Tomato Protein Phosphatase 2C (SlPP2C3) negatively regulates fruit ripening onset and fruit gloss

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Highlight: SlPP2C3 regulate tomato fruit ripening

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26   **Abstract**

27   Absciscic acid (ABA) plays a vital role in coordinating physiological processes during
 28   fresh fruit ripening. ABA can bind to ABA receptors which interacts and inhibits their
 29   co-receptors type 2C phosphatases (PP2Cs). However, the dissected mechanism of
 30   PP2C during fruit ripening is unclear. In this study, we identify the role of *SIPP2C3*, a
 31   tomato type 2C phosphatase, as a negative regulator of ABA signaling and fruit
 32   ripening. *SIPP2C3* selectively interacted with monomeric ABA receptors and
 33   *SlSnRK2.8* kinase in both yeast and tobacco epidermal cells. Expressions of *SIPP2C3*
 34   were observed in all tissues, and it negatively correlated with the fruit ripening which
 35   was induced by exogenous ABA. Tomato plants with suppressed *SIPP2C3* expression
 36   exhibited enhanced sensitivity to ABA, while *SIPP2C3* over-expressed plants were
 37   less sensitive to ABA. Meaningfully, lack of *SIPP2C3* expression causes the
 38   acceleration of fruit ripening onset via the alternation of ABA signaling activity, and
 39   the fruit gloss is affected by the changes of outer epidermis structure. RNA-seq
 40   analysis found significant different expression of cuticle-related genes in pericarp
 41   between wild-type and *SIPP2C3* suppressed lines. Taken together, our finding
 42   demonstrate that *SIPP2C3* plays an important role in the regulation of fruit ripening
 43   and fruit appearance quality in tomato.

44

45   **Keywords** :Protein phosphatase 2C, ABA signaling, Fruit ripening, fruit gloss, cuticle,
 46   tomato.

47

48 Introduction

49   Phytohormone ABA plays roles in various physiological processes during plant
50   lifecycle, including seed dormancy and germination, root growth, stomata movement,
51   leaf senescence and fruit ripening (Schroeder *et al.*, 2001; Finkelstein *et al.*, 2002;
52   Zhang *et al.*, 2009a,b). Plant ABA response is largely relied on its signal transduction
53   pathway (Cutler *et al.*, 2010). A dominant ABA signaling pathway are composed of
54   PYR/PYL/RCAR family receptor proteins, group A type 2C protein phosphatases
55   (PP2Cs), and subfamily 2 of the sucrose nonfermenting 1-related kinases (SnRK2s)
56   (Fujii *et al.*, 2009; Ma *et al.*, 2009; Park *et al.*, 2009; Umezawa *et al.*, 2009; Vlad *et*
57   *al.*, 2009). The canonical ABA signaling model could be summarized as following:
58   ABA binding to PYR/PYL/RCAR receptors induces a conformational change of
59   receptor and a compete SnRK2 kinase from PP2C inhibition. Released SnRK2s can
60   be activated by Raf-like kinases, then downstream substrates can be phosphorylated,
61   such as transcription factor or ion channel protein to elicit ABA response (Cutler *et al.*,
62   2010; Weiner *et al.*, 2010; Hauser *et al.*, 2011).

63   Protein phosphatases 2C is the earliest identified key component in ABA
64   signaling pathway (Koornneef, 1984). *Arabidopsis* group A PP2C members ABI1 and
65   ABI2 were cloned from *abi1-1* and *abi1-2* mutants with ABA-insensitive phenotypes,
66   and were shown to play a negative role in ABA signaling regulation with overlapped
67   functions (Leung *et al.*, 1994; Meyer *et al.*, 1994; Leung *et al.*, 1997; Gosti *et al.*,
68   1999; Merlot *et al.*, 2001). So far, nine genes encoded PP2C were identified as ABA
69   signaling pathway components in *Arabidopsis*, including ABI1, ABI2, HAB1, HAB2,

AHG1, AHG3/AtPP2CA, and three HAIs (Saez *et al.*, 2004; Kuhn *et al.*, 2006; Yoshida *et al.*, 2006; Nishimura *et al.*, 2007; Bhaskara *et al.*, 2012). PP2Cs interact and phosphorylate SnRK2s, the activity of PP2Cs is inhibited by ABA receptors PYR/PYL/RCAR proteins in both ABA-dependent and ABA-independent manners (Hao *et al.*, 2011; He *et al.*, 2014; Nemoto *et al.*, 2018). Structural studies indicate that Ser residues on the side chain of PYR/PYL/RCAR receptor insert into the PP2C active site and competitively occlude the access of the substrates (Melcher *et al.*, 2009; Miyazono *et al.*, 2009; Yin *et al.*, 2009). In this model, two residues are important for the interaction. One is Gly-180 of ABI1 or Gly246 of HAB1, this Gly residue is next to the PP2C active site and G160D mutation in ABI1 or Gly-Asp mutation in the corresponding position (Sheen, 1998; Robert *et al.*, 2006; Umezawa *et al.*, 2009; Yin *et al.*, 2009). Another important residue is Trp-300 of ABI1 or other corresponding Trp of the related PP2Cs which is the only residue in PP2C approaching ABA molecule in the interaction complex which plays an important role in complex stabilization (Melcher *et al.*, 2009; Dupeux *et al.*, 2011). Several studies show selective interaction among PP2C and PYR/PYL/RCAR receptors (Antoni *et al.*, 2012; Bhaskara *et al.*, 2012; Zhao *et al.*, 2013; Fuchs *et al.*, 2014), but the underlying mechanism remains unclear. Most of the PP2Cs have redundant function and show additive effect on ABA signaling. Single PP2C loss-of-function mutants exhibit mild phenotypic effects, while double or triple mutants show constitutive ABA response (Rubio *et al.*, 2009).

Although physiological function of PP2C function have been described in model

92 plant *Arabidopsis* and rice (Saez *et al.*, 2004; Kuhn *et al.*, 2006; Yoshida *et al.*, 2006;
93 Nishimura *et al.*, 2007; Bhaskara *et al.*, 2012), the function of PP2C on fresh fruit
94 development and ripening is unknown. Recent studies suggested ABA played a role in
95 metabolism in tomato fruit ripening regulation (Galpaz *et al.*, 2008; Zhang *et al.*, 2009;
96 Sun *et al.*, 2012a; Sun *et al.*, 2012b; Sun *et al.*, 2017). However, seldom is known on
97 the function of ABA signaling components during fresh fruit ripening. Overexpression
98 of tomato ABA receptor gene *SIPYL9* leads to early fruit ripening, while
99 *SIPYL9*-RNAi affects the mesocarp thickness and petal abscission (Kai *et al.*, 2019).
100 In addition, the function of *SIPP2C1* was reported in tomato. *SIPP2C1* suppressed the
101 early fruit ripening of plants but led to abnormal flower development, which may
102 reduce the fruit yield (Zhang *et al.*, 2018).

