Running Title: Trichome, cuticle and systemic immunity

* Correspondence to:
Pradeep Kachroo
Department of Plant Pathology
University of Kentucky
Lexington, KY, 40546
Email: pk62@uky.edu;
Phone: 859-257-7445 x 80729; Fax: 859-323-1961
The *glabra1* mutation affects cuticle formation and plant responses to microbes

Ye Xia¹, Keshun Yu¹, Duroy Navarre², Kenneth Sebold¹, Aardra Kachroo¹, Pradeep Kachroo¹,*

¹ Department of Plant Pathology, University of Kentucky, Lexington, KY 40546
² U.S. Department of Agriculture—Agricultural Research Service, Washington State University, Prosser, WA 99350

* Correspondence to: Email: pk62@uky.edu; Phone: 859-257-7445 x 80729; Fax: 859-323-1961

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Abstract

Systemic acquired resistance (SAR) is a form of defense that provides resistance against a broad-spectrum of pathogens in plants. Previous work indicates a role for plastidal glycerolipid biosynthesis in SAR. Specifically, mutations in *FATTY ACID DESATURASE 7* (*FAD7*), which lead to reduced trienoic FA levels and compromised plastidal lipid biosynthesis, have been associated with defective SAR. We show that the defective SAR in *fad7-1* plants is not associated with a mutation in *FAD7*, rather a second-site mutation in *GLABRA* (*GL*) 1, a gene well-known for its role in trichome formation. The compromised SAR in *gl1* plants is associated with impairment in their cuticles. Furthermore, mutations in two other components of trichome development, *GL3* and *TRANSPARENT TESTA GLABRA 1*, also impaired cuticle development and SAR. This suggests an overlap in the biochemical pathways leading to cuticle and trichome development. Interestingly, exogenous application of gibberellic acid (GA) not only enhanced SAR in wt plants, but also restored SAR in *gl1* plants. In contrast to GA, the defense phytohoromes salicylic acid or jasmonic acid were unable to restore SAR in *gl1* plants. GA application increased levels of cuticular components but not trichome formation on *gl1* plants, thus, implicating cuticle, but not trichomes, as an important component of SAR. Our findings question the prudence of using mutant backgrounds for genetic screens and underscore a need to reevaluate phenotypes previously studied in the *gl1* background.
INTRODUCTION

Plants have evolved a large array of defense mechanisms to resist infection by pathogens. Upon recognition, the host plant initiates one or more signal transduction pathways that activate various plant defenses and thereby prevent pathogen colonization. In many cases, resistance is associated with increased expression of defense genes, including the pathogenesis-related (PR) genes and the accumulation of salicylic acid (SA) in the inoculated leaf. Induction of these responses is accompanied by localized cell death at the site of pathogen entry, which can often restrict the spread of pathogen to cells within and immediately surrounding the lesions. This phenomenon known as the hypersensitive response (HR) is one of the earliest visible manifestations of induced defense responses and resembles programmed cell death in animals (Dangl et al., 1996, Gray, 2002; Glazebrook, 2005; Kachroo and Kachroo, 2006). Concurrent with HR development defense reactions are triggered in sites both local and distal from the primary infection. This phenomenon, known as systemic acquired resistance (SAR), is accompanied by a local and systemic increase in SA and jasmonic acid (JA) and a concomitant upregulation of a large set of defense genes (Durrant and Dong, 2004; Truman et al., 2007; Vlot et al., 2009).

SAR involves the generation of a mobile signal in the primary leaves which, upon translocation to the distal tissues, activates defense responses resulting in broad-spectrum resistance. The production of the mobile signal takes places within 3-6 h of avirulent pathogen inoculation in the primary leaves (Smith-Becker et al., 1998) and the inoculated leaf must remain attached for at least 4 h after inoculation for immunity to be induced in the systemic tissues (Rasmussen et al., 1991). Mutations compromising SA synthesis or impairing SA, JA, or auxin signaling, abolish SAR (Durrant and Dong, 2004; Truman et al., 2007; Truman et al., 2010). SAR is also dependent on the SA-binding protein 2 (SABP2)-catalyzed conversion of methyl SA to SA in the distal tissues (Kumar and Klessig, 2003). Recent studies have suggested that methyl SA is the mobile signal required to initiate SAR in distal tissues in tobacco (Park et al., 2007). However, ability to produce methyl SA does not appear to coincide with SAR in several Arabidopsis mutants (Attaran et al., 2009). Furthermore, the time point of requirement of SABP2 activity (between 48-72h post-inoculation, Park et al., 2009) does not coincide with the early generation and/or translocation of the mobile signal into distal tissues (within 6 h post-
inoculation).

Mutations in acp4, lacs2, or lacs9, which are impaired in FA/lipid flux (Schnurr et al., 2004; Xia et al., 2009), also compromise SAR (Xia et al., 2009). Detailed characterization has shown that the SAR defect in acp4, lacs2, and lacs9 mutants correlates with their defective cuticles. Analysis of the SAR response in acp4 plants has shown that these plants can generate the mobile signal required for inducing SAR, but are unable to respond to it. It is likely their defective cuticle that impairs perception of the SAR signal, because mechanical abrasion of cuticles disrupts SAR in wild-type plants (Xia et al., 2009). This SAR-disruptive effect of cuticle abrasion is highly specific because it does not alter local defenses and hinders SAR only during the time frame during which the mobile signal is translocated to distal tissues.

SAR is also compromised in plants that contain a mutation in glycerol-3-phosphate (G3P) dehydrogenase (G3Pdh; Nandi et al., 2004). The G3Pdh (GLY1) reduces dihydroxyacetone phosphate to generate G3P, an obligatory component and precursor for the biosynthesis of all plant glycerolipids. Consequently, a mutation in GLY1 results in reduced carbon flux through the prokaryotic pathway of lipid biosynthesis, which leads to a reduction in the hexadecatrienoic (16:3) fatty acids (FA) (Miquel et al., 1998; Kachroo et al., 2004). Carbon flux and SAR are also impaired in plants containing mutations in FAD (FATTY ACID DESATURASE) 7 (Chaturvedi et al., 2008). The FAD7 enzyme desaturates 16:2 and 18:2 FA species present on plastidial lipids to 16:3 and 18:3, respectively. Consequently, the fad7 mutant plants accumulate significantly reduced levels of trienoic FAs (16:3 and 18:3). Compromised SAR in mutants affected in certain plastidial FA/lipid pathways has prompted the suggestion that plastidial FA/lipids participate in SAR (Chaturvedi et al., 2008). Such a tempting conclusion is also favored by the fact that SAR requires the DIR1 encoded non-specific lipid transfer protein, which is required for generation and/or translocation of the mobile signal (Maldonado et al., 2002). In addition, azelaic acid, a dicarboxylic acid, was recently shown to prime SA biosynthesis and thereby SAR (Jung et al., 2009). The fact that azelaic acid is derived from oleic acid, a FA well known for its role in defense (Kachroo et al., 2003; 3004; 2005; 2007; 2008; Chandra-Shekara et al., 2007; Venugopal et al., 2009; Xia et al., 2009; Jiang et al., 2009) further suggests that FA/lipids might participate in SAR.
This study was undertaken to reexamine the role of the FA/lipid pathways in SAR and to
determine the nature of the FA/lipid species mediating SAR in \textit{fad7-1} plants. Our results show
that impaired FA/lipid flux is not associated with compromised SAR in \textit{fad7-1} plants but, rather,
an abnormal cuticle, which is the result of a non-allelic mutation in the \textit{GL} (\textit{GLABRA}) 1 gene.
Besides \textit{GL1}, other mutations affecting trichome formation also compromised cuticle and
thereby SAR. A compensatory effect of exogenous gibberellic acid on \textit{gl1} plants suggests that
GA might participate in resistance to bacterial pathogens by restoring cuticle formation.

\textbf{RESULTS}

\textbf{SAR is compromised in the \textit{fad7-1} plants but not in other \textit{fad} mutants}

To determine which, if any, of the desaturated FA species contributed to SAR, we first
evaluated SAR in all \textit{fad} mutants that are defective in the desaturation of various FA species
present on plastidial or extraplastidial membrane lipids (see Supplemental Figure 1 for the
respective FAD-catalyzed activities, reviewed in Kachroo and Kachroo, 2009). The \textit{fad2-1}, \textit{fad3-2},
\textit{fad4-1}, \textit{fad5-1}, \textit{fad6-1}, and \textit{fad7-1} mutants were first inoculated with MgCl$_2$ or avirulent
bacteria (\textit{avrRpt2}) followed by a second inoculation with virulent bacteria on distal tissues at 48
h post primary inoculation. The growth of the virulent bacteria was monitored at 0, 3, and 6 days
post inoculation (dpi) (Figure 1). As expected, MgCl$_2$ infiltrated leaves of wild-type (wt; Col-0
ecotype) plants supported more growth of the secondary virulent pathogen than the plants that
were pre-infected with the \textit{avrRpt2} strain, indicating appropriate induction of SAR (Figure 1).
Similar to wt plants, the \textit{fad2-1}, \textit{fad3-2}, \textit{fad4-1}, \textit{fad5-1}, and \textit{fad6-1} mutants also showed proper
induction of SAR. In agreement with a previous report (Chaturvedi et al., 2008), the \textit{fad7-1}
mutant plants showed compromised SAR; plants infiltrated with MgCl$_2$ or \textit{avrRpt2} bacteria were
equally susceptible to the secondary virulent bacteria in the distal tissues. Together, these data
suggested that the FAD7-catalyzed desaturation of 18:2 to 18:3 on plastidial membrane lipids
might be important for the proper induction of SAR.

