

SYMPLESMIC CONTINUUM FROM THE OUTER INTEGUMENT TO THE NUCELLUS IN THE DEVELOPING COTTON SEED

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Abstract

In cotton seeds, epidermal layers (outer and inner) surrounded both integuments at most parts of the seed, except at the chalaza. Dye movement studies using carboxyfluorescein showed that the epidermal layers (palisade layer, fringe layer) separated the outer integument from the inner integument and the inner integument from the nucellus symplasmically. Dye movement from the outer integument to the nucellus occurred through the integumentary connective and the transition zone. The phenol-containing cells of chalazal cap could protect the primary solute pathway (between integumentary connective and hypostase) against pathogens. The hypostase appears to be a control site for solute transfer to the endosperm.

Introduction

In cotton (*Gossypium hirsutum* L.) seeds, the maternal tissues consist of two integuments (outer and inner) and the nucellus with embryo sac. Epidermal layers (outer and inner) that surround both integuments at most parts of the seed may provide symplasmic barriers between the maternal tissues. However, at the chalaza, parenchymatic tissue connects the outer integument and the inner integument and the inner integument and the nucellus; these are termed the integumentary connective (formerly known as 'plug;' Pearson, 1939) and the transition zone, respectively (Jacobsen and Jernstedt, submitted). The integumentary connective and the transition zone are located in the center of openings in the palisade layer and in the fringe layer, respectively.

Based on our anatomical analyses of cotton seeds, we hypothesize that the maternal tissues form a symplasmic continuum and the integumentary connective and transition zone are essential passageways for nutrients that may control solute translocation and allocation to support embryo growth and development. The objective of this study was to trace the movement of solutes in cotton seeds using fluorescent dyes and show that the integumentary connective and transition zone are primary pathways for solutes and that the palisade layer and the fringe layer represent barriers to solute movement.

Materials and Methods

Developing cotton bolls, *Gossypium hirsutum* 'Acala SJ2' and the mutant *red-fiberless* (*rfl*), were collected from greenhouse-grown plants and either prepared for microscopy or used for transpiration and translocation experiments. Seeds from 0 to 38 dpa (days post anthesis) were chemically fixed and embedded in Histo-resin (Jung, Leica Instruments GmbH, Heidelberg). Serial sections were cut at 2 to 3 μ m and stained for cytochemistry as described in Weis et al. (1999). Aqueous 0.05% w/v Na-fluorescein (Sigma) or 10 mM CF (carboxyfluorescein, Sigma) was applied to the funiculus of seeds at 25 dpa. After various incubation intervals, seeds were hand sectioned and examined using an Olympus SZ H10 stereo-dissecting microscope or a BH-2 compound microscope (Southern Micro Instruments, Atlanta, GA) with the reflected light fluorescence attachment and filter combination G providing excitation at 495 nm. In addition, seeds at 29 dpa were cut in halves cross-wise and embryo and endosperm were removed. The vacated cavities were filled with the fluorescent dyes to trace the symplasmic and apoplasmic transfer pathways.

Results

In cotton seeds, *Gossypium hirsutum* 'Acala SJ2' and the mutant *red-fiberless* (*rfl*), an outer and an inner epidermal layer surrounded both integuments at most parts of the seed, except at the chalaza, where the palisade layer and the fringe layer were interrupted around the integumentary connective and the transition zone (ic, tz, Fig. 1), respectively. In the outer integument (oi), the outer part of the integumentary connective was positioned inside the branching point of the vascular bundles diverging from the funiculus. Files of cells surrounded by air spaces connect multiple vascular bundles with the integumentary connective (ic, Fig. 2). Likewise, files of cells radiating from the inner part of the integumentary connective unite with inner integument cells. Translocation studies with carboxyfluorescein applied to the funiculus showed that the fluorescent dye moved in the vascular tissue of the funiculus (Fig. 3A) toward the chalazal end and from there in the diverging vascular bundles throughout the outer integument. However, the dye did not cross the palisade layer (pl, Fig. 3B). Dye movement into the inner integument occurred through the integumentary connective (ic, Fig. 3B). Likewise, dye applied to the embryo sac after removing the embryo entered the inner integument through/via the transition zone and did not cross the fringe layer (Fig. 4A). The primary pathway for dye movement from the embryo sac into maternal tissues included began at the transition zone from where the dye moved through the integumentary connective (ic, Fig. 4AB) into the outer integument.

