horticulturae

MDPI

Article

Changes of Fruit Abscission and Carbohydrates, Hormones, Related Gene Expression in the Fruit and Pedicel of Macadamia under Starvation Stress

Weihai Yang [1,2,*] and Peijin Xiang [2,3]

[1] College of Life Science and Resources and Environment, Yichun University, Yichun 336000, China
[2] South Subtropical Crops Research Institute, Chinese Academy of Tropical Agricultural Sciences, Zhanjiang 524091, China; x943647809@163.com
[3] College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan 430070, China
* Correspondence: yangwh2012@163.com

Abstract: In order toexplore the regulation mechanism of macadamia fruitlet abscission induced by 'starvation stress', a treatment of girdling and defoliation was applied to the bearing shoots of macadamia cultivar 'H2' at the early stage of fruit development, simulating the starvation stress induced by interrupting carbon supply to fruit. The levels of carbohydrates, hormones, and related gene expression in the different tissues (husk, seed, and pedicel) were investigated after treatment. The results showed that a severe fruit drop occurred 3~5 d after starvation stress treatment. The contents of glucose, fructose, and sucrose in both the husk and the seed were significantly decreased, as well as the fructose and sucrose in the pedicel; this large reduction occurred prior to the massive fruit shedding. Starvation stress significantly reduced the GA_3 and ZR contents and enhanced the ABA level in the pedicel and the seed, whereas it did not obviously change these hormones in the husk. After treatment, IAA content decreased considerably in both the husk and seed but increased remarkably in the pedicel. In the husk, the expression of genes related to sugar metabolism and signaling (*NI*, *HXK2*, *TPS*, and *TPP*), as well as the biosynthesis of ethylene (*ACO2* and *ACS*) and ABA (*NCED1.1* and *AAO3*), was significantly upregulated by starvation stress, as well as the stress-responsive transcription factors (*AP2/ERF*, *HD-ZIP12*, *bZIP124*, and *ABI5*), whereas the *BG* gene associated with ABA accumulation and the early auxin-responsive genes (*Aux/IAA22* and *GH3.9*) were considerably suppressed during the period of massive fruit abscission. Similar changes in the expression of all genes occurred in the pedicel, except for *NI* and *AP2/ERF*, the expression of which was significantly upregulated during the early stage of fruit shedding and downregulated during the period of severe fruit drop. These results suggest that complicated crosstalk among the sugar, IAA, and ABA signaling may be related to macadamia fruitlet abscission induced by carbohydrate starvation.

Keywords: macadamia; fruit abscission; starvation stress; carbohydrate; hormone

Citation: Yang, W.; Xiang, P. Changes of Fruit Abscission and Carbohydrates, Hormones, Related Gene Expression in the Fruit and Pedicel of Macadamia under Starvation Stress. *Horticulturae* **2022**, *8*, 398. https://doi.org/10.3390/horticulturae8050398

Academic Editor: Daniela Farinelli

Received: 31 March 2022
Accepted: 27 April 2022
Published: 1 May 2022

1. Introduction

Macadamia (*Macadamia integrifolia* Maiden and Betche) is a typical evergreen nut crop that is widely cultivated for its premium edible kernels in the subtropical regions of the world. Usually, macadamia plants blossom in early spring and produce mass flowers. A full-grown tree can generate more than 10,000 racemes, each constituted by 100 to 300 flowers [1]. However, less than 10% of these flowers can successfully set young fruit at 2 weeks post-anthesis, and more than 80% of the immature fruits may abscise in the following 8 weeks [2], which often results in low tree productivity and poses a major challenge to the commercial production of macadamia fruit.

Fruit abscission is a complex programmed process regulated synergistically by multiple factors, including the environment, metabolism, and gene expression [3]. Generally,

physiological fruit drop is a result of the bearing tree regulating the growth balance between vegetative and reproductive organs by exerting its own genetic mechanism to ensure it has enough nutrients to meet the normal development of the remaining fruits on the tree [4]. Fruit abscission caused by various stresses is an inevitable manifestation of a tree coping with adverse conditions, as it is designed to concentrate limited carbon nutrition to guarantee the survival of partial fruits by equating the supply and demand in carbohydrates [4]. It is well-known that fruit development also involves the regulation of hormone signals excepting an adequate provision of carbohydrates [5]. Usually, the IAA, GA, and CTK positively regulate fruit set, and the ABA and ethylene are the key hormones and signal molecules in regulating fruit drop [6]. Thus, fruit abscission has been attributed to the status of carbohydrates and phytohormones.

