Arabidopsis GLASSY HAIR genes promote trichome papillae development

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Abstract

Specialized plant cells form cell walls with distinct composition and properties pertinent to their function. Leaf trichomes in Arabidopsis form thick cell walls that support the upright growth of these large cells and, curiously, have strong light-reflective properties. To understand the process of trichome cell-wall maturation and the molecular origins of this optical property, mutants affected in trichome light reflection were isolated and characterized. It was found that GLASSY HAIR (GLH) genes are required for the formation of surface papillae structures at late stages of trichome development. Trichomes in these mutants appeared transparent due to unobstructed light transmission. Genetic analysis of the isolated mutants revealed seven different gene loci. Two—TRICHOME BIREFRINGENCE (TBR) and NOK (Noeck)—have been reported previously to have the glassy trichome mutant phenotype. The other five glh mutants were analysed for cell-wall-related phenotypes. A significant reduction was found in cellulose content in glh2 and glh4 mutant trichomes. In addition to the glassy trichome phenotype, the glh6 mutants showed defects in leaf cuticular wax, and glh6 was found to represent a new allele of the eceriferum 10 (cer10) mutation. Trichomes of the glh1 and glh3 mutants did not show any other phenotypes beside reduced papillae formation. These data suggest that the GLH1 and GLH3 genes may have specific functions in trichome papillae formation, whereas GLH2, GLH4, and GLH6 genes are also involved in deposition of other cell-wall components.

Key words: Cell wall, light reflection, papillae, trichomes.

Introduction

Plant cell walls perform a variety of essential functions, including mechanical support of individual cells and plant organs, protection against pathogens and abiotic environmental damage, and control of anisotropic cell expansion. These functions depend on physical and chemical properties of cell-wall components that provide strength and plasticity, form a protective surface, and recognize and transduce signals (Carpita and Gibeaut, 1993; Braam, 1999; Jones and Takemoto, 2004; Vorwerk et al., 2004; Ellis et al., 2010). It is estimated that about 10% of plant genes are devoted to synthesis, remodelling, or turnover of cell-wall components (McCann and Rose, 2010).

Although many genes involved in cell-wall metabolism have been identified, there are still many gaps in our understanding of cell-wall formation, including coordination of different biochemical activities, temporal and spatial regulation of biosynthetic processes, targeted deposition of wall components, and three-dimensional organization. Also the generic model of cell-wall organization and composition is not universal, and cell walls are strongly divergent depending on the type of differentiated cells (Keegstra, 2010). Analysis of cell-wall formation in different cell types is important to uncover developmental control mechanisms of cell-wall formation and to reveal molecular processes underlying natural variation in cell-wall organization.

Abbreviations: CAPS, cleaved amplified polymorphic sequences; EMS, ethyl methanesulfonate; MS, Murashige and Skoog; TB, toluidine blue.

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Arabidopsis trichomes are specialized leaf epidermal cells that grow up to 0.5 mm in height and form a cell wall of an impressive 1 μm thickness (Marks et al., 2008). Large trichome size, stereotypic cell shape, accessibility for macroscopic observations, and dispensability for plant growth have facilitated the identification and functional characterization of >40 genes with loss-of-function trichome phenotypes (Marks et al., 2009). Genetic screens for trichome phenotypes have been instrumental in identification of genes involved in trichome development, as well as genes with essential functions in plant growth and development.

Arabidopsis trichomes provide an excellent model system to study the cell wall. Histochemical staining, transmission electron microscopy, and monosaccharide analyses have indicated that trichome cell walls are rich in pectin and cellulose, contain lignin and mannos-containing polysaccharides, and are covered with cuticular wax (Marks et al., 2008). Transcriptome analysis studies have shown that genes involved in cell-wall function, biosynthesis, and structure are expressed at high levels in trichomes (Jakoby et al., 2008; Marks et al., 2009).

One peculiar feature of a mature trichome cell wall is the presence of primary cell-wall characteristics, including high pectin content (Marks et al., 2008) and activity of primary-wall CesA genes (Betancur et al., 2010). Consequently, mutations in secondary-wall CesA genes had no effect on thickening or birefringence of trichome walls (Betancur et al., 2010). On the other hand, similar to other cells, trichome secondary-cell-wall thickening involves the formation of crystalline cellulose microfibrils that can be detected with polarized light as light birefringence (Potikha and Delmer, 1995; Nishikawa et al., 2008; Betancur et al., 2010). This mixture of primary and secondary cell-wall features in Arabidopsis trichomes provides an opportunity to uncover essential mechanisms of primary cell-wall formation, as well as to analyse processes important for the secondary cell wall, such as cellulose organization, lignin formation, and cuticular wax deposition.

