



Commentary on Real-time imaging of leaf apoplastic pH dynamics in response to NaCl stress.

Doug Van Hoewyk

Journal Name:	Frontiers in Plant Science
ISSN:	1664-462X
Article type:	General Commentary Article
Received on:	29 Aug 2011
Accepted on:	30 Aug 2011
Provisional PDF published on:	30 Aug 2011
Frontiers website link:	www.frontiersin.org
Citation:	Van hoewyk D(2011) Commentary on Real-time imaging of leaf apoplastic pH dynamics in response to NaCl stress. . 2:52. doi:10.3389/fpls.2011.00052
Article URL:	http://www.frontiersin.org/Journal/FullText.aspx?s=1220&name=plant%20nutrition&ART_DOI=10.3389/fpls.2011.00052 <small>(If clicking on the link doesn't work, try copying and pasting it into your browser.)</small>
Copyright statement:	? 2011 Van hoewyk. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.

This Provisional PDF corresponds to the article as it appeared upon acceptance, after rigorous peer-review. Fully formatted PDF and full text (HTML) versions will be made available soon.

Commentary on Real-time imaging of leaf apoplastic pH dynamics in response to NaCl stress.

In vivo imaging has made significant contributions to plant biology over the past decade. Notable advances in bioimaging have enabled a more comprehensive understanding of many plant-related topics, including photosynthetic electron transport (Ehlert and Hinch, 2008), viral pathogenesis (Tilsner and Oparka, 2010), and the role of reactive oxygen species (Swanson et al, 2011). Now it appears that the benefits of *in planta* imaging have been extended to the realms of the nonliving. Geilfus and Mühlh (2011) report in *Frontiers in Plant Nutrition* a novel non-invasive technique that allows for the direct quantification of ion and pH dynamics in the apoplast. Perhaps most noteworthy, the authors demonstrate the ability to quantify pH fluctuation in different apoplastic compartments in the leaves less than three hours after roots are subjected to salt stress.

The newly developed method reliably measures apoplastic pH both temporally and spatially in a living plant. Briefly, the technique uses a diffusible and fluorescent dye (Oregon Green 488) that is loaded into the apoplast of intact leaves in the common bean (*Vicia faba*). The pH sensitive, photostable dye is conjugated to a dextran molecule that prevents its movement into the symplast. Fluorescence microscopy is used to estimate apoplastic pH using the fluorescence ratio of F₄₉₅/F₄₄₄. Such ratio imaging is possible, because while the fluorescence of the dye at F₄₉₅ is pH dependent, the concentration of Oregon Green is corrected by the fluorescence at F₄₄₄, which is not sensitive to H⁺ concentration. Therefore, the *in situ* fluorescence ratio of the two wavelengths only provides a direct estimation of apoplastic pH, and is not affected by the dye concentration.

The functions of the apoplast in plant physiology and development varies enormously (Sattelmacher, 2001). Similarly, the potential application of this new method could extend far beyond the studies pertaining to crop stress physiology; the newly described technique to measure apoplastic pH both temporally and spatially could be transformative to a variety of disciplines in plant biology. First, the new approach to study changes in apoplastic pH could improve plant nutrient acquisition. For example, although the apoplast provides a continuum for the bulk flow of water and essential plant nutrients, membrane-bound proton pumps can greatly affect the acidity in a localized regions of the apoplast. H⁺ ATPases can therefore create a polarized microenvironment in the apoplast that can (i) drive cations down their electrochemical gradient into the symplast or (ii) facilitate the movement of anions across the cell membrane via co-transporters. Therefore, a better conceptual framework of apoplastic pH dynamics may potentially allow the screening and development of new crop varieties with enhanced nutritional content. Secondly, because accumulation of cations is dependent upon apoplastic pH, advances in the phytoremediation of soils or groundwater containing excessive amounts of Al, Cu, Fe, and Zn may also be

possible due to knowledge gleaned by utilizing the new technique developed by Geilfus and Mühlhling (2011). Lastly, a decrease in apoplastic pH is associated with cell-wall relaxation and cell elongation. In view of the acid growth theory, enhancing our understanding of apoplastic pH dynamics is relevant to plant growth and biomass. In this context, knowledge of apoplastic pH fluctuations could be far-reaching and applied to research in agriculture or cellulosic ethanol.

A reliable method to measure apoplastic pH in intact leaves has long been awaited. A review and assessment of techniques to quantify apoplastic pH notes the limitations of previously employed methods (Yu et al., 2000). The new technique by Geilfus and Mühlhling (2011) overcomes difficulties encountered with previous methods to estimate apoplastic pH, and comes with the added bonus that pH can be discriminated both spatially and temporally. The new protocol presented by Geilfus and Mühlhling (2011) ushers in the coming of age for the *in planta* imaging of apoplastic pH dynamics, and perhaps with it a better understanding of the extracellular space in plants.

References

Ehlert, B., and Hinch, D.K. (2008). Chlorophyll fluorescence imaging accurately quantifies freezing damage and cold acclimation responses in Arabidopsis leaves. *Plant Methods* 4,12

Geilfus, C.M., and Mühlhling, K.H. (2011). Real-time imaging of leaf apoplastic pH dynamics in response to NaCl stress. *Frontiers in Plant Science* 2, 1-11

Sattelmacher, B. (2001). The apoplast and its significance for plant mineral nutrition. *New Phytol.* 49, 167–192

Swanson, S.J., Choi, W.J., Chanoca, A., Gilroy, S. (2011). In Vivo Imaging of Ca²⁺, pH, and Reactive Oxygen Species Using Fluorescent Probes in Plants. *Ann Rev Plant Biol* 62, 273-297

Tilsner, J., and Oparka, K.J. (2010). Tracking the green invaders: advances in imaging virus infection in plants. *Biochem J.* 430, 21-37.

Yu, Q., Tang, C., Kuo, J. (2000.) A critical review on methods to measure apoplastic pH in plants. *Plants and Soil* 219, 29-40.