\textit{fad7-1} plants are compromised in the pathogen-induced accumulation of SA
Since SA plays a critical role in SAR, we first tested if the fad7-1 mutant plants were competent in pathogen responsive accumulation of SA. SA levels in wt and fad7-1 plants were determined before and after inoculation of *P. syringae* containing *avrRpt2*. As expected, wt plants inoculated with avirulent pathogen showed a significant increase in both free SA and SA glucoside (SAG) in their primary as well as systemic tissues. Although the fad7-1 plants also showed an increase in SA and SAG levels in the primary tissues, levels of SA/SAG were significantly lower in their distal leaves in comparison to wt plants (Figure 2A). Thus, impaired SAR in fad7-1 plants correlated with their inability to accumulate SA in the distal tissues. To confirm this, we tested the effect of exogenously supplied SA on SAR in fad7-1 plants. The wt and fad7-1 leaves were infiltrated with MgCl₂ (mock) or *avrRpt2* bacteria. Vascular exudates collected from these infiltrated leaves were mixed with water or the SA analog BTH [benzo(1,2,3)thiadiazole-7-carbothioic acid] and injected into the leaves of a fresh set of wt and fad7-1 plants. The distal leaves of this second set of plants were then inoculated with virulent bacteria and proliferation of the virulent bacteria monitored at 0 and 3 dpi (Figure 2B). Notably, BTH containing exudate from MgCl₂-infiltrated Col-0 plants conferred SAR on Col-0 plants but not on fad7-1 plants. In comparison, the BTH-containing exudate from MgCl₂-infiltrated fad7-1 was unable to confer SAR on either Col-0 or fad7-1 plants. Interestingly, the exudate from *avrRpt2* infiltrated fad7-1 plants conferred SAR on Col-0 plants but not on fad7-1 plants. The BTH-containing exudate from *avrRpt2*-infiltrated Col-0 or fad7-1 plants produced better SAR on Col-0 but not on fad7-1, suggesting that exogenous BTH had an additive effect on SAR on Col-0. Together, these results suggested that exogenously supplied BTH was unable to restore the defective SAR in fad7-1 plants.

To determine if pre-treatment of the whole plant with BTH restored SAR in fad7-1 plants, we treated Col-0 and fad7-1 plants with BTH for two consecutive days followed by inoculation of primary leaves with MgCl₂ (mock) or *avrRpt2* bacteria. The virulent bacteria were then infiltrated into the distal leaves 48 h after primary infiltration (MgCl₂/avrRpt2) and growth of virulent bacteria monitored at 3 and 6 dpi (Figure 2C). Whole plant application of BTH conferred enhanced resistance to virulent pathogen in both wt as well as fad7-1 plants. However, the BTH-treated fad7-1 plants continued to support higher growth of virulent bacteria compared to BTH-treated Col-0, suggesting that BTH application on fad7-1 enhanced resistance but not to wt-like levels. To determine if this was due to a partial insensitivity to SA, we compared the
levels of the SA-inducible marker, PR-1, in fad7-1 plants. Similar levels of PR-1 transcript were induced in response to BTH in both Col-0 and fad7 plants (Figure 2D), suggesting that fad7-1 plants are able to induce SA-derived marker gene expression.

fad7-1 plants are compromised in pathogen induced accumulation of JA

The FAD7 enzyme catalyzes the desaturation of 18:2 to 18:3 FA on membranous lipids and a mutation in FAD7 reduces trienoic FA’s levels in the plant (Figure 3A). Notably, the fad7-1 plants accumulated reduced levels of basal and pathogen-induced JA (Figure 3B), which correlated well with reduced levels of the JA precursor 16:3 and 18:3 FAs. To determine if reduced accumulation of JA was responsible for compromised SAR, we tested if exogenously supplied JA restored SAR in fad7 plants. The wt and fad7-1 leaves were infiltrated with MgCl₂ (mock) or avrRpt2 bacteria. Vascular exudates collected from the MgCl₂- or avrRpt2-infiltrated leaves were mixed with water or JA and injected into the leaves of a fresh set of wt and fad7-1 plants. The distal leaves of this second set of plants were then inoculated with virulent bacteria and their proliferation monitored at 0 and 3 dpi (Figure 3C). Unlike BTH, addition of JA to exudates from MgCl₂-infiltrated Col-0 or fad7-1 plants did not reduce the growth of virulent bacteria. Furthermore, addition of JA to exudate from avrRpt2-infiltrated Col-0 plants also did not increase the potency of SAR in Col-0. Application of exudate on the fad7-1 plants did not induce SAR, regardless of the source of the exudate (Col-0 or fad7-1) plants. Together, these data suggested that the inability to accumulate JA likely did not contribute to the compromised SAR in fad7-1 plants. This was further confirmed by whole plant application of JA (Figure 3D). The Col-0 and fad7-1 plants were treated with JA for two consecutive days followed by inoculation of primary leaves with MgCl₂ (mock) or avrRpt2 bacteria. The virulent bacteria were inoculated on distal leaves 48 h after primary infiltrations and their growth monitored at 3 and 6 dpi. JA-treated fad7-1 plants showed a marginal reduction in the growth of the virulent pathogen but they did not show SAR. Additionally, similar to results obtained in the exudate-related experiment (Figure 3C), whole plant pretreatment with JA did not improve SAR in either mock- or avrRpt2-inoculated wt plants. Together, these results argue against reduced JA as the cause of defective SAR in fad7-1 plants.
A *gl1* mutation in the *fad7-1* plants is responsible for their defective SAR

In addition to FAD7, the Arabidopsis FAD8 enzyme also catalyzes the desaturation of 18:2 to 18:3 on plastidial lipids. This is evident in the *fad7-2 fad8-1* double mutant plants, which are more severely reduced in their trienoic FA levels. To test if trienoic FAs contribute to SAR, we assayed SAR in the *fad7-2 fad8-1* double mutant plants. As expected, the *fad7-2 fad8-1* plants showed negligible levels of 16:3 and a significant reduction in 18:3 levels (Figure 4A). Surprisingly, the *fad7-2 fad8-1* plants showed normal SAR (Figure 4B). One possibility was that the *fad8* mutation restored SAR in the *fad7-2 fad8* double mutant. However, this was rather unlikely given the fact that both FAD7 and FAD8 catalyze the same desaturation event (18:2 to 18:3). It was also not easily testable since the *fad8* single mutation has no detectable effect on overall FA composition (McConn et al., 1994). We therefore retested the requirement of FAD7 in SAR induction by evaluating SAR in the *fad7-1* allelic mutant, *fad7-2*. Both *fad7-1* and *fad7-2* showed similar profile of FA’s and resulted in a similar decrease in the levels of trienoic FA’s (Figure 4A). However, unlike *fad7-1*, the *fad7-2* plants showed normal SAR (Figure 4B). Together, these data suggested that compromised SAR in *fad7-1* plants was not associated with their reduced trienoic FA levels or their altered FA/lipid profile.

A comparison of the genetic backgrounds used for isolating *fad7-1* and *fad7-2* mutants revealed that *fad7-1* was isolated in the *gabra* (**gl**) background, whereas *fad7-2* was isolated in the wt background. The *GL1* gene encodes the R2R2-MYB transcription factor, which is required for trichome differentiation in Arabidopsis (Oppenheimer et al., 1991). A mutation in the *GL1* gene results in a glabrous phenotype due to a lack of trichomes on leaves. Indeed, similar to leaves from *gl1* plants, *fad7-1 gl1* leaves were devoid of any trichomes (Figure 4C). On the other hand, wt, *fad7-2*, and *fad7-2 fad8-1* leaves showed normal trichomes (Figure 4C). Quantitative FA profiling showed a wt-like profile of FA’s in *gl1* plants (Figure 4A), suggesting that, unlike *fad7-1*, the *gl1* mutation was not associated with an altered FA profile. However, similar to *fad7-1*, the *gl1* plants were compromised in SAR (Figure 4B). Together, these data suggested that the *gl1* mutation was responsible for compromised SAR in *fad7-1* plants.
To confirm this further, we expressed a genomic copy of the FAD7 gene in fad7-1 gl1 plants. All the T1 plants showed wt-like levels of trienoic acid but were still devoid of trichomes, suggesting that these transgenic plants were complemented for fad7-1 but not for the gl1 mutation. Three independent T1 lines were analyzed in the T2 generation and these segregated for fad7-1 mutation in a ~ 3 wt-like: 1 fad7-like manner; the fad7-1 plants were scored based on their genotype as well as FA profile (Figure 4D, 4E). However, all the T2 plants lacked trichomes (Supplemental Figure 2), further confirming that the FAD7 transgene complemented only the fad7-1 and not the gl1 mutation. The fad7:FAD7 transgenic plants showed compromised SAR, like fad7-1 and gl1 plants (Figure 4F), further reiterating the fact that the fad7-1 mutation was not responsible for compromised SAR.

