Comparative Study on *Pseudaulacaspis Pentagona* Resistance of Four Different Cultivars of Kiwifruit

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Research Article

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Abstract

Background:

Kiwifruit is a common and popular fruit around the world. However, white peach scale (*Pseudaulacaspis pentagona)* [Targioni-Tozzetti], a scale insect with a wide range of hosts, seriously affects the yield and quality of kiwifruit. To investigate the differences in resistance of different kiwifruit cultivars to *Pseudaulacaspis pentagona,* cellular structure and gene expression assays were used to explain the mechanism.

Results:

In this study, based on the stability of the rate of injury fruit, we selected four cultivars from fifty kiwifruits for in-depth study, including “LC-04285”, “CF-3”, “DA-7B” and “Hayward”. By analyzing the differences in the anatomical structure of the canes of these cultivars, we found that the resistant cultivar "LC-04285" had thicker cuticle, denser epidermis and cortex. The real-time quantitative PCR data indicated that the expression levels of genes related to cuticle synthesis and formation of epidermis and cortex are also higher in “LC-04285”. Jasmonic acid (JA) is an important hormone involved in plant defense against many insect pests. In this study, we found that the expression levels of JA receptor *COI1* were higher in “LC-04285”. However, the expression levels of *AcJAZs*, which played negative role in JA signaling, were higher in susceptible cultivar “Hayward”. Besides, the expression levels of *AcICS*, *AcPAL4*, *AcPAL5*, and *AcNPRs*, which were involved in salicylic acid (SA) synthesis and SA response, were also higher in “LC-04285”.

Conclusions:

Our results revealed the mechanism of kiwifruit resistance to *P. pentagona* at the molecular and cellular levels. This study provided useful guidance for breeding insect-resistant kiwifruit in future.

Background

Kiwifruit is a popular fruit due to it’s unique flavor and high nutritive value [1]. According to the data from Food and Agriculture Organization of the United Nations (FAO) in 2019, the china’s production of kiwifruit is 2,196,727 tonnes [2]. Although the cultivated area of kiwifruit is ever-increasing each year, varities of pests, including scale insects, Metcalfa pruinosa, brown marmorated stinkbug and lepidopteran, destroy kiwifruit’s leaf, stem, and fruit, causing enormous economic loss [3–6]. White peach scale (*Pseudaulacaspis pentagona)* [Targioni-Tozzetti] is a destructive scale insect and causes severe kiwifruit yield reduction [7]. *P. pentagona* damages the kiwifruit directly by sucking sap from the leaves, stems and canes, affecting the quality and quantity of kiwifruit produced [8].

The cane of woody plant has six layers, which are, from outside to inside, epidermis, cortex, cambium, phloem, xylem and pith. Among them, the epidermis and cortex are located on the outside and play a vital

role in protecting and supporting plant growth. As the outermost barrier of plants, epidermis acts a pivotal part in protecting plants [9]. In the model plant rice, epidermis controls rice's growth process and protects rice against biotic and abiotic stress [10]. The epidermis differentiates into trichomes, which are closely related to plant resistance to insects [11, 12]. Cortex is a thin-walled tissue between the epidermis and the vascular bundle [13]. Cortical cells usually contain resins, essential oils, tannins, which confer partially resistance to pest [14–16]. Cuticle, which mainly contained wax, coatings the outside of the epidermal cells. It has been well documented that thicker cuicle and epidermis contribute to insect resistance in cacti [17].

Jasmonic acid (JA) and salicylic acid (SA) are representative hormones for plant resistance [18–20]. Many studies have reported its defensive effects on pests [21–23]. The silencing of the ICS1 gene, which regulates SA biosynthesis, reduces the insect resistance of tobacco [24]. The expression of *ICS1* and *NPR1* in rice can be induced after infesting by white-backed planthopper [25]. SA accumulation in wheat is also increased when suffering the Russian wheat aphid [26]. In the JA signaling pathway, COI1 induces the degradation of JAZ, which in turn activates the defense response of plants. It has been shown that silencing the expression of *NaCOI1* make tobacco more vulnerable to *Manduca sexta* [27]. Arabidopsis JAZ mutants display a high level of resistance to insect [28]. The JA defective mutant of arabidopsis is hyper-susceptible to *Meloidogyne hapla* [29]. Furthermore, exogenous application of JA or SA can significantly improve the insect resistance of plants [30, 31]. The ability of pest resistance in plant can be regulated by changing the expression levels of JA/SA-related genes [32, 33].

Although mineral oils and organophosphate sprays have been widely used for *P. pentagona* management, breeding of new resistant kiwifruit cultivars still shown great promise. In this study, we focuse on four kiwifruit cultivars with different *P. pentagona*-resistance ability after a large-scale screening. According to analyzing the difference of cell structure of cane and some JA/SA-related genes' expression levels among these cultivars, we preliminarily analyzed the *P. pentagona*-resistance mechanisms of resistant kiwifruit cultivars. These results will provide new thoughts and methods for insect prevention in kiwifruit and breeding of new resistant kiwifruit cultivars.

Methods

Growth conditions and the infestation rate measurement

In total fifty kiwifruit cultivars were planted outside in the field. The plants spaced 2 m apart in rows, and rows were placed 3 m apart. The kiwifruit plants had been mature and began to bear fruit in 2007. Then fruits of fifty kiwifruit cultivars were harvested in late August 2008, 2010 and 2011, and infestation rate was counted. Forty plants of each cultivar were harvested and thirty fruits of each cultivar were randomly sampled .

