ULTRASTRUCTURAL ANALYSIS OF SEED TESTA IN DEVELOPING *LUPINUS PILOSUS* SEEDS

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ABSTRACT

Cross sections of the testa and the testa surfaces of *Lupinus pilosus* seeds were testaed with gold and observed with scanning electron microscope. The following tissue layers could be identified on cross sections of *Lupinus pilosus* seed testa (testa): macrosclereids, osteosclereids (microsclereids) and parenchyma cells. The seed testa surface was cristate-papillate, formed by elongated polygonal cells. In *L. pilosus* seeds roughness of the testa surface could be detected starting at 26 days after flowering. At this stage pits in macrosclereid layer could also be observed, approx. 30 µm deep, whereas the cuticle layer was nearly intact. At this time seed dehydratation was proceeding quickly. It seems therefore, that the dehydratation of macrosclereid layer is the background of seed testa roughness trait commonly found in *L. pilosus*.

KEYWORDS

Lupinus pilosus, testa seed, ultrastructural, development of 'rough-seed'

INTRODUCTION

Lupins occur over a wide range of geographical and ecological conditions in both the 'New' and the 'Old World'. Based on seed testa (i.e. coat) structure, lupins are divided into two separate groups: *Scabrispermae*—rough seeded and *Malacospermae*—smooth seeded (Heyn and Herrnstadt, 1977). *Lupinus pilosus* is regarded a representative of *Scabrispermae*. Seed testa plays an important and active role in developing legume seeds. In angiosperm seeds, the embryo and endosperm are surrounded by the seed testa, which protects the embryo from mechanical damage, pathogen attack, and UV damage and maintains the dehydrated dormant stage until proper germination conditions exist.

The seed testa also functions as the maternal conduit to the embryo and some metabolites flow from the seed testa to the embryo (Wobus and Weber, 1999). The testa can prevent water imbibition, provide mechanic protection, interfere with gaseous exchanges and prevent or promote the release of inhibitors related to establishing or breaking of seed dormancy (Bewley and Black, 1994). Kontos and Spyropoulos (1996) suggested that the testa of controls the production of α-galactosidase and endo-β-mannanase in the endosperm of developing seeds. Testa appears to play a role during storage mobilisation in the legume seed of *Sesbania virgata*, probably by participating in the control of the production, modification and/or storage of the hydrolases (Tonini *et al.* 2007). At the beginning of seed development it is the main seed component and it may affect access of nutrients, as well as potentially harmful compounds (e.g. herbicides) to the embryo (Adomas *et al.* 2008). In mature seeds of some species structure of the seed testa has systematic and diagnostic significance (Michalczyk *et al.* 2006).

The aim of this study was to characterise development of the 'rough-seeded' trait in *L. pilosus* seeds.

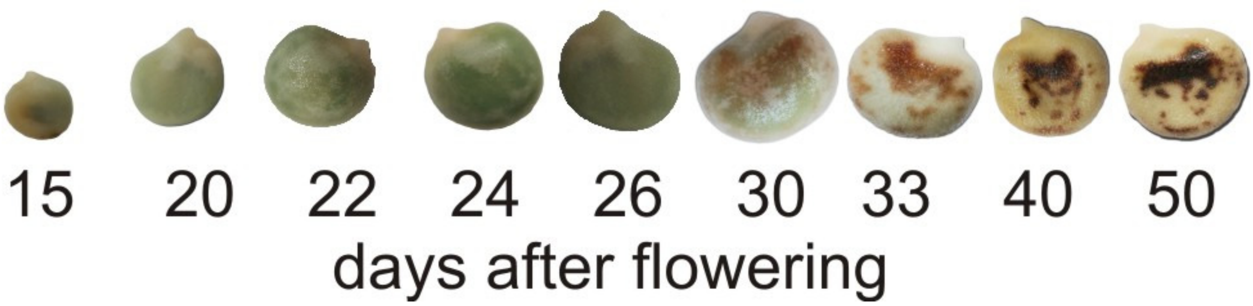

Fig. 1. Visual characteristics of developing seeds.

