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Increasing temperature changes the flux into the multiple biosynthetic pathways for 2-phenylethanol in model systems of tea (Camellia sinensis) and other plants

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ABSTRACT

2-Phenylethanol (2PE) is a representative aromatic aroma compound in tea (*Camellia sinensis*) leaves. However, its formation in tea remains unexplored. In our study, feeding experiments of [$^2H_8$]L-phenylalanine (Phe), [$^2H_5$]phenylpyruvic acid (PPA), or (*E*/*Z*)-phenylacetaldoxime (PAOx) showed that three biosynthesis pathways for 2PE derived from L-Phe occurred in tea leaves, namely, pathway I (*via* phenylacetaldehyde (PAld)), pathway II (*via* PPA and PAld), and pathway III (*via* (*E*/*Z*)-PAOx and PAld). Furthermore, increasing temperature resulted in increased flux into the pathway for 2PE from L-Phe *via* PPA and PAld. In addition, tomato fruits and petunia flowers also contained the 2PE biosynthetic pathway from L-Phe *via* PPA and PAld, and increasing temperatures led to increased flux into this pathway, suggesting that such a phenomenon might be common among most plants containing 2PE. This represents a characteristic example of changes in flux into the biosynthesis pathways of volatile compounds in plants in response to stresses.

KEYWORDS: tea; *Camellia sinensis*; aroma; 2-phenylethanol; phenylpyruvic acid; volatile

INTRODUCTION

Tea (*Camellia sinensis*) aroma compounds determine tea aroma quality.[1–3] Tea aroma compound formation and regulation are hot topics in tea-related research.[2–4] Since the isolation and identification of some glycosidically bound aroma compounds from tea leaves have been achieved, the transformations between glycosidically bound aroma compounds and free aroma compounds have become of great interest.[2,5] In recent years, with developments in tea plant biology, aroma compound formation from *de novo* enzymatic reactions in live tea leaves has attracted increasing research attention.[4] Biochemical pathways, enzymes, or genes regulating the formation of several tea characteristic aroma compounds, for example indole, jasmine lactone, (*S*)-linalool, (*E*)-nerolidol, β-ocimene, and (*Z*)-3-hexenal have been studied and elucidated.[6–13] Furthermore, tea aroma compound formation under stresses during the preharvest and postharvest tea stages have become of increasing interest, with the specific relationships between stresses and typical aromas considered key to the effective and safe improvement of tea aroma quality.[4] For example, biotic stresses (such as attack by tea green leafhoppers) can enhance volatile terpene compounds, such as linalool and diendiol,[4,10,14] while abiotic stresses (such as continuous wounding, and dual stress of wounding and low temperature) can lead to the high accumulation of, for example, jasmine lactone, (*E*)-nerolidol, and indole.[7,9,12] However, most studies have concentrated on how stresses affect the contents of volatile compounds in plants, while little is known about their effect on the flux of plant volatile compounds into biosynthesis pathways. In contrast to volatile terpenes and volatile fatty acid derivatives, there is few research on the biosynthesis of aromatic aroma compounds, volatile phenylpropanoids/benzenoids (VPBs), and their formation in response to stresses, in tea leaves.

In most tea cultivars, fresh tea leaves contain a relatively high percentage of VPBs, such as methyl salicylate, benzyl alcohol, benzaldehyde, phenylacetaldehyde (PAld), and 2-phenylethanol (2PE).[2,15] Among these VPBs, 2PE is a rose-like aromatic alcohol and representative aroma compound found in tea leaves.[2] Presently, in tea leaves, the formation of VPBs such as 2PE refers to studies on other plants, such as petunia flowers, rose flowers, and tomatoes.[16–21] Based on these reports, several pathways for 2PE synthesis have been identified in plants, as follows: (i) Derivation from L-phenylalanine (Phe) *via* PAld;[17,18] (ii) derivation from L-Phe *via* phenylpyruvic acid (PPA) and PAld;[21–23] and (iii) derivation from L-Phe *via* (*E*/*Z*)-phenylacetaldoxime (PAOx) and PAld, which was recently discovered in *Plumeria*.[24] However, the 2PE synthesis pathway(s) in tea leaves are unknown. In this study, we aimed to investigate how many pathways synthesize 2PE in tea leaves and, if more than one 2PE synthesis pathway was present, whether the flux into the different pathways was affected by stresses. Accordingly, [$^2H_8$]L-Phe (stable isotope-labeled) was used to confirm the occurrences of 2PE biochemical pathway(s) in tea leaves. We chemically synthesized [$^2H_5$]PPA (stable isotope-labeled) and (*E*/*Z*)-PAOx for tracing in a model system of tea leaves to confirm alternative pathways for 2PE synthesis. Furthermore, the effect of increasing temperature on flux into the different 2PE pathways in tea leaves was investigated. Finally, other plants, including tomato fruits and petunia flowers, were also investigated to determine whether the identified 2PE biochemical pathways and change in flux in response to increasing temperature were specific to tea leaves or common in most plants. This study aimed to elucidate 2PE formation in tea leaves, including biochemical pathways and their flux changes in response to increasing temperature stress. This is a characteristic example of changing flux into the biosynthesis pathways of volatile compounds in plants in response to stresses.

MATERIALS AND METHODS

Chemical reagents

Ethyl *n*-decanoate (purity, ≥99%) and sodium phenylpyruvate (purity, ≥98%) were obtained from Aladdin Industrial Co., Ltd (Shanghai, China). PAld (purity, ≥90%) was obtained from Sigma-Aldrich Company, Ltd. (Louis, MO, USA). [$^2H_8$]L-Phe (purity, ≥98%) and [$^2H_6$]benzaldehyde (≥ 98%) were obtained from Cambridge Isotope Laboratories Inc. (Cambridge, MA, USA). 4-Phenylimidazole (purity, ≥98%) was purchased from Aikon Biomedical Research and Development Co., Ltd (Jiangsu, China). 2PE (≥ 98%) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). [$^2H_5$]PPA (purity, ≥95%) and (*E*/*Z*)-PAOx (purity, ≥95%) were synthesized in our laboratory.

Plant materials and growth conditions

C. sinensis cv. 'Jinxuan' are widely cultivated in South China and suitable for making oolong tea. These tea plants were grown at the Tea Research Institute (Yingde Tea Experimental Station, Yingde, China), Guangdong Academy of Agricultural Sciences. Tea branches with one bud and three leaves from nearly twenty-year-old *C. sinensis* plants were used in this study.

Solanum lycopersicum cv. 'Micro Tom' and *Petunia* × *hybrida* cv. 'Mitchell Diplod' were planted in a greenhouse with controlled conditions (relative humidity, 70±2%; temperature, 25±2 °C; dark/light photoperiod, 8 h/16 h). Developing petunia flowers at about stages 6–7 based on the previous standard,[25] and tomato fruits (immature and mature) were used in this study.

Chemical synthesis of [$^2H_5$]PPA

[$^2H_5$]PPA was synthesized using the method of Liu et al.,[26] as shown in Figure 1A. The first step was the synthesis of [$^2H_6$](*Z*)-2-methyl-4-(phenylmethylene)oxazol-5(4H)-one (**3**). A solution of sodium acetate (10 mmol, 820 mg) dissolved in 4 mL acetic anhydride, [$^2H_6$]benzaldehyde (**1**, 2 mmol, 224 mg) and *N*-acylglycine (**2**, 2.4 mmol, 280 mg) were added in one portion. Then, the mixture was heated at 120 °C for 7 h under a nitrogen atmosphere and analyzed by thin-layer chromatography (TLC). When most of starting materials had disappeared, the reaction mixture cooled to room temperature. The resulting solution was stored in a refrigerator at 0 °C overnight to acquire a yellowish solid. After further filtration and washing with cold water, the resulting yellow solid was collected and subjected to drying under vacuum to obtain crude product [$^2H_6$](*Z*)-2-methyl-4-(phenylmethylene)oxazol-5(4H)-one (**3**, 310 mg). The second step was the synthesis of [$^2H_5$](*Z*)-2-acetamido-3-(phenyl)acrylic acid (**4**). Crude product [$^2H_6$](*Z*)-2-methyl-4-(phenylmethylene)oxazol-5(4H)-one (**3**, 310 mg) was redissolved in an acetone/H_2O mixture (5:7, 12 mL). Then, the solution was heated to reflux and kept at that time for 7 h under a nitrogen atmosphere. After most of starting material was consumed, the solvent was dried by evaporation to acquire crude product [$^2H_5$](*Z*)-2-acetamido-3-(phenyl)acrylic acid (**4**, 367 mg) as a residual solid. The third step was the synthesis of [$^2H_5$]PPA (**5**). Crude product [$^2H_5$](*Z*)-2-acetamido-3-(phenyl)acrylic acid (**4**, 367 mg) from the previous step was directly added to 4 mL HCl aqueous solution (1 mM). Then, the mixture was heated to dissolve the starting material (solid) and analyzed by TLC. After most of the starting material had been consumed and the reaction mixture cooled to room temperature, the mixture was purified using 10 mL ethyl acetate for 4 times, and the organic layers were collected and combined. It was dried with anhydrous Na_2SO_4, and filtrated with buchner funnel,

and the solvent was removed to obtain an oil (slight yellow) under reduced pressure *via* rotary evaporation. This crude product was further re-crystallized from hexane to obtain pure product (**5**, 0.64 mmol, 108 mg, 32% yield).

