variability of *C. roseus* based on the morphological, anatomical characters and chlorophyll contents, 2) to know the differences in morphological, anatomical characteristics and chlorophyll contents of *C. roseus* found in Banyumas Regency. The research used a survey method, with a purposive sampling technique. Preparation of leaf for anatomical analysis was based on paraffin method. The results showed that *C. roseus* in Banyumas Regency were grouped into eight varieties. The morphological characters observed consisted of 21 characters, 8 quantitative characters, and 13 qualitative characters. Most of the quantitative morphological and anatomical characters were significantly diverse ($p < 0.05$) except for petiole length, corolla number, stomata width and number of trichomata. The eight varieties of *C. roseus* have different chlorophyll a, chlorophyll b, and total chlorophyll contents but their anthocyanin contents were not significantly different.

Keywords: Anatomical character, *Catharanthus roseus*, chlorophyll, morphological character, variability

INTRODUCTION

Indonesia has a high diversity of medicinal plant's germplasm, one of which is *Catharanthus roseus*. This high diversity germplasm was obtained through exploration efforts. To be successfully utilized in plant breeding programs, the germplasm must be identified for the important characters, and expected to have a broad diversity. *C. roseus* is a complex and varied taxon consists of many varieties. This plant has traditionally been used to treat malaria, constipation, diuretics, diabetes mellitus and hypertension. *C. roseus* is commonly grown as an ornamental plant and medicine. This plant contains flavonoids, saponins, tannins, and various kinds of anti-cancer alkaloids such as vinblastine (VLB), vincristine (VCR) and leurosin (Jaleel et al. 2008; Pandiangan 2012; Nejat et al. 2015).

Catharanthus roseus has a purple, red, pink or white corolla (Plaizier 1981). According to Kumar et al. (2013) and Nejat et al. (2015), there are five variations of flower's color, namely white-yellow, white-red, pink-red, pink-white, and red-white. The leaves are widely used as alkaloid-producing medicinal plant materials (Renault et al. 1999). Several studies revealed that the leaves of *C. roseus* are able to treat various diseases. The existence of the *C. roseus* variety is a very valuable asset as part of germplasm conservation (Adnan et al. 2016).

Morphological dan anatomical characters have an important role in plant taxonomy and identification. The analysis of variance of morphological and anatomical characters revealed significant differences for majority of the tested traits, indicating there was variability among the taro accessions (Pitoyo et al. 2018). Environmental conditions trigger various plant responses including changes in morphology, anatomy, gene expression, cell metabolism and growth and productivity (Anjum et al. 2011). Pantilu et al. (2012) added that the mechanism of adaptation to light intensity is anatomical and morphological changes of leaves to maximize light absorption and photosynthesis efficiency. The physiological responses include increase in leaf area, ratio of chlorophyll a/b and anthocyanin pigment while anatomical changes include decreased leaf thickness, cuticle thickness, and trichomata density.

Information on morphological and anatomical characters of *C. roseus* is needed for development and improvement of this medicinal plant species so that its full potential can be optimally utilized. The objective of this research was to determine the variability of *C. roseus* plants in Banyumas Regency based on the morphological, anatomical characters and chlorophyll contents.

MATERIALS AND METHODS

Study area

The research employed a survey method, with purposive sampling in the Banyumas District, Central Java Province. Indonesia, including five subdistricts, namely: Sokaraja, Banyumas, Rawalo, Ajibarang, Baturraden, as shown in the following location map (Figure 1).

