

Involvement of Arabidopsis Acyl Carrier Protein 1 in PAMP-triggered immunity

Zhenzhen Zhao¹, Jiangbo Fan^{1,2}, Piao Yang¹, Zonghua Wang³, Stephen Obol Opiyo¹, David Mackey⁴, Ye Xia^{1,*}

¹Department of Plant Pathology, The Ohio State University, 2021 Coffey Road, Columbus, OH 43210, U.S.A.

²School of Agriculture and Biology, Shanghai Jiao Tong University, 800 Dongchuan Rd., Shanghai, 200240, China

³State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou, 350002, China

⁴Department of Horticulture and Crop Science, The Ohio State University, Columbus, OH 43210, U.S.A.

* Corresponding author: Ye Xia; Email: xia.374@osu.edu

Keywords: acyl carrier protein, fatty acids, plant immunity, PTI, plant hormones

Funding: This project was supported by the Hatch Project from USDA-NIFA-OHO01392; the Ohio Agricultural Research and Development Center (OARDC) seed grant OHOA1591; startup fund from OARDC and Ohio State University to YX.

21 **Abstract:**

22 Plant fatty acids (FAs) and lipids are essential in storing energy and act as structural
23 components for cell membranes and signaling molecules for plant growth and stress responses.
24 Acyl Carrier Proteins (ACPs) are small acidic proteins that covalently bind the fatty acyl
25 intermediates during the elongation of FAs. The *Arabidopsis thaliana* ACP family has eight
26 members. Through reverse genetic, molecular, and biochemical approaches, we have discovered
27 that ACP1 localizes to the chloroplast and limits the magnitude of pattern-triggered immunity (PTI)
28 against the bacterial pathogen *Pseudomonas syringae* pathovar tomato (*Pto*). The mutant *acp1*
29 plants have reduced levels of linolenic acid (18:3), which is the primary precursor for the
30 biosynthesis of the phytohormone jasmonic acid (JA), and a corresponding decrease in the
31 abundance of JA. Consistent with the known antagonistic relationship between JA and salicylic
32 acid (SA), *acp1* mutant plants also accumulate higher level of SA and display the corresponding
33 shifts in JA- and SA-regulated transcriptional outputs. Moreover, the methyl JA and linolenic acid
34 treatments cause an apparently enhanced decrease of resistance against *Pto* in *acp1* mutants than
35 that in wild-type plants. The ability of ACP1 to prevent this hormone imbalance likely underlies
36 its negative impact on PTI in plant defense. Thus, ACP1 links FA metabolism to stress hormone
37 homeostasis to be negatively involved in PTI in *Arabidopsis* plant defense.

38

39 **Keywords:** acyl carrier protein, fatty acids, plant immunity, PTI, plant hormones

40

41

42

43 **INTRODUCTION:**

44 *Pseudomonas syringae* pathovar tomato (*Pto*) is an agriculturally important pathogen that
45 causes the bacterial speck disease on tomato plants resulting in yield and economic losses (Bashan,
46 1997; Bashan and de-Bashan, 2002). *Pto* strain DC3000 (*Pto* DC3000) is also pathogenic to the
47 model plant *Arabidopsis thaliana*, which provides a classic system to study the pathogenesis
48 mechanisms and dynamics of plant-pathogen interactions (Xin et al., 2018). In addition to *Pto*,
49 more than 50 different *P. syringae* pathovars can infect more than 200 plant species and cause
50 destructive diseases, from leaf speck to stem cankers (Buttimer et al., 2017). Plants defend
51 themselves against *P. syringae* and the other pathogenic microbes through multi-layered innate
52 immunity (Jones and Dangl, 2006). Basal defense constitutes a first layer that often protects plants
53 entirely or limits the extent of pathogen growth. Critical to basal defense is the pattern triggered
54 immunity (PTI), which is activated upon the interaction of microbe-associated molecular patterns
55 (MAMPs) with transmembrane pattern recognition receptors (PRRs), such as between a 22 amino
56 acid epitope of the flagellin protein (flg22) and the kinase receptor FLAGELLIN SENSITIVE2
57 (FLS2) (Jones and Dangl, 2006). Pathogens secrete virulence effectors, such as secreted proteins
58 and toxins, limiting the basal defense and thus facilitating infection. The second layer of plant
59 defense, effector triggered immunity (ETI), is elicited when a resistance (R) protein directly or
60 indirectly recognizes a corresponding effector (Takemoto and Jones, 2005; Jones and Dangl, 2006;
61 Jones et al., 2016). Emerging experimental evidence indicates the significant mutual influences
62 and the overlapping downstream defense outputs between PTI and ETI, suggesting the intricate
63 and integrative interactions between these two layers of plant immunity (Ngou et al., 2021; Yuan
64 et al., 2021b; Yuan et al., 2021a).

Plant defense responses include reactive oxygen species (ROS) production, cell wall fortification, shifts in hormone levels and gene expression patterns, *etc.* (Spoel and Dong, 2012). ROS is produced rapidly upon the MAMP engagement of a PRR (Liu et al., 2009). In addition to providing antimicrobial activity, ROS are signaling molecules that influence plant defense (Dat et al., 2000; Mittler et al., 2004; Kwak et al., 2006). For example, the exogenous H₂O₂ enhances the accumulation of SA, which further upregulates ROS production (Shirasu et al., 1997). Cell wall fortification, which includes the deposition of the polysaccharide callose, is a somewhat later output of defense activation (Luna et al., 2011). Plant hormones play critical roles in plant defense as well. For example, SA-signaling activates both local and systemic defense responses against *Pto* (Gao et al., 2015). Conversely, JA-signaling increases the susceptibility of Arabidopsis to *Pto* (Zheng et al., 2012). These observations are consistent with numerous studies demonstrating the antagonism between SA- and JA-signaling (Xin and He, 2013). The interplay between fatty acid and lipid biosynthetic pathways with these defense hormones is a significant knowledge gap for understanding the plant immune responses.

Fatty acids (FAs) and lipids are critical for many biological processes of plants. Their involvement in energy storage is critical for plant growth and development. They are also important structural components of plant membranes (Brown et al., 2009). FAs and lipids have been implicated as signaling molecules contributing to systemic acquired resistance (SAR). For instance, glycerol-3-phosphate, a phosphoric ester of glycerol and a critical component of glycerophospholipids, is a mobile inducer of the broad-spectrum and long-lasting plant disease resistance characteristic of SAR (Chanda et al., 2011; Lim et al., 2020). FAs and lipids also play diverse functions in plant defense (Zoeller et al., 2012; Gao et al., 2014; Gao et al., 2015). SSI2 encodes a stearyl-acyl carrier protein desaturase, which mediates the conversion of stearic acid

(18:0) to oleic acid (18:1) to regulate the levels of unsaturated fatty acids in Arabidopsis plants. The reduced levels of oleic acid (18:1 FA) in Arabidopsis plants with a mutation of *SSI2* correlate with the increased ROS, SA, callose deposition levels, spontaneous cell death, and enhanced resistance against *Pto* (Kachroo et al., 2001). A mutation in Acyl Carrier Protein 4 (ACP4) restored the levels of 18:1 FA in *ssi2* plants, which in turn suppresses all the *ssi2*-triggered physiological and defense phenotypes. The ability of ACP4 to affect SSI2 related function was due to its effect on the glycerol-3-phosphate (G3P) acyltransferase (ACT1)-catalyzed reaction (Xia et al., 2009).

Acyl Carrier Proteins (ACPs) are central components for fatty acid biosynthesis. In plants, *de novo* FA biosynthesis is carried out by the conserved reactions starting with acetyl-CoA in the chloroplast (Byers and Gong, 2007). The synthesis process is performed via multiple elongation cycles (Marrakchi et al., 2002). The ACPs are small (~9kD) acidic polypeptides that covalently bind the fatty acyl intermediates through a phosphopantetheine linker during the elongation process (Chan and Vogel, 2010). The fatty acyl chain is extended by acetyl-CoA, two carbon units per cycle, until C16 or C18 acyl-ACPs are formed and exported for the long-chain FAs and lipid biosynthesis. Several ACP isoforms have been characterized in multiple plant species, including spinach (Ohlrogge et al., 1979), soybean (Ohlrogge and Kuo, 1985), barley (Hansen and von Wettstein-Knowles, 1991), cotton (Song and Allen, 1997), rapeseed (SAFFORD et al., 1988), olive (Cultrera et al., 2014) and Arabidopsis (Hloušek-Radojčić et al., 1992). Although the critical roles of ACPs in FA biosynthesis have been demonstrated, the roles of various ACP isoforms in plants remain unclear (Bonaventure and Ohlrogge, 2002). Of the eight genes encoding ACPs in the genome of the model plant Arabidopsis, five were predicted to be localized to the plastid [ACP1 (At3g05020), ACP2 (At1g54580), ACP3 (At1g54630), ACP4 (At4g25050), ACP5 (At5g27200)] and three to the mitochondria [mtACP1 (At2g44620), mtACP2 (At1g65290), and

mtACP3 (At5g47630)] through the use of antibodies and mass spectrometry (Meyer et al., 2007; Meier et al., 2010). The transcript and protein levels of ACPs in plants are influenced by diverse biotic and abiotic stresses (Ruuska et al., 2002). For instance, phosphate starvation increases the protein abundance of plastidal ACP isoforms (ACP1/2/3/4) in roots (Lan et al., 2012). ACP4 contributes to SAR dependent on its critical role for the cuticle formation in Arabidopsis leaves (Xia et al., 2009; Lim et al., 2020). It was reported that ACP5 could contribute to the plant resistance against salt stress, possibly through an influence of FAs on the Na⁺/K⁺ ratio. Indeed, overexpression of *ACP5* increased the levels of palmitic acid (16:0) and decreased the levels of oleic acid (18:1) (Huang et al., 2017). The functions of the other ACP isoforms in fatty acid biosynthesis and biotic or abiotic stresses are poorly understood.

In this study, we demonstrate that ACP1 limits the magnitude of PTI by influencing fatty acid biosynthesis. Specifically, the reduced levels of linolenic acid (18:3 FA) in the leaves of *acp1* mutant plants underlie the enhanced resistance against *Pto* DC3000 through the effects on the JA and SA accumulation and the related signaling pathways.

RESULTS

Identification and characterization of *acp1* mutant plants.

ACP is an essential cofactor during the synthesis of fatty acids in bacteria and plants. The phosphopantetheine binding domain (PP-binding superfamily), conserved in ACP proteins, is the prosthetic group of ACP, which acts as a linker for the attachment of the activated fatty acid and amino-acid groups via a phosphodiester bond (Roujeinikova et al., 2002; Crawford et al., 2008; Meier et al., 2010). All eight Arabidopsis ACP isoforms share this conserved domain (**Figure 1a**). To further investigate the relationships of the ACPs, the protein sequences of all 8 ACP isoforms

were uploaded to Clustal Omega for phylogenetic analysis by multiple sequence alignments. The protein sequence of ACP2 is 90% similar to ACP3. Furthermore, ACP1 is more closely related to ACP5 than the other ACP isoforms (**Figure 1b**).

Here, the roles of ACPs against biotic stress, especially in response to the bacterial pathogen *Pto* DC3000 were studied. The altered gene expression of *ACPs* in infected plant leaves might indicate their contribution to plant defense responses. The abundance of *ACP* transcripts was measured in the wild type (WT) Col-0 leaves before and 6 hours after the *Pto* DC3000 inoculation, which revealed that the expression of multiple *ACPs* changed in response to the pathogen infection. The basal expression of different isoforms was variable in WT Col-0 leaves. *ACP4* was expressed the highest in Arabidopsis leaves. The *Pto* infection reduced the transcript levels of *ACP1*, *ACP4*, and *ACP5* but increased *ACP3*, *mtACP2*, and *mtACP3* (**Figure S1**). The T-DNA insertion lines of all the 8 Arabidopsis *ACP* genes were obtained from ABRC (Arabidopsis Biological Resource Center at The Ohio State University), and the homozygous lines were identified using the protocol designed by the Salk Institute Genomic Analysis Laboratory (<http://signal.salk.edu/tdnaprimers.2.html>). The resistance levels of all the eight *acp* mutant plants were investigated against the *Pto* DC3000 infection. It was found that *acp1* mutant plants showed the enhanced resistance against *Pto* DC3000 compared to WT plants (unpublished data). Based on the enhanced resistance to *Pto*, the role of ACP1 in plant immunity was further investigated. For *acp1* mutant lines, the insertion sites of the two T-DNA insertion lines (*acp1-1* and *acp1-2*) are within the promoter and first intron, respectively (**Figure S2**). The transcript levels of *ACP1* were compared between the two *acp1* homozygous mutants and WT Col-0 plants by quantitative real-time PCR (qPCR). The *ACP1* transcript levels were significantly lower in the mutant lines than WT (**Figure 1c**).

FAs are critical constituents of cellular membranes, which maintain cellular structure and function. FAs are also essentially involved in the precursor formations of suberin, cell wall, cutin, and wax, which are pivotal for constructing the structural barriers and vital for plant growth and development (Beisson et al., 2007; Maejima et al., 2014). In our study, the visual examination revealed no effects of either *acp1* mutant allele on plant growth or development. Physiological parameters, such as biomass, plant height, and chlorophyll content, did not differ between the *acp1* mutants and WT plants (**Figure S3&S4**). Therefore, the defective function of *ACP1* does not appear to impair the growth and development of Arabidopsis plants significantly.

Subcellular localization of ACP1 in the transiently transformed *Nicotiana benthamiana*.

ACP1 is annotated as a plastidial ACP (Hloušek-Radojčić et al., 1992; Meyer et al., 2007; Meier et al., 2010). And it was predicted to be localized in chloroplast, which contains a chloroplast transit peptide with 30 amino acids as an N-terminal sorting signal based on the analysis results using the software of TargetP (<https://services.healthtech.dtu.dk/service.php?TargetP-2.0>) and iPSORT (<https://ipsort.hgc.jp/how.html>). To confirm the subcellular prediction, we constructed a GFP (green fluorescent protein)-tagged version of full-length ACP1 (ACP1-GFP) to determine the subcellular localization of ACP1 *in planta*. The empty vector pYBA1122-35S-GFP was applied as a negative control. ACP1-GFP and empty vector-GFP were co-expressed in *N. benthamiana* leave with PT-mCherry, a plastid organelle marker, respectively (Nelson et al., 2007). The fluorescence signals were checked three days after the infiltration through the confocal laser scanning microscopy. The green fluorescence signal of ACP1-GFP was found to overlap with the red fluorescence signal of PT-mCherry (**Figure 2a**). Meanwhile, the green signal of the empty vector-GFP was diffusely distributed (**Figure 2b**). Thus, it indicated that ACP1 localizes to the chloroplast in Arabidopsis plants.

179 Responses of *acp1* plants to the *Pto* infection.

180 To confirm the defense phenotype of *acp1* mutant plants, two *acp1* mutants and WT plants
181 were syringe infiltrated with a suspension of 5×10^5 CFU (colony forming unit)/ml of *Pto* DC3000.
182 WT Col-0 plants showed more severe chlorotic and necrotic disease symptoms than *acp1* mutant
183 plants three days after the inoculation (**Figure 3a**). Meanwhile, the bacterial growth was measured
184 at 0- and 3-days post-inoculation. The *Pto* DC3000 proliferated significantly less in the *acp1*
185 mutants than WT at 3 days post-inoculation (**Figure 3b**). To further investigate the role of *ACP1*
186 in PTI, the expression levels of three PTI marker genes, including *FRK1*, *Atlg51890*, and
187 *At2g17740* (Cheng et al., 2013; Guo et al., 2014), were monitored upon *Pto* DC3000 infection.
188 Notably, the induction of all the three PTI marker genes was significantly enhanced in *acp1*
189 mutants at 6 and 48 hours post-inoculation, compared with WT plants (**Figure 3c**). These results
190 indicate that *ACP1* is negatively involved in Arabidopsis resistance against the *Pto* DC3000
191 infection.

192 The *acp1* mutant plants showed reduced levels of C18:3 fatty acid.

193 As ACPs are the critical factors for FA synthesis, the abundance of total FAs was measured
194 from the 4-week-old *acp1* mutants and WT plant leaves following the established procedures (Xia
195 et al., 2009). Of the C16 and C18 FAs measured, only C18:3 FA differed significantly in the *acp1*
196 mutants compared to WT plants (**Figure 4a**). The reduced accumulation of C18:3 FA in the mutant
197 plants indicates a requirement of *ACP1* for the biosynthesis of this class of FA in Arabidopsis
198 leaves. To further investigate the level change of C18:3 FA in WT and *acp1* mutant plants against
199 the pathogen infection, the abundance of C18:3 FAs from *acp1* mutants and WT plant leaves were
200 measured 48 hours after the *Pto* DC3000 and buffer $MgCl_2$ inoculation, respectively. We observed
201 that *acp1* mutant plants accumulated significantly lower levels of C18:3 FA than WT plants with

buffer MgCl_2 treatment, which is similar to the condition without any treatment (**Figure 4b**). And the difference of C18:3 FA abundance between *acp1* mutants and WT plants was more apparent in response to the pathogen infection (**Figure 4b**). These data suggest that ACP1 displays a significant role in C18:3 FA accumulation in response to the *Pto* pathogen infection. Since linolenic acid (18:3 FA) is the primary precursor for the biosynthesis of plant hormone jasmonic acid (JA) (Mueller et al., 1993; Santino et al., 2013), thus *acp1* alleles may have a lower JA accumulation/response, and this may affect the outcome of the interaction with *Pto* with increased resistance level against the *Pto* DC3000 in *acp1* mutants.