Another peculiarity of trichome cell walls is the formation of surface papillae. Papillae form as subcuticular depositions of paracrystalline cellulose microfibrils that can be detected with polarized light as light birefringence (Potikha and Delmer, 1995; Nishikawa et al., 2008; Betancur et al., 2010). This mixture of primary and secondary cell-wall features in Arabidopsis trichomes provides an opportunity to uncover essential mechanisms of primary cell-wall formation, as well as to analyse processes important for the secondary cell wall, such as cellulose organization, lignin formation, and cuticular wax deposition.

Genetic characterization of mutants

Mutants with transparent leaf trichomes were isolated using ×10 magnification in a stereomicroscope. M2 sibling families collected from 2400 individually harvested ethyl methanesulfonate (EMS)-mutagenized Col-0 plants were screened. Mutants with glassy phenotypes were crossed with each other for complementation tests. To create a double mutant of hgd2 and gh3, which have indistinguishable glassy phenotypes and similar papillae defects, we screened the F2 generation for plants with glassy phenotype that were hemizygous for a T-DNA insertion in the HDG2 gene. Double mutants were selected in the F3 generation using PCR to identify segregating hgd2 homozygous plants with primers flanking the T-DNA insertion in the HDG2 gene: HDG2-LP1′, 5′-CACTCCAGCGCAGCCCTAATA-3′, and HDG2-RP2′, 5′-CTCGTTCCACACTGAAGATCTTGG-3′. The hgd2 gh3 double-mutant genotype was verified by crossing with both parent mutant plants.

For genetic mapping, gh3 mutants were crossed with Ler plants. Plants with a glassy phenotype were selected in F2 populations. Genomic DNA was isolated from 20–50 F2 plants. The map positions of the glassy mutations were determined relative to simple sequence length polymorphisms and cleaved amplified polymorphic sequence markers (CAPS) markers from the Arabidopsis Information Resource (TAIR) database (http://www.arabidopsis.org/servlets/Search?action=new_search&type=marker), or using newly designed CAPS and derived CAPS (dCAPS) markers (dCAPS finder 2.0 program; http://helix.wustl.edu/dfinder/dfinder.html) based on single-nucleotide polymorphisms between Col-0 and Ler found at the TAIR webpage (http://www.arabidopsis.org/).

Trichome isolation

To ensure that plant material was from the same developmental stage, fully expanded rosette leaves were collected from plants that had not bolted but already had flower buds. Trichomes were isolated...
from these leaves using a previously described method (Marks et al., 2008). After isolation, trichomes were mixed thoroughly in PBS buffer. Three replicates of 10 μl each were pipetted from the suspension and trichomes were counted to estimate cell density. An estimated 2000 trichomes were aliquotted for trichome cellulose testing experiments.

Cellulose quantification

Relative cellulose amounts were quantified using the Updegraff method (Updegraff, 1969) as follows. Prior to collection of material, plants were placed in the dark for 60–72 h to reduce starch content. One fully expanded leaf of 4-week-old plants was used for leaf cellulose quantification. Leaves were incubated in 70% ethanol at 98 °C, dried in acetone, and their weight measured. To remove soluble contents, leaves and isolated trichomes were placed in 300 μl of acetic-nitric reagent at 98 °C for 30 min. The plant material was then washed in water followed by two acetone washes. For the trichome cellulose detection experiment, trichome numbers in each tube were determined. Dried plant material was treated with 30 μl of 67% H2SO4 and vortexed to dissolve the samples. Next, 150 μl of water was added and mixed thoroughly, and the samples were placed on ice. Anthrone reagent (Acros Organics) was prepared on the day of the experiment, and 300 μl was added to each tube, mixed, and heated at 98°C for exactly 5 min, and then transferred back to ice to stop the reaction. For leaf cellulose testing, 100 μl of the above solution was diluted with 700 μl of 67% H2SO4. For trichome cellulose testing, 150 μl of the solution was diluted with 650 μl of 67% H2SO4. Absorbance at a wavelength of 620nm was measured using a spectrophotometer.

