

Morphological and physiological insights into coordination of anthocyanin deposition and cuticle formation in leaf development of *Rosa chinensis*

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Abstract

Juvenile leaves exhibit a red coloration due to the presence of anthocyanins, which assist plants in resisting various environmental stresses. This characteristic is common and visually striking among many woody species across various higher plant families. However, the mechanisms underlying leaf color change during development and the defense mechanisms of mature leaves remain unclear. In this study, we analyzed the mechanism of color change from red to green in Chinese Rose (*Rosa chinensis*) leaves and the development of cuticular wax in their natural state. The results show that anthocyanins and cuticular waxes are deposited at different stages of leaf development. During the red and young stage, anthocyanins are abundant in both the upper and lower epidermal cells of the leaves, while no cuticular wax is observed. As the leaves develop, the content of anthocyanins gradually decreases, leading the leaves to turn green. Anthocyanins first disappear from the upper epidermis, while the accumulation of cuticular wax begins at this stage. By the time the leaves are fully differentiated and green, anthocyanins have completely disappeared, and cuticular wax is deposited on both the upper and lower epidermis. The anthocyanins accumulated in the epidermal cells are primarily cyanidin, which is present in significantly higher content during the early stages of development compared to the later stages. Using gas chromatography-mass spectrometry (GC-MS) to determine the composition and content of leaf waxes, we found that alkanes and esters are the most prominent components present in the leaves. The content of waxes increases significantly in the later stages of development. In summary, our results indicate that the coordinated development of anthocyanins and cuticular wax provides a strategic mechanism to ensure the protection and functionality of *Rosa chinensis* leaves.

1 Introduction

It is widely recognized that leaves of different plant species, as well as those of the same species at various developmental stages, exhibit distinct colors ranging from deep green to light green, and even including red or purple. These vibrant colors serve as crucial indicators of plant health, environmental defense, and adaptation, while also holding significant ornamental and aesthetic value^[1]. In plants, leaf color changes primarily occur in two ways. The first type involves new leaves that are green, with their shapes altering with the seasons, thereby providing ornamental value. Examples of such plants include smoke trees (*Cotinus coggygia*), sweetgum (*Liquidambar formosana*), and Chinese pistache (*Pistacia chinensis*)^[2–4]. The second type encompasses leaf color changes that are not affected by seasonal variations, in which young tender leaves are red and gradually turn green as they mature, as observed in Chinese Rose (*Rosa chinensis*) and *Acer tutcheri*^[5]. While the first type has been extensively studied, the morphological developmental characteristics and mechanisms^[6], functions associated with the second type of leaf color change remain unclear.

Three types of pigments, chlorophylls, carotenoids, and flavonoids, are recognized as the primary pigments responsible for color changes in plant leaves^[7]. Carotenoids are present in leaves throughout the year, but their visibility is obscured by the green hue of chlorophyll in mature leaves. In autumn, the breakdown of chlorophyll into colorless metabolites allows the carotenoids to become visible^[8]. Red leaf coloration in

plants is often attributed to the presence of betalains or anthocyanins^[9]. Betalains are commonly found in plants of the caryophyllales, whereas anthocyanins are detected in all other flowering plants. These two pigments are mutually exclusive and have not been observed co-occurring in the same plant^[10, 11]. Among the most prevalent and biologically active types of anthocyanins found in nature are six primary forms, each characterized by distinct colors: pelargonidin (PelC), which displays an orange-red hue; cyanidin (CC), ranging from red to purple; delphinidin (DC), exhibiting a blue-purple color; peonidin (PeoC), a methylated derivative of cyanidin; petunidin (Pt), known for its deep purple shade; and malvidin (Mv), a methylated derivative of delphinidin. These anthocyanins are not only visually striking but also play vital roles in plant defense and attracting pollinators^[12]. These pigments impart various hues to plants, including pink, purple, red, and blue. Notably, the phenomenon of red coloration in nutrient organs, particularly in leaves, is the most prevalent^[13].

Anthocyanins play a crucial role in leaf color changes, possess antioxidant capabilities, neutralize free radicals, and slow cellular aging^[14]. Under strong light, anthocyanins can absorb excess light energy and convert it into heat, thereby protecting red young leaves from photoinhibition and photooxidative damage^[15, 16]. In addition to shielding visible light, anthocyanins also play a protective role by filtering or absorbing ultraviolet light^[17]. Conversely, UV-B radiation can enhance anthocyanin content in leaves^[18]. Anthocyanins also play important roles in antioxidant activities, osmoregulation, and pest resistance^[19–21], providing strong support for plant growth.

The cuticular layer on the surface of leaves plays a crucial role in leaf development. The cuticle is a thin waterproof layer covering the surface of plant leaves and is primarily composed of long-chain fatty acids, alcohols, and esters^[22, 23]. The primary function of the cuticle is to provide a physical barrier for plant leaves, inhibiting water loss from the plant surface, which is essential for plant survival in dry or stressful environments^[24]. Additionally, cuticular wax serves the functions of pest and disease resistance; it reduces the retention of water droplets, decreases the incidence of plant diseases, and contains antibacterial components that protect plants from pathogens^[25]. Furthermore, it can reflect sunlight, reduce leaf temperature, prevent damage to plants under intense sunlight, and increase leaf hardness and resilience, thereby enhancing resistance to environmental influences^[26]. However, research on the synergistic relationship between cuticular waxes and anthocyanins during leaf development remains relatively limited. In the future, as research in plant biology and biochemistry advances, we may gain a deeper understanding of the interactions and relationships between these two compounds within plants.

Chinese Roses (*Rosa chinensis* Jacq.) are shrubby plants in the Rosaceae family. They have pinnate compound leaves, with young leaves initially red that gradually turn green as they develop. Mature leaves are dark green and glossy on the surface. During the developmental stages of rose leaves, the question arises: How do the leaves transition from red to green, and why does this shift occur? What connection does this transformation have with the plant's adaptation to a variable external environment? Based on this, we measured the main pigments in rose leaves at different developmental stages and found that the color changes in rose leaves are primarily due to the accumulation of anthocyanins. Additionally, we observed the formation process, types, and content of the cuticular layer in the leaves. We discovered that

anthocyanins and the cuticular layer alternately appear during the early and late stages of leaf development, respectively, demonstrating continuity and extension in developmental terms.

