A *phi* Layer in Roots of *Ceratonia siliqua* L.

E. Pratikakis, Sophia Rhizopoulou, and G. K. Psaras

Institute of General Botany, University of Athens, Athens 15784, Greece

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Abstract: The central cylinder of the primary root of the carob tree (*Ceratonia siliqua*) is encircled by a layer of cells with wall thickenings, known as a *phi* (Φ) cell layer. The development of the Φ layer and the chemical composition of the cell wall thickenings have been studied in roots of *C. siliqua*. The results reveal the presence of condensed tannins in the mature *phi* thickenings and that the development of the Φ layer is asynchronous: at 0–1 cm from the root tip Φ thickenings appear before endodermis differentiation at the sites opposite phloem, at 1–4 cm new Φ thickenings are developed at the sites opposite xylem, at 4–7 cm the Φ layer consists of two layers of cells and it completely encloses the central cylinder.

Key words: *Ceratonia siliqua*, *phi* layer, root anatomy, water flux.

Introduction

Phi (Φ) thickenings are formed on the walls of certain cell layers in the root cortex of several species of Gymnosperms and of a few species of Angiosperms (Van Tieghem, 1887; Esau, 1943 and references therein; Guttenberg, 1961, 1968). *Phi* thickened cells usually consist of a multicellular sheath under the rhizodermis. However, Φ thickenings forming a single layer of cells have been observed in the hypodermis of *Pyrus* seedlings (Riedhart and Guard, 1957), in deeper layers of the cortex and near the endodermis of *Pyrus* and *Pelargonium* (Esau, 1943; Haas et al., 1976; Mackenzie, 1979; Peterson et al., 1981; Weerdenburg and Peterson, 1983). Occasionally, the Φ layer has been erroneously referred to as a Φ-type endodermis (Van Fleet, 1961) and Φ thickenings as Casparian strips (references cited in Guttenberg, 1968).

It has been suggested that the Φ layer plays a mechanical supporting role to the primary root (Guttenberg, 1961, 1968; Haas et el., 1976; Weerdenburg and Peterson, 1983) which is in agreement with early reports (Russow, 1875; Van Tieghem, 1887). *Phi* bands, in the hypodermis and in deeper cortical cell layers, consist mainly of cellulose and lignin, but they lack suberin (Kroemer, 1903; Haas et al., 1976). Mackenzie (1979), studying both the endodermis and the adjacent Φ layer in apple root cortex, argued that the Φ layer may regulate the inflow of water and nutrients to the stele. It is likely that *phi* thickenings do not function as barriers to the transport of relatively small molecules *via* the apoplast to the stele (Peterson et al., 1981).

Ceratonia siliqua L. (Caesalpiniaceae) is an evergreen sclerophyll species widespread as a native plant in the Mediterranean Basin. *C. siliqua* (carob tree) is an economically important plant due to the value of the seeds and the pulp of carob beans (Ortiz et al., 1995), as well as a valuable resource for afforestation and soil conservation in semi-arid regions (Catarino, 1996). Deep penetration of tap roots of *C. siliqua* can supply a substantial amount of water to the shoots throughout the year and this might lengthen the growing season for leaves (Rhizopoulou and Davies, 1991).

We report here on the development, structure and chemical composition of the Φ layer in *Ceratonia siliqua* roots.

Materials and Methods

Plant material and growth conditions

Seeds of *C. siliqua*, collected from a tree stand near the campus of Athens University, were germinated in petri dishes on moist filter paper. After germination, seedlings were transferred into pots containing perlite. When the first leaves appeared, plants were transplanted into tubes (5.0 cm diameter, 50 cm length), slit along two sides, sealed with insulating tape and filled with soil collected from the site where the mother trees grow. The tubes were then transferred into a growth cabinet (GB 48, Conviron, Canada), equipped with 48 incandescent lamps (Sylvania, 50 A19, 50 W) and 28 fluorescent tubes (Sylvania Cool White, FR96 T12/CW/VHO-235/1). Programmed temperature and light simulated Mediterranean autumn conditions (for details see Thanos et al., 1991). Plants were watered to soil capacity, twice a week for a two-month period.

Sectioning, staining and microscopy

Root samples were collected from 3-month-old plants grown in the cabinet, as well as from three-year-old, well-watered plants grown outdoors in large pots. After a 3-month period, roots penetrating deep into the soil profile reach a depth of 50 cm. In order to facilitate root harvesting, the tape was removed from the tubes, while pots were destroyed.

