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Anatomical characterization of *Portulaca grandiflora* Hook: Root, stem, leaf, and pollen structures

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A B S T R A C T

Portulaca grandiflora Hook. is a succulent plant commonly used as an ornamental as well as a medicinal plant. This species is capable of growing well under suboptimal climatic conditions and in poor soil compositions. This study aims to identify the tissue composition of various organs of *P. grandiflora* Hook., which can be utilized in the processes of identification, classification, and understanding its ecological adaptability. Anatomical observations were carried out using the free-hand section method to examine cross-sectional anatomy of roots, stems, and the epidermal tissue of leaves on both the adaxial and abaxial surfaces. Additionally, the acetolysis method was employed to observe the pollen structure. The results showed that the root cross-section consists of epidermal tissue, cortex, endodermis, pericycle, xylem, and phloem. The stem cross-section includes epidermal tissue, cortex with starch-containing parenchyma cells, pith, and vascular bundles of the open collateral type. The leaf epidermis, both on the adaxial and abaxial surfaces, consists of polyhedral epidermal cells with undulating cell walls. The type of stomata observed was parasitic, with an amphistomatic distribution. Pollen observation revealed that the pollen grains are spherical, have echinate ornamentation, and possess a monoporate aperture.

Keywords: Anatomy; Acetolysis; Free-Hand Section; *Portulaca grandiflora* Hook

1. INTRODUCTION

Portulaca grandiflora Hook. is an herbaceous plant from the Portulacaceae family. It can be used as both an ornamental and medicinal plant (Sari, Karno and Anwar, 2017). *P. grandiflora* Hook. has a prostrate stem growth habit and a rapid propagation process (Husnawati, Purwanto and Rispriandari, 2020). This succulent dicot plant has reddish-green leaves measuring 2–4 cm in length, flowers with a diameter of 3–5 cm, and plant height ranging from 7 to 15 cm (Cruz *et al.*, 2019). The leaves are oblong to cylindrical with pointed tips, and the flowers are bisexual with either actinomorphic or zygomorphic floral symmetry (Setiawan, Aisyah and Krisantini, 2016). The flowering phase of *P. grandiflora* begins with the emergence of floral buds, small buds, yellowish-green buds, enlarged buds, blooming flowers, wilting, drying, and petal drop, lasting 20 to 26 days. The flowers generally remain open from approximately 08:00 to 16:00 local time. During the flowering period, weather conditions from peak bloom to petal drop tend to remain stable. Fallen petals do not develop into fruits, leaving only the floral base, which eventually dries and turns brown (Rianita and Murni, 2023). Ambient temperature can affect the timing of first flower emergence, the number of flowers, and flower size (Khotimah, Sudiana and Pratiknya, 2022). The anatomical structure of plant leaves is used to indicate the effectiveness of plant growth in post-mining areas. Increasing fertilizer dosage can affect changes in the leaf anatomy, including stomatal density, stomatal index, upper epidermis thickness, and vascular bundle size(Sanputra and Irwanto, 2024). *Portulaca grandiflora* Hook demonstrates high tolerance to various stress conditions and can grow easily in a wide range of environments (Kumar *et al.*, 2021).

There are significant differences in flower color, root, and branching patterns between *P. grandiflora* and *P. oleracea* (Setiawan, Aisyah and Krisantini, 2016), Polyploid *P. grandiflora* plants have a chromosome number of 2n = 4x = 36, larger stomatal length and width, lower stomatal density, and overall larger morphology compared to diploid

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plants (Sari, Karno and Anwar, 2017). Polyploid *P. grandiflora* plants have a chromosome number of 2n = 4x = 36, larger stomatal length and width, lower stomatal density, and overall larger morphology compared to diploid plants. The concentration of phenolic and flavonoid compounds varies across different organs of *P. grandiflora* Hook. (Husnawati, Purwanto and Rispriandari, 2020). Total phenolic and flavonoid content is highest in the leaves (113.26 ± 3.85 mg GAE/g and 97.99 ± 1.28 mg QE/g, respectively), but the highest antioxidant activity is found in the mature stems, with an IC50 value of 122.15 ± 1.30 ppm, categorized as moderate antioxidant activity. P. *oleracea* L. contains vitamins and minerals known to help treat various ailments such as appendicitis, constipation, acute diarrhea, leucorrhea, jaundice, and intestinal worms. It has also been identified as an excellent source of alpha-linolenic acid (Astuti *et al.*, 2018). According (Kumar *et al.*, 2022) and (Mishra *et al.*, 2019), *Portulaca* is appreciated in Mediterranean and Asian cuisine for its crisp, fresh taste and is regarded as a "future superfood" due to its exceptional nutritional content and pharmacological properties.