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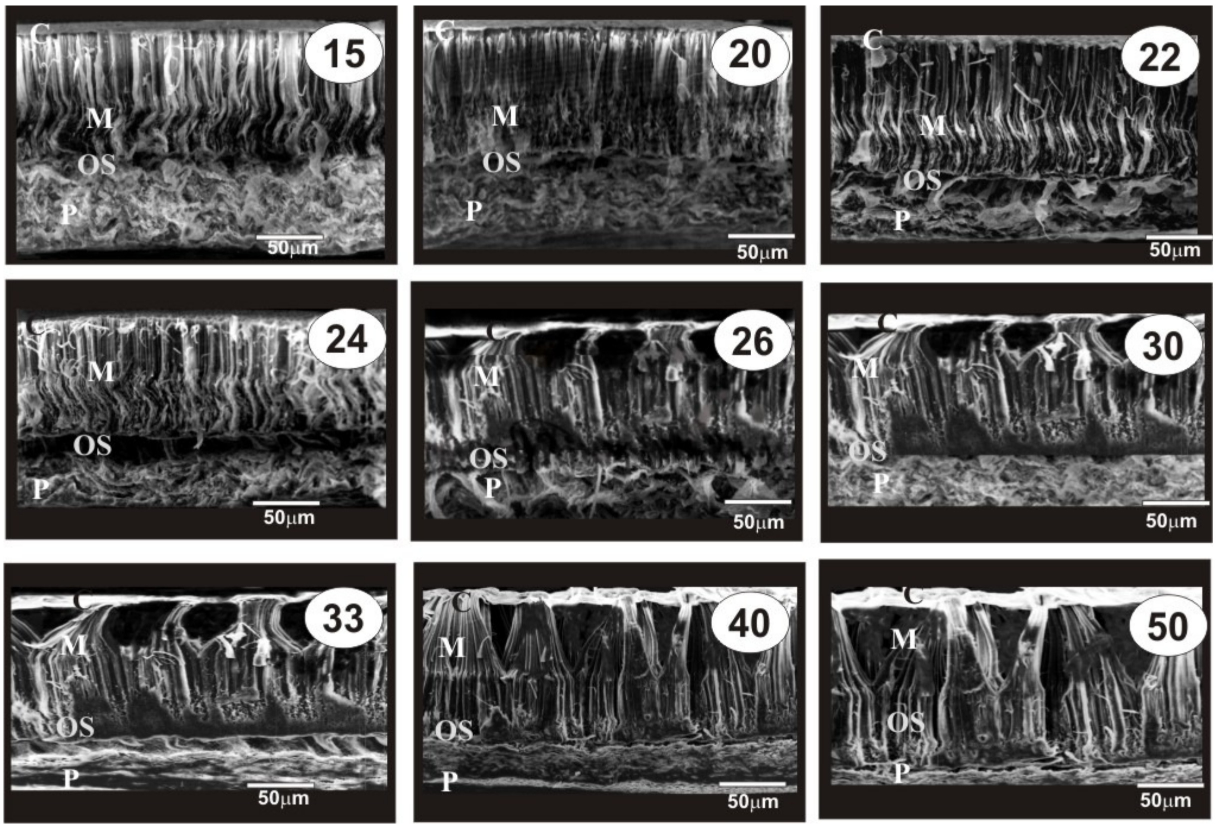

Fig. 2. Scanning electron micrographs of developing *Lupinus pilosus* testa seeds. C – cuticule, M – macrosclereid layer; Os – osteosclereid layer; P – parenchyma cells. Number showed days after flowering. Bar = 50 µm.