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Table 1 Reaction of *C. siliqua* roots to histochemical tests.

Treatment / Distance from the root apex		phloroglucinol	toluidine blue O	nitroso reaction	$FeCl_3$
< 1 cm	Φ bands	+	+	–	–
< 1 cm	endodermis	–	–	–	–
< 1 cm	tracheary elements	+	+	–	–
1 – 4 cm	Φ bands	+	+	–	–
1 – 4 cm	endodermis	–	–	–	–
1 – 4 cm	tracheary elements	+	+	–	–
4 – 7 cm	Φ bands	–	–	+	+
4 – 7 cm	endodermis	–	–	–	–
4 – 7 cm	tracheary elements	+	+	–	–
> 10 cm	Φ bands	+	–	+	+
> 10 cm	endodermis	–	–	–	–
> 10 cm	tracheary elements	+	+	–	–

Free-hand sections were made and observed with a Zeiss Axioplan microscope equipped with an Osram, HBO 50 W mercury lamp, epifluorescence and polarized light optics; for UV optics a G 365 (as exciter filter), an FT 395 (as chromatic beam splitter) and an LP 420 (as barrier filter) were used. Colour transparencies were recorded on Ektachrome 64 ASA film.

Lignin was identified with the phloroglucinol–HCl reaction according to Jensen (1962); in a second test indicating the presence of lignin, buffered solution of toluidine blue O was used (O'Brien et al., 1964).

Suberin was detected by staining with fluorol yellow 088 (Sigma–Aldrich Chemie GmbH, Germany) according to Brundrett et al. (1991), and with fluorescent berberine (Brundrett et al., 1988).

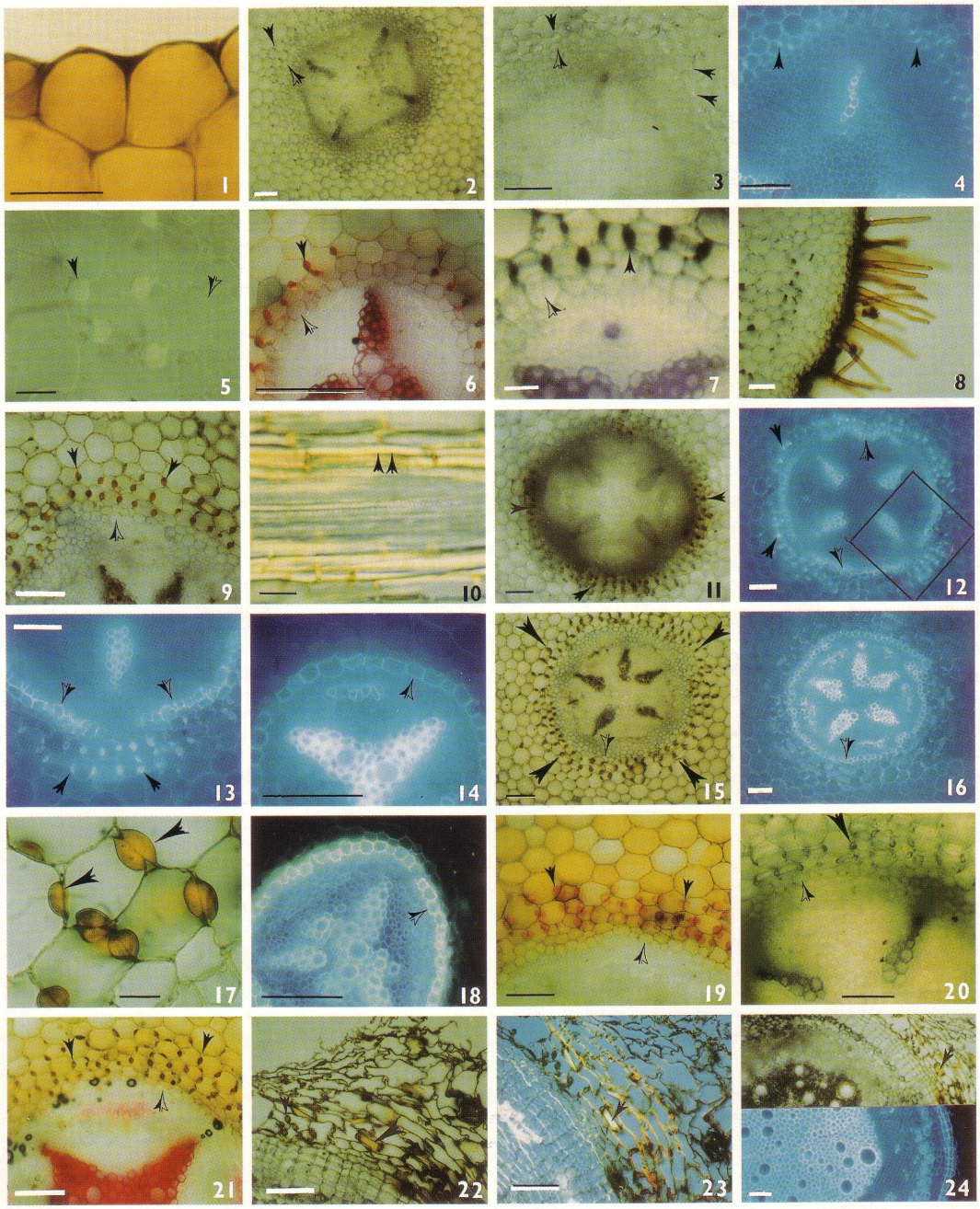
Two tests were conducted to detect the presence of condensed tannins: (i) condensed tannins were stained red, with the nitroso reaction (Reeve, 1951), and (ii) a precipitate indicated their presence, using the ferric sulfate reaction (Reeve, 1959).

