

Article

Comparative Analysis of the Cuticular Wax Morphology, Composition and Biosynthesis in Two Kumquat Cultivars During Fruit Development

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Abstract

Cuticular wax plays an important role in the quality of kumquat (*Fortunella crassifolia* Swingle) fruit. In this study, the wax morphology, compositional profile of epi- and intracuticular wax, and crucial gene expression in ‘Rongan’ kumquat (RAK) and ‘Huapi’ kumquat (HPK) were analyzed during fruit development. The results showed that the surfaces of two kumquat fruits were covered with an amorphous wax layer containing a small number of platelets. Compared to RAK, HPK contained more abundant and larger wax crystals during fruit development. In two kumquat fruits, the epicuticular wax and its major compositions consistently displayed significantly higher levels than the intracuticular wax. Additionally, their main wax composition shifted from alkanes in the early developmental stages to triterpenoids at harvest in both layers, while aldehydes were specifically enriched in the epicuticular wax. During the fruit development from 90 to 180 DAF, HPK fruit exhibited significantly higher levels of epicuticular wax and its majority fractions than RAK fruit. Meanwhile, the intracuticular wax contents of HPK from 90 DAF to 150 DAF were significantly higher than those in RAK, with triterpenoids accounting for the largest proportion of this increase. qRT-PCR results indicated that the up-regulation of wax-related genes in HPK was linked to its increased epicuticular wax deposition during the development. Overall, this study provided a comprehensive overview of the morphology, composition, and biosynthesis of cuticular wax in kumquat fruit during development.

Keywords: fruit development; cuticular wax morphology; wax composition; gene expression



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1. Introduction

The cuticular wax is an important determinant in defending plants against pre- and postharvest stresses [1–3]. In most plants, cuticular waxes are composed of aliphatics (e.g., fatty acids, alkanes, aldehydes, ketones, primary and secondary alcohols) and cyclic compounds (e.g., sterols, triterpenoids, etc.) [4]. Cuticular waxes are divided into two layers: an intracuticular wax embedded within the cutin, and an epicuticular wax located on the surface [5,6]. Epi- and intracuticular waxes differ significantly in composition and function across various plant species. Cyclic components and polar aliphatics are predominantly localized in intracuticular wax, whereas aliphatics are primarily accumulated in epicuticular wax [6–8].

In most species, fruit maturation was accompanied by a sustained increase in cuticular wax deposition, as observed in apple (*Malus domestica*) [9,10], citrus (*Citrus sinensis*) [11,12], bayberry (*Myrica pensylvanica*) [13], and mango (*Mangifera indica*) [14]. However, in many berry species, cuticular wax underwent rapid accumulation during the early developmental stages, while the wax content per unit area declined in later phases due to fruit expansion, such as sweet cherry (*Prunus avium*) [15], grape (*Vitis vinifera*) [16,17], and blueberry (*Vaccinium corymbosum*) [18]. Distinct patterns of cuticular wax accumulation also developed within the same species during fruit development. For instance, the total cuticular wax content in cherry tomato exhibited an early accumulation phase followed by a decline [19]. In contrast, medium-sized cultivars (e.g., ‘Ailsa Craig’ and ‘Micro Tom’) reached peak wax levels at the orange ripening stage and subsequently stabilized [20,21], while ‘RZ 72-00’ demonstrated a continuous increase in wax deposition throughout ripening [22].

The kumquat (*Fortunella crassifolia* Swingle) is the smallest-sized citrus fruit and the only variety within the citrus family characterized by an edible peel [23]. The ‘Huapi’ kumquat (HPK) originated as a seedling mutant of the ‘Rongan’ kumquat (RAK) [24,25]. In our earlier reports, the wax morphology and chemical composition of HPK and RAK during cold storage were investigated [26,27]. Although HPK has a noticeably thinner peel than RAK fruit [27], our prior studies found that HPK exhibited reduced rates of weight loss and decay under cold storage conditions. Further research has demonstrated that the wax crystals of HPK were larger and the total amount of wax was higher throughout cold storage, which contributes to post-harvest storability [27]. Given that kumquats encounter biotic or abiotic stresses, such as water loss and pathogenic microorganisms, during both their pre- and postharvest stages, do HPK and RAK exhibit wax accumulation patterns during development that resemble those observed during storage? To address this question, we evaluated the crystal morphology, the epi- and intracuticular wax compositions, and essential gene expression involved in wax synthesis, export and regulation in HPK and RAK throughout fruit development in this study.

2. Materials and Methods

2.1. Plant Materials

HPK and RAK fruit, free from physical damage, were handpicked from the 13-year-old plants in the same orchard in Guangxi Province, China. Samples were collected at 30-day intervals, starting from the young fruit stage (60 d after flowering, 60 DAF) until fruit maturity (180 d after flowering, 180 DAF). Trees were spaced 2 m × 3.5 m apart, resulting in a planting density of approximately 1400 trees/ha. The orchard management was conducted following the usual cultural practices of the producing region. The annual average precipitation and temperature are 1700~1900 mm and 19 °C, respectively. At every developmental time point, fruit was picked from 5 randomly selected trees, with 10 fruit sampled from each of the 4 principal directions of every tree. After transportation to the laboratory, fruit with uniform size and color was chosen for further study.

2.2. Scanning Electron Microscopy (SEM)

Pericarp pieces of 5 HPK or RAK fruit per replicate at 60, 90, 120, 150 and 180 DAF were sampled by a blade [27]. In total, 3 pericarp pieces (2 mm × 3 mm) of each fruit were used for SEM analysis. The pieces were frozen in liquid nitrogen for 6 h. The freeze-dried samples were sputter-coated with gold particles using Eiko IB-3 (Tokyo, Japan) and subsequently examined and photographed with a JEOL JSM-840 SEM (Tokyo, Japan).

