A DTX/MATE-Type Transporter Facilitates Abscisic Acid Efflux and Modulates ABA Sensitivity and Drought Tolerance in *Arabidopsis*

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ABSTRACT **Abscisic acid (ABA) regulates numerous physiological and developmental processes in plants. Recent studies identify intracellular ABA receptors, implicating the transport of ABA across cell membranes as crucial for ABA sensing and response. Here, we report that a DTX/Multidrug and Toxic Compound Extrusion (MATE) family member in *Arabidopsis thaliana*, AtDTX50, functions as an ABA efflux transporter. When expressed heterologously in both an *Escherichia coli* strain and *Xenopus* oocyte cells, AtDTX50 was found to facilitate ABA efflux. Furthermore, *dtx50* mutant mesophyll cells preloaded with ABA released less ABA compared with the wild-type (WT). The *AtDTX50* gene was expressed mainly in the vascular tissues and guard cells and its expression was strongly up-regulated by exogenous ABA. The AtDTX50::GFP fusion protein was localized predominantly to the plasma membrane. The *dtx50* mutant plants were observed to be more sensitive to ABA in growth inhibition. In addition, compared with the WT, *dtx50* mutant plants were more tolerant to drought with lower stomatal conductance, consistent with its function as an ABA efflux carrier in guard cells.**

Key words: hormone biology; molecular transport; *Arabidopsis*.

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INTRODUCTION

Abscisic acid (ABA) plays significant roles in various aspects of plant growth and development, including seed germination, senescence, and responses to abiotic stresses (Finkelstein et al., 2002). Drought is the most damaging abiotic stress that affects crop productivity worldwide. Under drought conditions, the ABA level in plants increases rapidly, leading to stomatal closure and induction of stress genes to cope with the stress (Hetherington and Woodward, 2003; Xie et al., 2006). Therefore, ABA is widely believed to serve as the central player in drought-stress responses (MacRobbie, 1998; Blatt, 2000; MacRobbie, 2000; Schroeder et al., 2001).

Research on ABA in the past several decades has been focused on the biosynthesis and signaling. Studies in both areas suggest that ABA transport is crucial for the function of this hormone. In higher plants, ABA is synthesized from precursors including carotenoids and the xanthophylls (Schwartz et al., 1997; Taylor et al., 2000; Milborrow, 2001). The synthetic enzymes such as ABA2, NCED3, and AAO3 were found to accumulate in vascular tissues and/or guard cells (Cheng et al., 2002; Tan et al., 2003; Koiwai et al., 2004; Endo et al., 2008), suggesting that ABA synthesis takes place in these cell types. Therefore, synthesized ABA needs to be transported to other target tissues. Concerning ABA signal transduction, recent studies identified PYR/PYL/RCAR proteins as ABA receptors that are localized in the cytosol and nucleus. It is now widely accepted that ABA binds to PYR/PYL/RCAR proteins to initiate signal transduction pathways leading to stomatal closure and activation of stress genes (Raghavendra et al., 2010; Joshi-Saha et al., 2011). As PYR/

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PYL/RCAR are intracellular soluble receptors, ABA transport across the cell membranes becomes critical in the control of ABA concentration and thus signaling strength in the cell.

Historically, ABA is thought to be synthesized in roots and transported up to leaves (including guard cells) through xylem mass flow (Hartung et al., 2002), triggering stomatal closure. More recent studies show that guard cells are autonomous in ABA synthesis and stomatal closure is triggered by ABA synthesized in guard cells (Endo et al., 2008; Bauer et al., 2013). However, identification of several ABA transporters supports the general notion that ABA is transported throughout the plant. Two ATP-binding cassette (ABC) transporter family members in *Arabidopsis*, AtABCC25 and AtABCG40, were found to work as ABA transporters (Kang et al., 2010; Kuromori et al., 2010). The *AtABCG25* gene is expressed mainly in vascular tissues, and AtABCG25 protein is localized to the plasma membrane working as an ABA exporter (Kuromori et al., 2010). While ABCG40 is also localized at plasma membrane, it has been shown to function as an ABA influx transporter. In yeast and BY2 cells expressing AtABCG40, ABA uptake was increased compared to the controls, whereas ABA uptake into *atabcg40* mutant cells was reduced when compared with the wild-type (WT) (Kang et al., 2010). Another type of ABA transporter, AIT1 (or NRT1.2 in the nitrate transporter family), was identified as an ABA influx facilitator at the site of ABA biosynthesis and regulates the stomatal aperture in inflorescence stems (Kanno et al., 2012). However, under growth conditions tested (Kang et al., 2010; Kuromori et al., 2010; Kanno et al., 2012), disruption of these above ABA transporters individually did not drastically alter ABA responses in plants, suggesting that these individual transporters may have synergistic functions in ABA transport. It is also possible that other unidentified transporter(s) are involved in ABA movements in plants.