Reduced levels of JA in *acp1* mutant plants disrupt the balance of JA- and SA-signaling.

Synthesis of JA, its methyl ester (MeJA), and its active isoleucine conjugates (JA-Ile/Leu) mostly begin with the C18:3 FA in the chloroplast (Ruan et al., 2019; Wasternack and Strnad, 2019). The reduced levels of C18:3 FA led us to hypothesize that the *acp1* mutants: 1) would have the reduced level of JA biosynthesis, and 2) as a result of antagonism, the SA level would be consequently increased. To test these hypotheses, the free JA, JA-Ile/Leu (JA-isoleucine/leucine), free SA, and SAG (SA-glycoside) levels in the *acp1* mutants and WT plants were quantified before and after the *Pto* pathogen inoculation. The related hormones and hormone-conjugate levels did not differ between the *acp1* mutants and WT plants before the *Pto* DC3000 treatment. However, the *acp1* mutants accumulated significantly lower JA and JA-Ile/Leu (**Figure 5a&5b**) and higher levels of SA and SAG following the challenges with the *Pto* DC3000 (**Figure 5c&5d**).

The altered levels of SA and JA are likely associated with the differential transcriptional outputs. To further characterize the influence of ACP1 on JA- and SA-signaling, the transcriptional levels of several genes in JA- and SA-related pathways were analyzed and compared between *acp1* mutants and WT plants by qPCR. Firstly, the expression of the SA biosynthesis gene (*SID2*) and

SA response gene (*PR1*) were analyzed. Although the SA and SAG accumulation did not show significant differences between *acp1* mutants and WT plants without the *Pto* DC3000 inoculation, *acp1* mutants had significantly higher *SID2* and *PR1* transcriptional levels than WT plants. Consistent with the hormone results, significantly higher transcriptional levels of *SID2* and *PR1* were present in *acp1* mutants, relative to WT plants, at 6- and 48-hours post-inoculation with the *Pto* DC3000 (**Figure 6a&6b**). Next, the expression levels of the JA receptor gene (*COII*) and a JA response gene (*PDF1.2*) were analyzed between the *acp1* mutants and WT plants. Again, despite the lack of difference in JA levels in unchallenged *acp1* mutants, it was found that the transcript levels of *PDF1.2* were significantly lower in *acp1* mutants, relative to WT, before the pathogen challenge. At 6- and 48-hours post-inoculation with *Pto* DC3000, the *acp1* mutants showed decreased *PDF1.2* and *COII* gene expression than WT (**Figure 6c&6d**). The transcription factor MYC2 has been well studied as a critical component in the crosstalk of SA and JA, which generally positively regulates JA signaling but suppresses the SA signaling against the bacterial pathogen *Pto* DC3000 (Cui et al., 2018; Yang et al., 2019; Gautam et al., 2021). It was reported that the *myc2* mutant displayed the enhanced resistance against *Pto* DC3000 with the increased SA level and upregulated *PR1* expression (Laurie-Berry et al., 2006). In our study, the basal transcript level of *MYC2* was decreased in *acp1* mutants than in WT plants before *Pto* DC3000 treatment. And the reduced gene expression of *MYC2* maintained in *acp1* mutants after *Pto* DC3000 infection (**Figure 6e**). We also found that the basal transcript levels of several other JA synthesis-related genes were significantly decreased in *acp1* mutants than in WT plants (**Figure S5**). Altogether, the hormone levels and expression of hormone pathway-related genes are consistent with the interpretation that *acp1* mutants may enhance the resistance against *Pto* DC3000 by repressing the JA-signaling pathway and upregulating the SA-signaling pathway.

248 **Methyl JA and linolenic acid treatments decreased the resistance against *Pto* in *acp1* mutants.**

249 The *acp1* mutants with the enhanced resistance against *Pto* showed a significantly reduced
 250 level of C18:3 FA in response to the *Pto* DC3000 infection, accompanied by the decrease in JA
 251 and JA-Ile/Leu accumulation as well as gene expressions associated with the JA pathway. To
 252 further investigate the role of ACP1 in the accumulation of C18:3 FA and JA against *Pto*,
 253 exogenous MeJA (methyl JA, 50 μ M) and linolenic acid (C18:3 FA, 0.1 mM) were sprayed onto
 254 WT and *acp1* mutants leaves 24 hours before the pathogen *Pto* DC3000 inoculation (Mata-Pérez
 255 et al., 2015; Zhao et al., 2020). The bacterial population was monitored three days after the
 256 inoculation. As expected, the MeJA treatment increased the susceptibility of WT plants to *Pto*
 257 DC3000 (**Figure 7a**) (Zhao et al., 2020). Also, MeJA treatment increased the plant susceptibility
 258 to *Pto* in *acp1* mutants. Eventually, the *acp1* mutants showed similar bacterial *Pto* multiplication
 259 as in WT plants with the MeJA treatment (**Figure 7a**). We also observed that the linolenic acid
 260 treatment increased the susceptibility of WT and mutant plants to the *Pto* infection (**Figure 7b**).
 261 Notably, although *acp1* showed less bacterial multiplication than WT, the magnitude of the
 262 enhanced susceptibility of *acp1* mutant plants, relative to WT plants, was increased following the
 263 linolenic acid application (**Figure 7b**). Consistent with the previous results of the changes of FAs
 264 and hormone levels against the pathogen infection, these results further indicated the role of ACP1
 265 in the accumulation of C18:3 FA and JA in the defense response to the *Pto* infection. The negative
 266 impact of ACP1 on PTI possibly links FA metabolism to the stress hormone homeostasis in plant
 267 defense.

268 **Enhanced PTI responses in *acp1* mutant plants.**

269 Because SA-signaling contributes positively to PTI, we speculated that the heightened PTI
 270 might be observed in *acp1* mutant plants. An early PTI response elicited by the exposure to

271 MAMPs is a burst of ROS. To further examine the role of ACP1 in PTI, the real-time quantification
272 of H₂O₂ from *acp1* mutants and WT plants exposed to flg22 was measured. The H₂O₂ burst
273 detected in *acp1* mutants was of greater magnitude compared to WT plants (**Figure 8a**). The H₂O₂
274 accumulation was also monitored after the *Pto* DC3000 inoculation by staining the plant leaves
275 with 3,3-diaminobenzidine tetrahydrochloride (DAB), which is oxidized by H₂O₂ to generate a
276 dark brown precipitate (Liu et al., 2014). The *acp1* mutants and WT plants were sprayed with a
277 suspension of *Pto* DC3000 or buffer and then stained with DAB 24 hours later. As observed by
278 following the flg22 treatment, the *acp1* mutants also accumulated more H₂O₂ than WT plants
279 following the treatment with *Pto* DC3000 (**Figure 8b&8c**), indicating *acp1* mutants could generate
280 much more H₂O₂ than WT plants in response to the *Pto* DC3000 inoculation.

281 Another commonly examined readout of PTI is the deposition of callose into defensive cell
282 wall reinforcements (Kim et al., 2005). The callose deposition was compared between *acp1*
283 mutants and WT plants 16 hours after the infiltration with buffer, flg22, *Pto* DC3000, or a T3SS-
284 deficient mutant of *Pto* DC3000 (*Pto* DC3000 *hrcC*⁻). The callose deposition levels were similar
285 between *acp1* mutants and WT plants with the buffer treatment. However, consistent with the
286 enhanced SA-signaling, more callose deposition was observed in the *acp1* mutants relative to WT
287 plants, following the infiltration with flg22, *Pto* DC3000, and *Pto* DC3000 *hrcC*⁻ (**Figure 9a&9b**).
288 The enhanced production of H₂O₂ and the deposition of callose may collectively contribute to the
289 enhanced resistance of the *acp1* mutants against *Pto* DC3000.

290 DISCUSSION

291 **ACP1 is required for Arabidopsis fatty acid biosynthesis without affecting plant growth and**
292 **development.**

Members of gene families often carry out discrete functions that can be challenging to tease apart. Our research reveals that ACP1, one of 8 Arabidopsis ACP isoforms, is involved in plant resistance to biotic stress. As mentioned above, ACPs are essential cofactors for fatty acid biosynthesis. Different ACP isoforms are likely required for the specific FA biosynthetic pathways (Xia et al., 2009; Huang et al., 2017). It was previously reported that overexpression of *ACP1* in Arabidopsis leaf tissues led to an increase in C18:3 FA and a decrease in C16:3 FA (Branen et al., 2001). For this study, our data indicate that a mutation in *ACP1* resulted in the significantly decreased levels of C18:3 FA in Arabidopsis plant leaves but did not affect the levels of the other FAs. And the lower level of C18:3 FA was even more apparent in *acp1* mutants than in WT plants in response to *Pto* DC3000 infection (**Figure 4a&4b**). Therefore, the data indicate that *ACP1* specifically affects C18:3 FA accumulation in Arabidopsis leaves, which may play a critical role in plant defense.

ACP1 was considered expressed at different plant tissues based on the Arabidopsis eFP Browser platform (<http://bar.utoronto.ca/eplant/>). It was predicted that *ACP1* was expressed in leaf, root, and seed tissues of Arabidopsis plants based on the published gene profiles (**Figure S6**). Of all the Arabidopsis ACP isoforms, ACP4 has been reported to be abundant in Arabidopsis leaf and play a significant role in leaf FA synthesis, with the reduced levels of C16:0, C16:1, C16:3, and C18:3 FAs in the mutants. Accordingly, the *acp4* mutant plants exhibited severe impaired growth and development, including the pale and undersized rosette leaves (Xia et al., 2009). In our study, the two *acp1* mutants are knock-down mutant lines with the significantly decreased gene expression of *ACP1* (**Figure 1c**). The mutants have the reduced levels of C18:3 FA (**Figure 4**). However, the effects of the *ACP1* mutation on plant growth and development were not apparent. For instance, the plant weight, height, and chlorophyll content of the mutants were similar to WT

plants (**Figure S3&S4**). Consequently, the reduced levels of C18:3 FA hypothetically contributed to the other phenotypes of the *acp1* mutants. All these results led ACP1 to be a promising candidate for studying its roles in plant responses to biotic and possibly additional stresses.

ACP1 influences plant immunity by controlling the defense hormone levels through FA accumulation.

Roles of FAs and lipids are emerging in plant immunity. For instance, the mutation of Acyl CoA Binding Protein 3 (ACBP3) of Arabidopsis, a member of the ACBP family which may regulate the intracellular transport of FAs and lipids, impaired the normal development of the leaf cuticle and affected both the basal and R gene-mediated defense against multiple pathogens (Xia et al., 2012). FAs and lipids and their associated factors also function as the plant systemic acquired resistance (SAR) signaling molecules. For example, azelaic acid (AzA), derived from the hydrolysis of unsaturated C18 FAs, is a mobile activator of SAR in Arabidopsis plants, at least in part by inducing the expression of genes involved in the biosynthesis of SAR inducer glycerol-3-phosphate (Jung et al., 2009; Yu et al., 2013; Wang et al., 2014). Furthermore, the oxidized fatty acids, such as oxylipins, directly inhibit the mycelial growth and spore germination of diverse fungal pathogens and stimulate the plant defense gene expression in response to the *Pto* infection (Prost et al., 2005; Montillet et al., 2013). Plant sphingolipids are bioactive lipids that play roles in defense against abiotic and biotic stresses (Li et al., 2021). Ceramides are key intermediates in sphingolipids metabolism. Ceramides could be phosphorylated by the ceramide kinase ACD5 or catabolized by alkaline ceramidase (ACER) (Bi et al., 2014; Wu et al., 2015; Li et al., 2021). A recent study found that the JA pathway was only activated in ceramide kinase mutant *acd5* mutant at the later growth stage with increased JA pathway gene expression and enhanced JA and JA-Ile/Leu accumulation. MeJA treatment enhanced the ceramide accumulation and cell death in *acd5*

mutant. However, the resistance to JA-related biotic stress, such as insect herbivory attack and necrotrophic pathogen *B.cinerea*, was not detected in the *acd5* mutant (Huang et al., 2021). Another report stated that double mutant *acer acd5* showed the enhanced SA pathway, oxidative stress pathway, and ceramide accumulation in response to flg22 treatment, which indicates ceramide plays a positive role in plant basal defense (Li et al., 2021). Therefore, FAs and lipids display diverse roles in plant immunity differently.

Here, we discover that *acpl* mutants display the enhanced resistance against *Pto* DC3000. The plastid localized ACP1 either directly or indirectly regulates the accumulation of C18:3 FA. Notably, C18:3 FA is the primary precursor for the biosynthesis of phytohormone JA (Melotto et al., 2006; Jiang et al., 2013; Geng et al., 2014; Gimenez-Ibanez et al., 2014; Yang et al., 2019). SA is well studied to promote the effective plant resistance responses against biotrophic and hemibiotrophic pathogens such as *Pseudomonas syringae*, including the full manifestation of PTI, ETI, and further development of SAR (Vlot et al., 2009; Pieterse et al., 2012; Kim et al., 2014; Ding and Ding, 2020). The importance of JA is, at least in part, related to its antagonistic crosstalk with SA in regulating the balance between plant growth and defense responses against diverse pathogens (Xin and He, 2013; Li et al., 2019; Yang et al., 2019; Liu and Timko, 2021). Numerous studies have reported the mechanisms underlying the SA-JA antagonism, most of which are focused on SA inhibiting JA (Gupta et al., 2020). Fewer cases deciphered the decrease or loss of JA could enhance SA accumulation (Zheng et al., 2012; Liu and Timko, 2021). In our study, the *acpl* mutant plants accumulated lower levels of C18:3 FA in response to the *Pto* pathogen infection (**Figure 4b**). Correspondingly, lower levels of free JA and JA-Ile/Leu and higher levels of free SA and SAG were accumulated in the mutants upon the pathogen infection, consistent with the altered transcriptional levels of JA- and SA-synthetic and responsive genes (**Figure 5&6**). The

enhanced susceptibility in *acp1* mutants was more apparent than in WT plants after the exogenous treatments of C18:3 and MeJA (**Figure 7**). Taken together, all these results indicate that the lower levels of C18:3 FA in *acp1* mutant plants may underlie a shift in the balance of JA- and SA-abundance and related signaling in response to the *Pto* DC3000 infection. Although the interplay between these defense hormones during the plant immune responses has been well documented, how they are linked to FAs and lipids biosynthesis is not well understood. Our findings highlight a critical role of ACP1 in influencing C18:3 FA accumulation specifically, which is associated with the plant defense hormone JA accumulation and its antagonistic interactions with SA.

The altered SA and JA levels enhanced the PTI-related responses against the *Pto* infection in *acp1* mutant plants.

Plants can induce diverse immune responses rapidly against microbial infections to protect themselves from severe damage or death. ROS is also one of the earliest plant defense responses, which plays an essential role in plant immunity (Hirt, 2016). Reinforcement of the plant cell wall, including callose deposition, is a later and hallmark defense response elicited during PTI (Malinovsky et al., 2014). The defense hormones such as SA and JA are vital for innate immunity and connect to the other biotic stress defense pathways. The changes in the amounts and compositions of SA and JA promote plant defense responses (Yang et al., 2019; Gupta et al., 2020). Generally, SA positively affects FLS2-mediated responses, such as callose deposition and ROS burst. JA negatively regulates FLS2-mediated responses (Yi et al., 2014). *ACP1* limits the extent of both ROS production and deposition of callose (**Figure 8&9**). The role of defense hormones regulated by *ACP1* in these outputs of PTI points to a likely underlying mechanism. The upregulated SA-related pathway might contribute to these defense responses within the host immune system.

385 **The broader perspectives of ACP1 for plant defense.**

386 The ACP1 homologs are distributed across plant species (**Table S1**), such as spinach,
 387 tomato, olive, cotton, and various brassicaceous plants. The potential role of ACP1 in plant defense
 388 across this broad range of plant species, coupled with its lack of effects on plant growth and
 389 development, points to a possible means for breeding or engineering disease-resistant plant
 390 varieties for numerous economically significant crops. In the future, gene editing approaches to
 391 modify the expression levels and function of *ACP1* in diverse crops may enhance their disease
 392 resistance against bacteria and potentially the other classes of pathogens. The detailed mechanisms
 393 of ACP1 in PTI need further investigations. For instance, the investigation of the interconnected
 394 networks of ACP1 in plant immunity as well as the related crosstalk pathways in different pathogen
 395 systems and plant species will strengthen our understanding of the functions of ACP1 and possibly
 396 the other ACP isoforms in plant immunity and facilitate their applications in enhancing plant health
 397 and yield. Furthermore, the ability of ACP1 to keep the hormone homeostasis between SA and JA
 398 indicates that ACP1 might also play critical roles in the other biotic and abiotic stresses, which
 399 deserve further investigation in the future.