[$^2H_5$]PPA (**5**) was white solid with a purity of ≥95%. The purity of [$^2H_5$]PPA was determined by its ^{1}H nuclear magnetic resonance (NMR) spectra (Figure S1A). Detailed NMR data for **5** was as follows: ^{1}H NMR (500 MHz, CD_3OD) δ_H 6.51 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ_C 110.1, 127.1, 127.3, 127.5, 128.7, 128.9, 129.1, 134.8, 134.8, 140.8, 140.9, 167.0, 172.0. HRESIMS *m/z* 503.2436 $[M–H]^-$ (calcd. for $C_{31}H_{35}O_6$, 503.2439).

Chemical synthesis of (*E*/*Z*)-PAOx

The synthesis of (*E*/*Z*)-PAOx was conducted using a literature method,[27] as shown in Figure 1B. Sodium carbonate (0.6 mmol, 63 mg) and hydroxylamine hydrochloride (1.2 mmol, 83 mg) were dissolved in a mixed solvent (4 mL, methanol/H_2O, 1:1) and stirred for 10 min with a magnetic stir bar. PAld (**6**, 1 mmol, 120 mg) was then added in one portion at 0 °C, and the mixture was further subjected to 24 h-stirring under a nitrogen atmosphere (room temperature), and analyzed by TLC. After most of starting materials were consumed, the reaction mixture was extracted with ethyl acetate (5 mL × 5). The organic layers were combined, dried with anhydrous Na_2SO_4, and filtrated with buchner funnel, and the solvent was removed to obtain a slight yellow solid under reduced pressure *via* rotary evaporation. Purification of the yellow slight solid was used flash column chromatography (silica gel, hexane/ethyl acetate, 5:1), and desired product **7** (120 mg, 89% yield) was obtained as a pair of *cis* and *trans* isomers with a ratio of about 0.3:1.

(*E*/*Z*)-PAOx (**7**) was a yellow solid with purity of ≥95%. The purity of (*E*/*Z*)-PAOx was determined by its ^{1}H NMR spectra (Figure S1B). Detailed NMR data for **7** was as follows: ^{1}H

NMR (500 MHz, $CDCl_3$) δ_H 3.57 (d, J = 6.30, 0.6H), 3.78 (d, J = 5.33, 2H), 6.93 (t, J = 5.33, 1H), 7.20–7.40 (m, 6.5H), 7.57 (t, J = 6.30, 0.3H); HRESIMS *m/z* 503.2436 $[M–H]^-$ (calcd. for $C_{31}H_{35}O_6$, 503.2439).

Feeding experiments using [$^2H_8$]L-Phe in different plants and under different temperature treatments

Tea branches from *C. sinensis* cv. 'Jinxuan' plants plucked in October 2016 were used to conduct the feeding experiment with [$^2H_8$]L-Phe. The tea branches were cultivated in [H_8]L-Phe solution (12 mM) (Figure S2A)[28] and the tea leaves were then kept in an incubator under controlled conditions (humidity, 70%; dark/light photoperiod, 8 h/16 h) at 15, 25, or 35 °C. The suitable growth temperature of tea plant is 20–25 °C. Therefore, the lower, medium, and higher temperature were set at 15, 25, and 35 °C, respectively. After treating for 3 d and 7 d, the samples were stored at –80 °C until further analysis after freezing with liquid N_2. Three samples were processed in parallel as replicates.

Fruits of *S. lycopersicum* cv. Micro-Tom plucked in January 2017 were used to conduct a feeding experiment with [$^2H_8$]L-Phe. Injecting the solution into tomato fruit was difficult using an injector, especially for mature fruit. Therefore, some part of the fruit was removed, and the solution was added, as shown in Figure S2B. However, the wounded tomato fruit could not be kept for long periods, especially at high temperatures. Therefore, the fruit was first subjected to different temperature treatments for 2 d, and then fed with [$^2H_8$]L-Phe and cultivated at the corresponding temperature for another 1 d. First, the fruit was kept in an incubator under controlled conditions (humidity, 70%; dark/light photoperiod, 8 h/16 h) at 10, 25, or 35 °C. The suitable growth temperature of tomato is 13–28 °C. Therefore, the lower, medium, and higher

temperature were set at 10, 25, and 35 °C, respectively. In contrast to tea plant, tomato is more resistant to low temperature. Therefore, the lower temperature in tomato treatment was set at 10 °C. After treating for 2 d, every fruit was fed with [$^2H_8$]L-Phe solution (300 μL, 12 mM) and continually treated in the corresponding incubator for another 1 d.[29] After treatment, the samples were stored at −80 °C until further analysis after freezing with liquid N_2. Three samples were processed in parallel as replicates for immature fruit, and two samples were processed in parallel as replicates for mature fruit.

Flowers of *P. hybrida* cv. 'Mitchell Diplod' plucked in May 2017 were used to conduct the feeding experiment with [$^2H_8$]L-Phe. Each flower was cultivated in [$^2H_8$]L-Phe solution (12 mM) (Figure S2C)[28,29] and then kept in an incubator under controlled conditions (humidity, 70%; dark/light photoperiod, 8 h/16 h) at 25 °C for 1 d. The samples were then divided into three parts. The first was kept in the incubator at 25 °C, the second was transferred to the incubator at 10 °C, and the third was transferred to the incubator at 35 °C. The suitable growth temperature of tomato is 13–24 °C. Therefore, the lower, medium, and higher temperature were set at 10, 25, and 35 °C, respectively. In contrast to tea plant, petunia is more resistant to low temperature. Therefore, the lower temperature in petunia treatment was set at 10 °C. After treating for a further 1 d, headspace solid-phase microextraction was applied to collect the aroma compounds emitted from the flowers. After collection, the samples were stored at −80 °C until further analysis after freezing with liquid N_2. Three samples were processed in parallel as replicates.

Feeding experiments using PPA, [$^2H_5$]PPA, (*E*/*Z*)-PAOx, and 4-phenylimidazole combined with [$^2H_8$]L-Phe in tea leaves

Tea branches from *C. sinensis* cv. 'Jinxuan' plants plucked in April 2019 were used to conduct

the feeding experiment with PPA. Branches with one bud and three leaves were cultivated in two different solutions (Figure S2A): (i) H_2O (Control 1) and (ii) 6 mM PPA (PPA Treatment). In PPA Treatment, each branch (nearly 700–800 mg) absorbed 1.5 mL PPA solution *via* transpiration, and was then cultivated in H_2O. As PPA is water insoluble, commercial sodium phenylpyruvate (water soluble) was used to prepare the PPA solution.

Tea branches from *C. sinensis* cv. 'Jinxuan' plants plucked in May 2019 were used to conduct the feeding experiment with [$^2H_5$]PPA. Branches with one bud and three leaves were cultivated in two different solutions (Figure S2A): (i) 0.1 mM phosphate buffer (pH 8) (Control 2) and (ii) 6 mM [$^2H_5$]PPA resolved in sodium bicarbonate buffer (0.1 mM, pH 8) ([$^2H_5$]PPA Treatment). In [$^2H_5$]PPA Treatment, each branch (nearly 700–800 mg) absorbed 1.5 mL [$^2H_5$]PPA solution *via* transpiration, and was then cultivated in phosphate buffer (0.1 mM, pH 8). As PPA is water insoluble, a slightly alkaline phosphate buffer (pH 8) was used to dissolve [$^2H_5$]PPA.

Tea branches from *C. sinensis* cv. 'Jinxuan' plants plucked in May 2019 were used to conduct the feeding experiment with (*E*/*Z*)-PAOx. The branches with one bud and three leaves were cultivated in two different solutions (Figure S2A): (i) H_2O containing 1.5% dimethyl sulfoxide (DMSO) (Control 3) and (ii) 6 mM (*E*/*Z*)-PAOx dissolved in H_2O containing 1.5% DMSO (PAOx Treatment). In PAOx Treatment, each branch (nearly 700–800 mg) absorbed 1.5 mL PAOx solution *via* transpiration, and was then cultivated in H_2O containing 1.5% DMSO. Based on the phenotype of tea leaves after PAOx treatment (data not shown in the study), it could be speculated that the concentration of 2PE after PAOx treatment was tolerable for tea branches.

