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Intact leaf gas exchange provides a robust method for measuring the kinetics of stomatal conductance responses to abscisic acid and other small molecules in Arabidopsis and grasses

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摘要	Background Guard cells perceive external and internal stimuli and regulate stomatal conductance in plants. With the use of gas exchange analyzers, time-resolved stomatal conductance responses to light intensity, [CO2] concentration and relative humidity changes can be measured. This is more difficult to achieve when measuring stomatal responses to small soluble molecules such as the plant hormone abscisic acid (ABA) or the bacterial peptide flagellin 22 (flg22), in particular when investigating mutants with response phenotypes. Results A method to evaluate the dynamic effects of small molecules on stomatal conductance in a time-resolved fashion using gas exchange analyzers is presented here. ABA-induced stomatal closure was investigated by adding ABA to the transpiration stream of intact leaves placed in a microcentrifuge tube containing water. Strong ABA responses were resolved in time- and in a dose-dependent manner in wild-type Arabidopsis leaves, whereas the same response was not observed in leaves of the ABA-insensitive mutant open stomata 1-3 (ost1-3). Moreover, when leaves of the Plasma membrane Intrinsic Protein (PIP) aquaporin quadruple mutant pip1;1 pip1;2 pip2;1 pip2;2 were tested, robust wild-type-like responses to ABA were observed. When the bacterial peptide flg22 was added to the transpiration stream of intact wild-type leaves, a strong flg22-induced stomatal closure effect was observed. Finally, the proposed technique was further developed and optimized for evaluation of stomatal conductance responses to small molecules in leaves of grasses using the reference plant Brachypodium distachyon.

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Due to the variable size of stomata in Arabidopsis and the limited dynamic response of stomata in isolated epidermal strips, evaluation of the effect of small molecules on stomatal physiology has been challenging and has led in some cases to inconsistent results. Moreover, potential signals from the mesophyll are missing when using epidermal peels to evaluate stomatal aperture responses. Here we propose a less invasive technique which allows for time-resolved measurements of stomatal conductance responses to small molecules optimized for both Arabidopsis and Brachypodium distachyon leaves.

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