400 **EXPERIMENTAL PROCEDURES**401 **Plant growth and homozygous line verification.**

402 The seeds of the mutant lines and WT Col-0 were ordered from the Arabidopsis Biological
 403 Resource Center at Columbus, USA (<http://www.arabidopsis.org/>). The Arabidopsis T-DNA
 404 insertion mutant lines of *ACP1* (At3g05020) were used in this study: Salk_075428 (*acp1-1*) and
 405 CS827601(*acp1-2*). The Arabidopsis plants were grown in the growth room with 60% humidity at
 406 22 °C under 10 hours of light and 14 hours darkness photoperiod (Fan et al., 2018). The same

growth condition was used for the pathogen inoculation experiments. The T-DNA insertion of the *acp* homozygous lines was identified following the instructions from Salk Institute Genomic Analysis Laboratory (<http://signal.salk.edu/tdnaprimers.2.html>). The primers used for genotyping and transcript confirmation are listed in **Table S2**. The *Nicotiana benthamiana* plants were grown in the same conditions as described above.

Sequence Analysis.

The protein sequences of all eight ACP isoforms were obtained from NCBI Gene-Bank (<http://www.ncbi.nlm.nih.gov>), which were uploaded to Clustal Omega via EMBL-EBI platform (<https://www.ebi.ac.uk>) for multiple protein sequence alignments. Simple Phylogeny via EMBL-EBI was used for the phylogenetic tree construction. The tree shown with straight branches and cladogram was based on the alignment with default parameters. The subcellular localization and chloroplast signal peptide was predicted using TargetP and iPSORT (<https://services.healthtech.dtu.dk/service.php?TargetP-2.0>; <https://ipsort.hgc.jp/how.html>) (Wang et al., 2021).

Gene expression analysis.

The total RNA for the quantitative RT-qPCR analysis was extracted from the leaf tissues. Total RNA was extracted by using Trizol (Sigma-Aldrich). The first-strand cDNA was synthesized using the Reverse Transcriptase (catalog number: 4368814, Applied Biosystem, Waltham, MA, USA). The RT-qPCR was performed using the 96-well blocks (Bio-Rad) with the CFX96 real-time PCR detection system (Bio-Rad, USA). The *ACTIN* or *UBIQUITIN* gene was used as the reference gene (Huang et al., 2017). The gene-specific primers were listed in Supplemental **Table S2**.

429 Chlorophyll content measurement.

430 The plant leaves at the 4-week-old growth stage were immersed in 80% ethanol (around 30
431 ml) in the glass tubes wrapped in the foil at room temperature. The supernatant (1 ml) was taken
432 from the tube every 30 mins (up to 150 mins) and finally terminated 48 hours after the treatment.
433 The absorbance of the supernatant at 664 and 647 nm was measured by using the spectrometer
434 (Thermo scientific Genesys 10S VIS, USA). The total micromoles chlorophyll was calculated
435 based on the equation developed previously. The total leaf chlorophyll per gram of fresh leaf
436 samples equals to $7.93(A_{664}) + 19.53(A_{647})$ (Lolle et al., 1997).

437 Pathogen inoculation and bacterial growth quantification.

438 The *Pto* DC3000 and 4-week-old plants were used for the plant pathogen infection. The *Pto*
439 DC3000 was cultured overnight on the plates with King's B medium (adding rifampin as the
440 selective antibiotic). The bacterial cells were obtained from the plates and diluted in 10 mM MgCl₂.
441 The suspension at the concentration of 5×10^5 CFU (colony forming unit)/ml was syringe infiltrated
442 into the *Arabidopsis* leaves. The residues were removed from the leaves using clean facial tissue.
443 And then, the plants were returned to the growth room 0.5-1-hour post-inoculation. The bacterial
444 growth was quantified three days after the inoculation as previously described (Kachroo et al.,
445 2003; Zhao et al., 2020). For each technical replicate, three-leaf discs were collected and ground
446 to homogeneity with a serial dilution in 10 mM MgCl₂. The serial dilutions were further plated
447 onto the King's B plate with rifampin antibiotic. The number of colonies on the plate was recorded
448 to calculate the CFU per cm². The log-transformed means from five technical replicates were
449 calculated. Three independent biological repeats were applied for analysis.

450

451 Exogenous Methyl JA and linolenic acid treatments

452 The Methyl JA (MeJA) (Sigma-Aldrich Lot#MKBZ8786V) and linolenic acid (Acros
453 Organics Lot#A0430295) were dissolved in ethanol and prepared as 10 mM and 3 M stock
454 solutions , respectively. The plant leaves were evenly sprayed with mock control treatment (1%
455 ethanol), 50 μ M MeJA, and 0.1 mM linolenic acid using the vacuum-aided spray nozzle (vacuum
456 pressure of 25 psi) (Mata-Pérez et al., 2015; Zhao et al., 2020; Huang et al., 2021). The plants
457 were put back in the growth room when the leaves were dry.

458 Subcellular localization of ACP1.

459 To locate ACP1 in plant cells, the full-length coding sequence of *ACP1* was amplified from
460 the cDNA of WT plants using specific primers (**Table S2**). The pYBA1122 vector with
461 Kanamycin selection maker includes the CaMV 35S promoter and eGFP sequence. The *ACP1*
462 amplified fragment and the pYBA1122 vector were digested with *EcoRI* and *Sall* enzymes. The
463 *ACP1* fragment was ligated and inserted into the pYBA1122 vector as the ACP1-GFP. The binary
464 plasmid of the mCherry-labeled plastid marker (PT-mCherry) was ordered from ABRC (ABRC
465 stock name: CD3-999 with kanamycin selection). The vectors (empty vector pYBA, GFP-ACP1,
466 and PT-mCherry) were transformed into the *A. tumefaciens* GV3101 by the electroporation method
467 (Rossi et al., 1993). The *N. benthamiana* leaves were infiltrated with *A. tumefaciens* GV3101
468 harboring the PT-mCherry with ACP1-GFP or empty vector pYBA, respectively, by following the
469 protocol described previously (Fan *et al.*, 2018). After three days of incubation, the fluorescence
470 signals were detected under a confocal laser scanning microscopy (Nikon Eclipse 80i epi-
471 fluorescent microscope, Japan).

472

473 Fatty acid level analysis.

474 FA analysis was done by placing 500 ug fresh leaf tissues of 4-week-old Arabidopsis plants
475 in the glass vials. Methylated H₂SO₄ (2 ml), which contains 3% H₂SO₄ in methanol and 0.001%
476 BTH was added to the glass vials. The C17:0 FA (SIGMA Lot#SLBR3733V) was added as the
477 internal standard. The leaf tissues were immersed in the solution. The glass vials were incubated
478 in a water bath at 80 °C for about 30 mins. The samples were cooled down for 5-10 mins. The n-
479 Hexane (1 ml) with 0.001% BTH was added to each glass vial. The vials were vortexed briefly
480 and stood still for 5-10 mins. The top transparent Hexane layer was removed into the GC vials and
481 set for GC analysis as described before (Kachroo et al., 2003). Six biological replicates were
482 applied for each genotype.

483 The determination of hormone levels.

484 The plant hormone extraction and detection methods were done as described previously
485 (Forcat et al., 2008; Peng et al., 2020; Zhao et al., 2020). Arabidopsis plant leaves were infiltrated
486 with buffer (10mM MgCl₂) or *Pto* DC3000 solution (at the concentration of 5×10⁵ CFU/ml). The
487 leaf samples (110 mg fresh leaf weight per sample) were collected at 0 and 48 hours post-
488 inoculation. Each leaf sample was extracted with a 400 µl extraction buffer which contained 10%
489 methanol and 1% acetic acid. Meanwhile, the isotope-labeled internal standards were added,
490 including d₄-SA (15 ng ²H₄-SA, part #D-1156, CDN Isotopes, Pointe-Claire, QC, Canada) and d₅-
491 JA (150 ng ²H₅-JA, part #D-6936, CDN Isotopes, Pointe-Claire, QC, Canada). The tubes with
492 extraction buffer and internal standards were incubated on ice for 30 minutes and centrifuged for
493 10 minutes at 4 °C (13000 g). The supernatant was collected into new tubes. Moreover, the pellets
494 were re-extracted with the same amount of extraction buffer without the internal standard. After
495 two times of extraction, the pooled supernatant was further analyzed by UPLC/ESI/MS through

496 the Thermal Fisher Ultimate 3000 system (Thermal Fisher, Waltham, MA, USA). The C18 (3 μ m,
497 100 mm $\times$ 2.0 mm) column (Waters company, Milford, MA, USA) was used for UPLC separation.
498 The mobile phase with a continuum gradient from (94.9% H₂O: 5% CH₃CN: 0.1% CHOOH) to (5%
499 H₂O: 94.9% CH₃CN: 0.1% CHOOH) was set for around 20 mins. Multiple Reaction Monitoring
500 (MRM) of ion pairs in negative ion mode was applied to analyze the compound. The retention
501 time and mass transitions for SA and JA were determined using authentic standards. The transition
502 settings for SA and JA were ²H₄-SA 141.0 (97.0), SA 137.0 (93.1), SAG 299.0 (93.1), ²H₅-JA
503 214.0 (61.0), JA 209.0 (59.1), and JA-Ile/Leu 322.0 (130.0). The daughter masses were denoted
504 in the brackets listed above. Five technical replicates were applied for each treatment.

505 **Determination of the callose deposition.**

506 Callose deposition was tested by following the infiltration of leaves of the *acp1* mutants and
507 Col-0 plants with the buffer (10 mM MgCl₂), 100 μ M flg22 (microbe-associated molecular
508 patterns (MAMPs)), *Pto* DC3000 at 10⁸ CFU/ml, and *Pto* DC3000 *hrcC*- at 10⁸ CFU/ml. The assay
509 was performed as described previously (Kim and Mackey, 2008). To observe the spatial patterns
510 of the callose deposition, the infected leaves at 16 hours post-inoculation were cleared in the
511 lactophenol and 80% ethanol for one day to bleach the chlorophyll at room temperature. Then the
512 leaves were submerged in the aniline blue staining solution (150 mM K₂HPO₄, 0.01% w/v aniline
513 blue, Cat #: 415049, Sigma-Aldrich) in the dark overnight. The leaves were mounted on the glass
514 slides in 50% sterile glycerol for microscopic assessment. The samples were analyzed by
515 fluorescence microscopy (Nikon Eclipse 80i epi-fluorescent microscope, Japan), using the UV
516 excitation (327-427 nm) and the DAPI/Aniline blue emission filter (emission 417-477 nm). At
517 least six individual leaves were applied for the analysis of each treatment. The pictures were

captured from each leaf, and the numbers of callose deposition were performed using the ImageJ software for the batch analysis (Jin and Mackey, 2017).

H₂O₂ Burst Assay and DAB staining for testing the H₂O₂ accumulation.

An oxidative burst assay for testing the H₂O₂ level was carried out as described previously (Wang et al., 2018) with minor modifications. The leaf discs (5mm in diameter) were cut and suspended in sterile water overnight to diminish the effects of wounds. The pre-treated leaf discs were used for the oxidative burst assay. The leaf disks were immersed in 100 ul reaction solution with the luminol substrate (Immuno-Star horseradish peroxidase substrate 170-5040, Bio-Rad), 1.0 µl peroxidase (1 mg/ml), and 1.0 µM flg22. After adding all the components, the samples were immediately quantified for H₂O₂ production. The Luminescence was recorded continuously for 1 s at 10 s intervals for up to 30 mins with a Glomax 20/20 single well luminometer (Promega) (Park et al., 2012). Three technical replicates were applied for each treatment. The result showed the H₂O₂ burst curve in real-time upon the 200 nM flg22 induction or water treatment. The related experiments had been carried out for three times with the consistent results.

According to the previous report, the staining test using 3,3-diaminobenzidine tetrahydrochloride (DAB) was also applied to detect H₂O₂ accumulation (Xiao et al., 2003). Arabidopsis plants at the 4-week-old growth stage were sprayed with the buffer (10 mM MgCl₂) or *Pto* DC3000 solution (10⁷ CFU/ml). Around 24 hours after the treatment, the leaf samples were collected and submerged in 1mg/ml of acidic 3,3-diaminobenzidine tetrahydrochloride (DAB) solution and incubated overnight in the dark cleared using 1:1 ethanol/acetic acid clearing solution. The images were captured after the chlorophyll clearing with a light camera (Nikon) (Xiao *et al.*, 2003).

540 **ACKNOWLEDGEMENTS.** We greatly appreciate Arabidopsis Biological Resource Center
541 (ABRC) for providing the Arabidopsis seeds and constructs. We gave our special thanks to
542 Christophe Cocuron and Ana Paula Alonso from BioDiscovery Institute and the Department of
543 Biological Sciences at the University of North Texas to help with the fatty acid level analysis. We
544 also appreciate the help with the fatty acid analysis by Dr. Keshun Yu and Pradeep Kachroo from
545 the Plant Pathology Department at the University of Kentucky. We are grateful to Dr. Mingzhe
546 Shen for his help on this project.

547 **LITERATURE CITED**

548 Bashan, Y. 1997. Alternative strategies for controlling plant diseases caused by *Pseudomonas*
549 *syringae*. Pages 575-583 in: *Pseudomonas syringae* pathovars and related pathogens,
550 Springer.

551 Bashan, Y., and de-Bashan, L.E. 2002. Reduction of bacterial speck (*Pseudomonas syringae* pv.
552 tomato) of tomato by combined treatments of plant growth-promoting bacterium,
553 *Azospirillum brasilense*, streptomycin sulfate, and chemo-thermal seed treatment.
554 European Journal of Plant Pathology 108:821-829.

555 Beisson, F., Li, Y., Bonaventure, G., Pollard, M., and Ohlrogge, J.B. 2007. The acyltransferase
556 GPAT5 is required for the synthesis of suberin in seed coat and root of Arabidopsis. The
557 Plant Cell 19:351-368.

558 Bi, F.-C., Liu, Z., Wu, J.-X., Liang, H., Xi, X.-L., Fang, C., Sun, T.-J., Yin, J., Dai, G.-Y., and
559 Rong, C. 2014. Loss of ceramide kinase in Arabidopsis impairs defenses and promotes
560 ceramide accumulation and mitochondrial H₂O₂ bursts. The Plant Cell 26:3449-3467.

561 Bonaventure, G., and Ohlrogge, J.B. 2002. Differential regulation of mRNA levels of acyl carrier
562 protein isoforms in Arabidopsis. Plant physiology 128:223-235.

- 563 Branen, J.K., Chiou, T.-J., and Engeseth, N.J. 2001. Overexpression of acyl carrier protein-1 alters
564 fatty acid composition of leaf tissue in Arabidopsis. *Plant physiology* 127:222-229.
- 565 Brown, A.P., Slabas, A.R., and Rafferty, J.B. 2009. Fatty acid biosynthesis in plants—metabolic
566 pathways, structure and organization. Pages 11-34 in: *Lipids in photosynthesis*, Springer.
- 567 Buttner, C., McAuliffe, O., Ross, R.P., Hill, C., O'Mahony, J., and Coffey, A. 2017.
568 Bacteriophages and bacterial plant diseases. *Frontiers in microbiology* 8:34.
- 569 Byers, D.M., and Gong, H. 2007. Acyl carrier protein: structure–function relationships in a
570 conserved multifunctional protein family. *Biochemistry and Cell Biology* 85:649-662.
- 571 Chan, D.I., and Vogel, H.J. 2010. Current understanding of fatty acid biosynthesis and the acyl
572 carrier protein. *Biochemical Journal* 430:1-19.
- 573 Chanda, B., Xia, Y., Mandal, M.K., Yu, K., Sekine, K.T., Gao, Q.M., Selote, D., Hu, Y., Stromberg,
574 A., Navarre, D., Kachroo, A., and Kachroo, P. 2011. Glycerol-3-phosphate is a critical
575 mobile inducer of systemic immunity in plants. *Nat Genet* 43:421-427.
- 576 Cheng, C., Gao, X., Feng, B., Sheen, J., Shan, L., and He, P. 2013. Plant immune response to
577 pathogens differs with changing temperatures. *Nat Commun* 4:2530.
- 578 Crawford, J.M., Vagstad, A.L., Ehrlich, K.C., Udway, D.W., and Townsend, C.A.J.C. 2008.
579 Acyl-Carrier Protein–Phosphopantetheinyltransferase Partnerships in Fungal Fatty Acid
580 Synthases 9:1559-1563.
- 581 Cui, H., Qiu, J., Zhou, Y., Bhandari, D.D., Zhao, C., Bautor, J., and Parker, J.E. 2018. Antagonism
582 of Transcription Factor MYC2 by EDS1/PAD4 Complexes Bolsters Salicylic Acid
583 Defense in Arabidopsis Effector-Triggered Immunity. *Mol Plant* 11:1053-1066.