Microscopy and histology

For trichome opacity determination, fully expanded leaves of 3-week-old plants were mounted on a small drop of water and covered with a cover slip. Images were taken from trichomes positioned on the leaf edge so that the light was transmitted only through the trichome and not though the leaf. Trichomes with stronger light reflection formed a darker image. ImageJ software was used to measure the light differential between trichomes and the background. All images that compared mutants with wild type were taken under identical light and camera settings.

Before observing isolated trichomes by polarized light microscopy, trichomes were pre-treated with an acetic-nitric reagent. This treatment removes cuticular waxes, as they form crystalline structures (Koornneef et al., 1989; Haslam et al., 2012) and can potentially interfere with birefringence from crystalline cellulose. Trichomes were placed on a microscope slide between the crossed polarizer and analyser. Samples were rotated relative to polarization filters to allow the maximum amount of light to be transmitted, and images were acquired with a CCD camera.

For quantitative analysis of trichome birefringence, mean pixel grey values for the middle part of trichome branches were measured and background values were subtracted.

For scanning electron microscopy trichome imaging, fully expanded leaves from 3-week-old plants were used. Images were taken under low-vacuum at 0.98 torr pressure, 12.5 kV, and 3.0 μm pore size. Plant tissue was placed on the stage cooled to 2 °C. Magnification of ×200 was used for whole-trichome images, ×800 for papillae density, and ×250 for papillae coverage.

Toluidine blue (TB) staining of cell-wall polysaccharides was performed as described previously (Tanaka et al., 2004). Plants were grown on MS plates for about 2 weeks, and 0.05% (w/v) solution of filtered (0.2 μm pore size) Toluidine Blue O (Sigma) aqueous solution was then poured into the plates until the plants were submerged. The TB solution was removed after 2 min, and plates were washed with water to remove the residual TB.

In all statistical tests, a Mann–Whitney non-parametric two-tailed test was used to determine the significance of measured differences.

Results

Isolation of glassy hair mutants

To uncover genes involved in trichome cell-wall maturation, we screened EMS-mutagenized populations of Arabidopsis for plants with glassy-appearing trichomes of normal size and morphology. These selection criteria focused our screen on identification of genes with a function during the trichome maturation phase, as opposed to genes required for progression through earlier stages of trichome development. We isolated ten glh mutants from the 2400 M2 sibling families of individually harvested EMS-mutagenized Col-0 plants. Crossing into the Col-0 wild type revealed that all glh mutants segregated with a 1:3 (mutant:wild type) ratio in the F2 generation, consistent with recessive and monogenic mutations.

These mutants were divided into three groups based on their trichome shape. The first group of six mutants had glassy trichomes of wild-type size and shape. Complementation tests within this group showed that the mutants were affected in five different genes: GLH1, GLH2, GLH3, GLH4, and GLH5.

To test for genetic complementation with previously described glassy mutants with wild-type trichome shape and size, mutants of the first group were crossed with tbr (Potikha and Delmer, 1995) and with hdg2 (Marks et al., 2009) mutants. The glh5 mutation did not complement the tbr mutation, suggesting that it represents a new allele of this mutant. Hence, we renamed glh5 as tbr-2. Curiously, we found that mature trichomes collapsed in the tbr-2 mutants (Fig. 1A), suggesting that trichome cell walls could not support the upright stature of these cells. We could also find occasional collapsing trichomes in the original tbr mutants, suggesting that tbr-2 is a stronger loss-of-function allele of the TBR gene.

The second group contained three mutants that showed variable phenotypes, ranging from wild-type-like to small swollen trichomes with rounded tips (Fig. 1A and 2F). Genetic complementation tests revealed that all three mutants were affected in the same gene, which we named GLH6.

One glassy mutant had trichomes of wild-type size with increased branch number, resembling the nok mutant (Folkers et al., 1997; Jakoby et al., 2008). Genetic cross with the nok-122 mutant allele did not show complementation, suggesting identification of a new nok allele, which we named nok-2.

Taken together, genetic analysis suggested that the GLH1, GLH2, GLH3, GLH4, and GLH6 loci represent separate gene loci with unreported functions during trichome cell-wall maturation.

The glassy trichome phenotype correlates with papillae development defects in glh mutants

We hypothesized that the visually perceived glassiness of trichomes reflects increased transparency of the cells. To test this hypothesis we measured the intensity of light transmitted through wild-type and glh mutant trichomes.