**gl1 plants are impaired in the perception, but not the generation, of the mobile signal**

We next assessed whether GL1 contributed to mobile signal production or perception in the SAR response. We evaluated the response of wt and gl1 and fad7-1 gl1 plants to vascular exudates collected from pathogen-inoculated wt, gl1, or fad7-1 gl1 leaves. The wt, gl1 or fad7-1 gl1 leaves were infiltrated with MgCl2 or avrRpt2 bacteria and vascular exudates collected from the inoculated leaves were injected into the leaves of a fresh set of wt or gl1, or fad7-1 gl1 plants. Distal leaves of the exudate-infiltrated plants were then inoculated with virulent bacteria and proliferation of virulent bacteria monitored at 0 and 3 dpi (Figure 5A). As expected, exudates from avrRpt2-infected wt plants conferred protection against virulent pathogen in wt plants. Similar to wt, exudates from avrRpt2-infected gl1 or fad7-1 gl1 plants also conferred resistance against virulent pathogen in wt plants. In comparison, the same exudates (wt, gl1 or fad7-1 gl1 plants) conferred only marginal SAR on gl1 or fad7-1 gl1 plants. These data also correlated with PR-1 gene expression; the fad7-1 gl1 plants showed basal levels of PR-1 transcript in response to exudate application compared to wt plants (Figure 5B). Together, these results suggested that gl1 plants are competent in generating the mobile SAR signal(s), but are defective in its perception.

**The gl1 plants are defective in their cuticle**

The inability of the \textit{fad7-1 gl1} plants to perceive SAR signal(s) was reminiscent of phenotypes associated with \textit{acp4} plants (Figure 5A and Xia et al., 2009), which are defective in cuticle formation. These similarities prompted us to investigate whether the \textit{fad7-1 gl1} and \textit{gl1} plants also contained defective cuticles, similar to \textit{acp4} plants. The \textit{fad2-1, fad3-2, fad4-1, fad5-1, fad6-1, fad7-2, fad7-2 fad8-1} mutants were also included in this analysis. The known cuticle impaired mutant \textit{gpat4 gpat8} was included as positive control (Figure 6A; Li et al., 2007).

Toluidin blue, a hydrophilic dye that only penetrates leaves with permeable cuticles (Tanaka et al., 2004), stained leaves in \textit{fad7-1 gl1, gl1}, and \textit{gpat4 gpat8} mutants. Furthermore, \textit{fad7-1 gl1}, and \textit{gl1} also showed increased water loss (Figure 6B) and chlorophyll leaching (Figure 6C), further supporting their defective cuticle phenotype.

We also analyzed the outermost cell wall of the epidermis of \textit{fad7-1 gl1}, and \textit{gl1} leaves by transmission electron microscopy (TEM). As expected, the cuticle of the wt leaf appeared as a continuous and regular electron-dense osmiophilic layer outside the cell wall (Figure 6D). In comparison, \textit{fad7-1 gl1} mutants showed electron-opaque cuticles (Figure 6D, marked by arrows). Comparison of the scanning electron micrographs (SEM) of wt and \textit{fad7-1 gl1} and \textit{gl1} leaf surfaces showed that the mutants each had an uneven surface, which was greatly folded compared to that of wt leaves (Figure 6E, data not shown for \textit{gl1}).

To determine if this defect in cuticle structure was associated with alterations in the content and/or composition of cuticular waxes or cutin polyester monomers, we compared levels of waxes and cutin monomers between wt and \textit{fad7-1 gl1} and \textit{gl1} leaves (Supplemental Figure 3). The \textit{fad7-1 gl1}, and \textit{gl1} leaves showed reduction in several FA, alkane and primary alcohol species compared to wt plants (Supplemental Figure 3A). The \textit{gl1} leaves showed a ~32\% overall decrease in cutin aliphatic monomer content. In case of \textit{gl1}, the decrease was more pronounced in two major monomers [16:0-, 18:1-, and 18:2- DCA and 16-OH-16:0 (Supplemental Figure 3B)]. Taken together, these results show that GL1 is required for the biosynthesis of the cuticular wax and cutin polymers in leaves.

\textbf{The} \textit{gl1} \textbf{plants show compromised resistance to fungal pathogens}
Since cuticle plays an important role in defense against fungal pathogens (Bessire et al., 2007; Chassot et al., 2007; Kurdyukov et al., 2006; Li et al., 2007; Mang et al., 2009), we next evaluated the response of fad7-1 gl1 and gl1 plants to the necrotrophic pathogen Botrytis cinerea and a hemibiotrophic fungal pathogen Colletotrichum higginsianum. Interestingly, both fad7-1 gl1 and gl1 plants showed enhanced susceptibility to B. cinerea and C. higginsianum; spray and spot inoculations showed significantly larger lesions on fad7-1 gl1 and gl1 leaves (Figure 7, Supplemental Figure 4). Unlike fad7-1 gl1 and gl1 plants, the fad7-2 and fad7-2 fad8 plants showed wt-like response to B. cinerea and C. higginsianum, arguing against a role for trienoic FA’s in resistance to necrotrophic or hemibiotrophic fungal pathogens.

Mutations in GL3 and TRANSPARENT TESTA GLABRA (TTG) 1 impair the cuticle and compromise SAR

Since the GL1 gene is involved in trichome formation, we next tested if trichome formation was generally associated with a defective cuticle and thereby impaired SAR. The cuticular phenotypes were assessed in two known mutants, gl3 and ttg1, which are affected in the differentiation of trichomes (Supplemental Figure 5). The GL3 and TTG1 genes encode a basic helix-loop-helix protein and WD40 repeat protein, respectively. GL3 and TTG1 along with GL1 are thought to form a combinatorial regulatory complex during trichome differentiation (Morohashi et al., 2007; Payne et al., 2000; Walker et al., 1999; Zhang et al., 2003). Indeed, similar to gl1, both gl3-1 and ttg1 mutants do not form any trichomes on their leaves (Supplemental Figure 5). Interestingly, the gl3 and ttg1 leaves rapidly stained with toluidine blue (Figure 8A) and also showed increased chlorophyll leaching (Figure 8B). These results suggest that gl3 and ttg1 mutants contain a defective cuticle. Consistent with the proposed role for cuticle in SAR both gl3 and ttg1 plants were unable to induce proper SAR (Figure 8C).

Gibberellic acid (GA) application is known to stimulate trichome formation (Chien and Sussex, 1996; Perazza et al., 1998). Since our results indicated that trichome differentiation was associated with cuticle formation, we next tested whether GA can restore trichome and/or cuticle formation on gl1 leaves. Exogenous application of GA to wt and gl1 plants caused a significant enhancement in their growth (Supplemental Figure 6A). Although GA application was unable to
induce trichome formation on *gl1* leaves (Supplemental Figure 6A), the GA-treated *gl1* plants showed a significant reduction in the toluidine blue-stained areas (Figure 9A). Furthermore, GA-treated wt and *gl1* plants showed a significant reduction in chlorophyll leaching from these leaves (Supplemental Figure 6B). These data suggested that GA application led to enhancement of the cuticle in both wt and *gl1* plants. Consistent with toluidine blue staining and chlorophyll leaching phenotypes, the GA-treated wt and *gl1* plants showed a pronounced increase in the levels of cuticular components (Figure 9B). Interestingly, GA-treated wt plants showed significantly better SAR compared to water-treated plants (Figure 9C). Exogenous application of GA also improved SAR on *gl1* plants but the effect was less pronounced compared to wt plants, likely because the cuticle might not be completely restored. Nonetheless, unlike SA or JA, GA was able to partially restore defective SAR in *gl1* plants.

**Discussion**

The *gl1* mutant was first isolated based on its glabrous phenotype (Koornneef et al., 1982), and this phenotype has served as a useful marker for numerous mutant isolations and/or screenings. A cursory database search identified over 200 articles in which the *gl1* background has been used to study molecular, genetic, and biochemical aspects of diverse aspects of plant physiology (Liu and Zhu, 1997; Aarts et al., 1998; Cecchini et al., 1998; Xie et al., 1998; Yi and Jack, 1998; Bieza and Lois, 2001; Ellis and Turner, 2001; Laby et al., 2001; Bender and Fink, 1998; Devoto et al., 2002; He et al., 2002; Collins et al., 2003; Quiel and Bender, 2003; Barkan et al., 2006; Zhu et al., 2007; Speth et al., 2009; Jung and Niyogi, 2010; Rowe et al., 2010). Notably, many of the mutants that were isolated or crossed into the *gl1* background have been studied for the defense physiologies. Interestingly, most studies were conducted with the assumption that the *gl1* mutant itself does not affect any phenotype but the loss of trichomes. Our work shows that this is clearly not the case and that *gl1* plants are compromised in defense to both bacterial as well as fungal pathogens. Additionally, the *gl1* mutation affects cuticular development. Our findings question the prudence in using mutant backgrounds to design genetic screens and underscore the need to reevaluate phenotypes that were previously studied in the *gl1* background.
This is clearly the case for the fad7-1 mutant, which was originally isolated in the gl1 background (Browse et al., 1986). Subsequent studies suggested a role for glycerolipid synthesis in SAR due to the inability of the fad7-1 (fad7-1 gl1) plants to induce SAR (Chaturvedi et al., 2008). However, normal SAR phenotype in allelic fad7-2 and the fad7-2 fad8 double mutant argue against a role for trienoic FAs/ lipid metabolism in SAR. This is further reiterated by the fact that trienoic FA levels but not the ability to induce SAR were restored in fad7-1 transgenic lines expressing a wt copy of FAD7. Characterization of the fad7-1 mutant showed that its impaired SAR is associated with a second-site mutation in the GL1 gene. Notably, a mutation in the GL1 gene impaired cuticle development, thereby compromising the plant’s ability to induce SAR. Interestingly, besides GL1, two other mutations affecting trichome formation (gl3 and ttg1) also contained deformed cuticles and were defective in inducing SAR. These data suggest that trichome differentiation and cuticle development may involve an overlapping biochemical basis. However, a correlation between the lack of trichomes and presence of a defective cuticle was not observed in transgenic plants expressing the FATTY ACID ELONGATION 1 gene specifically in the epidermis; these plants do not contain trichomes but have a normal cuticle (Reina-Pinto et al., 2009).

