

Seed coat ultrastructure of hard-seeded and soft-seeded varieties of *Vicia sativa*

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Abstract: The mature seed coat structure in hard-seeded and soft-seeded varieties of *Vicia sativa* L. was examined by scanning and transmission electron microscopy to investigate differentiation in cytological and morphological features between these varieties. The outer layer of the seed coats was composed of macrosclereid (Malpighian) cells in the hard-seeded and soft-seeded varieties of *Vicia sativa*. The walls of the macrosclereid cells were thickened, and the cell vacuoles were filled with tannin. The cytoplasm of the macrosclereids contained small- and large-sized vacuoles and ribosomes, mitochondria, and endoplasmic reticulum. Under this layer there was a hypodermal layer composed of osteosclereids separated by large intercellular spaces. The bottom layer consisted of parenchyma cells. Thin-walled parenchyma cells were filled with amyloplasts that contained large starch grains, protein bodies, and a few organelles. Seed size and colour are important characteristics for distinguishing between hard-seeded and soft-seeded varieties. The hard seeds of *Vicia sativa* are smaller than the soft seeds.

Key words: Seed coat, ultrastructure, *Vicia sativa*

1. Introduction

Vicia L. (Fabaceae) is a widespread, moderately large genus of ± 150 species with 64 species in Turkey (Davis & Plitmann, 1970; Maxted, 1989). It is a forage plant of economic importance and is cultivated in Turkey for forage or grain production, as a pasture plant, or as a green fertiliser plant. Its green, dry hay is of high quality and nutritious. The seeds are high in protein and are used as animal feed. The species is naturally widespread in the Mediterranean region and Asia Minor.

Soft-seeded summer and hard-seeded winter lines of *Vicia sativa* were developed by Elçi (1975) via selection, reformation, and breeding from a single plant. While stand establishment is easy in the summer line, it is difficult in the winter line. The winter line has a waxy substance on the surface of the seed coat. The objective of this study was to examine the ultrastructure of the seed coat in these 2 lines using light microscopy (LM) and transmission electron microscopy (TEM).

2. Materials and methods

Seed of both lines were obtained from the Ankara University Faculty of Agriculture, Department of Crop Science.

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From each line, 10 seeds (20 seeds total) were measured for length and width under a binocular lens to the nearest 0.1 mm. In addition, 10 seeds from each line were pooled and weighed. For TEM studies, mature seeds were cut into small segments (1–2 mm³) and fixed in 3% glutaraldehyde buffered with 0.1 M phosphate (pH 7.2) for 3 h at room temperature. The tissue was washed 3 times in 0.1 M phosphate buffer and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2) for 3 h at room temperature. Samples were then dehydrated in an ethanol series, transferred to 100% propylene oxide, and embedded in epoxy resin. Tissue for TEM was sectioned on a Reichert ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a JEOL CX-100 transmission electron microscope at 80 kW. Epoxy-embedded material for LM was cut at 1.5 or 2 μ m and stained with 1% methylene blue.

For scanning electron microscopy (SEM) studies, dry seeds were mounted on stubs and coated with gold for 4 min before viewing with a JEOL 3000 scanning electron microscope at 30 kW. Terminology followed that of Brochmann (1992). SPSS-PC and the Simpson and Roe graphical test (Van der Pluyma & Hideux, 1997) were used for statistical analysis.

3. Results

The seed coats of both hard-seeded and soft-seeded lines of *Vicia sativa* are similar in structure to the seed coats of other legumes (Harris, 1984, 1987; Manning & Van Staden, 1985; Serrato Valenti et al., 1989, 1993; Algan & Bakar Büyükkartal, 2000). The outer epidermis of the mature seed coat is composed of macrosclereid (Malpighian) cells, a hypodermal layer of osteosclereids, and several inner layers of crushed parenchyma cells (Figure 1).