103 To further understand ABA signaling during tomato fruit ripening, we identified
104 the roles of *SIPP2C3* in tomato fruit. It is found that *SIPP2C3* may play a crucial role
105 in fruit maturity and quality by affecting gene expressions of ethylene and cuticle.

106

107 **Materials and methods**

108

109 *Plant materials*

110 Tomato plants (*Solanum lycopersicum* L. cv. Micro Tom), including wild-type
111 plants and transgenic lines, were grown under standard greenhouse conditions. For
112 gene expression analysis, fruits were sampled according to the developmental stages,
113 including mature green (MG), three days after breaker (B+3), ten days after breaker

114 (B+10).

115

116 *Generation of SIPP2C3 transgenic tomato lines*

117

118 For *SIPP2C3*-OE tomato transformation, *SIPP2C3* (Soly06g076400.2.1) CDS
119 was cloned into pRI101-AN plasmid (TAKARA). For *SIPP2C3*-RNAi tomato
120 transformation, a fragment of the N-terminus specific sequence was amplified from
121 the *SIPP2C3* cDNA and was cloned in both sense and anti-sense orientation into
122 pCambia1305.1 plasmid to form a hairpin structure for RNA interference. The
123 primers were shown in Table S2, in which *SIPP2C3*-OE and *SIPP2C3*-RNAi
124 constructs were introduced into tomato (Micro Tom) via *Agrobacterium tumefaciens*
125 LBA4404-mediated transformation. OE lines were identified through Kanamycin
126 resistance and PCR detection. Positive *SIPP2C3*-RNAi transgenic plants were
127 identified through hygromycin B resistance and GUS staining. Lines RNAi4 and
128 RNAi13 were selected for further studies due to the suppression of *SIPP2C3*
129 expression. T0 plants were self-pollinated to generate T1 and T2 plants. Phenotypic
130 and molecular analyses of plants and fruits were performed on T2 plants after selfing
131 of a single homozygote T1 plant, while drought resistance, seed germination and
132 primary root growth assay were performed on T3 seeds obtained from selfing of
133 homozygote T2 plants.

134

135 *Subcellular localization and bimolecular fluorescence complementation (BiFC) assay*

136

137 To investigate the subcellular localizations of SIPP2Cs, the coding ORF
138 sequences of *SIPP2Cs* tagged with GFP at the C-terminus were cloned into the
139 pCambia1300 vector. The specific primers are listed in [Table S3](#). *Agrobacterium*
140 *tumefaciens* GV3101 carrying pCambia1300-SIPP2Cs-GFP was injected into the
141 5-6-week-old *Nicotiana benthamiana* leaves. 2 days after cultured in dark, the
142 infiltrated leaves were examined, and the GFP signal was monitored by a fluorescence
143 microscope (Olympus BX51, Japan) with an optical wavelength of 470 nm at 200x
144 magnification.

145 For bimolecular fluorescence complementation, the cDNA of *SIPP2C3* was
146 pSPYCE(M), and *SIPYLs* and *SlSnRK2s* were cloned into pSPYNE173 ([Waadts et al.,](#)
147 [2008](#)). The primers are listed in [Table S3](#). *A. tumefaciens* GV3101 carrying
148 pSPYCE(M) or pSPYNE173 fusion constructs were co-infiltrated into 5-6-week-old
149 *N. benthamiana* leaves. After 2 days, the infiltrated leaves were examined via
150 fluorescence microscope (Olympus BX51, Japan).

151

152 *Yeast two-hybrid (Y2H) assay*

153

154 The full-length, site-directed mutagenic, and catalytic core (residues 105–411) of
155 *SIPP2C3* were cloned into Y2H ‘prey’ vector pGADT7 (Clontech). Full-length
156 *SIPYLs* and *SlSnRK2s* were cloned into the ‘bait’ vector pGBKT7 (Clontech). Prey
157 and bait vectors were co-transformed into yeast strain Y2HGold (Clontech) and were

grown on SD -Leu/-Trp plates at 30 °C for 3 days. The interactions were examined on the control media SD -Leu/-Trp and the selective media SD -Leu/-Trp/-His/-Ade in the presence or absence of 10 μ M ABA. Yeast containing interest protein in combination with empty pGADT7 or pGBKT7 were used as controls. The primers used for Y2H assay are listed in [Table S4](#).

Site-directed mutagenesis

Mutants were created using the Fast Mutagenesis System (Transgen) according to the manufacturer's instructions. Mutagenesis reactions were conducted with pGADT7-SIPP2C3 as template. All mutagenesis primer sequences are provided in [Table S5](#).

Seed germination and primary root growth assays

Wild-type and transgenic T3 generation seeds obtained from T2 homozygous plants, were harvested, air dried, and stored at 4 °C for three weeks. After surface sterilization, the seeds (about 50 seeds for each replicate) were sown onto 1/2 MS medium containing 0 or 3 μ M ABA. Then the seeds were germinated in the dark at 25°C for 4 or 10 d. Seed germination rates were investigated daily. Radicle emergence >1 mm indicated the success germination. Three replicate plates were used in each treatment. For primary root growth assay, wild-type, *SIPP2C3*-OE, and

180 *SIPP2C3*-RNAi seedlings were grown on a 1/2 MS medium for 3 d, and then the
181 seedlings with roots of the same length were transferred onto another 1/2 MS medium
182 supplemented with 0 or 10 μ M ABA and growth for 5 d. The root length was recorded
183 from the base of the hypocotyl to the root tip. Experiments were performed three
184 times.

185

186 *Drought stress treatment, measure of relative water content and detached leaf water*
187 *loss*

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189 16-day-old WT and two independent lines of OE4 and RNAi13 were under a
190 22-day drought stress treatment by halting irrigation, and then the plants were
191 subsequently re-watered for 5 days. The survival rate of plants was measured.
192 Photographs were taken 0, 11, 15, 19, 22 d after the drought treatment and 5 d after
193 re-water. The relative water contents (RWC) in the leaves of the plants were measured
194 0, 11, 15, 19, 22 days after the treatment. RWC was calculated by [(fresh weight – dry
195 weight) / (turgid weight – dry weight)]. For the assay of water in detached leaves,
196 healthy leaves were detached from each line and placed abaxial side up in an open
197 plate under 25°C, 35% relative humidity condition. The water loss was measured over
198 a period of 8 h. All experiments were repeated three times.

199

200 *qRT-PCR analysis*

201

202 Total RNA was extracted from tomato tissues by the improved hot borate method
 203 ([Wan and Wilkins, 1994](#)). DNase treated RNA was used for cDNA synthesis through
 204 PrimeScript II 1st Strand cDNA Synthesis Kit (Takara) according to the
 205 manufacturer's instructions. qRT-PCR was performed via SYBR Premix Ex Taq
 206 (Takara) and Rotor-Gene 3000 system (Corbett Research). qRT-PCR was conducted
 207 in three biological replicates, and each sample was analyzed at least thrice and was
 208 normalized using the *SAND* gene (Soly03g115810) as an internal control. The
 209 relative fold expression for each gene was calculated by Rotor-Gene Q software using
 210 the delta Ct method. Primers used for the qRT-PCR analysis are provided in [Table S6](#).