Samples collection and pre-processing

P. pentagona was cultured in the insect rearing facility of The Sichuan Provincial Academy of Natural Resource Science. Twelve-hour newborn female eggs of *P. pentagona* on pumpkin or potato were collected before the test. One-year-old canes (approximately 1.0-1.5 m long and 1-2 cm in diameter), including “LC-04285”, “CF-3”, “DA-7B” and “Hayward”, were collected in winter (middle of December in China). The canes were wrapped in black plastic and held in a cool store at 0-2℃, RH60-70%. The laboratory experiment was set up 1-2 months later (Jan. or Feb.) in a controlled environment room at kiwifruit laboratory, Chengdu. The environmental conditions were 25℃, 70±10% Relative Humidity (RH), with a 14 h light:10 h dark photoperiod regime.

The kiwifruit canes were removed from the cold store, marked and cut into 60 cm with removing the bottom of 10 cm. Each cane length was labelled and the buds were cut off. All wounds were sealed with Vaseline. Each cane was gently wrapped 6-9 turns with wool, providing a place for the eggs and crawlers to colonize [56]. Each kiwifruit cultivar had five replicates.

Colonization rate and survival rate measurement

The female eggs (about 200) of *P. pentagona* were gently transfer to the sites which wrapped turns on branches using stroke pen. The branches were placed at an angle of 30-50 ° to ensure that the eggs would not fall. Additionally, a large number of male eggs were inoculated into the other two branches, so that the males could mate with the females on the branches after hatching. All branches were placed in an industrial climate box (23 ± 0.5 ° C, 60 ± 5% RH, 14 hours of light, and 10 hours of darkness). The water in the beaker was replaced every week. The buds were cut off and sealed with Vaseline weekly. The specific number of transferred female eggs was recorded under a stereomicroscope. After 7 days of egg hatching, the wool was cut off, the number of colonies of the crawlers was recorded, and a marker was used to mark the sides. The number of live and dead pests on the branch were recorded when the eggs hatch for about 40 days (the pests were in the adult stage). And then, the survival rate was calculated. The specific measurement methods referred to Hill’s report [56].

Scale area measurement

The scale’s area in three stages, including early second instar stage, early adult stage and oviposition stage, was measured by a transparent template which had circular scale. The area of template was 0.4-1.4 mm^2 and increased by 0.2 mm^2. The specific measurement methods referred to Hill’s report [56]. If the female scale’s area was larger than 1.2 mm^2, the software, ImageJ, was used to measure the area. Thirty female scales were measured each time.

Anatomic characterization

The sites of another canes, which are same as insect inoculation site, were chosen. The canes samples were cut into 2 mm thick slices using a scalpel. The samples were paraffin-embedded and sectioned as described previously [11]. A JS5BS stereo microscope was used to observe the samples. Culticle was measured under a microscope at 400-fold magnification. The cell length of pith, cortex and epidermis were measured under a microscope at 40-fold, 100-fold or 400-fold magnification, respectively. The

number of epidermis and cortex cells in an area of 1.5 cm × 1.5 cm was counted under a microscope at ×100 magnification.

RNA extraction and real-time quantitative PCR

Canes that were not infested by *P. pentagona* were used to extract RNA. RNAprep Pure Plant Plus Kit (TIANGEN) was used to isolate total RNA. SYBR Green Pro Taq HS (Accurate Biology) was used to qRT-PCR. The methods were performed as previously described [57]. The sequences of primers used for qRT-PCR are listed in Table S2.

Statistical analysis

All data in our study are presented as the mean ± SD of at least three independent experiments. SPSS software version was used to analyze the data. The Student's t-test was used to calculate the P value and analyze the significant differences between two groups of data. One-way ANOVA was used to calculate the P value and analyze the significant differences between multiple groups of data.

Results

Different survival situations of P. pentagona **in four kiwifruit cultivars**

For the purpose of screening kiwifruit cultivars with excellent *P. pentagona* resistant trait and breeding potential, fruits of fifty kiwifruit cultivars were collected outside and infestation rate by *P. pentagona* was counted respectively in 2008, 2010 and 2011. Considerable variability among cultivars for resistance to *P. pentagona* was revealed (Table S1). *P. pentagona* was not found in "JXFujun-CK-04227", "LC-04285", "SF-2", "CF-3" and "Me05-01" among these years, suggesting their potential resistance to *P. pentagona*. Based on the growth status, "LC-04285" (wild) and "CF-3" (wild) were used for further study. Meanwhile, the other two susceptible cultivars, "DA-7B" (wild) and "Hayward" (commercial), were also tested as controls.

To further investigate the *P. pentagona* resistant trait of these kiwifruit cultivars, the canes of these four cultivars were collected and inoculated with *P. pentagona* indoors. The colonization rate, survival rate and scale area were counted and used as indicators of the *P. pentagona* resistance.(Table 1). The canes infestation by *P. pentagona* was significantly different between resistant cultivars and susceptible cultivars. Average colonization rate was lower in "LC-04285" (9.92%), "CF-3" (7.48%), "DA-7B" (14.37%), and higher in "Hayward" (24.18%). After 40 days incubation, all nymphs in "LC-04285" did not survived. Although particial crawlers in "CF-3" and "DA-7B" survived, the scale area of all crawlers in "CF-3" was less than 1.2 mm^2, investigating the potential inhibition of somatic development by "CF-3". "Hayward" had the highest survival rate of crawlers at 65.2%, and all of the crawlers had a scale area of more than 1.2 mm^2. These results indicated that "LC-04285" and "CF-3" had significant *P. pentagona* resistant trait.

Table 1
Summary of colonization rate, survival rate and scale area.