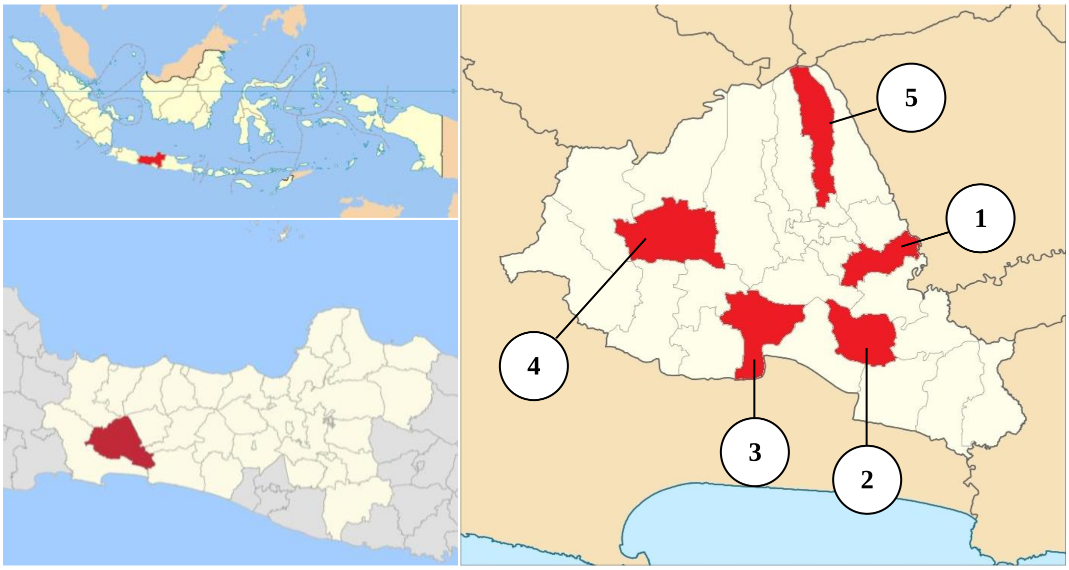

Figure 1. Map of research sampling area in Banyumas District, Central Java, Indonesia. Note: 1. Sokaraja Sub-district, 2. Banyumas Sub-district, 3. Rawalo Sub-district, 4. Ajibarang Sub-district, 5. Baturraden Sub-district

Plant materials

Catharanthus roseus plants were taken in several areas in Banyumas District including Sub-districts of Sokaraja, Banyumas, Rawalo, Ajibarang and Baturraden (Figure 1). Living plants of eight varieties of *C. roseus* were collected in 24 sites during the field survey. The flowering plants from each variety were collected and then transferred to a polybag and placed in the greenhouse of the Faculty of Biology, Jenderal Soedirman University, for one month. Plants were plantlet under controlled conditions at a temperature of 30º/27º C day/night, and relative humidity of 64%. Each plant sample was used for observation of morphological characters, including leaves and flowers. The 5th leaf from the shoot bud was taken to observed the anatomical character of the chlorophyll content. Flower samples were observed for anthocyanin content.

Morphological characters

Morphological characters measurements were taken on leaves and flowers. Qualitative characters included leaf shape, apex folii, base folii, margo folii, leaf surface color, leaf surface color, leaf surface texture, leaf stalk color, vernatio, inflorescence, flower symmetry, flower location, corolla color, and corolla diameter. Quantitative characters observed were leaf length, leaf width, petiole length, number of corolla, pedicle length, corolla diameter, corolla length, and width. All morphological characters were analyzed descriptively to determine the diversity of *C. roseus*.

Anatomical characters

The observed anatomical characters included cuticle thickness, epidermis thickness, mesophyll thickness, stomata size, stomata and trichomes density per 1 mm^2 area of epidermis of leaves. The 5th leaf from the shoot bud was taken and cut into one cm pieces, and then was subjected to fixation in FAA solution (FAA: 10% formalin, 5% acetic acid, 50% ethyl alcohol and aquadest 35%) for 24 hours. Preparation of leaf anatomy was based on the paraffin method, staining was done using safranin (1%) in 70% alcohol. Observation of anatomical characters was performed using a binocular microscope, Olympus CH-20. Measurement of anatomical characters was done using a calibrated ocular micrometer (Hammed et al. 2013; Pitoyo et al. 2018; Samiyarsih et al. 2018).

The measurement of chlorophyll and anthocyanin contents

Leaf samples were weighed 0.1 mg and extracted with 85% 10 mL acetone solution in a 1: 100 weight ratio. The extract was filtered with a filter paper and analyzed by using a UV Vis spectrophotometer on wavelengths of 644 nm and 663 nm. Chlorophyll content (mgL^{-1}) was determined based on the formula from the method of Gloria and Iswari (2015) and Setiari and Nurchayati (2009) as presented below:

Chlorophyll a = 1.07 (OD 663) - 0.094 (OD 644)

Chlorophyll b = 1.77 (OD 644) - 0.28 (OD 663)

Total Chlorophyll = 0.79 (OD 663) + 1,076 (OD 644)

Data analysis

The observed data of leaf morphological and anatomical characters were analyzed descriptively and used to describe and determine the differences in leaf anatomical characters. Quantitative data including chlorophyll content were subjected to analysis Variance (ANOVA), followed by a Duncan's New Multiple Range Test (DMRT) at a 95% confidence level to see the variability of the characters and the difference between their means.

RESULTS AND DISCUSSION

Morphological characters of *C. roseus*

Catharanthus roseus plants obtained in this research were classified into eight varieties based on the color of the corolla flowers (Table 1x). *C. roseus* is known to be tolerant to abiotic stresses such as drought and salinity, and the plant can survive in a variety of habitats such as sand, bushes, dryland, vineyards, roadsides, and beaches. This plant can grow at altitudes of 0 to 900 meters above sea level. Morphological characters of the eight varieties of *C. roseus* consisted of 21 characters; eight were quantitative characters and 13 were qualitative characters.