The Detoxification Efflux Carriers (DTX)/Multidrug and Toxic Compound Extrusion (MATE) transporters are conserved from bacteria to plants and animals (Omote et al., 2006). *Arabidopsis* genome encodes 58 members in the DTX/MATE family (Li et al., 2002), and several members have been reported to function as transporters of organic acids and secondary metabolites. AtDTX1 was shown to serve as an efflux carrier of plant-derived alkaloids and antibiotics (Li et al., 2002). FRD3 is a citric acid efflux transporter involved in effective Fe^{3+} uptake (Durrett et al., 2007). EDS5 is involved in the SA-dependent pathogen response pathway, and has been recently found to work as an SA efflux transporter involved in SA release from chloroplasts (Nawrath et al., 2002; Serrano et al., 2013). In other plant species, SbMATE1 and HvACC1 were both found to be citrate transporters required for aluminum tolerance in *Sorghum bicolor* and *Hordeum vulgare*, respectively (Furukawa et al., 2007; Magalhaes et al., 2007). Here, we report that an *Arabidopsis* DTX/MATE family member, DTX50, functions as an ABA efflux transporter and plays a role in ABA-mediated growth inhibition and responses to drought conditions.

RESULTS

Disruption of *DTX50* Gene Causes Growth Retardation and ABA Hypersensitivity

After initial characterization of the DTX/MATE family (Li et al., 2002), we carried out a comprehensive reverse genetic analysis of all the family members using T-DNA insertion mutants in *Arabidopsis.* Homozygous insertion line(s) of 12 genes were initially identified and compared with the WT plants. When grown on ½ MS medium or in the soil under normal greenhouse conditions, most of these mutant lines did not show any obvious difference from the WT (Supplemental Figure 1, showing part of the screening). However, an insertion mutant of the *DTX50* gene was found to be significantly smaller and more yellowish when grown in the soil (Figure 1C and Supplemental Figure 1). This mutant did not show much phenotypic difference from the WT when grown on ½ MS medium (Supplemental Figure 1). The *dtx50* mutant carries a T-DNA insertion in the only exon of the *At5g52050* gene (Figure 1A). RT–PCR showed that *DTX50* mRNA was not detectable in the mutant line (Figure 1B), and the phenotype was complemented by transforming the mutant plants with *DTX50* complementation construct (Figure 1C).

Since the MATE/DTX family was previously reported to transport toxic compounds and organic acids, we further tested the *dtx50* mutant on growth medium supplied with several organic acids including citrate, malate, anthocyanidin, tetramethylammonium, and alkaloids, and no difference was observed. But, interestingly, when tested on growth medium supplied with plant hormones, including gibberellic acid (GA), JA, auxins, and ABA, which all fall into the organic acid category, *dtx50* plants showed a more severe growth inhibition by ABA as compared to the WT, but no difference was found on other hormones tested. When 2-week-old seedlings were transferred onto ½ MS medium containing ABA (at concentration of 5 μM and 20 μM), *dtx50* plants displayed bleached leaves, shorter primary roots, and fewer lateral roots. The complementation line *DTX50/dtx50* showed a similar phenotype to the WT (Figure 1C–1E and Supplemental Figure 2). Further, on ABA-containing medium, the *dtx50* mutant died much earlier than the WT and complementation plants, namely the aerial parts of *dtx50* plants, turned brown and dried sooner than the WT (Supplemental Figure 2).

To further investigate ABA responses in the mutant, we performed germination assays. On ½ MS medium without ABA, the *dtx50* mutant seeds germinated more slowly as compared with the WT seeds in a time course analysis (Figure 1F, left). On ½ MS medium containing ABA, the