211

212 *Measurement of ABA and ethylene*

213

214 The extraction and measurement of ABA was performed according to the
 215 description previously ([Sun et al., 2017](#)). Briefly, three grams of tissues were ground
 216 and extracted by 80% (v/v) methanol at -20°C for 18 h. After centrifugation, pellet was
 217 extracted by 80% methanol twice. The supernatant was dried under vacuum, and the
 218 residue was dissolved in a 0.02 M phosphate buffer (pH 8.0). Petroleum ether was
 219 used to remove pigments and the insoluble PVPP was used to remove polyphenols.
 220 ABA was extracted three times by ethyl acetate. The ethyl acetate phase was dried
 221 under vacuum and dissolved in 50% methanol (v/v). The ABA content was
 222 determined via HPLC (Agilent LC 1200, CA, USA) through a 4.8 x 150 mm C18
 223 column (Agilent Technologies, CA, USA), and (±)-abscisic acid (Sigma) was used as

standards. Three replicates were conducted for each sample.

Ethylene production was measured by enclosing two to four fruits in an 50-mL airtight containers for 2 h at 25°C, then 1 ml of the headspace gas was withdrew and injected into a gas chromatograph (Agilent GC 6890N, CA, USA) fitted with a flame ionization detector and an activated alumina column (Zhang *et al.*, 2009).

RNA-seq

Total RNA was extracted from the fruit skin of WT and *SLPP2C3*-RNAi4 at mature green stage. RNA degradation and contamination were monitored on 1% agarose gels. RNA integrity was assessed through the RNA 6000 Pico Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). A total amount of 3µg RNA per sample was used for mRNA purification and library construction via the Truseq™ RNA Sample Prep Kit (Illumina, CA, USA) followed the manufacturer's instructions. The samples were sequenced on an Illumina HiSeq™ 2000 (Illumina). Each sample yielded more than 6 G of data.

Raw data of fastq format was firstly processed through in-house perl scripts. Clean data was obtained by removing reads containing adapter, reads containing ploy-N and low-quality reads from raw data. Filtered RNA-seq clean reads were aligned to the reference tomato genome releasing SL2.50 through TopHat v2.0.13 (Kim *et al.*, 2013). HTSeq v0.5.3 was used to count the reads numbers mapped to each gene. And then the RPKM of each gene was calculated based on the length of

the gene and reads count mapped to this gene. Differential expression analysis was performed using the DEGSeq R package (1.12.0) and Cufflinks (Trapnell *et al.*, 2012). Corrected P-value of 0.001 and log2 (Fold change) of 1 were set as the threshold for significant differential expression.

250

251 *Statistical analysis*

252

The data were statistically analyzed with SPSS software using one-way analysis of variance and Duncan's test of significance. *t-test P value < 0.05; **t-test P value < 0.01.

256

257 **Results**

258

259 *Phylogenetic analyses of tomato Group A PP2C family*

260

To explore the entire family members of group A PP2C in tomato, predicted *Arabidopsis* PP2C protein sequence (Schweighofer *et al.*, 2004) was used as query to BLAST against the SGN database (<https://solgenomics.net/>). Total 93 putative protein phosphatase 2C family members were identified in tomato genome. Phylogenetic analyses indicated that tomato PP2Cs were divided into 12 groups (A-K) (Fig. S1), which was consistent with previous study in *Arabidopsis* and rice (Schweighofer *et al.*, 2004; Xue *et al.*, 2008). Fourteen tomato PP2Cs were identified as members of group

268 A, which have been shown to participate in ABA signaling. We named these 14
269 tomato members as SIPP2C1-14 (Table S1). Further amino acid alignment indicated
270 the conserved catalyze domain of 14 PP2Cs with *Arabidopsis* homologues and
271 diverse N-terminal (Fig. S2). All tomato PP2Cs have conserved active-site residues
272 and PYL-interaction residues, except SIPP2C7 in which some motifs in catalyzed
273 domain is missing. Interestingly, SIPP2C11-14 lack the conserved tryptophan residue
274 which acts as a “lock” in the interaction with ABA receptor, similar to *Arabidopsis*
275 AtAHG1 (Antoni *et al.*, 2012). It is worth noting that SIPP2C3 is the single ortholog
276 of *Arabidopsis* AtHAI1/2/3 (Fig. S3). In *Arabidopsis*, three AtHAIs have redundant
277 function during drought resistance, leaf senescence and seed dormancy (Bhaskara *et*
278 *al.*, 2012; Zhang *et al.*, 2012; Kim *et al.*, 2013) which implied the non-redundant
279 function of SIPP2C3 in tomato physiological processes.

280

281 *SIPP2C3 selectively interact with monomer ABA receptors and SlSnRK2.8*

282

283 Our previous study reported the interactions of SIPP2C1-5 and SIPYLs or
284 SlSnRK2s which showed that SIPP2C3 is the only member interacted with SlSnRK2
285 in yeast two hybrid assay (Chen *et al.*, 2016). In this work, we further study the
286 interaction manner of SIPP2C3 and ABA receptors. Full length of SIPP2C3 interacted
287 with subfamily I and subfamily II monomeric receptors in yeast cell. The interactions
288 between SIPP2C3 and SIPYL1, 4, 7, 9 or 11 was ABA dependent while the
289 interactions with SIPYL2, 3, 10 or 13 were observed as ABA independent. In addition,

290 SIPP2C3 did not interact with subfamily III dimeric receptor members SIPYL5 and 8
291 even in the presence of 10 μ M ABA (Fig. 1A). For SLSnRK2s, SIPP2C3 can only
292 interact with SLSnRK2.8 (Fig. 1B), consistent with our previous results (Chen *et al.*,
293 2016).

294 Catalyzed domain of SIPP2C3 (105-411 amino acid) acted similar as the full
295 length. However, the Gly-160Asp (corresponding to AtABI1^{G180D} encoded by *abil-1*)
296 mutation abolished the interaction between SIPP2C3 and all SIPYLs (Fig. 1A).
297 Further, bimolecular fluorescence complementation (BiFC) assay was performed to
298 confirm the SIPP2C3-SIPYLs/SLSnRK2.8 interaction *in-planta*. Fluorescent signal
299 was detected in tobacco leaf which co-expressed in SIPP2C3 and SIPYL1, 3, 9, 10, 13
300 or SLSnRK2.8 (Fig. 2A). The interaction between SIPP2C3 and SIPYL1/9 may due to
301 the sufficient endogenous ABA level in leaf. Most interactions occur in nucleus and
302 cytoplasm, except for SIPP2C3 and SIPYL3, which only occur in nucleus. The
303 interaction localization was further validated by subcellular location of SIPP2C3 (Fig.
304 2B). Both *in-vitro* and *in-planta* assay indicate that SIPP2C3 cannot interact with
305 dimer receptors belonging to subfamily III (Fig. 1A; Fig. 2A).