Observed qualitative characters included shape of leaf, apex folii, base folii, margo folii, abaxial surface of leaf, leaf surface texture, stalk of leaf, vernatio, inflorescence, flower symmetry, corolla color, does not have high variability (Table 2). *C. roseus* plants that have different leaf shapes, where varieties B and E have an obovatus leaf shape while the other six varieties have an ovalis leaf shape. In addition, apex folii in variety D was obtusus while that of seven other varieties was folii mucronatus. Analysis of variance for morphological characters showed a significant difference ($p<0.05$) in almost all observed characters of the eight varieties *C. roseus* except for pedicle length and corolla number. Meanwhile, the quantitative characters of leaf length, leaf width, petiole length, corolla diameter, and corolla length varied significantly among the varieties (Table 3).

Qualitative characteristics of eight varieties of *C. roseus* were actinomorphic flower symmetry, axillary flower, compound inflorescence, pinnate leaf, green petiole color, shiny leaf surface, green leaf color, dark green leaf surface, blunt leaf base and oval leaf shape (Figure 2). The qualitative characteristics that distinguished the eight varieties of *C. roseus* were the color of corolla (petala), which is milky white with a red radiating eye, small red center; white with a white eye, yellow center; purple-blue with a white large radiating base, yellow center; pale pink with red radiating eye, red center, pink with a dark pink radiating eye, pink center; pinkish red with a pink radiating eye, yellow center, and whitish pink with a pink radiating eye, yellow center (Figure 3). According to Shaw et al. (2009) and El-Domyati et al. (2012), detection of different varieties was based on the color of the petals and eyes of the flower and the middle color.

Table 1. The classification eight varieties of *Catharanthus roseus* based on the color of the corolla flowers

Code	Name of varieties	Petal color eye color
A	Milky white	Red radiating eye with small red center
B	White	White eye with yellow center
C	Purple blue	White large radiating base with yellow center
D	Pale pink	White eye with yellow center
E	Pink	Dark pink radiating eye with pink center
F	Pinkish red	Pink radiating eye with yellow center
G	Pinkish red	Red radiating eye with yellow center
H	Whitish pink	Pink radiating eye with yellow center

Table 2. The qualitative morphological characters of eight varieties of *Catharanthus roseus*

Var.	Leaf shape	Apex folii	Base folii	Margo folii	Leaf abaxial surface	Leaf surface texture	Leaf stalk	Vernatio	Inflorescence	Flower symmetry	Flower location
A	Ovalis	Mucronatus	Obtusus	Integer	Dark-green	Shiny	Light green	Pinnate	Compound	Actinomorph	Axilaris
B	Obovatus	Mucronatus	Obtusus	Integer	Dark-green	Shiny	Light green	Pinnate	Compound	Actinomorph	Axilaris
C	Ovalis	Mucronatus	Obtusus	Integer	Dark-green	Shiny	Light green	Pinnate	Compound	Actinomorph	Axilaris
D	Ovalis	Obtusus	Obtusus	Integer	Dark-green	Shiny	Light green	Pinnate	Compound	Actinomorph	Axilaris
E	Obovatus	Mucronatus	Obtusus	Integer	Dark-green	Shiny	Light green	Pinnate	Compound	Actinomorph	Axilaris
F	Ovalis	Mucronatus	Obtusus	Integer	Dark-green	Shiny	Light green	Pinnate	Compound	Actinomorph	Axilaris
G	Ovalis	Mucronatus	Obtusus	Integer	Dark-green	Shiny	Light green	Pinnate	Compound	Actinomorph	Axilaris
H	Ovalis	Mucronatus	Obtusus	Integer	Dark-green	Shiny	Light green	Pinnate	Compound	Actinomorph	Axilaris

Note: qualitative morphological characters of leaves and flowers of *C. roseus*. Note: Varieties of A-H were distinguished based on the color of the corolla as indicated in Table 1

Table 3. The average of quantitative characters of the eight varieties of *Catharanthus roseus*

Varieties	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Pedicle length (cm)	Corolla diameter (cm)	Corolla length (cm)
A	5.76±1.60b	2.64±0.58b	0.72±0.15b	2.56±0.05a	4.54±0.05b	2.24±0.05b
B	4.92±1.50a	1.84±0.41c	1.76±0.10c	2.66±0.05a	5.16±0.05c	2.56±0.05b
C	6.02±1.61b	2.70±0.60b	0.56±0.16a	2.34±0.05a	3.82±0.07a	1.98±0.07a
D	5.58±1.51b	2.16±0.74d	0.48±0.13a	2.32±0.04a	4.24±0.05b	2.06±0.05b
E	5.70±1.23b	2.36±0.47b	0.70±0.20b	2.44±0.05a	4.66±0.05b	2.28±0.04b
F	4.78±1.11a	1.70±0.42c	0.74±0.16b	3.54±0.05b	4.14±0.05b	2.10±0.06b
G	4.26±1.02c	1.32±0.29a	0.66±0.16b	2.44±0.05a	4.22±0.04b	2.06±0.05b
H	4.44±1.03c	1.34±0.33a	0.70±0.09b	2.44±0.08a	4.26±0.05b	2.04±0.05b

