

Sulfate is an important trigger of abscisic acid biosynthesis and stomata closure

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Sulfur is an important macronutrient of all plants. Its uptake in form of sulfate from the soil is facilitated by plasma-lemma localized high-affinity sulfate transporters that are specifically regulated in response to internal demand and environmental challenges. Surprisingly, sulfate has been reported as the first metabolite whose concentration in the xylem is altered upon drought in the xylem. It was also shown to affect stomata closure by serving as substrate and activator of the quick anion channel 1 (Ernst et al. 2010; Malcheska et al. 2017). Furthermore, the external sulfate supply is known to impinge on ABA steady state level in Arabidopsis seedling (Cao et al. 2014).

In the current study, we uncover the molecular mechanism of sulfate-induced stomata closure and provide *bona fide* evidence for the role of sulfate in ABA biosynthesis: We show that feeding of physiologically relevant sulfate concentrations via the petiole trigger stomata closure by induction of plasma-lemma localized NADPH oxidases that produce reactive oxygen species (ROS). Since ROS production is a known response of stomata towards ABA stimulus, we tested if sulfate can promote stomata closure and ROS production in the ABA biosynthesis mutant, *aba3*, and the ABA insensitive mutant, *abi2*. Sulfate failed to induce stomata closure and production of ROS in both mutants. Furthermore, loss of the ABA-activated slow anion channel 1 (SLAC1) impaired sulfate-induced stomatal closure. Application of the ABAleon2;1 sensor, a FRET-based probe for non-invasive determination of ABA by live cell imaging, ultimately proved that sulfate application rapidly increases cytosolic ABA levels in guard cells. In view of the fact that the molybdenum cofactor sulfurylase ABA3 uses cysteine as substrate for activation of ABA biosynthesis (Bittner et al. 2001), we tested the ability of sulfate to close stomata of the *sir1-1* and the *serat tko* mutants, which are both impaired in cysteine biosynthesis. As expected, sulfate was unable to induce stomata closure and ROS production in these mutants. In agreement with a promoting role of cysteine in ABA synthesis, application of ABA or cysteine to *sir1-1* and *serat tko* induced ROS production and stomata closure. The latter results proved that ABA signaling is still functional in both cysteine synthesis depleted mutants. Intriguingly, guard-cell autonomous production of ABA by ABA3 was sufficient for sulfate-induced stomata closure. Taken together our results promote sulfate as a xylem-delivered root-to-shoot signal that upon early drought induces ABA production in guard cells resulting in stomata closure.

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