306 Our previous study showed that subfamily III receptors SIPYL5 and SIPYL8
307 interacted with SIPP2C5, ortholog of *Arabidopsis* HAB1. To understand molecular
308 mechanism of these selective receptor interaction, we compared the amino acid
309 sequences of SIPP2C3 and SIPP2C5, and found some diverse amino acid residues in
310 PP2C-PYL interaction region between SIPP2C3 and SIPP2C5 (Fig. 3A) (Melcher *et*
311 *al.*, 2009; Miyazono *et al.*, 2009). We conducted the site-mutagenesis on SIPP2C3 to

switch these residues to the SIPP2C5 version, reasoning that the mutant of these residues could mimic SIPP2C5 (Fig. 3A). Our results revealed that S295F mutation in SIPP2C3 increased the interactions of SIPP2C3-SIPYL5/8, while R285K mutation increased SIPP2C3-SIPYL8 interaction in the presence of ABA (Fig 3B). These results partially explained the preferred interaction of SIPP2Cs and SIPYLs.

SIPP2C3 expression is negatively correlated with fruit ripening and induced by ABA

SIPP2C3 was highly transcribed in seed, however, the expression in seedling, root and stem were relatively low. During flowering and fruit development, *SIPP2C3* mRNA level was high in flower and immature fruit and it decreased during ripening, which indicated that *SIPP2C3* might play a negative role in fruit ripening (Fig. 4A).

To examine whether *SIPP2C3* transcription is regulated by ABA, we performed qRT-PCR to investigate the expressions of *SIPP2C3* during seed imbibition and leaf dehydration. These two physiological processes have been shown to accompany by the decreasing or increasing of endogenous ABA level, respectively. The expression of *SIPP2C3* decreased during seed imbibition, on the other hand, *SIPP2C3* was induced in dehydrated leaf (Fig. 4B-C). Furthermore, *SIPP2C3* were induced by exogenous ABA treatment in seed and mature green fruit (Fig. 4D-E). These results indicated that the expressions of *SIPP2C3* is positively regulated by ABA.

Altered expression of SIPP2C3 resulted in the change of ABA sensitivity

334

335   Considering the expression and interaction pattern, we are interested in the
336 physiological function of *SIPP2C3* in *planta*. To gain insight into the role of *SIPP2C3*,
337 the expressions of *SIPP2C3* through RNA interference were suppressed or
338 overexpressed in Micro-Tom tomato, respectively. Independent *SIPP2C3*-OE (OE4
339 and OE33) and *SIPP2C3*-RNAi (RNAi4 and RNAi13) transgenic lines with
340 dramatically increased or decreased *SIPP2C3* expression were used for further
341 investigation (Fig. S4).

342   *SIPP2C3*-OE and *SIPP2C3*-RNAi plants were similar to WT in plant architecture
343 (Fig. S5A-D). The leaves of transgenic lines exhibited similar shape, color and size to
344 WT (Fig. S5F-G). These data suggested that *SIPP2C3* played a limited role in plant
345 vegetative growth.

346   *SIPP2C3* is a putative core component in ABA signaling, therefore, we tested
347 whether ABA sensitivity can be altered by *SIPP2C3* manipulation. The
348 ABA-mediated inhibition of seed germination was compared among the transgenic
349 lines and WT. It is found that the seed germination of *SIPP2C3*-RNAi lines was
350 delayed which is more sensitive to exogenous ABA, while *SIPP2C3*-OE lines
351 exhibited oppositely (Fig. 5A-B). Furthermore, the primary root growth of
352 *SIPP2C3*-OE seedling was less sensitive to exogenous ABA treatment compared to
353 WT. However, *SIPP2C3*-RNAi seedling was more sensitive to exogenous ABA
354 treatment (Fig. 5C-D). These results demonstrated that *SIPP2C3* might act as a
355 negative regulator in ABA signaling.

356

357 *SIPP2C3 plays a negative role in drought tolerance*

358

359 To understand whether *SIPP2C3* plays a role in ABA-mediated drought tolerance,
 360 16-days-old transgenic lines and WT plants were exposed to a 22-day drought stress
 361 treatment. 15 d after the treatment, *SIPP2C3*-OE line showed more wilted leaves than
 362 WT, while *SIPP2C3*-RNAi exhibited significant improvement under drought
 363 tolerance compared to the WT (Fig. 6A). 5 days after re-watering, *SIPP2C3*-OE plants
 364 showed 6% survival rate compared to 30% for the WT, while the survival rate of
 365 *SIPP2C3*-RNAi plants was nearly 60% (Fig. 6B). Consistent with the above result,
 366 the relative water content in *SIPP2C3*-OE leaves was significantly lower than that of
 367 WT, while the relative water content in *SIPP2C3*-RNAi leaves were the highest (Fig.
 368 6C). Leaves from *SIPP2C3*-RNAi exhibited lower water loss rate than WT which
 369 implied the lower transpiration rate of *SIPP2C3*-RNAi leaves (Fig. 6D). These assays
 370 suggested that *SIPP2C3* played a negative role in plant drought tolerance.

371

372 *SIPP2C3 affects fruit ripening schedule*

373

374 Given the role of ABA in fruit ripening regulation and the negative correlated
 375 expression pattern of *SIPP2C3* with fruit ripening, we investigated whether *SIPP2C3*
 376 played a role in fruit ripening. It was observed that the *SIPP2C3*-OE fruits required
 377 two more days from anthesis to the breaker stage than WT, while the *SIPP2C3*-RNAi

fruits required 2-3 fewer days (Fig. 7A-B). The breaker time of WT is from 37 to 42 days, while the breaker times of *SIPP2C3*-OE and *SIPP2C3*-RNAi fruits are 39-44 days and 34-40 days, respectively. The ethylene and ABA production peaks of *SIPP2C3*-OE / *SIPP2C3*-RNAi fruits were delayed / advanced (Fig. 7C-D). Moreover, the transcription of some 1-aminocyclopropane-1-carboxylic acid oxidase genes which involved in ethylene biosynthesis and several ethylene receptor genes were up-regulated in *SIPP2C3*-RNAi fruits (Fig. S6; Fig. 10B).

To determine whether fruit quality was altered by *SIPP2C3*-OE or *SIPP2C3*-RNAi, several typical fruit quality parameters were measured. The fruit shape index (the ratio of fruit vertical diameter to horizontal diameter) of *SIPP2C3*-OE (especially OE33) was higher than WT (Fig. 8C). The fruit weight and seed numbers of *SIPP2C3*-OE were lower than WT, while *SIPP2C3*-RNAi showed no significant difference (Fig. 8D, F). Fruit firmness, contents of soluble solids, total sugar content and the titratable acid in the transgenic fruits were similar to WT (Fig. 8E, H; Fig. S7D-E). The number of OE33 fruits in 2 locule of OE33 was significantly higher than that of WT, while the number of OE33 fruits in 3 locule was less (Fig. 8G). The total carotenoids and lycopene of *SIPP2C3*-OE fruits were higher than WT, however, the *SIPP2C3*-RNAi fruit showed similar level to WT (Fig. S7A-C). These results suggest that *SIPP2C3* play a role in fruit developed and metabolic processes.