Note: Numbers followed by the same letters in the same column are not significantly different ($p>0.05$). Note: Varieties of A-H were distinguished based on the color of the corolla as indicated in Table 1

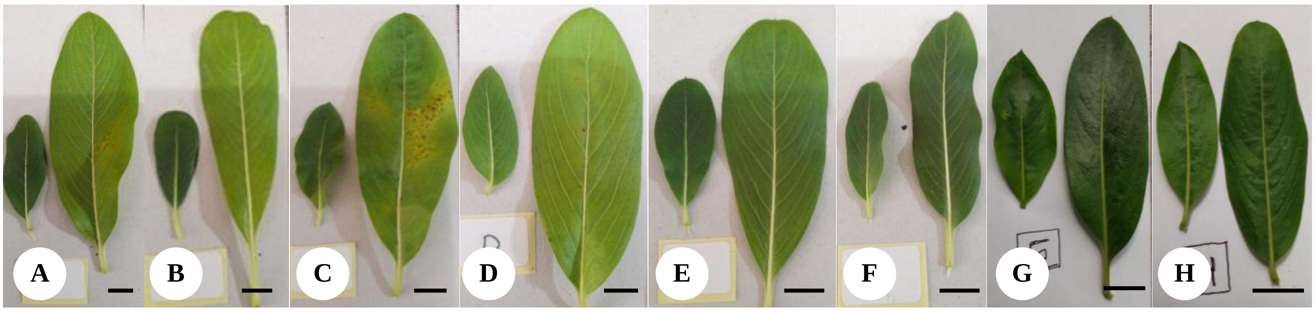

Figure 2. Morphology of leaves of eight varieties of *Catharanthus roseus* (L.). Bar = 1 cm. Note: Varieties of A-H were distinguished based on the color of the corolla as indicated in Table 1

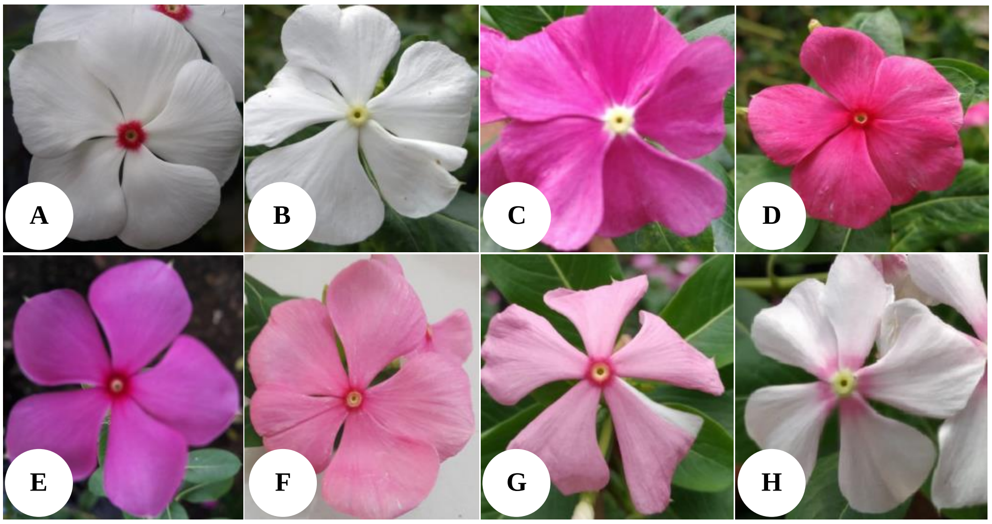

Figure 3. Photographs of the color flowers the eight varieties of *Catharanthus roseus*. Note: Varieties of A-H were distinguished based on the color of the corolla as indicated in Table 1

Table 4. The average of anatomical character of eight varieties of *Catharanthus roseus*

Varieties	Cuticle thickness adaxial (µm)	Cuticle thickness abaxial (µm)	Epidermis thickness adaxial (µm)	Epidermis thickness abaxial (µm)	Mesophyll thickness (µm)
A	4.75±0.37ab	3.25±0.34a	13.40±1.07a	10.75±0.68a	169.5±5.86bcd
B	5.25±0.37ab	3.00±0.34a	22.50±1.07d	16.70±0.68c	170.0±5.86bcd
C	5.70±0.37a	2.85±0.34a	18.75±1.07bc	12.50±0.68ab	149.0±5.86a
D	4.25±0.37b	1.75±0.34b	17.50±1.07b	11.25±0.68a	176.5±5.86bcd
E	2.75±0.37c	1.75±0.34b	21.50±1.07cd	14.00±0.68b	185.5±5.86d
F	3.00±0.37c	1.25±0.34b	24.00±1.07cd	17.50±0.68c	181.5±5.86cd
G	2.25±0.37c	2.25±0.34ab	24.00±1.07d	16.00±0.68c	165.5±5.86abc
H	2.00±0.37c	1.50±0.34b	24.00±1.07d	17.00±0.68c	162.5±5.86ab