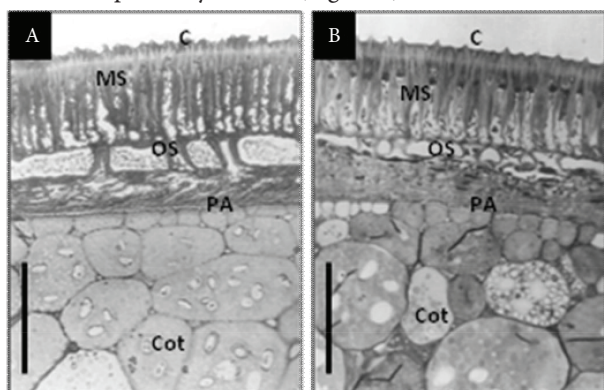


Figure 1. Light micrographs of longitudinal semithin sections of A) hard-seeded and B) soft-seeded lines of *Vicia sativa* L. MS = macrosclereid layer, OS = osteosclereid layer, PA = parenchyma cells, C = cuticle, Cot = cotyledon. Scale bar = 50 μm .

In the hard-seeded line, the mean length of the macrosclereid cells is 37.62 μm and the width is 4.69 μm . A thick cuticle is developed (Figure 2), and the radial as well as tangential walls of the macrosclereids are thickened (Figure 2). The walls of the macrosclereid cells have suberised caps that are embedded in a pectic cuticular matrix that makes up the subcuticular layer. The epidermis is continuous with macrosclereid cells across the hilar region, which has similarly thickened cells. The macrosclereids are densely cytoplasmic and contain small and large vacuoles filled with tannin (Figure 2). The osteosclereid layer is composed of large vacuolated cells and develops thicker walls adjacent to the intercellular spaces that were formed distally in this cell layer in the hard-seeded line (Figure 2). The average length of the osteosclereid cells is 6.84 μm and the width is 18.9 μm . These cells are separated by large intercellular spaces and contain fewer organelles than the underlying cotyledon parenchyma cells. The cytoplasm of the cotyledon parenchyma cells contains amyloplasts with large starch grains and numerous small or several larger vacuoles (Figure 2). Parenchyma cells are separated by prominent intercellular spaces, and the walls of the cells are electron-translucent. The endoplasmic reticulum is poorly developed. Small lipid bodies are also present in

