CellPress
PARTNER JOURNAL

Molecular Plant
Research Article

Arabidopsis Squalene Epoxidase 3 (SQE3) Complements SQE1 and Is Important for Embryo Development and Bulk Squalene Epoxidase Activity

Sara Laranjeira[1], Vitor Amorim-Silva[2], Alicia Esteban[2], Monserrat Arró[3,4], Albert Ferrer[3,4], Rui Manuel Tavares[1], Miguel Angel Botella[2], Abel Rosado[5,*] and Herlânder Azevedo[6,*]

[1]Center for Biodiversity, Functional & Integrative Genomics (BioFIG), Plant Functional Biology Center, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

[2]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Departmento de Biología Molecular y Bioquímica, Universidad de Málaga, Campus Teatinos, 29071 Málaga, Spain

[3]Department of Molecular Genetics, Centre for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB), Bellaterra-Cerdanyola del Vallés, 08193, Spain

[4]Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain

[5]Canada Research Chair in Plant Physiology and Cellular Dynamics, Department of Botany, Faculty of Sciences, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

[6]CIBIO, InBIO - Research Network in Biodiversity and Evolutionary Biology, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

***Correspondence: Herlânder Azevedo (hazevedo@cibio.up.pt), Abel Rosado (abel.rosado@botany.ubc.ca)**

http://dx.doi.org/10.1016/j.molp.2015.02.007

ABSTRACT

The existence of multigenic families in the mevalonate pathway suggests divergent functional roles for pathway components involved in the biosynthesis of plant sterols. Squalene epoxidases (SQEs) are key components of this pathway, and Squalene Epoxidase 1 (SQE1) has been identified as a fundamental enzyme in this biosynthetic step. In the present work, we extended the characterization of the remaining SQE family members, phylogenetically resolving between true SQEs and a subfamily of SQE-like proteins that is exclusive to Brassicaceae. Functional characterization of true SQE family members, Squalene Epoxidase 2 (SQE2) and Squalene Epoxidase 3 (SQE3), indicates that SQE3, but not SQE2, contributes to the bulk SQE activity in *Arabidopsis*, with *sqe3-1* mutants accumulating squalene and displaying sensitivity to terbinafine. We genetically demonstrated that SQE3 seems to play a particularly significant role in embryo development. Also, SQE1 and SQE3 both localize in the endoplasmic reticulum, and SQE3 can functionally complement SQE1. Thus, SQE1 and SQE3 seem to be two functionally unequal redundant genes in the promotion of plant SQE activity in *Arabidopsis*.

Key words: *Arabidopsis*, embryo development, MVA pathway, squalene epoxidase, sterol biosynthesis

Laranjeira S., Amorim-Silva V., Esteban A., Arró M., Ferrer A., Tavares R.M., Botella M.A., Rosado A., and Azevedo H. (2015). *Arabidopsis* Squalene Epoxidase 3 (SQE3) Complements SQE1 and Is Important for Embryo Development and Bulk Squalene Epoxidase Activity. Mol. Plant. **8**, 1090–1102.

INTRODUCTION

Isoprenoids constitute the most functionally and structurally diverse set of plant metabolites, comprising over 40 000 molecules (Bohlmann and Keeling, 2008). These molecules have multiple agricultural and industrial applications as pharmaceuticals, pesticides, disinfectants, flavors, fragrances and, more recently, petrochemicals (Bohlmann and Keeling, 2008; Vranová et al., 2013). Isoprenoids are essential to all living organisms and are implicated in various plants processes, including respiration and photosynthesis, growth and reproduction, and the response to both environmental stimuli and biotic stress challenges (Tholl and Lee, 2011; Hemmerlin et al., 2012). Higher plants are capable of synthesizing thousands of isoprenoid-derived compounds using the five-carbon building unit isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (Bouvier-Navé et al., 2010). Unlike other living organisms, plant isoprenoid precursors are synthesized by two independent pathways: the

Published by the Molecular Plant Shanghai Editorial Office in association with Cell Press, an imprint of Elsevier Inc., on behalf of CSPB and IPPE, SIBS, CAS.

plastid-localized methylerythritol phosphate (MEP) pathway (Vranová et al., 2013) and the cytosolic-localized mevalonate (MVA) pathway. The latter is responsible for the biosynthesis of plant hormones such as brassinosteroids and cytokinins, the prenyl groups used for protein posttranslational modifications and, more importantly for our study, the biosynthesis of plants sterols (Benveniste, 2004; Phillips et al., 2006; Schaller, 2010).

Sterols are globally important for plant development, acting specifically as essential structural components of the membrane lipid bilayer, modulating membrane permeability and fluidity, and the activity of membrane-bound proteins (Hartmann, 1998; Benveniste, 2004; Phillips et al., 2006). Several studies have highlighted the significance of correct sterol composition, implicating sterols in plant cell division, elongation and polarity (Schrick et al., 2000; Willemsen et al., 2003; Men et al., 2008), embryonic, vascular, and stomatal patterning (Jang et al., 2000), hormonal regulation (Souter et al., 2002; Souter et al., 2004), production of reactive oxygen species (ROS) (Posé et al., 2009), activity of plant microRNAs (Brodersen et al., 2012), and tolerance to low temperature and drought (Posé et al., 2009; Senthil-Kumar et al., 2013). Together with callose, sterol biosynthesis is also involved in the maintenance of the ploidy level in the premeiotic germ lineage (De Storme et al., 2013).

