ABSTRACT

GENETIC ANALYSIS OF TOCOPHEROL FUNCTIONS IN ARABIDOPSIS AT LOW TEMPERATURES

By

Wan Song

Tocopherols (vitamin E) are lipid-soluble antioxidants that are synthesized only by photosynthetic organisms. Molecular dissection of the tocopherol biosynthetic pathway in Arabidopsis and Synechocystis and the availability of various mutants containing different amounts and compositions of tocopherols have greatly facilitated studies directed at elucidating tocopherol functions in photosynthetic organisms. Blockage in phloem source-to-sink transportation is a common phenotype shared by multiple tocopherol-deficient plant species but the molecular mechanism behind the phenomenon has remained enigmatic. The phenotype in Arabidopsis thaliana tocopherol deficient vte2 mutant is inducible under low temperature (LT) treatment and thus provides an ideal system to study the relevant tocopherol functions. A series of events occurs in vte2 mutant, including abnormal transfer cell wall development, vascular callose deposition, impaired photoassimilate export capacity, sugar and starch accumulation, which eventually led to growth inhibition during LT adaptation. A forward genetic screen for mutations that suppress the vte2 LT-induced phenotypes was undertaken in order to understand the genetic basis of the vte2 phenotype and determine links between tocopherol deficiency and the vte2 LT-induced phenotypes. Seven independent sve (suppressor ofyte2 low temperature-induced phenotype) lines were identified from EMS mutagenized vte2 population and three lines were selected for further detailed analysis

and molecular cloning based on biochemical characterization of the primary sve lines. svel completely suppressed all vte2 LT phenotypes and was found to be a novel allele of fad2, the endoplasmic reticulum-localized oleate desaturase. sve2 showed partial suppression and was found to be a new allele of trigalactosyldiacylglyceroll (tgdl), a component of the ER-to-plastid lipid ATP-binding cassette (ABC) transporter. Introduction of tgdl, tgd3, and tgd4 mutations into the vte2 background similarly suppressed the vte2 LT phenotypes, indicating a key role for lipid transport in this process, svel partially suppressed all vte2 LT phenotypes without impacting fatty acid and lipid metabolism at permissive temperature. Analyses of the acyl composition of ERand plastid-derived lipids before and after LT treatment demonstrated the elevation of 18:2 in phosphotidylcholine is an early and key component in vte2 LT-induced responses as all suppressors attenuated this change. Identification and characterization of sve loci highlights the involvement of ER lipid metabolism in tocopherol function in plants. A global transcript profiling study was carried out to further investigate the transcriptional effect of tocopherol deficiency and understand the vte2 LT-induced phenotypes. By comparing vte2 and wild type under different time period of LT treatment, it was shown that tocopherol deficiency had no effect on gene expression at permissive conditions but affected a limited number of specific genes after 48h of LT treatment. Based on gene expression profiles, tocopherol deficiency appeared to result in some degree of oxidative stress response and influenced the expression of genes involved in cell wall modification and solute transport in LT-treated vte2. Statistical analyses also highlighted several potentially important target genes for future studies. These results together provide new insights into tocopherol functions in LT adaptation in plants.