Is there a connection between trichome formation and cuticle development? To begin with, epidermal cells are involved in both of these processes. Trichomes differentiate from the pluripotent epidermal cells in a patterned manner, which is determined by genetic interactions between trichome-promoting or trichome-repressing factors (reviewed in Schellmann and Hulskamp; 2005; Ishida et al., 2008). Proteins encoded by GL1, GL3, and TTG1 act as positive regulators and mutations in these genes affect trichome development. Both GL1 and GL3 act as transcriptional regulators and are known to regulate genes involved in various other processes besides trichome development (Morohashi and Grotewold, 2009). Interestingly, one of the direct targets of GL3/GL1 includes FIDDLEHEAD (FDH), which encodes a protein related to β-ketoacyl-CoA synthase involved in cuticle development (Pruitt et al., 2000; Voisin et al., 2009). Notably, the fdh mutant shows a significant reduction in the number of trichomes (Yephremov et al., 1999). Similarly, several other mutants, including lcr (LACERATA), bdg (BODYGUARD), yre (YORE-YORE, allelic to WAX2/CER3), dso (DESPERADO/AtWBC11), which are defective in cuticle formation exhibit abnormal differentiation of trichomes and reduced trichome numbers (Wellesen et al., 2001; Kurata et al., 2003; Kurdyukov et al., 2006; Panikashvili et al., 2007).
Suppression of ketoacyl reductase activity, which catalyzes the first reduction step leading to synthesis of very-long-chain-FAs, also results in abnormal trichomes, and correlates with reduced cuticular wax levels (Beaudoin et al., 2009). These findings, together with our results, support the notion that overlapping pathways involving GL1, GL3 and TTG1, might regulate cuticle development as well as trichome formation. This is further supported by the fact the defective trichome phenotypes of the yre gl2 and yre ttg1 double mutant plants are more severe than the yre, ttg1 or gl2 single mutants (Kurata et al., 2003).

The ability of exogenous GA to complement the SAR defect in gl1 plants but not restore trichome formation suggests that trichomes might not play a role in SAR. This is further supported by fact that compromised SAR in acp4 plants is not associated with trichome formation; acp4 plants show wt-like trichomes on their leaves (supplemental Figure 7). It appears that GA might participate in systemic immunity by affecting cuticle development. This is based on the fact that GA application reduced chlorophyll leaching by increasing the levels of cuticular components. Increased incorporation of palmitic acid and oleic acid into cutin monomers was also seen in GA-treated rice and pea plants (Bowen and Walton, 1988; Hoffmann-Benning and Kende, 1994), suggesting that GA-mediated increased carbon flux into cuticle might be a common phenomenon in plants. GA application also induced stronger SAR in wt plants. GA application is known to derepress its signaling pathway by inducing the degradation of DELLA proteins (Achard and Genschik, 2009), which negatively regulates accumulation of reactive oxygen species (ROS) (Achard et al., 2008). Notably, GA treatment, in the absence of pathogen inoculation, did not alter ROS levels; ROS levels in uninoculated, GA-treated wt or gl1 plants were similar to that in water-treated plants. This suggests that the GA-triggered increased carbon flux into cuticle is not mediated via ROS. However, it does not rule out a role for ROS in GA-triggered SAR. The fact that exogenous application of H2O2 is unable to improve the resistance response of wt plants to Pseudomonas discounts a role for ROS in GA-triggered gl1 plants. This is further supported by the result that exogenous application of SA, which potentiated defense response leading to increased accumulation of ROS (Shirasu et al., 1997), was unable to trigger SAR in gl1 plants. Similarly, SA and JA treatments were also unable to complement defective SAR in other cuticle-deficient mutant, acp4 (Xia et al., 2009). Together, these observations support the notion that GA-mediated restoration of cuticle might be responsible for restored SAR in gl1 plants. More importantly, these and our earlier results (Xia et
al., 2009), reconfirm the fact that cuticle-deficient mutants are impaired in their ability to perceive mobile signal.

Methods

Plant growth conditions and complementation analysis

Plants were grown in MTPS 144 Conviron (Winnipeg, MB, Canada) walk-in chambers at 22 °C, 65% relative humidity and 14 hour photoperiod. For complementation, a ~3.5 Kb region encompassing FAD7 coding region was amplified from Col-0 genomic DNA using BamHI and XbaI linkered primers, AAATTCATGCGGAATCAGAGAACG and TCTATGTTCGATACTGAAGC primers, respectively, and cloned into the pBAR1 binary vector. After confirmation of the DNA sequence, the binary vector was transformed into fad7-1 plants using floral dip method. The transgenic plants were selected on soil sprayed with the herbicide Basta. The complementation was confirmed by FA and genotype analysis of the T1 plants and by analyzing the segregation of FA phenotypes in the T2 generation. The fad7-1 mutation was identified as a C to T transition that converted amino acid at position 253 from proline to leucine. The coding region of FAD7 was amplified using ATGGCGAACTTGGTCTTATCAGAA and GAGGTCAAAGTAAGAGCAGATTGA primer. Genomic CAPS for the fad7-1 was performed by amplifying a 481 bp fragment, using primers GAGGAGTCTCCATTGGAGGAA and CATGTTGCTAGTAGACCAACCC, which was digested with Sau3AI.

RNA extraction and northern analyses

Small-scale extraction of RNA from one or two leaves was performed in the TRIzol reagent (Invitrogen, Gaithersburg, MD) following the manufacturer’s instructions. Northern analyses and synthesis of random primed probes was carried out as described before (Kachroo et al., 2005).
SA, FA, and JA quantifications

SA and SA glucoside (SAG) were extracted and measured from ~ 0.3 g of fresh weight leaf tissue, as described before (Chandra-Shekara et al., 2006). Measurements were performed in triplicates and the experiment was repeated twice.

FA extraction was carried out by placing leaf tissue (n=6) in 2 ml of 3% H₂SO₄ in methanol. After 30 min incubation at 80°C, 1 ml of hexane with 0.001% butylated hydroxytoluene (BHT) was added. The hexane phase was then transferred to vials for gas chromatography (GC) analysis. One-microliter samples were analyzed by GC on a Varian FAME 0.25 mm x 50 m column and quantified with flame ionization detection. For quantification of FAs, leaves (50 mg) were extracted together with an internal standard 19:0 and the FA levels were calculated based on the detected peak areas corresponding to the FA retention time relative to the areas of the internal standard. FA quantifications were repeated three times.

For JA estimations, leaves (1g) were ground in liquid nitrogen and extracted in 100% methanol using dihydro-JA (DJA) as an internal standard. The extract was acidified to pH ≤ 4 with 1M HCl and passed through C-18 Sep-Pak columns (Waters: 500mg: 3mL), which were pre-equilibrated with 75% methanol containing 0.2% Acetic acid. The column purified extract was saturated with sodium chloride and re-extracted with diethyl ether. The ether extract was completely dried under a stream of nitrogen gas and methylated with diazomethane. The oxylipins were solublized in 0.5 mL hexane, dried to ~10 μl under a gentle stream of nitrogen gas and 1μL was injected into GC-MS as described above. The JA peaks were identified using mass spectrometry. For quantitative analysis of JA, the mass spectrometer was run in selective ion monitoring (SIM) mode for m/z 224 and 226. The ratio of m/z 224 to 226 in the peak area (retention time between 9.5 min to 11.0 min) was used to calculate the JA levels based on the abundance of m/z 224 and 226 generated by standard JA, DJA and their equal level mixture. Measurements were performed in triplicates and the experiment was repeated twice.

For JA levels in vascular exudates, samples were extracted using a solution containing glacial acetic acid, methanol, chloroform and potassium chloride (0.9%) (1:4:8:8, vol/vol) and 17:0 as internal standard. The lower phase was removed and dried under a stream of nitrogen gas.
and samples were derivatized with diazomethane, dried and reconstituted in MTBE, transferred to a glass insert and dried again under a stream of nitrogen gas and reconstituted in a minimum volume of acetonitrile. Samples (1 µl) were analyzed with GC-MS as described above. Measurements were performed in triplicates and the experiment was repeated twice.

SA, JA, and GA treatments

SA, JA and GA (GA4 and GA7) treatments were carried out by spraying 500 µM, 50 µM or 100 µM solutions, respectively. JA treated plants were covered with a transparent plastic dome to maximize exposure to JA.

Pathogen infections

Inoculations with bacterial pathogen *Pseudomonas syringae* were conducted as described before (Kachroo et al., 2005). The bacterial cultures were grown overnight in King’s B medium containing rifampicin and/or kanamycin. The cells were washed and suspended in 10 mM MgCl₂. The bacterial suspension was injected into the abaxial surface of the leaf using needleless syringe. Three discs from the inoculated leaves were collected and homogenized in 10 mM MgCl₂. The extract was diluted and appropriate dilutions were plated on King’s B medium. For analysis of SAR, the primary leaves were inoculated with MgCl₂ or the avirulent bacteria (10⁷ CFU ml⁻¹) and 48 h later the systemic leaves were inoculated with vir bacteria (10⁵ CFU ml⁻¹). Unless noted otherwise, samples from the systemic leaves were harvested at 3 dpi. All SAR experiments were repeated at least three times.