Results obtained from plants grown in the growth cabinet were identical to those from plants grown in pots outdoors.

Results

Observation of unstained cross sections of the lower white zone of the root (0 – 1 cm from the tip) reveals that the root is

Figs. 1 – 24 Freehand sections of *C. siliqua* roots showing the development of the Φ layer and the histochemistry of the wall thickenings. Open arrowheads indicate endodermis; closed arrowheads indicate Φ thickenings. Magnification bars represent 10 μm, except Figs. **1**, **5**, **7**, **10** and **17**, where bars represent 50 μm.
Figs. 1 – 7 Cross sections of the root apex (0 – 1 cm). **Fig. 1** Unstained section of root epidermis; external walls are slightly thickened. **Fig. 2** Unstained section showing the vascular cylinder and the sites of the first appearance of Φ thickenings. **Fig. 3** Unstained section viewed under white light; xylem and endodermis are difficult to identify. **Fig. 4** The same section, as seen in Fig. **3**, viewed with UV light; xylem and Φ thickenings autofluoresce, while endodermis is difficult to identify. **Fig. 5** Unstained section showing colourless Φ thickenings. **Fig. 6** Φ thickenings and xylem elements stained red with phloroglucinol. **Fig. 7** Φ thickenings stained blue with toluidine blue O.
Figs. 8 – 14 Sections at 1 – 4 cm distance from the root tip. **Fig. 8** Unstained cross section showing the root hairs. **Fig. 9** Unstained section showing mature, brownish Φ thickenings. **Fig. 10** Longitudinal root section viewed with polarized light, Φ thickenings appear bright on the walls of the elongated cells of the Φ layer (arrowheads). **Fig. 11** Unstained section in white light; Φ thickenings located opposite phloem appear brown, whereas those opposite xylem appear colourless. **Fig. 12** the same section as in Fig. **11**, viewed with UV light. The brown Φ thickenings opposite phloem ceased to autofluoresce. At the same sites, endodermis possesses autofluorescent walls. **Fig. 13** The area indicated in Fig. **12** in higher magnification under UV light; Φ thickenings located opposite xylem autofluoresce. **Fig. 14** Section stained with berberine-aniline blue and viewed with UV light; endodermis can be discerned clearly.
Figs. 15 – 21 Cross sections taken 4 – 5 cm from the root tip. **Fig. 15** Unstained section in white light; both the endodermis and the Φ layer are complete. *Phi* thickenings are brown. **Fig. 16** In the same section as in Fig. **15** under UV light, endodermis autofluoresces, while Φ layer ceased to autofluoresce. **Fig. 17** The Φ bands are well developed, they look brownish and occasionally additional half *phi* thickenings (*ϕ*) appear. **Fig. 18** Mature endodermis; a berberine and aniline blue-stained section viewed under UV light. **Fig. 19** *Phi* thickenings appear red in a cross section stained by the nitroso-reaction for identification of condensed tannins. **Fig. 20** Φ thickenings appear blue in a cross section stained with ferric sulfate. **Fig. 21** Cross section stained with phloroglucinol-HCl; in differentiated primary roots Φ bands do react positively.
Figs. 22 – 24 Cross sections through roots in secondary growth. **Fig. 22** *Phi* layer is shed with the primary cortex. **Fig. 23** The same section as Fig. **22** viewed under polarized light; Φ thickenings look bright and have maintained their structure. **Fig. 24** The two halves presented here have been obtained from the same section; the upper part shows the section under white light and the lower part under UV light. Cork cells possess autofluorescent walls.