- 584 Cultrera, N.G., Alagna, F., Mariotti, R., De Marchis, F., Pompa, A., Bellucci, M., and Baldoni, L.
585 2014. Isolation and molecular characterization of three acyl carrier protein genes in olive
586 (*Olea europaea* L.). *Tree genetics & genomes* 10:895-909.
- 587 Dat, J., Vandenabeele, S., Vranova, E., Van Montagu, M., Inzé, D., and Van Breusegem, F. 2000.
588 Dual action of the active oxygen species during plant stress responses. *Cellular and*
589 *Molecular Life Sciences CMLS* 57:779-795.
- 590 Ding, P., and Ding, Y. 2020. Stories of salicylic acid: A plant defense hormone. *Trends in Plant*
591 *Science*.
- 592 Fan, G., Yang, Y., Li, T., Lu, W., Du, Y., Qiang, X., Wen, Q., and Shan, W. 2018. A *Phytophthora*
593 *capsici* RXLR effector targets and inhibits a plant PPIase to suppress endoplasmic
594 reticulum-mediated immunity. *Molecular plant* 11:1067-1083.
- 595 Forcat, S., Bennett, M.H., Mansfield, J.W., and Grant, M.R. 2008. A rapid and robust method for
596 simultaneously measuring changes in the phytohormones ABA, JA and SA in plants
597 following biotic and abiotic stress. *Plant Methods* 4:16.
- 598 Gao, Q.-M., Zhu, S., Kachroo, P., and Kachroo, A. 2015. Signal regulators of systemic acquired
599 resistance. *Frontiers in plant science* 6:228.
- 600 Gao, Q.M., Yu, K., Xia, Y., Shine, M.B., Wang, C., Navarre, D., Kachroo, A., and Kachroo, P.
601 2014. Mono- and digalactosyldiacylglycerol lipids function nonredundantly to regulate
602 systemic acquired resistance in plants. *Cell Rep* 9:1681-1691.
- 603 Gautam, J.K., Giri, M.K., Singh, D., Chattopadhyay, S., and Nandi, A.K. 2021. MYC2 influences
604 salicylic acid biosynthesis and defense against bacterial pathogens in *Arabidopsis thaliana*.
605 *Physiol Plant* 173:2248-2261.

- 606 Geng, X., Jin, L., Shimada, M., Kim, M.G., and Mackey, D. 2014. The phytotoxin coronatine is a
607 multifunctional component of the virulence armament of *Pseudomonas syringae*. *Planta*
608 240:1149-1165.
- 609 Gimenez-Ibanez, S., Boter, M., Fernández-Barbero, G., Chini, A., Rathjen, J.P., and Solano, R.
610 2014. The bacterial effector HopX1 targets JAZ transcriptional repressors to activate
611 jasmonate signaling and promote infection in *Arabidopsis*. *PLoS biology* 12.
- 612 Guo, W., Zuo, Z., Cheng, X., Sun, J., Li, H., Li, L., and Qiu, J.L. 2014. The chloride channel
613 family gene CLCd negatively regulates pathogen-associated molecular pattern (PAMP)-
614 triggered immunity in *Arabidopsis*. *J Exp Bot* 65:1205-1215.
- 615 Gupta, A., Bhardwaj, M., and Tran, L.P. 2020. Jasmonic Acid at the Crossroads of Plant Immunity
616 and *Pseudomonas syringae* Virulence. *Int J Mol Sci* 21.
- 617 Hansen, L., and von Wettstein-Knowles, P. 1991. The barley genes Acl1 and Acl3 encoding acyl
618 carrier proteins I and III are located on different chromosomes. *Molecular and General*
619 *Genetics* MGG 229:467-478.
- 620 Hirt, H. 2016. Aquaporins link ROS signaling to plant immunity. *Plant physiology* 171:1540-1540.
- 621 Hloušek-Radojčić, A., Post-Beittenmiller, D., and Ohlrogge, J.B. 1992. Expression of constitutive
622 and tissue-specific acyl carrier protein isoforms in *Arabidopsis*. *Plant physiology* 98:206-
623 214.
- 624 Huang, J., Xue, C., Wang, H., Wang, L., Schmidt, W., Shen, R., and Lan, P. 2017. Genes of ACYL
625 CARRIER PROTEIN family show different expression profiles and overexpression of
626 ACYL CARRIER PROTEIN 5 modulates fatty acid composition and enhances salt stress
627 tolerance in *Arabidopsis*. *Frontiers in plant science* 8:987.

- 628 Huang, L.Q., Chen, D.K., Li, P.P., Bao, H.N., Liu, H.Z., Yin, J., Zeng, H.Y., Yang, Y.B., Li, Y.K.,
629 Xiao, S., and Yao, N. 2021. Jasmonates modulate sphingolipid metabolism and accelerate
630 cell death in the ceramide kinase mutant *acd5*. *Plant Physiol* 187:1713-1727.
- 631 Jiang, S., Yao, J., Ma, K.-W., Zhou, H., Song, J., He, S.Y., and Ma, W. 2013. Bacterial effector
632 activates jasmonate signaling by directly targeting JAZ transcriptional repressors. *PLoS*
633 *pathogens* 9.
- 634 Jin, L., and Mackey, D.M. 2017. Measuring callose deposition, an indicator of cell wall
635 reinforcement, during bacterial infection in *Arabidopsis*. Pages 195-205 in: *Plant Pattern*
636 *Recognition Receptors*, Springer.
- 637 Jones, J.D., and Dangl, J.L. 2006. The plant immune system. *nature* 444:323-329.
- 638 Jones, J.D., Vance, R.E., and Dangl, J.L. 2016. Intracellular innate immune surveillance devices
639 in plants and animals. *Science* 354:aaf6395.
- 640 Jung, H.W., Tschaplinski, T.J., Wang, L., Glazebrook, J., and Greenberg, J.T. 2009. Priming in
641 systemic plant immunity. *Science* 324:89-91.
- 642 Kachroo, A., Lapchyk, L., Fukushige, H., Hildebrand, D., Klessig, D., and Kachroo, P. 2003.
643 Plastidial fatty acid signaling modulates salicylic acid–and jasmonic acid–mediated
644 defense pathways in the *Arabidopsis* *ssi2* mutant. *The Plant Cell* 15:2952-2965.
- 645 Kachroo, P., Shanklin, J., Shah, J., Whittle, E.J., and Klessig, D.F. 2001. A fatty acid desaturase
646 modulates the activation of defense signaling pathways in plants. *Proceedings of the*
647 *National Academy of Sciences* 98:9448-9453.
- 648 Kim, M.G., and Mackey, D. 2008. Measuring cell-wall-based defenses and their effect on bacterial
649 growth in *Arabidopsis*. Pages 443-452 in: *Innate Immunity*, Springer.

- 650 Kim, M.G., Da Cunha, L., McFall, A.J., Belkhadir, Y., DebRoy, S., Dangl, J.L., and Mackey, D.
651 2005. Two *Pseudomonas syringae* type III effectors inhibit RIN4-regulated basal defense
652 in *Arabidopsis*. *Cell* 121:749-759.
- 653 Kim, Y., Tsuda, K., Igarashi, D., Hillmer, R.A., Sakakibara, H., Myers, C.L., and Katagiri, F.
654 2014. Mechanisms underlying robustness and tunability in a plant immune signaling
655 network. *Cell host & microbe* 15:84-94.
- 656 Kwak, J.M., Nguyen, V., and Schroeder, J.I. 2006. The role of reactive oxygen species in hormonal
657 responses. *Plant Physiology* 141:323-329.
- 658 Lan, P., Li, W., and Schmidt, W. 2012. Complementary proteome and transcriptome profiling in
659 phosphate-deficient *Arabidopsis* roots reveals multiple levels of gene regulation.
660 *Molecular & Cellular Proteomics* 11:1156-1166.
- 661 Laurie-Berry, N., Joardar, V., Street, I.H., and Kunkel, B.N. 2006. The *Arabidopsis thaliana*
662 JASMONATE INSENSITIVE 1 gene is required for suppression of salicylic acid-
663 dependent defenses during infection by *Pseudomonas syringae*. *Molecular Plant-Microbe*
664 *Interactions* 19:789-800.
- 665 Li, J., Yin, J., Wu, J.X., Wang, L.Y., Liu, Y., Huang, L.Q., Wang, R.H., and Yao, N. 2021.
666 Ceramides regulate defense response by binding to RbohD in *Arabidopsis*. *Plant J.*
- 667 Li, N., Han, X., Feng, D., Yuan, D., and Huang, L.-J. 2019. Signaling Crosstalk between Salicylic
668 Acid and Ethylene/Jasmonate in Plant Defense: Do We Understand What They Are
669 Whispering? *International Journal of Molecular Sciences* 20.
- 670 Lim, G.-H., Liu, H., Yu, K., Liu, R., Shine, M.B., Fernandez, J., Burch-Smith, T., Mobley, J.K.,
671 McLetchie, N., Kachroo, A., and Kachroo, P. 2020. The plant cuticle regulates apoplastic
672 transport of salicylic acid during systemic acquired resistance 6:eaaz0478.

- 673 Liu, H., and Timko, M.P. 2021. Jasmonic Acid Signaling and Molecular Crosstalk with Other
674 Phytohormones. *Int J Mol Sci* 22.
- 675 Liu, J., Elmore, J.M., Fuglsang, A.T., Palmgren, M.G., Staskawicz, B.J., and Coaker, G. 2009.
676 RIN4 functions with plasma membrane H⁺-ATPases to regulate stomatal apertures during
677 pathogen attack. *PLoS biology* 7.
- 678 Liu, Y.-H., Offler, C.E., and Ruan, Y.-L. 2014. A simple, rapid, and reliable protocol to localize
679 hydrogen peroxide in large plant organs by DAB-mediated tissue printing. *Frontiers in*
680 *plant science* 5:745.
- 681 Lolle, S.J., Berlyn, G.P., Engstrom, E.M., Krolkowski, K.A., Reiter, W.-D., and Pruitt, R.E. 1997.
682 Developmental Regulation of Cell Interactions in the *Arabidopsis* fiddlehead-1 Mutant: A
683 Role for the Epidermal Cell Wall and Cuticle. *Developmental biology* 189:311-321.
- 684 Luna, E., Pastor, V., Robert, J., Flors, V., Mauch-Mani, B., and Ton, J. 2011. Callose deposition:
685 a multifaceted plant defense response. *Molecular Plant-Microbe Interactions* 24:183-193.
- 686 Maejima, E., Watanabe, T., Osaki, M., and Wagatsuma, T. 2014. Phosphorus deficiency enhances
687 aluminum tolerance of rice (*Oryza sativa*) by changing the physicochemical characteristics
688 of root plasma membranes and cell walls. *Journal of plant physiology* 171:9-15.
- 689 Malinovsky, F.G., Fangel, J.U., and Willats, W.G. 2014. The role of the cell wall in plant immunity.
690 *Frontiers in plant science* 5:178.
- 691 Marrakchi, H., Zhang, Y.-M., and Rock, C. (2002). Mechanistic diversity and regulation of Type
692 II fatty acid synthesis (Portland Press Ltd.).
- 693 Mata-Pérez, C., Sánchez-Calvo, B., Begara-Morales, J.C., Luque, F., Jiménez-Ruiz, J., Padilla,
694 M.N., Fierro-Risco, J., Valderrama, R., Fernández-Ocaña, A., and Corpas, F.J. 2015.

- 695 Transcriptomic profiling of linolenic acid-responsive genes in ROS signaling from RNA-
696 seq data in Arabidopsis. *Frontiers in plant science* 6:122.
- 697 Meier, J.L., Haushalter, R.W., and Burkart, M.D. 2010. A mechanism based protein crosslinker
698 for acyl carrier protein dehydratases. *Bioorganic & medicinal chemistry letters* 20:4936-
699 4939.
- 700 Melotto, M., Underwood, W., Koczan, J., Nomura, K., and He, S.Y. 2006. Plant stomata function
701 in innate immunity against bacterial invasion. *Cell* 126:969-980.
- 702 Meyer, E.H., Heazlewood, J.L., and Millar, A.H. 2007. Mitochondrial acyl carrier proteins in
703 *Arabidopsis thaliana* are predominantly soluble matrix proteins and none can be confirmed
704 as subunits of respiratory Complex I. *Plant molecular biology* 64:319-327.
- 705 Mittler, R., Vanderauwera, S., Gollery, M., and Van Breusegem, F. 2004. Reactive oxygen gene
706 network of plants. *Trends in plant science* 9:490-498.
- 707 Montillet, J.-L., Leonhardt, N., Mondy, S., Tranchimand, S., Rumeau, D., Boudsocq, M., Garcia,
708 A.V., Douki, T., Bigeard, J., and Lauriere, C. 2013. An abscisic acid-independent oxylipin
709 pathway controls stomatal closure and immune defense in *Arabidopsis*. *PLoS biology* 11.
- 710 Mueller, M.J., Brodschelm, W., Spannagl, E., and Zenk, M.H.J.P.o.t.N.A.o.S. 1993. Signaling in
711 the elicitation process is mediated through the octadecanoid pathway leading to jasmonic
712 acid 90:7490-7494.
- 713 Nelson, B.K., Cai, X., and Nebenführ, A. 2007. A multicolored set of in vivo organelle markers
714 for co-localization studies in *Arabidopsis* and other plants. *The Plant Journal* 51:1126-1136.
- 715 Ngou, B.P.M., Ahn, H.K., Ding, P., and Jones, J.D.G. 2021. Mutual potentiation of plant immunity
716 by cell-surface and intracellular receptors. *Nature* 592:110-115.

- 717 Ohlrogge, J.B., and Kuo, T.M. 1985. Plants have isoforms for acyl carrier protein that are
718 expressed differently in different tissues. *Journal of Biological Chemistry* 260:8032-8037.
- 719 Ohlrogge, J.B., Kuhn, D.N., and Stumpf, P. 1979. Subcellular localization of acyl carrier protein
720 in leaf protoplasts of *Spinacia oleracea*. *Proceedings of the National Academy of Sciences*
721 76:1194-1198.
- 722 Park, C.-H., Chen, S., Shirsekar, G., Zhou, B., Khang, C.H., Songkumarn, P., Afzal, A.J., Ning,
723 Y., Wang, R., and Bellizzi, M. 2012. The *Magnaporthe oryzae* effector AvrPiz-t targets the
724 RING E3 ubiquitin ligase APIP6 to suppress pathogen-associated molecular pattern–
725 triggered immunity in rice. *The plant cell* 24:4748-4762.
- 726 Peng, Q., Wang, Z., Liu, P., Liang, Y., Zhao, Z., Li, W., Liu, X., and Xia, Y. 2020. Oxathiapiprolin,
727 a novel chemical inducer activates the plant disease resistance. *International journal of*
728 *molecular sciences* 21:1223.
- 729 Pieterse, C.M., Van der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S.C. 2012.
730 Hormonal modulation of plant immunity. *Annual review of cell and developmental biology*
731 28:489-521.
- 732 Prost, I., Dhondt, S., Rothe, G., Vicente, J., Rodriguez, M.J., Kift, N., Carbonne, F., Griffiths, G.,
733 Esquerré-Tugayé, M.-T., and Rosahl, S. 2005. Evaluation of the antimicrobial activities of
734 plant oxylipins supports their involvement in defense against pathogens. *Plant physiology*
735 139:1902-1913.
- 736 Rossi, L., Escudero, J., Hohn, B., and Tinland, B. 1993. Efficient and sensitive assay for T-DNA-
737 dependent transient gene expression. *Plant Molecular Biology Reporter* 11:220-229.

- 738 Roujeinikova, A., Baldock, C., Simon, W.J., Gilroy, J., Baker, P.J., Stuitje, A.R., Rice, D.W.,
 739 Slabas, A.R., and Rafferty, J.B.J.S. 2002. X-ray crystallographic studies on butyryl-ACP
 740 reveal flexibility of the structure around a putative acyl chain binding site 10:825-835.
- 741 Ruan, J., Zhou, Y., Zhou, M., Yan, J., Khurshid, M., Weng, W., Cheng, J., and Zhang, K. 2019.
 742 Jasmonic acid signaling pathway in plants. *International journal of molecular sciences*
 743 20:2479.
- 744 Ruuska, S.A., Girke, T., Benning, C., and Ohlrogge, J.B. 2002. Contrapuntal networks of gene
 745 expression during Arabidopsis seed filling. *The Plant Cell* 14:1191-1206.
- 746 SAFFORD, R., WINDUST, J.H., LUCAS, C., DE SILVA, J., JAMES, C.M., HELLYER, A.,
 747 SMITH, C.G., SLABAS, A.R., and HUGHES, S.G. 1988. Plastid-localised seed
 748 acyl-carrier protein of Brassica napus is encoded by a distinct, nuclear multigene family.
 749 *European journal of biochemistry* 174:287-295.
- 750 Santino, A., Taurino, M., De Domenico, S., Bonsegna, S., Poltronieri, P., Pastor, V., and Flors, V.
 751 2013. Jasmonate signaling in plant development and defense response to multiple (a)biotic
 752 stresses. *Plant Cell Rep* 32:1085-1098.
- 753 Shirasu, K., Nakajima, H., Rajasekhar, V.K., Dixon, R.A., and Lamb, C. 1997. Salicylic acid
 754 potentiates an agonist-dependent gain control that amplifies pathogen signals in the
 755 activation of defense mechanisms. *The Plant Cell* 9:261-270.
- 756 Song, P., and Allen, R.D. 1997. Identification of a cotton fiber-specific acyl carrier protein cDNA
 757 by differential display. *Biochimica et Biophysica Acta (BBA)-Gene Structure and*
 758 *Expression* 1351:305-312.
- 759 Spoel, S.H., and Dong, X. 2012. How do plants achieve immunity? Defence without specialized
 760 immune cells. *Nature reviews immunology* 12:89-100.