2 MATERIALS AND METHODS

2.1 Plant Materials and Growth Condition

Seedlings of *Rosa chinensis* Jacq. (Rosaceae) were cultivated in a natural environment at the Ornamental Center of Henan Normal University, Xinxiang, China (35°2′N, 113°8′E). For stereomicroscope photography, the development of *Rosa chinensis* was captured in a horizontal orientation using a Canon digital camera EOS5D (Canon Instech Co. Ltd, Tokyo, Japan) (Supplement Fig. 1).

2.2 Pigments content analysis

Chlorophylls were extracted in 2 mL of 80% acetone from fresh leaf samples at room temperature in the dark for 24 h. The absorbance of the chlorophyll extracts was recorded at 470 nm, 645 nm, and 663 nm using a UV-Visible spectrophotometer (UV754, Shimadzu Corporation, Japan). The chlorophyll and carotenoid concentrations were calculated according to Wellburn^[27]. At least three replicates were performed.

pH differential method for anthocyanin^[28]: Dissolve 20 mg of the sample with 10% ethanol, and then dilute with distilled water to a final volume of 10 mL in a volumetric flask to prepare the test solution. Transfer 1 mL of the test solution into a 10 mL volumetric flask each time. Use KCl/HCl buffer (pH 1.0) and sodium acetate buffer (pH 4.5) to dilute to the mark. React in the dark for 1 h. Measure the absorbance at both 510 nm and 700 nm. Perform parallel tests for each sample and calculate the anthocyanin content using the following formula: Anthocyanin content (mg/L) = $A \times MW \times DF \times 1000 / \epsilon$, where $A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}$; MW (Molecular Weight): g/mol of cyanidin-3-glucoside (449.2 g/mol); DF: Dilution factor; ϵ : Molar absorptivity of cyanidin-3-glucoside [26900 L/(mol·cm)]. The experiment should be repeated at least three times to ensure reliable results.

ESI-HPLC-MS/MS analysis of anthocyanin: A 0.5 g portion of lyophilized powder was transferred to a 3 mL extraction buffer composed of 95% ethyl alcohol and 1.5 M HCL in a ratio of 85:15. The solution was sonicated for 30 min and centrifuged at 10,000g for 5min at 4°C. The supernatant was collected, and 3 mL of extraction solution was added to the sediment for further extraction. The combined supernatants were then treated with 1 mL of concentrated HCl and heated in a water bath at 90°C for 40 min. After natural cooling, the solution was diluted with acetonitrile to a final volume of 10 mL. The extract was filtered through a 0.22 µm PTFE syringe filter. The filtrate was analyzed using HPLC (Agilent 1290, Agilent Technologies, USA) and ESI-LC/MS (SCIEX-6500Qtrap, Applied Biosystems, USA). HPLC analyses were performed at a flow rate of 1.0 mL/min with a column oven temperature of 35°C and a detector wavelength of 292 nm. The solvent system employed consisted of acetonitrile and water/formic acid (99:1, v/v) in a volume ratio of 3: 7. For the identification of individual anthocyanins, the MS analysis was conducted using an ESI interface. The MS operating conditions were as follows: ion spray voltage of 4.5 kV; curtain gas (N₂) at 30 psi; nebulizing gas and heating gas (N₂) at 65 psi; and heating gas temperature of 400°C.

2.3 Measurement of enzyme activity

Phenylalanine ammonia-lyase (PAL) activity: The fresh materials weighing 0.5 g were placed in a mortar, followed by the addition of 2 mL of 0.1 M boric acid extraction buffer before thoroughly grinding the mixture on an ice bath. The homogenate was then centrifuged at 4°C, 10,000 g for 20 min. A portion of 0.5 mL of the supernatant was taken, and 3 mL of 0.05 M boric acid buffer and 0.5 mL of 20 mM L-phenylalanine were added before incubation at 37°C for 60 min. After incubation, 0.1 mL of 6 M HCl was immediately added to terminate the reaction. The enzyme extract was then placed in a boiling water bath for 5 min and used as a control, after which the enzyme extract was measured at 290 nm using a UV-Visible spectrophotometer (UV754, Shimadzu Corporation, Japan).

Chalcone isomerase (CHI) activity was measured following the method with minor modifications^[29]. CHI was extracted from 2 g of samples in 5 mL of 100 mM sodium acetate buffer (pH 5.2) containing 1 mM EDTA, 5 mM β -mercaptoethanol and 0.1% (w/v) ascorbic acid. After centrifugation at 12000 g for 15 min at 4°C, the supernatants were collected for CHI determinations. The reaction mixture consisted of 2 mL of 50 mM Tris-HCl buffer (pH 7.4) containing 7.5 mg/mL BSA and 50 mM NaHSO₃, along with 0.2 mL of 1 mg/mL chalcone. Changes in the absorbance of the reaction solution were monitored at 381 nm.

2.4 Leaf morphology

For the scanning electron microscope (SEM), leaves at different stages were fixed in 2.5% glutaraldehyde (pH 7.2) and dehydrated in a graded ethanol series as follows: 30%, 50%, 70%, 80%, 90%, and 100% ethanol for 15–20 min each. Samples were transferred into Tert-butyl alcohol for 20 min and frozen in liquid nitrogen for 5–10 min. Samples were critically dried using a freeze dryer (Christ, Germany) for 2–3 h. After being sputter-coated with gold, the dried petals were examined using a scanning electron microscope (Hitachi TM3030 plus, Japan), which operated at 5 KV voltage.

