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41 - Divergent roles of RBOHD, AtCuAO β , and GLR3.3 in systemic wound signaling

Ilaria Fraudentali⁽¹⁾ - Chiara Pedalino⁽¹⁾ - Adriana Furlani⁽¹⁾ - Margherita Gagliano⁽¹⁾ - Riccardo D'Incà⁽¹⁾
- Paraskevi Tavladoraki⁽¹⁾ - Alessandra Cona⁽¹⁾

⁽¹⁾ *Università degli Studi Roma Tre, Dipartimento di Scienze, Roma, Italia*

Reactive oxygen species (ROS) function as crucial second messengers in long-distance wound responses, coordinating systemic stomatal closure. In this study, we dissect the relative contributions of two ROS-generating enzymes—the plasma membrane NADPH oxidase RBOHD and the apoplastic amine oxidase AtCuAO β —and the glutamate receptor-like ion channel GLR3.3, to both local (wounded leaf) and systemic (distal leaf) ROS accumulation and Ca²⁺ dynamics in Arabidopsis. In wild-type (WT) seedlings, leaf wounding triggers rapid ROS accumulation both within guard cells—detected by confocal microscopy using CM-H₂DCFDA—and leaf tissues, observed *via* whole-seedling fluorescence imaging. They also exhibit a fast-propagating Ca²⁺ wave, detected using the red-shifted R-GECO1 indicator. *Atcuao β* mutants fail to exhibit any detectable ROS increase, either locally or distally, yet retain a WT-like Ca²⁺ wave, indicating an essential role for AtCuAO β in both local and systemic ROS production. *rboh*d mutants show WT-like levels of local ROS but reduced distal ROS accumulation and delayed Ca²⁺ wave propagation, highlighting the involvement of RBOHD mainly in long-distance signaling. *glr3.3* mutants exhibit attenuated ROS accumulation in both local and distal tissues, underscoring the importance of GLR3.3 in Ca²⁺-dependent amplification of ROS signals. Overall, these findings provide new insights into the role of ROS–Ca²⁺ crosstalk in the coordination of local and systemic wound signaling.