Species	Colonization rate (%)	Survival rate (%)	Rate of scale area ≥ 1.2 mm^2 (%)
LC-04285	9.92 ± 6.29 a	0.00 ± 0.00 a	0.00 ± 0.00 a
CF-3	7.48 ± 10.69 a	6.40 ± 9.05 a	0.00 ± 0.00 a
DA-7B	14.37 ± 8.51 ab	1.80 ± 2.50 a	1.50 ± 2.12 b
Hayward	24.18 ± 5.97 b	65.2 ± 10.63 b	100.00 ± 0.00 c

Data are represent the mean ± standard errors (n = 3). Values with different lower case letters are significantly ($p < 0.05$) different.

"LC-04285" had thicker cuticle than other kiwifruit cultivars

To investigate the reasons for the difference in resistance to *P. pentagona* among different kiwifruit cultivars, we performed anatomical analysis using paraffin sections of the canes of these four kiwifruit cultivars. As the outermost barrier for plants, cuticle protect plants from biotic stresses [34]. Interestingly, "LC-04285" had thicker cuticle than other cultivars (Fig. 1). Then, we analyzed the expression levels of *AcSHN1*, *AcKCS3*, *AcKCS4*, *AcCER4*, *AcCER5* and *AcGPAT1* in the canes of four kiwifruit cultivars by real-time quantitative PCR (qRT-PCR), which were involved in the synthesis of cuticle. As show in Fig. 2, the expression levels of these genes in "LC-04285" were higher than those of other kivifruit cultivars. These results showed that thicker cuticle conferred greater resistance to "LC-04285" against *P. pentagona*.

"LC-04285" had denser epidermis and cortex than other kiwifruit cultivars

In order to further analyze the relationship between anatomical structures and *P. pentagona* resistance, we counted the number of cell layer, cell number and cell length. No difference in number of cell layer and cell length was detected among these four kiwifruit cultivars (Fig. 3a-c). However, the densities of epidermal and cortical cells in "LC-04285" were higher than the others (Fig. 3a, d). We also analyzed the expression levels of genes related to the formation of the epidermis and cortex. As show in Fig. 4, the expression level of positive regulator *AcSHR1* in "LC-04285" was higher, while the expression levels of nagative regulators, *AcAGO4* and *AcSCL3*, were lower. These results indicated that denser epidermis and cortex protected kiwifruit by preventing *P. pentagona* from sucking plants' sap.

Resistant cultivar "LC-04285" had a stronger response to JA.

Previous studies demonstrated that JA played an important role in pest resistance [22, 35]. Changes in the expression of JA-related genes affected the pest resistant trait of plants [27]. *COI1* was the key gene in response to JA signal. As show in Fig. 5a, the expression levels of *AcCOI1* in "LC-04285" were higher than that of other cultivars. This result indicated that "LC-04285" could response to JA signal fast when being fed by *P. pentagona*. JAZ family genes were plant-specific JA response genes and nagative regulation of JA signal. Suppression of JAZ family genes in plants could enhance the biotic stresses

resistance [36]. In the above research, we had found that “Hayward” had the worst resistance to *P. pentagona* (Table 1). Similarly, the expression levels of *AcJAZ8*, *AcJAZ11*, *AcJAZ12*, *AcJAZ13*, *AcJAZ14* and *AcJAZ15* in “Hayward” were higher than those of other kiwifurit cultivars (Fig. 5b-h). In contrast, these genes were lowly expressed in “LC-04285”, which had strong resistance to *P. pentagona*. The above results indicated that the strong response to JA conferred great *P. pentagona* resistance in resistant cultivar “LC-04285” .

Resistant cultivar “LC-04285” had higher expression levels of SA-related genes.

SA played an important role in pest resistance as it was involved in the inducing of various defensive responses [26]. SA is synthesized via two routes by two crucial enzymes, isochorismate synthase (ICS) and phenylalanine ammonia-lyase (PAL) [19]. To determine whether more SA was synthesized in “LC-04285”, we analyzed the expression levels of SA biosynthesis-related genes. As show in Fig. 6a-c, the expression levels of *AcICS*, *AcPAL4* and *AcPAL5* in “LC-04285” were higher than those in other kiwifurit cultivars. These results indicated that “LC-04285” had more SA accumulation and leaded to *P. pentagona* resistance. Further, NPR genes were SA receptor and regulated some resistant genes’ expression [37]. As show in Fig. 6d-f, the expression levels of *AcNPR1*, *AcNPR2* and *AcNPR3* in “LC-04285” were higher than that in other kiwifurit cultivars. Collectively, the above results indicated that resistant cultivar “LC-04285” possessed a stronger ability to synthesize SA and a higher level of resistance mediated by SA.

Discussion

China is the origin of kiwifruit, and there are a large number of wild kiwifruits in the mountains. For the purpose of screening kiwifruit with insect resistance and breeding potential, we investigated the rate of fruit injury of 336 kiwifruits in 2008, 2010 and 2011, and sellected fifty cultivars with stable tolerance performance to *P. pentagona*. In this study, we selected four representative cultivars for study, including “LC-04285”, “CF-3”, “DA-7B” and “Hayward”. By analyzing their anatomical structure of cane, we found that resistant cultivars, “LC-04285”, had thicker cuticle, denser epidermis and cortex (Fig. 1, 3). The qRT-PCR data indicated that the expression levels of some genes related to cuticle synthesis and formation of epidermis and cortex are higher in “LC-04285” (Fig. 2, 4). Some studies have reported that the cuticle have a certain relationship with plant insect resistance [38, 39]. For example, Kosma et al. found that wheat with intact cuticles was more resistant to Hessian fly larvae [40]. The olive trees with thicker epidermis and cuticle were more resistant to eriophyid mites [41]. As a result, thicker cuticle and denser epidermis and cortex prevent *P. pentagona* from sucking plant juice.

