Phi thickenings in Brassica roots - an adaption to water stress?

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Phi thickenings are unusual bands of secondary cell wall found in the radial cell walls of the root cortex, where only thin, primary cell walls normally occur. These bands, present in diverse angiosperms and gymnosperms, are coordinated between adjacent cells so that the thickenings appear similar to the Greek letter phi (Φ) when viewed in cross-section. Although first described in the 19th century, surprisingly little is known about phi thickenings even though their formation can be induced in the roots of some species by water stress and salinity. Various functions have been proposed for phi thickenings, with the most likely being stabilisation of root architecture during stress responses. However, directed research into the development of phi thickenings and their function(s) has been lacking.

We have investigated phi thickenings in two crops important to Australian agriculture, *Brassica oleracea* (broccoli and relatives) and *B. napus* (canola). We selected these species because 19th century reports suggest that phi thickenings are widespread within the Brassicaceae (1) (although they do not, apparently, occur in *Arabidopsis*), and because phi thickenings can be induced in *B. oleracea* roots by 14-day hydroponic treatment with raised salt (2, 3). Simplifying these experiments, we found that phi thickenings can be induced in both species in 5 day-old seedlings grown on agar plates containing salt, and that salt-induced induction occurs within 24 h. Furthermore, induction is not specific to salt stress, as induction also occurred under sucrose-induced water stress that did not inhibit root elongation, thus demonstrating that root growth inhibition and phi thickening induction are not directly linked.

Using confocal microscopy and 3D reconstructions, we show that phi thickenings form a continuous, lignified ring around the inner cortex of *Brassica* roots, immediately outside the endodermis. More interestingly, these reconstructions also show a delicate, reticulate network of secondary walls along the inner face of these cells adjacent to the endodermis. This structure, reported by van Tieghem in 1887 (1), has not subsequently been described in the literature. We are now attempting to image phi thickenings in roots with other advanced imaging techniques, including light-sheet microscopy and X-ray microtomography, to characterise larger sections of root tissue in order to model how the phi thickenings may change root mechanics. More significantly, however, our imaging has shown that phi thickening induction in both *B. oleracea* and *B. napus* is cultivar-dependent. Testing more than 20 commercial *B. oleracea* varieties and 5 commercial *B. napus* cultivars, we have observed that some completely lacked inducible phi thickenings whereas others show strongly induced phi thickenings, with the patterns formed within the reticulate network being variable. We anticipate that quantification of our microscopic observations of phi thickening induction, including that of the reticulate network, across a large collection of different cultivars, may allow discovery of genetic loci linked to water stress-induced phi thickening development in the Brassicaceae.

1 Van Tieghem (1887) Bull Soc Botanique France 34: 125
2 Lòpez-Pérez et al (2007) Int J Plant Sci 168: 1141
3 Fernandez-Garcia et al (2009) New Phytol. 181: 347