Figs. 1–24

covered by a uniseriate epidermis with slightly thickened external walls (Fig. **1**). The central cylinder is surrounded by young endodermis cells with thin walls (Fig. **2**). As seen under high magnification, Φ thickenings can be observed in one or two layers of the thin-walled cortical cells, outside the endodermis and only opposite the phloem (Fig. **3**). *Phi* bands, as seen under UV, show autofluorescence that indicates the presence of phenol-containing polymers, as opposed to endodermal cell walls (Fig. **4**). Striking features of this stage are the colourless Φ thickenings (Fig. **5**) and a Φ layer interrupted opposite the xylem poles (Figs. **3**, **4**, **6**). Tests for lignin were positive (Figs. **6**, **7** respectively; Table **1**), while tests for condensed tannins (Table **1**) and suberin were negative for both the Φ thickenings and the endodermis.

The study of cross sections 1 – 4 cm from the root tip, reveals that the external walls of the epidermal cells are brown, while the other root tissues are colourless (Fig. **8**). At this stage of development, the xylem elements are well developed, and a complete cylinder of Φ thickened cells has been formed (Fig. **9**). Thickenings resemble Φ bands and they are localized on the radial and transverse walls of one to two cell layers of the cortex near the endodermis (Fig. **9**). The shape of the Φ cells is cylindrical; their long axes are two to three fold their diameters.

Presumably, Φ thickenings consist of cellulose microfibrils, as seen under polarized light (Fig. **10**). The endodermal cell layer opposite the phloem, colourless in white light (Fig. **11**), shows autofluorescence under UV (Fig. **12**); this indicates the presence of suberin lamellae, with the exception of the areas opposite the xylem. The colourless Φ thickenings opposite the xylem autofluoresce, whereas the brownish Φ bands have ceased to autofluoresce (Figs. **12**, **13**). The phloroglucinol and toluidine blue tests were positive for Φ bands (Table **1**). The berberine reaction was positive only for the endodermis (Fig. **14**), indicating the presence of phenol-containing polymers.

Observation of cross sections 4 – 7 cm from the root tip, under white light, reveals that the Φ layer consists of two layers of cells and completely encloses the central cylinder (Fig. **15**); Φ bands appear brownish. As seen under UV light, Φ bands ceased to autofluoresce whereas the cell walls of the endodermis show bright autofluorescence (Fig. **16**). Occasionally, larger cells of the Φ layer, forming additional thickenings that resemble half a Φ (i.e. φ), are found on the common wall with neighbouring cells (Fig. **17**). The berberine fluorescent staining was positive for endodermis cell walls and negative for the Φ bands (Fig. **18**). The nitroso reaction and the reaction to ferric sulfate gave positive results, for Φ thickenings (Figs. **19**, **20**), indicating the presence of condensed tannins. The phloroglucinol test gave reddish to brown colour and seems to be positive for lignin in Φ thickenings (Fig. **21**).

The Φ layer is shed with the rest of the primary cortex during the secondary growth (> 10 cm distance from the tip) of the root (Fig. **22**). Until then, Φ thickenings, as seen under white and polarized light, maintain their structure (Fig. **23**). Cell walls of the periderm, rich in suberin, show bright blue autofluorescence (Fig. **24**).

Discussion

A layer with Φ bands is developed in the hypodermal cell layer of the roots of some mesophytes and xerophytes (Guttenberg, 1968), though it may appear in deeper cell layers of the root cortex (Haas et al., 1976). In the case of *C. siliqua* roots, a sheath of cells with Φ bands is developed externally, adjacent to the endodermis, as in the apple tree. It should be noted that the Φ layer is developed in *C. siliqua* plants growing in soil. In a previous work, carob seedlings were grown in perlite saturated with Hoagland solution and Φ bands were not observed (Christodoulakis and Psaras, 1987). This may be attributed to a relationship between the supporting role of wall thickenings and the mechanical impedance of the soil.