2.3. Epicuticular and Intracuticular Wax Extraction and Analysis

In total, 30 fruits at each of the 5 developmental stages were used for the extraction of epi- and intracuticular wax. Epicuticular wax was extracted using gum arabic (Sigma-Aldrich, Saint Louis, MO, USA) and chloroform (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) [28]. Briefly, the 90% gum arabic aqueous solution was used as an adhesive to remove the epicuticular wax. After drying for about 5 h, the polymer film was delaminated into pieces and extracted twice in chloroform and water (3:1, *v/v*). And the kumquat surface area was calculated by $S = (M1 \times D)/M2$, where M1 represented the weight of the fruit pericarp, D denoted the area of the twenty disks (13 mm diameter), and M2 indicated the weight of the twenty disks [29]. Following the removal of epicuticular wax, intracuticular wax was extracted twice with chloroform as previously described [27]. The wax constituents were identified and quantified using GC-MS as described by Lü et al. [30]. The wax composition content was quantified by determining GC-MS peak areas relative to the internal standard n-tetracosane.

2.4. Gene Expression Analysis

A total of 30 RNA samples were extracted from flavedo tissues of HPK and RAK fruit with FastPure Universal Plant Total RNA Isolation Kit (Cat. RC411, Vazyme, Nanjing, China), and the synthesis of first-stand cDNA was performed using PrimeScript™ FAST RT reagent Kit with gDNA Eraser (Cat. RR092A; Takara Bio, Dalian, China), according to the manufacturer's instructions. qRT-PCR was conducted utilizing TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) Mix (Cat. RR820A; Takara Bio, Dalian, China) on a Bio-Rad CFX96 real-time PCR system (Berkeley, CA, USA) with 3 biological replicates. The 10 µL reaction system and the qPCR cycling conditions were performed according to Zhao et al. [31]. Primers were designed based on the Hong Kong kumquat (*Fortunella hindsii*) genome (<http://citrus.hzau.edu.cn>, accessed on 26 August 2024) using the Primer 5.0 software (Premier, Irvine, CA, USA). The primer sequences are shown in Table S1. All results were normalized to the citrus β-actin gene.

2.5. Statistical Analysis

The results were shown as mean ± standard error from 3 replicates. All statistical analyses were conducted using SPSS 22.0. A Student's *t*-test was applied to assess the cultivar effect on wax composition and wax-related gene transcription at each developmental stage.

3. Results

3.1. Crystal Morphology of HPK and RAK Fruit During Development

Developmental dynamics of fruit appearance of HPK and RAK cultivars were presented in Figure 1A. At 60 DAF, the surfaces of HPK and RAK fruit were covered with an amorphous wax layer containing a small number of platelets (Figure 1B). During fruit development, two kumquat cultivars exhibited progressive accumulation of cuticular wax crystals on their fruit surfaces. Notably, the wax crystals on the surface of HPK fruit were larger and more densely packed compared to RAK fruit during fruit development.

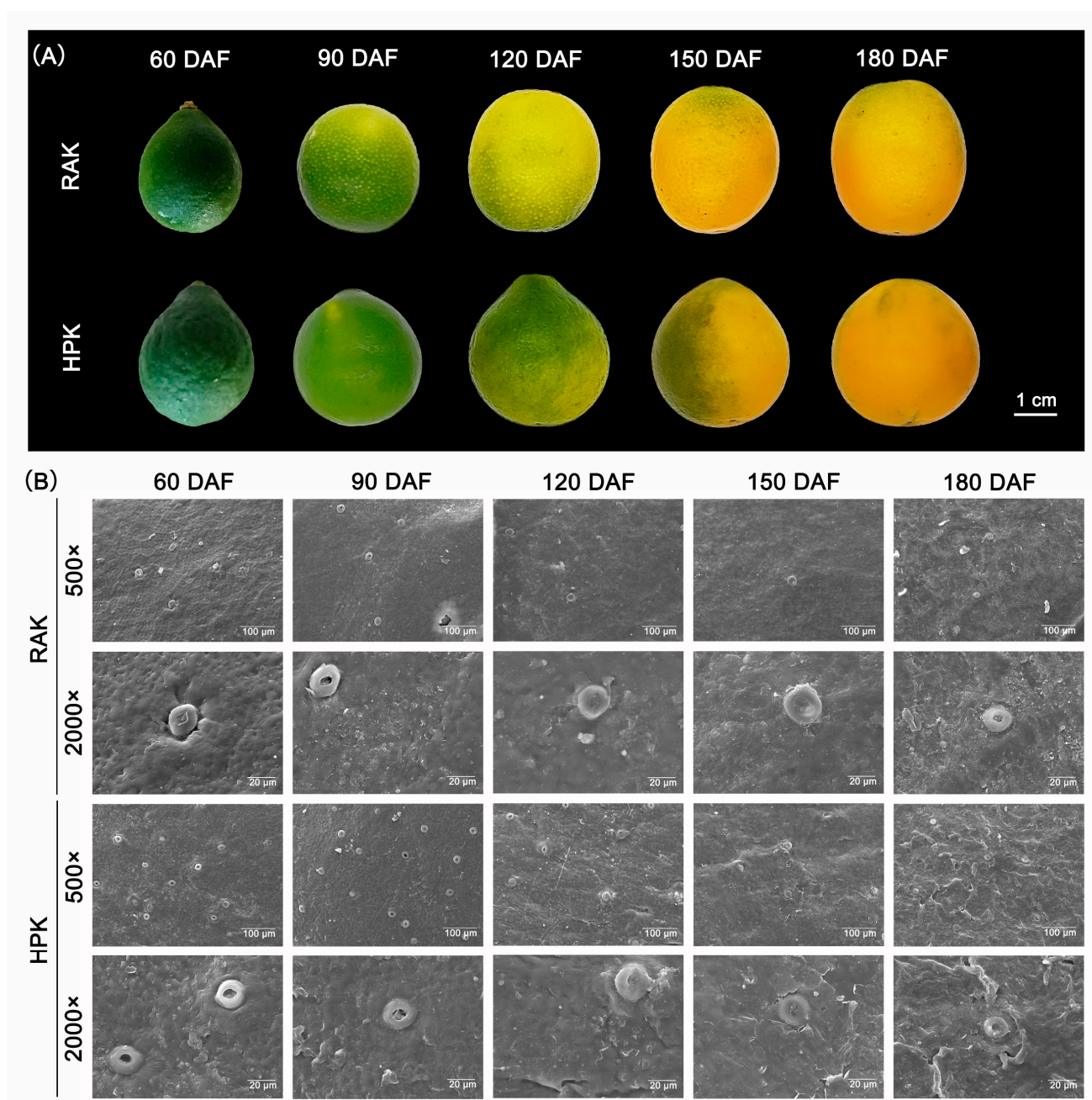


Figure 1. Changes in HPK and RAK phenotype and wax morphology at 5 developmental stages. (A) Fruit phenotype; (B) wax morphology.