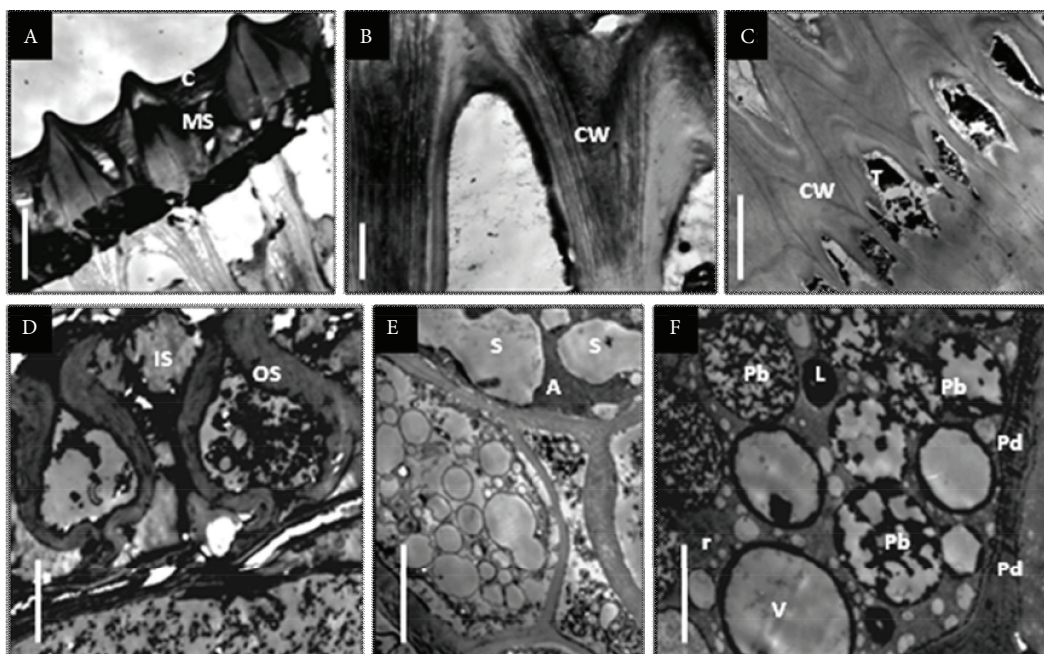


Figure 2. Electron micrographs of the mature seed coat in the hard-seeded line of *V. sativa* L. A) Epidermal outer wall of macrosclereid cells (MS). Note that the cuticle (C) is thickened. Scale bar = 5 μm . B) Mature macrosclereid showing more vacuolated cytoplasm and central cell walls (CW). Scale bar = 3 μm . C) Cytoplasm of the macrosclereid cell. Note that cell vacuoles contain tannin (T). Scale bar = 5 μm . D) Electron micrograph showing the osteosclereid cells (OS). Note that cell walls adjacent to the intercellular spaces (IS) are thickened. Scale bar = 5 μm . E) Cytoplasm of the cotyledon parenchyma cells showing amyloplast (A) containing starch grains (S). Scale bar = 5 μm . F) Details of cytoplasm in cotyledon parenchyma cells showing r = ribosomes, L = lipid, Pb = protein bodies, V = vacuole, and Pd = plasmodesmata. Scale bar = 3 μm .

the cytoplasm. Large vacuole-like vesicles become visible and seem to be entirely filled with protein (Figure 2). Plasmodesmata occur in the common walls between the parenchyma cells.

In the soft-seeded line of *Vicia sativa*, macrosclereids had secondary walls and little cytoplasm remained in the lumen. Macrosclereid cells have an average length of 39.24 μm and a width of 5.75 μm . A thin cuticle is present (Figure 3). The outer tangential wall is thinner and more electron-translucent than that of the hard-seeded line.

The cytoplasm contains a centrally located nucleus and several organelles (Figure 3), and the major part of the cytoplasm is vacuolated. These vacuoles are completely or incompletely filled with tannin (Figure 3). The osteosclereid cells are 7.38 μm long and 17.73 μm wide. The osteosclereid layer develops thicker walls adjacent to the intercellular spaces, which have formed distally in this cell layer (Figure 3). The ultrastructural features of the cells are essentially the same as those of the hard-seeded line.

Thin-walled cotyledon parenchyma cells are filled with amyloplasts that contain large starch grains and electron-dense cytoplasm. Small intercellular spaces were observed between the cells of the parenchyma layer. Occasionally, a layer of endosperm (aleurone layer) remained attached

to the parenchyma layer. Numerous protein bodies were observed in all cells and were visible as electron-dense material in the vacuoles (Figure 3). Ribosome density in the parenchyma cells is higher than that of the hard-seeded variety. Some amorphous materials are also present within the intercellular air spaces. Endoplasmic reticulum cisternae are reduced and fragmented and are not very electron-dense (Figure 3).

3.1. Seed macromorphology

Hard- and soft-seeded lines of *Vicia sativa* are similar in shape but differ significantly in length, width, and mass (Table; Figure 4). The largest and heaviest seeds occur in the soft-seeded line, with a mean size of 5.35 \times 4.29 mm and a mean mass of 0.064 g. The seeds are smaller in the hard-seeded line, with a mean size of 4.78 \times 3.53 mm and a mean mass of 0.036 g. The seeds of the soft-seeded line are light brown while seeds of the hard-seeded line are black in colour (Figure 5).