In our research, we found that the “CF-3” had yellow epidermal cells (Fig. 1a). We supposed the cells were rich in flavonoids. Furthermore, the qRT-PCR results show that the expression levels of *AcC4H*, *AcFLS7* and *AcLDOX2* in “CF-3” were higher (Fig. S1). Flavonoids are present in various tissue cells of the plant, including epidermal cells. Among the petal epidermal cells of lisianthus flowers, yellow-colored cells had higher levels of flavonoids [42]. The orange color of tomato fruit was also due to the accumulation of flavonoids [43]. Flavonoids were closely related to plant biological stress. Genistein was a kind of

flavonoid found mainly in legumes, which could help protect legumes from Piezodorus guildiniiIn [44]. Tomatoes with higher flavonoids are more resistant to whitefly Bemisia tabaci [45]. However, it is still necessary to identify the structure and function of the flavonoids in “CF-3” in the future work.

In this study, we also analyzed the expression level of JA/SA-related genes. we found that the genes involed in SA synthesis were expressed higher in “LC-04285”, which has stronger resistance to *P. pentagona* (Fig. 6a). It has been reported that spraying the fruits with certain concentration of exogenous SA can increase the content of the endogenous SA in fruit and the resistance to pathogenes without affecting the quality of fruit [46, 47]. In addition, the expression levels of NPR genes in “LC-04285” are still increased (Fig. 6b-f). NPR family genes are closely related to the response of plants to biological stress [37, 48, 49]. The resistances to various pathogens in different plants are increased through heterologously expressing *NPR1* of Arabidopsis thaliana [50–52]. Studies have shown that overexpression of *NPR1* of Arabidopsis thaliana in tobacco increases the resistance of tobacco to Spodoptera litura [53]. More interestingly, it is known that SA-signaling pathway can be elicited by many sap-sucking insects, and, simultaneously, the JA-signaling pathway is suppressed [54, 55]. However, we found that the expression levels of *AcCOI1* in “LC-04285” were higher than that in other kiwifurit cultivars (Fig. 5a). Furthermore, the expression levels of some JAZ repressors in “Hayward” were higher (Fig. 5b-h). These results indicate that in addition to SA, JA also plays a crucial role in plant resistance to *P. pentagona*. However, the mechanism of JA and SA against *P. pentagona* needs further investigation.

Conclusions

In general, “LC-04285” has a thicker cuticle and denser epidermis and cortex, which can resist the sucking of *P. pentagona*. Furthermore, “LC-04285” has stronger SA/JA synthesis and response ability. These results provide more new ideas for the development of insect resistant cultivars

Abbreviations

FAO: Food and Agriculture Organization of the United Nations; *P. pentagona*: *Pseudaulacaspis pentagona*;JA: Jasmonic acid; SA: Salicylic acid; qRT-PCR: Real-time quantitative PCR; ICS: Isochorismate synthase; PAL: Phenylalanine ammonia-lyase.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare no competing interests.

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Author Contributions

QZ, WD, and XZ designed the experiments. XZ, QL, QZ, and KT performed the experiments. XZ and GW analyzed the data. QZ, WD, and XZ wrote the manuscript text.

