

HISTO-ANATOMICAL FEATURES OF GARDEN LUPIN (*LUPINUS POLYPHYLLUS* LINDL.)

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Abstract

Lupinus polyphyllus Lindl. (garden lupin, large-leaved lupin) is a perennial herbaceous legume, used for ornamental purposes, for improving soil quality, as forage or in human food products. However, little is known about anatomical features of this species. Lupin vegetative organs (roots, petioles, leaf blades) were collected, cross sectioned and analyzed by bright-field microscopy. Lupin roots showed primary and incipient secondary structures, with a cortex featuring large-sized cells with rich cellular content and granular solid inclusions. Leaf petioles had rare peltate hairs and large sub-stomatal lacunes, a large medullary lacune and rich parenchyma. Leaf blades had typical bifacial structure, with three-layered palisade tissue and less prominent midrib, with a collateral bundle, protected by small caps of collenchyma.

Introduction

Lupinus is one of the largest genera belonging to Fabaceae, with around 280 currently accepted species. Taxonomically, it is a member of the order Fabales, family Fabaceae, tribe Genistae, being one of the few herbaceous plants in this tribe. Lupin species are found in both the Americas and the Old World (Office of the Gene Technology Regulator 2013).

One of the most widespread species is *Lupinus polyphyllus* Lindl., commonly known as garden lupin, large-leaved lupin, or Washington lupin, a native of the Pacific Northwest, from British Columbia to California and up to Alberta and Montana in the east (Beuthin 2012)

L. polyphyllus has a deep (1-2 m) taproot, with characteristic bacteria-bearing nodules found in all legumes. The endosymbiont in all lupin species belongs to the *Bradyrhizobium* genus, specifically to slow-growing but acid-tolerant strains common to many ruderal legumes, including several invasive weeds.

Aboveground organs form a central crown mostly made up of long-petiolated (10-40 cm) palmate compound leaves (9-16 leaflets, each having 5-15 cm and a pointed apex) (Fig. 1A).

Stems usually become visible only in the flowering season (usually in spring and summer – May to August – but garden lupine can flower in any time of the year, if conditions are suitable, since it is indifferent to photoperiodism and vernalisation). Reproductive stems can grow up to 1.5 m, bearing racemes of white-pink to purple-blue flowers (Fig. 1B). Fruits are small (2.5-5 cm) pods, each containing an average of 9 seeds (Beuthin 2012, Ryan-Salter 2019).

Like other species of lupin, it contains quinolizidine and other related alkaloids, thus limiting its usage as forage. However, alkaloid content in *L. polyphyllus* is much lower than in other species and it declines even further during spring and autumn. Low-alkaloid varieties are commonly grown as pasture plants (Ryan-Salter 2019).

It is also used for soil improvement in nitrogen-deficient and sandy soils, ornamental purposes, for attracting pollinators or even in human consumption, as a proteic supplement in meat products, salads or for producing a low-gluten flour.

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Due to its hardiness, garden lupin is often an invasive species and a noxious weed in many parts of the world (Beuthin 2012, Office of the Gene Technology Regulator 2013, Hassani *et al.* 2021).

While some studies were conducted to assess biochemical properties of some lupin species, little research has been done on the anatomy of this genus and of *L. polyphyllus* in particular. The present study was aimed to characterize the species' vegetative organs from an anatomic point of view.



Fig. 1. *Lupinus polyphyllus* Lindl.: A) vegetative organs; B) flowers.

Material and Methods

Samples of *L. polyphyllus* were collected from an experimental farm located in Izvoarele, Tulcea County, Romania. Samples consisted of fresh vegetative organs: roots, petioles and leaf blades. After being cut into small pieces, they were fixed in formalin, glacial acetic acid and alcohol in a ratio of 5 : 5 : 90.

Cross sections were done manually and stained with iodine green and alum carmine. (Bercu and Jianu 2003). Anatomical observations and micrographs were performed with a BIOROM-T bright field microscope, equipped with an Omegon Telemikro USB electronic camera. Resulting images were assembled and analyzed using BEL MicroImage Analyzer software.

Results and Discussion

Lupin roots showed a primary structure and incipient secondary structure. The single-layered epidermis was covered by a thick cuticle. The cortex was well-developed, formed of multiple layers of large cells, rich cellular content and granular solid inclusions (likely starch grains), while other cortical cells only had massive central vacuoles (Fig. 2B).

The stele and, in some root samples, also outer layers, showed an incipient secondary structure. Large xylem vessels are located centrally, surrounded by phloem. Medullary rays are made up of large-sized parenchymatous cells (Fig. 2A). These findings are consistent with those of Bramley *et al.* (2009). In one of the few anatomical studies conducted on lupin – in this case, on *L. angustifolius* – the authors found a predominantly typical primary structure, with two xylem bundles (diarch xylem) made up of 5-6 vessels, growing towards the root center and forming a continuous band, a pattern still visible in the present cross-section (Fig. 2C). From this point, a secondary structure, with larger vessels, proliferated laterally from the initial xylem band, giving the root section a flattened shape. A secondary structure, with a similar arrangement as that found in this case is known in *Lupinus luteus* (Łotocka 2008).