Tea branches from *C. sinensis* cv. 'Jinxuan' plants plucked in May 2019 were used to conduct the feeding experiment with [$^2H_8$]L-Phe or 4-phenylimidazole and [$^2H_8$]L-Phe. Branches with one bud and three leaves were cultivated in two different solutions (Figure S2A): (i) 12 mM

200 [$^2H_8$]L-Phe resolved in H_2O containing 0.5% ethanol and (ii) 12 mM [$^2H_8$]L-Phe and 1 mM
201 4-phenylimidazole resolved in H_2O containing 0.5% ethanol. 4-Phenylimidazole is an inhibitor
202 of CYP450 enzymes.[24]
203 All samples were placed in an incubator under controlled conditions (humidity, 70%;
204 dark/light photoperiod, 8 h/16 h) at 25 °C for 2 d. After treatment, the samples were stored at
205 −80 °C until further analysis after freezing with liquid N_2. Four samples were processed in
206 parallel as replicates.
207
208 **Determination of 2PE in samples**
209 Organic reagent extraction was used to extract endogenous 2PE from tissues based on a
210 previous method with some modifications.[12] All samples were ground into powder with liquid N_2
211 before extraction. Dichloromethane (1.8 mL) was selected to extract aroma compounds from tea
212 leaves or tomato fruit (500 mg, fresh weight), and ethyl *n*-decanoate (internal standard, 5 nmol)
213 was added to the mixture. Dichloromethane (1.5 mL) was selected to extract aroma compounds
214 from petunia flowers (100 mg, fresh weight), and ethyl *n*-decanoate (internal standard, 3 nmol)
215 was added to the mixture. A horizontal shaker kept at room temperature was used to conduct
216 oscillating extraction of samples overnight. After oscillating extraction, the extract was gathered,
217 dried with anhydrous Na_2SO_4, and concentrated to 100 μL using gaseous N_2. An aliquot of the
218 extract (1 μL) was then separated and analyzed by gas chromatography–mass spectrometer
219 (GC–MS).
220 The collection of 2PE emitted from petunia flowers was achieved using solid-phase
221 microextraction (SPME) fibers (2 cm, 50/30 μm DVB/CarboxenTM/PDMS Stable FlexTM,
222 Supelco Inc., Bellefonte, PA, USA), with a method similar to that published elsewhere.[30] A

petunia flower was kept in a sealed container (1 L) and the emitted aroma compounds were collected at 25±2 °C using a SPME fiber for 1 h. The aroma compounds absorbed in SPME fiber were separated and analyzed by GC–MS QP2010 SE.

The injection port of GC was kept at 230 °C for 1 min, all injections were conducted in splitless mode, and a velocity of 1 mL/min helium was used. For the separation of aroma compounds, a SUPELCOWAX 10 column of 30 m × 0.25 mm × 0.25 μm (Supelco Inc., Bellefonte, PA, USA) was selected. Initial temperature of GC oven was 60 °C after maintaining for 3 min, which was then up to 240 °C (at 4 °C/min) and held at 240 °C for 20 min. A full-scan mode with a mass range of *m/z* 40–200 was used for mass spectrometry. Both non-labeled or labeled 2PE were identified and quantitatively analyzed based on the non-labeled 2PE authentic standard.

Expression level analyses of the related genes

The gene expression level was measured using quantitative real time PCR (qRT-PCR) according to the previous study.[30] The internal reference gene was *encoding elongation factor1* (*EF1*).[31] The specific primers of the related genes used for qRT-PCR analysis are listed in Table S1. Detailed steps are provided in supplementary materials.

Analysis of glycosidically conjugated 2-phenylethanol (2PE-Gly) in tea samples

Extraction and analysis of 2PE-Gly in tea samples were referred to the previous studies with some modifications.[30,32] Change in the content of non-labeled and labeled 2PE-Gly was monitored by GC–MS combined with enzymatic hydrolysis. Detailed steps are provided in supplementary materials. The relative content of 2PE-Gly was based on the content of the 2PE

246 from enzymatic hydrolysis of 2PE-Gly.

247

248 **Statistical analysis**

249 One-way analysis of variance (ANOVA) or student's *t*-test were used to determine statistical
250 significance performing on SPSS software version 18 (SPSS Inc., Chicago, IL, USA).
251 Differences among three groups were evaluated using ANOVA, followed by Duncan's multiple
252 comparison test. Differences of $p \leq 0.05$ were defined as significant. Difference between two
253 groups was evaluated using a two-tailed Student's *t*-test.

254

255 **RESULTS AND DISCUSSION**

256

257 **Identification of 2PE biosynthesis pathways in tea leaves**

258 To determine the biochemical pathway(s) for the synthesis of 2PE in tea leaves, we first
259 treated the tea leaves with [$^2H_8$]L-Phe. Two types of deuterium-labeled 2PE products, namely,
260 [$^2H_7$]2PE and [$^2H_8$]2PE, were detected, suggesting that there was more than one pathway for the
261 synthesis of 2PE in tea leaves (Figure 2). To confirm the occurrence of proposed pathway II
262 (Figure 2A) in the tea leaves, we first chemically synthesized [$^2H_5$]PPA, which was not
263 commercially available (Figure 1A). After supplementation of the tea leaves with [$^2H_5$]PPA,
264 [$^2H_5$]2PE was detected (Figure 2B). Furthermore, after supplementation of the tea leaves with
265 non-labeled and labeled PPA, the 2PE and [$^2H_5$]2PE contents were both increased (Figures 3A
266 and 3B). These results indicated that pathway II (Figure 2A) occurred in tea leaves. To confirm
267 the occurrence of the proposed pathway III (Figure 2A) in tea leaves, we also chemically
268 synthesized (*E*/*Z*)-PAOx, which are not commercially available (Figure 1B). After

supplementing the tea leaves with (*E*/*Z*)-PAOx, the 2PE content was dramatically increased (Figure 3C). Furthermore, we supplied 4-phenylimidazole, an inhibitor of CYP450 enzymes potentially related to the pathway derived from L-Phe to PAOx, to combine with [$^2H_8$]L-Phe in the tea leaves. The ratio of [$^2H_8$]2PE to [$^2H_7$]2PE decreased (Figure 3D), suggesting that the flux into [$^2H_8$]2PE decreased in tea leaves when fed with 4-phenylimidazole.

VPBs are ubiquitous in plant species and rank the second most ubiquitous among all volatile classifications.[33] In most cases, volatile terpenes and volatile fatty acid derivatives are proposed to be mainly used for defense against herbivores, while VPBs seem to be primarily used for pollinator attraction.[34] In addition to ecological functions, VPBs have abundant value for economic applications, such as in improving flavor and memory, sedation, and food storage.[33,35] Current knowledge regarding VPB biosynthesis in plants has mainly been obtained from researches on flowers from *Petunia*, which is a model plant species for studying VPBs because metabolic background of *Petunia* is comparatively simple.[16] Furthermore, some ornamental flowers, such as rose flowers, contain abundant VPBs.[18,19,22,23] Most VPBs belong to the shikimate pathway and are generally derived from L-Phe. The most-studied first step is catalytic conversion into *trans*-cinnamic acid (CA) from L-Phe by L-phenylalanine ammonia lyase (PAL).[33] Some VPBs are synthesized *via* non-β-oxidation, a coenzyme A (CoA) independent pathway, or a β-oxidation CoA-dependent pathway.[16] The β-oxidation pathway involves CA activation through cinnamoyl-CoA ester formation, the formation of 3-hydroxy-3-phenylpropionyl-CoA from CoA ester hydration, and the formation of 3-oxo-3-phenylpropionyl-CoA by hydroxyl group oxidation.[16] In general, studies on the biosynthesis of VPBs have mostly focused on the last biosynthetic steps of VPBs, such as functional characterization of the involved enzymes and genes. Recently, several studies on VPB

upstream pathways have led to important discoveries. Firstly, 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase has been proposed to be a critical enzyme controlling flux *via* the shikimate pathway based on an expression study on a 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (bacterial feedback-insensitive) involved in the shikimate pathway in *Arabidopsis*.[36] Secondly *Chorismate mutase 1* was reported to be related to the formation of VPBs derived from L-Phe in *Petunia* flower using an RNAi suppression system.[37] Thirdly, the synthesis of L-Phe *via* arogenate from prephenate under catalysis by prephenate aminotransferase and arogenate dehydratase is a dominant pathway in *Petunia* flowers.[38,39] Furthermore, similar to some microorganisms, plants can synthesize L-Phe from PPA.[40] Finally, CoA ligases have been found to play important roles in benzenoid biosynthesis in *Petunia* flowers. A close positive correlation was detected between the expression of petunia *cinnamate:CoA ligase* (Ph-*CNL*) and emission of some VPBs, such as methylbenzoate, phenylethylbenzoate, and benzylbenzoate. In addition, during β-oxidative pathway, formation of cinnamoyl-CoA under the action of peroxidosomal-located Ph-*CNL* was the key procedure, and was closely involved in benzoic acid formation from L-Phe.[41] However, direct investigations of VPB biosyntheses in tea plants have been limited.

Among VPBs in plants, 2PE has been widely studied. The biosynthesis of other VPBs, such as PAld and 2PE, competes with PAL for L-Phe utilization and is not derived from CA. Previous studies on other plants, such as tomato and rose flowers, found that 2PE was derived from L-Phe *via* another VPB compound, PAld, under the action of two enzymes, namely, aromatic phenylacetaldehyde synthase (PAAS)/amino acid decarboxylase (AADC), and phenylacetaldehyde reductase (PAR).[17,18,42] In tea plants, PAAS/AADC has yet to be functionally characterized, but a PAR that can catalyze PAld to 2PE has been identified.[29]

Although the pathway for 2PE derived from L-Phe *via* PAld in tea plants is generally accepted, further *in vivo* evidence is required (Figure 2). In recent years, another 2PE biosynthesis pathway has been identified in rose flowers and melon, starting from L-Phe *via* PPA and PAld under catalysis by aromatic amino acid aminotransferase (AAAT), phenylpyruvic acid decarboxylase (PPDC), and PAR.[22,23,43] AAAT1 in tea leaves showed L-Phe transaminase functions and convert L-Phe into PPA using *in vitro* and *in vivo* systems.[44] Furthermore, based on evidence obtained from the present study, the 2PE biosynthesis from L-Phe *via* PPA and PAld was proposed to occur in tea plants (Figure 2). In 2019, a third pathway for 2PE formation was discovered in *Plumeria*, derived from L-Phe *via* (*E*/*Z*)-PAOx and PAld. Furthermore, a cytochrome P450 enzyme PrCYP79D73 was isolated, identified, and functionally characterized to contribute to 2PE production.[24] In the present study, based on the feeding experiments of (*E*/*Z*)-PAOx and inhibitor 4-phenylimidazole combined with [$^2H_8$]L-Phe (Figures 3C and 3D), the third pathway from L-Phe *via* PAOx and PAld was also confirmed in a model system of tea plants (Figure 2A).