3.2. Seed micromorphology (SEM)

Both seed lines have a papillate testa surface. The papillae are conical, multiribbed, and moderate in size and density. The soft-seeded line has 7–8 papillae per 100 μm^2 , and the hard-seeded line has 10–12 papillae per 100 μm^2 (Figure 6).

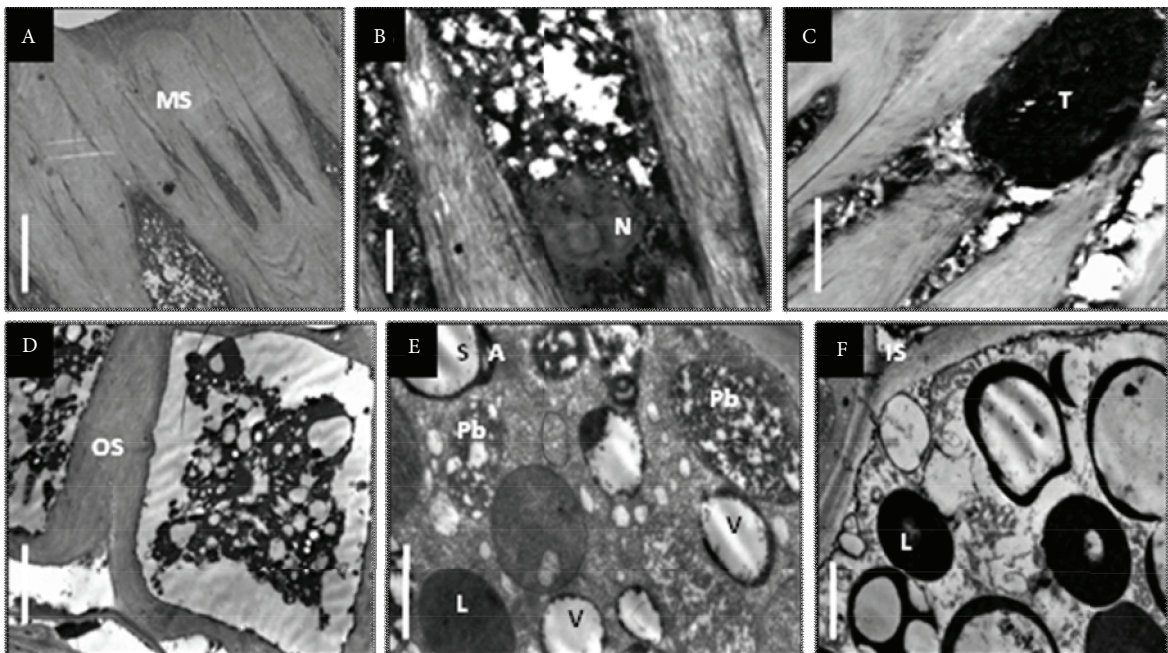


Figure 3. Electron micrographs showing the mature seed coat in the soft-seeded line of *V. sativa* L. A) Outer walls of the macrosclereid cells (MS) are covered by a thin cuticle (C). Scale bar = 5 μm . B) Cytoplasm of the macrosclereid cells showing centrally located nucleus (N). Scale bar = 1 μm . C) Cells contain dense deposits of tannin (T). Scale bar = 2 μm . D) Electron micrographs showing the osteosclereid cells (OS). Scale bar = 3 μm . E) Cytoplasm of the parenchyma cells containing amyloplast (A), which contains large starch grain (S), protein bodies (Pb), vacuole (V), ribosomes (r), and lipid (L). Scale bar = 2 μm . F) The parenchyma cell contains endoplasmic reticulum cisternae (ER) and lipid (L). Note that intercellular air spaces contain amorphous materials (arrow). Scale bar = 2 μm .

Table. Seed characteristics of soft-seeded and hard-seeded lines of *V. sativa*. Seed length and width (mm) are based on mean values; seed mass is the mean mass of 30 seeds from each form.