SIPP2C3-RNAi led to a bad fruit gloss due to altered outer epidermis

400 It is noticed that *SIPP2C3*-RNAi fruits exhibit dull fruit surface compared to the
401 WT (Fig. 9A). Further scanning electron microscope study revealed a verrucous outer
402 epidermis surface of *SIPP2C3*-RNAi fruit, which may imply an alter in cuticle/wax
403 metabolism (Fig. 9B). To understand the underlying mechanism, a global gene
404 expression analysis of *SIPP2C3*-RNAi fruit skin was performed (Dataset S1). 374
405 up-regulated and 519 down-regulated genes of *SIPP2C3*-RNAi fruit skin were
406 screened and compared to the WT (Dataset S2). Among them, two transcription
407 factors and 24 structural genes related to cuticle formation were identified (Table 1),
408 and the expressions of some genes were further verified by qRT-PCR (Fig. 9D).
409 R2R3-type MYB transcription factor *SIMYB96* (Soly03g116100) were
410 down-regulated in *SIPP2C3*-RNAi fruits, while *SIMYB106* (Soly02g088190) were
411 up-regulated. The orthologs in *Arabidopsis* were shown to regulate gene expressions
412 in cutin and wax biosynthesis (Seo *et al.*, 2011; Oshima *et al.*, 2013). Out of 18
413 up-regulated genes, 11 were involved in cutin biosynthesis, including one *LACS* gene
414 *SILACS2* which was putatively required for the acyl activation of fatty acid (Schnurr
415 *et al.*, 2004), four *CYP450s* genes (*SICYP77A1*, *SICYP77A2*, *SICYP86A68* and
416 *SICYP86A69*) which were involved in midchain and terminal hydroxylation of fatty
417 acid (Li-Beisson *et al.*, 2009; Shi *et al.*, 2013), four *SIHTH* genes in which the
418 ortholog in *Arabidopsis* was proved to be important in the formation of dicarboxylic
419 fatty acids (Kurdyukov *et al.*, 2006) and two Glycerol-3-phosphate acyltransferase
420 genes *SIGAPT4* and *SIGAPT6* were mediated in the last step of cutin monomers
421 biosynthesis (Li *et al.*, 2007), a cuticle transporter gene *SIABCG32*, and a cutin

422 synthase gene *SICD1* which was required for cutin polymerization (Bessire *et al.*,
423 2011; Yeats *et al.*, 2014; Fabre *et al.*, 2016). On the other hand, 7 out of 9
424 down-regulated genes were involved in wax biosynthesis. These data indicated that
425 low *SIPP2C3* expression may change the cuticle composition of fruit outer epidermis.

426 In order to assess the relationship between ABA signaling and cuticle formation,
427 we examined the expression of ABA signaling core component genes in fruit skin of
428 both *SIPP2C3*-RNAi lines and WT (Fig. 9C; Fig. 10C). Expressions of ABA receptor
429 genes *SIPYL3* and *SIPYL8* were up regulated in transgenic lines. *SIPP2C1*, the only
430 group A PP2C member, showed mild downregulated except for *SIPP2C3*. Among
431 eight *SnRK2* members, expressions of *SlSnRK2.4*, *SlSnRK2.6* and *SlSnRK2.8* were
432 significantly up regulated in transgenic lines. In addition, *SIPYL3* and *SIPP2C3*
433 expressions were enriched in epidermis, and were co-expressed with *SIMYB96* and
434 *SIMYB106* (Fig. S7). These data imply that the altered ABA signaling transduction in
435 *SIPP2C3*-RNAi lines may be responsible for the changes of cuticle metabolism genes.

436

437 Discussion

438

439 Preferential interaction of *SIPP2C3* with monomeric receptors

440

441 ABA initiates signal transduction by forming ABA-PYL-PP2C ternary complex.
442 Both PYLs and PP2Cs are multigene families with redundant function. According to
443 oligomeric state, PYL receptors were divided into dimeric receptor and monomeric

receptor (Hao *et al.*, 2011). Group A PP2Cs were clustered into two subfamilies (Fig. S3) which determined the complexity of PYL-PP2C interaction. In our data, SiPP2C3 showed no interaction with dimeric receptors SIPYL5 and SIPYL8 in both yeast and plant cell. Similar result was obtained in our previous work as well (Chen *et al.*, 2016). Phylogenetic analyses indicated that SiPP2C3 is the ortholog of *Arabidopsis* AtHAI1/2/3. In *Arabidopsis*, HAI PP2Cs have marked preference for interactions with monomeric PYLs and showed no interaction with dimeric receptors AtPYR1, AtPYL1 and AtPYL2 (Bhaskara *et al.*, 2012). However, Antoni *et al.* showed that AtHAI1 activity could be inhibited by dimeric receptors *in vitro* (Antoni *et al.*, 2012). The molecular mechanism of the selective interaction between HAI-like PP2Cs and dimeric receptors is unknown. In our data, by generating a series mutant on PP2C-PYL interaction key residues SiPP2C3, we identified Arg-285 and Ser-295 might play an important role in preventing SiPP2C3 from interaction with dimeric receptors (Fig. 3). This result partially explain the mechanism of PP2C preference. However, more evidence from other members of subfamily II PP2Cs are required.

459

SiPP2C3 negatively regulates ABA signaling and affects fruit ripening

461

In our study, SiPP2C3 acts as a negative regulator in tomato ABA signaling which was supported by two pieces of evidence: (1) SiPP2C3 interact with multiple SIPYLs and SiSnRK2.8 (Fig. 1; Fig. 2A) and (2) *SiPP2C3*-RNAi plants were hypersensitive to ABA while in *SiPP2C3*-OE plants, ABA were less sensitive in

several ABA mediated processes (Fig. 5; Fig. 6). *SIPP2C3* acts negatively in ABA signaling which is similar to other group A PP2Cs reported in several plant species. (Gosti *et al.*, 1999; Merlot *et al.*, 2001; Saez *et al.*, 2006; Rubio *et al.*, 2009; Li *et al.*, 2015; Zhang *et al.*, 2018).