During fruit development, carbohydrates are not only used directly as carbon nutrients but also function as signals in regulating fruit drop [7–9]. Studies on citrus [10,11], longan [12], and mango [13] showed that fruit shedding is triggered when the sugar content decreases below the critical threshold required for fruit growth. Many experiments on the fruit abscission induced by starvation stress, such as tree shading [14–16], defoliation [17], and girdling plus defoliation [18,19], revealed that the decreased glucose, fructose, and sucrose contents in fruit were associated closely with fruit drop. An increase in available carbohydrates for the rapidly developing fruits lessened immature fruit abscission in macadamia [20,21]. Furthermore, a decrease in total soluble sugar and sucrose in pedicel was also related to fruit abscission [11,19]. Huang et al. [22] found that the reinforced activity of neutral invertase (NI) and acid invertase, accelerating sucrose consumption in fruit, was one of the causes of citrus fruit drop. Guo et al. [23] reported that increased activities of acid invertase and sucrose synthase were closely related to the shedding of young almond fruits. At the molecular level, the largest group of functional genes involved in fruit abscission under carbohydrate starvation stress was connected with sugar metabolism [15,16,24]. However, few studies have investigated the metabolic process of carbohydrates in macadamia fruit under sugar starvation conditions.

Control of fruit abscission requires the coordination of different hormones. Studies on citrus [11,17], apple [16,25], and litchi [15,26] showed that ethylene and ABA were involved in fruit abscission induced by an imbalance of carbohydrate metabolism. Gómez-Cadenas et al. [17] pointed out that ABA accumulation in young fruits might be a response to sugar stress and participate in the abscission process activated by ethylene. Carbohydrate starvation stress induced upregulated expressions of ABA biosynthesis genes (*NCED* and *AAO*) in pericarp and the abscission zone (AZ), which led to ABA accumulation and resulted in fruit drop [16,19,27]. In macadamia, main-branch girdling [21] and raceme soaking with CPPU [20] treatments alleviated early fruit drop by increasing the IAA, GA_3, and ZR (a type of CTK) contents and decreasing the ABA level in fruitlet. However, information is currently limited concerning the link between hormonal signals and macadamia fruit abscission under starvation stress.

The objectives of this study were: (1) to test the effect of starvation stress induced by a girdling and defoliation treatment on fruit abscission during macadamia early fruit development and (2) to determine the changes in the levels of carbohydrates, hormones, and their related gene expression in the fruit and pedicel under starvation stress, with an attempt to reveal the connection of carbohydrates and hormones with fruit drop and provide valuable information for exploring the regulation mechanism of fruit abscission in macadamia.

2. Materials and Methods

2.1. Plant Materials

The experiment was carried out in a mature macadamia orchard located in Wangmo County (106°04′ E, 25°06′ N; 550 m a.s.l.), Guizhou, China, in May 2017. The climate is categorized as subtropical wet monsoon, with a mean annual rainfall of 1230 mm and a mean annual temperature of 19.5 °C. Trees of macadamia variety 'H2' (*Macadamia*

integrifolia) grafted on 'O.C' rootstock were grown at 5 by 6 m in an orchard with a weak acidic Fluvent of medium fertility. Six 8-year-old trees with similar canopy size and initial fruit set were selected for the test, which was performed with 3 replicates (n = 3) using two trees as an experimental block. Irrigation and fertilization were performed according to local practices, and pests were controlled when necessary.

2.2. Starvation Stress Simulationby Girdling and Defoliation Treatment and Sample Collection

After successful pollination and fertilization, young macadamia fruits grow and develop rapidly within 3–10 weeks after anthesis, during which the abscission of immature fruit occurs mainly at 6–7 and 10 weeks post-anthesis [2]. Thus, 25 bearing shoots with similar diameters (0.6–0.8 cm) and initial fruit set (5–6 racemes each consisting of 30–40 fruitlets) at different positions of the canopy were chosen and tested from each tree on day 32 after anthesis. Immediately, 15 of these were treated with the girdling and defoliation, and the other 10 were used as the control. Girdling was performed about 5–6 cm away from the base of the bearing shoot using a single-blade knife. Two separate circular cuts were carefully made about 0.8 cm apart, and the outer bark and phloem tissues around the shoot were removed. Then, the leaves above the cuts were defoliated. For each treatment in a tree, five bearing shoots were used to record the fruit number on each raceme, and the remaining shoots were used for sampling fruit and pedicel. The sampled fruit was immediately separated into the husk and seed, and then all the samples were frozen and ground to fine powder in liquid nitrogen and stored at −80 °C for analyses.