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Figures

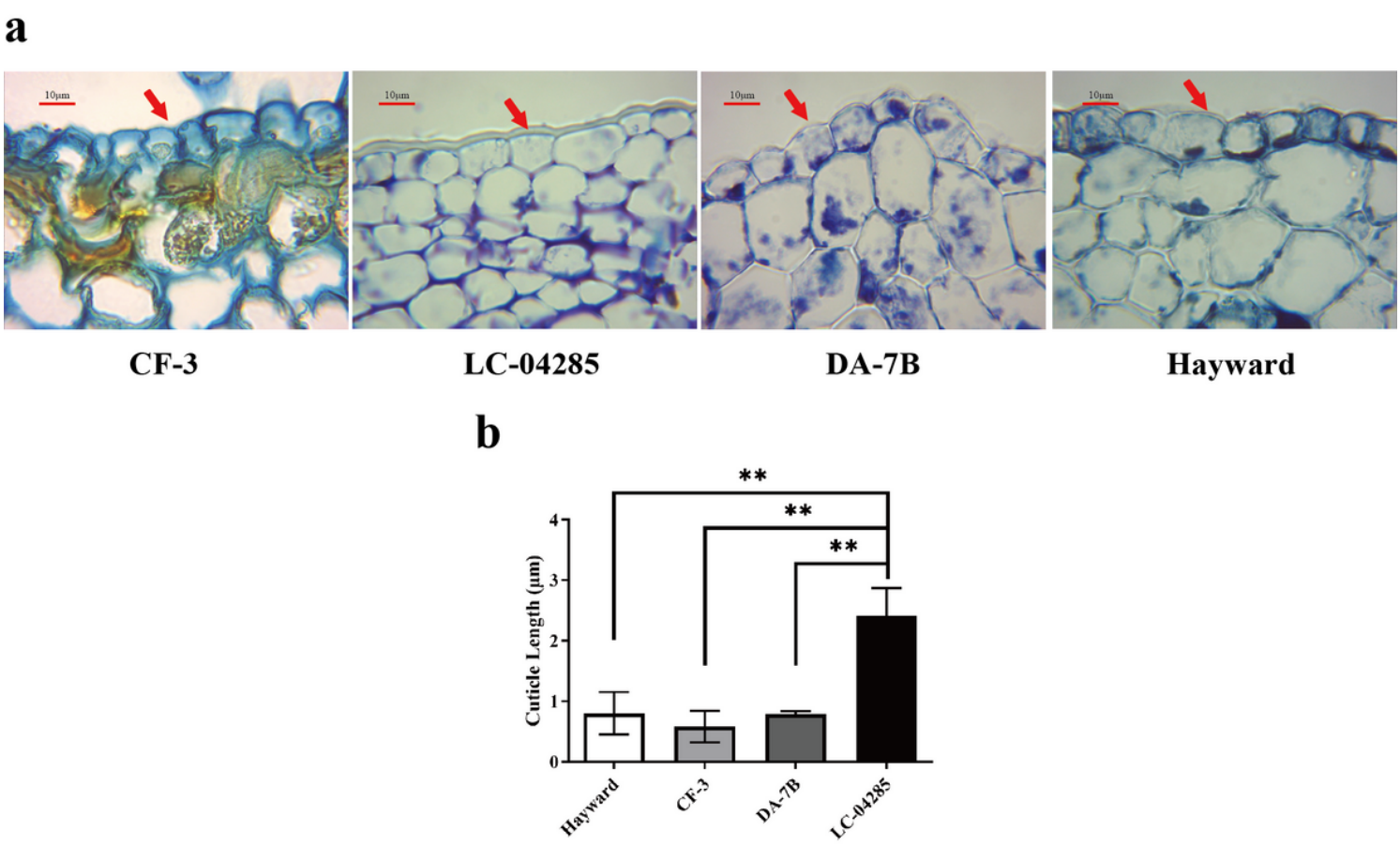

Figure 1

Cuticle and epidermal cells observation of cane cells in different kiwi cultivars. (a) Microscopic structure of paraffin section samples of four kiwifruit species. Red arrows represents the cuticle. (b) Cuticle length of the four kiwifruit species. Data are presented as the mean ±SD of ten independent experiments. Asterisks indicate significant differences between “LC-04285” and other kiwifruit cultivars (**P<0.01), as determined by Student’s t test. Scale bars, 10 μm.

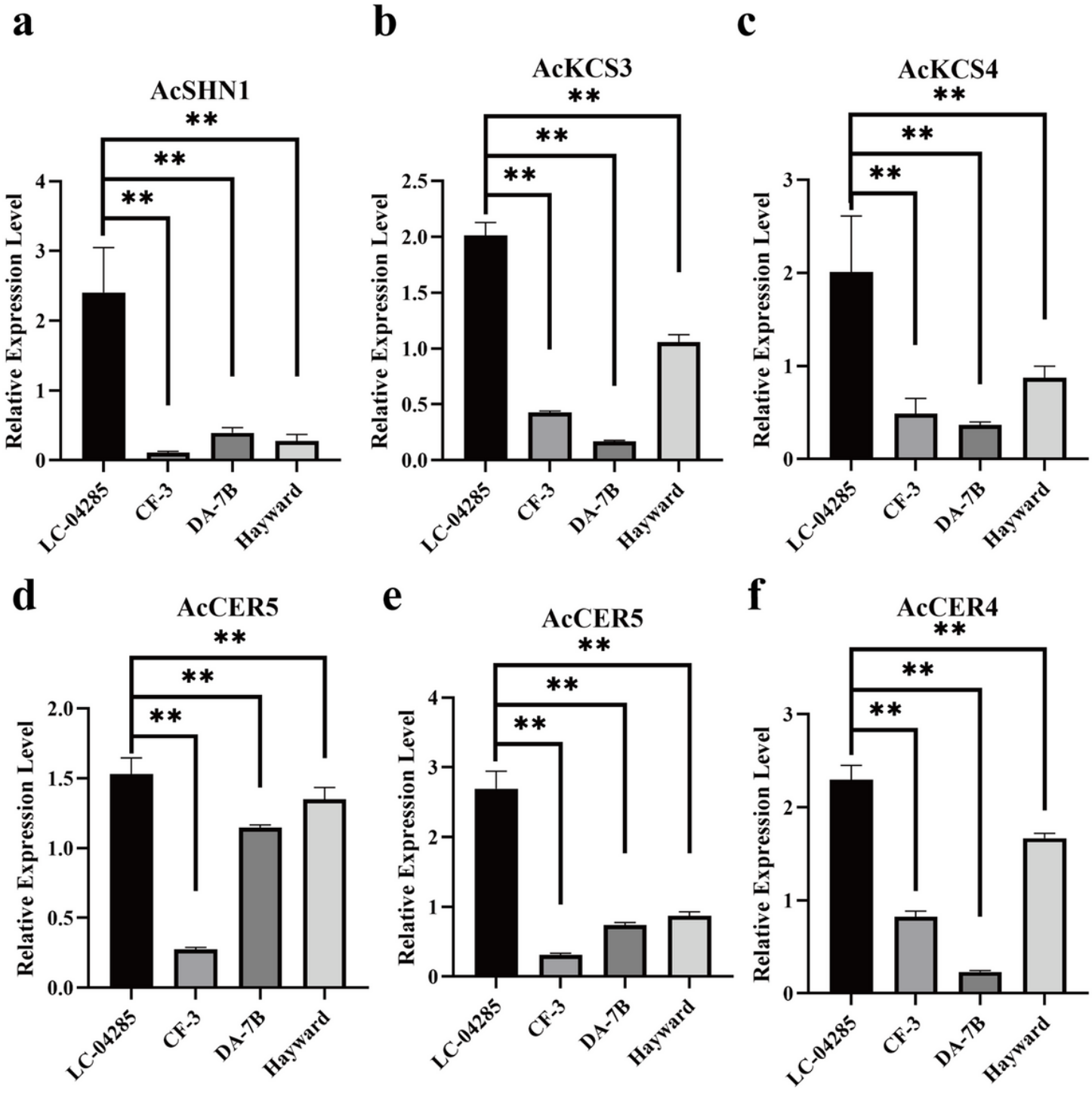

Figure 2

Differential expression levels of cuticle synthesis genes in these four kiwifruit cultivars. Data are presented as the mean ±SD of three independent experiments. Asterisks indicate significant differences between “LC-04285” and other kiwifruit cultivars (**P<0.01), as determined by Student’s t test.

