

# Chapter 2

## Role of *Phi* Cells Under Abiotic Stress in Plants

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### 2.1 Introduction

#### 2.1.1 *Phi* Thickening Definition, Description and Discovery

The definition of *phi* thickening is not always present in the most consulted texts on plant anatomy. In many, the definition is not complete or is poorly defined. The last edition of the famous book on plant anatomy ‘Esau’s Plant Anatomy 3rd edition’ defined *phi* thickening as ‘*Phi* thickenings are reticulate or band-like wall thickenings on cortical cells of certain gymnosperms (Ginkgoaceae, Araucariaceae, Taxaceae, and Cupressaceae (Gerrath et al. 2002)) and a few species of angiosperms such as *Ceratonia siliqua*, *Pyrus malus* (*Malus domestica*), and *Pelargonium hortorum* (Peterson et al. 1981)’. The reading of this definition suggests that *phi* thickening is an exception in the root anatomy. However, we have found in the literature 16 different families, covering more than 100 species, which present the *phi* thickening in the roots. All species described presenting *phi* thickenings are dicotyledons. However, there is one exception, the presence of *phi* thickenings in the rhizodermis of *Zea mays* (a monocotyledon) treated with a solid-waste slag. In Table 2.1, we show the different families and species that present *phi* thickenings in the roots. Some of these species are also very relevant for agriculture, e.g. *Prunus avium*, *Prunus dulcis*, *M. domestica*, *Pyrus communis*, *Brassica oleracea*, *Brassica napus*, *Raphanus sativus*, *Sinapis alba*, *Alnus glutinosa*, etc.

The *phi* thickening was first described by Van Tieghem in 1871 in roots of *Taxus baccata* (see Fig. 2.1). Van Tieghem called this cell wall thickening ‘*reseau sus-endodermique*’. However, the name ‘*phi* thickening’ was first proposed by Russow in 1875, who commented that the transverse sections of the thick walls

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**Table 2.1** List of families and species showing *phi* thickenings in the roots