Trichomes in wild-type plants appeared darker and we measured higher light differentials between trichomes and the background light, indicating higher opacity than in glh mutants (Fig. 1A–C). The lowest light differentials were found
in glh1, glh2, and tbr mutant trichomes, correlating with the stronger glassy appearance of the mutants. Trichomes of glh3, glh4, and glh6 mutants appeared less glassy under the stereomicroscope and were more opaque than glh1 and glh2 trichomes (Fig. 1A, C).

It has been hypothesized that a reduction in papillae causes the glassy trichome phenotype (Jakoby et al., 2008). Papillae are numerous round-shaped bumps on trichome walls that can increase light scattering due to an uneven cell surface. In their absence, trichome walls will be smoother and scatter less light. Previous studies showed that nok, gl3-sst, and hdg2 mutants with a glassy appearance of trichomes formed fewer trichome papillae (Esch et al., 2003; Jakoby et al., 2008; Marks et al., 2009).

We found that all glh mutants were defective in papillae formation (Fig. 2A–F). To quantify the defects, we measured papillae density and coverage of trichome branches in glh mutants (Fig. 2G–J).

On average, glh1 trichomes formed 1.6 papillae per 100 μm² trichome area, and glh2 trichomes formed 1.3 papillae. This corresponds to a 5 fold (for glh1) and a 6 fold (for glh2) reduction compared with wild type (7.85 papillae per 100 μm²). The less transparent glh3 and glh4 mutants formed 3.6 and 6.5 papillae 100 per μm² corresponding to a 2.2- and a 1.3-fold reduction in density compared with the wild type.

The density of papillae correlated strongly with trichome opacity (Fig. 2H). The most transparent glh1 and glh2 trichomes showed the strongest defects in papillae development, while the weaker glassy mutants glh3 and glh4 showed a moderate decrease in papillae density and coverage.

The papillae coverage of glh1 trichome branches was decreased by 55% compared with that of the wild type. Underdeveloped papillae in glh2 were not discernable in ×250 magnification scanning electron microscopy images, precluding us from measuring papillae coverage in the mutant. Papillae coverage was decreased by 13% in the glh3 mutant and by 39% in the glh4 mutant, and this difference was statistically significant for the glh4 mutant (Fig. 2I). In addition to defects in papillae density and coverage, the glh1, glh2, glh4,
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and glh6 mutants formed less-developed flattened papillae (Fig. 2B′, C′, E′, F′).

Papillae density and coverage showed strong variation in glh6 mutant trichomes (Fig. 2F, G, J). This mutant also showed high variation in trichome branching and size (Supplementary Figs S1 and S2, and Supplementary Table S1 at JXB online) suggesting that papillae formation defects in the glh6 mutant trichomes may be an indirect consequence of developmental arrest at various stages.

Weaker papillae defects in the glh3 mutant resembled those of the hdg2 glassy trichome mutant, where papillae develop at a low density (Marks et al., 2009). To test the genetic relationships between these two weak glassy mutants, we created double mutants and inspected their trichome papillae. Compared with single mutants, trichomes of hdg2 glh3 double mutants had more-severe papillae defects, forming fewer papillae (Fig. 3). This additive double-mutant phenotype suggested that the HDG2 and GLH3 genes promote papillae formation independently and that the GLH3 gene is not a probable target of the HDG2 transcription factor.

Taken together, our data suggested that GLH genes promote papillae development and that papillae confer light-reflective properties on trichomes.

### Trichomes of glh2 and glh4 mutants deposit less cellulose

The glassy trichome mutants tbr and cpr5 have been shown to have reduced birefringence and cellulose deposition (Potikha and Delmer, 1995; Brininstool et al., 2008; Bischoff et al., 2010).

To test whether glh mutations affected birefringence, which is caused by highly ordered crystalline cellulose in plant cell walls, we used polarized light microscopy. Trichomes of the tbr-2 mutant were used as a control and showed the strongest birefringence defect. Birefringence was also visibly reduced in glh2 and glh4 but not in glh1, glh3, and glh6 mutants (Fig. 4A, B). These observations suggested that cellulose deposition in trichomes may be affected by glh2 and glh4 mutations.