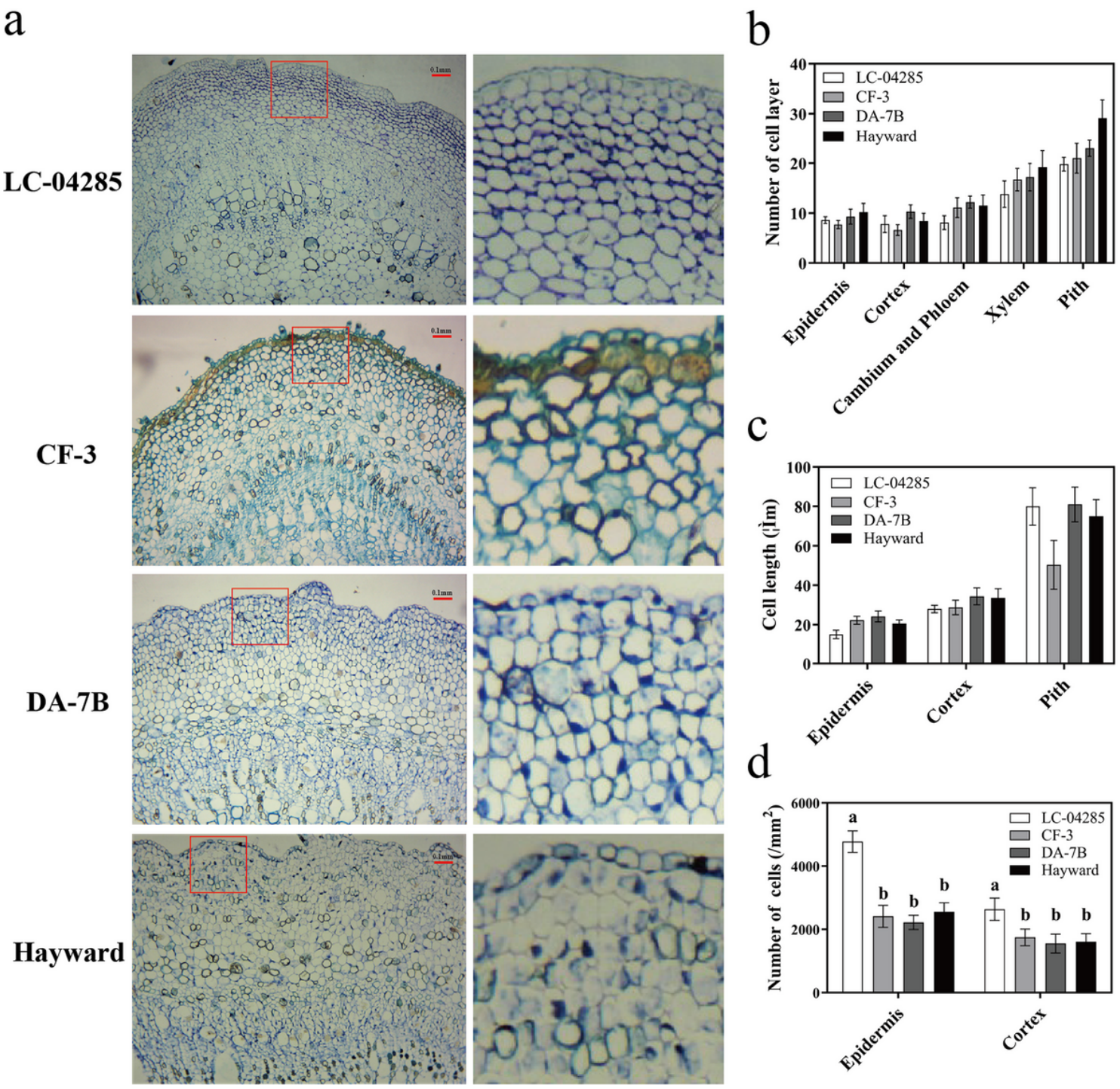

Figure 3

Anatomical Structure Analysis of Cane Cells in Different Kiwi Cultivars. (A) Microscopic structure of paraffin section samples of four kiwifruit species. The red box represents epidermis and cortex in the same area. (B) Number of cell layer of the epidermis, cortex, cambium and phloem, xylem and pith. (C) Cell length of epidermis, cortex and pith. (D) Number of cells of the epidermis and cortex. Different letters indicate significant differences between genotypes at $P < 0.05$. Data are presented as the mean ±SD of ten independent experiments. Scale bars, 0.1 mm.