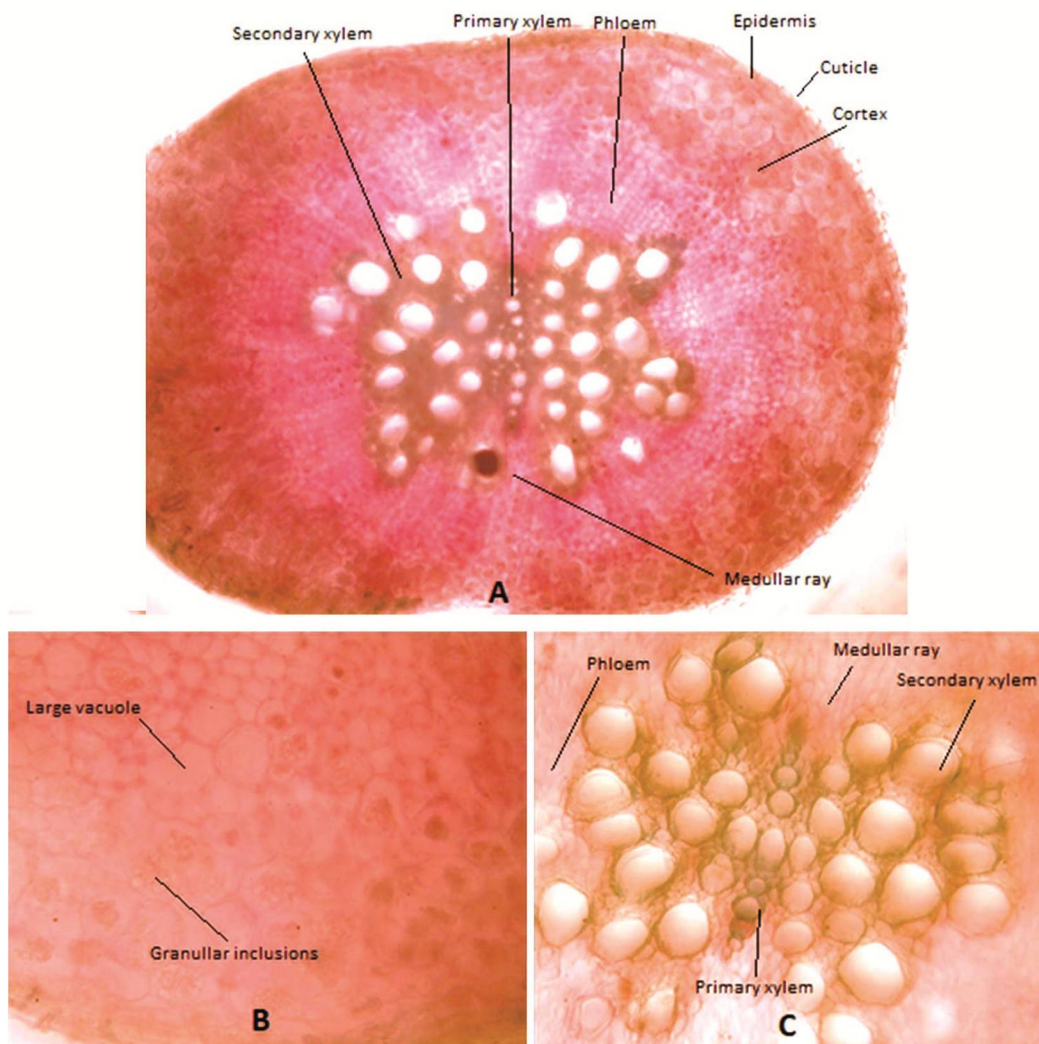


Fig. 2. Lupin root anatomy: A) cross-section; B) parenchymal tissue, with granular inclusions; C) vascular bundles.

Leaf petioles had an epidermis featuring sparse, round-shaped, peltate hairs and large substomatal lacunes. A large medullary lacune was located at the center. The rich parenchymatous cortex consisted of large cells, featuring massive vacuoles (Fig. 3A).

Vascular bundles were collateral, fan-shaped, each with an outer cap of sclerenchyma (Fig. 3B). Anatomic analyses done by Zorić *et al.* (2015) in *L. albus* revealed a similar structure for floriferous stems, with multiple vascular bundles protected by large sclerenchymal caps, but with an incipient secondary structure – bundles eventually joining to form a continuous ring.