Note: Numbers followed by the same letters in the same column are not different significant at the level of $p < 0.05$. Varieties of A-H were distinguished based on the color of the corolla as indicated in Table 1

Table 5. The average of length, width, stomata and trichomes density of eight varieties *Catharanthus roseus*

Varieties	Length of adaxial stomata (µm)	Length of abaxial stomata (µm)	Width of adaxial stomata (µm)	Width of abaxial stomata (µm)	Density of stomata (mm^2)	Density of trichomes (mm^2)
A	23.60±0.54a	19.10±0.89b	7.10±0.33a	7.95±0.29b	17.00±0.14b	3.80±0.28b
B	21.75±0.54b	20.25±0.89b	6.70±0.33ab	7.85±0.29b	16.40±0.14b	4.60±0.28a
C	17.25±0.54c	17.40±0.89b	5.65±0.33cd	7.65±0.29b	14.40±0.14b	3.60±0.28b
D	14.30±0.54d	16.75±0.89a	6.60±0.33abc	6.10±0.29a	18.00±0.14a	3.80±0.28b
E	16.50±0.54c	17.90±0.89a	7.00±0.33a	6.35±0.29a	15.00±0.14a	4.80±0.28a
F	23.50±0.54a	20.30±0.89b	5.55±0.33d	8.10±0.29c	13.80±0.14b	4.60±0.28a
G	20.35±0.54b	17.45±0.89c	5.80±0.33bcd	9.10±0.29bc	15.00±0.14c	3.80±0.28b
H	16.40±0.54c	19.10±0.89bc	6.30±0.33abcd	8.40±0.29c	16.80±0.14bc	3.60±0.28b

Note: Numbers followed by the same letters in the same column are not different significant at the level of $p < 0.05$. Varieties of A-H

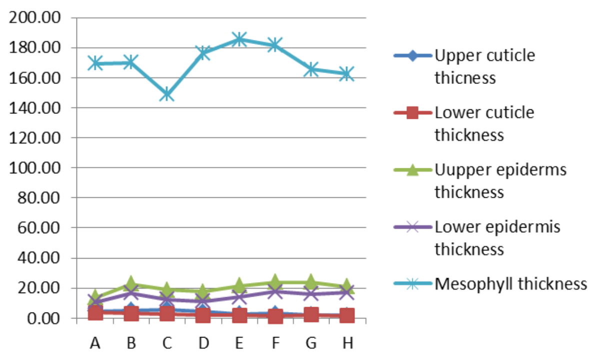

Figure 4. Thickness of cuticles, epidermis and mesophyll leaves of eight *C. roseus* varieties

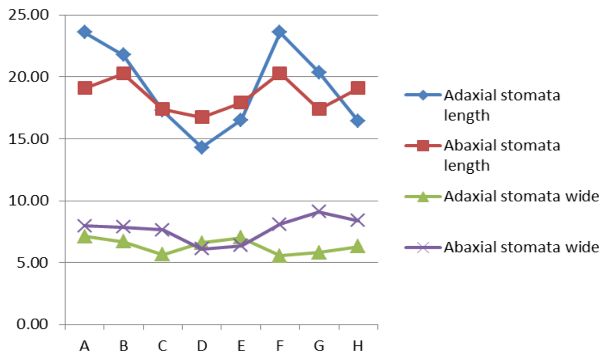

Figure 5. The length and width of the stomata of the leaves of eight *C. roseus* varieties

In general, the results showed that *C. roseus* had low variability in qualitative characters such as leaf shape, apex folii, base folii, surface of leaf, vernatio, inflorescence, and symmetry of flower and location of flower. Morphological characters such as plant height, leaf length, leaf width, petiole length, flower diameter, and corolla length were highly variable. This shows that morphological characters can be used to distinguish variability of *C. roseus*. Leaf characters are often ignored by taxonomists when identifying and classifying plants because of their high phenotypic plasticity. Leaf characters, especially venation patterns, are genetically improved so that they can be used as taxonomic tools (Masungsong et al. 2019). Morphological characters are still routinely used for preliminary evaluation because they are fast, simple, cheap and can be used as a general approach to assessing plant genetic diversity (Jingura and Kamusoko 2015). Pitoyo et al. (2018) reported that taro accessions showed high variability in morphological characters. Morphological characters, therefore, can be used to distinguish the variability of varieties.