3.2. Changes in the Epicuticular Wax Compositions During Fruit Development

Epicuticular wax accumulation on HPK fruit showed an overall upward trend during fruit development, reaching $30.97 \mu\text{g}/\text{cm}^2$ at harvest (180 DAF) (Figure 2A). Differently, epicuticular wax content on RAK fruit demonstrated a decreasing trend from 60 to 120 DAF, followed by a significant increase beginning at 150 DAF, ultimately reaching $21.08 \mu\text{g}/\text{cm}^2$ at harvest (180 DAF) (Figure 2A). The epicuticular waxes of both kumquats were composed of fatty acids, primary alcohols, alkanes, aldehydes, sterols, and triterpenoids. With the exception of aldehydes, sterols and triterpenoids in RAK, as well as alkanes in HPK, the contents of the identified wax compositions in both cultivars had the same changing trends as the total epicuticular wax during the fruit development (Figure 2B–G).

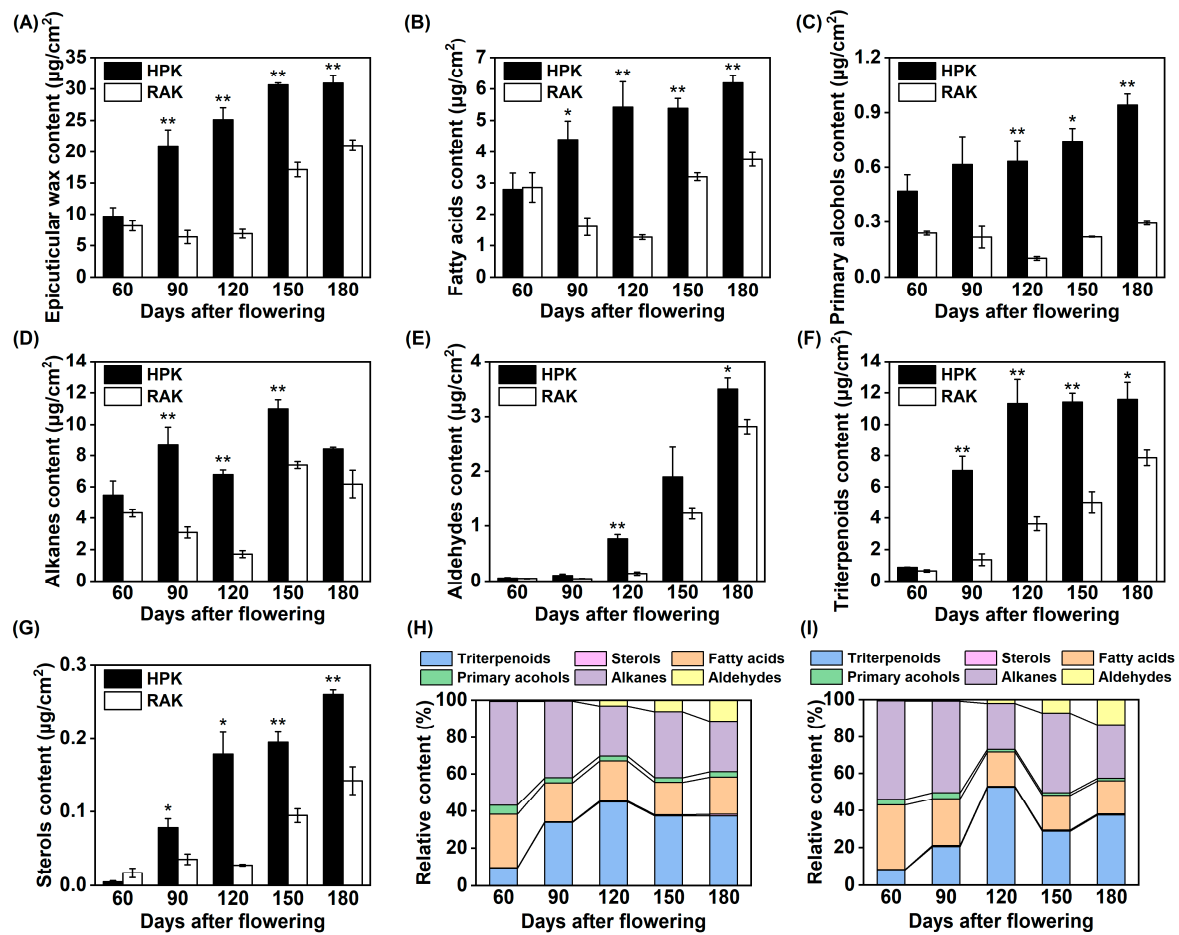


Figure 2. Total epicuticular wax content, fractional abundances, and relative proportions in HPK and RAK at 5 developmental stages. (A) Epicuticular wax; (B) fatty acids; (C) primary alcohols; (D) alkanes; (E) aldehydes; (F) triterpenoids; (G) sterols; (H) the relative percentage (%) of different epicuticular wax composition in HPK; (I) the relative percentage (%) of different epicuticular wax composition in RAK. Data represent mean $\pm$ SE ($n = 3$). Asterisks identify statistical differences (* $p < 0.05$, ** $p < 0.01$).

The change trends of relative percentages (%) of epicuticular wax fractions during fruit development were remarkably similar between HPK and RAK (Figure 2H,I). At 60, 90 and 150 DAF, alkanes were the dominant fraction, accounting for 35.84%~56.38% of the total epicuticular wax in HPK and 49.63%~53.44% in RAK, respectively. At 120 and 180 DAF, triterpenoids emerged as the dominant wax fraction, accounting for 37.37%~44.79% of the total epicuticular wax in HPK and 37.41%~52.58% in RAK, respectively. The relative percentages of fatty acids and primary alcohols decreased obviously from 60 DAF to 120 DAF, whereas those of aldehydes and sterols increased significantly from 120 DAF to 180 DAF.