To test whether GLH genes play a role in cellulose deposition we quantified relative cellulose amounts in the trichomes...
and leaves of glh mutants using a modified Updegraff method (Updegraff, 1969) and by normalizing the trichome cellulose amounts by adjusting to the numbers of isolated trichomes. As the glh1, glh2, glh4, and glh6 mutants grew and developed slower than the wild type (Supplementary Fig. S2), leaves of the same developmental stage (rosette plants at the beginning of bolting) were used for trichome isolation. The tbr mutant, which was shown previously to have less cellulose in trichomes (Bischoff et al., 2010), was used as a control. In our experiments, tbr trichomes contained 34% of the wild-type cellulose level (Fig. 4C), which is similar to previously published data. We did not detect a significant change in trichome cellulose in glh1 and glh3 mutants. However, glh2 and glh4 mutants showed a significant reduction in trichome cellulose, containing 64 and 59% of the wild-type levels, respectively (P<0.01 for both mutants). Trichomes of the glh6 mutant showed a moderate reduction in cellulose to 84% of the wild-type content, but this difference was not statistically significant (P=0.08).

The observed cellulose reduction in glh2, glh3, and glh6 trichome could be caused either by decreased cellulose content in trichome walls or by a decrease in trichome size. To distinguish between these two possibilities, we measured trichome length in all glh mutants. We found that glh mutations did not affect trichome size, except for the glh6 mutant trichomes, which showed a 31% reduction in combined length of trichome stem and branches (Supplementary Fig. S1). This suggested that the observed cellulose reduction in glh6 trichomes could, at least partially, be due to a decrease in trichome size. Conversely, we concluded that the GLH2 and GLH4 genes promote cellulose deposition in trichome cell walls.

Measurements of the cellulose content in leaves did not show significant changes in glh mutants (Fig. 4D), suggesting that the GLH2 and GLH4 genes primarily affect cellulose deposition in trichomes.

The glh6 mutant is affected in cuticle formation

The outer covering layer of the epidermal cell wall, the cuticle, has light-reflective properties, and mutants defective in cuticle formation, such as ecferiferum, appear bright green due to reduced light scattering by the cell surface (Koornneef et al., 1989; Rashotte et al., 2004). To test cuticle integrity in glh mutants, we stained leaves with TB dye, which binds to cell-wall polysaccharides but cannot permeate the intact cuticle layer (Tanaka et al., 2004). We found that glh6 mutant trichomes and leaves were stained with TB (Supplementary Fig. S3 at JXB online), suggesting a role for GLH6 in cuticle formation. The determined chromosomal interval of the glh6 mutation (Fig. 5) contains the ECERIFERUM10 (CER10) gene, which was shown previously to be involved in cuticle formation (Zheng et al., 2005). Therefore, we performed a complementation test between the glh6 and cer10-2 mutants. No complementation was found, suggesting that glh6 is a new cer10 allele.

The GLH1, GLH2, GLH3, and GLH6 genes are involved in trichome branching

In addition to glassy cell walls, trichomes in some of the glh mutants showed cell morphogenesis defects. On average, trichomes on glh1, glh2, and glh6 leaves formed fewer branches. Compared with wild-type leaves with 79% three-branched and 21% four-branched trichomes, glh1 and glh2 mutants had 1 and 3% two-branched trichomes, respectively, 1% four-branched trichomes, and the majority (96–97%) were...
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three-branched (Supplementary Fig. S2 and Supplementary Table S1). In contrast, glh3 mutants formed more trichome branches exhibiting 29% four-branched and 1% five-branched trichomes.

Although on average glh6 mutant trichomes formed fewer branches, we also observed trichomes forming with up to five branches (Supplementary Fig. S2). This suggests that the GLH6 gene is important for formation of trichomes with a typical number of three or four branches, having functions in both promoting branching and suppressing overbranching.

GLH gene mapping

To confirm that glh mutants represented distinct genetic loci, we determined the chromosomal position of the GLH genes using PCR-based markers (Lukowitz et al., 2000). In the first step, GLH genes were positioned on chromosomes using bulked segregant analysis. Narrow intervals were then determined for each of the five GLH genes (Fig. 5). Analysis of the TAIR database and Arabidopsis loss-of-function mutant dataset (Lloyd and Meinke, 2012) did not reveal any genes with a reported glassy mutant phenotype in the indentified intervals. This suggested that the GLH1, GLH2, GLH3, and GLH4 loci are not yet functionally characterized or, as is the case for the GHL6/CER10 gene, the trichome cell-wall phenotypes have not been reported.