| Gymnosperms                                                                                                                                                            | Angiosperms                                                                                                                                                                                    | Angiosperms                                                                                                                                                                            |                      |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| <b>Cycadaceae</b>                                                                                                                                                      | <b>Rosaceae</b>                                                                                                                                                                                | <i>Koniga</i>                                                                                                                                                                          | } Van Tieghem (1888) |
| <i>Cycas circinalis</i> (Van Tieghem 1888)                                                                                                                             | <i>Prunus avium</i> L. (Soukup et al. 2004)                                                                                                                                                    | <i>Farsetia</i>                                                                                                                                                                        |                      |
| Fossil: <i>Antarcticycas</i> (Millay et al. 1987); <i>Radiculites</i> type (Strullu-Derrien et al. 2009)                                                               | <i>Prunus dulcis</i> × <i>P. persica</i> (Marin et al. 2009)                                                                                                                                   | <i>Berberoa</i>                                                                                                                                                                        |                      |
| <b>Ginkgoaceae</b>                                                                                                                                                     | <i>Eriobotrya japonica</i> Lindl. (Nii et al. 2004; Pan et al. 2006)                                                                                                                           | <i>Vesicaria</i>                                                                                                                                                                       |                      |
| <i>Ginkgo biloba</i> (Gerrath et al. 2002)                                                                                                                             | <i>Malus domestica</i> (Mackenzie 1979; Weerdenburg and Peterson 1983)                                                                                                                         | <i>Hirschfeldia</i>                                                                                                                                                                    |                      |
| <b>Taxaceae</b>                                                                                                                                                        | <i>Pyrus communis</i> (Essau 1943)                                                                                                                                                             | <i>Thlaspi</i>                                                                                                                                                                         |                      |
| <i>Taxus cuspidata</i> (Gerrath et al. 2002), <i>T. baccata</i> (Van Tieghem 1871; Von Guttenberg 1961)                                                                | <i>Dryas integrifolia</i> Vahl. (Melville et al. 1987)                                                                                                                                         | <i>Clypeola</i>                                                                                                                                                                        |                      |
| <i>Torreya nucifera</i> (Van Tieghem 1888)                                                                                                                             | <b>Fabaceae</b>                                                                                                                                                                                | <i>Isatis</i>                                                                                                                                                                          |                      |
| <b>Cupressaceae</b>                                                                                                                                                    | <i>Cerantonia siliqua</i> L. (Pratikakis et al. 1998; Rhizopoulou 2004)                                                                                                                        | <i>Crambe</i>                                                                                                                                                                          |                      |
| <i>Callitris quadrivalvis</i> (Noelle 1910)                                                                                                                            | <i>Arachis hypogaea</i> (Tajima et al. 2008)                                                                                                                                                   | <i>Enarthrocarpus</i>                                                                                                                                                                  |                      |
| <i>Chamaecyparis lawsoniana</i> , <i>C. obtuse</i> , <i>C. pisifera</i> , <i>C. sphaeroidea</i> (Noelle 1910)                                                          | <i>Caesalpinia peltophoroides</i> (Henrique et al. 2010)                                                                                                                                       | <i>Erucaria</i>                                                                                                                                                                        |                      |
| <i>Cryptomeria japonica</i> (Gerrath et al. 2002; Noelle 1910)                                                                                                         | <i>Hedysarum pedicellare</i> (Van Tieghem 1888)                                                                                                                                                | <b>Adoxaceae</b>                                                                                                                                                                       |                      |
| <i>Cunninghamia lanceolata</i> (Gerrath et al. 2002), <i>C. sinensis</i> (Noelle 1910)                                                                                 | <b>Geraniaceae</b>                                                                                                                                                                             | <i>Viburnum</i> sp. (Schwendener 1874)                                                                                                                                                 |                      |
| <i>Cupressus macrocarpa</i> , <i>C. sempervirens</i> (Noelle 1910)                                                                                                     | <i>Pelargonium hortorum</i> Bailey (Haas et al. 1976; Peterson et al. 1981)                                                                                                                    | <b>Sapindaceae</b>                                                                                                                                                                     |                      |
| <i>Juniperus virginiana</i> (Gerrath et al. 2002), <i>J. communis</i> , <i>J. chinensis</i> , <i>J. excelsa</i> , <i>J. nana</i> , <i>J. sabina</i> (Noelle 1910)      | <i>Geranium</i> sp. (Van Tieghem 1888)                                                                                                                                                         | <i>Sapindus saponaria</i> (Van Tieghem 1888)                                                                                                                                           |                      |
| <i>Libocedrus decurrens</i> (Noelle 1910; Wilcox 1962)                                                                                                                 | <b>Brassicaceae</b>                                                                                                                                                                            | <i>Nephelium longana</i> (Van Tieghem 1888)                                                                                                                                            |                      |
| <i>Sciadopitys verticillata</i> (Noelle 1910)                                                                                                                          | <i>Thlaspi caerulescens</i> (Broadley et al. 2007; Zelko et al. 2008)                                                                                                                          | <i>Talisia</i> sp. (Van Tieghem 1888)                                                                                                                                                  |                      |
| <i>Sequoiadendron gigantea</i> (Gerrath et al. 2002), <i>S. sempervirens</i> (Noelle 1910; Von Guttenberg 1961)                                                        | <i>Brassica oleracea</i> (Fernandez-García et al. 2009)                                                                                                                                        | <b>Berberidaceae</b>                                                                                                                                                                   |                      |
| <i>Taxodium distichum</i> (Noelle 1910)                                                                                                                                | <i>B. napus</i> (Enstone et al. 2003), <i>Brassica carinata</i> , <i>B. nigra</i> (Van Tieghem 1888)                                                                                           | <i>Mahonia aquifolium</i> (Van Tieghem 1888)                                                                                                                                           |                      |
| <i>Thuja occidentalis</i> (Gerrath et al. 2002; Van Tieghem 1888; Brundett et al. 1990), <i>T. orientalis</i> , <i>T. plicata</i> , <i>T. standishii</i> (Noelle 1910) | <i>Sinapis alba</i> , <i>S. allionii</i> , <i>S. hispida</i> , <i>S. dissecta</i> , <i>S. pubescens</i> , <i>S. abyssinica</i> , <i>S. laevigata</i> , <i>S. geniculata</i> (Van Tieghem 1888) | <b>Betulaceae</b>                                                                                                                                                                      |                      |
|                                                                                                                                                                        | <i>Cheiranthus cheiri</i> (Van Tieghem 1888)                                                                                                                                                   | <i>Alnus glutinosa</i> (Massicotte et al. 1999)                                                                                                                                        |                      |
|                                                                                                                                                                        | <i>Lepidium sativum</i> (Van                                                                                                                                                                   | <i>Betula alleghaniensis</i> (Massicotte et al. 1988)                                                                                                                                  |                      |
|                                                                                                                                                                        |                                                                                                                                                                                                | <b>Fagaceae</b>                                                                                                                                                                        |                      |
|                                                                                                                                                                        |                                                                                                                                                                                                | <i>Quercus coccifera</i> L. (Christodoulakis and Psaras 1988)                                                                                                                          |                      |
|                                                                                                                                                                        |                                                                                                                                                                                                | <b>Orchidaceae</b>                                                                                                                                                                     |                      |
|                                                                                                                                                                        |                                                                                                                                                                                                | <i>Prosthechea alagoensis</i> , <i>P. bulbosa</i> , <i>P. caetensis</i> , <i>P. faresiana</i> , <i>P. fragrans</i> , <i>P. vespa</i> (Pires et al. 2003)                               |                      |
|                                                                                                                                                                        |                                                                                                                                                                                                | <i>Encyclia alboxanthina</i> , <i>E. amicta</i> , <i>E. dichroma</i> , <i>E. linearifolioides</i> , <i>E. longifolia</i> , <i>E. megalantha</i> , <i>E. randii</i> (Pires et al. 2003) |                      |
|                                                                                                                                                                        |                                                                                                                                                                                                | <i>Epidendrum crassifolium</i> (Pires et al. 2003)                                                                                                                                     |                      |
|                                                                                                                                                                        |                                                                                                                                                                                                | <i>Oncidium varicosum</i> (Pires et al. 2003)                                                                                                                                          |                      |
|                                                                                                                                                                        |                                                                                                                                                                                                | <b>Scrophulariaceae</b>                                                                                                                                                                |                      |
|                                                                                                                                                                        |                                                                                                                                                                                                | <i>Bacopa salzmännii</i> ,                                                                                                                                                             |                      |

(continued)