2.5 Analytics of cuticular wax

Sampling for all cuticular wax analyses consistently adhered to the same methodological pattern^[26]. For the cuticular wax extraction, 10 mL of chloroform/methanol (v/v = 3:1) filled tubes that were tightly placed onto the investigated leaf surface. The tubes underwent sonication for 45 s, and each sample were extracted three times to ensure a sufficient wax extraction. Three extracts per leaf were pooled to yield one biological replicate. To prepare the samples for gas chromatographic (GC) analysis, the sample volumes were completely evaporated under nitrogen at 60°C before derivatization with 60 μ L of BSTFA [N,O-bis(trimethylsilyl) trifluoroacetamide] and 60 μ L of pyridine for 40 min at 70°C. GC analysis was performed to identify wax compounds using a coupled mass spectrometer (GC-MS: 7890A-5975CMS, Agilent). Finally, the fragmentation patterns of wax compounds (GC-MS) were qualified using an in-house created mass spectral library, and the signal intensities of wax compounds (GC-FID) were quantified in relation to a known internal standard.

3 Results

3.1 Morphological and structural characteristics of Chinese Rose leaves in natural condition

According to the developmental process of the leaves, they are divided into four stages (Supplement Fig. 1). In the S1 stage, the leaves are not yet unfolded, and both the adaxial and abaxial surfaces are red. In the S2 stage, the leaves unfold, and the leaf area increases, while both the adaxial and abaxial surfaces remain red. In the S3 stage, the red color on the adaxial surface decreases and turns green, while the abaxial surface remains red. By the S4 stage, the leaves are completely green, and the leaf area remains relatively unchanged (Fig. 1A).

Leaf reddening is primarily due to the presence of anthocyanins. To gain insights into the distribution patterns of anthocyanins throughout plant growth, we manually sectioned and observed the leaves under a microscope. In cross sections of juvenile leaves, anthocyanins are distributed along the entire epidermal cells on both the adaxial and abaxial leaf surfaces. Thus, the red color of the S1 stage is attributed to anthocyanins. The mesophyll tissue was not differentiated into palisade parenchyma and spongy parenchyma (Fig. 1B). During the S2 stage, a reduction in anthocyanins is observed in the epidermis; however, they remain particularly evident in the adaxial epidermis (Fig. 1C). Along with leaf ontogeny and growth, the presence of anthocyanins was observed only in the abaxial epidermis in the S3 stage (Fig. 1D). Subsequently, anthocyanins in the abaxial epidermis disappeared. When the leaves matured to the green S4 stage, anthocyanins completely vanished from the epidermal cells (Fig.1E).

The color of leaves is primarily determined by the combined effects of chlorophyll, carotenoids, and anthocyanins. Therefore, we investigated the pigment content at different stages. The anthocyanin content was highest during the S1 stage and lowest during the S4 stage (Fig. 2A). In contrast, the contents of chlorophyll a, chlorophyll b, and carotenoids were lowest during the S1 stage and gradually increased throughout leaf development, reaching their maximum levels during the S4 stage (Fig. 2B-D). These findings suggest that during the early stages of leaf development, the red coloration of the leaves is primarily due to the presence of anthocyanins in the epidermal cells. As the leaves develop, the anthocyanin content decreases, causing the red color to fade, while the mesophyll tissue becomes fully developed, and the chlorophyll and carotenoid contents increase significantly, resulting in the leaves turning green.

3.2 Changes in anthocyanin types and contents in leaves during different developmental stages

Table 1 The difference in anthocyanin levels during leaf development

Stage	CC (µg/g FW)	PelC	PeoC	DC	Pt	Mv
S1	642.144±7.391 a	0.254±0.010 a	0.024±0.002 c	0.248±0.006 a	0.011±0.000 b	0.012±0.000 b
S2	443.427±6.440 b	0.127±0.002 b	0.400±0.008 a	0.289±0.008 a	0.022±0.000 a	0.098±0.064 a
S3	212.025±6.638 c	0.124±0.003 b	0.015±0.002 c	0.216±0.004 a	0.005±0.001 c	0.057±0.004 a
S4	22.584±1.124 d	0.127±0.002 b	0.143±0.007 b	0.201±0.007 a	0.004±0.000 c	0.025±0.004 ab

Note: ESI-HPLC-MS/MS analysis of anthocyanin. CC: Cyanidin; PelC: Pelargonidin; DC: Delphinidin; PeoC: Peonidin; Pt: Petunidin; Mv: Malvidin

To clarify why the leaves initially appear red, we employed liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) to analyze the types and content of anthocyanins in the leaves. Using this technique, we measured and analyzed six different anthocyanin pigments. During the S1 stage, the content of cyanidin, a specific anthocyanin, was the highest, reaching 642.144 µg/g FW, while the levels of other anthocyanins were minimal. This indicates that the red coloration of the leaves was primarily determined by cyanidin. As the leaves developed, the content of cyanidin decreased significantly, until it dropping to 22.584 µg/g FW in the S4 stage, when the leaves became fully green. This suggests that during the later stages of leaf development, the green coloration was achieved not merely by an increase in chlorophyll content but more significantly by a reduction in anthocyanin content.

The anthocyanin biosynthesis pathway is primarily divided into two major stages: phenylpropanoid metabolism and flavonoid synthesis, with PAL (Phenylalanine Ammonia Lyase) and CHI (Chalcone Isomerase) serving as key enzymes. PAL activity was highest during the S1 stage and gradually decreased as the leaf developed, reaching its lowest level by the S4 stage. The activity of CHI followed a similar trend to that of PAL, with both enzymes exhibiting the highest activity in the S1 stage and the lowest in the S4 stage. The activities of these key enzymes in the anthocyanin biosynthesis pathway were consistent with the anthocyanin content. This suggests that the reduction in the activity of these key enzymes leads to a decrease in anthocyanin levels.

3.3 Characterization of the cuticle in leaves during different developmental stages

The cuticular layer contains certain compounds that exhibit autofluorescence under ultraviolet light. Taking advantage of this feature, we observed the cuticular layers of leaves at different developmental stages using a fluorescence microscope. During the S1 stage, when the leaves were young, a large amount of anthocyanins accumulated in the epidermal cells, and no significant fluorescence was observed on the leaf surface, indicating that the cuticular layer had not yet formed (Fig. 1F). By the S3 stage, when the anthocyanins in the adaxial epidermal cells had almost disappeared, a weak fluorescence signal was detectable on the outer surface of the epidermal cells. Conversely, on the abaxial side, where some anthocyanins were still present in the epidermal cells, no fluorescence signal was observed on the outer

surface of the epidermal cells (Fig. 1H). By the S4 stage, the leaves were fully developed and green, with all anthocyanins having disappeared. At this stage, distinct fluorescence signals were observable on the outer surfaces of both the adaxial and abaxial epidermal cells (Fig. 1I). These results suggest that anthocyanins and the cuticular layer alternately play functional roles.