The anatomical character of *Catharanthus roseus* leaves

Anatomical character is one approach used to help solving taxonomic problems that are morphologically difficult to separate or still doubtful. Custódio et al. (2013) stated that the commonly used anatomical characters were epidermal thickness, mesophyll thickness, cuticle thickness, leaf mesophyll character (palisade and sponge), stomata density, stomata length, and width.

The analysis results showed that the eight observed varieties of *C. roseus* varied greatly in characters such as cuticle thickness, epidermis thickness, mesophyll thickness, stomata length and density of the observed varieties (Tables 2-3; Figures 4-5; Figures 6-7). Analysis of variance showed the eight *C. roseus* varieties were significantly different ($p<0.05$) for all observed anatomical characters, except for stomata width and trichome density per 1 mm^2 of leaf area. The cuticle of the leaves functions as a response to changes in environmental factors that are unfavorable to plants, and the thickness of the cuticle plays a role in reducing the rate of transpiration in the leaves (Tanzerina et al. 2013). The thickness of the cuticle layer is influenced by the presence of cutin compounds which are able to prevent the evaporation of water molecules from the leaves (Ali et al. 2009). Pitoyo et al. (2018) reported significant differences for the majority the anatomical characters of taro indicating a high variability among the taro accessions.

Mesophyll is located between the two epidermal layers, differentiated into palisade parenchymal tissue and spongy parenchyma. The palisade parenchyma is elongated and tightly arranged. The spongy parenchyma consists of cells with a lot of space between cells (Figure 6). The observations results showed that the eight *C. roseus* varieties significantly differed in the above quantitative anatomical characters. Mesophyll thickness of *C. roseus* leaves ranged between 149.00 - 185.50 µm. According to Salisbury and Ross (1995), palisade cells will elongate, thicken or form additional layers of palisade when the plants receive high light intensity. Pantilu et al. (2012) added that plants that receive high light intensity produce smaller leaf, thicker and compact palisade, and small cell space. The thickness of leaf mesophyll is also determined by marginal initial cell division and submarginal initials.

The ANOVA results of showed that the eight varieties were significantly different ($p<0.05$) in their stomata density. The highest stomata density (stomata per 1 mm^2 of leaf area) was observed in *C. roseus* with a Pale pink corolla color, red radiating eye with red center (D), while the lowest stomata density was found in *C. roseus* with a Pinkish red corolla color, pink radiating eye with yellow center (F) (13.6 stomata per 1 mm^2 of leaf area). The surface of the abaxial epidermis has a higher stomata density compared to the surface of the adaxial epidermis. The length and width of the stomata were also significantly different among the eight varieties ($p<0.05$). The longer and wider stomata will result in a high transpiration rate because more water molecules are released by the leaves, which will increase the absorption of nutrients from the soil. The absorbed nutrients are then used for photosynthesis, which causes an increase in the rate of photosynthesis, and hence, affects plant growth and development. Hameed et al. (2013) stated that the surface of the abaxial epidermis has a higher stomata density than the surface of the adaxial epidermis. Stomata density and size affect the transpiration process or the efficient use of O_2 and CO_2 in plants.