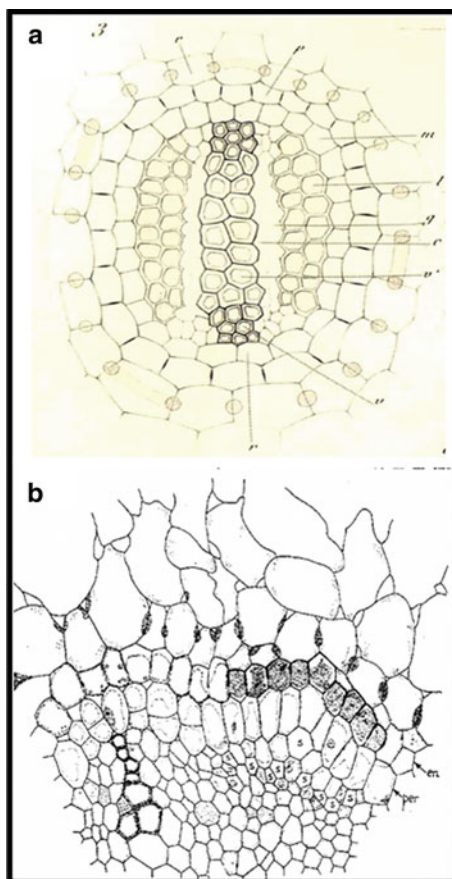
**Table 2.1** (continued)

| Gymnosperms                                                                                                                                                                  | Angiosperms                                                                                                  | Angiosperms                                      |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|--------------------------------------------------|
| <i>Thujaops dolabrata</i> (Noelle 1910)                                                                                                                                      | Tieghem 1888)                                                                                                | <i>B. monnierioides</i> (Bona and Morretes 2003) |
| <b><i>Araucariaceae</i></b>                                                                                                                                                  | <i>Alliaria officinalis</i> (Van Tieghem 1888)                                                               |                                                  |
| <i>Araucaria heterophylla</i> (Gerrath et al. 2002; Van Tieghem 1888), <i>A. brasiliana</i> , <i>A. excelsa</i> , <i>A. imbricata</i> , <i>A. cunninghamii</i> (Noelle 1910) | <i>Raphanus sativum</i> , <i>R. landra</i> (Van Tieghem 1888)                                                |                                                  |
|                                                                                                                                                                              | <i>Malcolmia intermedia</i> , <i>M. Africana</i> , <i>M. chia</i> (Van Tieghem 1888)                         |                                                  |
|                                                                                                                                                                              | <i>Sisymbrium hirsutum</i> , <i>S. bursifolium</i> , <i>S. binerve</i> , <i>S. sophia</i> (Van Tieghem 1888) |                                                  |
|                                                                                                                                                                              | <i>Cochlearia armoracia</i> (Van Tieghem 1888)                                                               |                                                  |
|                                                                                                                                                                              | <i>Alyssum saxatile</i> (Van Tieghem 1888)                                                                   |                                                  |

resembled the Greek letter *phi*. Van Tieghem (1888) classified them into three types based on their root cell location: type I, the most frequently found, *phi* cell layer is located in contact with the endodermis. *Phi* thickening could be observed in one or two cell layers, depending on the species and/or location in the root. Type II *phi* cell layer is located in contact with the epidermis and is frequently formed by only one cell layer. Type III *phi* cell layers are located in the inner cortical cells, but not in contact with either the epidermis or the endodermis; they can be composed of one or more *phi* cell layers. In general, we consider that this classification is up to date and can be used to describe the different locations of *phi* thickening in the root.

What is the origin of the cell wall thickenings? The occurrence of cell wall thickening in the radial cell walls of the cortical cells has been observed in fossil plants from the upper Triassic, Paleozoic and upper carboniferous periods (Millay et al. 1987; Strullu-Derrien et al. 2009; Cesari et al. 2012). Strullu-Derrien et al. (2009) observed in rootlets of the Radiculites type in Radiculites (upper carboniferous flora; samples were obtained from Grand Croix, France) the undoubted presence of *phi* thickenings. Similarly, Millay et al. (1987) have described the presence of *phi* thickenings in roots of the fossil family Antarticycas. These fossils show that in young roots a single cell layer with *phi* thickenings is present in both the tangential and the radial walls and in direct contact with the endodermis. Mature primary roots show well-developed *phi* thickenings that may occupy about 25 % of the walls. These interesting findings in fossil plants demonstrate that *phi* thickening was present in the ancestors of the present flora.

**Fig. 2.1** (a) Original picture of root section of *Taxus baccata* showing the first description of *phi* thickening [Memoire sur la racine, Van Tieghem (1871)]. (b) Picture of transverse section of *Pyrus communis* root showing *phi* thickening [Vascular differentiation in the pear root, Essau (1943)]



## 2.2 *Phi* Cell Ultrastructure and Chemical Composition of *Phi* Thickening

The ultrastructure of the *phi* cells has been poorly described in the literature. To our knowledge, the first ultrastructural description of *phi* thickening was published by Haas et al. (1976) in *Pelargonium hortorum*. The *phi* cells presented abundant microtubules parallel to the long axis of the *phi* thickenings. In some cases, the authors observed that *phi* thickenings of adjacent cells are occasionally misaligned to some extent. In this work, the authors observed that plasmodesmata were absent in the thicker region of the *phi* thickenings but were observed in their periphery. The histochemical analysis demonstrated that the *phi* thickenings were intensively lignified in matured cells.