To further determine whether the leaf surface possesses a cuticular layer, the leaves were subjected to dehydration, freeze-drying, and gold-sputtering, and then observed under a scanning electron microscope. During the S1 and S2 stages, no cuticular layer crystals were observed on either the abaxial or adaxial surfaces (Fig. 4A-D). In the S3 stage, a cuticular wax was observed on the adaxial surface of the leaf, but not on the abaxial surface. By the S4 stage, a distinct cuticular layer was observable on both the adaxial and abaxial surfaces. These results are consistent with our earlier observations of leaf cross-sections, indicating that the cuticular layer appears only during the later stages of leaf development.

3.4 Types and content of the cuticular waxes in leaves during different developmental stages

Through gas chromatography-mass spectrometry (GC-MS) technology, the components and content of leaf cuticular waxes were determined. A total of 68 compounds were identified in rose leaf cuticular waxes (Supplement Table 1). The main components include 35 alkanes, 8 fatty acids, 11 esters, and small amounts of olefins, phenols, aldehydes, and others (Supplement Table 1). In rose leaf cuticular waxes, alkanes and esters were the most prominent components. The levels of alkanes and esters were particularly notable during the S4 stage, with no significant differences observed during the other three stages (Fig. 5). Fatty acids were categorized into saturated and unsaturated fatty acids, both of which showed higher concentrations during the S4 stage. Among unsaturated fatty acids, β -tocopherol (Vitamin E) was not detected in the S1 stage, measured at 3.71 and 3.76 $\mu\text{g}/\text{cm}^2$ during the S2 and S3 stages, respectively, but surged to 36.06 $\mu\text{g}/\text{cm}^2$ during the S4 stage. Additionally, fatty acid derivatives had the highest content during the S1 stage but decreased during the later stages of leaf development. Olefins, phenols, and other compounds, such as alkanols and triterpene alcohols, also showed the highest concentrations during the S4 stage (Fig. 5).

4 Discussion

In the present study, we analyzed the distribution of anthocyanins and the development of cuticles in the leaves of *Rosa chinensis* under natural conditions. The results showed that anthocyanins accumulated in epidermal cells (adaxial and abaxial) appearing red on both adaxial and abaxial surfaces of leaves at the nascent stage. As the leaves underwent ontogeny and growth, anthocyanins initially faded from the adaxial surface, followed by the abaxial surface. Just as juvenile leaves were abundant in anthocyanins, the cuticles began to develop. The cuticles were not clearly observed until the anthocyanins disappeared. From these observations, we inferred that the leaves of *Rosa chinensis* may employ different strategies to protect against stress at different developmental stages.

Leaf color change is a striking phenomenon that relates not only to the aesthetic value of plants but also closely associates with their physiological functions. The main pigments present in plant leaves are chlorophyll, carotenoids, and anthocyanins. Different proportions of these pigments result in leaves

displaying a variety of colors^[10]. Anthocyanins are a group of water-soluble flavonoids that impart pink to purple colors in leaves and other plant organs. In both *Osmanthus fragrans* and walnuts, the red coloration of the leaves is attributed to a high abundance of anthocyanins^[30, 31]. However, there are significant differences in the deposition locations of anthocyanins within the leaf tissues. In *Quintinia serrata*, anthocyanins were observed inside the cell vacuoles of the epidermise, the palisade mesophyll, spongy mesophyll, and vascular parenchyma at the midrib of leaves. Statistical analysis has shown that anthocyanins appear more frequently in palisade mesophyll cells, with a very low probability of occurring in epidermal cells^[32]. Additionally, Gould observed 25 plant species and found anthocyanins present in combinations of two or more types of tissues. Only four species contained anthocyanins in the upper and/or lower epidermal cells. However, in these leaves, only the adaxial side exhibited pigmentation, which was not directly related to their developmental state^[33]. This finding is inconsistent with our observation that in rose leaves, anthocyanins are present exclusively in epidermal cells and accumulate solely during the young leaf stage. Similarly, Hughes observed red leaves in three deciduous plants (sweetgum, Red maple, Redbud) and found that anthocyanins accumulated in both the upper and lower epidermis of the Redbud leaves^[34]. This suggests that the cells in which anthocyanins accumulate differ among plant species, which may be closely related to the developmental stage of the leaves and the function of anthocyanins within them. Gould et al. found that anthocyanins in leaves are primarily based on cyanidin^[33]. This finding was subsequently confirmed in *Acer pseudosieboldianum*^[35]. However, petunidin, malvidin, and pelargonidin have also been detected in colored potatoes and bud mutants of *Populus*^[36, 37]. In rose leaves, we detected only cyanidin. These results indicate a strong relationship between cyanidin and the red coloration of young rose leaves.

Anthocyanins are involved in photoprotection, antioxidant protection, osmoregulation, and defense against herbivores and pathogens. Additionally, cyanidins display significant antioxidant activities and cytoprotective effects against various forms of oxidative stress^[38]. The evolutionary convergence for the ability to accumulate red pigments in vegetative tissue suggests that this provides an adaptive advantage. In rose, anthocyanins accumulate abundantly in young leaves, which have not yet fully developed, primarily because the mesophyll tissue has not fully differentiated. The transient accumulation of anthocyanins likely contributes to a short-term defense strategy to limit damage from biotic and abiotic stresses. Young, expanding organs or tissues that are more susceptible to photodamage of photosynthetic pigments than mature ones may be preserved in favor of more expendable components. As well, Ranjan investigates the photosynthetic efficiency of juvenile red and mature green leaves of *Jatropha curcas*, revealing that red leaves have lower photosynthetic efficiency due to immaturity but are protected from photoinhibition by anthocyanins, which facilitate harmless energy dissipation and faster recovery^[39]. In the developing leaves of deciduous trees (sweetgum, Red maple, Redbud), the reduction of anthocyanin correlates with advancements in photosynthetic maturation, leaf structural development, and pigmentation, suggesting that anthocyanins protect young tissues until photosynthetic processes mature to effectively balance light capture and utilization^[34]. Therefore, we propose that anthocyanins predominantly accumulated in epidermal cells may be effective in screening UV-B radiation during the early stage.