Obtaining direct evidence of secondary metabolite synthesis in tea *in vivo* is difficult because, to date, it is unavailable using a well-established genetic transformation system. Therefore, obtaining direct evidence of metabolic networks in plants lacking transformation systems based on the trace using stable isotope-labeled precursors in a model system is available and effective.[45] In previous studies, investigations into the pathways of secondary metabolites in tea have been successfully performed using the technique based on stable isotope labeling.[7,12,41,46] In our study, using this method, multiple pathways of 2PE formation in tea leaves were confirmed, including the pathways from L-Phe *via* PAld, *via* PPA and PAld, and *via* (*E*/*Z*)-PAOx and PAld (Figure 2A).

Effect of increasing temperature on flux into 2PE pathway from L-Phe *via* PPA and PAld

To study the effect of increasing temperature on flux into the pathways for 2PE synthesis, we supplied [$^2H_8$]L-Phe to the tea leaves at different temperatures, namely 15, 25, or 35 °C, for 3 d and 7 d, and then analyzed the ratio of [$^2H_8$]2PE to [$^2H_7$]2PE. The results showed that the ratio of [$^2H_8$]2PE to [$^2H_7$]2PE decreased with increasing temperature (Figure 4). [$^2H_7$]2PE was derived from [$^2H_8$]L-Phe *via* [$^2H_7$]PPA and [$^2H_7$]PAld (Figure 2A, pathway II), which suggested that increasing the temperature resulted in increased flux into the pathway of 2PE from L-Phe *via* PPA and PAld in tea leaves. To further determine whether this phenomenon specifically occurred in tea leaves or was commonly present in most plants, investigations into other plants, including tomato fruit and petunia flowers, have been performed. Non-labeled 2PE, [$^2H_8$]2PE, and [$^2H_7$]2PE were also measured, but the content of these compounds did not exhibit a changing tendency in these plants that was consistent with tea plants (Figures 4, 5, and S3). In our previous experiments, jasmonic acid, as a marker of wounding stress, in tea leaves that were cultivated in water remains at low level (nearly 20 ng per 1 g tea leaves), suggesting that cut branches may not be exposed to stress for several days. In addition, the comparison among different temperatures was at the same treatment time. Although 2PE contents in tea leaves were slightly higher after 7 d at 25 and 35 °C (Figure 4A), there were significant differences in 2PE content among the different temperature treatments. From the results (Figures 4A, 5A, 5D, S3A and S3D), it was found that the contents of 2PE in different plant species showed different change patterns under the increasing temperatures. Increasing temperatures resulted in an increase in 2PE in tea leaves (Figure 4A), while it led to a decrease in 2PE in petunia flowers (Figures 5D and S3D). Furthermore, 2PE showed no significant change in tomato fruit under increasing temperatures (Figures 5A and S3A). However, the ratio of [$^2H_8$]2PE to [$^2H_7$]2PE, either in mature or immature

fruit, and either endogenous 2PE or emitted 2PE, decreased with increasing temperature in these samples (Figures 5C, 5F, S3C, and S3F), suggesting that such a phenomenon might be common in most plants containing 2PE, regardless of different plants and tissues.

The expression level of genes, including *CsAADC*, *CsAAAT*, *CsCYP79D73*, *CsPPDC*, and *CsPAR* (being involved in 2PE synthesis derived from L-Phe) in tea leaves under increasing temperature were also investigated. The expression levels of *CsAAAT1* and *CsPPDC* (being involved in pathway II) increased, while other two genes, *CsAADC* and *CsCYP79D73* (being involved in pathway I and III) were not up-regulated under increasing temperature treatment for 7 d (Figure 6). Furthermore, expression level of *CsPARs* were also enhanced by higher temperature (Figure 6), which may contribute to increase in 2PE in tea leaves under increasing temperature. Change in the content of non-labeled and labeled 2PE-Gly was monitored by GC–MS combined with enzymatic hydrolysis. Relative contents of 2PE-Gly and [$^2H_7$]2PE-Gly also increased under the increasing temperature, while the ratio of [$^2H_8$]2PE-Gly to [$^2H_7$]2PE-Gly decreased (Figure 7), which were consistent with the changing tendencies of non-labeled and labeled 2PE (Figure 4).

2PE is abundant in tea products, such as green tea, oolong tea and black tea. Many studies have confirmed that 2PE is identified as an important aroma contributor to these tea products.[47–49] On the other hand, 2PE is a taxonomically widespread floral volatile compound and has been reported to be involved in plant-insect interactions.[50] Hence, the increased 2PE may also play a role in ecological function in tea plants. The suitable growth temperature varies with plant species (details were shown in materials and methods). In contrast to tea plant, tomato and petunia are more resistant to low temperature. Therefore, 15 °C was set as lower temperature treatment for tea leaves, and 10 °C was set as lower temperature treatment for tomato fruits and

petunia flowers. In addition, the medium and higher temperature were all set at 25 and 35 °C, respectively. Apart from the temperature, the treatment time was another factor affecting the different pathways into 2PE. In the present study, the ratio of [$^2H_8$]2PE to [$^2H_7$]2PE showed significant difference after different temperature treatment for 2-3 d. In tea leaves, there was the same changing tendency under increasing temperature for a longer treatment time (7 d) (Figure 4C). However, for tomato fruits and cut petunia flowers, the treatment time could not be too long, especially at higher temperature. Further studies are need to investigate the change in the preharvest tomato fruits and petunia flowers. In the present study, the different pathways always existed in three plant species, including tea, tomato and petunia, regardless of temperature. In tea leaves, increasing temperature resulted in an increase in [$^2H_7$]2PE, thus inducing a decrease in the ratio of [$^2H_8$]2PE to [$^2H_7$]2PE. In tomato fruits and petunia flowers, increasing temperature resulted in a decrease in [$^2H_8$]2PE, thus inducing a decrease in the ratio of [$^2H_8$]2PE to [$^2H_7$]2PE. Increasing temperature affected the content of [$^2H_7$]2PE in tea leaves, and content of [$^2H_8$]2PE in tomato fruit and petunia flowers. It did not terminate the pathway into [$^2H_8$]2PE. Therefore, there was only slight change in ratio of [$^2H_8$]2PE to [$^2H_7$]2PE under increasing temperature. Usually, plants have the ability to adapt to their environment, so a certain temperature difference is needed to make a difference in plant species. Ten degrees of temperature difference in these plant species may be too low to induce the significant difference (Figures 4C, 5C, 5F, S3C and S3F), and a greater temperature differences, such as twenty degrees or even twenty-five degrees were needed.

Under abiotic and biotic stresses, plants have evolutionarily formed a series of diverse protective mechanisms. For example, to adapt to environmental conditions, plants generally produce specialized metabolites, such as volatile metabolites.[51] In most cases, volatile fatty acid

derivatives and terpenes are closely related to several types of biotic interactions, while VPBs are mostly reported to be involved in abiotic interactions.[20,51,52] Temperature is one of mostly studied abiotic stresses. In the last several decades, the average global temperature has increased significantly, with the rate of increase accelerating. It has been shown that, in the last 1400 years, the average temperature of the northern hemisphere during the period from 1983 to 2012 might be the warmest period (30 years).[53] Furthermore, in the last four decades, springtime has advanced at the rate of 2.5 d/decade, while heat wave events and warmer nights have occurred more frequently.[53,54] Global climate change has attracted the attention of researchers regarding interactions between temperature and plant volatile compounds.[54] Many studies have investigated the effects of increasing temperature on VPB formation in *Petunia* flowers, but the changing tendency varied in previous studies. In *P. axillaris* flowers, augmenting the environmental temperature to 30 ℃ for one week could enhance the emission of floral VPBs.[55] Nonetheless, a high-temperature growth condition with long-term (one month, 28 ℃) negatively influenced the floral VPBs of *P. hybrida*.[54] In the cut flowers of *P. hybrida*, a high-temperature treatment with a shorter time, namely, at 28 ℃ for 10−12 h, reduced most VPB emissions, whatever the light conditions are.[20] The reduction in VPB emissions from petunia flowers under a high temperature treatment with long-term at 28 ℃ for one month was due to the down-regulation of genes related to the shikimate pathway, and up-regulation of the negative regulation factor of floral production.[54] Increasing the temperature short-term can also affect VPB emissions under regulation factors and structural genes.[20] In response to temperature changes in different seasons, 2PE formation *via* two different biosynthetic pathways changed in rose flowers.[23] In addition to the effects of seasons, the evaluated temperature can also activate the pathway into 2PE from L-Phe *via* PPA in postharvest cut rose flowers.[56] Most studies of

interactions between temperature and VPBs are reported for plant floral parts, while little is known about the vegetative parts. Furthermore, plants produce some specialized metabolites through more than one pathway. Currently, most studies have concentrated on the effects of stresses on plant volatile metabolite contents, while little is known about the effects of stresses on flux into their biosynthesis pathways. In tea plants, 2PE was not significantly affected by insect attacks and light wavelengths, but was significantly enhanced under dark conditions (shading treatment) owing to the increase in its precursor, L-Phe.[6,15,57] In the present study, increasing the temperature resulted in increased flux into the pathway of 2PE from L-Phe *via* PPA and PAld in tea leaves (pathway II, Figures 2A and 4). An increase in expression level of *CsAAAT1* and *CsPPDC* might be a reason to account for the phenomenon (Figure 6). Furthermore, this phenomenon might be common in most plants containing 2PE, regardless of different species and tissues (Figures 5 and S3). It would be interesting to know whether this plant response had some special biology functions for plants in response to high-temperature stress. Furthermore, in fresh tea leaves, the PAld content is generally much lower than the 2PE content, which led to PAld in tea plants receiving little attention. As the biological functions of both PAld and 2PE in tea plants are presently unknown, it would be interesting to investigate their roles in tea plants exposed to abiotic stresses.