It is clear from our results that the Φ layer differs from the endodermis, in agreement with Haas et al. (1976) and Weerdenburg and Peterson (1983). In dicotyledons the development of the endodermis is completed in three stages (Guttenberg, 1968; Sanderson, 1983 and literature therein; Watt et al., 1996). A Casparian strip is formed during the first stage; suberin is deposited on the whole cell wall during the second stage, and only during the third stage is the cell wall thickened. In *C. siliqua* roots suberin is deposited on the walls of the endodermis cells very early, whereas tertiary development of the endodermis was not observed. The very rapid deposition of suberin on the wall of the endodermis cells is to be expected, since *C. siliqua* is a slow-growing woody species and the sequence of developmental stages along the root would be compressed (Wilcox, 1962; Watt et al., 1996).

In root apices Φ thickenings first appear in the cortex cells opposite the phloem, then at the sites opposite the xylem. The same pattern is followed by the endodermis, although its differentiation takes place later. Wilcox (1962) noticed that the Φ layer is developed considerably in advance of the differentiation of the endodermis. In *C. siliqua*, Φ bands completed their development although the endodermis still lacked suberin lamellae. In this part of the root, a Φ layer interrupted at the sides opposite the xylem will facilitate water uptake. In root apices of *C. siliqua*, high water potentials (– 0.2 MPa, approximately) and local depletion rates sustain water flux to the leaves (Rhizopoulou and Davies, 1991), whereas the upper parts of the roots of *C. siliqua* develop a periderm with two to three layers of suberized cells (Fig. **24**). The amount of water absorbed through such regions tends to be negligible (Tinker, 1976; Drew, 1987). Cells near xylem must transport water necessary not only for their growth, but also for the growth of all cells beyond them up to the transpiring leaves (Cosgrove, 1986; Passioura, 1988). It is likely that a Φ layer does not act as a barrier to water and/or solute movement from the cortex towards the xylem. On the contrary, the lignification of the Φ bands opposite the phloem could retard centrifugal water leakage from the stele to the cortex. Also, the Φ sheath may be the site of resistance to diurnal shrinkage of a root growing in drying soil (Passioura, 1988), that will reduce the roots' abilitiy to extract water from the soil (less contact between the root cylinder and the soil). In *C. siliqua*, at 1 – 4 cm from the root tip, the Φ bands opposite the xylem are lignified, but those opposite the phloem contain condensed tannins. At the same time, the endodermis cell walls opposite the xylem lack suberin lamellae. A late deposition of suberin lamellae in the endodermal cells opposite xylem has been observed by Wilcox (1962) in cedar.

Phi bands in *C. siliqua* are composed of cellulose and lignins, in agreement with earlier reports (Guttenberg, 1961, 1968; Haas et al., 1976; Kroemer, 1903; Mackenzie, 1979; Weerdenburg and Peterson, 1983). Furthermore, in *C. siliqua* we found condensed tannins in the Φ thickenings of the mature sheath. Thus, the unknown material of the Φ thickenings mentioned by Haas et al. (1976), seems to contain condensed tannins. The cessation of lignin autofluorescence in Φ bands in mature Φ cells might be attributed either to changes of the heterogeneous lignin mixture or, more likely, to the presence of condensed tannins. On the walls of cortex cells outside the vascular tissues of the tap root of *C. siliqua*, lignins and condensed tannins are present. This structure is maintained next to the endodermis until the end of primary root development. The deposition of condensed tannins in the walls of root cells external to the stele of *C. siliqua*, as well as in the endodermis of *Quercus coccifera* (Christodoulakis and Psaras, 1988), would improve the resistance of the root to water flow and could be protective for the stele, as argued by Mckenzie and Peterson (1995 a, b).

It is assumed, based on our results, that the role of the Φ sheath is unique. During early root development the first lignified Φ thickenings may retard apoplastic movement opposite the phloem. This might result in a more sharp radial gradient in water potentials required to sustain water flux and growth of root apices during a prolonged drought period, as often experienced by *C. siliqua*. *Phi* thickenings may also impart rigidity to the walls, forming a supporting network in the primary root (Guttenberg, 1968; Haas et al., 1976; Weerdenburg and Peterson, 1983). Permeability tests for Φ cell layers in *C. siliqua* are required to fully understand the physiological significance of the Φ sheath.

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Dr. S. Rhizopoulou

Institute of General Botany
University of Athens
Panepistimiopolis
Athens, 15784
Greece
tel.: 00301 7284513, 7284659
fax: 00301723 4136
E-mail: srhizop@biology.db.uoa.gr

Section Editor: M. Melkonian