Since anthocyanins are crucial for leaves, why do they gradually diminish as the leaves mature? A significant amount of anthocyanins accumulates in the vesicles of the upper and lower epidermal cells of young red hazel (*Corylus avellana* L.) leaves, with up to 95% of the visible radiation entering the leaves absorbed by these pigments [40]. Research has demonstrated that the enrichment of anthocyanins in red walnuts, enhances fruit quality, but reduces growth potential^[31]. This suggests that anthocyanins may inhibit plant growth. Accumulation and maintenance of anthocyanins entail an energy cost, which may reduce light capture and ultimately carbon assimilation. This potential loss could be a negative factor associated with the possible benefits of accumulated anthocyanins. Therefore, acclimation to stress involves the replacement of anthocyanins with more physical barriers, such as cuticles and glandular hairs that reestablish homeostasis between the plant and the environment^[23]. In the leaves of roses, we found that when the mesophyll tissue of the leaves is fully developed, the chlorophyll content increases, while the anthocyanins completely disappear. This change ensures that the leaves can maximize their use of light for photosynthesis. This indicates that anthocyanins played a protective role for the leaves during the early stages of leaf development. Meanwhile, in the later stages of leaf development, cuticular layer, a new substance appears on the upper and lower epidermis of the leaves. It is speculated that the cuticular layer takes over the role of anthocyanins, serving both to protect the plant leaves and to not interfere with photosynthesis.

Cuticular waxes are essential for plant growth and defense mechanisms. The cuticular layer reflects and scatters intense light and ultraviolet rays, minimizing direct damage to the leaves, while also preventing water evaporation and damage from pests and insects^[24, 41, 42]. The cuticles, which serve as physical barriers that later protect mature leaves, are absent in nascent leaves. Since the adaxial surface of the leaves receives a higher level of light and/or UV-B radiation than the abaxial surface, cuticles are initially differentiated on the upper epidermis as the anthocyanins disappear. A survey of isolated cuticles from a range of species indicated that cuticles take a role in effectively screening the UV-B spectrum while allowing light with longer wavelengths that are photosynthetically active to transmit efficiently^[43]. Therefore, we speculate that the cuticles can protect mature leaves of *Rosa chinensis* from high light and/or UV-B radiation by reflecting, screening, and absorbing. It is noteworthy that during the development of rose leaves, the cuticular layer does not initially appear but gradually accumulates and emerges in the later stages of development as anthocyanins disappear. Results from scanning electron microscopy and gas chromatography support this perspective. This indicates that the cuticular layer primarily serves a protective role in the later stages of leaf development. However, wax crystals and cuticular structures may hinder leaf expansion if cuticles form during the early growth period, thus decreasing leaf area and reducing the gross biomass of plants. This may explain the apparent evolutionary convergence of red non-photosynthetic pigments and thick cuticles.

Anthocyanins and cuticular waxes are compounds found in plants that play significant roles in growth and development. Despite their vastly different chemical natures and properties, both are closely associated with the plant's ability to adapt to its environment. This study posits that the color change in rose leaves is a complex physiological process involving the interaction of various compounds, including chlorophyll, anthocyanins, and waxes. This process may be linked to the plant's response mechanisms to

environmental stress. Anthocyanins serve a protective function during the early stages of leaf development, while the cuticular wax layer assumes a defensive role once the leaf has matured. These mechanisms collectively assist the plant in adapting to ever-changing environmental conditions. Collectively, we propose that during the different developmental stages of *Rosa chinensis*, the alternating presence of anthocyanins and cuticles in leaves provides the optimal strategy for intercepting stress.

Declarations

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Author Contributions

Z.P. and Z.L. conceived the experiments, and Z.Y., G.S., W.D. and L.X. performed the experiments. Z.Y., Z.P., and Z.L. analyzed the data. Z.Y., Z.P., and Z.L. wrote and revised the manuscript. Z.P. and Z.L. critically edited the manuscript. All authors approved the final manuscript.

Conflict of Interest

The authors declare no competing interests.

Data availability

All relevant data can be found within the article and its supplementary information.