*Colletotrichum higginsianum* Sacc. (IMI 349063) and *Botrytis cinerea* were maintained on potato dextrose agar (PDA) and V8 medium, respectively. Four-week-old Arabidopsis plants were used for both spray and spot inoculations. Spore suspensions at concentrations of 10⁴-10⁶ spores/ml were used for various experiments. For spot inoculations, 10 µl of spore suspension was used to inoculate Arabidopsis leaves. After inoculations, the plants were transferred to a PGV36 Conviron walk-in chamber and covered with a plastic dome to maintain high humidity. Disease symptoms were scored between 4-11 dpi. A digital Vernier caliper was used to measure
lesion size in spot-inoculated leaves. Each experiment was repeated at least twice and each included 30-50 individual plants. Statistical significance was determined using Student’s t-test.

Collection of phloem exudate

Leaf exudate was collected as described earlier (Maldonado et al., 2002). In brief, plants were induced for SAR by inoculation with *P. syringae* containing *avrRpt2* (10^6 CFU ml\(^{-1}\)). Twelve-twentyfour hours later, petioles were excised, surface sterilized in 50% ethanol, 0.0006% bleach, rinsed in sterile 1 mM EDTA and submerged in ~1.9 ml of 1 mM EDTA and 100 μg ml\(^{-1}\) ampicillin. Exudates were collected over 48 h and infiltrated into healthy plants. Infiltrated leaves were harvested after two days for *PR-1* gene expression studies. For SAR studies, virulent pathogen was inoculated in the distal leaves two days after infiltration of exudate.

Microscopy, chlorophyll leaching and water loss

For SEM analysis both abaxial and adaxial surface of the leaf samples was mounted on sample holder with 12 mm conductive carbon tabs (Ted Pella Inc.), sputter-coated with gold-palladium and observed on a Hitachi S-3200 SEM with and without backscatter detector at 5 and 20 kV.

For TEM analysis leaves were fixed in paraformaldehyde and embedded in epon-araldite. Leaves were sectioned on a Reichert-Jung Ultracut E microtome with a Diatome diamond knife and observed under a Philips Tecnai Biotwin 12 TEM.

For chlorophyll leaching assays, 100 mg of leaves were weighed and gently agitated, in dark, at room temperature in tubes containing 80% ethanol. Absorbance of each sample was measured at 664 and 647 and micromolar concentration of total chlorophyll per gram of fresh weight was calculated using the formula: total micromoles chlorophyll = 7.93 (A\(_{664}\)) + 19.3 (A\(_{647}\)).

For water loss measurements, four-week old plants were either subjected to drought or kept moist. The leaf weight was measured from ~50 leaves.
Analysis of wax and cutin components

For analysis of the wax component, 500 mg of four-week old leaves were immersed in 10 ml of chloroform for 10 s. The leaves were rinsed once more with 10 ml of chloroform. An internal standard (100 μg of n-tetracosane) was added and the sample volume was evaporated under a gentle steam of nitrogen. The samples were dried under a stream of nitrogen gas and methylated with diazomethane, dried again and derivatized with 100 μl of acetic anhydride in 100 μl of pyridine and the sealed tubes were incubated for 60 minutes at 60 °C. The samples were again dried under a stream of nitrogen and dissolved in 1 ml of acetonitrile. Samples (1μl) were injected into an HP-5 column of GC equipped with flame ionization detector. The same samples were also run on an HP-5 column (30 m x 0.25 mm x 0.25 mm film thickness) on a GC equipped with mass spectrometer (MS). Various components were identified based on their retention time as compared to standards and by MS analysis. Quantification was based on flame ionization detector peak areas as compared to the peak areas of the internal standard tetracosane added prior to derivatization. Measurements were performed in triplicates and the experiment was repeated twice.

Cutin monomer composition and content were determined using sodium methoxide-catalyzed transmethylation method followed by acetylation of the hydroxyl groups with acetic anhydride and GC-MS slightly modified from previously described (Bonaventure et al., 2004; Molina et al., 2006). After methanolysis, the methylene dichloride extract of cutin monomers were washed with 0.9% potassium chloride instead of 0.5 M sodium chloride. For GC-MS analysis, the FAME capillary column used was as described in wax analysis with helium carrier gas at 1 ml min⁻¹. The mass spectrometer was run in scan mode over 35-450 amu (electron impact ionization). Measurements were performed in triplicates and the experiment was repeated twice.

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1. Aarts N, Metz M, Holub E, Staskawicz BJ, Daniels MJ, Parker JE (1998) Different requirements for EDS1 and NDR1 by disease resistance genes define at least two R gene-mediated signaling pathways in Arabidopsis. Proc Natl Acad Sci USA 95: 10306-10311.

2. Achard P, Genschik P (2009) Releasing the brakes of plant growth: how GAs shutdown DELLA proteins. J Exp Bot 60: 1085-1092.

3. Achard P, Renou J-P, Berthome R, Harberd NP, Genschik P (2008) Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. Current Biol 18: 656-660.

4. Attaran E, Zeier TE, Griebel T, Zeier J (2009) Methyl salicylate production and jasmonate signaling are not essential for systemic acquired resistance in Arabidopsis. Plant Cell 21: 954-971.

5. Barkan L, Vijayan P, Carlsson AS, Mekhedov S, Browse J (2006) A suppressor of fab1 challenges hypotheses on the role of thylakoid unsaturation in photosynthetic function. Plant Physiol 141: 1012-1020.

6. Beaudoin F, Wu X, Li F, Haslam, RP, Markham JE, Zheng H, Napier JA, Kunst L (2009) Functional characterization of the Arabidopsis B-Ketoacyl-Coenzyme A reductase candidates of the fatty acid elongase. Plant Physiol 150: 1174-1191.

7. Bender J, Fink GR (1998) A Myb homologue, ATR1, activates tryptophan gene expression in Arabidopsis. Proc Natl Acad Sci USA 95: 5655-5660.

8. Bessire MCC, Jacquot A-C, Humphry M, Borel S, Petitot JMC, Métraux J-P, Nawrath C (2007) A permeable cuticle in Arabidopsis leads to a strong resistance to Botrytis cinerea. EMBO J 26: 2158–2168.

9. Bieza K, Lois R (2001) An Arabidopsis mutant tolerant to lethal ultraviolet-B levels shows constitutively elevated accumulation of flavonoids and other phenolics. Plant Physiol 126: 1105-1115.

10. Bonaventure G, Ohlrogge JB (2002) Differential Regulation of mRNA Levels of Acyl Carrier Protein isoforms in Arabidopsis. Plant Physiol 128: 223-235.
11. **Bowen DJ, Walton TJ** (1988) Cutin composition and biosynthesis during gibberellic acid-induced stem extension of *Pisum sativum* var Meteor. Plant Sci 55: 115-127.

12. **Browse J, McCourt P, Somerville C** (1986) A mutant of *Arabidopsis* deficient in C18:3 and C16:3 leaf lipids. Plant Physiol 81: 859-864.

13. **Cecchini E, Al-Kaff N, Bannister A, Giannakou M, McCallum D, Maule A, Milner J, Covey S** (1998) Pathogenic interactions between variants of cauliflower mosaic virus and *Arabidopsis thaliana*. J Exp Bot 49: 731-737.

14. **Chandra-Shekara A C, Gupte M, Navarre D, Raina S, Raina R, Klessig D, Kachroo P** (2006) Light-dependent hypersensitive response and resistance signaling to turnip crinkle virus in Arabidopsis. Plant J 45: 320-335.

15. **Chandra-Shekara AC, Venugopal SC, Barman SR, Kachroo A, Kachroo P** (2007) Plastidial fatty acid levels regulate resistance gene-dependent defense signaling in Arabidopsis. Proc Natl Acad Sci USA 104: 7277-7282.

16. **Chaturvedi R, Krothapalli K, Makandar R, Nandi A, Sparks AA, Roth M, Welti R, Shah J** (2008) Plastid ω-3 desaturase-dependent accumulation of a systemic acquired resistance inducing activity in petiole exudates of *Arabidopsis thaliana* is independent of jasmonic acid. Plant J 54: 106-117.

17. **Chassot C, Nawrath C, Metraux J-P** (2007) Cuticular defects lead to full immunity to a major plant pathogen. Plant J 49: 972-980.

18. **Chien J C, Sussex IM** (1996) Differential regulation of trichome formation on the adaxial and abaxial surfaces by gibberellins and photoperiod in *Arabidopsis thaliana* (L) Heynh. Plant Physiol 111: 1321-1328.

19. **Collins NC, Thordal-Christensen H, Lipka V, Bau S, Kombrink E, Qiu J-L, Hückelhoven R, Stein M, Freialdenhoven A, Somerville SC, Schulze-Lefert P** (2003) SNARE-protein-mediated disease resistance at the plant cell wall. Nature 425: 973-977.

20. **Dangl JL, Dietrich RA, Richberg MH** (1996) Death don't have no mercy: cell death programs in plant-microbe interactions. Plant Cell 8: 1793-1807.