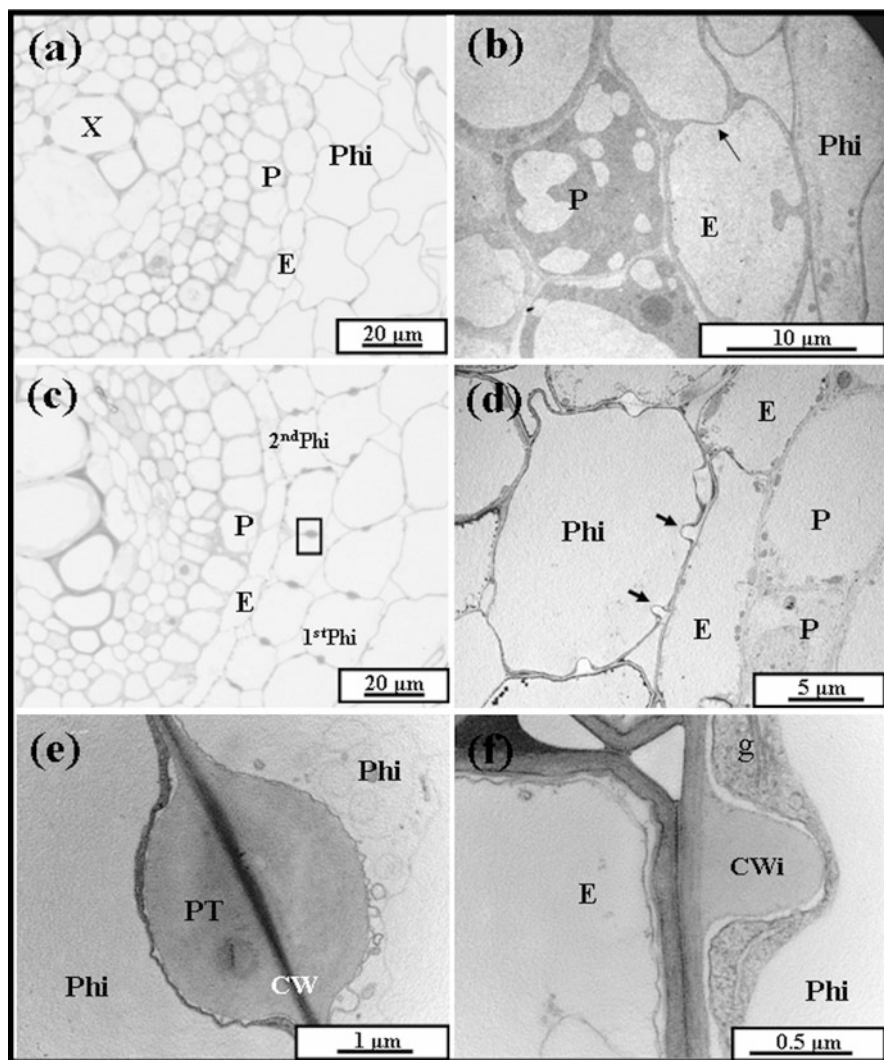
Recently, Fernandez-Garcia et al. (2009) have described the ultrastructure of *phi* cells in *Brassica oleracea*. In *Brassica oleracea*, the *phi* thickening is type I and can be composed of one or, less frequently, two cell layers (see Fig. 2.2). The *phi*

thickenings are present in the radial cell walls, and between two adjacent cells, they are frequently aligned, but in some cases, similarly to *Pelargonium*, they can also be misaligned. The ultrastructural study of the radial walls of the immature *phi* cells showed that these cell walls were rich in plasmodesmata connecting two neighbouring immature *phi* cells. Mature cells presented well-developed *phi* thickenings, and in many cases they were traversed by plasmodesmata. In some cases, the middle portion of the thickening seemed to be blocked, although it is highly probable that the plasmodesmata passed out of the plane of sectioning, as has been described in the literature. The *phi* cells of *Brassica oleracea* showed several cell wall ingrowths present only in the inner tangential cell walls (see Fig. 2.2). These cell wall ingrowths were never observed in the outer tangential cell walls of *phi* cells. In general, the cell wall ingrowths in the inner tangential cell walls were smaller in size compared with the *phi* thickenings (about 0.5  $\mu\text{m}$  at the base and 0.5–1.0  $\mu\text{m}$  long). The physiological role of these cell wall ingrowths is unknown. Fernandez-Garcia et al. (2009) have proposed on the basis of the differential distribution of the cell wall ingrowths, observed only in the inner tangential cell walls, that *phi* cells might present a possible polarity. Moreover, the increased number of the cell wall ingrowths supposes a significant increment of the plasmalemma in the inner cell wall of the *phi* cells. This may indicate different roles for the two cell walls. The authors suggest that, across the plasmalemma of the outer tangential cell walls, the *phi* cells can probably take up cations and anions actively for accumulation in the vacuoles, whilst the inner tangential wall can regulate the excretion of cations and anions to the apoplast between the *phi* cells and the endodermis.

*Phi* thickenings are structures of the secondary cell walls and could be lignified. Therefore, cell walls of *phi* cells may contain lignin and/or suberin. The chemical composition of *phi* thickening has been analysed using specific dyes for cell wall components, such as phloroglucinol for lignin or Sudan III for suberins. Some authors have observed that *phi* thickenings have abundant content of lignins (Haas et al. 1976; Soukup et al. 2004) and can be significantly lignified after environmental stresses (Fernandez-Garcia et al. 2009; Henrique et al. 2010). Suberin is typically found in the endodermal cells, but has not been found in the *phi* thickenings.