Although *P. grandiflora* Hook. has been extensively studied in terms of morphology and pharmacology, detailed studies on the anatomical structure of its organs such as root and stem cross-sections, leaf epidermal tissues, and pollen morphology have not yet been reported. The absence of anatomical data may hinder the accuracy of species identification, both morphologically and molecularly. Anatomical characterization can differentiate *P. grandiflora* from other *Portulaca* species, as anatomical structures are generally unaffected by environmental factors, thus enabling more accurate classification. Therefore, this study was conducted to determine the anatomical structure of *P. grandiflora* Hook., which can be utilized for identification and classification purposes (Azlina & Iriani, 2024). The representation of plant organ anatomy in 2D, 3D, and verbal forms is essential for understanding the tissue structures that compose plant organs (Ermayanti, Susanti, & Anwar, 2018).

2. MATERIAL AND METHODS

This study was conducted using two methods: the free-hand section method for observing the anatomy of roots, stems, and leaves, and the acetolysis method for examining pollen morphology.

2.1 Study area

The sample collection was conducted in the vicinity of the Faculty of Teacher Training and Education. Anatomical observations were carried out at the Biology Laboratory, Universitas Sriwijaya, from January to April 2025.

2.2 Specimen preparation and experimental design

List and describe the materials used in the study, including biological samples, chemicals, instruments, and other relevant items. The tools and materials used for observing the anatomy of stems, roots, and leaves included a razor blade, microscope slides, cover slips, test tubes, test tube racks, mounted needles, label, 50 ml beakers, a binocular microscope. A total of five samples for each plant organ (roots, stems, and leaves) were examined, with five replications conducted for each sample. Observations were performed using a binocular microscope at magnifications of 100× and 400×.The chemical reagents used included: distilled water (aqua dest), Formaldehyde Acetic Acid Alcohol (FAA) solution, alcohol solutions at concentrations of 50%, 70%, 96%, and 100%, alcohol–xylene mixtures in ratios of 3:1, 1:1, and 1:3, pure xylene (100%), 1% safranin solution, and Haupt's solution. The tools and materials used for observing the pollen of *P. grandiflora Hook*, included test tubes, test tube racks, centrifuge tubes, a centrifuge, mounted needles, microscope slides, cover slips, a binocular microscope. A total of five pollen samples were observed with five replications per sample. Observations were carried out using a

binocular microscope at 1000× magnifications. FAA, a mixture of glacial acetic acid and sulfuric acid in a 9:1 ratio, and distilled water.

2.3 Anatomical observation

The free-hand section method was performed by making thin transverse sections of the roots and stems, as well as paradermal sections of the leaves of *P. grandiflora* Hook. **Fixation**; Thin transverse sections were prepared from roots and stems, and paradermal sections from the adaxial and abaxial surfaces of the leaves. A total of five slides were prepared for each organ. Sections were initially examined under a binocular microscope to ensure appropriate thinness. Once selected, sections were placed into individually labeled test tubes and fixed in FAA solution for 24 hours. **Dehydration**; Following fixation, the FAA solution was discarded and replaced with 50% ethanol for one hour. Samples were then transferred to 70% ethanol containing 1% safranin and stored for an additional 24 hours. Subsequent dehydration was carried out using 96% and 100% ethanol, each for one hour. **Dealcoholization**; The sections were then transferred sequentially into alcohol–xylene mixtures in the ratios of 3:1, 1:1, and 1:3, followed by immersion in 100% xylene, each step lasting one hour. **Microscopic Observation***;* After preparation, the transverse sections of roots and stems and the paradermal sections of leaves were re-observed using a binocular microscope at magnifications of 100 400 and 1000. Parameters observed in the root and stem sections included tissue structure, while leaf sections were examined for epidermal cell morphology and the type of stomatal distribution.

The acetolysis method consisted of several stages. **Fixation**; Pollen grains were collected from fully bloomed flowers and fixed in Formaldehyde Acetic Acid Alcohol (FAA) solution for up to 24 hours. **Centrifugation**; The fixed samples were centrifuged at 1,000 rpm for 15 minutes. The supernatant was discarded. **Acetolysis**; The pellet was then replaced with a mixture of glacial acetic acid and sulfuric acid in a 9:1 ratio. The sample was centrifuged again at 1,000 rpm for 15 minutes. After centrifugation, the liquid was discarded. **Washing**; The sample was washed 2–3 times with distilled water. Each wash was followed by centrifugation at 1,000 rpm for 15 minutes to ensure proper removal of residual chemicals. **Microscopic Observation**; The final pollen sample was mounted on a microscope slide and observed under a binocular microscope at magnifications of 100×, 400×, and 1000×. The parameters observed included pollen ornamentation, aperture type, and pollen shape.

