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Ultrastructural Characterization of Seed Coat Maturation in *Cercis Siliquastrum L.*

By

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With 9 Figures

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Summary

BONALDO A., BALDAN B., RASCIO N., MARIANI P., GASTALDO P., PROFUMO P. & RIGGIO BEVILACQUA L. 1994. Ultrastructural characterization of seed coat maturation in *Cercis siliquastrum L.* – Phyton (Horn, Austria) 33 (2): 257–265, 9 figures. – English with German summary.

The development of the seed coat has been observed in the course of maturation of the *Cercis siliquastrum* seed. The inner integument as well as the nucellus underwent major morphological changes, and ultimately they degenerated, so that the seed coat was formed mainly by the modified outer integument. In the nucellar epidermis a thickening of the cuticle occurred; in the mature seed, together with the inner integument cuticle, it formed a thick, autofluorescent boundary around the endosperm. The role of both the cuticular layers, and the testa, in determining the impermeability of the seed coat is discussed.

Zusammenfassung

BONALDO A., BALDAN B., RASCIO N., MARIANI P., GASTALDO P., PROFUMO P. & RIGGIO BEVILACQUA L. 1994. Ultrastrukturelle Charakterisierung der Samenschalenreifung

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von *Cercis siliquastrum L.* – Phyton (Horn, Austria) 33 (2): 257–265, 9 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Bei Cercis siliquastrum wurde die Entwicklung der Samenschale im Verlauf der Samenreife untersucht. Sowohl das innere Integument als auch der Nucellus erfahren größere morphologische Veränderungen, bis sie schließlich degenerieren. Die Samenschale wird dann hauptsächlich durch das modifizierte äußere Integument gebildet. In der Epidermis des Nucellus verdickt sich die Cuticula und bildet in reifen Samen zusammen mit der Cuticula des inneren Integuments eine dicke Begrenzung um das Endosperm, welche Autofluoreszenz zeigt. Die Rolle der Cuticulaschichten und der Testa als bestimmende Faktoren der Undurchlässigkeit der Samenschale wird diskutiert.

Introduction

Cercis siliquastrum (*Cesalpinioideae,* Fabales) has a dry and dormant mature seed. According to RIGGIO BEVILACQUA & al. 1985, the seed coat prevents water uptake. The impediment to water uptake appears to be due to the remnants of the inner integument (RIGGIO BEVILACQUA & al. 1986, ROTI-MICHELOZZI & al. 1987). In fact, only when the seeds are scarified to the endosperm can water enter, causing them to swell.

In order to individuate cytological features of the cellular layers involved as a barrier to water uptake we have observed the changes of the seed coat at the ultrastructural level in the course of seed maturation.

Even though our results confirm some previous observations at the light microscopy level (GASTALDO & PROFUMO 1975, SERRATO-VALENTI & al. 1979), they give further information about the structural basis of the seed coat behaviour in preventing water uptake; in particular they suggest that the remnants of the nucellus are involved in this process too.

Materials and Methods

Cercis siliquastrum L. seeds were collected weekly till complete maturation during two vegetative cycles (April–November) from a tree grown in the Botanical Gardens in Padua.

For light and electron microscopy, samples were fixed overnight in 6% glutaraldehyde in 0.1 M Na cacodylate buffer (pH 6.9), post-fixed in 1% osmium tetroxide in