During fruit development, except for 60 DAF, the epicuticular wax contents of HPK were significantly higher than those on RAK fruit, with a range of 1.47- to 3.29-fold (Figure 2A). At 60 DAF, both the contents of total epicuticular wax and individual compositions exhibited no significant differences between HPK and RAK. In the following stages of fruit development, the total epicuticular wax content and most fractions were much higher in HPK fruit relative to RAK fruit, apart from the amounts of primary alcohols on 90 DAF, alkanes on 180 DAF and aldehydes on 90 and 150 DAF (Figure 2B–G).

3.3. Changes in the Epicuticular Wax Constituents During Fruit Development

A total of 31 epicuticular wax constituents were detected on the HPK and the RAK fruit at all five development stages, including 10 saturated fatty acids (C16–C34), 2 unsaturated fatty acids (C18:2 and C18:3), 3 primary alcohols (C18, C28, and C32), 9 alkanes (C23–C33), 4 aldehydes (C26, C28, C30, and C32), 1 sterol (stigmasterol) and 2 triterpenoids (α -amyrin and β -amyrin) (Figure 3). Among the fatty acids, the C16, C18, and C28 compounds showed higher abundance. Similarly, the C27, C29, and C31 alkanes, along with C28 primary alcohol and C28 aldehyde, were predominant within their respective groups. At 60 DAF, no significant differences were analyzed in the contents of epicuticular wax constituents between HPK and RAK fruit, except for C18:3 fatty acid and C23 alkane. From 90 DAF to 180 DAF, HPK fruit accumulated higher amounts of C32 fatty acid, C32–C33 alkanes, α -amyrin, and stigmasterol compared to RAK fruit.

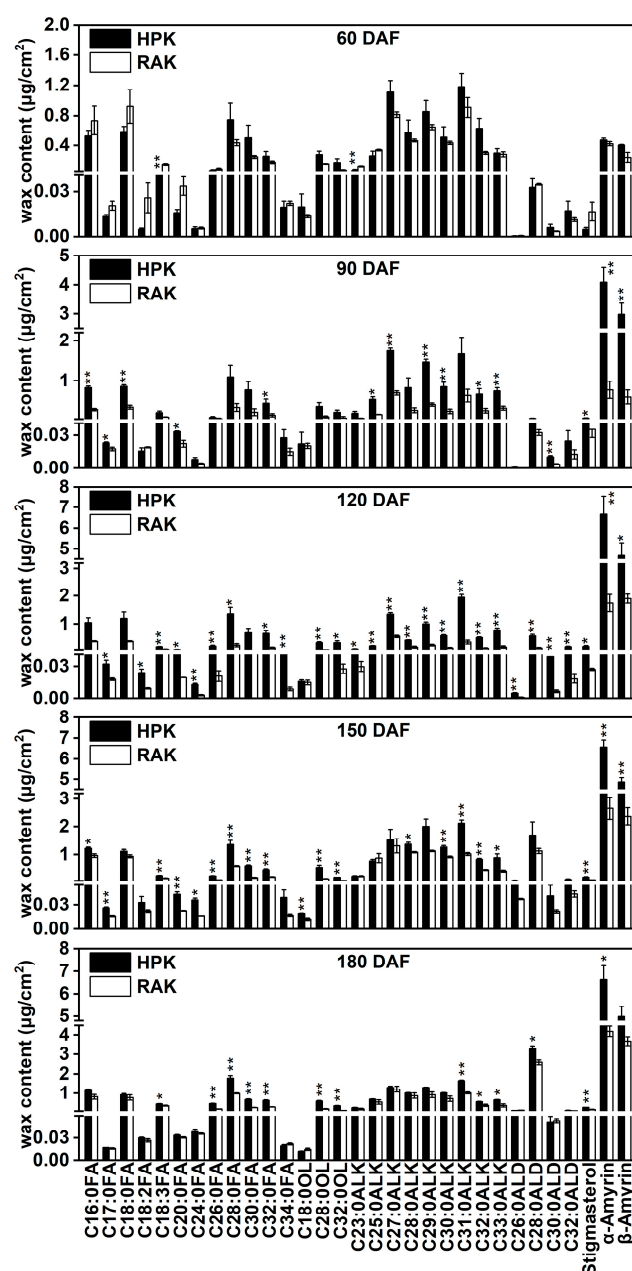


Figure 3. Concentrations of the epicuticular wax constituents of HPK and RAK at 5 developmental stages. FA: fatty acids; OL: primary alcohols; ALK: alkanes; ALD: aldehydes; data represent mean $\pm$ SE (n = 3). Asterisks identify statistical differences (* p < 0.05, ** p < 0.01).

3.4. Changes in the Intracuticular Wax Compositions During Fruit Development

The total intracuticular wax content in HPK exhibited minimal variation throughout fruit development, measuring $6.54 \mu\text{g}/\text{cm}^2$ at harvest (180 DAF) (Figure 4A). In RAK fruit, the intracuticular wax content decreased from 60 DAF to 120 DAF, followed by an increase from 120 DAF to 180 DAF, ultimately reaching $6.57 \mu\text{g}/\text{cm}^2$ at harvest (Figure 4A). Unlike epicuticular wax, aldehydes were not detected in the intracuticular wax of either kumquat cultivar.