It is worthy to notice that the fruit ripening schedule was affected by *SIPP2C3* expression (Fig. 7A, B). Previous studies demonstrated the role of endogenous ABA level and exogenous ABA treatment on tomato fruit ripening (Zhang *et al.*, 2009; Sun *et al.*, 2012b; Sun *et al.*, 2017; Liang *et al.*, 2020). More recent studies indicate that ABA signaling also plays a crucial role in regulating fruit ripening process. For example, the overexpression of tomato ABA receptor *SIPYL9* or suppression of *SIPP2C1* causes early fruit ripening (Zhang *et al.*, 2018; Kai *et al.*, 2019). In addition, ABA signaling inhibitor AA1 inhibits tomato fruit ripening (Ye *et al.*, 2017). Our data indicates that tomato fruit ethylene production is affected by *SIPP2C3* (Fig. 7C) and several studies show the cross talk between PP2C and ethylene production. For instance, in *Arabidopsis* AtABI1 interacts with AtACS2 or AtACS6 and inhibits their activity by dephosphorylation (Ludwikow *et al.*, 2014). ABA inhibits ethylene production through ABI4-mediated transcriptional repression of AtACS4 and AtACS8 (Dong *et al.*, 2015). In our transcription data, several genes are involved in ethylene biosynthesis (*SLACOs*) and signaling (*SIETRs*) was up-regulated in *SIPP2C3*-RNAi fruit (Fig. S6). Taken together, we propose that *SIPP2C3* negatively affects fruit ripening via the suppression of ethylene production.

488 *SlPP2C3* affects fruit outer epidermis cuticle formation

489

490 Fruit outer epidermis is the first barrier protecting fruit from desiccation and
 491 pathogen invasion, and it also plays a crucial role in fruit shelf life. ABA plays a role
 492 in cuticle formation and cuticle associated gene expressions under water deficit
 493 condition in *Arabidopsis* and tomato (Kosma *et al.*, 2009; Curvers *et al.*, 2010; Wang
 494 *et al.*, 2011). Recent study indicated that ABA core signal affected cuticle
 495 development in *Arabidopsis* not through transcription factors ABF2/3/4, which are the
 496 direct targets of SnRK2. This result implies the existence of a divergent pathway in
 497 the down-stream of SnRK2 kinase (Cui *et al.*, 2016). Martin et al. reported that
 498 tomato ABA deficient mutants exhibited reduced cutin and wax composition and
 499 down-regulated cuticle biosynthesis genes in leaf. However, ABA deficiency
 500 distinctly effected tomato fruit cuticle compared to leaf (Martin *et al.*, 2017). In our
 501 study, *SlPP2C3*-RNAi fruits exhibit altered epidermis surface (Fig. 9A), and further
 502 transcriptome data identified 2 MYB (*SIMYB96* and *SIMYB106*) transcription factor
 503 and 25 cuticle metabolism genes which were differentially expressed in
 504 *SlPP2C3*-RNAi fruit skin (Table 1). These MYB transcription factor may be the
 505 potential down-steam targets in ABA signaling pathway. Considering the similar
 506 expression patterns of *MYB96*, *MYB106*, *SIPYL3* and *SlPP2C3* as well as the unique
 507 localization of SIPYL3-SIPP2C3 complex (Fig. 2A; Fig. S8), we speculated that
 508 SIPYL3-SIPP2C3 mediated up-stream ABA signaling may be important in cuticle
 509 formation regulation. However, the precise mechanism remains open.

510

511 **Supplementary data**

512 Fig. S1. Phylogenetic analysis of tomato and *Arabidopsis* type 2C protein phosphatase
513 (PP2C) family members.

514 Fig. S2. Alignment of the amino acid sequences of tomato and *Arabidopsis* group A
515 PP2C members.

516 Fig. S3. Phylogenetic analysis of tomato and *Arabidopsis* group A PP2C family
517 members.

518 Fig. S4. Expressions of *SlPP2C3* in *SlPP2C3* transgenic lines.

519 Fig. S5. Vegetative phenotypes of *SlPP2C3* transgenic lines.

520 Fig. S6. Expression levels of genes related to ethylene synthesis and signaling in
521 *SlPP2C3*-RNAi and WT fruits.

522 Fig. S7. Carotenoids, sugar and titratable acid in WT and *SlPP2C3* transgenic fruits.

523 Fig. S8. Spatiotemporal expressions of *SlPP2C3*, *SlPYL3*, *SlMYB96* and *SlMYB106*
524 during M82 tomato fruit development and ripening.

525 Table S1. Basic information of tomato group A PP2Cs genes.

526 Table S2. Primers used for *SlPP2C3*-OE and *SlPP2C3*-RNAi plasmid construction.

527 Table S3. Primers used for bimolecular fluorescence complementation and subcellular
528 localization construct.

529 Table S4. Primers used for yeast two-hybrid assay.

530 Table S5. Primers used for site-directed mutagenesis.

531 Table S6. Primers used for qRT-PCR analysis.

532 Dataset 1. Total genes in the WT and *SIPP2C3*-RNAi fruit skin at mature green stage
533 detected by RNA-Seq.

534 Dataset 2. Genes expressed differentially in *SIPP2C3*-RNAi fruit skin compared to
535 WT by RNA-Seq.

536

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541

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815

816 **Figure legends**

817

818 **Fig. 1.** Interactions of SIPP2C3 with SIPYLs or SlSnRK2s in yeast two hybrid (Y2H)
819 assay.

820 Interactions were determined using a growth assay on media lacking Leu, Trp, His
821 and Ade (-LWHA), with or without 10 μ M ABA. The yeasts growth on media lacking
822 Leu and Trp (-LW) were used as control. (A) Interaction of SIPP2C3-AD and
823 SIPYLs-BD receptors. First lane, full length SIPP2C3; second lane, SIPP2C3 with

824 Gly-160 mutated into Asp (SIPP2C3^{G160D}); third lane, the catalytic core (residues
825 105–411) of SIPP2C3 (Δ NSIPP2C3); last lane, pGADT7 empty vector control. (B)
826 Interaction of SIPP2C3-AD and SLSnRK2s-BD.

827

828 **Fig. 2.** Interaction of SIPP2C3 with SIPYLs and SLSnRK2s in tobacco leave.

829 (A) SIPP2C3-YFPC and SIPYLs-YFPN or SLSnRK2s-YFPN were co-expressed in *N.*
830 *benthamiana* leaves through *A. tumefaciens* infiltration. The YFP signal was
831 monitored 2 days after co-infiltration. (B) Subcellular locations of SIPP2C3.
832 SIPP2Cs-GFP was transiently expressed in *N. benthamiana* leaves through *A.*
833 *tumefaciens* infiltration. The GFP signal was monitored 2 days after infiltration. The
834 left column shows fluorescent signal; the middle column shows the bright-field image;
835 and the right column shows the merged image.

836

837 **Fig. 3.** *SIPP2C3* mutations interact with dimeric receptors SIPYL5 and SIPYL8 in
838 Y2H assay.

839 (A) Alignment of SIPP2C3 and SIPP2C5 amino acid sequences. Location of the
840 SIPP2C3 mutations are marked by black asterisks. Black triangle indicated the
841 residues involved in PYLs interaction (Miyazono *et al.*, 2009). (B) Interaction of
842 SIPP2C3, SIPP2C5 or SIPP2C3 mutants (fused to the activating domain) and either
843 SIPYL5 or SIPYL8 (fused to the binding domain). Interaction was determined by
844 growth assay on medium lacking Leu, Trp, His and Ade (-LWHA), with or without 10
845 μ M ABA. The photographs were taken after 5 d growth at 30°C.