- 761 Takemoto, D., and Jones, D.A. 2005. Membrane release and destabilization of Arabidopsis RIN4
762 following cleavage by *Pseudomonas syringae* AvrRpt2. *Molecular plant-microbe*
763 *interactions* 18:1258-1268.
- 764 Vlot, A.C., Dempsey, D.M.A., and Klessig, D.F. 2009. Salicylic acid, a multifaceted hormone to
765 combat disease. *Annual review of phytopathology* 47:177-206.
- 766 Wang, C., El-Shetehy, M., Shine, M., Yu, K., Navarre, D., Wendehenne, D., Kachroo, A., and
767 Kachroo, P. 2014. Free radicals mediate systemic acquired resistance. *Cell Reports* 7:348-
768 355.
- 769 Wang, M., Rui, L., Yan, H., Shi, H., Zhao, W., Lin, J.E., Zhang, K., Blakeslee, J.J., Mackey, D.,
770 and Tang, D. 2018. The major leaf ferredoxin Fd2 regulates plant innate immunity in
771 Arabidopsis. *Molecular plant pathology* 19:1377-1390.
- 772 Wang, Q., Zhu, B., Chen, C., Yuan, Z., Guo, J., Yang, X., Wang, S., Lv, Y., Liu, Q., Yang, B.,
773 Sun, C., Wang, P., and Deng, X. 2021. A Single Nucleotide Substitution of GSAM Gene
774 Causes Massive Accumulation of Glutamate 1-Semialdehyde and Yellow Leaf Phenotype
775 in Rice. *Rice (N Y)* 14:50.
- 776 Wasternack, C., and Strnad, M. 2019. Jasmonates are signals in the biosynthesis of secondary
777 metabolites—Pathways, transcription factors and applied aspects—A brief review. *New*
778 *biotechnology* 48:1-11.
- 779 Wu, J.X., Li, J., Liu, Z., Yin, J., Chang, Z.Y., Rong, C., Wu, J.L., Bi, F.C., and Yao, N. 2015. The
780 Arabidopsis ceramidase At ACER functions in disease resistance and salt tolerance. *The*
781 *Plant Journal* 81:767-780.

- 782 Xia, Y., Yu, K., Gao, Q.-m., Wilson, E., Navarre, D., Kachroo, P., and Kachroo, A. 2012. Acyl
783 CoA binding proteins are required for cuticle formation and plant responses to microbes.
784 *Frontiers in plant science* 3:224.
- 785 Xia, Y., Gao, Q.-M., Yu, K., Lapchyk, L., Navarre, D., Hildebrand, D., Kachroo, A., and Kachroo,
786 P. 2009. An intact cuticle in distal tissues is essential for the induction of systemic acquired
787 resistance in plants. *Cell Host & Microbe* 5:151-165.
- 788 Xiao, S., Brown, S., Patrick, E., Brearley, C., and Turner, J.G. 2003. Enhanced transcription of the
789 *Arabidopsis* disease resistance genes RPW8. 1 and RPW8. 2 via a salicylic acid-dependent
790 amplification circuit is required for hypersensitive cell death. *The Plant Cell* 15:33-45.
- 791 Xin, X.-F., and He, S.Y. 2013. *Pseudomonas syringae* pv. tomato DC3000: a model pathogen for
792 probing disease susceptibility and hormone signaling in plants. *Annual review of*
793 *phytopathology* 51:473-498.
- 794 Xin, X.-F., Kvitko, B., and He, S.Y. 2018. *Pseudomonas syringae*: what it takes to be a pathogen.
795 *Nature Reviews Microbiology* 16:316.
- 796 Yang, J., Duan, G., Li, C., Liu, L., Han, G., Zhang, Y., and Wang, C. 2019. The Crosstalks
797 Between Jasmonic Acid and Other Plant Hormone Signaling Highlight the Involvement of
798 Jasmonic Acid as a Core Component in Plant Response to Biotic and Abiotic Stresses.
799 *Frontiers in plant science* 10.
- 800 Yi, S.Y., Shirasu, K., Moon, J.S., Lee, S.-G., and Kwon, S.-Y. 2014. The activated SA and JA
801 signaling pathways have an influence on flg22-triggered oxidative burst and callose
802 deposition. *PloS one* 9.
- 803 Yu, K., Soares, J.M., Mandal, M.K., Wang, C., Chanda, B., Gifford, A.N., Fowler, J.S., Navarre,
804 D., Kachroo, A., and Kachroo, P. 2013. A feedback regulatory loop between G3P and lipid

805 transfer proteins DIR1 and AZI1 mediates azelaic-acid-induced systemic immunity. Cell
806 Reports 3:1266-1278.

807 Yuan, M., Ngou, B.P.M., Ding, P., and Xin, X.F. 2021a. PTI-ETI crosstalk: an integrative view of
808 plant immunity. Curr Opin Plant Biol 62:102030.

809 Yuan, M., Jiang, Z., Bi, G., Nomura, K., Liu, M., Wang, Y., Cai, B., Zhou, J.M., He, S.Y., and
810 Xin, X.F. 2021b. Pattern-recognition receptors are required for NLR-mediated plant
811 immunity. Nature 592:105-109.

812 Zhao, Z., Yang, X., Lü, S., Fan, J., Opiyo, S., Yang, P., Mangold, J., Mackey, D., and Xia, Y.
813 2020. Deciphering the novel role of AtMIN7 in cuticle formation and defense against the
814 bacterial pathogen infection. International journal of molecular sciences 21:5547.

815 Zheng, X.-y., Spivey, N.W., Zeng, W., Liu, P.-P., Fu, Z.Q., Klessig, D.F., He, S.Y., and Dong, X.
816 2012. Coronatine promotes *Pseudomonas syringae* virulence in plants by activating a
817 signaling cascade that inhibits salicylic acid accumulation. Cell host & microbe 11:587-
818 596.

819 Zoeller, M., Stingl, N., Krischke, M., Fekete, A., Waller, F., Berger, S., and Mueller, M.J. 2012.
820 Lipid profiling of the Arabidopsis hypersensitive response reveals specific lipid
821 peroxidation and fragmentation processes: biogenesis of pimelic and azelaic acid. Plant
822 Physiol 160:365-378.

823

824

825

826

827 **Figure captions**

828 **Figure 1. Characterization of the ACPs family and identification of *acp1* mutants.** (a) The
 829 conserved domains of Arabidopsis Acyl Carrier Proteins (ACPs). Image modified from National
 830 Center for Biotechnology Information (NCBI). (b) Phylogenetic analyses of Arabidopsis ACP
 831 protein family members. Protein sequences are aligned by Clustal Omega. The Phylogenetic Tree
 832 was constructed from Simple Phylogeny via EMBL-EBI. The numbers shown indicated the
 833 distance values, which were the length of the branch immediately leading to the node. (c) Relative
 834 gene expression of *ACP1* in WT and *acp1* mutants and normalized to the expression of *ACTIN*.
 835 Data represent means $\pm$ SE ($n = 4$), which are from one of the three independent repeats with
 836 consistent results. Significant differences between the WT and *acp1* mutants were indicated by the
 837 asterisks determined from the Student's *t*-test ($p < 0.05$).

838 **Figure 2. The visualization of subcellular localization of ACP1 in the transiently transformed**
 839 *Nicotiana benthamiana*. (a) GFP signals of the ACP1-GFP fusion protein. (b) GFP signals of the
 840 empty vector pYBA1122-35S-GFP. Green fluorescence indicates GFP signal; red fluorescence
 841 indicates plastid autofluorescence; yellow fluorescence shows the merged images with two types
 842 of fluorescence. The signals of ACP1-GFP and PT-mCherry were overlapped to the exact
 843 localization at the plastids. Scale bar = 25 μ m.

844 **Figure 3. The responses of WT and *acp1* mutant plants to the bacterial pathogen *Pto* DC3000**
 845 **infection.** (a) Plant phenotypes of WT Col-0 and the *acp1* mutants in response to the *Pto* pathogen
 846 infection. WT Col-0, *acp1-1*, and *acp1-2* plant leaves were syringe infiltrated with 5×10^5 CFU/mL
 847 of *Pto* DC3000. Images were captured three days post- infiltration. (b) Bacterial multiplication in
 848 the leaves of WT and *acp1* mutants at 0 and 3 days after the inoculation. Data represent means $\pm$

849 SE ($n = 4$), which are from one of the three independent repeats with consistent results. Different
 850 letters a-c within the figure indicate the significant differences at $p < 0.05$, which was calculated
 851 by one-way analysis of variance (ANOVA) using SPSS ver. 21. (c) Relative PTI marker gene
 852 expressions in WT and *acp1* mutants plant leaves at 0, 6, and 48 hours after the inoculation with
 853 5×10^5 CFU/mL of *Pto* DC3000. The gene expression was quantified by qPCR and normalized to
 854 the expression of *UBIQUITIN*. The experiments were repeated three times with similar results.
 855 Data represent means $\pm$ SE ($n = 4$). Significant differences between the WT and *acp1* mutants were
 856 indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$).

857 **Figure 4. The levels of FAs in the 4-week-old WT and *acp1* mutant plant leaves.** (a) The
 858 abundance of the FAs at 4-week-old *acp1* mutants and WT plant leaves without any treatment.
 859 Data represent means $\pm$ SE ($n = 4$), which are from one of the three independent repeats with
 860 consistent results. Significant differences between the WT and *acp1* mutants were indicated by the
 861 asterisks determined from the Student's *t*-test ($p < 0.05$). (b) The abundance of C18:3 FAs at 4-
 862 week-old *acp1* mutants and WT plant leaves 48 hours after the *Pto* DC3000 and buffer MgCl_2
 863 inoculation. The FA analysis was extracted by placing leaf tissues in 2 ml of 3% H_2SO_4 in methanol
 864 with 17:0 FA as the standard. Data represent means $\pm$ SE ($n = 4$), which are from one of the three
 865 independent repeats with consistent results. Different letters a-c within the figure indicate the
 866 significant differences at $p < 0.05$, which was calculated by one-way analysis of variance (ANOVA)
 867 using SPSS ver. 21.

868 **Figure 5. The *acp1* mutants accumulated a higher level of salicylic acid (SA) and a lower level**
 869 **of jasmonic acid (JA) after the pathogen *Pto* DC3000 infection.** WT and *acp1* mutant plants
 870 were inoculated with the buffer (10 mM MgCl_2) or *Pto* DC3000 at 5×10^5 CFU/ml by leaf
 871 infiltration. Leaf samples were collected for quantification of free JA (a) and JA-Ile/Leu (JA-

isoleucine/leucine) (b), free SA (c), and SAG (SA-glycoside) (d) at 48 hours post-inoculation. Data represent means $\pm$ SE ($n = 4$), which are from one of the three independent repeats with consistent results. Different letters a-c within the figure (a-d) indicate the significant differences at $p < 0.05$, which was calculated by one-way analysis of variance (ANOVA) using SPSS ver. 21.

Figure 6. The *acp1* mutants showed the enhanced SA-related gene expression and reduced JA-related gene expression after the *Pto* DC3000 treatment. After the inoculation with *Pto* DC3000 at 5×10^5 CFU/ml by infiltration, the total RNA was extracted from the inoculated leaves of WT Col-0 and *acp1* mutants at the indicated times. The relative transcriptional levels of *SID2* (a), *PR1* (b), *PDF1.2* (c), *COII* (d), and *MYC2* (e) were determined by qPCR with *UBIQUITIN* as the internal reference gene. Data represent means $\pm$ SE ($n = 4$), which are from one of the three independent repeats with consistent results. Significant differences between the WT and *acp1* mutants were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$).

Figure 7. Effects of MeJA and C18:3 FA (linolenic acid) treatments on the plant defense of *acp1* mutants against *Pto* DC3000. Four-week-old WT Col-0 and *acp1* plants were sprayed with solvent control (0.1% ethanol) or 50 μ M MeJA or 0.1 mM linolenic acid. Plants were syringe infiltrated with 5×10^5 CFU/mL of *Pto* DC3000 24 hours after treatments. The bacterial multiplication in the leaves of WT and *acp* mutants was checked three days after the inoculation (a & b). Data represent means $\pm$ SE ($n = 4$), which are from one of three independent repeats with consistent results. Different letters a-c within the figures indicate the significant differences at $p < 0.05$, calculated by one-way analysis of variance (ANOVA) using SPSS ver. 21.

Figure 8. The *acp1* mutants showed the enhanced H_2O_2 accumulation upon the MAMP flg22 treatment or *Pto* DC3000 inoculation (a). The real-time chemiluminescence of H_2O_2 burst curve

894 upon the 100 nM flg22 induction or water treatment. Leaf discs (5 mm in diameter) were pre-
 895 treated with water overnight. Pre-treated leaf discs were treated with 100 nM flg22 in the buffer,
 896 which contained the luminol and horseradish peroxidase or mock treatment (water). Luminescence
 897 was recorded for 30 mins using the GLOMAX luminometer. Data represent means $\pm$ SE (n = 5),
 898 which are from one of the two independent repeats with consistent results. (b). WT Col-0 and *acp1*
 899 mutant plants were sprayed with *Pto* DC3000 (10^7 CFU/ml) or with buffer (10 mM MgCl₂) as the
 900 control. The H₂O₂ production was visualized by the 3,3-diaminobenzidine tetrahydrochloride
 901 (DAB) staining at 48 hours post-inoculation. The brown color indicated the H₂O₂ production site.
 902 (c) The production of H₂O₂ upon *Pto* DC3000 infection and buffer treatment is indicated by the
 903 DAB staining intensity. Data represent means $\pm$ SE (n = 3), which are from one of the three
 904 independent repeats with consistent results. Significant differences between the WT and *acp1*
 905 mutants were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$).

906 **Figure 9. The *acp1* mutants showed the enhanced callose deposition in response to the**
 907 **MAMP treatment and the pathogen *Pto* infection.** The leaves of *acp1* mutants and WT Col-0
 908 plants were infiltrated with the buffer (10 mM MgCl₂), 100 μ M flg22, *Pto* DC3000 at 10^8 CFU/ml,
 909 and *Pto* DC3000 *hrcC*- at 10^8 CFU/ml. Callose deposition quantification was determined by
 910 staining the leaves with 0.01% aniline blue solution 16 hours after inoculation. (a). The images of
 911 callose deposition in Arabidopsis leaves. (b). The number of callose deposition points was
 912 quantified by Image J software. The numbers shown are mean callose deposits per mm² from six
 913 images. Data represent means $\pm$ SE (n = 12), which are from one of the three independent repeats
 914 with consistent results. Significant differences between the *acp1* mutants and WT were indicated
 915 by the asterisks determined from the Student's *t*-test ($p < 0.05$).

916

917 **Supporting Information figure captions**

918 **Figure S1.** The relative transcriptional levels of *ACPs* in response to the bacterial pathogen *Pto*
 919 DC3000 infection. Col-0 leaves were infiltrated with buffer and 5×10^5 CFU/mL of *Pto* DC3000.
 920 Quantitative real-time PCR (qPCR) was used to measure the amount of *ACPs* transcript (relative
 921 to *ACTIN*) at 6 hours post-inoculation. Data represent means $\pm$ SE ($n = 4$), which are from one of
 922 the three independent repeats with consistent results. Significant differences between the WT and
 923 *acp1* mutants were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$).

924 **Figure S2. The diagram of the *ACP1* genomic structure and T-DNA insertion sites in two**
 925 ***acp1* mutants.** The position and orientation of primers used for PCR confirmation of the *acp1*-
 926 1 (F1 and R1) and *acp1*-2 mutants (F2 and R2) are marked by arrows. LBa1.3 and LB3 (left border
 927 primer) are specific for the T-DNA sequence of *acp1*-1 and *acp1*-2 mutants, respectively. The
 928 position and orientation of primers used for qRT-PCR are indicated as qPCRf and qPCRr.

929 **Figure S3. The physiological parameters of the *acp1* mutants and WT plants.** (a). Plant
 930 biomass (weight) of the *acp1* mutants and WT plants. (b). Plant height of the *acp1* mutants and
 931 WT plants. Data represent means $\pm$ SE ($n = 4$), which are from one of the two independent repeats
 932 with consistent results. Significant differences between the *acp1* mutants and WT were indicated
 933 by the asterisks determined from the Student's *t*-test ($p < 0.05$). NS represents no significance. (c).
 934 Morphological phenotypes of rosettes from 4-week-old *acp1* mutants and WT plants. (d).
 935 Morphological phenotypes of the 6-week-old *acp1* mutants and WT plants.

936 **Figure S4. Chlorophyll content and chlorophyll leaching rate of WT plant leaves (blue**
 937 **circles), *acp1*-1 mutant (orange circles), and *acp1*-2 mutant (grey circles).** The *acp1* mutants
 938 showed similar chlorophyll content and chlorophyll leaching rate to the WT Col-0. Data represent

means $\pm$ SE ($n = 3$), which are from one of the three independent repeats with consistent results. Significant differences between the *acp1* mutants and WT were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$).

Figure S5. Basal transcript levels of JA-related genes in 4-week-old *acp1* mutants and WT plant leaves. The JA biosynthesis genes *LOX3*, *AOS*, and JA downstream signaling genes *JAZ1* and *JAZ10* were quantified by qRT-PCR and normalized to the expression of *UBIQUITIN*. Data represent means $\pm$ SE ($n = 4$), which are from one of the three independent repeats with consistent results. Significant differences between the WT and *acp1* mutants were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$).