Discussion

The dye movement studies on the mutant *red-fiberless* confirmed that in cotton seeds palisade layer and fringe layer symplasmically separated the outer integument from the inner integument and the inner integument from the nucellus, respectively. Further, the experiments demonstrated that the maternal tissues form a symplasm in which integumentary connective and transition zone represent primary pathways for solute movement to the nucellus/embryo sac (Table 1). Anatomical (Fig. 1) and physiological data (Fig. 4a) illustrated that solute movement occurs in the phenolic-free cells in the center of the chalazal cap. The phenolic-free cells provide the shortest distance between the integumentary connective and hypostase. In the mutant *red-fiberless* nutrients were allocated primarily to the embryo and secondarily for the use in maternal tissues.

Our data also suggest that the hypostase, especially the tip, plays an important role in solute transfer. Endosperm cells inside the tip of the hypostase were much larger than elsewhere in the embryo sac and it appeared that the endosperm received nutrients through the tip/tip cells of the hypostase. These observations are consistent with the literature, which regards the hypostase as facilitating translocation of nutrients to the developing embryo (Netolitzky, 1926; Steyn et al., 1993). The dye movement pathways match Netolitzky's (1926) description, that nutrients enter the outer integument via the funiculus and transfer from the chalaza directly to the nucellus as long as the pathway through the hypostase is open. In this regard it seems plausible that the phenolic-containing cells of the chalazal cap are arranged funnel-like around the phenolic-free cells in the center of the cap and converge at the tip of the hypostase. The position of the hypostase near the solute conduits or pathways is another piece of evidence for its important role in solute transfer, as for example in the seed of *Indigofera parviflora*, in which the major vascular bundle ends at the hypostase (Manning and van Staden, 1987).

The major nutrient solutions mostly consist of sugars and other carbohydrates and therefore present an optimal target for pathogens such as fungi, which could enter the seed through the micropyle. The micropyle is especially vulnerable at anthesis when pollen tubes enter the ovules. Fungal hyphae could also enter the micropyle and migrate between the epidermal layers, inner epidermis of the outer integument and outer epidermis of the inner integument (palisade layer), towards the

integumentary connective and the phenolic-free cells. The phenolic compounds in chalazal cap cells may provide protection against such pathogens.

Summary

In developing cotton seeds, palisade layer and fringe layer separate the outer integument from the inner integument and the inner integument from the nucellus symplasmically. The maternal tissues form a symplasmic continuum in which integumentary connective and transition zone represent primary pathways for solute movement to the nucellus/embryo sac.

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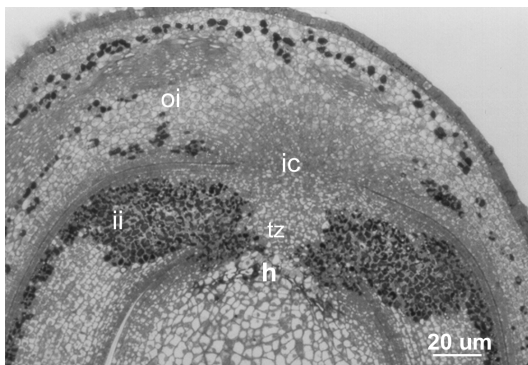


Figure 1. Longitudinal section of plastic-embedded chalazal tissues of an immature cotton seed at 0 dpa. Median section of the integumentary connective (ic). The inner integument (ii) parenchyma cells between the integumentary connective and the transition zone (tz)/hypostase (h) are free of phenolic compounds (dark blue). Outer integument (oi).