### 2.3 The *Phi* Thickening Is Altered by the Environment

Since its discovery, the function of *phi* thickening has been the subject of much speculation and experimentation, but it remains unclear. Van Tieghem (1888) proposed that the *phi* thickening can provide a mechanical support for the root. Although Weerdenburg and Peterson (1983) assumed that *phi* thickenings function primarily as supportive structures in the root cortex, they did not specify what force this mechanical support might oppose. Mackenzie (1979) suggested that they may act in a manner similar to a Casparian band.



**Fig. 2.2** (a) Semi-thin section of a control root of *Brassica oleracea*; *phi* cells are present in the innermost layer of the cortex (*Phi*). (b) Electron micrograph of a control root showing the endodermis and *phi* cell. (c) Semi-thin section of salt-treated root of *Brassica oleracea*; the *phi* cells are the two cortical cell layers adjacent to the endodermis. *Phi* thickenings are present in two cell layers in the radial cell walls. (d) Electron micrograph of salt-treated root showing the endodermis and *phi* cell (arrows indicate the cell wall ingrowths). (e) Electron micrograph of (d) showing a detail of *phi* thickening. (f) Electron micrograph of a tangential wall between a *phi* cell and endodermal cell showing a cell wall ingrowth. CW cell wall, CWi cell wall ingrowth, E endodermis, P pericycle cell, Phi *phi* cell, PT *phi* thickening. These images are a selection of Figs. 1, 2 and 6 from Fernández-García et al. (2009)

In the last decade, new studies have demonstrated that *phi* thickening can be altered by the environmental conditions. Different abiotic and biotic stresses may be modifying the distribution, structure and/or development of the *phi* thickening.

### 2.3.1 Solute Movement: Salt Stress

The solute movement in the roots can be via apoplast or symplast and is affected by the anatomy and the developmental stage of the roots (Enstone et al. 2003). The apoplast movement of solutes has been the most studied mechanism in roots. Therefore, one of the first interesting questions about the role of *phi* thickening was whether it was an apoplastic barrier for solute movement. Wilcox (1962) had shown that, in *Libocedrus decurrens*, the *phi* cell layer is not suberised, considering it unlikely that it would affect cell permeability. Peterson et al. (1981) have analysed the solute movement in the roots of *Pyrus* and *Pelargonium* using a brightener dye, Tinopal CBS-X. In these experiments, they showed that the apoplastic fluorescent dye was able to cross the *phi* thickenings but not the endodermis. But this experiment cannot exclude the possibility that *phi* thickenings could be reducing the rate of water and solute transport through the cell walls of the *phi* cell layers.

When *Brassica oleracea* is grown hydroponically, the *phi* cell layer is poorly developed, and *phi* thickenings are difficult to observe. However, when the plants were grown in NaCl, the *phi* thickenings were induced and lignified (Fernandez-Garcia et al. 2009; see Fig. 2.2). Interestingly, the authors demonstrated that the *phi* thickening may be acting at least as a partial barrier for ion movement from the cortex to the pericycle when *Brassica oleracea* plants were salt stressed. For this study, the authors followed a transmission electron microscopy technique and lanthanum ions that are known to be excellent apoplastic markers, and they have been used successfully to mark the pathway of ion movement across the apoplast of roots and leaves. In *Brassica oleracea* roots,  $\text{La}^{3+}$  can move freely in the apoplast of cortical cells, but in *phi* cells the  $\text{La}^{3+}$  movement was restricted to the periplasmatic apoplast of the *phi* radial cell wall thickenings. The authors suggested that the lignification of the *phi* thickening could prevent the movement of  $\text{La}^{3+}$  through the *phi* thickening. The greater deposition of  $\text{La}^{3+}$  in the apoplast between *phi* cells and endodermis of control roots, compared with salt-treated roots, could be due to the reduced presence of the *phi* thickenings in control roots. However, the deposition of  $\text{La}^{3+}$  in the apoplast between the *phi* cells and the endodermis of salt-treated roots indicates that the *phi* thickening is not a complete diffusion barrier for ions.

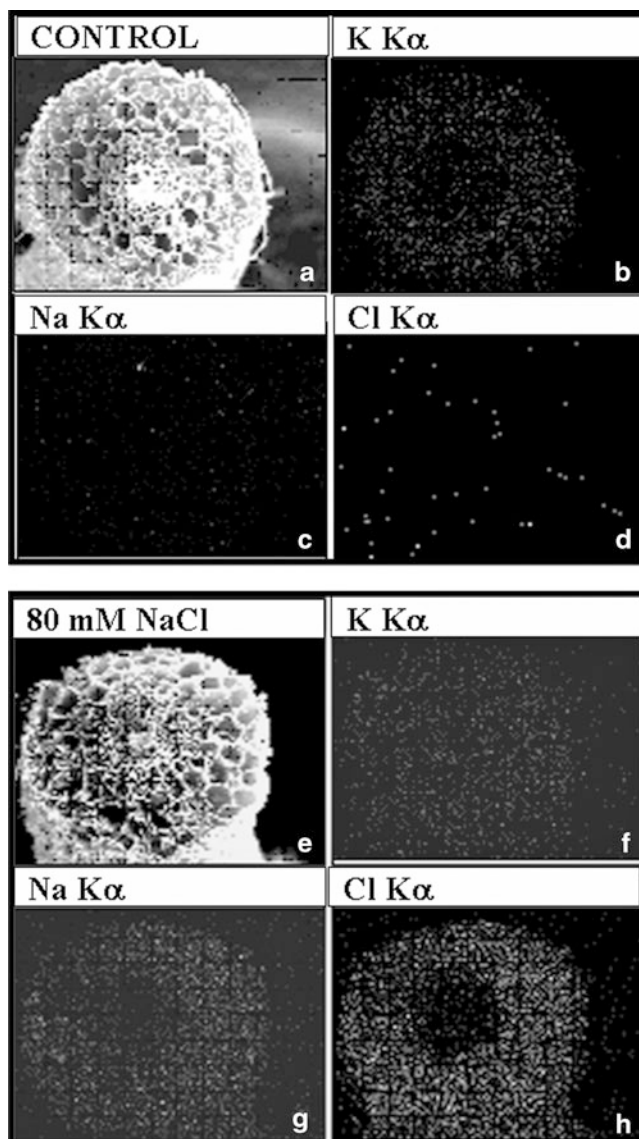
The symplastic movement of solutes is mediated by plasmodesmata between the different cell layers of the roots. The presence and distribution of plasmodesmata are critical for solute and ion movements via symplastic flux. Mackenzie (1979) observed a higher number of plasmodesmata in the outer tangential cell walls of the *phi* cells than between the *phi* layer and endodermis. He interpreted this as meaning that the *phi* cells can play a physiological role, in the accumulation of nutrients.