In the present study, we chemically synthesized precursors, including [$^2H_5$]PPA and (*E*/*Z*)-PAOx. [$^2H_8$]L-Phe and the as-synthesized precursors were applied to tea leaves to obtain evidence of 2PE biosynthesis in a model system and its response to increasing high-temperature stress. Three pathways toward the synthesis of 2PE in tea leaves were elucidated, namely, the pathways from L-Phe *via* PAld, *via* PPA and PAld, and *via* (*E*/*Z*)-PAOx and PAld. Furthermore, flux into the pathway of 2PE from L-Phe *via* PPA and PAld was increased by increasing the high

453 temperature stress, which might be common in most plants containing 2PE, regardless of
454 different species and tissues (Figure 8). The results obtained from this study not only provided
455 evidence of 2PE formation in a model system of tea leaves, which is not a model crop, but also
456 gave a characteristic example of changes in flux into the biosynthesis pathways of volatile
457 compounds in plants in response to stresses. The information will provide critical guidance for
458 improving the aroma quality of tea leaves and other agricultural and food products.
459

460 **ASSOCIATED CONTENT**

461 Supporting information

462 **Table S1** Primer Used for Quantitative Real Time PCR (qRT-PCR) in the Study.

463 **Figure S1** Identification of [$^2H_5$]Phenylpyruvic Acid (A) and (*E*/*Z*)-Phenylacetaldoxime (B)
464 Authentic Standard Based on Nuclear Magnetic Resonance Analysis.

465 **Figure S2** Feeding Methods of Tea Leaves (A), Tomato Fruit (B), and Petunia Flowers (C).

466 **Figure S3** Effect of Increasing Temperature on Endogenous 2-Phenylethanol (2PE) in Mature
467 Tomato Fruit and Emitted 2PE in Petunia Flowers.
468

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472 Author Contributions

473 # Lanting Zeng and Haibo Tan equally contributed to this work.

474 Notes

475 The authors declare no competing financial interest.

476

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483

484 **ABBREVIATIONS**

485 FW, fresh weight; PAld, phenylacetaldehyde; (*E*/*Z*)-PAOx, (*E*/*Z*)-phenylacetaldoxime; 2PE,
486 2-phenylethanol; 2PE-Gly, glycosidically conjugated 2-phenylethanol; L-Phe, L-phenylalanine;
487 PPA, phenylpyruvic acid; VPBs, volatile phenylpropanoids/ benzenoids.

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Figure Caption

Figure 1 Schematic presentation of synthesis of [$^2H_5$]phenylpyruvic acid (A) and (*E*/*Z*)-phenylacetaldoxime (B).

(A) 1, [$^2H_6$]benzaldehyde; 2, *N*-acylglycine; 3, [$^2H_6$](*Z*)-2-methyl-4-(phenylmethylene)oxazol-5(4H)-one; 4, [$^2H_5$](*Z*)-2-acetamido-3-(phenyl)acrylic acid; 5, [$^2H_5$]phenylpyruvic acid. (B) 6, phenylacetaldehyde; 7, (*E*/*Z*)-phenylacetaldoxime.

Figure 2 Proposed biosynthetic pathways into 2-phenylethanol (2PE) (A) and identification of labeled 2PE (B) in tea leaves.

(A) L-Phe, L-phenylalanine; PPA, phenylpyruvic acid; (*E*/*Z*)-PAOx, phenylacetaldoxime; PAld, phenylacetaldehyde; 2PE, 2-phenylethanol; AADC, aromatic amino acid decarboxylase; AAAT1, aromatic amino acid aminotransferase 1; PPDC, phenylpyruvic acid decarboxylase; PAR, phenylacetaldehyde reductase. (B) Mass spectrum and chromatography of 2PE standard and labeled 2PE from tea leaves fed with [$^2H_5$]PPA or [$^2H_8$]L-Phe.

Figure 3 Identification of the biosynthetic pathways into 2-phenylethanol (2PE) from phenylpyruvic acid (PAA) (A and B) and (*E*/*Z*)-phenylacetaldoxime (PAOx) (C and D).

Data are expressed as mean ± SD (n =4). * $p \leq 0.05$; ** $p \leq 0.01$, comparison between control and treatment. FW, fresh weight. (A) Change in content of 2PE in tea leaves fed with PPA. Control 1, sum of 2PE content from tea leaves and PPA standard. PPA Treatment, 2PE content from tea leaves fed with PPA. (B) Change in content of [$^2H_5$]2PE in tea leaves fed with

[$^2H_5$]PPA. Control 2, sum of [$^2H_5$]2PE content from tea leaves and [$^2H_5$]PPA standard. [$^2H_5$]PPA Treatment, content of [$^2H_5$]2PE from tea leaves fed with [$^2H_5$]PPA. (C) Change in content of 2PE in tea leaves fed with (*E*/*Z*)-PAOx. Control 3, sum of 2PE content from tea leaves and (*E*/*Z*)-PAOx standard. PAOx Treatment, 2PE content from tea leaves fed with (*E*/*Z*)-PAOx. (D) Change in ratio of [$^2H_8$]2PE to [$^2H_7$]2PE in tea leaves fed with [$^2H_8$]L-phenylalanine (Phe) or co-fed with [$^2H_8$]L-Phe and 4-phenylimidazole.

Figure 4 Effect of increasing temperature on endogenous 2-phenylethanol (2PE) in tea leaves.

Change in content of endogenous 2PE (A), endogenous [$^2H_8$]2PE and endogenous [$^2H_7$]2PE (B), and ratio of endogenous [$^2H_8$]2PE to endogenous [$^2H_7$]2PE (C) in tea leaves fed with [$^2H_8$]L-phenylalanine (Phe). Data are expressed as mean ± SD (n =3). Means with different letters are significantly different from each other at the same treatment time ($p \leq 0.05$). FW, fresh weight.

Figure 5 Effect of increasing temperature on endogenous 2-phenylethanol (2PE) in immature tomato fruit and petunia flowers.

Data are expressed as mean ± SD (n =3). Means with different letters are significantly different from each other ($p \leq 0.05$). FW, fresh weight. Change in content of endogenous 2PE (A), endogenous [$^2H_8$]2PE and endogenous [$^2H_7$]2PE (B), and ratio of endogenous [$^2H_8$]2PE to endogenous [$^2H_7$]2PE (C) in immature tomato fruit fed with [$^2H_8$]L-phenylalanine (Phe). Change in content of endogenous 2PE (D), endogenous [$^2H_8$]2PE and endogenous [$^2H_7$]2PE (E), and ratio of endogenous [$^2H_8$]2PE to endogenous [$^2H_7$]2PE (F) in petunia flowers fed with [$^2H_8$]L-Phe.

Figure 6 Effect of increasing temperature on expression level of genes involved in the 2-phenylethanol synthesis derived from L-phenylalanine in tea leaves.

AADC, *aromatic amino acid decarboxylase*; *AAAT1*, *aromatic amino acid aminotransferase 1*; *PPDC*, *phenylpyruvic acid decarboxylase*; *PAR*, *phenylacetaldehyde reductase*. Data are expressed as mean ± SD (n =3). Means with different letters are significantly different from each other at the same treatment time ($p \leq 0.05$).

Figure 7 Effect of increasing temperature on glycosidically conjugated 2-phenylethanol (2PE-Gly) in tea leaves.

Change in content of 2PE-Gly (A), [$^2H_8$]2PE-Gly and [$^2H_7$]2PE-Gly (B), and ratio of [$^2H_8$]2PE-Gly to [$^2H_7$]2PE-Gly (C) in tea leaves fed with [$^2H_8$]L-phenylalanine (Phe). The relative content of 2PE-Gly was based on the content of the free 2PE from enzymatic hydrolysis of 2PE-Gly. Data are expressed as mean ± SD (n =3). Means with different letters are significantly different from each other at the same treatment time ($p \leq 0.05$). FW, fresh weight.

Figure 8 Change in flux of biosynthetic pathway into 2-phenylethanol (2PE) in plants under increasing temperature.