846

847 **Fig. 4.** Expression pattern of *SlPP2Cs* genes in tomato.

848 (A) Absolute quantification of *SlPP2C3* in various tomato tissues. S, seed; Se,
849 seedling; R, root; St, stem; L, Leaf; Fl, flower; fruit pericarp at IM (immature, 15
850 DAF), MG (mature Green, 25 DAF), B (break), B5 (5 days after break) and B10 (10
851 days after break). Error bars represent $\pm$ SD of biological triplicates. (B) Absolute
852 quantification of *SlPP2C3* in tomato seeds which were soaked and imbibed in water
853 for indicated time. (C) Relative expression of *SlPP2C3* in tomato detached leaves. (D)
854 Relative expression of *SlPP2C3* in tomato seeds which were treated with exogenous
855 ABA or NaCl for 48 h. (E) Relative expression of *SlPP2C3* in mature green tomato
856 pericarp disks treated by 100 μ M exogenous ABA. For all expression analysis,
857 *SISAND* gene was used as an internal control. The error bars indicate $\pm$ SD of
858 biological triplicates. *P value t-test < 0.05; **P value t-test < 0.01.

859

860 **Fig. 5.** Altered sensitivity to ABA-mediated inhibition of seed germination, primary
861 root growth in *SlPP2C3* transgenic lines compared to WT.

862 (A, B) Approximately 50 seeds of *SlPP2C3*-OE, *SlPP2C3*-RNAi lines and WT (three
863 independent experiments) were sown on 1/2 MS medium with or without 3 μ M ABA.
864 Seeds germination were scored every day. Values are means $\pm$ SE. *t-test P value <
865 0.05; **t-test P value < 0.01. (C, D) Primary root length and photographs of tomato
866 seedlings 5 days after transferring 3-day-old seedlings into 1/2 MS medium with or
867 without 10 μ M ABA. OE: OE4 seeds mixed with OE33 seeds. RNAi: RNAi4 seeds

868 mixed with RNAi13 seeds. OE-0/OE-10: OE seedlings 5 days after transferring
869 3-day-old seedlings into 1/2 MS medium with or without 10 μ M ABA. Percentages
870 represent the ratio of root length of seedlings treated with 10 μ M ABA to that treated
871 without ABA. Values are means $\pm$ SD. *t-test P value < 0.05; **t-test P value < 0.01.

872

873 **Fig. 6.** Drought resistance of *SLPP2C3* transgenic lines.

874 (A) 16-days-old *SLPP2C3* transgenic lines and WT plants were subjected to drought
875 stress withholding water for 22 days and then re-watered for 5 days. The experiments
876 were repeated three times. (B) Survival rates were scored 5 days after re-watering. (C)
877 Relative water contents in *SLPP2C3* transgenic lines and WT leaves after drought
878 stress. Values are means $\pm$ SE. *t-test P value < 0.05; **t-test P value < 0.01. (D)
879 Water loss analysis for the detached leaves. Values are means $\pm$ SD for three
880 independent experiments. *t-test P value < 0.05; **t-test P value < 0.01.

881

882 **Fig. 7.** The phenotype of *SLPP2C3* transgenic and WT fruits.

883 (A) The ripening time of fruits from *SLPP2C3* transgenic lines compared to WT fruits.
884 (B) Days from anthesis to break. (C-E) Changes of ethylene release and ABA content
885 in WT and *SLPP2C3* transgenic fruits during fruit ripening. n > 15, values are means $\pm$
886 SD. *t-test P value < 0.05; **t-test P value < 0.01.

887

888 **Fig. 8.** Physiological parameters related to fruit in *SLPP2C3* transgenic and WT fruits.

889 (A) Horizontal diameters of B10 fruits. (B) Vertical diameters of B10 fruits. (C) Fruit

890 shape index of B10 fruits. (D) Single fruit weight of B10 fruits. (E) Soluble solid
891 content (Brix) of B10 fruits. (F) Seed number of each fruit. (G) Locule number rate of
892 fruit. (H) Fruit firmness during fruit ripening. Values are means $\pm$ SD. Letters
893 represent significant differences, t-test P value < 0.05.

894

895 **Fig. 9.** Reduced fruit brightness and altered gene expressions in cuticle metabolism
896 and ABA signaling of *SIPP2C3*-RNAi fruits skin.

897 (A) Photographs of *SIPP2C3*-RNAi lines and WT tomato fruit at MG and B+10
898 stages showing surface brightness. (B) Outer epidermis surface of *SIPP2C3*-RNAi
899 lines and WT tomato fruits at B stage by scanning electron microscope. (C)
900 Expressions of cuticle metabolism genes in transgenic and WT fruit skin at the MG
901 stage. (D) Expressions of ABA signaling genes (*SIPYLs*, *SIPP2Cs* and *SlSnRK2s*) in
902 transgenic and WT fruit skin at MG stage. *SISAND* gene was used as internal
903 reference. The data indicates the mean $\pm$ SD of three biological replicates. *t-test P
904 value < 0.05; **t-test P value < 0.01.

905

906 **Fig. 10.** *SIPP2C3* affects the transcription of genes involved in ethylene and ABA
907 pathway.

908 (A) Differential expressed genes between *SIPP2C3*-RNAi4 and WT fruits at mature
909 green stage shown by volcano plot. (B, C) Log2 fold change values of genes involved
910 in ethylene and ABA pathway in *SIPP2C3*-RNAi4 fruits compared to the WT. The
911 statistically significant changes in color: red indicates up-regulation, green indicates

912 down-regulation. Fold change $> |1|$, adjusted p value < 0.001 , N.D., not detectable.

913

914

A

R127P D128E
**
SIPP2C3 115 PKFGFASVCGRRRDMEDAVAIHPSFI140-151HYFAVDYDGHGCSHVATKCKERLHELVEEL
SIPP2C5 152 PLWGSVSICGRPEMEDALVVVPNF177-202HFYGVYDGHGCSQVADYCRKRVLHLAVEEL
▲▲▲▲▲▲▲▲

D227E A228T
**
SIPP2C3 181-219CELHTPECDAVGSTAVVAVV 239-283GGRVIYWDPGRVSGVLAMSRAIGDNYLKPVI
SIPP2C5 233-284CTSEPIAPETVGSTAVVAVI 304-348GGKVIQNGHRVFGLAMSRSIGDRYLKPWI
▲▲▲▲▲▲▲▲▲▲▲▲▲▲▲▲▲▲

R285K Y288Q D290N S295F
* * * *
SIPP2C3 239-283GGRVIYWDPGRVSGVLAMSRAIGDNYLKPVI
SIPP2C5 304-348GGKVIQNGHRVFGLAMSRSIGDRYLKPWI
▲▲▲▲▲▲▲▲▲▲▲▲▲▲▲▲▲▲