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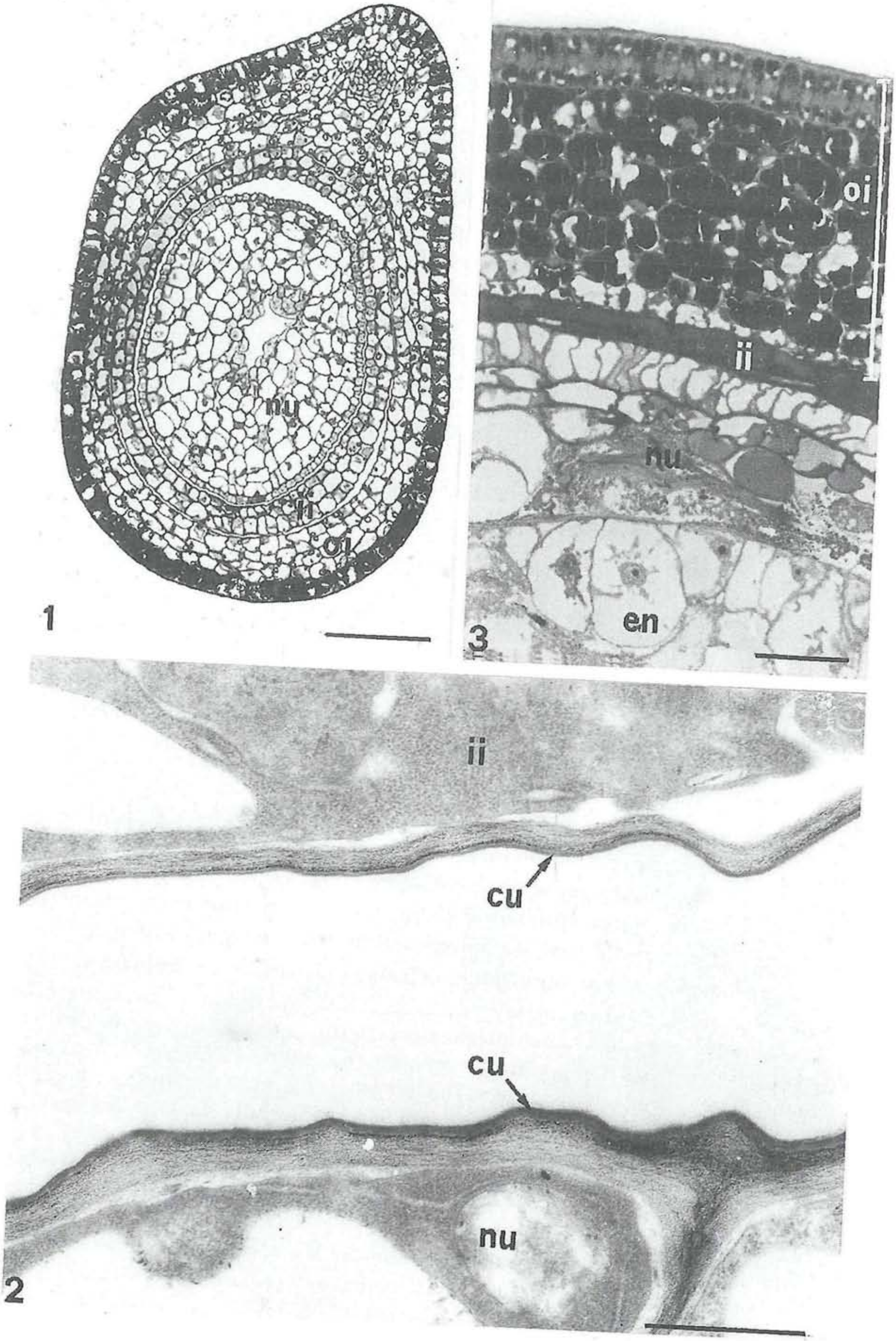
Fig. 1. Fertilized ovule: the two integuments and the nucellus are evident. A space separates the outer integument from the inner one and the latter from the nucellus. (oi = outer integument, ii = inner integument, nu = nucellus). Bar 100 µm.

Fig. 2. Both the inner integument and the nucellus show a distinct cuticle. (cu = cuticle). Bar 0.5 µm.