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Figures

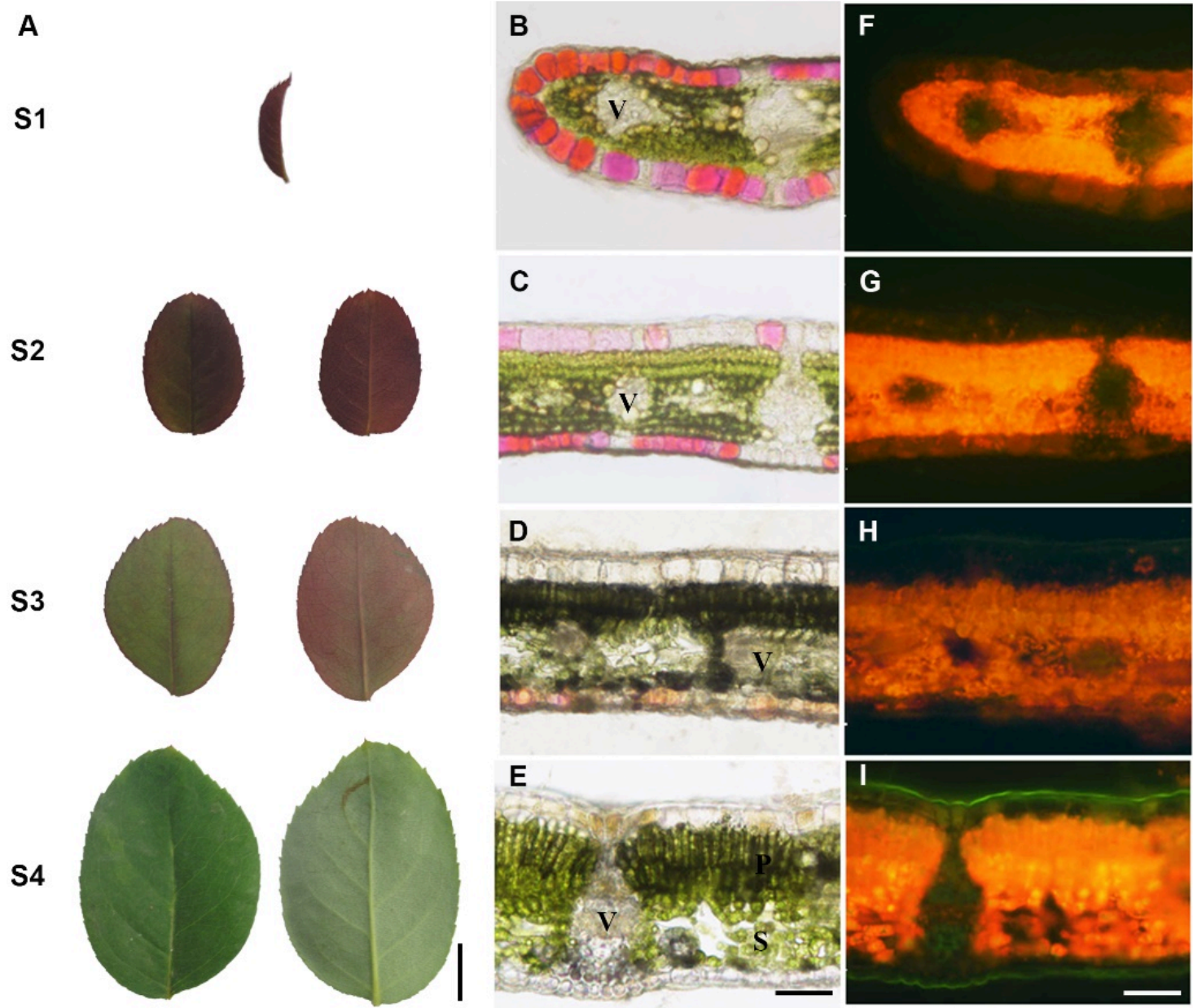


Figure 1

The developmental process and micromorphology of leaves from the Chinese Rose

(A) The process of leaf development was captured using a camera. The adaxial and abaxial images of the leaves are shown on the left and right. Scale bar = 1 cm. (B) Transverse sections from leaf development were generated by freehand dissection. Bar = 50 μ m. V = vascular bundle; P = palisade parenchyma; S = spongy parenchyma.

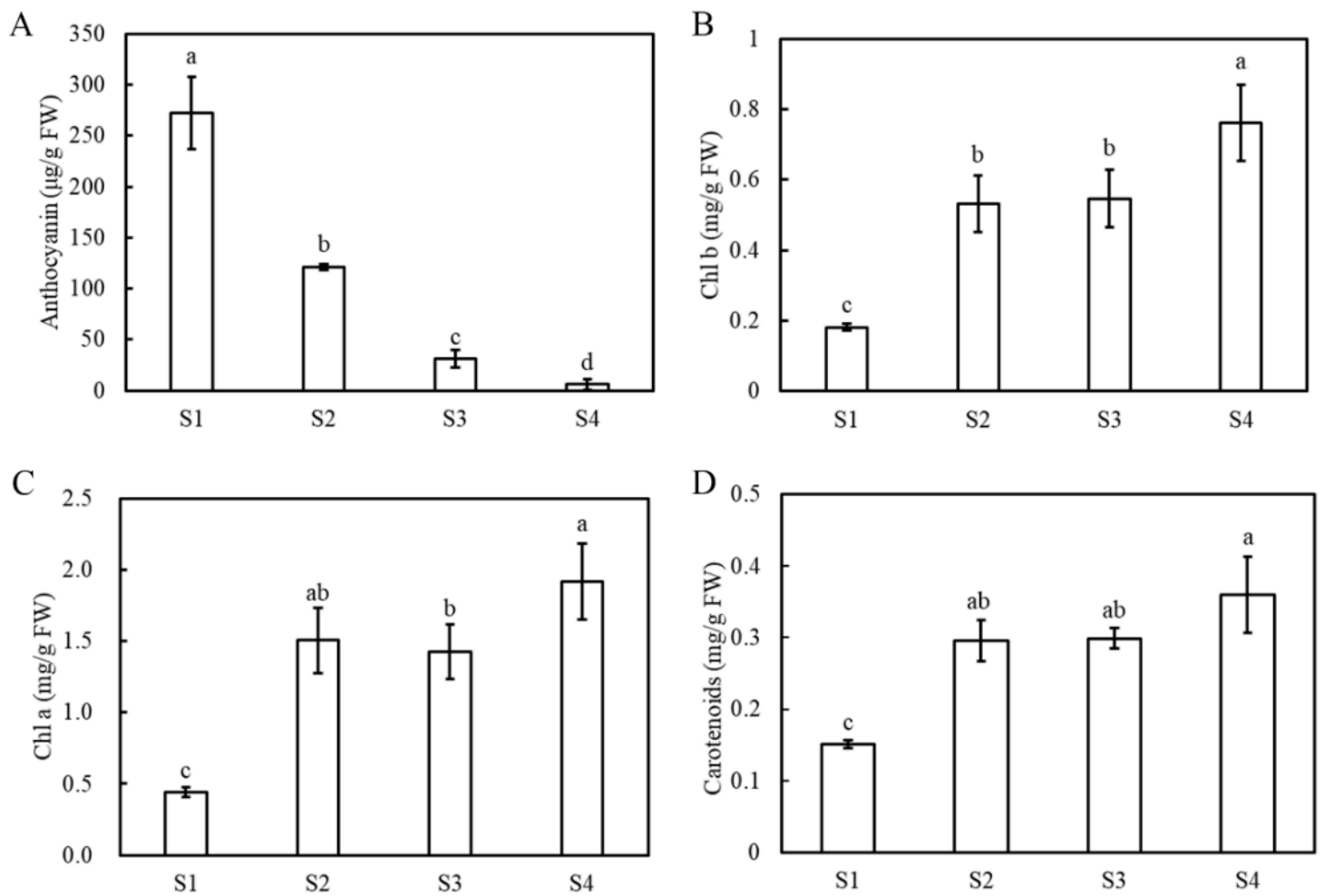


Figure 2

The pigment contents in the leaves of Chinese Rose

It presents the content of anthocyanin (A), chlorophyll b (B), chlorophyll a (C), and carotenoid (D).