21. **Devoto A, Nieto- Rostro M, Xie D, Ellis C, Harmston R, Patrick E, Davis J, Sherratt L, Coleman M, Turner JG** (2002) COII links jasmonate signaling and fertility to the SCF ubiquitin-ligase complex in Arabidopsis. Plant J 32: 457-466.
22. Durrant WE, Dong X (2004) Systemic acquired resistance. Annu Rev Phytopathol 42: 185-209.

23. Ellis C, Turner JG (2001) The Arabidopsis mutant cev1 has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. Plant Cell 13: 1025-1033.

24. Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 43: 205-227.

25. Gray WM (2002) Plant defense: a new weapon in the arsenal. Curr Biol 12: R352-R354.

26. He Y, Fukushige H, Hildebrand DF, Gan S (2002) Evidence supporting a role of jasmonic acid in Arabidopsis leaf senescence. Plant Physiol 128: 876-884.

27. Hoffmann-Benning S, Kende H (1994) Cuticle biosynthesis in rapidly growing internodes of deepwater rice. Plant Physiol 104: 719-723.

28. Ishida T, Kurata T, Okada K, Wada T (2008) A genetic regulatory network in the development of trichomes and root hairs. Annu Rev Plant Biol 59: 365-386.

29. Jarvis P, Dörmann P, Peto CA, Lutes J, Benning C, Chory J (2000) Galactolipid deficiency and abnormal chloroplast development in the Arabidopsis MGD synthase 1 mutant. Proc Natl Acad Sci USA 97: 8175-8179.

30. Jiang CJ, Shimono M, Maeda S, Inoue H, Mori M, Hasegawa M, Sugano S, Takatsuji H (2009) Suppression of the rice fatty-acid desaturase gene OsSSI2 enhances resistance to blast and leaf blight diseases in rice. Mol Plant-Microbe Interact 22: 820-829.

31. Jung H-S, Niyogi KK (2010) Mutations in Arabidopsis YCF20-like genes affect thermal dissipation of excess absorbed light energy. Planta 231: 923-937.

32. Jung HW, Tschaplinksi TJ, Wang L, Glazebrook J, Greenberg J (2009) Priming in systemic plant immunity. Science 324: 89-91.

33. Kachroo A, Daqi F, Havens W, Navarre D, Kachroo P, Ghabrial S (2008) An oleic acid–mediated pathway induces constitutive defense signaling and enhanced resistance to multiple pathogens in soybean. Mol. Plant-Microbe Interact 21: 564-575.

34. Kachroo A, Kachroo P (2006) Salicylic Acid-, Jasmonic Acid- and Ethylene-Mediated Regulation of Plant Defense Signaling In Genetic Regulation of Plant Defense Mechanisms, Ed Jane Setlow, Springer pubs, 28: 55-83.
35. **Kachroo A, Kachroo P** (2009) Fatty acid derived signals in plant defense. Ann Rev Phytopath **47**: 153-176.

36. **Kachroo A, Lapchyk L, Fukushima H, Hildebrand D, Klessig D, Kachroo P**. (2003) Plastidial fatty acid signaling modulates salicylic acid- and jasmonic acid-mediated defense pathways in the Arabidopsis ssi2 mutant. Plant Cell **12**: 2952-2965.

37. **Kachroo A, Shanklin J, Lapchyk L, Whittle E, Hildebrand D, Kachroo P** (2007) The Arabidopsis stearoyl-acyl carrier protein-desaturase family and the contribution of leaf isoforms to oleic acid synthesis. Plant Mol Biol **63**: 257-271.

38. **Kachroo A, Venugopal SC, Lapchyk L, Falcone D, Hildebrand D, Kachroo P** (2004) Oleic acid levels regulated by glycerolipid metabolism modulate defense gene expression in Arabidopsis. Proc Natl Acad Sci USA **101**: 5152-5257.

39. **Kachroo P, Venugopal SC, Navarre DA, Lapchyk L, Kachroo A** (2005) Role of salicylic acid and fatty acid desaturation pathways in ssi2-mediated signaling. Plant Physiol **139**: 1717-1735.

40. **Koornneef M, Dellaert LWM, van der Veen JH** (1982) EMS- and radiation-induced mutation frequencies at individual loci in Arabidopsis thaliana (L) Heynh. Mutat Res **93**: 109-123.

41. **Kumar D, Klessig DF** (2003) The high-affinity salicylic acid-binding protein 2 is required for plant innate immunity and has salicylic acid-stimulated lipase activity. Proc Natl Acad Sci USA **100**: 16101-16106.

42. **Kurata T, Kawabata-Awai C, Sakuradani E, Shimizu S, Okada K, Wada T** (2003) The YORE-YORE gene regulates multiple aspects of epidermal cell differentiation in Arabidopsis. Plant J **36**:55-56.

43. **Kurdyukov S, Faust A, Nawrath C, Bär S, Voisin D, Efremova N, Franke R, Schreiber L, Saedler H, Métraux J-P, Yephremov A** (2006) The epidermis-specific extracellular BODYGUARD controls cuticle development and morphogenesis in Arabidopsis. Plant Cell **18**: 321–339.

44. **Laby RJ, Kim D, Gibson SI** (2001) The ram1 mutant of Arabidopsis exhibits severely decreased β-amylase activity. Plant Physiol **127**: 1798-1807.
45. Li Y, Beisson F, Koo AJK, Molina I, Pollard M, Ohlrogge J (2007) Identification of acyltransferases required for cutin biosynthesis and production of cutin with suberin-like monomers. Proc Natl Acad Sci USA 104: 18339-18344.

46. Liu J, Zhu J-K (1997) An *Arabidopsis* mutant that requires increased calcium for potassium nutrition and salt tolerance. Proc Natl Acad Sci USA 94: 14960-14964.

47. Maldonado AM, Doerner P, Dixon RA, Lamb CJ, Cameron RK (2002) A putative lipid transfer protein involved in systemic resistance signaling in *Arabidopsis*. Nature 419: 399-403

48. Mang HG, Laluk KA, Parsons EP, Kosma DK, Cooper BR, Park HC, AbuQamar S, Boccongelli C, Miyazaki S, Consiglio F, Chilosi G, Bohnert HJ, Bressan RA, Mengiste T, Jenks MA (2009) The *Arabidopsis* *RESURRECTION1* gene regulates a novel antagonistic interaction in plant defense to biotrophs and necrotrophs. Plant Physiol 151:290-305.

49. McConn M, Hugly S, Browse J, Somerville C (1994) A mutation at the *fad8* locus of *Arabidopsis* identifies a second chloroplast ω-3 desaturase. Plant Physiol 106: 1609-1614.

50. Miquel M, Cassagne C, Browse J (1998) A new class of *Arabidopsis* mutants with reduced hexadecatrienoic acid fatty acid levels. Plant Physiol 117: 923-930.

51. Molina I, Bonaventure G, Ohlrogge, J, Pollard M (2006) The lipid polyester composition of *Arabidopsis thaliana* and *Brassica napus* seeds. Phytochemistry 67: 2597-2610.

52. Morohashi K, Grotewold E (2009) A system approach reveals regulatory circuitry for *Arabidopsis* trichome initiation by the GL3 and GL1 selectors. PLOS Genetics 5: e1000396

53. Morohashi K, Zhao M, Yang M, Read B, Lloyd A (2007) Participation of the *Arabidopsis* bHLH factor GL3 in trichome initiation regulatory events. Plant Physiol 145: 736-746

54. Nandi A, Welti R, Shah J (2004) The *Arabidopsis thaliana* dihydroxyacetone phosphate reductase gene SUPPRESSOR OF FATTY ACID DESATURASE DEFICIENCY1 is
required for glycerolipid metabolism and for the activation of systemic acquired resistance. Plant Cell 16: 465-477.

55. Oppenheimer DG, Herman PL Sivakumaran S, Esch J, Marks MD (1991) A myb gene required for leaf trichome differentiation in Arabidopsis is expressed in stipules. Cell 67: 483-493.

56. Panikashvili D, Savaldi-Goldstein S, Mandel T, Yifhar T, Franke RB, Höfer R, Schreiber L, Chory J, Aharoni A (2007) The Arabidopsis DESPERADO/AtWBC11 transporter is required for cutin and wax secretion. Plant Physiol 145:1345-1360.

57. Park S-W, Kaimoyo E, Kumar D, Mosher S, Klessig, DF (2007) Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science 318: 113-116.

58. Park SW, Liu PP, Forouhar F, Vlot AC, Tong L, Tietjen K, Klessig DF (2009) Use of a synthetic salicylic Acid analog to investigate the roles of methyl salicylate and its esterases in plant disease resistance. J Biol Chem 284: 7307-7317.

59. Payne CT, Zhang F, Llyod AM (2000) GL3 encodes a bHLH protein that regulates trichome development in Arabidopsis through interaction with GL1 and TTG1. Genetics 156: 1349-1362.

60. Perazza D, Vachon G, Herzog M (1998) Gibberellins promote trichome formation by up-regulating GLABROUS1 in Arabidopsis. Plant Physiol 117: 375-383.

61. Pruitt RE, Vielle-Calzada JP, Ploense SE, Grossniklaus U, Lolle SJ (2000) FIDDLEHEAD, a gene required to suppress epidermal cell interactions in Arabidopsis, encodes a putative lipid biosynthetic enzyme. Proc Natl Acad Sci USA 97: 1311-1316.

62. Quiel JA, Bender J (2003) Glucose conjugation of anthranilate by the Arabidopsis UGT74F2 glucosyltransferase is required for tryptophan mutant blue fluorescence. J Biol Chem 278: 6275-6281.