Fig. 3. Early stage of the endosperm cellularisation: in the outer integument the epidermal cells are radially elongated, covered by a cuticle; the parenchymatous cells have vacuoles with dense contents. The cells of the inner integument are tangentially elongated and tightly packed. The nucellus is partially degenerated. (en = endosperm). Bar 50 µm.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2

0.1 M Na cacodylate buffer (pH 6.9) for 2 h, dehydrated in a graded series of ethanol and propilene oxide, embedded in Araldite. Ultra-thin sections were observed after uranyl acetate and lead citrate staining with a Hitachi H 300 EM operating at 75 kV. Semi-thin sections (1µm) were stained with Toluidine Blue 1%. Some histochemical tests were performed on unstained semithin sections. To give evidence to cell wall polysaccharides PAS staining was performed according to Jensen 1962.

To test the presence of tannins, according to Gahan 1984 transversal sections obtained by a cryostate were treated with a ferric chlorure 1% in HCl 0.1 N solution for 15' and immediately observed; tannins were blue-black stained.

In order to evidentiate autofluorescence of the cuticular layers, transversal sections (10 µm) obtained with a cryostate were observed with a U.V. light fluorescence microscope (Zeiss Universal Photo microscope, filters 0365 and LP 420).

Results

Just after the fertilization, in the outer integument a differentiation of the external cellular layer was evident (Fig. 1). This layer later give rise to the epidermal (malpighian) layer. Peculiar features of this layer were the cellular shape and the electrondense vacuolar contents.

The subepidermal zone was formed by roundish cells with a large vacuole.

The inner integument, spatially separated from the outer one, was formed by 2–3 cellular layers with large vacuoles. The cellular layer towards the nucellus had an evident but thin cuticle (Fig. 2). The nucellus was separated from the inner integument by a space; it was pluristratified and healthy (Fig. 1). In the nucellar epidermal cells the outer tangential wall was rather thickened and covered by an evident cuticle (Fig. 2).

At the early stage of cell wall formation in the nuclear endosperm, the epidermal layer of the outer integument showed further differentiation: cells were radially elongated (palisade-like cells), with PAS-positive cell walls (not shown) and thickened outer and inner tangential ones; the light-line (linea lucida) was sometimes evident. A distinct cuticle covered the palisade cells on the outer side; cellular contents were polarized sho-