Figure S6. The *ACPI* gene expressions in different tissues of Arabidopsis plants. The *ACPI* gene expression at different tissues of the Arabidopsis plant is shown in different colors. Related information is obtained from Arabidopsis eFP Browser. <http://bar.utoronto.ca/eplant/>.

Table S1. The accession number list of ACPI homologs

Table S2. The primer list for the PCR and RT-qPCR

Figure 1.

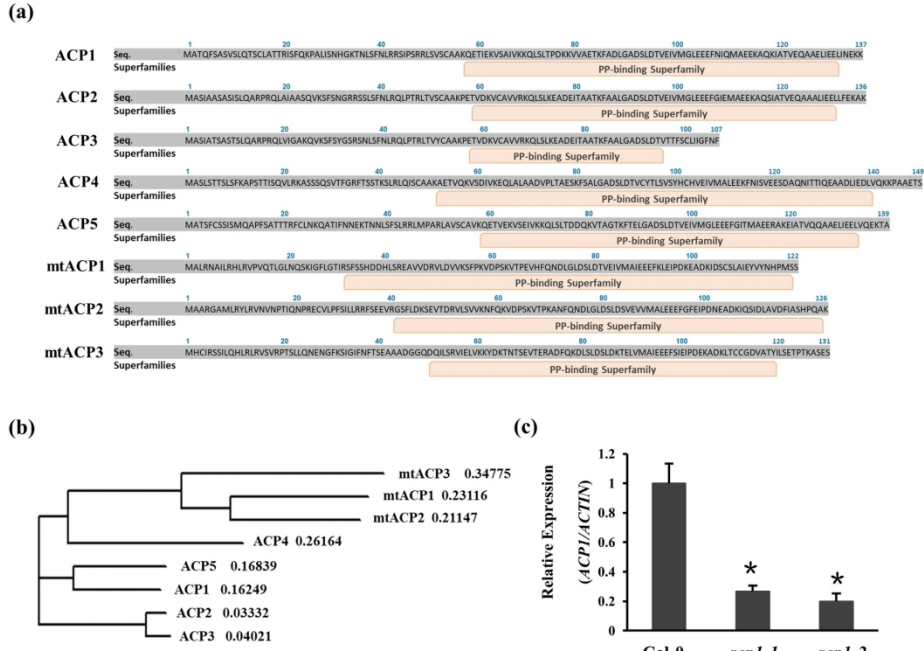


Figure 1. Characterization of the ACPs family and identification of *acp1* mutants. (a) The conserved domains of Arabidopsis Acyl Carrier Proteins (ACPs). Image modified from National Center for Biotechnology Information (NCBI). (b) Phylogenetic analyses of Arabidopsis ACP protein family members. Protein sequences are aligned by Clustal Omega. The Phylogenetic Tree was constructed from Simple Phylogeny via EMBL-EBI. The numbers shown indicated the distance values, which were the length of the branch immediately leading to the node. (c) Relative gene expression of *ACP1* in WT and *acp1* mutants and normalized to the expression of *ACTIN*. Data represent means $\pm$ SE ($n = 4$), which are from one of the three independent repeats with consistent results. Significant differences between the WT and *acp1* mutants were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$).

176x138mm (300 x 300 DPI)

Figure 2.

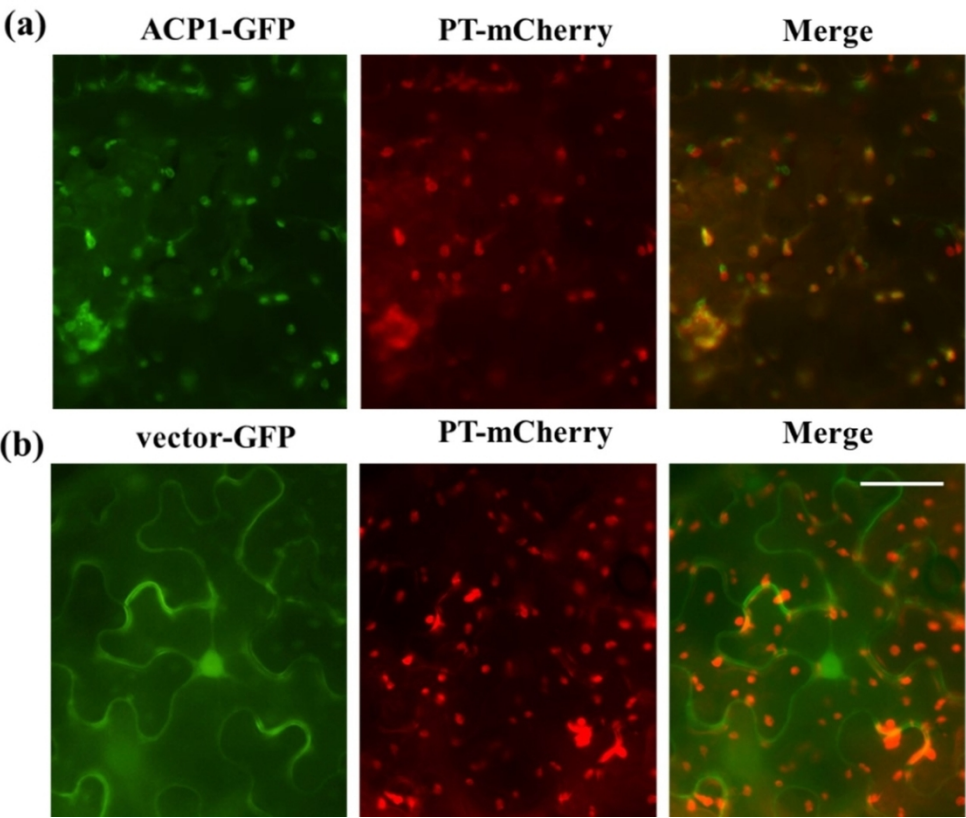


Figure 2. The visualization of subcellular localization of ACP1 in the transiently transformed *Nicotiana benthamiana*. (a) GFP signals of the ACP1-GFP fusion protein. (b) GFP signals of the empty vector pYBA1122-35S-GFP. Green fluorescence indicates GFP signal; red fluorescence indicates plastid autofluorescence; yellow fluorescence shows the merged images with two types of fluorescence. The signals of ACP1-GFP and PT-mCherry were overlapped to the exact localization at the plastids. Scale bar =25 μ m.

101x97mm (300 x 300 DPI)

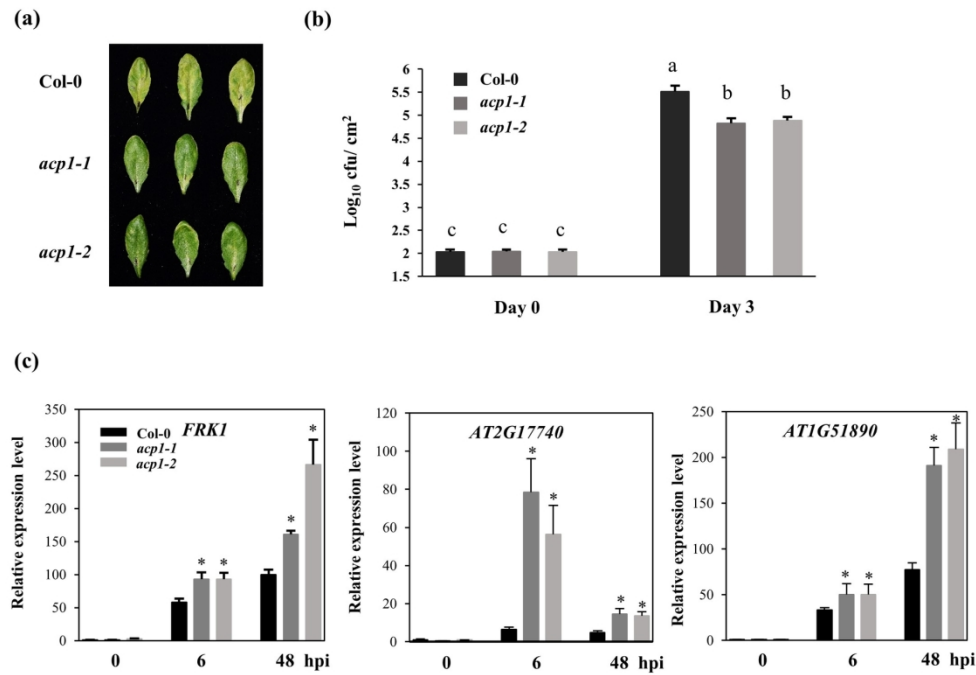
Figure 3.

Figure 3. The responses of WT and *acp1* mutant plants to the bacterial pathogen *Pto* DC3000 infection. (a) Plant phenotypes of WT Col-0 and the *acp1* mutants in response to the *Pto* pathogen infection. WT Col-0, *acp1-1*, and *acp1-2* plant leaves were syringe infiltrated with 5×10^5 CFU/mL of *Pto* DC3000. Images were captured three days post-infiltration. (b) Bacterial multiplication in the leaves of WT and *acp* mutants at 0 and 3 days after the inoculation. Data represent means $\pm$ SE ($n = 4$), which are from one of the three independent repeats with consistent results. Different letters a-c within the figure indicate the significant differences at $p < 0.05$, which was calculated by one-way analysis of variance (ANOVA) using SPSS ver. 21. (c) Relative PTI marker gene expressions in WT and *acp1* mutants plant leaves at 0, 6, and 48 hours after the inoculation with 5×10^5 CFU/mL of *Pto* DC3000. The gene expression was quantified by qPCR and normalized to the expression of *UBIQUITIN*. The experiments were repeated three times with similar results. Data represent means $\pm$ SE ($n = 4$). Significant differences between the WT and *acp1* mutants were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$).

169x135mm (300 x 300 DPI)

Figure 4.

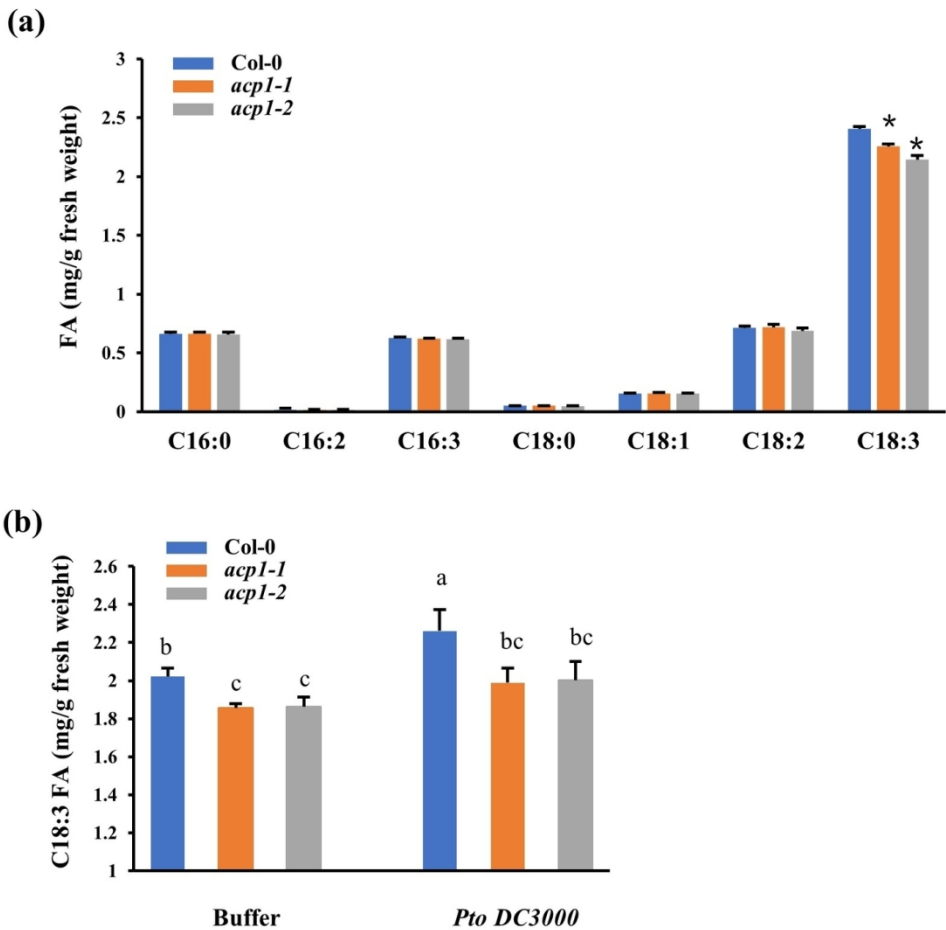


Figure 4. The levels of FAs in the 4-week-old WT and *acp1* mutant plant leaves. (a) The abundance of the FAs at 4-week-old *acp1* mutants and WT plant leaves without any treatment. Data represent means $\pm$ SE ($n = 4$), which are from one of the three independent repeats with consistent results. Significant differences between the WT and *acp1* mutants were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$). (b) The abundance of C18:3 FAs at 4-week-old *acp1* mutants and WT plant leaves 48 hours after the *Pto DC3000* and buffer $MgCl_2$ inoculation. The FA analysis was extracted by placing leaf tissues in 2 ml of 3% H_2SO_4 in methanol with 17:0 FA as the standard. Data represent means $\pm$ SE ($n = 4$), which are from one of the three independent repeats with consistent results. Different letters a-c within the figure indicate the significant differences at $p < 0.05$, which was calculated by one-way analysis of variance (ANOVA) using SPSS ver. 21.

Figure 5.

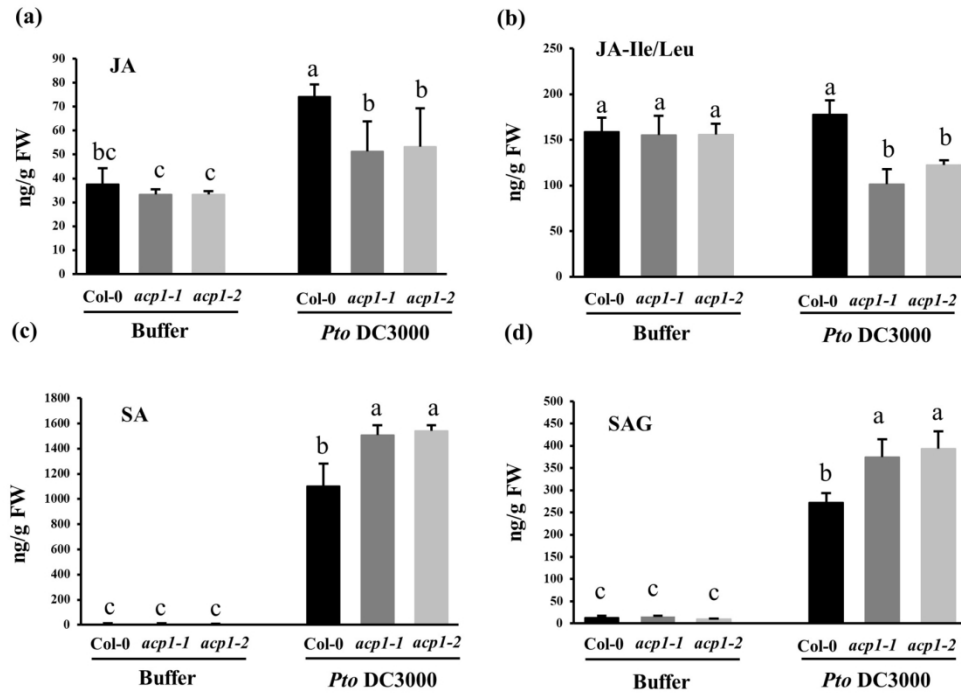


Figure 5. The *acp1* mutants accumulated a higher level of salicylic acid (SA) and a lower level of jasmonic acid (JA) after the pathogen *Pto* DC3000 infection. WT and *acp1* mutant plants were inoculated with the buffer (10 mM MgCl_2) or *Pto* DC3000 at 5×10^5 CFU/ml by leaf infiltration. Leaf samples were collected for quantification of free JA (a) and JA-Ile/Leu (JA-isoleucine/leucine) (b), free SA (c), and SAG (SA-glycoside) (d) at 48 hours post-inoculation. Data represent means $\pm$ SE ($n = 4$), which are from one of the three independent repeats with consistent results. Different letters a-c within the figure (a-d) indicate the significant differences at $p < 0.05$, which was calculated by one-way analysis of variance (ANOVA) using SPSS ver. 21.

164x135mm (300 x 300 DPI)

Figure 6.