Taxa	Length (mm)				Width (mm)				Length/width	Mass (g)			Colour
	Min	Max	Mean	SD	Min	Max	Mean	SD	Mean	Min	Max	Mean	
Soft line	4	6	5.35	0.053	4	4.5	4.29	0.037	1.24	0.031	0.082	0.036	light brown
Hard line	3.5	6	4.78	0.078	3	4	3.53	0.019	1.35	0.02	0.052	0.036	black

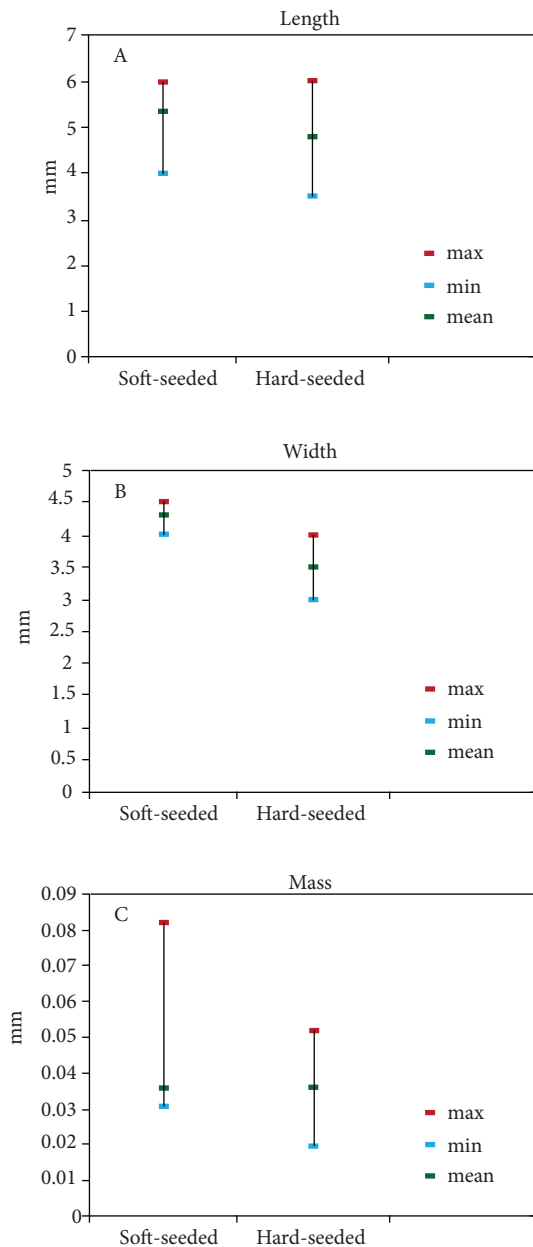


Figure 4. A) Seed length, B) width, and C) mass in soft- and hard-seeded lines of *V. sativa*.

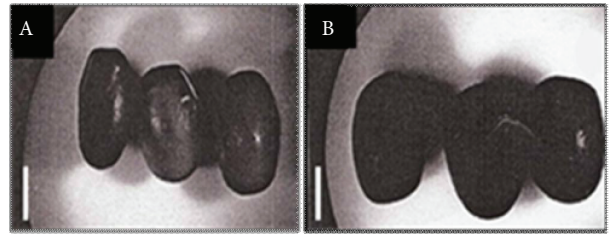


Figure 5. A) Seeds of the soft line of *V. sativa* L. are light brown; scale bar = 4 mm. B) Seeds of the hard line of *V. sativa* L. are black; scale bar = 2 mm.

4. Discussion

Seed coat or testa morphology is known to be an important character for taxonomic and evolutionary studies in Angiospermatophyta (Riahi & Zarre, 2009; Kaya et al., 2011; Fagundez & Izco, 2011).

Both hard and soft lines of *Vicia sativa* have similar major seed coat characteristics; notably, they both have thickened secondary epidermal macrosclereid walls and tanniferous cell vacuoles.