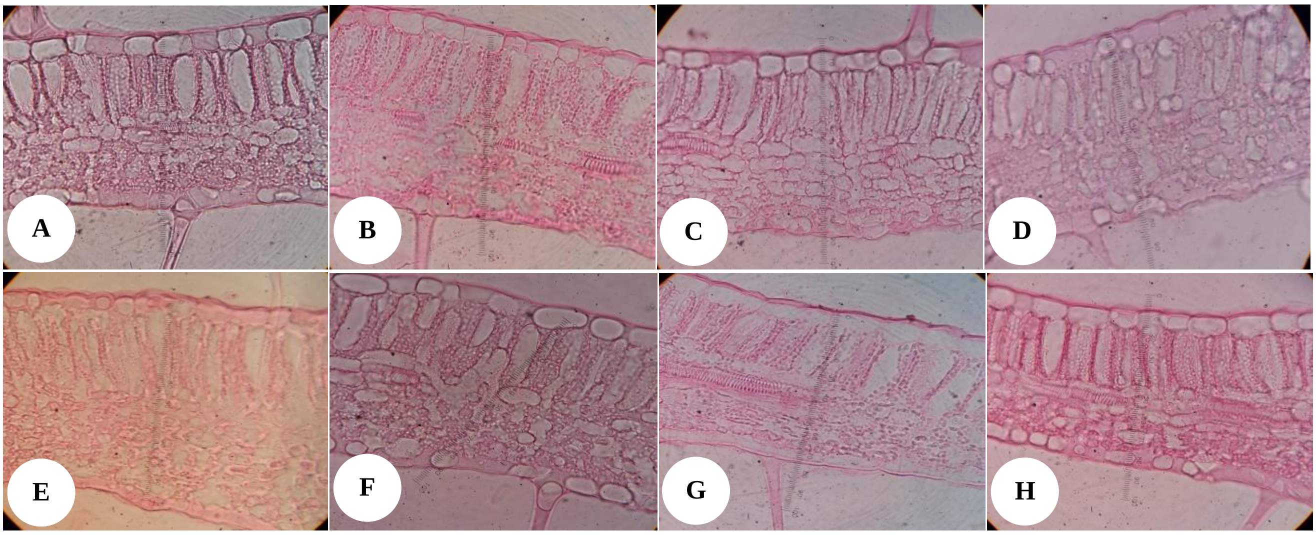

Figure 6. Cross-section of leaf eight varieties *Catharanthus roseus*, magnification of 400x. Note: Varieties of A-H were distinguished based on the color of the corolla as indicated in Table 1

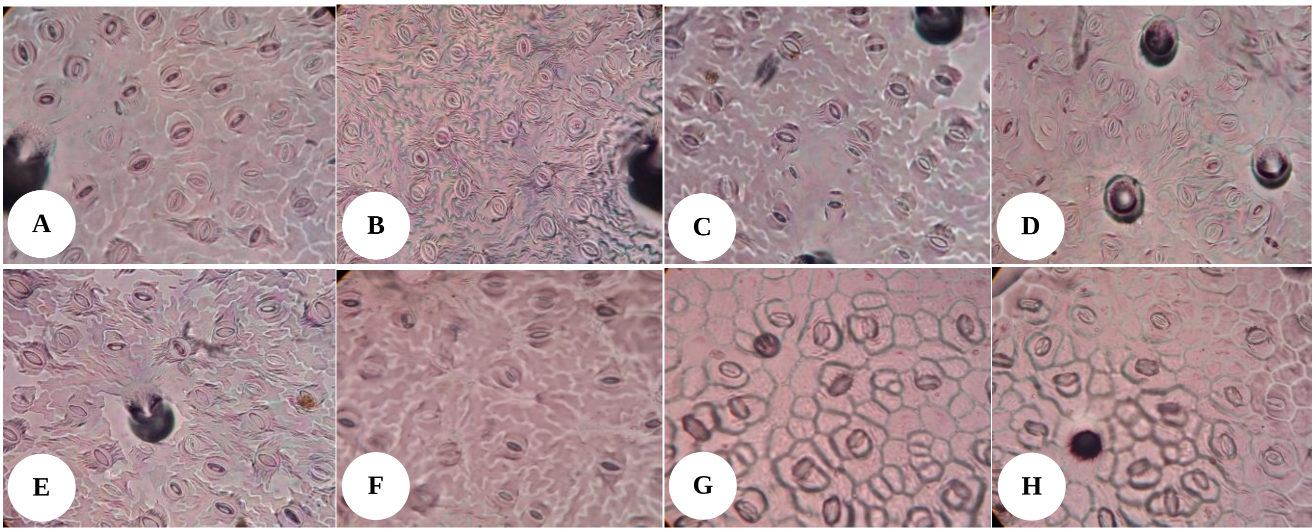

Figure 7. The stomata dan trichomes of eight *Catharanthus roseus* varieties, magnification of 400x. Note: Varieties of A-H were distinguished based on the color of the corolla as indicated in Table 1

Table 6. Average chlorophyll a, b, total chlorophyll and anthocyanin contents of eight *Catharanthus roseu.* varieties.

Variety	Chlorophyll a (mgL^{-1})	Chlorophyll b (mgL^{-1})	Total chlorophyll (mgL^{-1})	Anthocyanin (mgL^{-1})
A	8.99±0.21cd	3.81±0.11cd	0.79±0.49b	0.19±0.018
B	9.80±0.21cd	3.88±0.11cd	0.88±0.49bc	0.20±0.018
C	12.24±0.21e	4.82±0.11d	0.47±0.49a	0.18±0.018
D	6.41±0.21ab	3.30±0.11ab	0.89±0.49bc	0.20±0.018
E	8.66±0.21bc	3.60±0.11bc	1.11±0.49d	0.22±0.018
F	7.96±0.21a	3.10±0.11a	0.97±0.49cd	0.22±0.018
G	8.05±0.21cd	3.64±0.11cd	0.78±0.49b	0.17±0.018
H	11.16±0.21d	3.98±0.11d	0.74±0.49bc	0.22±0.018

Note: Numbers followed by the same letters in the same column are not significantly different at the level of $p < 0.05$. Columns that are not followed by letters indicate that treatment had no significant effect. Varieties of A-H were distinguished based on the color of the corolla as indicated in Table 1x