Fernandez-Garcia et al. (2009) have analysed the distribution and frequency of plasmodesmata in control and salt-treated plants of *Brassica oleracea*. The authors observed that plasmodesmata are scarce in the outer tangential walls of the *phi* cells or between the *phi* cell layer and endodermis. However, radial cell walls of *phi* cells showed several plasmodesmata connecting the two adjacent *phi* cells in control plants. But, when *phi* thickenings were observed in salt-treated plants, the majority of the plasmodesmata from the radial cell walls were occluded by the new cell wall formed. The authors suggested that the lack of plasmodesmata in the tangential cell walls of the *phi* cells clearly indicates that the symplastic flow via plasmodesmata is abolished. However, the radial flow between *phi* cells is possible in control plants but should be highly reduced in salt-treated plants due to the very low number of functional plasmodesmata observed. If *phi* cell layers are acting as a physiological barrier for ion movement, it can be expected retention of ions in these cell layers. Using X-ray microanalysis coupled to a scanning electron microscope, the authors demonstrated that the *phi* cell layer was a barrier when plants were grown with NaCl; the *phi* cell layer showed a significative build-up of  $\text{Na}^+$  and  $\text{Cl}^-$  (Fernandez-Garcia et al. 2009, see Fig. 2.3).

### 2.3.2 Water Logging and Soil Compaction

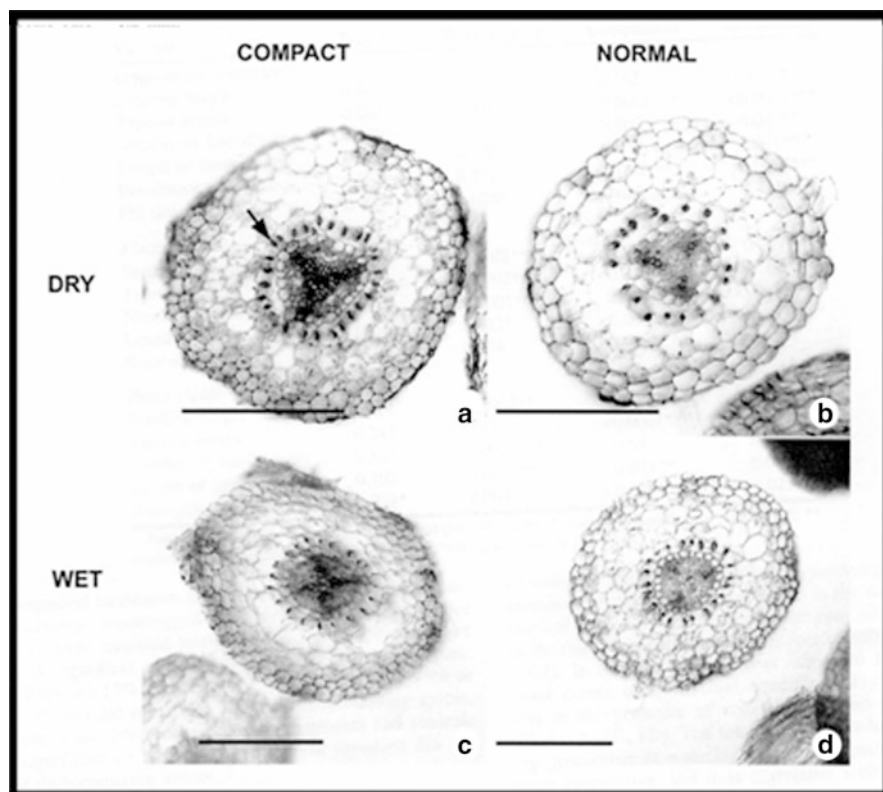
Gerrath et al. (2005) have developed different experiments to demonstrate whether *phi* thickenings can be altered and/or induced by the environment. For these experiments, they used three different gymnosperms, *Cryptomeria japonica*, which presents *phi* thickenings, and two *Pinus* species, *P. aristata* Engel. and *P. rigida* Mill. whose *phi* thickenings were absents. Plants were subjected to water logging and soil compaction to observe the effect of the environment in the roots. The first conclusion of these experiments was that the presence or absence of *phi* thickening cannot be induced by the environment. The two *Pinus* species never developed *phi* thickenings in the different ambient conditions. For *C. japonica*, the area of *phi* thickenings relative to the total root area was reduced under waterlogged and compacted soil (see Fig. 2.4). However, there was no significant interaction between the effects of water logging and soil compaction, and they were independent of the maturity of the root. These authors suggest that the reduction of *phi* thickening density in waterlogged and compacted soil might correlate with a lower plant growth and a general reduction in plant vigour. The authors have also analysed the distribution of *phi* thickening and the phylogenetic tree for the gymnosperm families. Interestingly, they propose that the *phi* thickening presence appeared early on and/or repeatedly in gymnosperm evolution. They observed that the capacity to develop *phi* thickening disappeared twice: once in the Gnetales-Pinaceae clade and once in the Podocarpaceae.