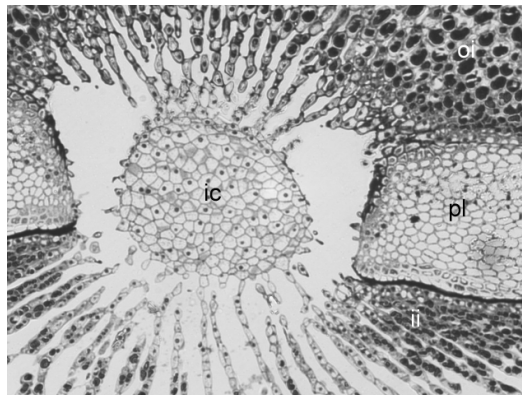


Figure 2. Transverse (oblique) sections (plastic-embedded) of the integumentary connective and adjacent tissues in a cotton seed at 14 dpa. Files of cells radiate from the integumentary connective (ic) to the inner integument. Palisade cells (pl) show some cell wall ingrowths. Phenolic compounds (blue) are present in the inner (ii) and outer (oi) integument cells.

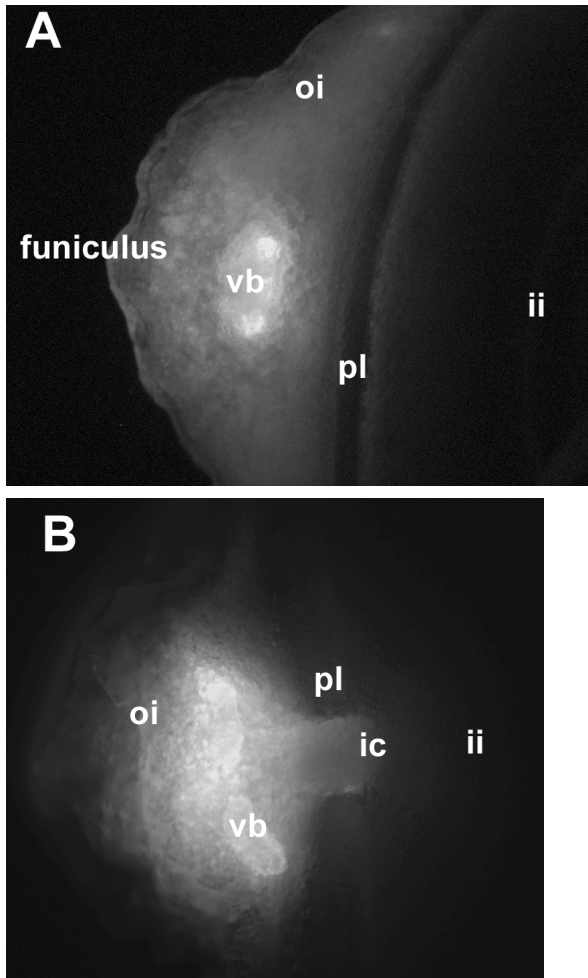


Figure 3. Tracing the translocation pathways in cotton seeds using carboxyfluorescein (CF). Seeds of *rfl* (red fiberless) mutants (at 25 dpa) were removed from the boll and fluorescent dye was applied to the funiculus. **A**) Transverse section at the medial region of a seed shows fluorescent transversely cut vascular bundles (vb) in the outer integument. Palisade layer (pl) of the inner integument. **B**) Transverse section cut at the median level of the integumentary connective (ic). The fluorescent dye moves from the vascular bundles in the outer integument towards the inner integument through the opening in the palisade layer (pl) via the integumentary connective (ic).

