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Bringing the dead compartment of a plant cell to life: a novel imaging technique resurrects the dynamic nature of the apoplast

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A commentary on

Real-time imaging of leaf apoplastic pH dynamics in response to NaCl stress
by Geilfus, C. M., and Mühling, K. H. (2011). Real-time imaging of leaf apoplastic pH dynamics in response to NaCl stress. Front. Plant Sci. 2:13. doi: 10.3389/fpls.2011.00013

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 Hincha, 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 non-living. Geilfus and Mühling (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 3 h 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 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 Mühling (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 Mühling (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 Mühling (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.

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