L-Phe, L-phenylalanine; PPA, phenylpyruvic acid; PAld, phenylacetaldehyde; (*E*/*Z*)-PAOx, (*E*/*Z*)-phenylacetaldoxime.

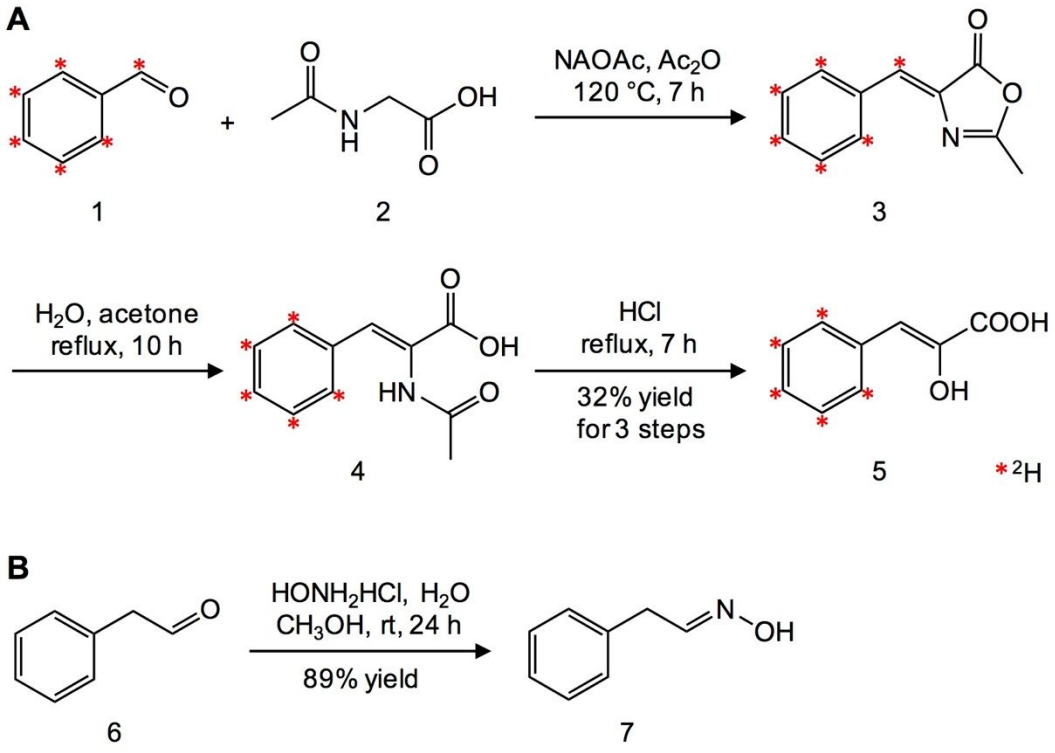

A
NAOAc, Ac2O
120 °C, 7 h
1
2
3
H2O, acetone
reflux, 10 h
HCl
reflux, 7 h
32% yield
for 3 steps
4
5
*2H
B
HONH2HCl, H2O
CH3OH, rt, 24 h
89% yield
6
7

Figure 1

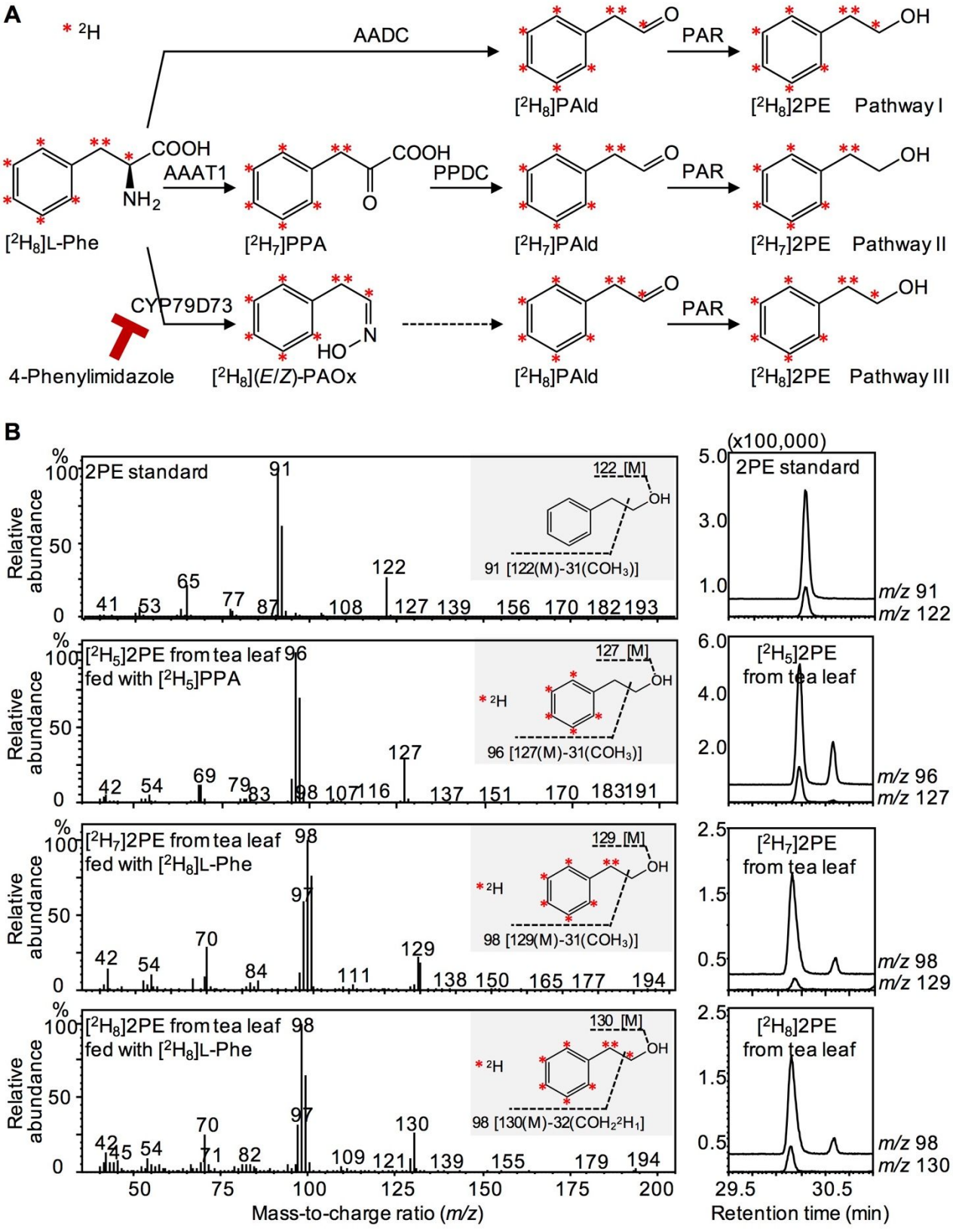

A
* ²H
AADC
PAR
[²H8]PAld
[²H8]2PE
Pathway I
COOH
AAAT1
NH2
PPDC
[²H8]L-Phe
[²H7]PPA
[²H7]PAld
[²H7]2PE
Pathway II
CYP79D73
4-Phenylimidazole
HO
N
[²H8](E/Z)-PAOx
[²H8]PAld
[²H8]2PE
Pathway III
B
2PE standard
122 [M]
91 [122(M)-31(COH3)]
[²H5]2PE from tea leaf fed with [²H5]PPA
127 [M]
96 [127(M)-31(COH3)]
[²H7]2PE from tea leaf fed with [²H8]L-Phe
129 [M]
98 [129(M)-31(COH3)]
[²H8]2PE from tea leaf fed with [²H8]L-Phe
130 [M]
98 [130(M)-32(COH2²H1]
Relative abundance
Mass-to-charge ratio (m/z)
(x100,000)
m/z 91
m/z 122
m/z 96
m/z 127
m/z 98
m/z 129
m/z 98
m/z 130
Retention time (min)

Figure 2

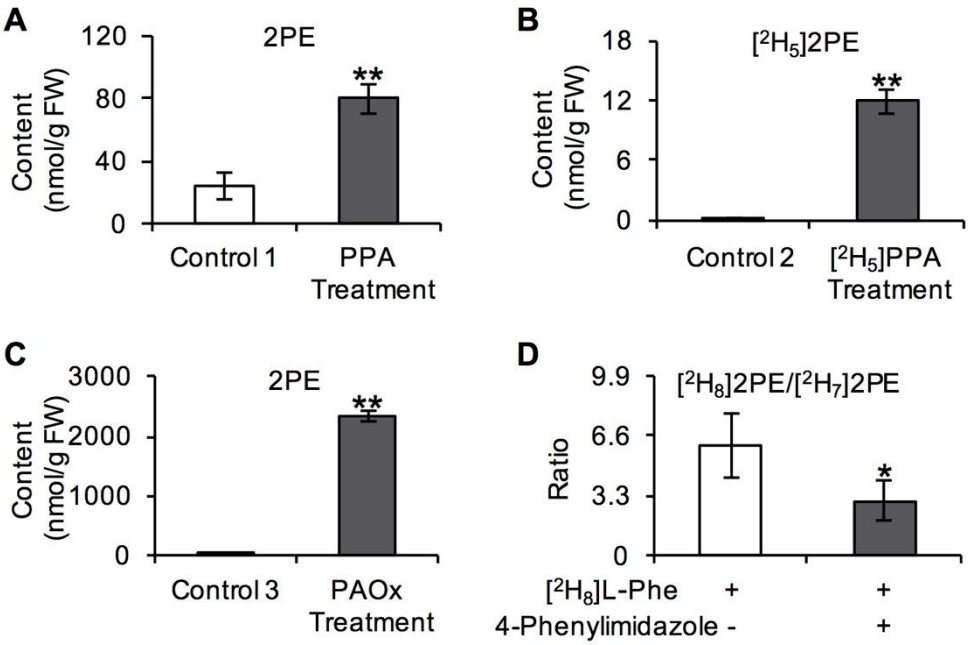

A
2PE
Content (nmol/g FW)
120
80
40
0
**
Control 1
PPA
Treatment
B
[2H5]2PE
Content (nmol/g FW)
18
12
6
0
**
Control 2
[2H5]PPA
Treatment
C
2PE
Content (nmol/g FW)
3000
2000
1000
0
**
Control 3
PAOx
Treatment
D
[2H8]2PE/[2H7]2PE
Ratio
9.9
6.6
3.3
0
*
[2H8]L-Phe + +
4-Phenylimidazole - +