MVA-dependent sterol biosynthesis requires the conversion of IPP to the linear 30-carbon intermediate squalene (SQ) (Phillips et al., 2006; Rasbery et al., 2007), a polyunsaturated triterpene containing six isoprene units (Spanova and Daum, 2011). SQ is further oxidized to 2,3-oxidosqualene in a reaction catalyzed by squalene epoxidase (SQE) (Phillips et al., 2006; Rasbery et al., 2007). At this point, plants use a sterol biosynthetic pathway that favors the cyclization of 2,3-oxidosqualene to cycloartenol rather than lanosterol, the intermediate present in animals and fungi (Schaller, 2003; Benveniste, 2004; Schaller, 2004; Spanova and Daum, 2011). A lanosterol pathway was recently shown to exist in plants, accounting for approximately 1.5% of cyclized squalene in *Arabidopsis* (Ohyama et al., 2009). In plants, cycloartenol biosynthesis ultimately leads to the formation of 24-methyl sterols, which include campesterol and brassinosteroids, and 24-ethyl sterols, which include the structural sterols sitosterol and stigmasterol (Clouse, 2002).

Yeast and mammal genomes encode single copies of squalene epoxidases, while plant SQEs form multicopy gene families. In *Arabidopsis thaliana*, six predicted *SQEs* were reported based on a homology search (*SQE1-6*) (Benveniste, 2002; Rasbery et al., 2007; Posé et al., 2009), however only the products of *SQE1*, *SQE2*, and *SQE3* were able to functionally complement the *Saccharomyces cerevisiae* SQE mutant *erg1* (Rasbery et al., 2007). Functional studies to uncover the biological roles of *Arabidopsis* SQEs have focused on *SQE1*. Null *SQE1* mutants show increased squalene levels and pleiotropic phenotypes that include defective root, hypocotyl and stem elongation, and unviable seeds, indicating that the activity of SQE1 is essential for different developmental processes (Phillips et al., 2006; Rasbery et al., 2007). By using the hypomorphic and fertile *Arabidopsis drought hypersensitive 2* (*dry2/sqe1-5*) mutant allele, it was shown that *dry2/sqe1-5* was defective in root but not shoot sterol composition and displayed altered stomatal responses (Posé et al., 2009). Furthermore, both the *dry2/sqe1-5* root and stomatal defects were associated with altered NADPH oxidase activity and defective ROS production, establishing a novel link between the MVA pathway and ROS homeostasis (Posé et al., 2009). In a recent study, the *dry2/sqe1-5* mutant was used in a genetic screening for second-site suppressor mutations, leading to the identification of *SUpressor of dry2 Defects 1* (*SUD1*), a putative E3 ubiquitin ligase that recovers most of the *dry2/sqe1-5* defects by modulating the activity of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) upstream of SQEs (Doblas et al., 2013).

The presence of several SQE genes in most plants and their phylogenetic diversity suggest new evolutionary roles involving plant SQEs. In the present work, we reconstructed the phylogeny of plant SQEs to reveal that SQE-like proteins are restricted to Brassicaceae, while three true SQEs are likely to retain standard SQE function. Since most studies have concentrated on *SQE1*, we have investigated the role of *SQE2* and *SQE3* using physiological, biochemical, and genetic analyses. We found that *SQE2* expression is restricted to very specific tissues and is unlikely to influence the overall sterol composition at the whole plant level. Most significantly, we showed that SQE3 activity has a role in sterol biosynthesis and is predominantly important over SQE2, with *sqe3-1* mutants accumulating squalene and displaying sensitivity to terbinafine, which is indicative of the SQE activity of SQE3. Like SQE1, SQE3 is targeted to the endoplasmic reticulum (ER) and complements the *sqe1-5* mutation. However, phenotypic and genetic analyses suggest that SQE3 is only partially redundant with SQE1, likely due to tissue-specific functionalization.

RESULTS

In Brassicaceae, SQEs Are Divided between True SQEs and SQE-like Proteins

SQEs catalyze the first oxygenation step in phytosterol and triterpenoid saponin biosynthesis and represent one of the rate-limiting enzymes in this pathway (Posé et al., 2009; Bouvier-Navé et al., 2010; Doblas et al., 2013). Even though previous reports demonstrated the importance of *Arabidopsis SQE1* in plant development through the analysis of different alleles of the *sqe1* mutant (Rasbery et al., 2007; Posé et al., 2009), the role of the remaining members of the *SQE* gene family has not been established. In the present study, we analyzed the distribution of *SQE* homologs in representative plant species using the Phytozome and PLAZA platforms (Proost et al., 2009; Goodstein et al., 2012; Van Bel et al., 2012). This analysis rendered a single putative *SQE* gene in Chlorophyta and primitive land plants such as *Selaginella moellendorffii* and *Physcomitrella patents,* no more than two putative *SQEs* in monocots, and several putative *SQE* genes in dicot genomes (Figure 1A). This suggests that SQE retention through plant evolution is required, and that in higher plants (particularly in dicots), functional specialization of plant *SQE* paralogs might exist. In *Arabidopsis thaliana*, six putative *SQE* genes (*SQE1–SQE6*) were reported (Rasbery et al., 2007; Posé et al., 2009). The phylogenetic inference shown in Figure 1A resolved their protein products into two clades, one containing SQE1, SQE2, and SQE3 and a second containing SQE4, SQE5, and SQE6. In a previous study, it was shown that SQE1, SQE2, and SQE3 could functionally complement the yeast SQE mutant (*erg1*) while SQE4, SQE5, and SQE6 proteins could not (Rasbery