2.4 Data analysis

The observation results were analyzed using a qualitative descriptive method by describing the findings obtained during the research.

3. Results and Discussion

3.1 Root anatomy

In the transverse section of the root of *P. grandiflora* Hook shown in **Figure 1**, the root tissues are arranged from the outside to the inside, consisting of the epidermis, cortex, endodermis, pericycle, xylem, and phloem. The epidermis is the outermost layer composed of a single layer of cells. The cortex lies beneath the epidermis and consists of several layers of cells that are larger than epidermal cells. This is because the cortex is made up of parenchyma tissue, which can store water reserves to tolerate environmental conditions. All *Portulaca* genotypes are considered "ion includers," meaning they absorb and accumulate high amounts of Na^+ and Cl^- ions under salinity stress conditions. However, *P. grandiflora* has proven to be more salt-tolerant, showing only slight growth reduction under stress conditions, increased flower production, and the lowest K^+/Na^+ ratio in its leaves compared to other genotypes (Borsai *et al.*, 2020). The endodermis acts as a boundary between the cortex and the central cylinder of

the root. Within the vascular tissue, the xylem is larger in size than the phloem. The xylem functions in transporting water and nutrients from the soil, while the phloem transports photosynthesis products from photosynthetic organs.

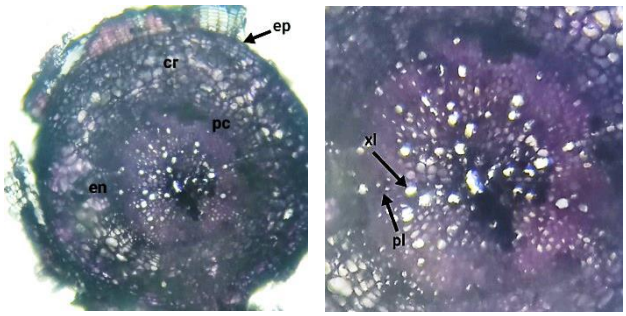

Figure 1. Transverse section of the root of *P. grandiflora* Hook. at 100× magnification.
Note: ep (epidermis); cr (cortex); en (endodermis); pc (pericycle); xl (xylem) and pl (phloem)

3.2 Steam anatomy

In the cross-section of the *P. grandiflora* Hook stem **Figure 2**, the tissue structure consists of an epidermis and a cortex made up of parenchyma cells, which have thin cell walls and large cell sizes. Starch grains are present in each cell, functioning to store water as a food reserve within the stem. The central region contains the pith. The vascular bundles are of the open collateral type, with xylem located on the inner side and phloem on the outer side, separated by the cambium. The presence of starch-storing parenchyma in the stem indicates a strategy for conserving energy and water under environmental stress. Such storage tissues support survival during drought periods by sustaining essential physiological processes and enabling regrowth once conditions improve (Taiz *et al.*, 2015). This feature is commonly found in succulent and xerophytic species inhabiting semi-arid ecosystems.

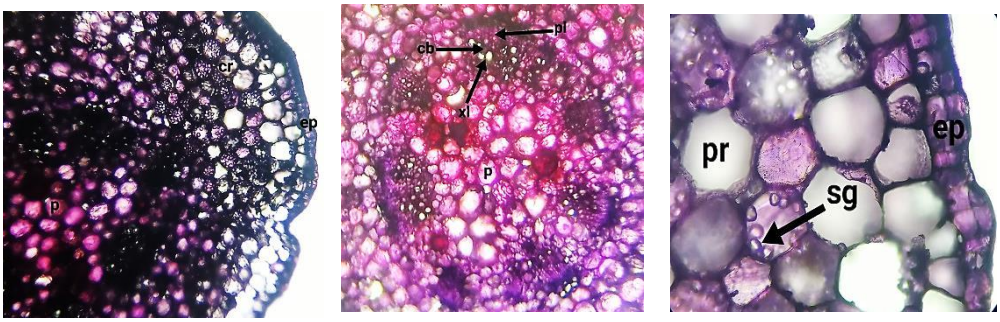

Figure 2. Transverse section of the stem of *P. grandiflora* Hook at 100× magnification.
Note: ep (epidermis); cr (cortex); pr (parenchyma); sg (starch grain); p (pith); xl (xylem); pl (phloem) and cb (cambium)