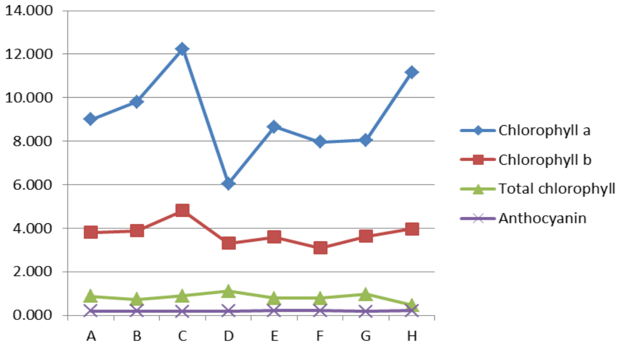

Figure 8. The contents of chlorophyll a, b and total chlorophyll, and anthocyanin of eight *C. roseus* varieties

Plants have the ability to respond to their needs, especially during their life cycle, if environmental factors are not supportive. This response can be in morphological, physiological or anatomical forms. Maghsoudi and Moud (2008) stated that light intensity, air humidity, and CO_2 concentration are environmental factors that influence changes in stomata size. Stomata have an important role in respiration, photosynthesis and transpiration processes in plants. Opening of stomata on the leaf surface is to release air in the form of steam into the atmosphere (Saadu et al. 2009). According to Haryanti (2010) stomata have a mechanism of adaptation to changes in groundwater content, which is affected by the capacity of the soil in water storage. Adams et al. (2011) stated that the most beneficial adaptation of plants in responding to the effect of environmental factors is to maintain the rate of transpiration by reducing the density and size of stomata.

The chlorophyll and anthocyanin contents

ANOVA results showed that chlorophyll and anthocyanin were not significantly different ($p<0.05$) among the eight *C. roseus* varieties. Chlorophyll that is synthesized in leaves is different in number for each type of plant depending on environmental and genetic factors. One of the internal factors that affect the rate of photosynthesis is the leaf chlorophyll content. The higher the chlorophyll content, the more efficient it will be in capturing sunlight energy for photosynthesis. The results of measurement of chlorophyll and anthocyanin content in eight varieties of *C. roseus* are presented in Table 6 and Figure 8.

Based on Table 6 and Figure 8, the highest chlorophyll a and chlorophyll b contents in the leaves were observed in *C. roseus* verities with purple blue (C), respectively, 12.24 mgL^{-1} and 4.82 mgL^{-1}. The chlorophyll a and chlorophyll b contents in plants affect growth and development processes. The higher the chlorophyll a and chlorophyll b contents, the higher the rate of photosynthesis will be, which causes a more optimal absorption of nutrients from the soil, which will stimulate plant growth and development. There was a linear relationship between leaf mesophyll thickness and the total chlorophyll content, where the thicker the mesophyll, the higher the total chlorophyll content. Variety E (pink) has the thickness mesophyll (185.5 μm) and the highest total chlorophyll content (1.11 mgL^{-1}). According to Taiz and Zieger (1998), chlorophyll a and chlorophyll b are very good at absorbing the red spectrum. This red spectrum with a wavelength of 630 - 675 nm will later be used to produce energy in photosystem I and photosystem II. The energy produced will be used for the growth process. Zen et al. (2016) reported that photosynthesis occurs perfectly because there are thick palisades that contain lots of chlorophyll, so photosynthesis rates increase, plant growth increases and productivity also increases. Factors that influence chlorophyll synthesis include light, water, temperature, sugar or carbohydrates, genetic factors and elements such as nitrogen, magnesium, iron, manganese, Cu, Zn, sulfur, and oxygen.

The study results showed no relationship between anthocyanin content and corolla color in the eight varieties. Varieties E (Pink), F (Pinkish red) and H (Whitish pink) have the highest anthocyanin content (0.22 mgL^{-1}) and the anthocyanin content of variety G (Pinkish red) was 0.17 mgL^{-1}. This shows that the G (Pinkish red) variety with sharp corolla color has the lowest anthocyanin content, while the H (Whitish pink) variety with pale corolla has the highest anthocyanin content. The difference in anthocyanin content can also be caused by differences in their ability to synthesize anthocyanin. Mlodzinska (2009) stated that the biosynthetic ability of each plant in producing color pigments is not the same, resulting in different color of the plant organs. Hasidah reported that the low anthocyanin level in plants was due to the inhibition of anthocyanin synthesis activity by chlorophyll synthesis.

To conclude, 21 morphological characters (8 quantitative and 13 qualitative characters) were observed in eight varieties of *Catharanthus roseus* in Banyumas Regency. Almost all quantitative morphological and anatomical characters were significantly diverse ($p <0.05$) among the eight varieties, except for petiole length, corolla number, stomata width and trichomata number. The eight varieties of *C. roseus* have different contents of chlorophyll a, chlorophyll b, and total chlorophyll contents, but their anthocyanin content was not significantly different.

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