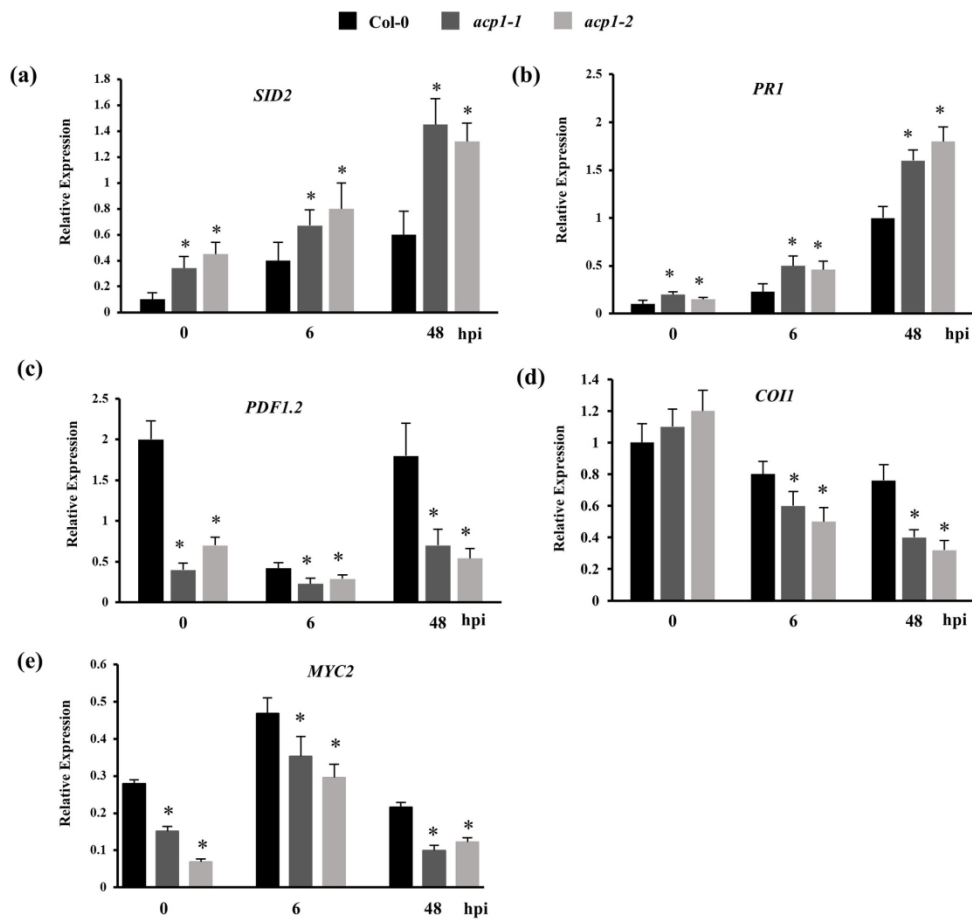


Figure 6. The *acp1* mutants showed the enhanced SA-related gene expression and reduced JA-related gene expression after the *Pto* DC3000 treatment. After the inoculation with *Pto* DC3000 at 5×10^5 CFU/ml by infiltration, the total RNA was extracted from the inoculated leaves of WT Col-0 and *acp1* mutants at the indicated times. The relative transcriptional levels of *SID2* (a), *PR1* (b), *PDF1.2* (c), *COI1* (d), and *MYC2* (e) were determined by qPCR with *UBIQUITIN* as the internal reference gene. Data represent means $\pm$ SE (n = 4), which are from one of the three independent repeats with consistent results. Significant differences between the WT and *acp1* mutants were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$).

163x175mm (300 x 300 DPI)

Figure 7.

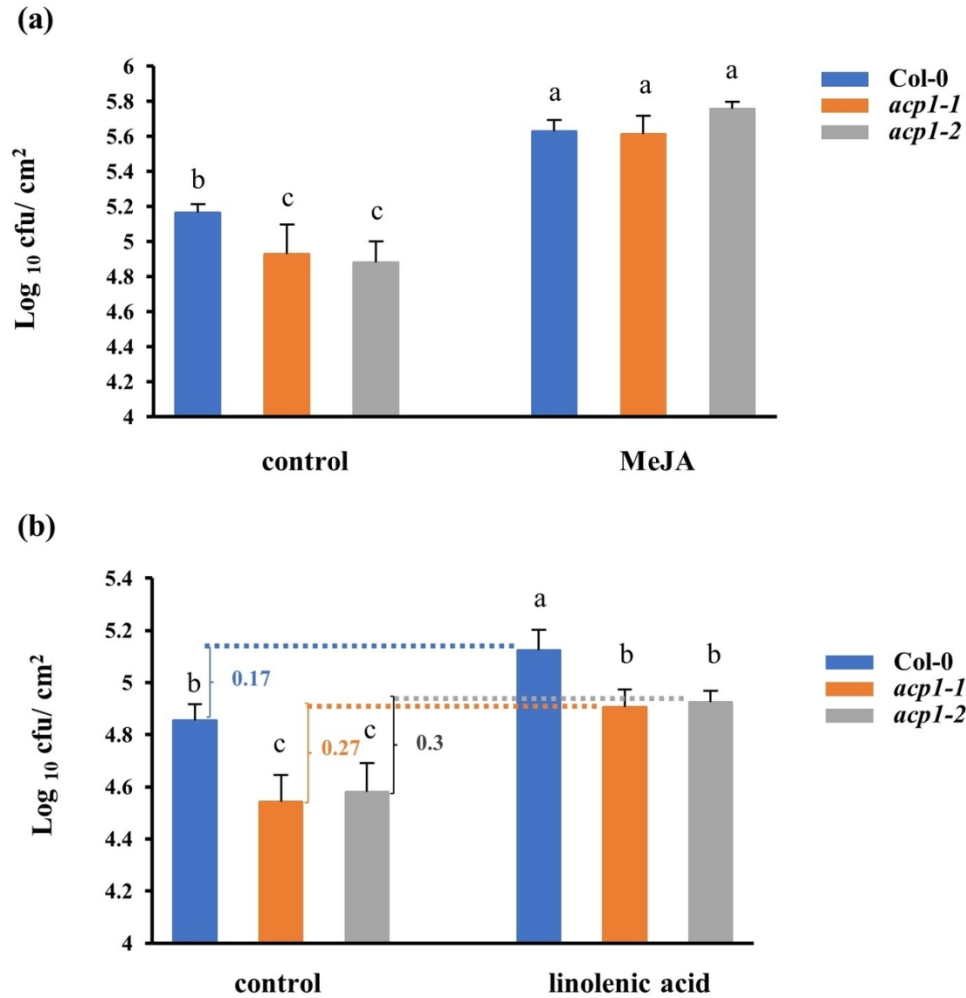


Figure 7. Effects of MeJA and C18:3 FA (linolenic acid) treatments on the plant defense of *acp1* mutants against *Pto* DC3000. Four-week-old WT Col-0 and *acp1* plants were sprayed with solvent control (0.1% ethanol) or 50 μM MeJA or 0.1 mM linolenic acid. Plants were syringe infiltrated with 5×10^5 CFU/mL of *Pto* DC3000 24 hours after treatments. The bacterial multiplication in the leaves of WT and *acp1* mutants was checked three days after the inoculation (a & b). Data represent means $\pm$ SE ($n = 4$), which are from one of three independent repeats with consistent results. Different letters a-c within the figures indicate the significant differences at $p < 0.05$, calculated by one-way analysis of variance (ANOVA) using SPSS ver. 21.

109x131mm (300 x 300 DPI)

Figure 8.

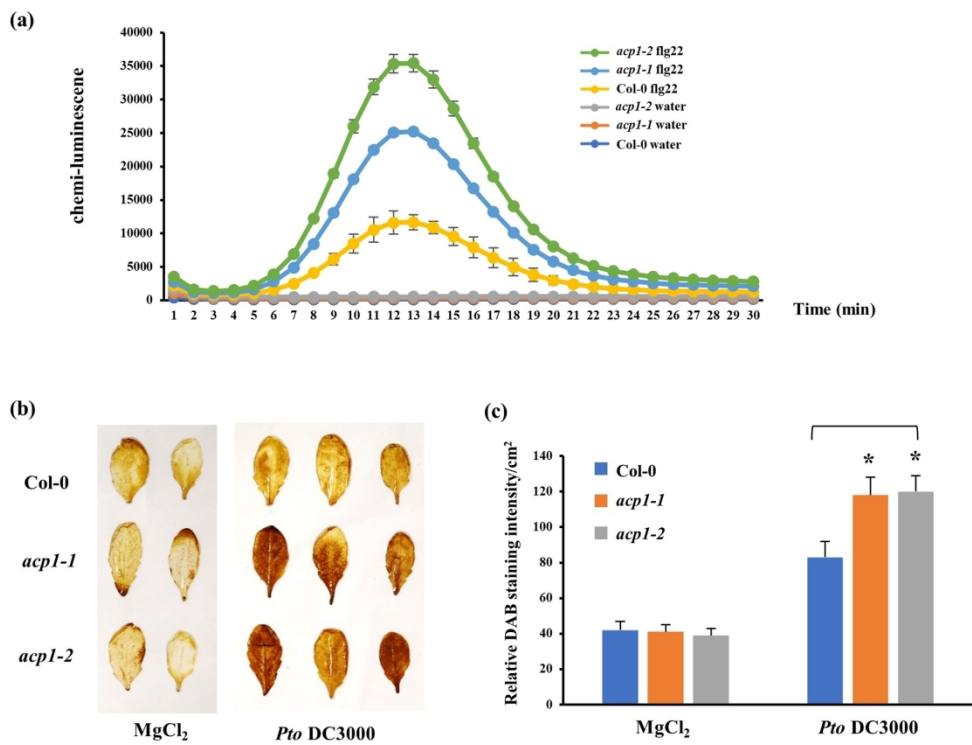


Figure 8. The *acp1* mutants showed enhanced H₂O₂ accumulation upon the MAMP flg22 treatment or *Pto* DC3000 inoculation (a). The real-time chemiluminescence of H₂O₂ burst curve upon the 100 nM flg22 induction or water treatment. Leaf discs (5 mm in diameter) were pre-treated with water overnight. Pre-treated leaf discs were treated with 100 nM flg22 in the buffer, which contained the luminol and horseradish peroxidase or mock treatment (water). Luminescence was recorded for 30 mins using the GLOMAX luminometer. Data represent means $\pm$ SE ($n = 5$), which are from one of the two independent repeats with consistent results. (b). WT Col-0 and *acp1* mutant plants were sprayed with *Pto* DC3000 (10^7 CFU/ml) or with buffer (10 mM MgCl₂) as the control. The H₂O₂ production was visualized by the 3,3-diaminobenzidine tetrahydrochloride (DAB) staining at 48 hours post-inoculation. The brown color indicated the H₂O₂ production site. (c) The production of H₂O₂ upon *Pto* DC3000 infection and buffer treatment is indicated by the DAB staining intensity. Data represent means $\pm$ SE ($n = 3$), which are from one of the three independent repeats with consistent results. Significant differences between the WT and *acp1* mutants were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$).

162x141mm (300 x 300 DPI)

Figure 9.

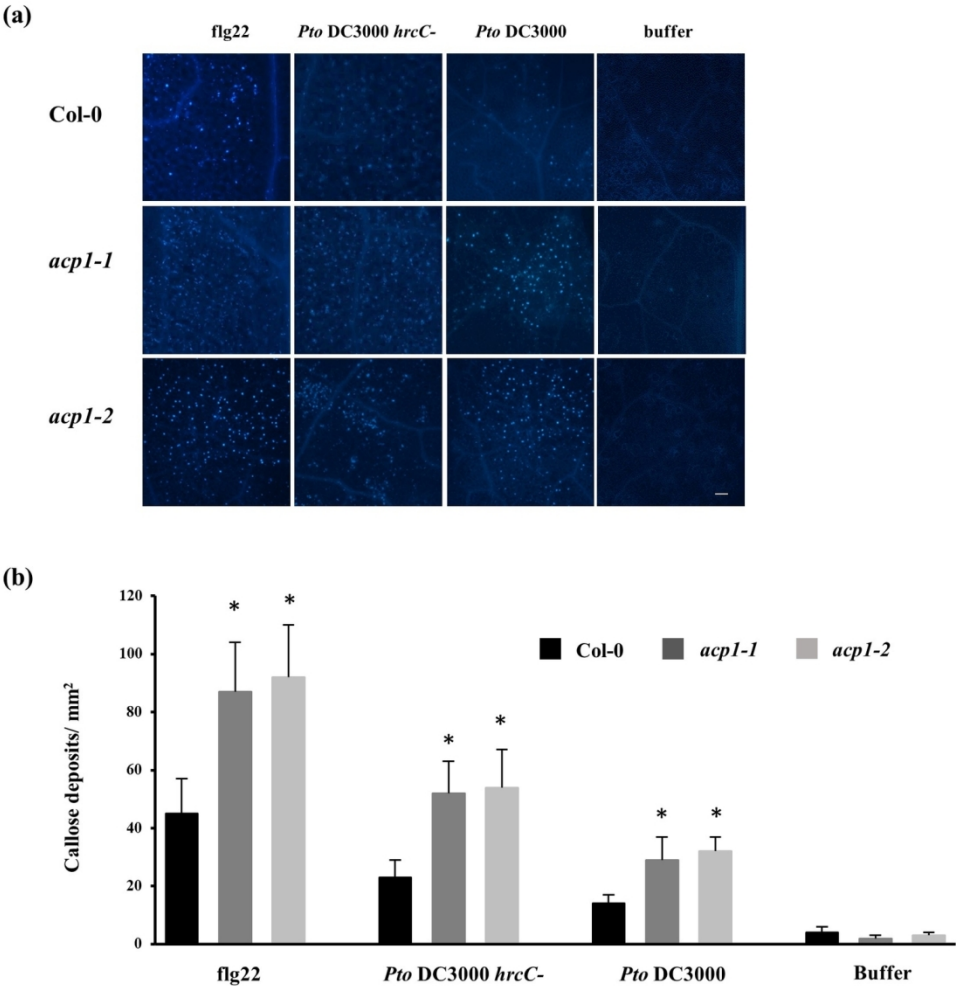


Figure 9. The *acp1* mutants showed the enhanced callose deposition in response to the MAMP treatment and the pathogen *Pto* infection. The leaves of *acp1* mutants and WT Col-0 plants were infiltrated with the buffer (10 mM MgCl₂), 100 μM flg22, *Pto* DC3000 at 10⁸ CFU/ml, and *Pto* DC3000 *hrcC*⁻ at 10⁸ CFU/ml. Callose deposition quantification was determined by staining the leaves with 0.01% aniline blue solution 16 hours after inoculation. (a). The images of callose deposition in Arabidopsis leaves. (b). The number of callose deposition points was quantified by Image J software. The numbers shown are mean callose deposits per mm² from six images. Data represent means ± SE (n = 12), which are from one of the three independent repeats with consistent results. Significant differences between the *acp1* mutants and WT were indicated by the asterisks determined from the Student's *t*-test (*p* < 0.05).

137x158mm (300 x 300 DPI)

Supporting Material

Involvement of Arabidopsis Acyl Carrier Protein 1 in PAMP-triggered immunity

Zhenzhen Zhao¹, Jiangbo Fan^{1,2}, Piao Yang¹, Zonghua Wang³, Stephen Obol Opiyo¹, David Mackey⁴, Ye Xia^{1,*}

¹Department of Plant Pathology, The Ohio State University, 2021 Coffey Road, Columbus, OH 43210, U.S.A.

²School of Agriculture and Biology, Shanghai Jiao Tong University, 800 Dongchuan Rd., Shanghai, 200240, China

³State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou, 350002, China

⁴Department of Horticulture and Crop Science, The Ohio State University, Columbus, OH 43210, U.S.A.

* Corresponding author: Ye Xia; Email: xia.374@osu.edu

Keywords: acyl carrier protein, fatty acids, plant immunity, PTI, plant hormones

Author contributions: Conceptualization, Z.Z., D.M., and Y.X.; methodology, Z.Z., D.M., and Y.X.; software, S.O.; formal analysis, Z.Z.; investigation, Z.Z., J.F., P.Y., Z.W., and X.Y.; data curation, Z.Z.; writing—original draft preparation, Z.Z.; writing—review and editing, Z.Z., D.M., and Y.X.; visualization, Z.Z.; supervision, Z.Z., D.M., and Y.X.; project administration, Z.Z. and Y.X.; and funding acquisition, D.M. and Y.X. All authors have read and agreed to the published version of the manuscript.

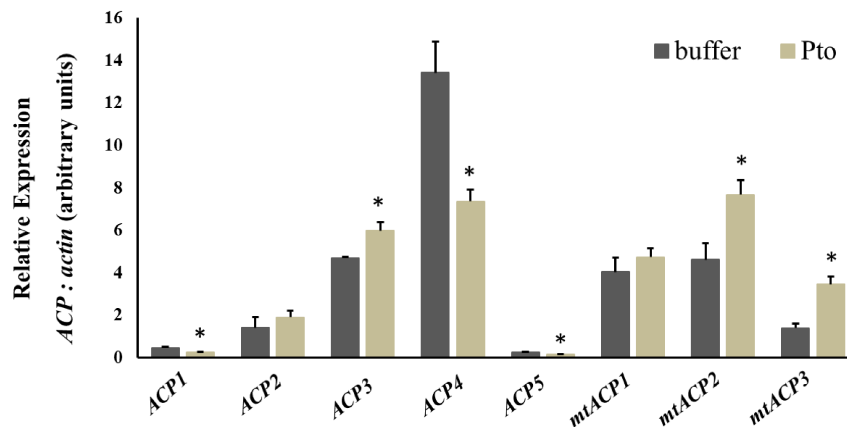


Figure S1. The relative transcriptional levels of *ACPs* in response to the bacterial pathogen *Pto* DC3000 infection. Col-0 leaves were infiltrated with buffer and 5×10^5 CFU/mL of *Pto* DC3000. Quantitative real-time PCR (qPCR) was used to measure the amount of *ACPs* transcript (relative to *ACTIN*) at 6 hours post-inoculation. Data represent means $\pm$ SE ($n = 4$), which are from one of the three independent repeats with consistent results. Significant differences between the WT and *acp1* mutants were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$).