2.3. Investigation of Fruit Set

After treatment, the fruit number on each raceme in the tagged bearing shoot was recorded every day until 5 days after treatment, and the accumulative fruit drop rate was determined. The accumulative fruit drop rate is the percentage of the total number of fruit abscissions from the day of treatment versus the initial fruit set.

2.4. Determination of Carbohydrate Composition

The extraction and measurement of carbohydrates was carried out according to the protocol of Zeng et al. [20]. Briefly, a sample (pericarp, 1.0 g; seed, 0.5 g; or pedicel, 0.2 g) was homogenized with 5 mL of 90% ethanol, the homogenate was centrifuged at 8000× g for 15 min at 4 °C, and the precipitate was extracted again with 5 mL 90% ethanol following the procedures mentioned above. The supernatants were extracted twice and combined and evaporated at 85 °C to remove ethanol. The condensate was diluted to 3 mL with distilled water and then filtered for HPLC analysis using an LC-20A HPLC system (Shimadzu Co., Kyoto, Japan) equipped with a refractive index detector. The HPLC conditions were as follows: NH_2 chromatographic column (Agilent Zorbax, Santa Clara, CA, USA), 5.0 μm, 250 mm × 4.6 mm; mobile phase, 70% acetonitrile; injection volume, 10 μL; flow speed, 1.0 mL/min; column temperature, 35 °C. Carbohydrates were quantified according to external standard solution calibration. Standard sugars (sucrose, fructose, glucose, and inositol) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.5. Determination of Endogenous Hormones

Hormones (IAA, GA_3, ZR, and ABA) were extracted, purified, and determined by ELISA [20]. In brief, a sample (pericarp, 1.0 g; seed, 0.5 g; or pedicel, 0.2 g) was homogenized with the appropriate phosphate buffer solution (100 mmol/L, pH 7.4) according to the ratio of material to liquid 1:9 (m/v). After centrifuging, the supernatant was passed through a C_{18} Sep-Pak cartridge (Waters Corp., Millford, MA, USA) and then eluted with methanol. The hormone fractions were dried under N_2 and dissolved with 2 mL phosphate buffer solution for analysis by ELISA. Hormones were quantified using the standard curves, which were all generated at high coefficients of quadratic correlation ($R^2 > 0.998$).

2.6. Gene Expression by qRT-PCR Analysis

To explore the crosstalk between sugar and hormone signaling during macadamia young fruit shedding induced by carbohydrate stress, 15 genes associated with sugar and hormone metabolism and its signaling were subjected to qRT-PCR analysis, and the primers for these genes were designed by Primer 5.0 (Premier Biosoft, Montréal, QC, Canada) and described in Table 1. Total RNA from samples was extracted with an SK8661 kit (Shanghai Sangon Biochemical Technology Co., Ltd., Shanghai, China) and applied to synthesized cDNA using a reverse transcriptase kit EP0733 (Thermo Fisher Technology (China) Co., Ltd., Shanghai, China). qRT-PCR was performed on a LightCycler® 480 real-time PCR system (Roche Medical Instrument Co., Basel, Switzerland) using a SybrGreen Fast qPCR master mix kit (Roche Medical Instrument Co., Basel, Switzerland). The reaction programs were as follows: 95 °C for 3 min and 45 cycles of 95 °C for 7 s, 57 °C for 10 s, and 72 °C for 15 s. The relative expression values of genes were calculated according to the $2^{-\Delta\Delta CT}$ method against internal reference *NADPH5*. Three biological replicates and two technical replicates were conducted for each sample.

Table 1. Specific primers used for qRT-PCR in this study.