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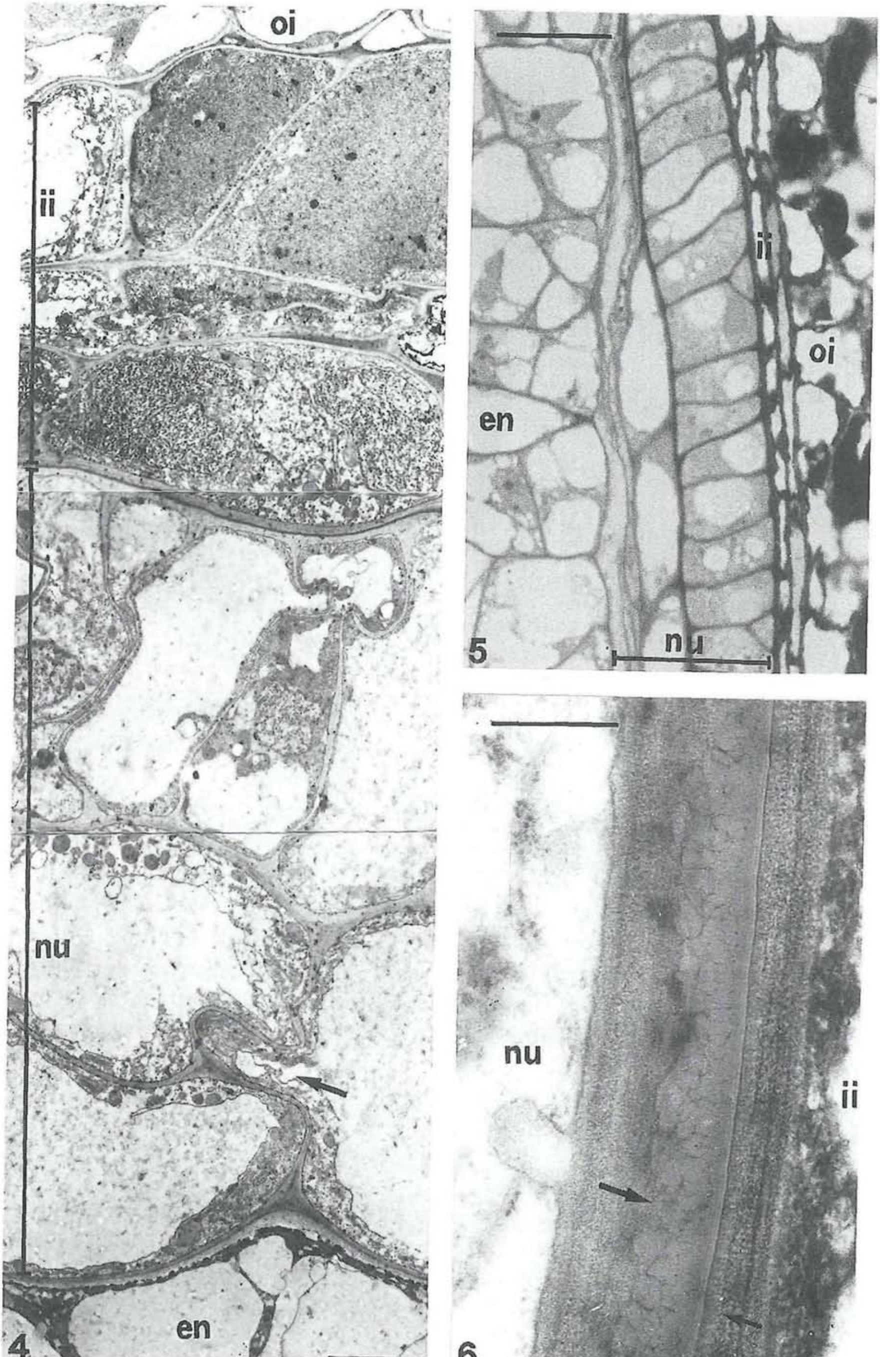
Fig. 4. From top to bottom: inner integument cells (ii), tangentially flattened, with a degenerated cytoplasm and a granular vacuolar content; nucellus (nu) with an epidermal and two sub-epidermal layers. The last cellular layer of the outer integument (oi) as well as the first one of the endosperm (en) are partially shown. Note the cell wall folding in the nucellus (arrow). Bar 3 µm.

Fig. 5. Complete endosperm cellularisation. The inner integument cells are very close and degenerated; only the epidermal cells of the nucellus appear healthy, the inner ones are reduced to remnants. Bar 20 µm.

Fig. 6. High magnification shows the two cuticular layers (that of the inner integument and the nucellar one) close together. Note that a thickening of the nucellar cuticle has occurred. Bar 0.5 µm.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ii

wing a central cytoplasm and dense vacuoles localized at the both cellular poles (Fig. 3).

The inner cells of the outer integument were isodiametric with thin cell walls and an electron-dense vacuolar content. A cytochemical test for tannin resulted positive (not shown). Deep changes occurred at inner integument level (Fig. 3): cells were tangentially stretched, tightly packed; vacuoles were filled with electron-dense material and partially degenerated cytoplasm (Fig. 4). At this stage some nucellar layers were still recognizable: an epidermis adjacent very close to the inner integument (space shown in Fig. 1 disappeared) with radially elongated cells, and some parenchymatous layers (Fig. 3), the innermost degenerating. The stretching of the inner integument cells, the disappearance of the space between inner integument and nucellus, and the presence of partially folded nucellar cell walls might to be due to the increase in the seed internal mass.

At the end of the endosperm cellularisation the modifications of the outer integument were very limited; the cells of the inner integument were partially degenerated and compressed to give a very flattened layer (Fig.5).

The nucellus was reduced to the epidermis and the remnants of the underlying cells (Fig. 5). An evident thickening of the cuticle occurred; the nucellar cuticle was adjacent very close to the cuticular layer of the inner integument (Fig. 6).