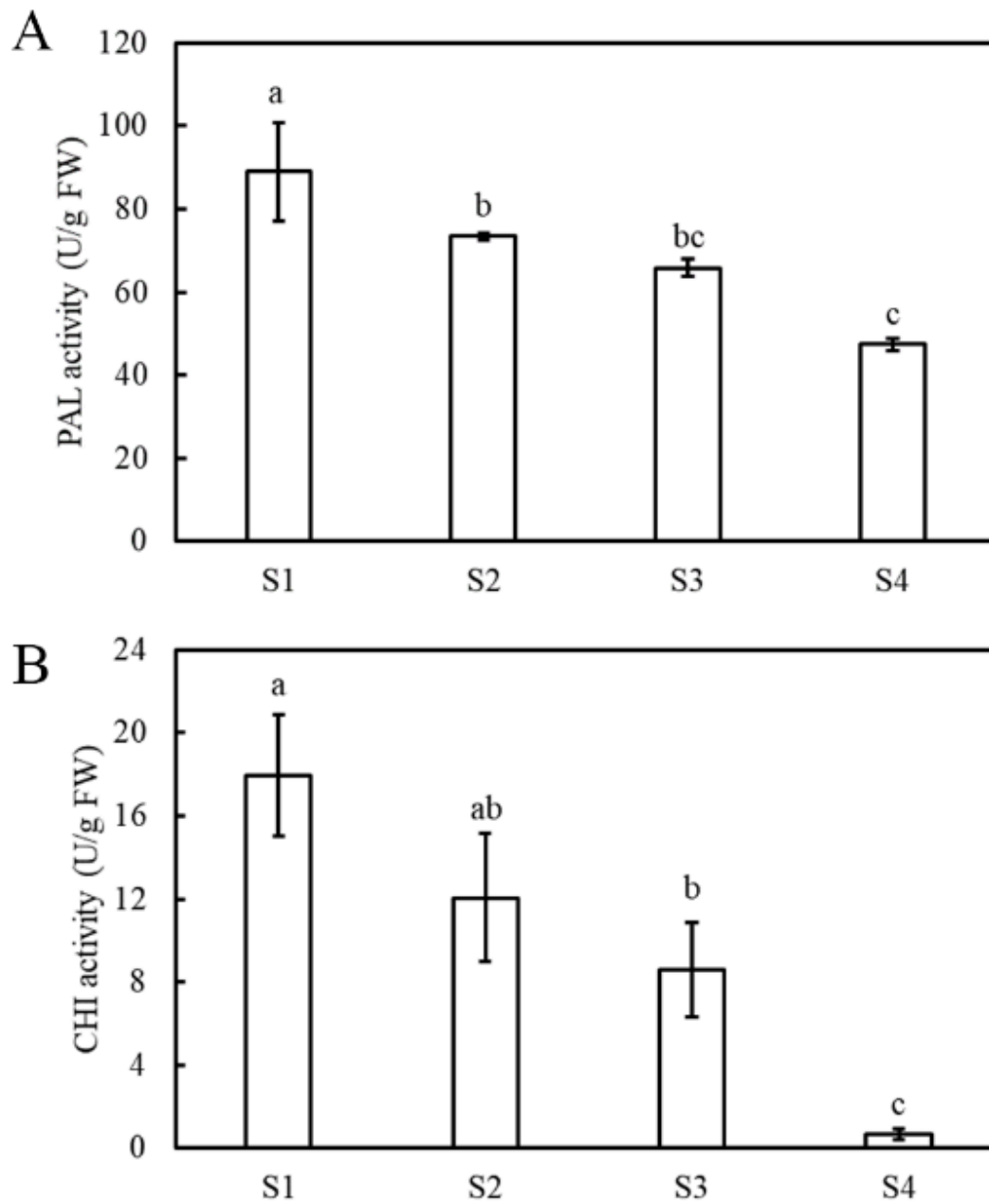


Figure 3

Changes in PAL and CHI activity in leaves

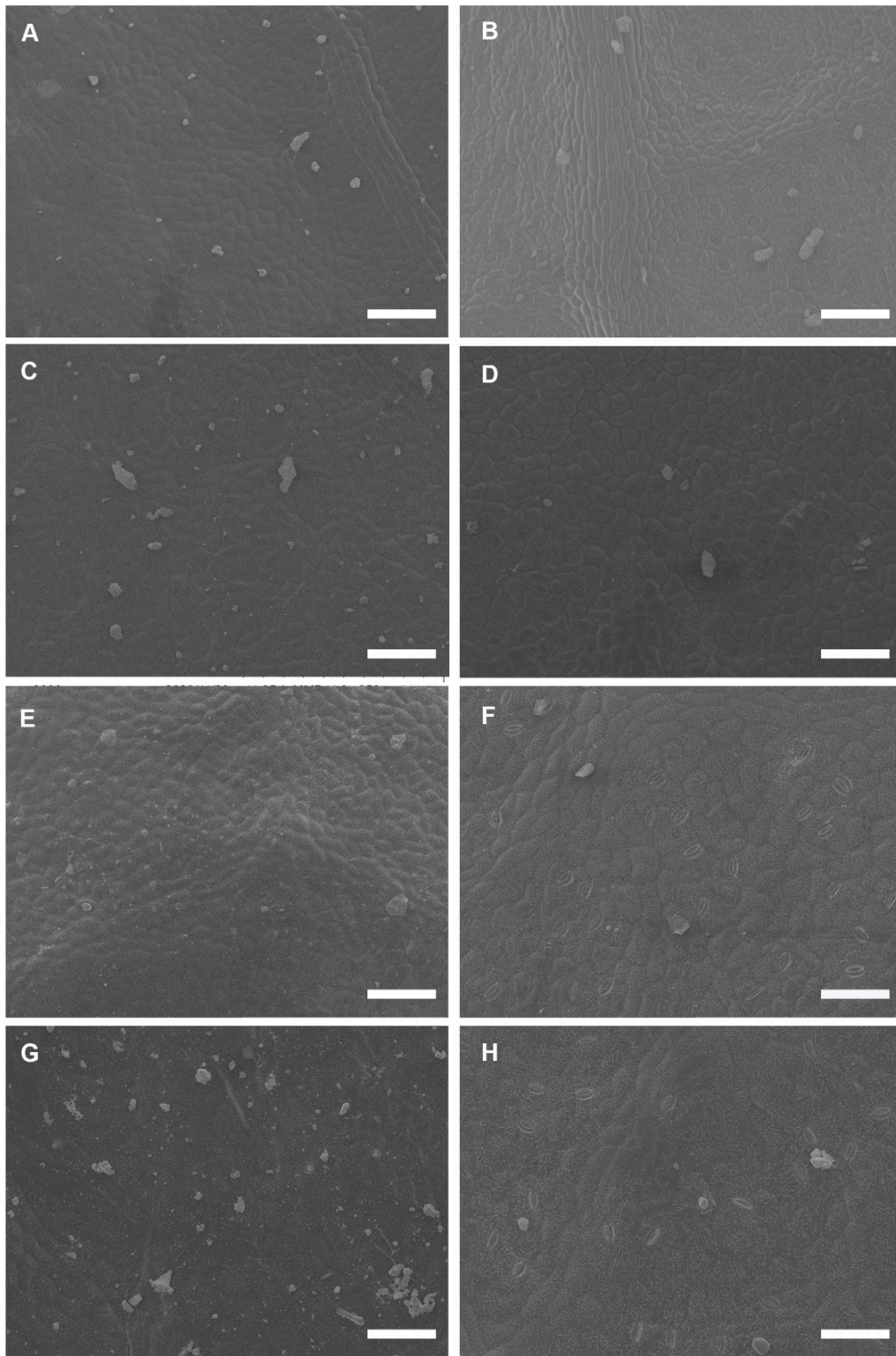


Figure 4

Leaf surfaces of the adaxial and abaxial epidermis at different stages

A-B: S1 stage; C-D: S2 stage; E-F: S3 stage; G-H: S4 