This study suggests that the macrosclereid and osteosclereid layers contribute to the mechanical strength of the seed coat in both hard-seeded and soft-seeded lines of *Vicia sativa*. Development of an electron-translucent cap (light line) occurs in the outer wall of all macrosclereid cells in the hard-seeded line.

Manning and Van Staden (1985) suggested that the light line in *Erythrina lysistemon* Hutch. was an optical phenomenon linked to structural discontinuity at the boundary between lignified and unlignified parts of the cell walls. The macrosclereid layer is generally considered responsible for water impermeability in Fabaceae seeds (Serrato Valenti et al., 1986, 1989, 1993; Riggio Bevilacqua et al., 1989).

Rangaswamy and Nandakumar (1985) indicated that the hilum and micropyle were important structures for water absorption. The impermeability of seeds has been attributed to different structures such as contracted macrosclereid cells and thick cuticles and to substances such as tannins, waxes, lignin, hemicellulose, suberin, cutin, callose, and phenolics (Marbach & Mayer, 1974; Slattery et al., 1982; Gulz & Hangst, 1983).

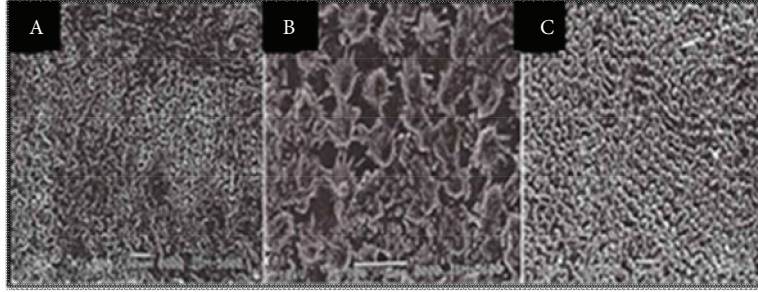


Figure 6. SEM of seed coat surface in A, B) soft and C) hard lines of *V. sativa*.

Considering the macrosclereid layers of the 2 different seed lines of *V. sativa*, it has been determined that cell size is larger and cuticle thickness is greater in the hard-seeded line. In both seed lines the osteosclereid cell walls became very thick, and the cell contents were almost completely degraded at maturity. The development of the macrosclereids and osteosclereids in both lines of *V. sativa* was similar to the development pattern observed in *Pisum sativum* L. (Harris, 1984), *Glycine max* Merr. (Harris, 1987), *Erythrina lysistemon* Hutch. (Manning & Van Staden, 1985), and *Trifolium pratense* L. (Algan & Bakar Büyükkartal, 2000).

A layer of parenchyma tissue, derived from the crushed inner epidermis of the testa and the entire tegmen, is present in all Fabaceae; however, little is known about the possible role of this layer in metabolite translocation (Yeung & Clutter, 1978; Yeung & Cavey, 1988). One of the significant observations of this study was the presence of electron-dense amorphous materials within the intercellular air spaces (Figure 3).

Seed size and colour are important characteristics for distinguishing between hard-seeded and soft-seeded lines (Juan et al., 1994; Brochmann, 1992). The hard seeds of *Vicia sativa* are smaller and lighter than the soft seeds. The

soft seeds are light brown in colour while the hard seeds are black, indicating some degree of chemical or mechanical difference between them. The seed types have similar seed coat surfaces.

This study indicates that the basic testa cytology of hard-seeded and soft-seeded lines of *Vicia sativa* is similar. However, the outer wall of the seed coat contains large amounts of lanoline substances, and a light line occurs in the outer wall of macrosclereid cells in the hard-seeded line. This study indicates that there are 3 major differences in seed coat anatomy between the 2 seed lines. In the hard-seeded line the cuticle is significantly thicker and the macrosclereids are larger with a well-developed light line in the outer wall.

These chemico-structural differences are possibly attributable to the differences in seed coat permeability that characterise the hard- and soft-seeded lines of *Vicia sativa*.

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