During the integument maturation a gradual degeneration till the death of all the cells was observed, as well as a further compression of the nucellar remnants.

In the mature seed (Fig. 7) the integument rough organization remained unchanged. The only layer with mechanical strength was the external one of the outer integument (exotestal seed coat, BOESEWINKEL & BOUMAN 1984); nevertheless the cell wall did not lignify, contrary to the common feature of the Fabales seeds (BOESEWINKEL & BOUMAN 1984, PANDEY & JHA 1990). The remaining parietal layers of the nucellus (Fig. 8) were tightly pressed together and they made up a boundary between integuments and endosperm; they remained stuck to the endosperm during specimen sampling.

Fig. 9 shows the U.V. bright autofluorescence of the boundary between the seed coat and the endosperm. A faint autofluorescence is also detecta-

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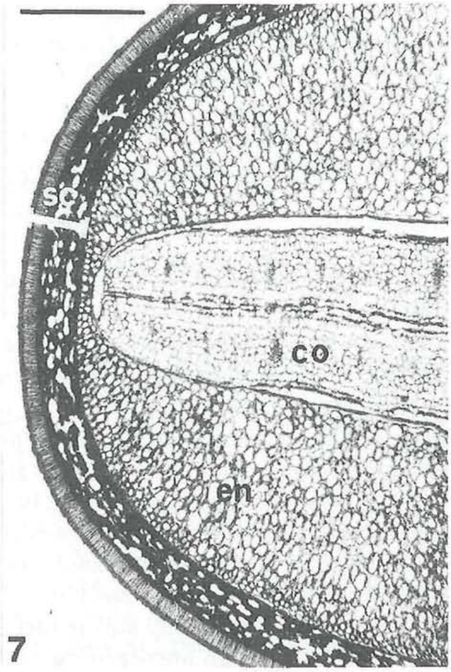
Fig. 7. Mature seed transection. The seed coat appears formed mainly by the outer integument. (en = endosperm; co = cotyledons; sc = seed coat). Bar 300 µm.

Fig. 8. Particular of the remnants of the nucellar cells, with walls closely packed together. Note the very thick cuticle. Bar 1 µm.

Fig. 9. U.V. micrograph showing the bright autofluorescence of the boundary between the seed coat and the endosperm. The walls of the seed coat cells are also faintly autofluorescent. Bar 50 µm.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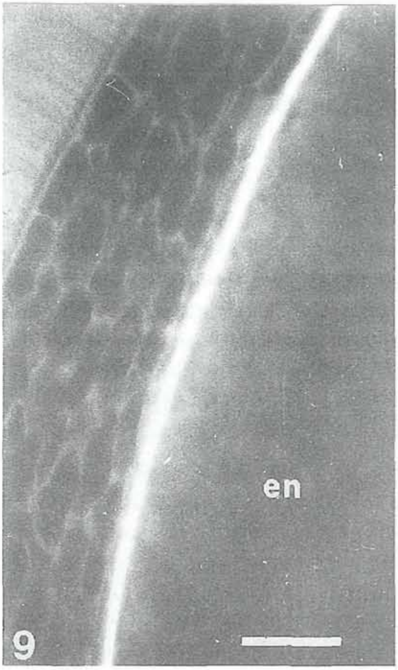
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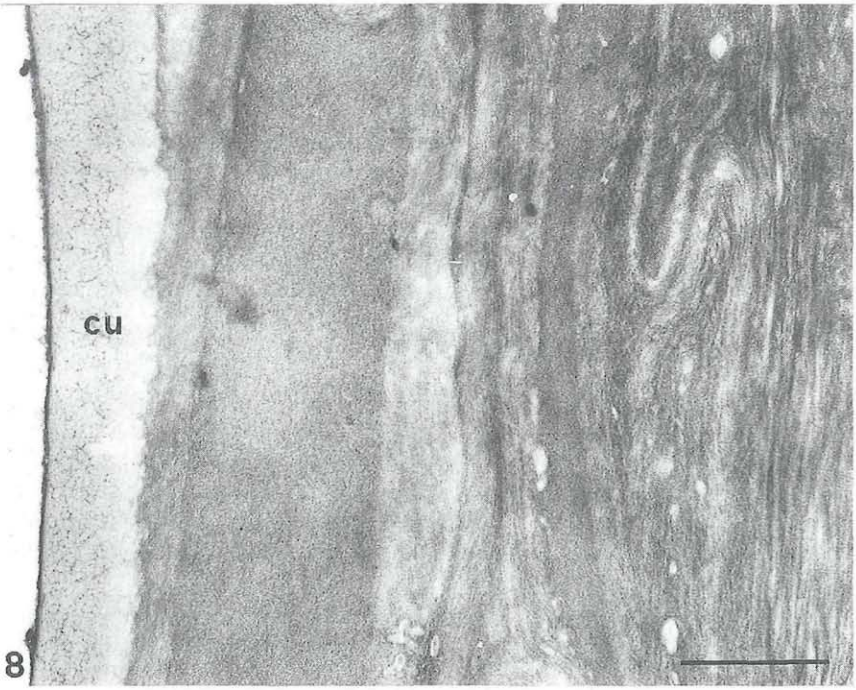
cu
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ble in the seed coat. At the ultrastructural level a continuous, thin osmiophylic layer was evident between the cell walls and was probably responsible for the autofluorescence emission.