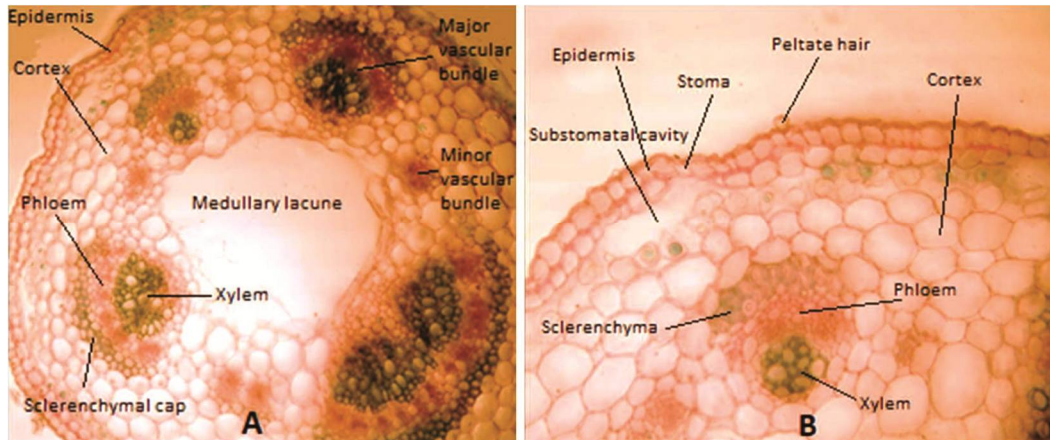


Fig. 3. Lupin leaf petiole: A) cross-section; B) detail with vascular bundle and peltate hair.

A study on four Mexican species showed a high variability of petiole anatomy. Primary structure with discontinuous bundles similar to the present findings was determined in *L. montanus* and *L. reflexus*. Continuous bundle rings were found in *L. exaltatus*, while an intermediate situation, with a discontinuous, but compact ring of bundles was found in *L. aschenbornii* (Zamora-Natera and Terrazas 2012).

Leaf blades had typical bifacial structure, with three-layered palisade tissue and less prominent midrib, with a collateral bundle, protected by small caps of collenchyma. Leaves were amphistomatic, with stomata found on both the upper and lower surfaces, with a higher occurrence on the lower one. Upper leaf epidermis was composed of larger cells and had a thicker cuticle (Fig. 3A).

Palisade tissues were composed of 2-3 cell layers, with a similar thickness to that of lacunar tissue. Vascular bundles were collateral, covered by a thin sheath of sclerenchyma and angular collenchyma and a thicker sheath of large parenchymal cells. A thin cap of collenchyma was located beneath the epidermis in the midrib zone of the lower leaf surface (Fig. 3B).

Leaf blade structure in lupine species is another variable character, and especially the presence or absence of mechanical tissue around vascular bundles. For instance, Zamora-Natera and Terrazas (2012) did not find any sclerenchyma or collenchyma in *L. aschenbornii*, *L. exaltatus*, *L. montanus* and *L. reflexus*, while the latter three species had sheaths of gelatinous fibers. Midrib bundle structure can be semicircular like in *L. polyphyllus* (in *L. reflexus* and *L. montanus*) or ring-shaped in *L. aschenbornii* and *L. exaltatus*.

Palisade tissue consistency is a feature that some authors consider to distinguish xeromorphic species (with a more compact tissue) from the rest of the genus with a lax tissue, containing air

spaces. *L. polyphyllus* leaf blade had a compact palisade tissue. However, in some lupine species this character is known to show significant variation among local population – the case of *L. bilineatus* and *L. mexicanus* (Sandoval-Zapotitla *et al.* 2024).

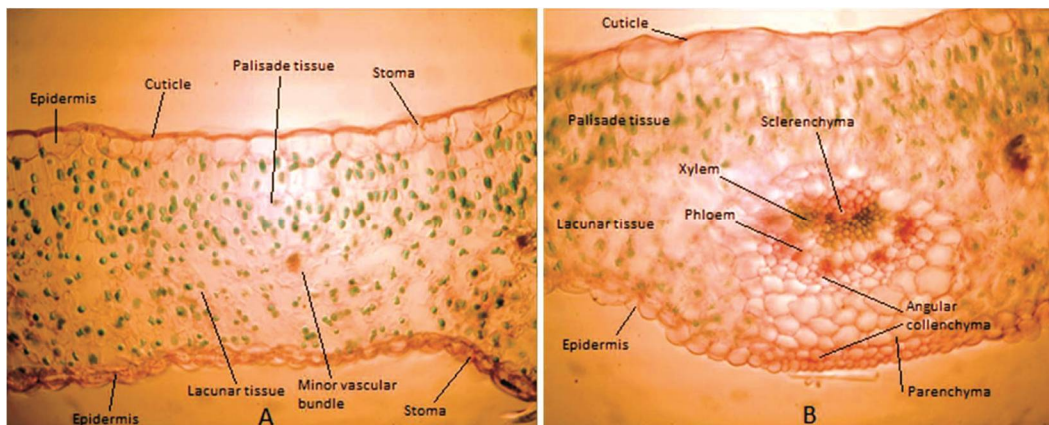


Fig. 4. Lupin leaf blade: A) mesophyll; B) midrib with vascular bundle.

Root, petiole and leaf structures were typical for a herbaceous species with incipient secondary structure. The presence or absence of the secondary structure in root is a variable character among lupine species and it relates *L. polyphyllus* to *L. luteus* among studied species. On the other hand, petioles had a more typical primary structure. Another distinctive feature was the presence of both sclerenchyma and angular collenchyma around leaf blade vascular bundles. Lupine anatomy was remarkable through the abundant organic granules in root parenchyma and low-development of mechanical tissues in leaf blades.

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