Figure 3

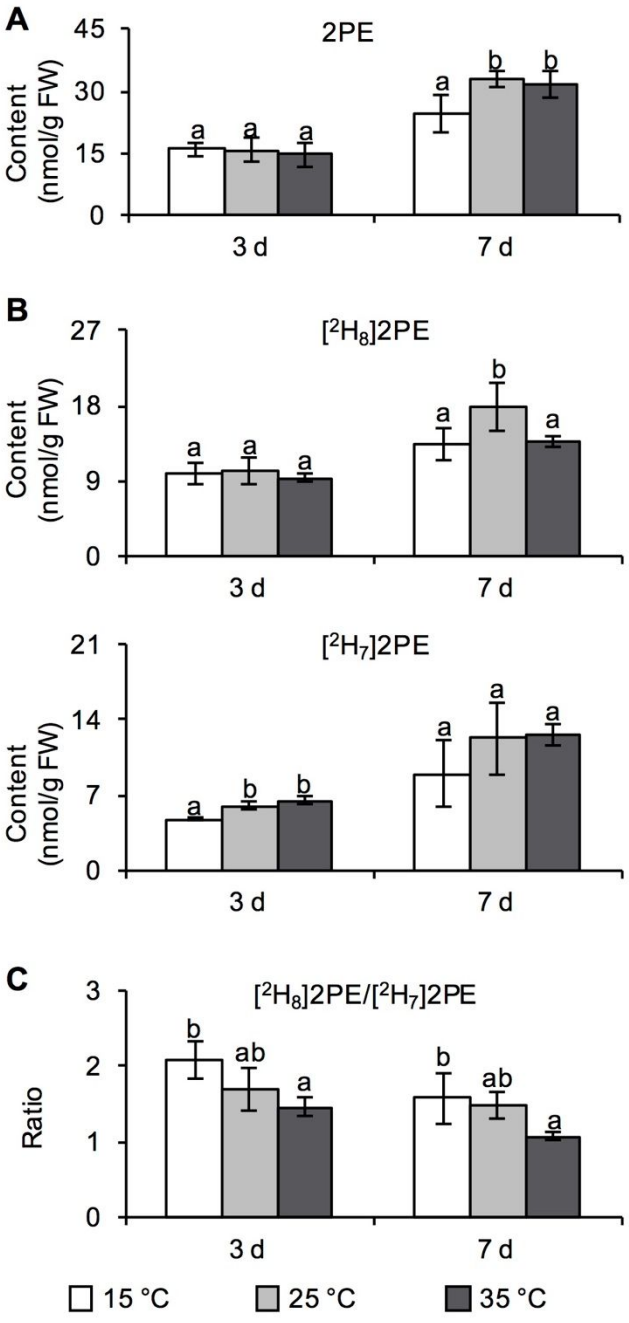

A
2PE
Content (nmol/g FW)
45
30
15
0
a a a b b
3 d
7 d
B
[2H8]2PE
27
18
9
0
a a a b a
[2H7]2PE
21
14
7
0
a b b a a a
C
[2H8]2PE/[2H7]2PE
Ratio
3
2
1
0
b ab a b ab a
15 °C
25 °C
35 °C

Figure 4

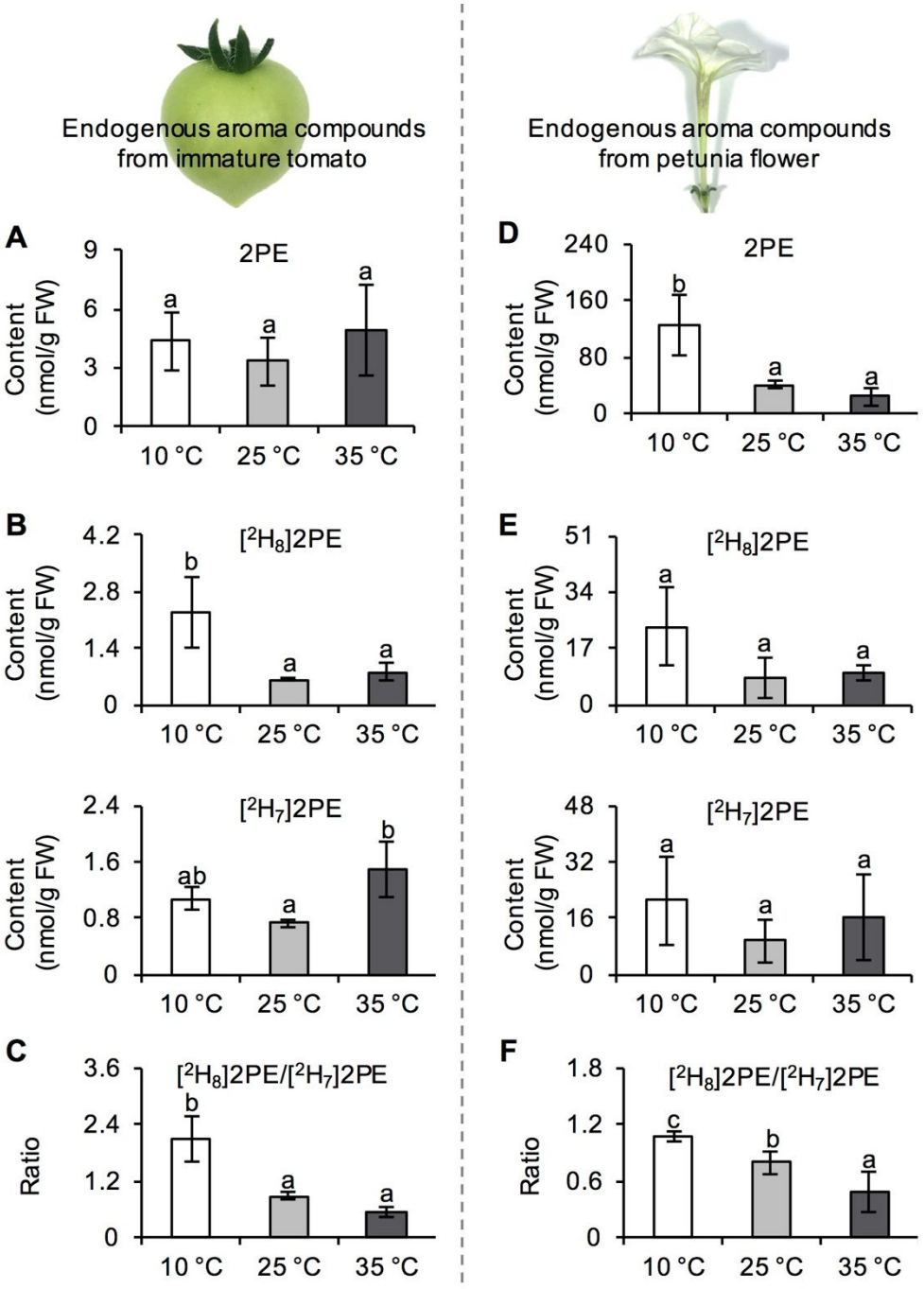

Endogenous aroma compounds from immature tomato
Endogenous aroma compounds from petunia flower
A
2PE
Content (nmol/g FW)
9
6
3
0
a
a
a
10 °C
25 °C
35 °C
D
2PE
240
160
80
0
b
a
a
B
[2H8]2PE
4.2
2.8
1.4
E
[2H8]2PE
51
34
17
[2H7]2PE
2.4
1.6
0.8
ab
[2H7]2PE
48
32
16
C
[2H8]2PE/[2H7]2PE
Ratio
3.6
2.4
1.2
F
[2H8]2PE/[2H7]2PE
1.8
1.2
0.6
c