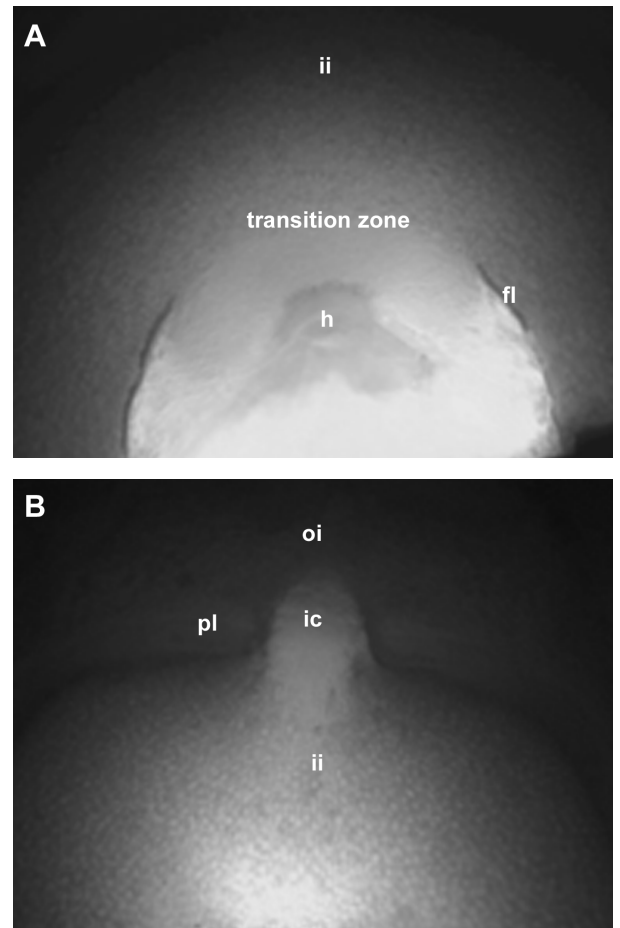


Figure 4. Tracing dye movement in the chalazal half of the seed using carboxyfluorescein (CF). The seed was cut in halves and the embryo removed. Dye was applied to the vacated cavity. **A, B**) Longitudinal sections of the chalazal half of the seed. **A**) The dye moved from the embryo sac via hypostase (h) and transition zone into the inner integument (ii) towards the outer integument (oi). **B**) Fluorescent dye has entered the integumentary connective (ic). The fringe layer (fl, in **A**) and the palisade layer (pl, in **B**) appear to be barriers.

Table 1. Anatomical analyses, hypotheses, and experimental evidence

Anatomical analyses:	Hypotheses:	Experimental evidence:
Epidermal layers surrounded the integuments (Fig. 1) at most parts of the seed.	Epidermal layers may provide a symplasmic barrier between the maternal tissues (oi, ii, nu).	Palisade layer (Figs. 6B-D, 7A) and fringe layer (Fig. 7B) were barriers to solute movements.
At the chalaza, parenchymatic connections (integumentary connective [ic], transition zone [tz]) were observed between the maternal tissues.	Integumentary connective and transition zone may control solute translocation and allocation.	Fluorescent dye moved via integumentary connective (Figs. 6C, 7A) and transition zone (Fig. 7B).
In the outer integument (oi), the outer part of the integumentary connective was observed inside the branching point of the vascular bundles diverging from the funiculus (Fig. 3A), and files of cells surrounded by air spaces connected multiple vascular bundles with the integumentary connective.	The position of the integumentary connective allows optimal access to imported nutrients and therefore may function as a conduit for nutrients. Files of cells may represent symplasmic pathways for nutrients between vascular tissue and integumentary connective.	Fluorescent dye moved through the integumentary connective into the inner integument (Fig. 6C).
Parenchyma cells in the center of the chalazal cap (cc, Fig. 1), between the integumentary connective (ic) and the tip of the hypostase (h) did not contain phenolic compounds (Fig. 2A).	Phenolic-free cells may provide the pathway between the integumentary connective and the hypostase (and nucellus).	Fluorescent dye primarily moved in the center of the chalazal cap (Fig. 7A).