stage. A, C, E, and G show the adaxial sides, while B, D, F, and H show the abaxial sides. Bar = 100 μm .

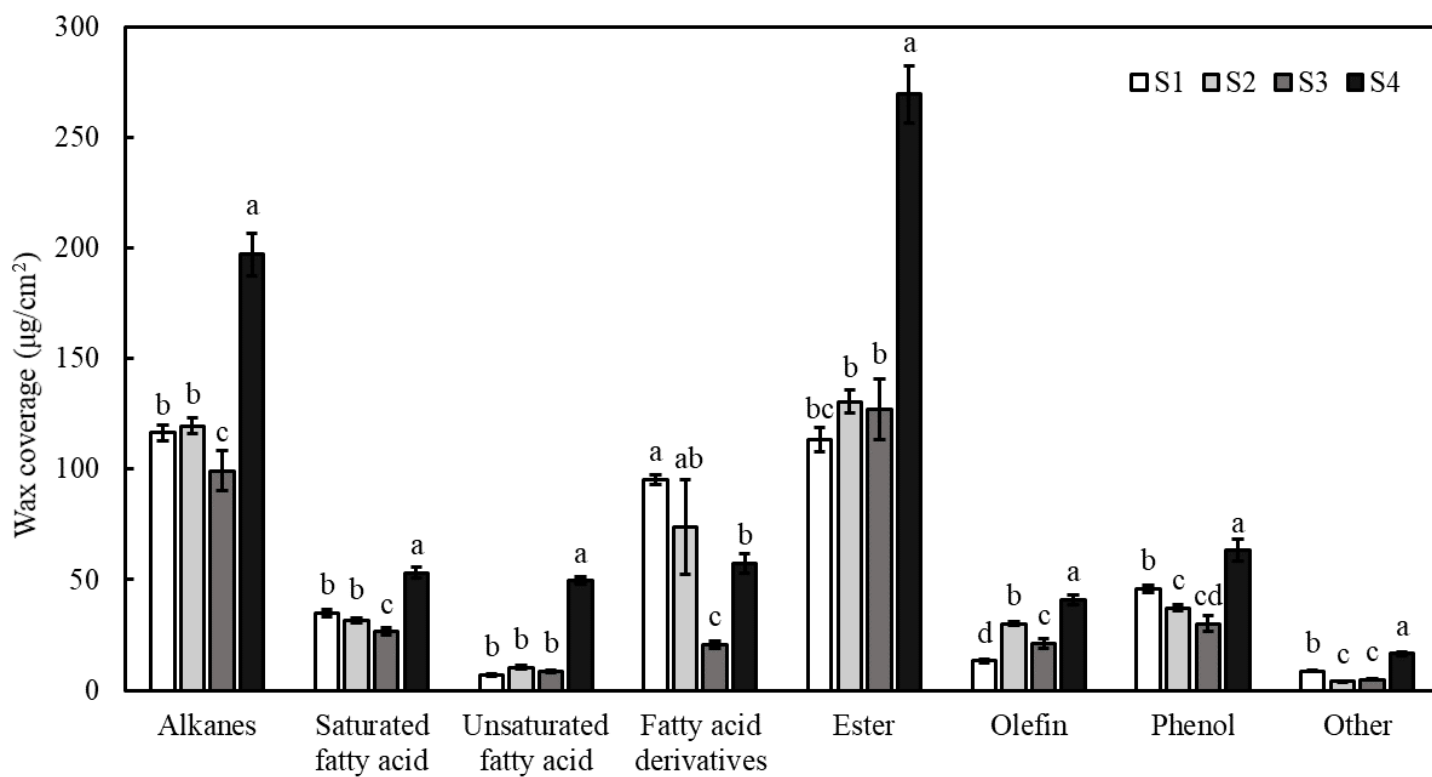


Figure 5

The content of cuticular waxes in leaves

Supplementary Files

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