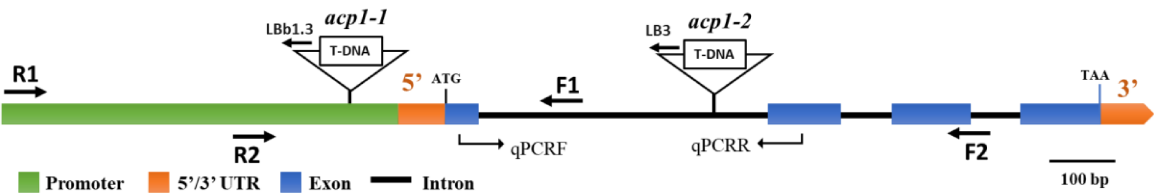


Figure S2. The diagram of the *ACP1* genomic structure and T-DNA insertion sites in two *acp1* mutants. The position and orientation of primers used for PCR confirmation of the *acp1-1* (F1 and R1) and *acp1-2* mutants (F2 and R2) are marked by arrows. LBa1.3 and LB3 (left border primer) are specific for the T-DNA sequence of *acp1-1* and *acp1-2* mutants, respectively. The position and orientation of primers used for qRT-PCR are indicated as qPCRf and qPCRr.

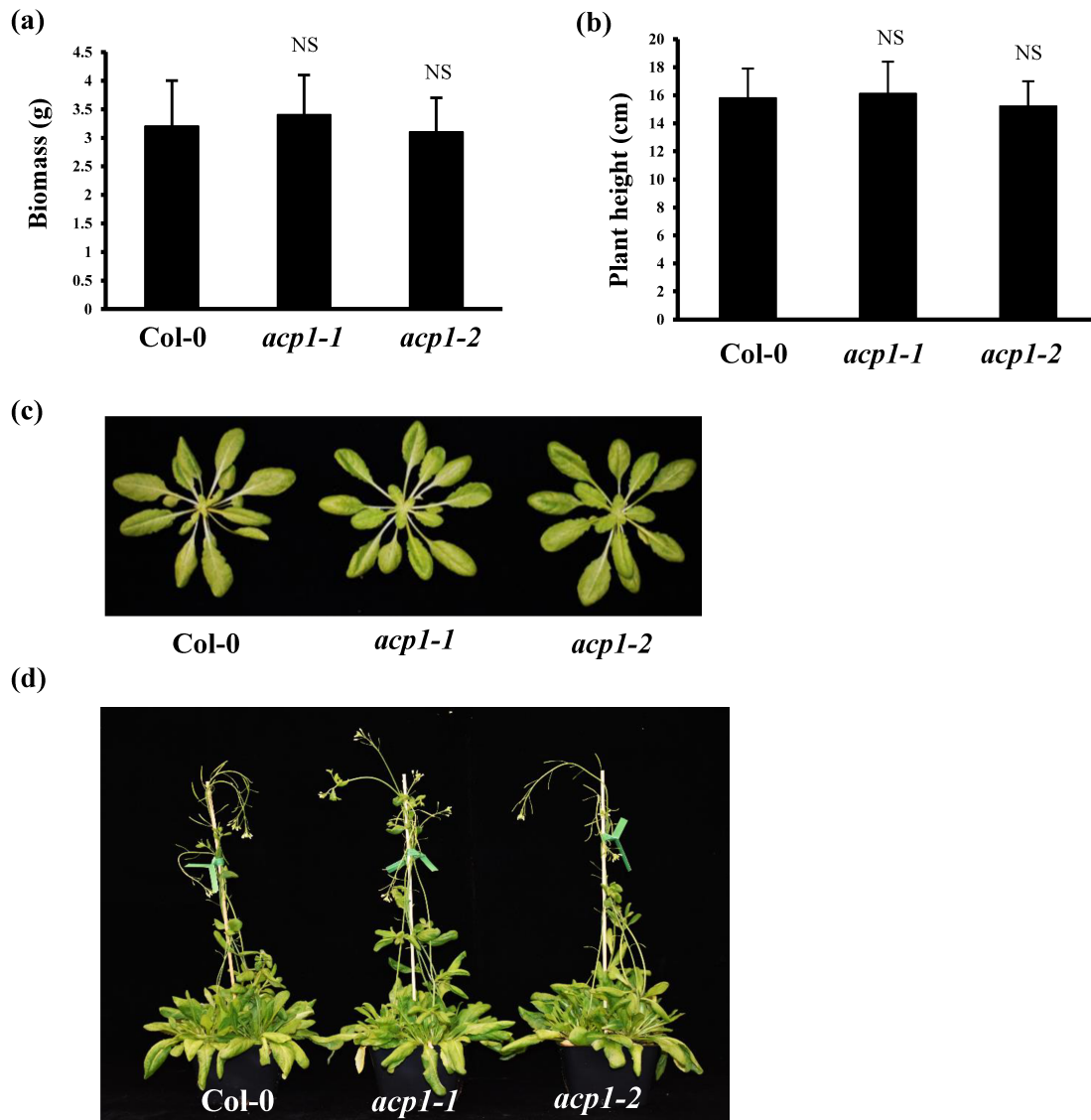


Figure S3. The physiological parameters of the *acp1* mutants and WT plants. (a). Plant biomass (weight) of the *acp1* mutants and WT plants. (b). Plant height of the *acp1* mutants and WT plants. Data represent means $\pm$ SE ($n = 4$), which are from one of the two independent repeats with consistent results. Significant differences between the *acp1* mutants and WT were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$). NS represents no significance. (c). Morphological phenotypes of rosettes from 4-week-old *acp1* mutants and WT plants. (d). Morphological phenotypes of the 6-week-old *acp1* mutants and WT plants.

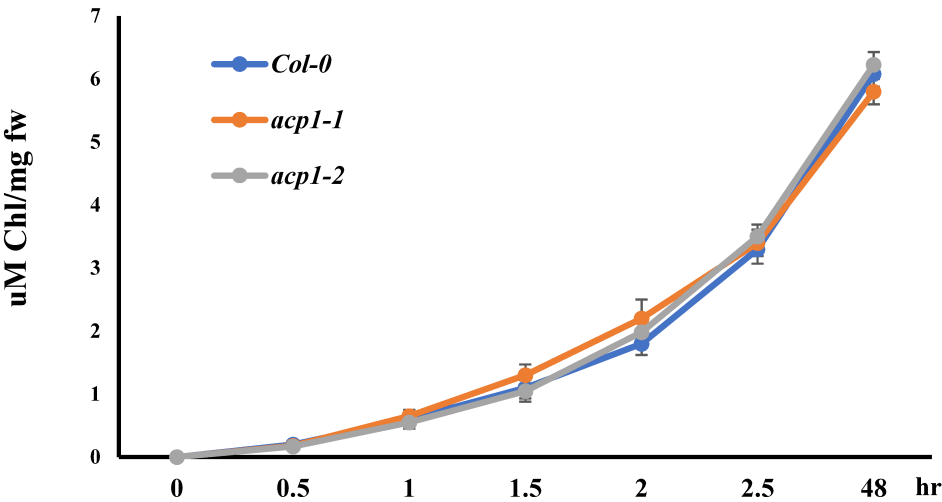


Figure S4. Chlorophyll content and chlorophyll leaching rate of WT plant leaves (blue circles), *acp1-1* mutant (orange circles), and *acp1-2* mutant (grey circles). The *acp1* mutants showed similar chlorophyll content and chlorophyll leaching rate to the WT Col-0. Data represent means $\pm$ SE ($n = 3$), which are from one of the three independent repeats with consistent results. Significant differences between the *acp1* mutants and WT were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$).

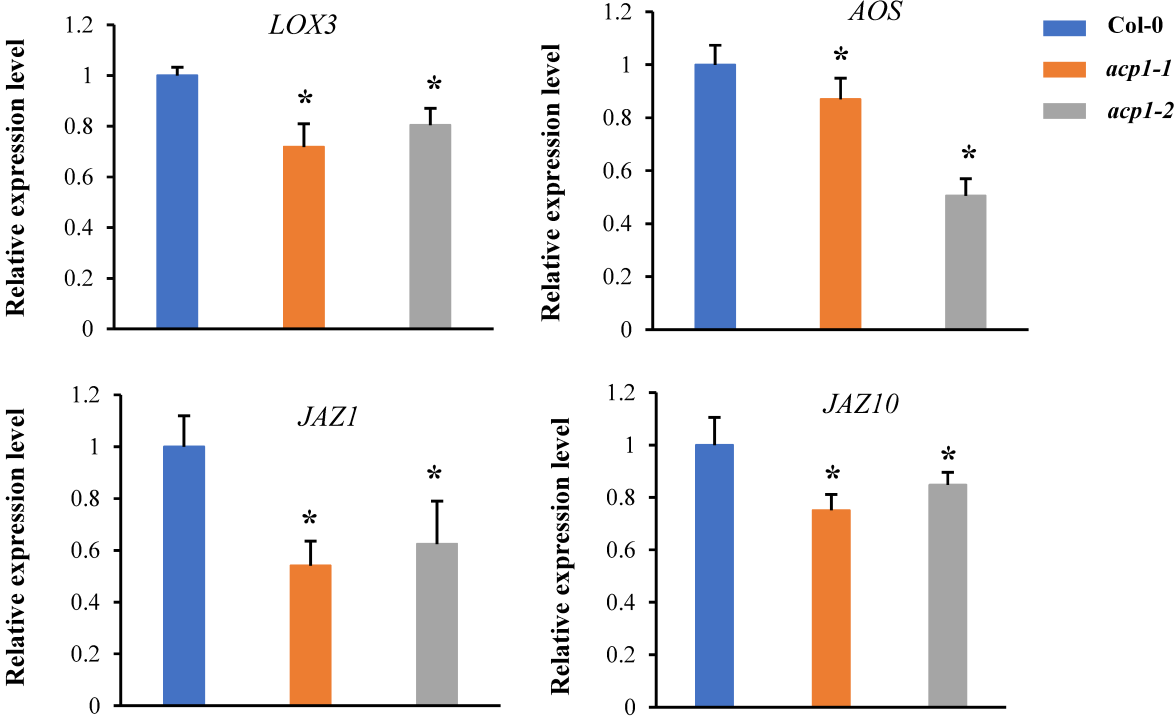


Figure S5. Basal transcript levels of JA-related genes in 4-week-old *acp1* mutants and WT plant leaves. The JA biosynthesis genes *LOX3*, *AOS*, and JA downstream signaling genes *JAZ1* and *JAZ10* were quantified by qRT-PCR and normalized to the expression of *ubiquitin*. Data represent means $\pm$ SE ($n = 4$), which are from one of the three independent repeats with consistent results. Significant differences between the WT and *acp1* mutants were indicated by the asterisks determined from the Student's *t*-test ($p < 0.05$).

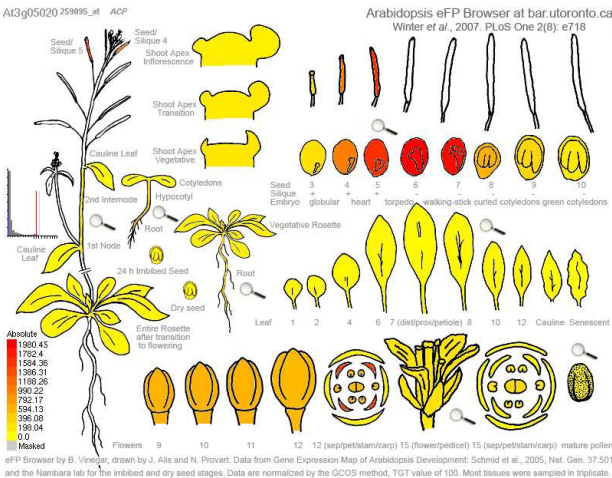


Figure S6. The *ACP1* gene expressions in different tissues of Arabidopsis plants. The *ACP1* gene expression at different tissues of the Arabidopsis plant is shown in different colors. Related information is obtained from Arabidopsis eFP Browser. <http://bar.utoronto.ca/eplant/>.

176 **Table S1. The accession number list of ACP1 homologs**

Species Name	Protein Sequence Accession Number in NCBI
<i>Arabidopsis thaliana</i>	NP_187153.1
<i>Arabidopsis lyrata subsp. Lyrata</i>	XP_020886365.1
<i>Capsella rubella</i>	XP_006299536.2
<i>Camelina sativa</i>	XP_010464003.1
<i>Arabis nemorensis</i>	VVA96518.1
<i>Eutrema salsugineum</i>	XP_006408119.1
<i>Brassica oleracea var. oleracea</i>	XP_013586447.1
<i>Brassica rapa</i>	XP_009124065.1
<i>Arabis alpine</i>	KFK37908.1
<i>Raphanus sativus</i>	XP_018438995.1
<i>Brassica rapa subsp. Chinensis</i>	ANS54496.1
<i>Brassica cretica</i>	RQL89968.1
<i>Brassica oleracea var. oleracea</i>	XP_013584173.1
<i>Tarenaya hassleriana</i>	XP_010524895.1
<i>Eutrema salsugineum</i>	XP_006394987.1
<i>Populus euphratica</i>	XP_011023489.1
<i>Theobroma cacao</i>	XP_007030153.1
<i>Parasponia andersonii</i>	PON73966.1
<i>Populus trichocarpa</i>	ABK93956.1
<i>Durio zibethinus</i>	XP_022718945.1
<i>Populus alba</i>	TKS10858.1

177
178
179
180
181
182
183
184
185
186
187
188
189
190
191

192 **Table S2. The primer list for the PCR and RT-qPCR**

Name	Sequence 5' to 3'	Purpose
acp1-1-LP (F1)	TTTTAATTCCATGTGTTGCCG	<i>acp1-1</i> T-DNA line genotyping
acp1-1-RP (R1)	AGTTTGATGATGCCTTCATGC	
acp1-2-LP (F2)	ACGATAACAAGAGCAGGCATG	<i>acp1-2</i> T-DNA line genotyping
acp1-2-RP (R2)	CTCCGACTGAGAGAAGCAGCC	
LB3	TAGCATCTGAATTCATAACCAATCTCGATACAC	T-DNA left border primer
LBb1.3	ATTTTGCCGATTCGGAAC	T-DNA left border primer
ACP1GFP-F	CACGCTCGAGGAATTCATGATGGGTTGTTTCGGTCTCGA AGATGACTCAATCTATCTGTTTCGTC	Clone full-length CDS of <i>ACP1</i> for subcellular localization
ACP1GFP-R	GCTCACCATGGTGTGTTGGTC	
ACP1-qF	GCGGAGGTTGAAGGATAGATTA	qRT-PCR for <i>ACP1</i> expression pattern
ACP1-qR	GCTTCTGTCTCATTGCAAACCTT	
ACP2-qF	GGCC TTCTTTTAATCTCCGCGCCG	qRT-PCR for <i>ACP2</i> expression pattern
ACP2-qR	GCCGGCACCAAGTGCAGCAAGCTG	
ACP3-qF	GCGCTTCTTTTAATCTTCGCGCCG	qRT-PCR for <i>ACP3</i> expression pattern
ACP3-qR	CCGCGGGAATCAGCACCAAGGCCG	
ACP4-qF	ACAATGGAGATAGTGATGGCGTTG	qRT-PCR for <i>ACP4</i> expression pattern
ACP4-qR	CGGCAAACGCTCTGAAGGCAAGAA	
ACP5-qF	CCGCGGCTACGATTTTCAACGCGC	qRT-PCR for <i>ACP5</i> expression pattern
ACP5-qR	CCGCTCGGTAAATTTAGTTCGGCC	
MTACP1-qF	GGCGTTTCTTCGCACGATGAGCCG	qRT-PCR for <i>MTACP1</i> expression pattern
MTACP1-qR	GCTGCCAGCTTGAATTCCTCGGCG	
MTACP2-qF	CGGCCGAGGAAGTTAGAGGCCGGC	qRT-PCR for <i>MTACP2</i> expression pattern
MTACP2-qR	GGGCAACCCAAATTCCTCCTCGCG	
MTACP3-qF	GGCGTTTCACATCAGAAGCACGGC	qRT-PCR for <i>MTACP3</i> expression pattern
MTACP3-qR	CCGCGAGAACTCTTCTTCAAGCCG	
SID2-qF	CACGGAGTGTCCCACTTCG	RT-qPCR test
SID2-qR	CGTCATGTCATCAGCGGTATC	

PR1-qF	GGCTCATATACCTCTGCACTCTA	RT-qPCR test
PR1-qR	TGGTTTAGATACTCTGCTACGGC	
PDF1.2-qF	AATAGGAATTGATCCAGTCGCAG	
PDF1.2-qR	CTTTCGTCGCCTTACACTCTTT	
ACTIN-qF	ATCCAATCCTCCCCAACACC	
ACTIN-qR	AACTCTGTCCTTTCTCTTCTCC	
UBIQUITIN-qF	AGATCCAGGACAAGGAGGTATTC	
UBIQUITIN-qR	CGCAGGACCAAGTGAAGAGTAG	

193
194
195
196