A functional cutin matrix is required for plant protection against water loss

Guoxiong Chen,1,2* Takao Komatsuda,2† Jian Feng Ma,3 Chao Li,1 Naoki Yamaji3 and Eviatar Nevo4

1Laboratory of Plant Stress Ecophysiology and Biotechnology; Cold and Arid Regions Environmental and Engineering Institute; Chinese Academy of Sciences; Lanzhou China; 2National Institute of Agrobiological Sciences; Tsukuba, Ibaraki, Japan; 3Institute of Plant Science and Resources; Okayama University; Kurashiki, Okayama, Japan; 4Institute of Evolution; University of Haifa; Mount Carmel, Haifa, Israel

†These authors contributed equally to this work.

Key words: ABC transporter, cuticle, cuticular wax, drought resistance, inclusion

The plant cuticle, a cutin matrix embedded with and covered by wax, seals the aerial organ’s surface to protect the plant against uncontrolled water loss. The cutin matrix is essential for the cuticle to function as a barrier to water loss. Recently, we identified from wild barley a drought supersensitive mutant, eibi1, which is caused by a defective cutin matrix as the result of the loss of function of HvABCG31, an ABCG full transporter. Here, we report that eibi1 epidermal cells contain lipid-like droplets, which are supposed to consist of cutin monomers that have not been transported out of the cells. The eibi1 cuticle is fragile due to a defective cutin matrix. The rice ortholog of the EIBI1 gene has a similar pattern of expression, young shoot but not flag leaf blade, as the barley gene. The model of the function of Eibi1 is discussed. The HvABCG31 full transporter functions in the export of cutin components and contributed to land plant colonization, hence also to terrestrial life evolution.

Inclusions in eibi1 Mutant Epidermal Cells

The drought-hypersensitive wild barley’s (Hordeum spontaneum Koch) naturally occurring mutant eibi1 suffers from a particularly severe level of water loss and displays a defective cuticle with reduced cutin deposition (~50% of the wild type) and a thin cuticle (~25% of the wild type) and a similar amount of the major wax component, 1-hexacosanol.2 Protrusions of cytoplasm into the vacuole were a specific feature of elongation-zone epidermis cells in eibi1 leaves. These protrusions may be caused by the inclusions of cutin monomers failed to be secreted. When the cells become as large as mature cells in the non-elongation zone and the emerged blade, the inclusions may remain in cytoplasm as indicated by lipid-like droplets in the epidermis (Fig. 1A). The lipid-like droplets were not found in wild-type epidermis (Fig. 1B) since no protrusions or inclusions exist in wild-type epidermis cells. Similar protrusions have been noted in the stem epidermal cells of the Arabidopsis thaliana atabcg11 and atabcg12 mutants.3,4 These mutants are unable to export cutin and wax from the epidermis cells, leading to an accumulation of intracellular lipid, which is responsible for the formation of protrusions. In Arabidopsis pec1 mutant, the eibi1 ortholog, the inclusions in petals observed by TEM are also observed by light microscopy with nile red staining.8

Fragile eibi1 Cuticle

A key role for EIBI1 is in cutin matrix formation. The EIBI1 protein was detected exclusively in the elongation zone where the cutin matrix was formed.2 The reduced cuticle thickness correlated well with the reduced amount of cutin in eibi1 mutant leaves. A fragile cuticle was observed in eibi1 leaves (Fig. 1C). The eibi1 cuticle was broken while the wild-type cuticle was kept intact during the process of sample preparation for SEM analysis (Fig. 1D). The thin and fragile cuticle might explain the excess water loss in eibi1 mutant leaves. Many Arabidopsis cutin mutants such as bdg, dcr, att1 and lacs2 have a disorganized cutin matrix and display increased cuticle permeability. A few cutin mutants in monocot species, such as the Sorghum bicolor bm2 (previously named bm22) mutants, show a reduced cuticle thickness and increased water loss.9,10 The rice wdl1 mutant shows increased water loss from wdl1 mutant leaves, which is associated with loose packing of the cuticle and an irregular thickness of the cell wall. These evidences demonstrate that a functional cuticle is required for water retention.

Expression of the Eibi1 Orthologous Gene from Rice

The Eibi1 gene was highly expressed in the elongation zone of the growing leaf (the site of cutin synthesis), and its gene product
These results indicate that the Eibi1 expression pattern is highly conserved not only in monocot but also in dicot plants.

A Model of Eibi1 Function

The deposition of cutin and wax are under independent control in monocots during leaf emergence. Eibi1 is hypothesized to function as a transporter involved in the secretion of cutin monomers or oligomers in elongating epidermal cells in a young growing leaf (Fig. 3). Cutin monomers or oligomers are transported out of the cells and across the cell wall to form a cutin matrix. A functional cuticle is synthesized by filling and covering the cutin matrix with waxes. In eibi1 mutant leaves, the cutin monomers or oligomers appear as inclusions in epidermis because they are not transported out of the cells when there is loss of function of eibi1, which leads to a thin and fragile cuticle. Therefore, eibi1 mutant leaves are unable to retain water as effectively as wild type can. Eibi1 encodes an ABCG full transporter. There are three half ABCG transporters that have been identified in Arabidopsis, AtABCG11, AtABCG12 and AtABCG13. ABCG11 is required for the export of cutin precursors as well as wax molecules.6-7 ABCG12 is required for the secretion of cuticular wax.3 ABCG13 is involved in cutin formation in flowers.11 However, ABCG32, the Eibi1 ortholog in Arabidopsis, has a function in the export of cuticular components distinct from the three half transporters.8 Homologs of HvABCG31 were found in green algae, moss and lycopods, indicating that this full transporter is highly conserved in land plants thereby contributing to land plant colonization and evolution.

Acknowledgments

This work was supported by the “One Hundred Talents” Project of the Chinese Academy of Sciences (O827751002), the National Natural Science Foundation of China (30970449, 31170369), Genomics for Agricultural Innovation Grant TRG1004 from the Ministry of Agriculture, Forestry and Fisheries of Japan, post-doctoral Grant P10511 from the Japan Society for the Promotion of Science, the Ancell Teicher Research Foundation for Genetics and Molecular Evolution.

References


**Figure 3.** Putative model of Eibi1 function in barley.