63. Rasmussen, JB, Hammerschmidt, R and Zook, MN (1991) Systemic induction of salicylic acid accumulation in cucumber after inoculation with Pseudomonas syringae pv syringae. Plant Physiol 97: 1342-1347.

64. Reina-Pinto JJ, Voisin D, Kurydakov S, Faust A, Haslam RP, Michaelson LV, Efremova N, Franke B, Schreiber L, Napier JA, Yephremov, A (2009) Misexpression of FATTY ACID ELONGATION1 in the Arabidopsis epidermis induces cell death and suggests a critical role for phospholipase A2 in this process. Plant Cell 21: 1252-1272
65. Rowe HC, Walley JW Corwin J, Chan EK-F, Dehesh K, Kliebenstein DJ (2010)
    Deficiencies in jasmonate-mediated plant defense reveal quantitative variation in Botrytis
    cinerea pathogenesis. PLOS Pathogen 6: e1000861.

66. Schellmann S, Hülskamp M (2005) Epidermal differntiation: trichomes in Arabidopsis
    as a model system. Int J Dev Bio 49: 579-584.

67. Schnurr J, Shockey J, Browse J (2004) The acyl-CoA synthetase encoded by LACS2 is
    essential for normal cuticle development in Arabidopsis. Plant Cell 16: 629–642.

68. Shirasu K, Nakajima H, Rajasekhar VK, Dixon RA, Lamb C (1997) Salicylic acid
    potentiates an agonist-dependent gain control that amplifies pathogen signals in the
    activation of defense mechanisms. Plant Cell 9: 261-270.

69. Smith-Becker J, Marois E, Huguet EJ, Midland SL, Sims JJ, Keen NT (1998)
    Accumulation of salicylic acid and 4-hydroxybenzoic acid in phloem fluids of cucumber
    during systemic acquired resistance is preceded by a transient increase in phenylalanine
    ammonia-lyase activity in petioles and stems. Plant Physiol 116: 231-238.

70. Speth EB, Imboden L, Hauck P, He SY (2009) Subcellular localization and functional
    analysis of the Arabidopsis GTPase RabE. Plant Physiol 149: 1824-1837.

71. Tanaka T, Tanaka H, Machida C, Watanabe M, Machida Y (2004) A new method
    for rapid visualization of defects in leaf cuticle reveals five intrinsic patterns of surface
    defects in Arabidopsis. Plant J 37: 139-146.

72. Truman W, Bennett MH, Kubigsteltig I, Turnbull C, Grant M (2007) Arabidopsis
    systemic immunity uses conserved defense signaling pathways and is mediated by
    jasmonates. Proc Natl Acad Sci USA 104: 1075-10780.

73. Truman W, Bennett MH, Turnbull CGN, Grant MR (2010) Arabidopsis auxin
    mutants are compromised in systemic acquired resistance and exhibit aberrant
    accumulation of various indolic compounds. Plant Physiol 152: 1562-1573.

74. Venugopal SC, Jeong R-D, Mandal M, Zhu S, Chandra-Shekara AC, Xia Y, Hersh
    M, Stromberg AJ, Navarre D, Kachroo A, Kachroo P (2009) ENHANCED DISEASE
    SUSCEPTIBILITY 1 and SALICYLIC ACID act redundantly to regulate resistance gene
    expression and low OLEATE-induced defense signaling. PLOS Genetics 5: e1000545.

75. Vlot AC, Dempsey DA, Klessig DF (2009) Salicylic acid, a multifaceted horomone to
    combat disease. Ann Rev Phytopath 47: 177-206.
76. Voisin D, Nawrath C, Kurdyukov S, Franke RB, Reina-Pinto JJ, Efremova N, Will I, Schreiber L, Yephremov A (2009) Dissection of the Complex Phenotype in Cuticular Mutants of Arabidopsis Reveals a Role of SERRATE as a Mediator. PLoS Genet 5: e1000703.

77. Walker AR, Davison PA, Bolognesi-Winfield AC, James CM, Srinivasan N, Blundell TL, Esch JJ, Marks MD, Gray JC (1999) The TRANSPARENT TESTA GLABRA1 locus, which regulates trichome differentiation and anthocyanin biosynthesis in Arabidopsis, encodes a WD40 repeat protein. Plant Cell 11: 1337-1350

78. Wellesen K, Durst F, Pinot F, Benveniste I, Nettesheim K, Wisman E, Steiner-Lange S, Saedler H, Yephremov A (2001) Functional analysis of the LACERATA gene of Arabidopsis provides evidence for different roles of fatty acid ω-hydroxylation in development. Proc Natl Acad Sci USA 98: 9694–9699.

79. Xia Y, Gao Q-M, Yu K, Lapchyk L, Navarre D, Hildebrand D, Kachroo A, Kachroo P (2009) An intact cuticle in distal tissues is essential for the induction of systemic acquired resistance in plants. Cell Host & Microbe 5: 151-165

80. Xie DX, Feys BF, James S, Nieto-Rostro M, Turner JG (1998) COII: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science 280: 1091-1094.

81. Yephremov A, Wisman E, Huijser P, Huijser C, Wellesen K, Saedler H (1999) Characterization of the FIDDLEHEAD gene of Arabidopsis reveals a link between adhesion response and cell differentiation in the epidermis. Plant Cell 11: 2187-2202.

82. Yi Y, Jack T (1998) An intragenic suppressor of the Arabidopsis floral organ identity mutant apetala3-1 functions by suppressing defects in splicing. Plant Cell 10: 1465-1478.

83. Zhang F, Gonzalez A, Zhao M, Payne CT, Lloyd A (2003) A network of redundant bHLH proteins functions in all TTG1-dependent pathways of Arabidopsis. Development 130: 4859-4869.

84. Zhu J, Fu Z, Koo YD, Zhu J-K, Jenny FEJr, Adams MWW, Zhu Y, Shi H, Yun D-J, Hasegawa PM, Bressan RA (2007) An enhancer mutant of Arabidopsis salt overly sensitive 3 mediates both ion homeostasis and the oxidative stress response. Mol Cell Biol 27: 5214-5224.
FIGURE LEGENDS

Figure 1. SAR response in fad mutants. Primary leaves were inoculated with MgCl₂ (gray bars) or *P. syringae* expressing *avrRpt2* (black bars) and the systemic leaves were inoculated 48 h later with a virulent strain of *P. syringae*.

Figure 2. SA levels and pathogen response of Col-0 and *fad7-1* plants after exogenous application of BTH. (A) SA and SAG levels in Col-0 and *fad7-1* plants inoculated with MgCl₂ or *P. syringae* expressing *avrRpt2*. (B) SAR response in Col-0 and *fad7-1* plants infiltrated with exudates collected from wt or *fad7-1* plants that were treated either with MgCl₂ or *P. syringae* expressing *avrRpt2*. Exudates (Ex) were mixed with water or 100 µM BTH prior to infiltration into a fresh set of plants. (C) SAR in Col-0 or *fad7-1* plants treated with water or BTH for 48 h prior to inoculation. (D) RNA gel blot showing transcript levels of *PR-1* gene in plants treated with water or BTH for 48 h.

Figure 3. FA and JA levels and pathogen response of Col-0 and *fad7-1* plants after exogenous application of JA. (A) Levels of FAs in four-week-old Col-0 and *fad7-1* leaves. The error bars represent SD. Asterisks denote a significant difference with Col-0 (*t* test, *p*<0.05). FW indicates fresh weight. (B) JA levels in Col-0 and *fad7-1* plants inoculated with MgCl₂ or *P. syringae* expressing *avrRpt2*. (C) SAR response in Col-0 and *fad7-1* plants infiltrated with exudates collected from wt or *fad7-1* plants that were treated either with MgCl₂ or *P. syringae* expressing *avrRpt2*. Exudates (Ex) were mixed with water or 50 µM JA prior to infiltration into a fresh set of plants. (D) SAR in Col-0 or *fad7-1* plants treated with water or JA for 48 h prior to inoculation.

Figure 4. FA levels, SAR response and trichome phenotypes in *fad7* and *fad7 fad8* plants. (A) Levels of FAs in four-week-old leaves. The error bars represent SD. Asterisks denote a
significant difference with Col-0 \((t\) test, \(P<0.05\)). FW indicates fresh weight. (B) SAR response in indicated genotypes. (C) Leaves from indicated genotypes showing presence or absence of trichomes. (D) Levels of FAs in indicated genotypes. At least 10 different T2 transgenic plants expressing a genomic copy of \(FAD7\) in the \(fad7-1\) background were analyzed and all showed a similar profile. The error bars represent SD. Asterisks denote a significant difference with Col-0 \((t\) test, \(P<0.05\)). FW indicates fresh weight. (E) Cleaved amplified polymorphic sequence analysis of indicated genotypes for \(fad7-1\) mutation. (F) SAR response in indicated genotypes. Two independent transgenic lines were analyzed and both showed compromised SAR.

**Figure 5. Responsiveness of \(gl1\) plants to petiole exudates collected from pathogen inoculated plants.** (A) SAR response in Col-0, \(fad7-1\) \(gl1\) and \(gl1\) plants infiltrated with exudates collected from wt or \(acp4\) plants that were treated either with MgCl\(_2\) or \(avrRpt2\). (B) RNA gel blot showing transcript levels of \(PR-1\) in Col-0 and \(fad7-1\) \(gl1\) leaves infiltrated with petiole exudates (Ex). \(PR-1\) transcript levels were analyzed 48h after treatments. M and avr indicate petiole exudates collected from leaves infiltrated with MgCl\(_2\) or \(P. syringae\) containing \(avrRpt2\).

