01-05: Fluorescence ratiometry and NIR transmission in combination allow in-situ analysis of leaf apoplastic pH under controlled changes of leaf water content

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Optical measurements have the potential to non-invasively study undisturbed physiological processes in plant tissues. Micro-environmental control of the living sample is necessary to investigate such cellular responses to environmental stimuli both under realistic and repeatable conditions. Here, an automated microscopic platform is presented, which offers various microscopic methods, like fluorescence ratiometry, FRET, BRET and confocal imaging. Ratiometric fluorescence imaging is used for continuous in-vivo monitoring of ionic concentrations in different cellular compartments. The instrument was complemented with a custom environmental leaf chamber allowing microscopic observation under controlled light and humidty conditions. Inclusion of a newly developed optical sensor based on NIR-transmission for leaf water content (LWC) allows continuous and precise observation of LWC-fluctuations. These occur passively as induced by changes in environmental conditions and also as a result of osmotic adjustment processes and stomatal responses. A control system was implemented, feeding back the LWC-signal into the humidity control of the cuvette enclosing the leaf and thus forming a feedback-loop which allows imposition of defined changes of leaf water content. This setup, for the first time enables defined and repeatable experimental control over LWC simultaneously with in-planta ion-measurements. Using the ratiometric dye Oregon Green loaded to the apoplast we studied the possible role of apoplastic pH-variations in signaling local tissue water status to the guard cells. Upon a decrease in LWC the apoplast consistently showed a substantial alkalisation preceding stomatal closure. Strength of the pH-response, timing and a consistent dose-response-relationship are in agreement with a role as a tissue signal involved in leaf water homeostasis.