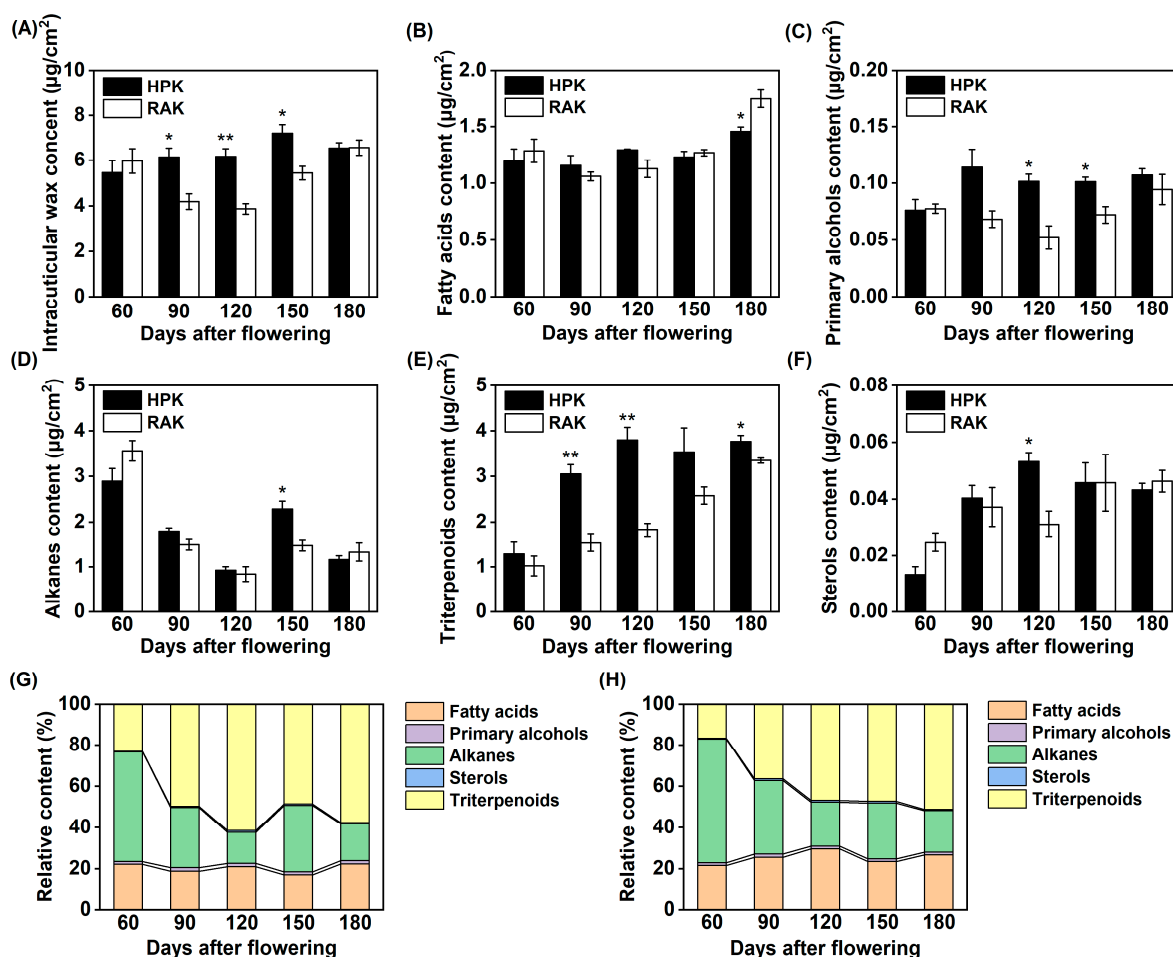


Figure 4. Total intracuticular wax content, fractional abundances, and relative proportions in HPK and RAK at 5 developmental stages. (A) Intracuticular wax; (B) fatty acids; (C) primary alcohols; (D) alkanes; (E) triterpenoids; (F) sterols; (G) the relative percentage (%) of intracuticular wax compositions in HPK; (H) the relative percentage (%) of intracuticular wax compositions in RAK. Data represent mean $\pm$ SE ($n = 3$). Asterisks identify statistical differences (* $p < 0.05$, ** $p < 0.01$).

At 60 DAF, alkanes were identified as the predominant fractions in intracuticular wax, accounting for 53.09% in HPK and 60.00% in RAK (Figure 4G,H). From 90 DAF to 180 DAF, triterpenoids were the major intracuticular wax fraction, accounting for 48.66%~61.45% in HPK and 36.35%~51.17% in RAK. The relative content (%) of fatty acids in HPK was 17.13%~23.31% during fruit development, and 21.58%~29.58% in RAK. Primary alcohols and sterols each constituted approximately 2% of the total intracuticular wax, indicating their minimal abundance.

During fruit development (90~150 DAF), the intracuticular wax content of HPK fruit was about 1.5-fold greater than that of RAK fruit. Further analysis was conducted to elucidate the differences in the accumulation of each wax fraction between HPK and RAK fruit. At 90, 120 and 180 DAF, HPK fruit accumulated significantly higher triterpenoid

content compared to RAK fruit (Figure 4E). In addition, HPK fruit exhibited significantly higher contents of primary alcohols at 120 and 150 DAF, alkanes at 150 DAF, and sterols at 120 DAF compared to RAK fruit (Figure 4C,D,F).

3.5. Changes in the Intracuticular Wax Constituents During Fruit Development

A total of 26 intracuticular wax constituents were detected on HPK and RAK fruit during fruit development (Figure 5). Compared with epicuticular wax, intracuticular wax constituents were devoid of all aldehydes and C34 fatty acid. Meanwhile, the quantity of wax constituents with varying wax contents in intracuticular wax was lower between the two kumquat cultivars during fruit development. Most types of differential fatty acids observed during fruit development were found to have lower contents in HPK than in RAK. Conversely, the amount of α -amyrin in HPK was significantly higher than that in RAK at 90, 120 and 180 DAF, while the β -amyrin content was higher at 90 and 120 DAF.