Sibipiruna (*Caesalpinia peltophoroides* Benth.) is a tree of the Fabaceae family which presents a *phi* thickening of type I (Henrique et al. 2010). This plant is from Brazil and is typically used for gardening. Henrique et al. (2010) have observed that



**Fig. 2.3** Images of Na<sup>+</sup>, Cl<sup>-</sup> and K<sup>+</sup> distribution in control (a–d) and salt-treated (e–h) *Brassica oleracea* roots, from map scanning of an X-ray. (a, e) Scanning electron micrographs. (b, f) K<sup>+</sup> images; (c, g) Na<sup>+</sup> images; (d, h) Cl<sup>-</sup> images. This figure is obtained from Fig. 9 of Fernandez-Garcia et al. (2009)

sibipiruna plants subjected to flooding showed a higher increment of *phi* thickening density. The authors suggest that the *phi* thickening may be acting as a physical barrier for water flux from the vascular cylinder to the cortical cells.



**Fig. 2.4** Transverse sections of *Cryptomeria japonica* roots, under different treatments as indicated on the figure. (a) Compact and mesic (dry) conditions. (b) Uncompacted (normal) and mesic (dry) conditions. (c) Compact and waterlogged (wet) conditions. (d) Uncompacted (normal) and waterlogged (wet) conditions. The roots were stained with phloroglucinol. The arrow points to a phi thickening. Scale bars = 0.2 mm. Figure obtained from '© 2005 Canadian Science Publishing or its licensors. Reproduced with permission, Gerrath et al. (2005)'

Plant micropropagation under *in vitro* conditions is a technique frequently used for plant propagators. Rooting in agar media is an effective part of this technology. Soukup et al. (2004) have analysed the roots of wild cherry (*Prunus avium* L.) growing in perlite or *in vitro* conditions. When *P. avium* was grown in perlite, the roots develop *phi* thickenings in the radial and traverse wall of the *phi* cell layer directly in contact with the endodermis and within 5 mm from the root tip. However, *P. avium* grown *in vitro* culture did not show the *phi* thickenings in the roots, even after prolonged period of *in vitro* cultivation allowing formation of secondary tissues. The authors consider that *phi* thickening formation is not constitutive, but could be regulated by the environmental factors.

### 2.3.3 Drought Stress

Drought stress is a serious problem in the world and generates a progressive reduction in vegetation cover, coupled with rapid soil erosion. Drought stress is characterised by specific changes in plant anatomy and ultrastructure. Pan et al. (2006) studied the alteration of *phi* thickening in loquat (*Eriobotrya japonica* Lindl.) roots under drought stress. In control plants, *phi* thickenings were observed at 10 mm from the root tip, and these are formed by one or two *phi* cell layers. However, in trees grown under drought stress, *phi* thickening had developed dramatically compared with normal conditions. *Phi* thickening in drought-stressed plants extended to three *phi* cell layers and in some cases appeared beyond the fourth layers. The authors suggested that *phi* thickening of the cortical cells in loquat roots may be regarded as a defence mechanism against water stress under drought.

### 2.3.4 Heavy Metals and Solid-Waste Slag

An interesting case of *phi* thickening is the presence of cell wall thickenings in heavy metal hyperaccumulator species. *Thlaspi caerulescens* is a zinc hyperaccumulator, but *Thlaspi arvense* is a non-hyperaccumulator. *Thlaspi caerulescens* is characterised by the presence of a ‘peri-endodermal’ cell layer presenting thickened inner tangential walls but not in *T. arvense* (Broadley et al. 2007; Zelko et al. 2008). These thickenings develop close to the root tip, about 400–600 µm from the root cap boundary. The thickening extends to the radial walls where the cells are attached to each other. However, the corresponding cell layer in *T. arvense* is composed of thin-walled cells without thickening. In *T. caerulescens*, the cell wall thickening was lignified (Zelko et al. 2008). These authors prefer to use the term ‘peri-endodermal’ because they consider that *phi* thickening does not adequately describe the position of this cell layer.

What is the role of cell wall thickenings in Zn hyperaccumulators? Broadley et al. (2007) suggested that the presence or not of the cell wall thickenings might have an impact on apoplastic fluxes of Zn into the root stele. Interestingly, Zelko et al. (2008) have proposed the differences between the two *Thlaspi* species, in radial transport of Zn across the root. Its loading into the xylem and transport to the shoot may be determined structurally. These authors consider that the cell wall thickenings are increasing the surface of plasma membrane, as observed in *Brassica oleracea phi* cells (Fernandez-Garcia et al. 2009). These cells might function as transfer cells, and the increase of the plasma membrane could be intensifying the transport capacity of these cells.