Discussion

The seed coat has an important role in embryo relationships with the mother plant during maturation and with the environment after the release of the mature seed from the plant.

Besides being of help in a correct interpretation of the mature seed coat, the developmental studies of the seed coat in *C. siliquastrum* allowed us to presume the functions carried out by these maternal tissues during the seed maturation and to follow the establishment of those characteristics which will give the coat its properties in mature seed.

Initially, when their cells were alive and both embryo and endosperm accumulate reserves, the integuments appear to act as intermediaries in the transport processes between the sinks and the maternal producing tissues. During late events in maturation structural modifications take part in preparing the seed for future interactions with the environment: the epidermal layer of the outer integument gradually acquires mechanical strength due to a cellulosic wall thickening. Simultaneously sub-epidermal cells accumulate phenolics in their vacuoles with an anti-feeding role and probably also an involvement in the inhibition of germination. At seed maturity the seed coat (testa) results mainly from the outer integument and, when cells are completely dead, its properties are to be ascribed to the cell walls and the vacuolar content.

We mainly focused our attention on the gradual formation of a barrier to prevent water uptake in the zone between the inner integument and the nucellus.

It was previously shown by GASTALDO & al. 1984 that the layer derived from the appressed cells of the inner integument was autofluorescent in U.V. light. This layer was also positive to the Sudan Black test (RIGGIO BEVILACQUA & al. 1986) indicating to be lipophilic. They suggest that these inner integument remnants are able to prevent water uptake within the seed.

Our further observations at the ultrastructural level supply evidence that also the nucellus (in particular the thick epidermal cuticle) may be involved in determining the seed impermeability to water and gases. The strict closeness of both the cuticular layers (that of the inner integument and the nucellar one) and the hydrophobic properties of the cuticle provide the seed with a thick and watertight boundary, which efficiently isolates the embryo from the environment.

The presence of cuticular layers in the integuments and / or in the nucellus is a common feature in several seeds (BOESEWINKEL & BOUMAN 1984). In the wheat caryopsis a situation similar to that observed in *C. siliqua-*

strum has been recognized; the nature of the lipophilic material has been examined and it resembles that of the leaf cuticle (MATZKE & RIEDERER 1990). The weak autofluorescence of the testa cell walls may be due to the deposit of a cutin-like substance in the intercellular regions and may explain the previously observed slight involvement of this region in determining the impermeability of the seed coat (RIGGIO BEVILACQUA & al. 1985).

Acknowledgements

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