

Analysis of the *Arabidopsis dry2/sqe1-5* mutant suggests a role for sterols in signaling

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Sterols play multi-faceted roles in all Eukaryotes. In plants, there are mounting evidences pointing to sterols, other than BRs, can act as signaling molecules. The *Arabidopsis dry2/sqe1-5* mutant has multiple developmental defects caused by a point mutation in the *SQE1* gene that generates a hypomorphic allele. *SQE1* encodes a squalene epoxidase, which converts squalene into 2,3-oxidosqualene the precursor of plant sterols. Genetic, molecular and biochemical analyses suggest that *dry2/sqe1-5* defective phenotypes cannot be simply explained by a depletion of bulk sterols but rather by altered ROS. It remains to be elucidated whether the altered ROS production of the mutant is caused by membrane composition, which in turn affect the lipid rafts composition and/or an altered signaling.

Plant sterols have been extensively studied in past years with a major focus on biosynthetic and biochemical aspects.¹ Sterols are important not only as structural components of eukaryotic cell membranes because of their role in membrane fluidity and permeability,² but also because they are the biosynthetic precursors of steroid hormones in animals, insects and plants.³⁻⁵ The role of animal steroids in the regulation of embryonic and postembryonic development along with adult homeostasis is well known.⁶ However cholesterol itself can also serve as a signaling molecule, without conversion to steroid hormones.⁷⁻⁹ Whereas in animals, cholesterol is the only structural sterol, plant membranes consist of a variable mixture of several phytosterols, being sitosterol the most abundant.⁴ Brassinosteroids (BRs) are the only sterol-derived steroid hormones in plants. The

diverse functions of BRs in growth and development have been investigated rigorously but little is known about the regulatory roles of other phytosterols.

We have identified the *Arabidopsis thaliana dry2/sqe1-5* mutant by its extreme drought sensitivity caused by a defective stomata function.¹⁰ In addition, *dry2/sqe1-5* also showed pleiotropic developmental defects, including altered root architecture, root hairs, diminished shoot size and chlorophyll content. The cause of all these defective phenotypes in *dry2/sqe1-5* is a point mutation in the *SQUALENE EPOXIDASE 1 (SQE1)* gene that likely produce an enzyme with reduced activity. Squalene epoxidase enzymes catalyse the conversion of squalene into 2,3-oxidosqualene, the precursor of all cyclic triterpenoids. The developmental defects in sterol biosynthetic mutants have been hypothesized to be caused by structural defects in cellular membrane networks due to sterol depletions.^{11,12} However, the strong developmental defects in *dry2/sqe1-5* shoots does not correlate with significant changes in sterol composition but rather are associated with defective reactive oxygen species (ROS) production and NADPH oxidase function.¹⁰ Defective polar localization of proteins such PIN2 has been reported in several sterol-biosynthesis mutants probably caused by altered endocytic turnover.¹²⁻¹⁴ However, while RHD2/AtbohC is de-localized in *dry2/sqe1-5* root hairs, PIN2 localization is correct. Hence general endocytosis caused by an altered composition in root sterols is not impaired in *dry2/sqe1-5* mutant.

The molecular mechanisms underlying the regulatory role of sterols other than BRs in plant development have not been

Key words: sterol, signaling, squalene epoxidase, *dry2/sqe1-5*, reactive oxygen species, arabidopsis

Abbreviations: ROS, reactive oxygen species; BRs, brassinosteroids

Submitted: 07/01/09

Accepted: 07/01/09

Previously published online:

www.landesbioscience.com/journals/psb/article/9425

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Addendum to: Posé D, Castanedo I, Borsani O, Nieto B, Rosado A, Taconnat L, et al. Identification of the *Arabidopsis dry2/sqe1-5* mutant reveals a central role for sterols in drought tolerance and regulation of reactive oxygen species. *Plant J* 2009; 59:63-76; PMID: 19309460; DOI: 10.1111/j.1365-3113X.2009.03849.x.

established and their putative role as signaling molecules remains highly speculative. The low concentrations of sterols present in plant tissues, their multifaceted roles, complex biosynthesis and regulation, and ability of movement between cells make difficult their functional analysis.^{1,4,15,16} Furthermore, the cell specific expression found for several sterol biosynthetic genes suggests that sterols are produced in specific cell types and therefore the concentration of the various sterols is predicted to be different between cells. The sterol analyses is further complicated because most mutations in sterol biosynthetic genes lead to cell division and expansion defects, as well as patterning defects such as multiple shoot meristems in embryogenesis.^{1,4}

Therefore a central question remains: Do plant sterols have only structural roles or do they also possess other roles as signaling molecules? Several lines of evidence support the latest hypothesis i.e., that plant sterols also have signaling roles independent of BRs. First, sterol deficient mutants show defects in embryogenesis while BRs-deficient mutants do not. In addition sterol mutants cannot be rescued by BRs treatment.³ Second, typical sterols such sitosterol and stigmasterol thought to have exclusively a structural role induce the specific expression of genes involved in cell expansion and division.¹⁷ Third, lipid/sterol-binding StAR-related lipid transfer (START) protein domains have been identified in plants.¹⁸ In fact, START domains are more common in plants than in animals and are primarily found within homeodomain (HD) transcription factors, suggesting a mechanism by which lipid/sterol ligands can directly modulate transcription in plants.³ Fourth, an intermediate sterol such obtusifoliol can be transported to distal parts of the plant away from the sprayed leaves.¹⁶ An alternative possibility is that *dry2/sqe1* show defects in lipid rafts. Lipid rafts are enriched in sphingolipids

and sterols creating a microenvironment in the membrane that activates signal transduction by promoting clustering of specific membrane-associated proteins or directly modulating their activity possibly by a modification of their conformation.^{19,20} The fact that the NADPH oxidase NtrbohD is associated with tobacco lipid rafts¹⁹ suggests the possibility that *dry2/sqe1* mutant has a defect in these microdomains, impairing the correct localization of RHD2/AtrbohC, possibly due to an impaired endocytic turnover, and the consequent proper polarized production of ROS.

Identification of the hypomorphic *dry2/sqe1-5* allele allowed the identification and characterization of physiological processes regulated by sterols that otherwise would be concealed. In a similar fashion, the discovery of the weak *cas1-1* allele allowed the identification of a role for sterols in plastid biogenesis.^{11,21} The fact that the *dry2/sqe1-5* mutant is fertile, in contrast to the null *sqe1-3* allele, allowed us to perform a suppressor screening leading to the identification of a large number of independent mutants that reverted the extreme drought sensitivity of *dry2/sqe1-5* and other developmental defects. The analysis of these mutants and the identification of the genes affected could determine whether or not sterols other than BRs have a signaling role in plant development and open the possibility to uncover such components.

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