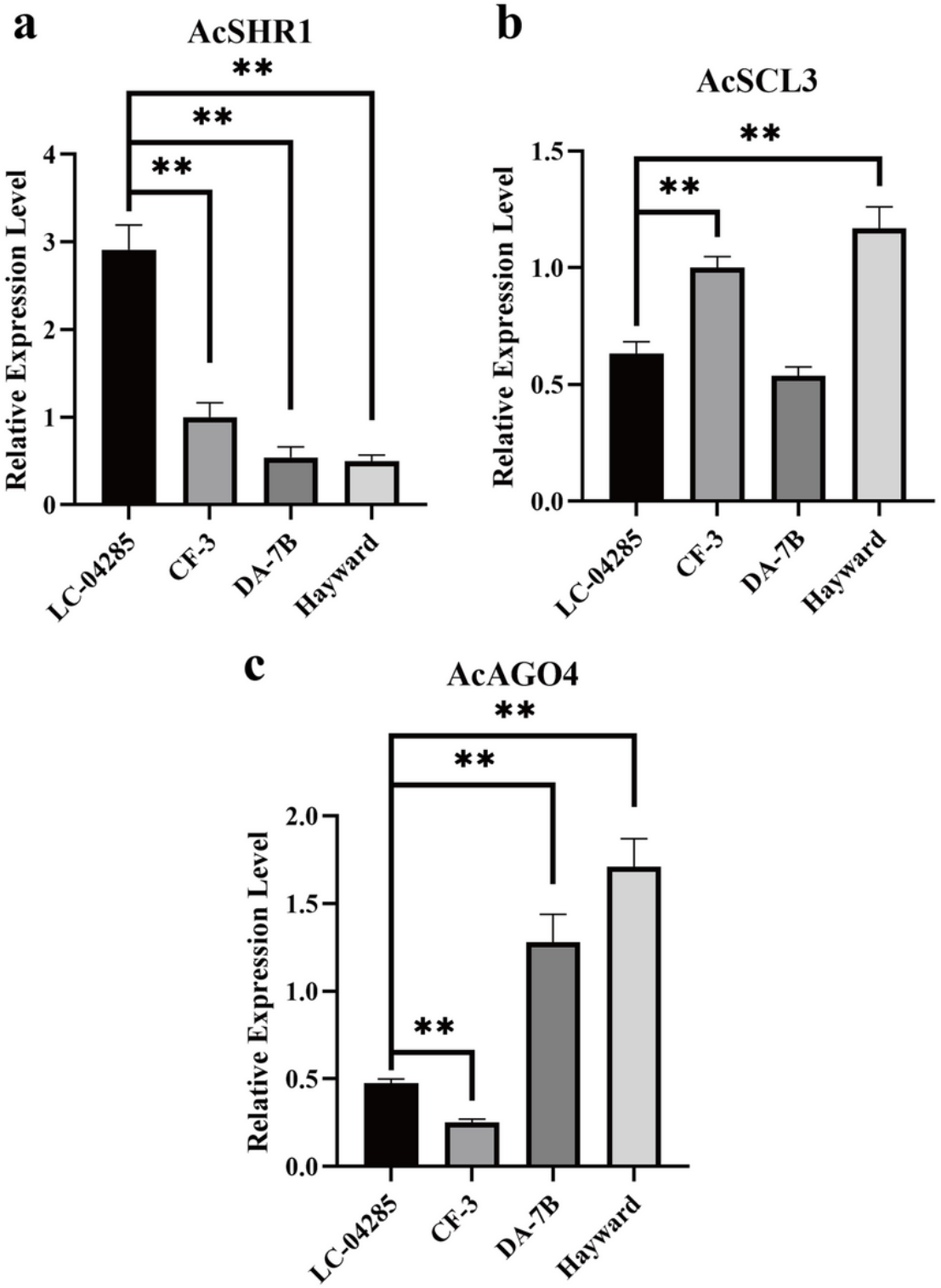

Figure 4

Differential expression levels of genes related to the formation of the epidermis and cortex. Data are presented as the mean ±SD of three independent experiments. Asterisks indicate significant differences between “LC-04285” and other kiwifruit cultivars (**P<0.01), as determined by Student’s t test.