3.3 Leaf anatomy

The paradermal leaf consists of the upper surface (adaxial) and the lower surface (abaxial). Figure 3 shows the adaxial and abaxial surfaces of the *P. grandiflora* Hook leaf. The leaf epidermal cells are polyhedral with undulating walls. Stomata with a paracytic type are present on both the adaxial and abaxial surfaces. The paracytic type is a stomatal type in which the guard cells are accompanied by one or more subsidiary cells positioned parallel to the stomatal pore's axis (Mella and Chatri, 2021). According to (Anu, Rampe and Pelealu, 2017), the paracytic type features guard cells flanked by one or more subsidiary cells whose long axes run parallel to those of the guard cells and the pore. The stomata distribution is amphistomatic, meaning that stomata are present on both the abaxial and adaxial surfaces of the leaf. Amphistomatic stomata are defined as those distributed on both leaf surfaces (Viranda, Ermayanti and Santri, 2024).

The presence of stomata on both adaxial and abaxial leaf surfaces (amphistomatic condition) can provide adaptive advantages, especially in high-light environments. Amphistomatic leaves enable more efficient gas exchange by allowing CO_2 diffusion from both sides of the leaf, thereby enhancing photosynthetic performance (Salisbury and Ross, 1992). However, this also increases the potential for water loss through transpiration. To compensate, such plants often exhibit xeromorphic features such as thick cuticles, compact mesophyll tissues, or sunken stomata as adaptations to arid conditions (Fahn, 1990).

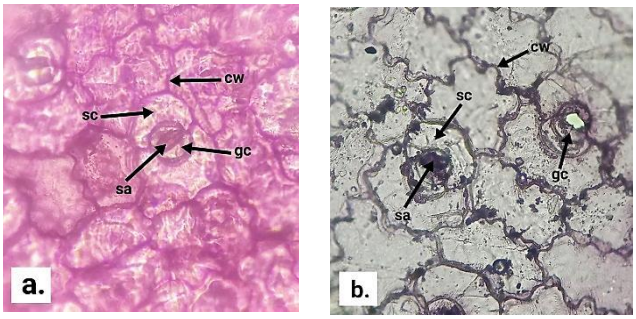

Figure 3. Surfaces of *P. grandiflora* Hook leaf at 400× magnification (a. Adaxial; b. Abaxial)
Note: sa (stomata); gc (guard cell); sc (subsidiary cell) and cw (cell wall)

3.4 Pollen morphology

Observation of the pollen of *P. grandiflora* Hook flower in Figure 4 shows that the pollen is spherical in shape with echinate ornamentation and apertures. Echinate pollen ornamentation is commonly associated with insect pollination. The spiny structures enhance the adhesion of pollen grains to the surface of pollinators, facilitating effective transfer between flowers (Erdtman, 1969). This suggests that *P. grandiflora* has evolved biotic pollination strategies, possibly indicating co-evolutionary relationships with specific pollinators (Pacini and Hesse, 2005).

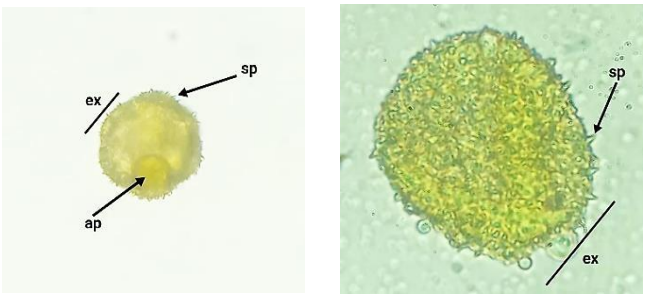

Figure 4. Pollen of. *P. grandiflora* Hook at 1000× magnification
Note: ex (exine); ap (aperture) and sp (spine)

4. CONCLUSION

The anatomical characterization study of *Portulaca grandiflora* Hook., which includes detailed observations of the root, stem, leaf, and pollen structures, presents systematic documentation and scientific interpretation of the plant's internal structures. These findings are relevant for taxonomic purposes, species identification, and understanding its ecological adaptations. Features such as amphistomatic leaves, echinate pollen ornamentation, and starch-storing parenchyma tissue in the stem indicate *P. grandiflora*'s adaptive strategies to arid conditions and its potential mutualistic relationships with specific pollinators. This study not only provides fundamental anatomical data for *P. grandiflora* but also serves as an important reference for comparative studies within the *Portulaca* genus.

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