Discussion

Trichome cells deposit large amounts of cell-wall material, forming conspicuously thick cell walls (Marks et al., 2008). Most of the cell-wall material is deposited during the cell-wall maturation phase (Szymanski et al., 1998), after trichomes have formed branches and finished radial expansion.

In mutants, such as gl2, midbin4, and cpr5, trichome growth and development stops at early stages and trichomes appear glassy (Kirik et al., 2007; Brininstool et al., 2008; Marks et al., 2009). In the gl3-sst mutant, where trichomes are not reduced in size but show early developmental arrest, trichomes also appear glassy and form fewer papillae on their cell walls (Esch et al., 2003). Thus, the glassy trichome phenotype may be caused by developmental arrest before the trichome cell-wall maturation phase.

In this EMS-mutagenesis screen, we selected glassy trichome mutants with no strong changes in trichome size or branch number. Therefore, GLH genes isolated here are likely to function at the last step in trichome development and may promote the transition from the stage of diffuse growth (stage 5) to the cell-wall maturation stage (stage 6) (Szymanski et al., 1998). Alternatively, rather than having developmental roles, GLH genes may be involved in metabolism or deposition of trichome cell-wall components.
GLH2, GLH4, and GLH6 genes have pleiotropic functions in trichome cell-wall maturation

Trichome cell walls in glh2 and glh4 mutants displayed distinct papillae defects and accumulated less cellulose: glh2 trichomes formed almost no papillae, whereas glh4 mutants formed smaller papillae at a normal density. Trichome length and branching were not strongly affected in these mutants, suggesting that trichome development was normal up to the cell-wall maturation stage. The glh4 mutant phenotype was more pleiotropic and plants were strongly stunted in growth. Phenotypic differences between these mutants suggested that the GLH2 and GLH4 genes function in separate pathways that regulate papillae formation and trichome cellulose deposition.

Both trichome cellulose deposition and papillae formation depend on the GLH2 function. A similar phenotype is caused by the Arabidopsis tbr mutation (Potikha and Delmer, 1995). Both mutants show reduced birefringence and cellulose content in trichomes (Fig. 4A–C) (Potikha and Delmer, 1995; Bischoff et al., 2010). TBR and its homologous TBR-like (TBL) genes encode putative transmembrane proteins with a plant-specific DUF231 domain. Although the molecular function of the TBR protein is not known, it was suggested that it might support secondary cell-wall formation by promoting pectin methylesterification, as etiolated hypocotyls of tbr and tbl3 mutants had increased pectin methylesterase activity and showed reduced pectin esterification (Bischoff et al., 2010). Pectins are present in large amounts in trichome cell walls (Marks et al., 2008) and may play a
role in cellulose deposition, as suggested by their binding to cellulose (Zykwinska et al., 2005). Thus, it is conceivable that reduced cellulose content in tbr mutants may be a secondary consequence of deficiency in non-cellulosic cell-wall polysaccharides. Previous data indicate complex relationships between components of the cell wall, so that changes in deposition, structure, or modification of one cell-wall component often affected others. For example, it has been shown that the IXR8/GAUT12 gene, which regulates production of glucoronoxylan and homogalacturonan cell-wall polysaccharides, is important for anisotropic cell expansion and cellulose content (Persson et al., 2007). Conversely, cellulose deposition can influence papillae formation, as several mutants deficient in cellulose production exhibited increased pectin contents (His et al., 2001). No members of the TBR/TLB family are present in the GLH2 mapping interval on chromosome 4, and therefore it is unlikely that the GLH2 gene belongs to this family. Identification of the GLH2 gene together with detailed phenotypic analysis of the mutant cell wall will address the relationship between the GLH2 and TBR genes and will help to elucidate molecular mechanisms regulating both papillae and cellulose deposition during trichome cell-wall maturation.