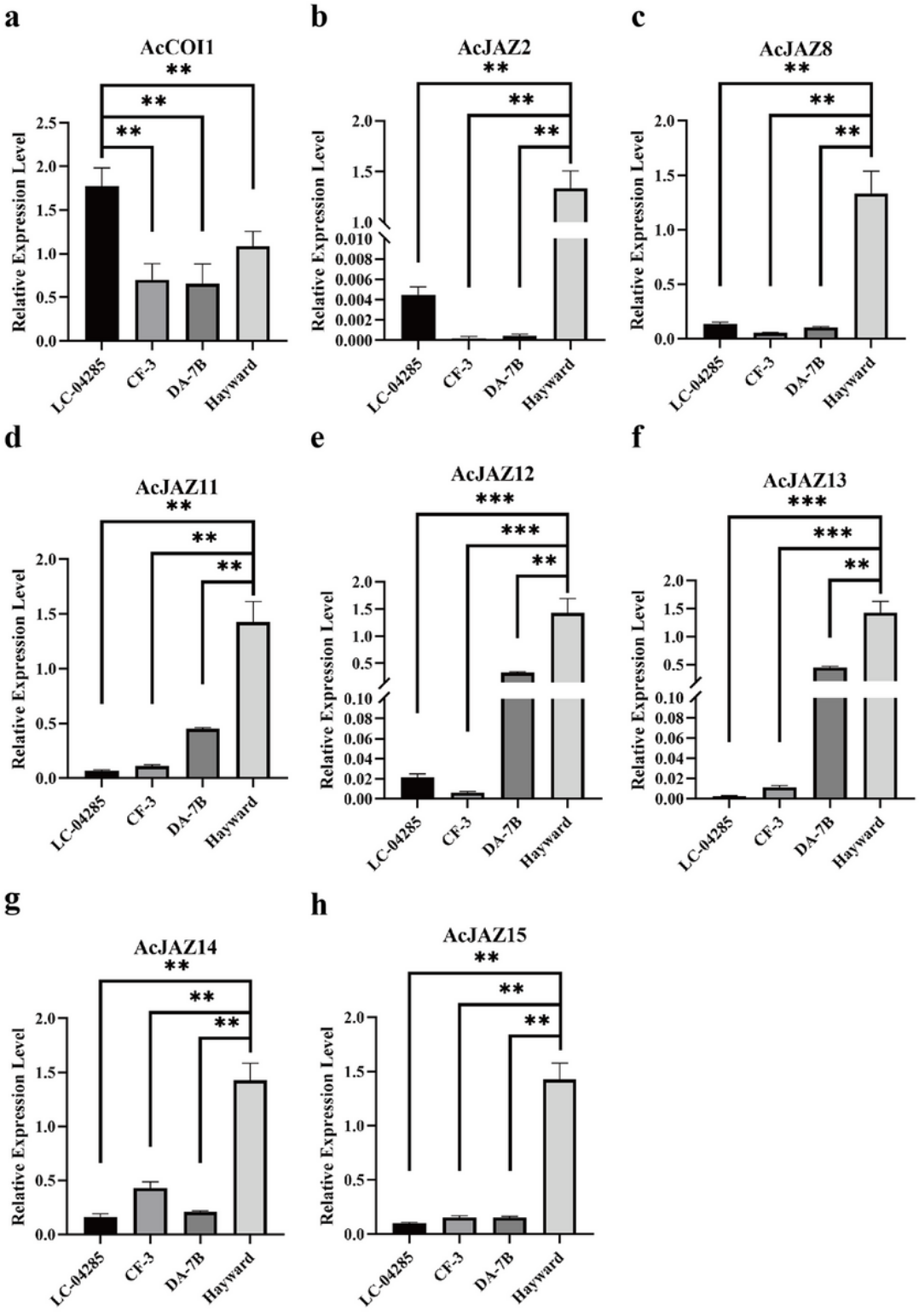

Figure 5

Differential expression levels of JA-related genes in these four kiwifruit cultivars. Data are presented as the mean ±SD of three independent experiments. (A) Asterisks indicate significant differences between “LC-04285” and other kiwifruit cultivars (**P<0.01), as determined by Student’s t test. (B) Asterisks indicate significant differences between “Hayward” and other kiwifruit cultivars (**P<0.01, ***P<0.001), as determined by Student’s t test.

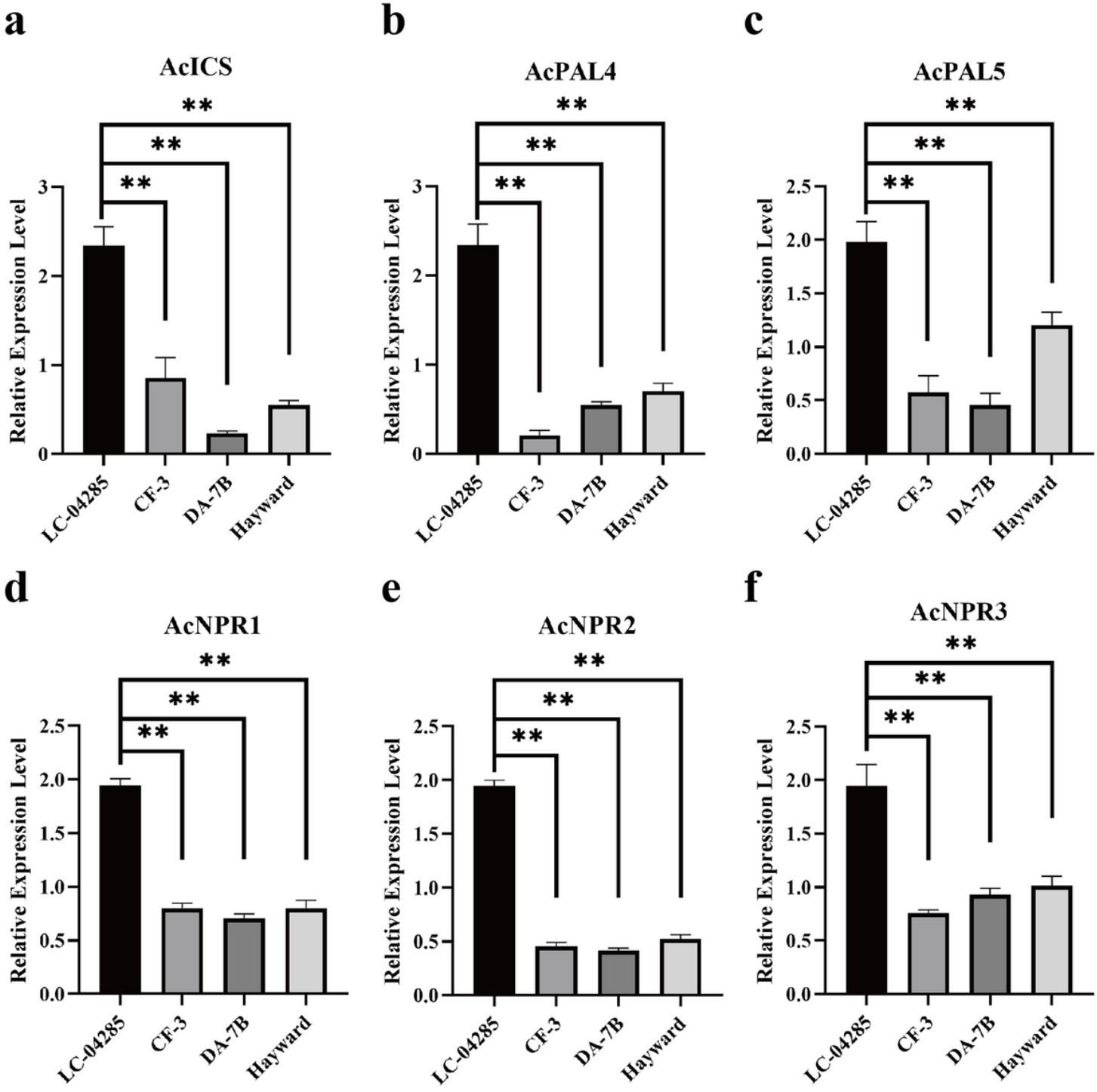

Figure 6

Differential expression levels of SA-related genes in these four kiwifruit cultivars. Data are presented as the mean ±SD of three independent experiments. Asterisks indicate significant differences between “LC-04285” and other kiwifruit cultivars (**P<0.01), as determined by Student’s t test.

Supplementary Files

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