

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

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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.³⁻⁷ 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

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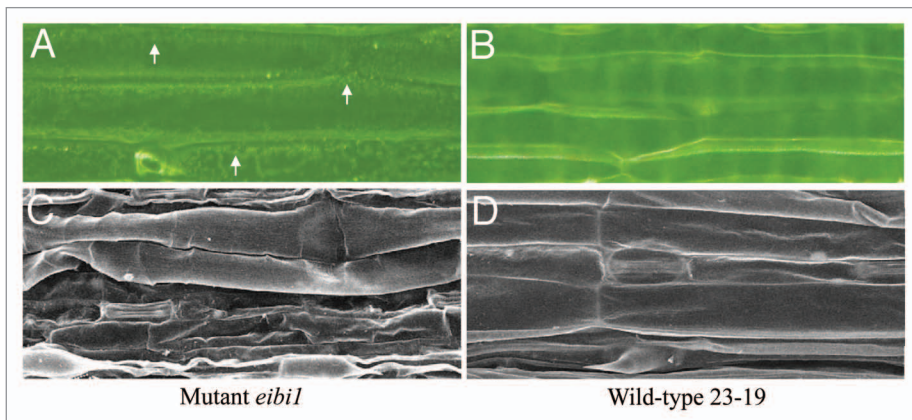


Figure 1. Light microscope (A and B) and scanning electron microscope (C and D) images of emerged leaf blades of *eibi1* mutant (A and C) and wild type (B and D).

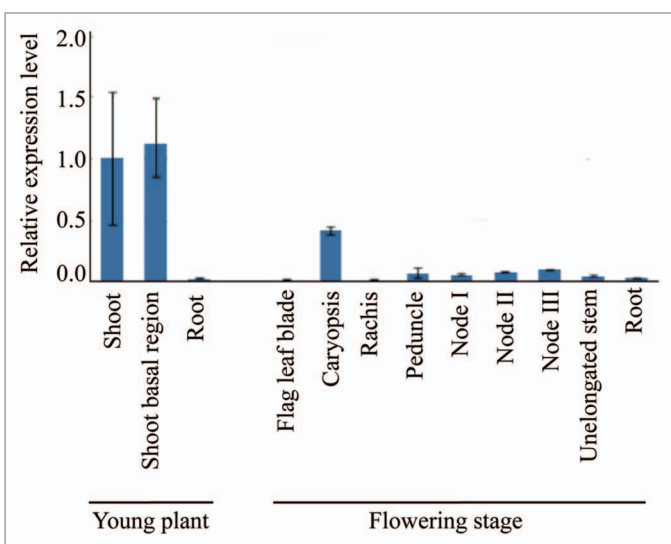


Figure 2. Quantitative RT-PCR analysis of *Eibi1* gene expression in different organs of rice plants.

was also localized in developing, but not in mature tissue in barley.² Similarly, the analysis of *Eibi1* expression in rice showed the presence of abundant transcripts in young shoot, but only traces in flag leaf blade, rachis and root (Fig. 2). The gene was also expressed in young caryopsis, peduncle, nodes and unelongated stem. *AtABCG32/Pec1*, the closest *Eibi1* homolog in Arabidopsis, is expressed in all organs of the shoot with a tendency toward higher expression levels in young, expanding tissues than in older

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ones.⁸ 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 synthesised 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.⁴⁻⁷ 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.

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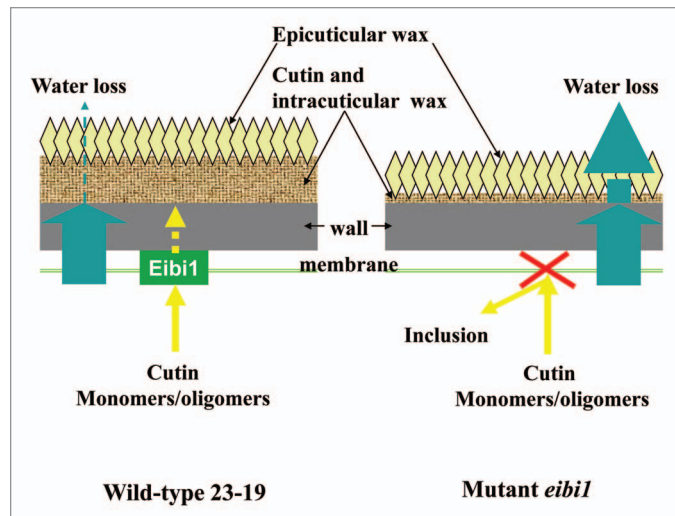


Figure 3. Putative model of Eibi1 function in barley.