Trichomes on glh6 mutants had fewer papillae and were variable in size, branch number, and branch shape, often forming rounded branch tips, indicating arrest at stages 3, 4, 5, and 6 of trichome development (Szymanski et al., 1998). This variation in phenotype was observed even within one leaf, with less-developed trichomes showing a stronger glassy phenotype. Mapping the glh6 mutation placed it into the 1.9 Mbp interval on the lower arm of chromosome 3. This interval contains the CER10 gene, which shows a similar loss-of-function phenotype with the glh6 mutant. Both mutant plants have stunted growth, produce sterile flowers, deposit less cuticular wax, and occasionally have fused trichomes when grown in high humidity (Supplementary Fig. S3B; Zheng et al., 2005). Our complementation tests revealed that glh6 is a new cer10 allele. Although the glassy trichome phenotype was not described for the cer10 mutant, the glh6 glassy phenotype is relatively weak and not uniform among the trichomes. Observed variability of glh6 mutant trichomes may explain why the glassy phenotype has not been reported for the cer10 mutants. The CER10 gene encodes the enoyl-CoA reductase, which is required for very-long-chain fatty acid synthesis (Zheng et al., 2005). It has been suggested that altered composition of very-long-chain fatty acid sphingolipids in cer10 mutants causes pleiotropic developmental changes.

Trichome papillae formation and function

Targeted cell-wall deposition events leading to formation of trichome papillae, molecular composition, and functions of papillae are not understood. To uncover the molecular mechanisms of cell-wall maturation in trichomes, genes involved in papillae formation need to be identified and characterized.

Trichomes of the glh1 mutant showed wild-type levels of cellulose and normal birefringence. At the same time, the glh1 trichomes had lost most of the surface papillae. This suggests that papillae development and formation of crystalline cellulose are independent of each other. However, these two processes are likely to share common upstream pathways that involve the TBR and GLH2 genes, as tbr and glh2 papillae-less mutants also have reduced cellulose levels in trichomes.

Hemicelluloses have been linked to papillae formation in several studies. Double mutants in the functionally redundant XXT1 and XXT2 genes, encoding xylosyltransferases, displayed collapsed papillae on their trichomes (Briggs et al., 2006; Cavalier et al., 2008). The papillae also appear wrinkled and collapsed on mnr2 and mnr3 trichomes (Vanzin et al., 2002; Madson, 2003). The MUR2 gene accounts for all of the xyloglucan fucosyltransferase activity in Arabidopsis, while MUR3 acts as a galactosyltransferase targeting the third xylose residue in the XXXG xyloglucan core structure. Scanning electron microscopy images of the trichome surface have shown that the papillae phenotype is less pronounced in mnr2 and mnr3 mutants than in glh mutants (Madson, 2003). Thus, the relatively weak papillae phenotype in the hemicellulose mutants suggests that hemicelluloses provide a minor contribution to trichome papillae formation.

Trichomes on glh1 and glh3 mutants displayed strong papillae defects, affecting both density and coverage of the trichome surface (Fig. 2). These mutants did not show any alterations in cellulose and cuticular wax. This suggests papillae-specific functions for the GLH1 and GLH3 genes during trichome cell-wall maturation. These genes may be required for synthesis or deposition of papillae components. Identification of GLH1 and GLH3 genes may uncover molecular mechanisms of trichome papillae formation, composition, and function.

Conclusions

Just as roughening of clear glass turns it into frosted glass by introducing surface irregularities, trichome papillae introduce surface non-uniformities that can scatter light. Indeed, all glh mutants displayed trichome defects in papillae, forming fewer or less-developed papillae. This suggests that the trichome glassy phenotype is caused by reduced light reflection due to diminished scattering on a smooth trichome surface of papillae-less glh trichomes. As a result, more light can pass through the glh trichomes (Fig. 1C).

Our data indicated that papillae increase light reflection of trichomes. This optical property may play an important role in previously suggested trichome functions in leaf temperature regulation (Dell and McComb, 1979; Klick, 2000), in protection against UV-B radiation (Manetas, 2003), and in protection of the photosystem II in young leaves against excessive light (Karabourniotis et al., 1992; Karabourniotis et al., 1993). The glh mutants provide a tool to address physiological functions of trichome papillae in future studies. Identification of the GLH genes will provide an insight into mechanisms of localized deposition of materials on the outer cell-wall surface and will expand our understanding of the cellulose deposition mechanisms in trichome cells.
Supplementary data

Supplementary data are available at JXB online.

Supplementary Table S1. Trichome branching in glh mutants.

Supplementary Fig. S1. Relative trichome length of glh mutants compared to wild type.

Supplementary Fig. S2. Effects of glh mutations on plant development.

Supplementary Fig. S3. glh6 mutants have cuticle defects.

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References


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