**Figure 6. Evaluation of cuticle-associated phenotypes in \(fad7-1\) \(gl1\) plants.** (A) Toluidine blue-stained leaves from four-week-old leaves of indicated genotypes. (B) Measurement of water lost from the leaves subjected to drought conditions for 4 days. (C) A time-course measurement of chlorophyll leaching in indicated genotypes. (D) Transmission electron micrographs showing the cuticle layer on the adaxial surface of leaves from indicated genotypes. Arrow indicates electron-opaque regions. CW indicates cell wall (scale bars, 50 nm). (E) Scanning electron micrographs showing adaxial surface of leaves from indicated genotypes (scale bars, 200 \(\mu\)M).

**Figure 7. Response of \(gl1\) to hemibiotrophic pathogen \(C. higginsianum\).** (A) Disease symptoms on indicated genotypes spot-inoculated with \(10^6\) spores/ml of \(C. higginsianum\). (B) Lesion size in spot-inoculated genotypes. The plants were spot-inoculated with \(10^6\) spores/ml of
C. higginsianum and the lesion size was measured from 20-30 independent leaves at 6 dpi. Statistical significance was determined using Students t-test. Asterisks indicate data statistically significant from that of control (Col-0) (P<0.05). Error bars indicate SD.

Figure 8. Evaluation of cuticle associated phenotypes and SAR response in gl3, and ttg1 plants. (A) Toluidine blue-stained leaves from four-week-old leaves of indicated genotypes. (B) A time-course measurement of chlorophyll leaching in indicated genotypes. FW indicates fresh weight. (C) SAR response in indicated genotypes.

Figure 9. Evaluation of cuticle associated phenotypes and SAR response in plants treated with GA. (A) Toluidine blue-stained leaves from four-week-old leaves of indicated genotypes. (B) Analysis of wax components from leaves of four-week old Col-0 and gl1 plants treated with water or GA. 16:0-30:0 are FAs, C27-C33 are alkanes, C26-OH-C32-OH are primary alcohols. (C) SAR response in Col-0 and gl1 plants pretreated with water or GA. Asterisks denote a significant difference with respective water-treated and MgCl2-infiltrated plants (t test, P<0.05).
Supplementary Figure Legends

Supplemental Figure 1. An Abbreviated scheme for fatty acid and lipid biosynthesis. *De novo* FA biosynthesis from acetyl-CoA occurs exclusively in the plastids of all cells (represented by oval). Acetyl-CoA carboxylase (ACC) and the fatty acid synthase (FAS) complex are key enzymes involved in biosynthesis of palmitic acid (16:0). Upon elongation to stearic acid (18:0), this FA undergoes desaturation to oleic acid (18:1). Desaturation of stearic acid (18:0)-ACP to 18:1-ACP catalyzed by the *SSI2/FAB2*-encoded stearoyl-acyl carrier protein (ACP) desaturase (SACPD), is one of the key steps in the FA biosynthesis pathway that regulates levels of unsaturated FAs in the cell. The 18:1-ACP generated in this reaction enters the prokaryotic pathway through acylation of glycerol-3-phosphate (G3P) and this reaction is catalyzed by the *ACT1*-encoded G3P acyltransferase. The 18:1-ACP is also exported out of plastids as a CoA-thioester to enter the eukaryotic pathway. Desaturation of 18:1 present on membrane glycerolipids (GL) is catalyzed by *FAD2* and *FAD6*-encoded ω6 desaturases that are present on the endoplasmic reticulum (ER) or plastid envelope, respectively. Desaturation of 18:2 present on membrane GL is catalyzed by *FAD3* and *FAD7/FAD8*-encoded desaturases that are present on the ER and plastid respectively. Desaturation of 16:0 to 16:1 is catalyzed by plastidal-localized desaturases FAD4 and FAD5. Symbols for various components are: Lyso-PA, acyl-glycerol 3-phosphate; PA, phosphatidic acid; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SL, sulfolipid; DAG, diacylglycerol.

Supplemental Figure 2. Leaves from indicated genotypes showing presence or absence of trichomes. Five different transgenic lines expressing genomic copy of *FAD7* in *fad7-1 gl1* background were tested and all showed absence of trichomes.

Supplemental Figure 3. Cuticular wax and cutin monomer profiles. (A) Analysis of wax components from leaves of indicated genotypes. 16:0-24:0 are FAs, C27-C33 are alkanes, C26-OH-C32-OH are primary alcohols. (B) Analysis of lipid polyester monomer content of four-
week-old plants. Error bars in A and B represent SD. Asterisks in A and B denote a significant difference with wt (Col-0) (t test, P<0.05). Symbols for various components are: 16:0-DCA, 1,16-hexadecane dioic acid; 16-OH-16:0, 16-hydroxyhexadecanoic acid; 10,16-OH-16:0, 10,16-dihydroxyhexadecanoic acid; 18:0-DCA, 1,18-octadecane dioic acid; 18:1-DCA, 1,18-octadecene dioic acid; 18:-OH-18:1, 18-hydroxyoctadecenoic acid, 18:2-DCA, 1,18-octadecadiene dioic acid; 18-OH-18:2, 18-hydroxyoctadecadienoic acid; 18-OH-18:3, 18-hydroxyoctadecatrienoic acid.

Supplemental Figure 4. Response of gl1 to necrotrophic pathogen B. cinerea. Lesion size in spot-inoculated genotypes. The plants were spot-inoculated with $10^6$ spores ml$^{-1}$ of B. cinerea and the lesion size was measured from 20-30 independent leaves at 6 dpi. Statistical significance was determined using Students t-test. Asterisks indicate data statistically significant from that of control (Col-0) (P<0.05). Error bars indicate SD.

Supplemental Figure 5. Leaves of indicated genotypes showing absence of trichomes on gl3 and ttg1 plants.

Supplemental Figure 6. Effect of GA treatment on trichome formation, leaf size and chlorophyll leaching. (A) Leaves of Col-0 and gl1 plants treated with water or GA. GA treatment increased the leaf size but did not induce trichome formation on gl1 plants. Scale bars, 2 cm. (B) A time-course measurement of chlorophyll leaching in Col-0 and gl1 plants treated with water or GA.

Supplemental Figure 7. Scanning electron micrographs showing trichome on adaxial surface of leaves from Nö and acp4 plants. Holes in acp4 leaf indicate ruptured cuticle.
Figure 1. SAR response in fad mutants. Primary leaves were inoculated with MgCl₂ (gray bars) or P. syringae expressing avrRpt2 (black bars) and the systemic leaves were inoculated 48 h later with virulent strain of P. syringae.
Figure 2. SA levels and pathogen response of Col-0 and fad7-1 plants after exogenous application of BTH. (A) SA and SAG levels in Col-0 and fad7-1 plants inoculated with MgCl₂ or P. syringae expressing avrRpt2. (B) SAR response in Col-0 and fad7-1 plants infiltrated with exudates collected from wt or fad7-1 plants that were treated either with MgCl₂ or P. syringae expressing avrRpt2. Exudates (Ex) were mixed with water or 100 µM BTH prior to infiltration into fresh set of plants. (C) SAR in Col-0 or fad7-1 plants treated with water or BTH for 48 h prior to inoculation. (D) RNA gel blot showing transcript levels of PR-1 gene in plants treated with water or BTH for 48 h.
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FIGURE 4

A

![Bar graph showing FA (mg/g FW)]

- Col-0
- fad7-1
- fad7-2
- fad7-1 fad7/8
- fad7 fad8
- gl1

B

![Bar graph showing Log CFU / Leaf disk]

- Mock
- avrRpt2

C

![Images of leaf samples at 0 dpi and 3 dpi]

- Col-0
- fad7-1
- gl1
- fad7-2
- fad7 fad8
Figure 4. FA levels, SAR response and trichome phenotypes in fad7 and fad7 fad8 plants.

(A) Levels of FAs in four-week-old leaves. The error bars represent SD. Asterisks denote a significant difference with Col-0 (t test, P<0.05). FW indicates fresh weight. (B) SAR response in indicated genotypes. (C) Leaves from indicated genotypes showing presence or absence of trichomes. (D) Levels of FAs in indicated genotypes. At least 10 different T2 transgenic plants expressing genomic copy of FAD7 in fad7-1 background were analyzed and all showed similar profile. The error bars represent SD. Asterisks denote a significant difference with Col-0 (t test, P<0.05). FW indicates fresh weight. (E) Cleaved amplified polymorphic sequence analysis of indicated genotypes for fad7-1 mutation. (F) SAR response in indicated genotypes. Two independent transgenic lines were analyzed and both showed compromised SAR.
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Figure 6. Evaluation of cuticle associated phenotypes in fad7-1 gl1 plants. (A) Toluidine blue stained leaves from four-week-old leaves of indicated genotypes. (B) Measurement of water lost from the leaves subjected to drought conditions for 4 days. (C) A time-course measurement of chlorophyll leaching in indicated genotypes. (D) Transmission electron micrographs showing cuticle layer on adaxial surface of leaves from indicated genotypes. Arrows indicate electron-opaque regions. CW indicated cell wall (scale bars, 50 nm). (E) Scanning electron micrographs showing adaxial surface of leaves from indicated genotypes (scale bars, 200 μM).
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