BD-SIPYL5

BD-SIPYL8

-LWHA

-LWHA

-LW

- + ABA

-LW

—

AD-SIPP2C3

AD-S|PP2C5

AD-R285K

AD-Y288Q

AD-D290N

AD-S295F

AD-R127P D128E

AD-D227E A228T

AD-R285K D290N

AD-D290N S295F

AD-R127P D128F R285K

AD-D227E A228T R285K

AD-R127P D128E S295F

AD-D227E A228T S295F

RNAi4 vs WT

B

Ethylene biosynthesis				Ethylene signaling			
SIACS1A	-0.78	SIACO1	1.60	SIETR1	0.33	SICTR1	0.34
SIACS1B	-1.15	SIACO2	-0.50	SIETR2	-0.76	SICTR3	0.18
SIACS2	2.16	SIACO3	3.42	SIETR3	2.20	SICTR4	-0.17
SIACS3	-0.70	SIACO4	2.97	SIETR4	2.91	SIEIN2	0.03
SIACS4	0.51	SIACO5	4.64	SIETR5	1.17	SIEIL1	0.14
SIACS5	N.D.	SIACO6	0.95	SIETR6	4.10	SIEIL2	-0.38
SIACS6	-0.14			SIETR7	0.54	SIEIL3	0.41
SIACS7	N.D.					SIEIL4	-0.19
SIACS8	N.D.					SIEBF1	0.67
SIACS10	-0.63					SIEBF2	2.59
SIACS12	-0.20						

C

ABA biosynthesis			ABA signaling				
<i>SIZEP</i>	-1.71	<i>SIPYL1</i>	-0.01	<i>SIPP2C1</i>	-1.65	<i>SISnRK2.1</i>	-3.87
<i>SINXD1</i>	-0.40	<i>SIPYL2</i>	0.22	<i>SIPP2C2</i>	-0.89	<i>SISnRK2.2</i>	-0.37
<i>SINCED1</i>	0.31	<i>SIPYL3</i>	1.04	<i>SIPP2C3</i>	-1.48	<i>SISnRK2.3</i>	-0.27
<i>SINCED2</i>	0.10	<i>SIPYL4</i>	1.83	<i>SIPP2C4</i>	-0.28	<i>SISnRK2.4</i>	0.10
<i>SINCED3</i>	N.D.	<i>SIPYL5</i>	-0.83	<i>SIPP2C5</i>	-0.05	<i>SISnRK2.5</i>	N.D.
<i>SITIENS</i>	0.38	<i>SIPYL6</i>	-0.26	<i>SIPP2C6</i>	0.29	<i>SISnRK2.6</i>	1.08
<i>FLACCA</i>	0.09	<i>SIPYL7</i>	0.41	<i>SIPP2C7</i>	0.26	<i>SISnRK2.7</i>	0.01
<i>SIAAO1</i>	-0.13	<i>SIPYL8</i>	-0.04	<i>SIPP2C8</i>	0.03	<i>SISnRK2.8</i>	0.13
<i>SIAAO2</i>	0.40	<i>SIPYL9</i>	-2.78	<i>SIPP2C9</i>	-0.05		
<i>SIAAO3</i>	1.97	<i>SIPYL10</i>	0.65	<i>SIPP2C10</i>	N.D.		
		<i>SIPYL11</i>	-0.28	<i>SIPP2C11</i>	N.D.		
		<i>SIPYL12</i>	N.D.	<i>SIPP2C12</i>	N.D.		
		<i>SIPYL13</i>	N.D.	<i>SIPP2C13</i>	N.D.		
		<i>SIPYL14</i>	N.D.	<i>SIPP2C14</i>	-0.48		

Table 1. Differential expressed genes related to cuticle metabolism in transgenic fruit peel compared with WT.

Gene ID	Gene Name	Annotation	Pathway	Log2 Fold Change	Adjusted P value
Up-regulated					
Solyc02g088190	MIXTA	MYB transcription factor	Regulation	1.70	7.30E-08
Solyc12g009490	SHN2	Ethylene responsive transcription factor	Regulation	1.88	2.32E-05
Solyc01g109180	LACS2	Long-chain-fatty-acid-CoA ligase	Cutin synthesis	2.51	7.18E-101
Solyc01g094750	CYP86A68	Cytochrome P450	Cutin synthesis	2.99	9.47E-05
Solyc08g081220	CYP86A69	Cytochrome P450	Cutin synthesis	2.46	8.12E-138
Solyc05g055400	CYP77A2	Cytochrome P450	Cutin synthesis	1.77	1.01E-152
Solyc11g007540	CYP77A1	Cytochrome P450	Cutin synthesis	2.77	1.49E-38
Solyc06g062600	HTH-like	Choline dehydrogenase	Cutin synthesis	2.63	9.18E-21
Solyc03g121600	HTH-like	Choline dehydrogenase	Cutin synthesis	2.01	5.85E-16
Solyc06g035580	HTH-like	Choline dehydrogenase	Cutin synthesis	2.08	4.99E-123
Solyc01g094700	GPAT4	Glycerol-3-phosphate acyltransferase	Cutin synthesis	1.63	1.39E-36
Solyc09g014350	GPAT6	Glycerol-3-phosphate acyltransferase	Cutin synthesis	3.06	4.99E-123
Solyc06g065670	ABCG32	ATP-binding cassette transporter	Cutin transport	1.34	1.39E-08
Solyc11g006250	CD1/CUS1	GDSL esterase/lipase	Cutin assembly	1.95	6.57E-191
Solyc01g088400	CER1-like	CER1 Fatty acid hydroxylase	Wax synthesis	1.05	5.52E-06
Solyc03g005320	KCS-like	Fatty acid elongase 3-ketoacyl-CoA synthase	Wax synthesis	3.27	7.59E-05
Solyc08g067260	KCS10	Fatty acid elongase 3-ketoacyl-CoA synthase	Wax synthesis	1.08	9.75E-35
Solyc11g067180	CER4-like	Fatty acyl coA reductase	Wax synthesis	5.99	2.44E-04
Down-regulated					
Solyc07g056320	GPAT1	ER glycerol-phosphate acyltransferase	Cutin synthesis	-2.40	1.17E-24
Solyc03g065250	CER1	CER1 Fatty acid hydroxylase	Wax synthesis	-1.72	1.12E-22
Solyc03g117800	CER1-like	CER1 Fatty acid hydroxylase	Wax synthesis	-1.64	8.16E-28
Solyc10g080840	MAH1	Cytochrome P450	Wax synthesis	-2.48	8.41E-27
Solyc09g083050	KCS2	Fatty acid elongase 3-ketoacyl-CoA synthase	Wax synthesis	-4.33	1.14E-65
Solyc10g009240	KCS1	Fatty acid elongase 3-ketoacyl-CoA synthase	Wax synthesis	-1.35	2.43E-04
Solyc06g074390	CER4/FAR3	Fatty acyl coA reductase	Wax synthesis	-3.19	3.74E-14
Solyc09g092270	CER2-like	Hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase	Wax synthesis	-1.59	8.39E-13