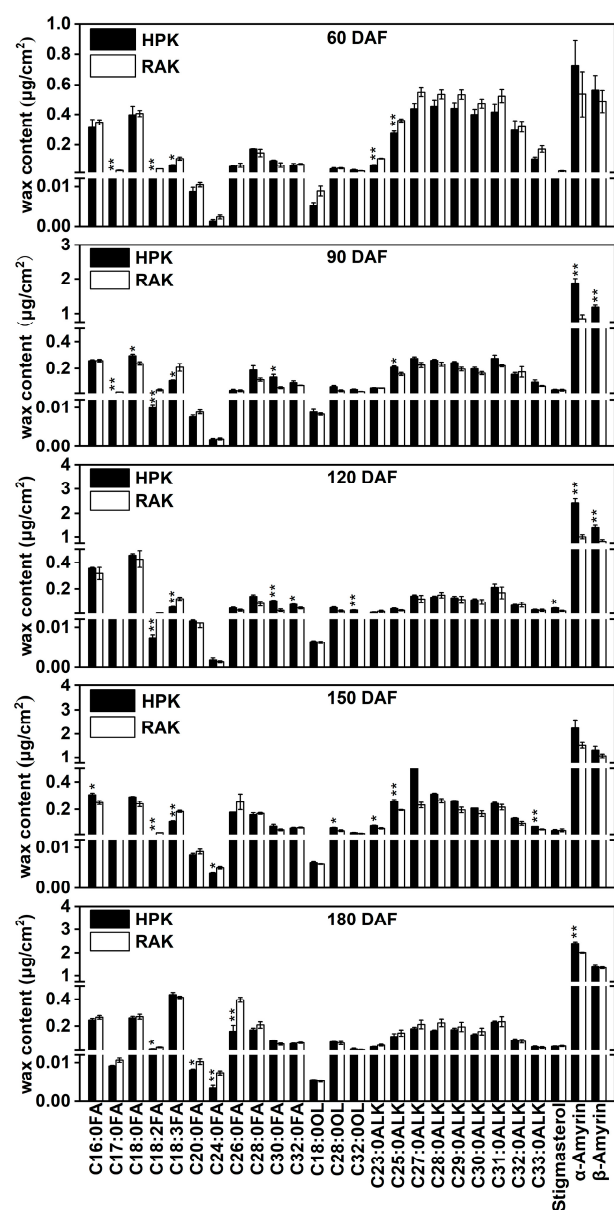


Figure 5. Concentrations of the intracuticular wax constituents of HPK and RAK at 5 developmental stages. FA: fatty acids; OL: primary alcohols; ALK: alkanes; each data is presented as mean $\pm$ SE (n = 3). Asterisks identify statistical differences (* $p < 0.05$, ** $p < 0.01$).

3.6. Expression Patterns of Genes Involved in Wax Formation During Fruit Development

During the fruit development of both HPK and RAK, genes participating in wax synthesis, transport, and regulation displayed distinct expression patterns (Figures 6 and S1). Most genes in HPK fruit exhibited a progressively upregulated expression pattern throughout the development period. However, the expression levels of *LACS4*, *CER4*, *FAR2*, *CER1*, *LUP4* and *ABCG11* decreased at 120 DAF and/or 180 DAF in HPK. In RAK, the transcriptional levels of six genes, including *CER3*, *LUP2*, *LUP4*, *ABCG11*, *MYB94* and *MYB96*, exhibited an increasing transcription pattern throughout fruit development. In contrast, the expression levels of other genes were down-regulated from 60 DAF to 120 DAF (Figure S1). It was noteworthy that the transcriptional levels of the majority of wax-related genes in HPK were significantly elevated compared to those in RAK throughout the development period (Figure 6). In this study, the epicuticular wax contents of HPK and RAK constituted 60% to 80% of the total wax contents. Therefore, we performed a joint analysis of the epicuticular wax fractions and the transcriptional levels of wax-related genes (Figure 7). The results suggested that the upregulation of wax metabolism genes in HPK might lead to an increased accumulation of each epicuticular wax fraction relative to RAK.