Solid-waste slag from contaminated municipal soil has been used as substrate for roads, banks or parking lots as top layer. This kind of soil has a high amount of heavy metals and low nitrogen. Degenhardt and Gimmler (2000) have used this

substrate to investigate its effect on root growth of *Z. mays*. We mentioned above that the *phi* thickening is only present in dicotyledon species, except in the monocotyledon *Z. mays*, which can be induced by treatment with solid-waste slag. These authors observed that slag-grown plants showed *phi* thickenings in the radial cell walls of the rhizodermis but the distribution was incomplete around the whole root circumference. However, when plants were treated with other stresses, such as salinity, or were grown in hydroponic and aeroponic cultures, this did not induce *phi* thickening in the roots. The authors suggest that *phi* thickenings may be acting mainly as mechanical support for the roots and not as a physical barrier for solute movement.

### 2.3.5 Interaction of Mycorrhiza and Phi Thickening

*Phi* thickenings have also been suggested as a possible physical barrier for fungal movement through the cortical cells. Yellow birch (*Betula alleghaniensis* Britton) roots can be infected by the mycorrhiza *Chloridium paucisporum*. The fungal hypha is able to penetrate the radial cell walls of the epidermis and develop the Hartig net that can be well extended in the external cortical cells. However, the penetration in depth seems to be limited by the presence of the *phi* thickening in the second layer of cortical cells (Wilcox and Wang 1987). *Dryas integrifolia* is a boreal member of the Rosaceae family and establishes an ectomycorrhizal association with *Hebeloma cylindrosporum*. Melville et al. (1987) have shown that *D. integrifolia* roots develop *phi* thickenings in the inner cortical cells near the apex of first-order lateral roots when they interact with *H. cylindrosporum*. In some first-order lateral roots, the *phi* thickening is composed of two cortical cell layers, which then becomes impervious to hyphal penetration. Moreover, the *phi* thickening was not developed in the older portion of the root, where the Hartig net was poorly developed. These authors suggest that the Hartig net is inhibited beyond cortical cell layers with *phi* thickenings in their radial cell walls.

Elsewhere, other authors have reported that *phi* thickening is not a physical barrier for fungal penetration (Massicotte et al. 1988, 1999). Yellow birch is also able to form an ectomycorrhizal association with *Pisolithus tinctorius*, forming a Hartig net only in the epidermis. However, primary roots at the base of first-order mycorrhizal lateral roots develop *phi* thickenings in the second cortical layer or in the second and third layer of cortical cells. Therefore, the presence of the *phi* thickening seems to be not necessarily correlated with the fungal infection and penetration. Similarly, European black alder (*Alnus glutinosa*) may be associated with the ectomycorrhiza *Paxillus involutus*. The roots of *A. glutinosa* develop in the subapical regions *phi* thickenings in the second layer of cortical cells. However, in this region of the roots, the hyphae did not penetrate between epidermal cells. Moreover, if we advance to the apical region, the *phi* thickenings are thinner and fewer in number. Finally, close to the apical meristem, the *phi* thickenings are not

present, but the mantle and the Hartig net are well developed (Massicotte et al. 1999).

## 2.4 *Phi* Thickening as Taxonomic Tool

Gerrath et al. (2002) proposed that *phi* thickening can be a useful tool for taxonomic classification in gymnosperms. These authors analysed the presence or absence of *phi* thickening in roots of 22 species of gymnosperms representing all the major groups. This study demonstrated that *phi* thickenings were absent in the Cycadaceae, Gnetaceae, Pinaceae, Ephedraceae and Podocarpaceae families and were present in the Ginkgoaceae, Araucariaceae, Taxaceae and Cupressaceae. The results of this study were confirmed by a previous description of *phi* thickenings in gymnosperm species.

Pires et al. (2003) have also developed a study to separate taxonomically two genera, *Prosthechea* and *Encyclia*, using the anatomy of leaves and roots. These authors observed the presence of large *phi* thickenings in the cortical cells of both genera. These *phi* thickenings were placed concentrically, generally forming a continuous sheath in the cortical region of the root. However, the *phi* thickenings did not show any correlation that could help to discriminate between the two genera studied.

## 2.5 Conclusions

*Phi* thickening in cortical cells of the roots was discovered in the nineteenth century. *Phi* thickenings of cell walls can be present in the different cell layers of the cortical cells forming in many cases a continuous cell layer. We have found that more than 100 species present *phi* thickening in roots, including angiosperms and gymnosperms. A mechanical role was originally suggested for this structure, although it has never been demonstrated. In the last decade, new studies have demonstrated that *phi* thickening can be altered by the environmental conditions. Different abiotic and biotic stresses may be modifying the distribution, structure and/or development of *phi* thickening. Abiotic stresses such as salinity, heavy metals and flooding are altering *phi* thickening distribution and lignification. A physiological role for *phi* thickenings has also been proposed, acting as a barrier, altering cation movement through the apoplast and regulating the symplastic ion movement through the plasmodesmata system.

From the nineteenth century, the presence of *phi* thickenings has been described in many different species, but the controversy about its role is still open. It is evident that *phi* thickenings might present several roles, including different physiological and/or mechanical functions. If we consider the different studies, it is possible to extract some general conclusion:

1. *Phi* thickening cannot be induced in species that *phi* thickening is not present.
2. *Phi* thickening may be acting as a partial barrier in solute movement from the cortex to the vascular cylinder.
3. *Phi* thickening appeared early in the plant evolution.
4. *Phi* thickening development can be affected by environmental factors like culture, media, drought stress, salinity, soil contaminant, etc.

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