Figure 5

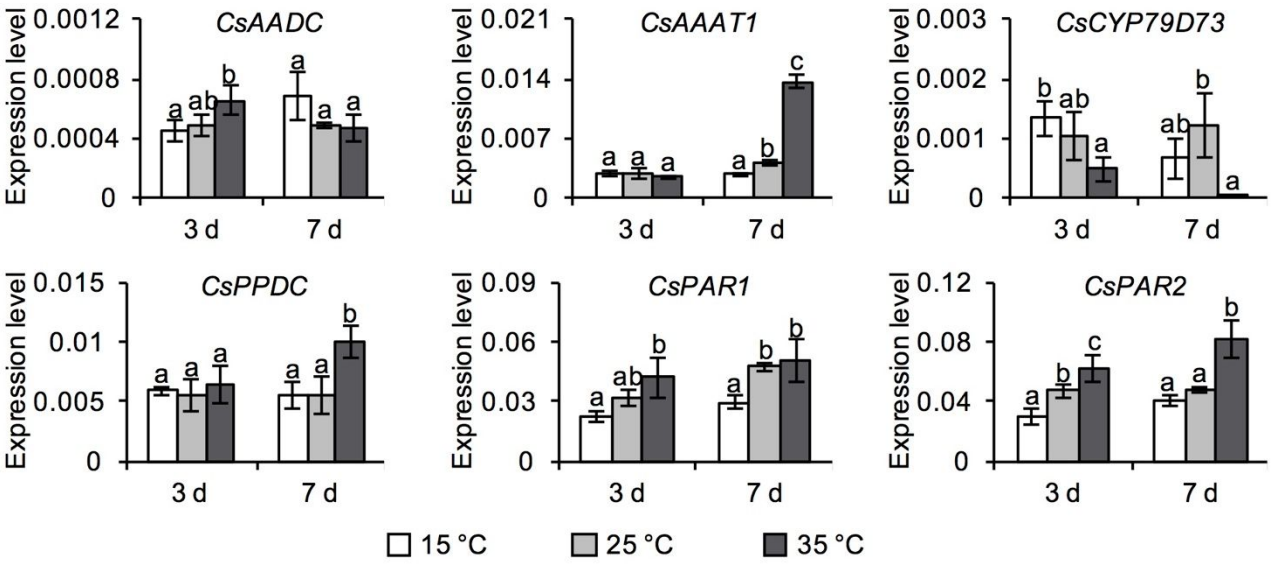

CsAADC
CsAAAT1
CsCYP79D73
CsPPDC
CsPAR1
CsPAR2
Expression level
0.0012
0.0008
0.0004
0
0.021
0.014
0.007
0.003
0.002
0.001
0.015
0.01
0.005
0.09
0.06
0.03
0.12
0.08
0.04
3 d
7 d
15 °C
25 °C
35 °C

Figure 6

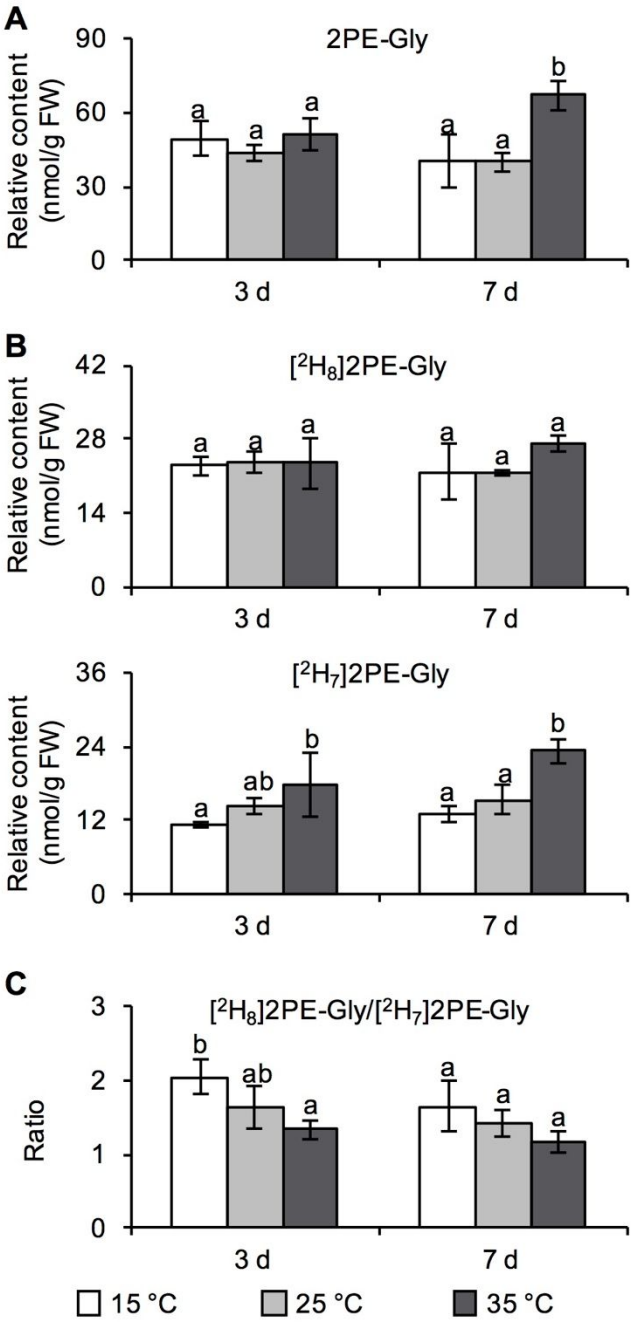

A
2PE-Gly
Relative content (nmol/g FW)
90
60
30
0
a
a
a
a
a
b
3 d
7 d
B
[2H8]2PE-Gly
Relative content (nmol/g FW)
42
28
14
0
a
a
a
a
a
a
3 d
7 d
[2H7]2PE-Gly
Relative content (nmol/g FW)
36
24
12
0
a
ab
b
a
a
b
3 d
7 d
C
[2H8]2PE-Gly/[2H7]2PE-Gly
Ratio
3
2
1
0
b
ab
a
a
a
a
3 d
7 d
15 °C
25 °C
35 °C

Figure 7

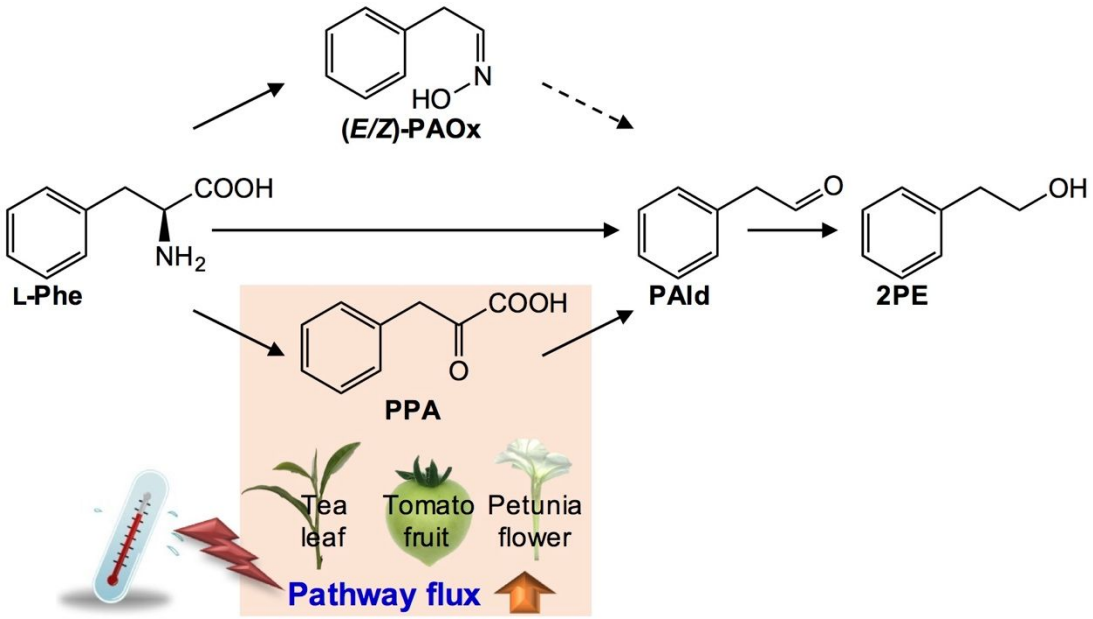
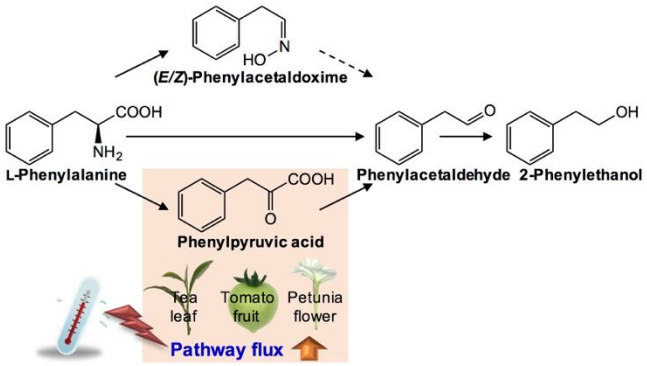

HO
N
(E/Z)-PAOx
COOH
NH2
L-Phe
O
OH
PAld
2PE
COOH
O
PPA
Tea leaf
Tomato fruit
Petunia flower
Pathway flux

Figure 8

TOC


(E/Z)-Phenylacetaldoxime
L-Phenylalanine
Phenylacetaldehyde
2-Phenylethanol
Phenylpyruvic acid
Tea leaf
Tomato fruit
Petunia flower
Pathway flux