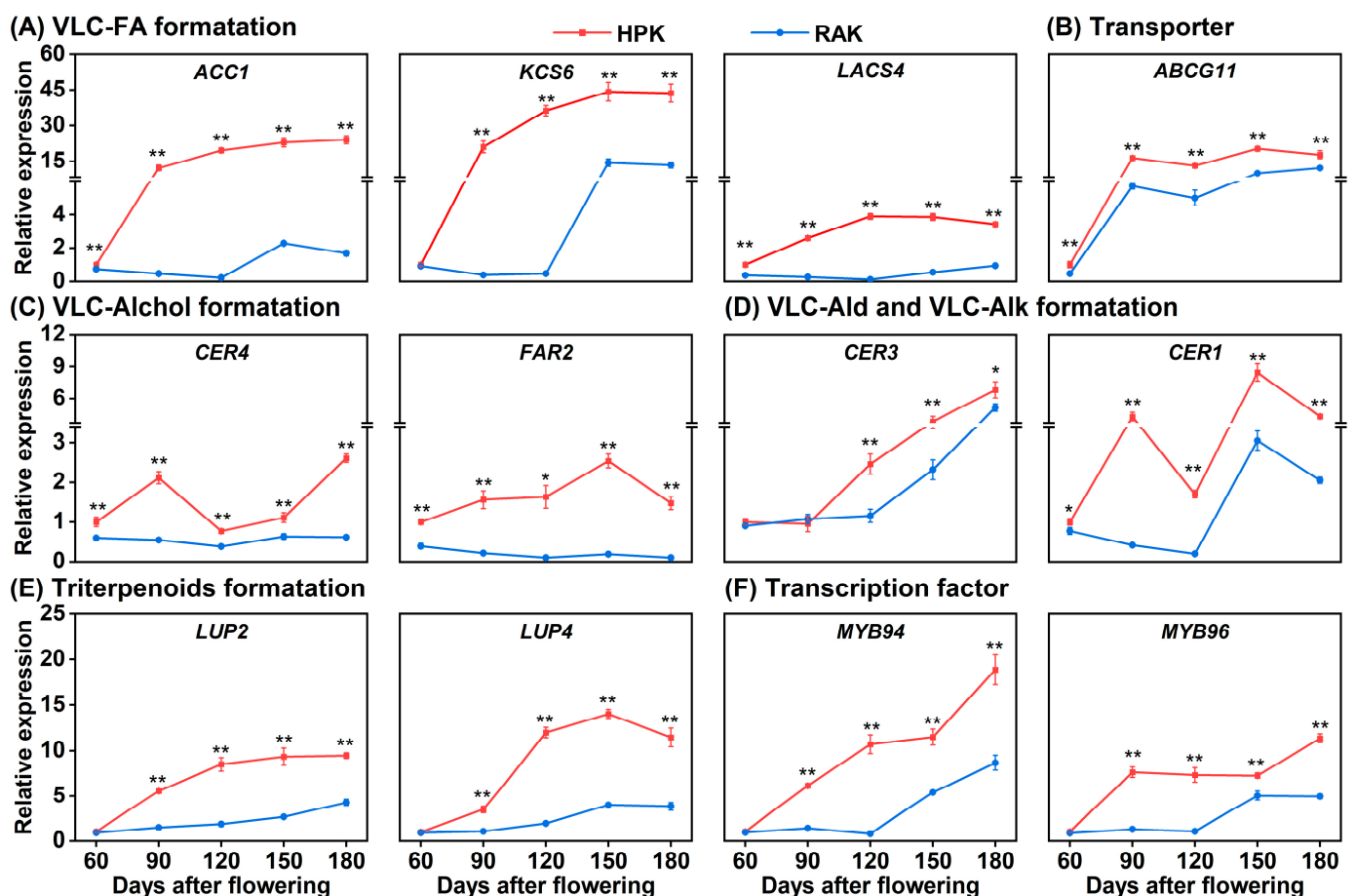


Figure 6. Expression analysis of wax-related genes in HPK and RAK at 5 developmental stages. The expression of genes for VLC-FA formation (A), wax transport (B), VLC-alcohol formation (C), VLC-aldehyde and VLC-alkane formation (D), triterpenoid formation (E), and transcription factor (F) was analyzed by qRT-PCR. Expression of each gene at 60 DAF in HPK was normalized to 1 for comparing gene expression. Each data point is presented as mean $\pm$ SE (n = 3). Asterisks identify statistical differences (*p < 0.05, **p < 0.01).

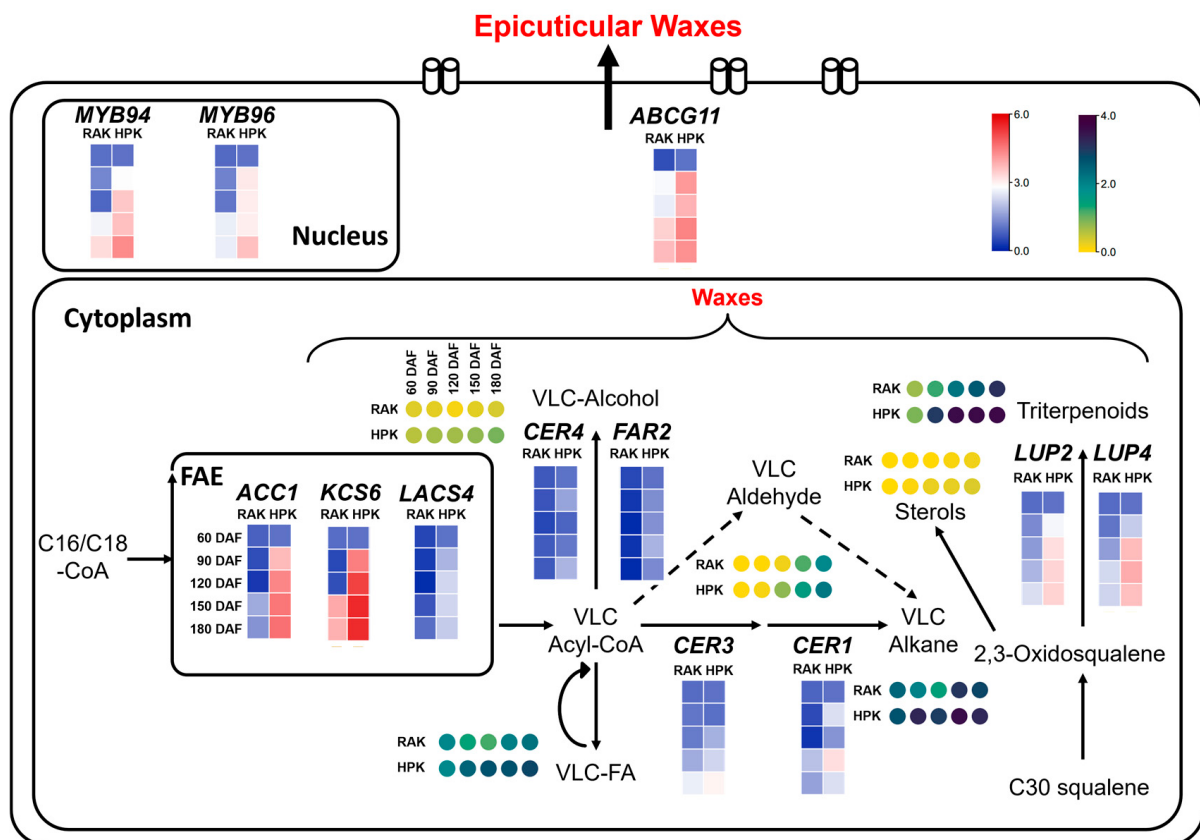


Figure 7. Heatmap of the epicuticular wax fraction content and the transcriptional levels of wax-related genes in HPK and RAK during development. The square boxes represent the transcription levels of genes related to wax metabolism in HPK and RAK. Circles indicate the wax fraction levels in HPK and RAK.

4. Discussion

A total of six epicuticular wax fractions and five intracuticular wax fractions were detected in HPK and RAK throughout fruit development (Figures 3 and 5). In citrus, the relative abundances and compositional profiles of cuticular wax exhibit significant varietal divergence [32–35]. Our prior investigations found that triterpenoids were the principal composition of kumquat fruit cuticular wax during postharvest [26,27]. In this study, triterpenoids have been further confirmed as the main components of both epi- and intracuticular wax layers at harvest. Notably, we found that this situation was not constant across the entire fruit development period. The proportions of alkanes in both epi- and intracuticular wax of HPK and RAK fruit were higher than those of triterpenoids at the early developmental stage (Figures 2 and 4). This pattern is similar to the developmental shift reported in ‘Newhall’ navel orange and its glossy mutant, where the predominant fraction of intracuticular wax shifted from fatty acids to triterpenoids [28].