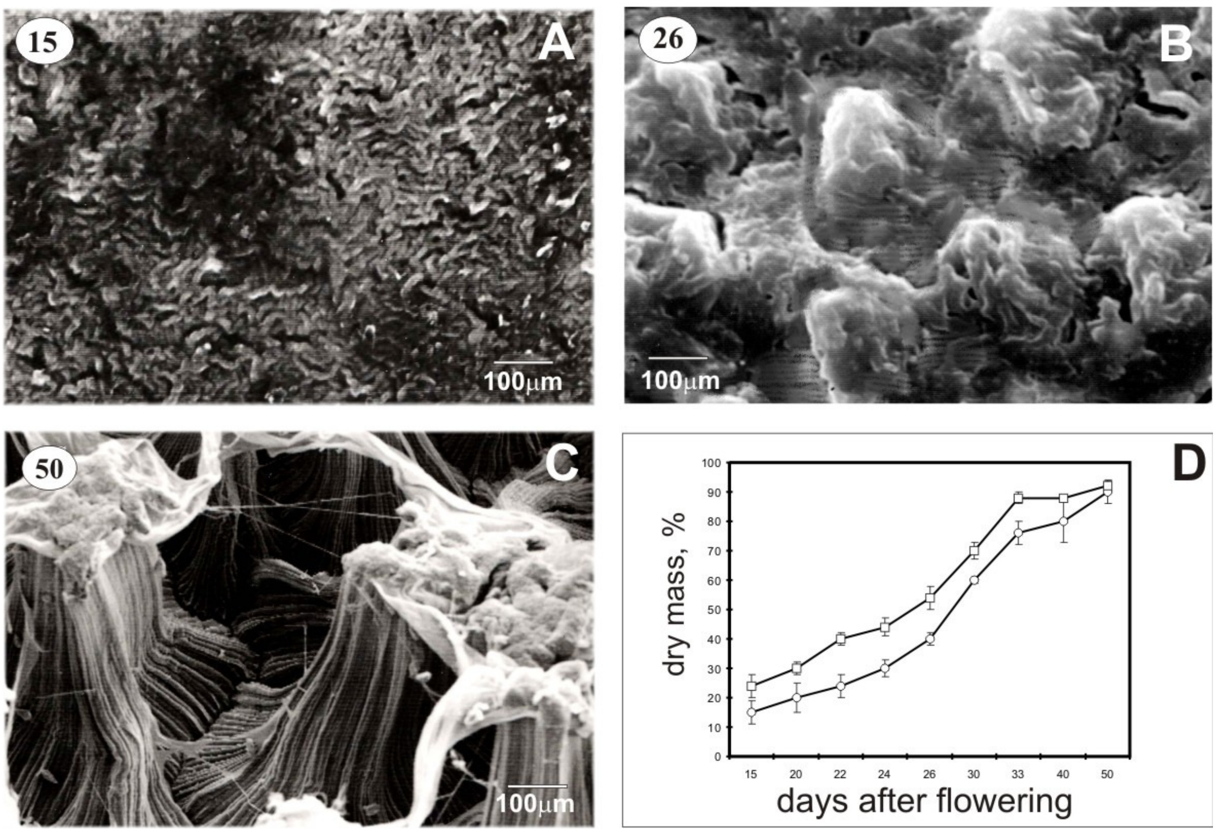

Fig. 3. SEM images of seed surface at 15 (panel A), 26 (panel B), and 50 (panel C) days after flowering. Dry mass [panel D, %] of seed (o) and testa (□) in maturing *Lupinus pilosus*.

MATERIALS AND METHODS

Lupinus pilosus plants were cultivated in greenhouse. Plants were grown in 10 L pots in a green house with a 12 h photoperiod at 23°C day/18°C night and 140 µmol photons m^{-2} s^{-1} irradiance. Mixture of peat, garden soil and sand (1:1:1, v/v/v) was used as substrate for plant growth. On the day of flowering the plants were labelled and seeds testa were collected in 15, 20, 22, 24, 26, 30, 33, 40 and 50 days after flowering. The maturing seeds testa were separated from the endosperm and developing cotyledons, cut into small segments. For observation in the scanning electron microscope (SEM) the seeds were testaed with gold using a JEOL JFC 1200 ion testaer and observed in a JEOL JSM-5310LV scanning electron microscope under a 20 kV. Dry mass were determined according to ISTA (1997).

RESULTS AND DISCUSSION

Ultrastructural and histochemical studies of the seed testa have been carried out in *Phaseolus vulgaris* (Yeung and Cavey, 1990), *Pisum sativum* (Harris, 1984), *Glycine max* (Miller *et al.* 1999) and *Lupinus angustifolius* (Serrato-Valenti *et al.* 1987), etc. The seed testa structure of *Lupinus pilosus* is similar to that of other legumes (Piotrowicz-Cieślak, 2005; Michalczyk *et al.* 2006). The mature seed testa (Fig. 1) is composed of three layers of cells: outer macrosclereid layer - covered by cuticule and supported by an osteosclereid layer (Fig. 2). The remainder of the testa was composed of a parenchymatous region. In transmission electron microscope (Algan and Baker Buyukkaral, 2000) developing macrosclereids of *Trifolium pratense* have a dense cytoplasm with organelles typical of actively metabolic cells, and the cytoplasm of the parenchyma cell contains amyloplasts, rough ER and ribosomes.

Macrosclereid cells divide anticlinally at the zygote stage of development (Algan and Baker Buyukkara, 2000). In *Lupinus pilosus* macrosclereid cells grow increasing their length from 150 µm at the 15 DAF to 350 µm at the 50 DAF. In *Lupinus pilosus* seed testa the thinnest layer (7-10 µm) was built by osteosclereids. These cells had massive central (proximal) walls, but their distal walls remained thin, allowing the terminal portions of cells to expand and become bulbous.

The cell walls of both macrosclereid and osteosclereid layers contribute to the mechanical durability of the seed testa (Harris, 1987). Scanning electron microscopy revealed pits in seed testas. The ratio of parenchyma layer thickness and macrosclereid layer in seed testa changed during *L. pilosus* seed maturation (Fig. 2). Roughness of the seed testa surface could be observed beginning from 26 days after flowering. At this moment pits in macrosclereid layer, approx. 30 mm deep, could also be observed, whereas the cuticule layer was nearly intact (Fig. 3B). At this moment seed dehydratation was proceeding quickly (Fig. 3D). It seems therefore that the dehydratation of macrosclereid layer is the background of seed testa roughness trait commonly found in *L. pilosus*.

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