The epicuticular wax plays a vital role in wax crystal formation, protecting the plant surface against pathogen infestation, insect attacks, ultraviolet radiation, and enhancing storage performance [5,36,37], while intracuticular wax contributes to the transpiration barrier and reduces microscopic cracking [38–41]. HPK exhibits fewer oil glands and lower essential oil content in fruit [24,42]. This characteristic makes HPK more susceptible to insect infestation during development [25]. In this study, it was found that the HPK fruit surface accumulated a higher content of total epicuticular wax and most of its compositions compared to RAK during fruit development (Figure 2). In addition, HPK displayed significantly higher intracuticular wax content than RAK during the fruit expansion and

ripening color transition stages (90 DAF to 150 DAF) (Figure 4). We speculate that the significant increase in cuticular wax on HPK fruit surfaces compared to RAK may represent an adaptive or compensatory mechanism. On one hand, it serves to fill the surface voids resulting from the oil gland reduction; on the other hand, it may offset the potential weakening of resistance functions caused by the decreased essential oil content.

Epicuticular wax exhibited accelerated accumulation compared to intracuticular wax during the fruit development of HPK and RAK, with its content at harvest reaching levels 4-fold higher than those observed in the intracuticular wax layer. Furthermore, the contents of most epicuticular wax compositions were also markedly higher than those in intracuticular wax across the entire developmental stages (Figures 2 and 4). The phenomenon of wax being enriched at higher concentrations in the epicuticular layers was also found in different organs of most species [8]. The close correlation between wax crystal formation and total wax content in kumquats has been confirmed in our previous storage study [27]. But wax crystals are microscopic structures formed by epicuticular wax [43]. In this study, the accelerated development of more and larger wax crystals on HPK fruit, compared with RAK, was consistent with its notably higher deposition of total epicuticular wax from 90 to 180 DAF (Figure 2). The specific chemical composition of epicuticular wax determines the morphology of its wax crystals. The platelet-like wax crystals on the citrus fruit surface have been reported to correlate with the enrichment of aldehydes and alkanes [12,44]. Since the contents of C32 and C33 alkanes in HPK were higher than those in RAK from 90 DAF to 180 DAF (Figure 3), we speculate that these two substances are related to the platelet-like wax crystal structure in kumquat. Additionally, four aldehyde constituents that were specifically enriched in the epicuticular wax may also provide evidence of their influence on the epidermal waxy crystals in the kumquat.

Integrated analysis revealed that wax-related genes exhibited a stronger correlation with epicuticular wax contents. The aliphatic compounds of kumquat include fatty acids, aldehydes, primary alcohols and alkanes. *ACC1*, *KCS6* and *LACS4* encode key enzymes that participate in fatty acid synthesis [45–47]. In the decarbonylation branch, the higher expression of *CER3* promoted the synthesis of aldehydes in the epicuticular wax of citrus [44]. Additionally, the heterologous expression of *CsCER1* enhanced the alkanes biosynthesis in *Arabidopsis* leaves [48]. In the acyl reduction branch, *FAR2* and *CER4* were characterized in the synthesis of primary alcohols [49]. In HPK and RAK, the dynamic transcription patterns of these seven genes (except for *CER4* in HPK) mirrored the changing trends of their corresponding epicuticular wax fractions in both cultivars throughout development. Moreover, the differential expression levels of the above genes related to aliphatic synthesis between HPK and RAK fruit were largely in agreement with the differences in corresponding wax composition accumulation (Figure 7). Therefore, we speculate that these genes contribute to the target wax composition biosynthesis during kumquat fruit development.

The oxidosqualene cyclases (OSCs) act as the rate-limiting enzymes in the anabolic pathways of triterpenoid backbones [50]. In *Arabidopsis*, the OSC gene *AtLUP4* and *AtLUP2* encoded monofunctional and multifunctional triterpene synthases, respectively [51]. The transcript levels of *LUP2* and *LUP4* showed an overall increasing trend with two kumquat maturation stages, except for *LUP4*, which decreased in HPK at 180 DAF. Moreover, except at 60 DAF, HPK demonstrated markedly greater transcript abundance of *LUP2* and *LUP4* compared to RAK. These findings may explain the rapid accumulation of triterpenoids during kumquat development, with a greater deposition observed in HPK fruit.

MYB94 and MYB96, two R2R3-MYB transcription factors, positively regulated wax biosynthesis genes in both *Arabidopsis*, citrus and other plants [52,53]. In this study, *MYB94* and *MYB96* were significantly up-regulated starting from 90 DAF in HPK and 150 DAF in RAK, respectively, which were in agreement with the changes in epicuticular wax amounts

in the two cultivars during development. ABCG11 has emerged as a key component mediating wax transport [54]. Meanwhile, compared to RAK, *ABCG11*, *MYB94* and *MYB96* showed much higher expression levels in HPK during fruit development, except at 60 DAF, which may explain the more accumulation of epicuticular and total wax in HPK.

5. Conclusions

In this study, two kumquat fruit surfaces were found to have similar waxy crystal structures and wax compositions throughout fruit development. Epicuticular wax demonstrated an accelerated accumulation compared to intracuticular wax, with its content at harvest reaching levels four times higher than those observed in the intracuticular wax layer. The main wax composition shifted from alkanes in the early developmental stages to triterpenoids at harvest in both layers, while aldehydes were specifically enriched in the epicuticular wax. In fruit development, HPK fruit surface contained more abundant and larger platelet-wax crystals, as well as higher contents of both epi- and intracuticular wax. In HPK, the upregulation of wax-related genes during fruit development, compared to RAK, was associated with the higher cuticular wax amount. These results provide valuable insights for future genetic studies and the breeding of kumquat varieties with enhanced fruit quality and postharvest preservation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae11121516/s1>, Table S1: Primers of wax-related genes used for qRT-PCR analysis. Figure S1: The expression trends of genes related to wax formation in HPK and RAK during development.

Author Contributions: Conceptualization, Y.L. and L.Y.; methodology, Y.H. and L.Q.; software, Y.H.; validation, Y.H. and L.Q.; formal analysis, Y.H.; investigation, L.Q.; resources, Y.L.; data curation, D.L., W.H. and Z.X.; writing—original draft preparation, Y.H.; writing—review and editing, L.Y.; visualization, Y.H., L.K. and J.S.; supervision, L.Y. and Y.L.; project administration, L.Y. and Y.L.; funding acquisition, Y.L. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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