Gene Name	Forward Primer Sequence (5′→3′)	Reverse Primer Sequence (5′→3′)	Length/bp
NADPH5	CAGTGCCAGAAGTATTCAACCA	CAATGCGACCAAAACCGT	116
NI	GCTTAGGCTTGGCTATCTTCTT	CCAGAATACTATGACGGGAAGAC	166
HXK2	ATCAAATGTTGCGGAATGGG	TTCTGAAGGCGGGAGTAAGC	195
TPS	TCCCCTTAAAATACCAGCGTG	CGGAGAACCCATCTTTGAGC	96
TPP	AGATGACCGAACAGATGAAGATG	AATGCGTTGCTTTCTTTTGG	99
ACO2	GTGATAGCCCAAACAGACGG	GGATAAACCACCTGGCATTG	158
ACS	TTTGGAGAAACTGGACATAGCC	CCCTTGAGAATAAGACCTTGGAT	91
NCED1.1	CTTCATTCTGTGATTTGGGCTAC	TGGAGGACTGGAGGAGTTTGT	103
AAO	GTGCTTCAAGACCTTCCGTG	CAGGAGGGAAGAACATAGGAAT	177
BG	GCCACGTCTCCATTGCTTT	TTCCACCAGGTTTCTATTTCG	148
GH3.9	AGACGAAGAAGATGAGGAGGTG	ACTGGGGTGCTTTGTTGTAGA	164
Aux/IAA22	TATGGCATCGGTGGGTTGT	TCCTTAGCCTTTTGCATGACTC	136
HDZIP12	TCCAGAAGTGAACCCGAACC	TGCCCAGACCACTCAGGAAT	110
bZIP124	TTAACGCAGGACTCCGTATCG	ACCCATCGTCAGTGAGCCAT	132
AP2/ERF	GTGGCTGGGGACATTTGAT	GTAACATAAGCGGCAGGCA	192
ABI5	CTACCGTGTATGCCTGTTTCC	AATGGGGAGTTGTTACAGGGT	164

2.7. Statistical Analysis

Statistical data analysis was performed by SPSS software (Version 16.0, SPSS Inc., Chicago, IL, USA). The difference between treatment and control was analyzed using the procedure of independent sample *t*-test, and the least significant difference ($p < 0.05$) was applied to compare data.

3. Results and Analysis

3.1. Effect of Starvation Stress on Young Fruit Abscission

The young fruit in the treated bearing shoot of macadamia shed obviously from day 3 after the girdling and defoliation treatment performed on day 32 after anthesis, and the cumulative fruit drop rate was significantly higher than that of the control. Until day 5 after treatment, most of the fruits had fallen off, and the cumulative fruit drop rate increased rapidly to about 90% (Figure 1). However, the fruit drop rate of the control did not change much during this period.

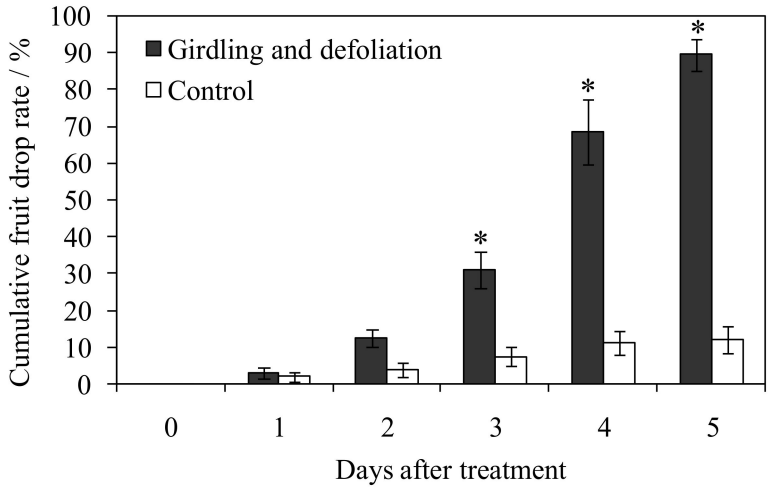

Figure 1. Effect of the girdling and defoliation treatment on fruit abscission of macadamia. The asterisks indicate significant differences between the treatment and control based on *t*-test ($p < 0.05$).

3.2. Effect of Starvation Stress on Carbohydrates in Fruit Tissues and Pedicel

The girdling and defoliation treatment significantly reduced the contents of various sugars in the husk (Figure 2A–C), seed (Figure 2D–F), and pedicel (Figure 2G–I) of macadamia, except for glucose in the pedicel (Figure 2G), which decreased slightly. Compared with the control, the contents of glucose and fructose in the husk were significantly decreased by the girdling and defoliation treatment in the period from 1 to 5 days after treatment, with a large decrease of 45.6% and 43.6% 4 days after treatment, respectively (Figure 2A,B). Furthermore, girdling and defoliation significantly decreased the sucrose content in the husk on days 1 and 3 after treatment; in particular, within 1 day after treatment, the decrease was about 50.0%, which is larger than respective decreases in glucose and fructose (Figure 2C). In the seed, glucose was significantly reduced by the girdling and defoliation 2 to 3 days after treatment, as well as fructose and sucrose 1 to 5 days after treatment (Figure 2D–F). The reduction in glucose, fructose, and sucrose contents in the seed was most obvious within 3 days after treatment, with reductions of 24.0%, 44.8%, and 38.4%, respectively. Meanwhile, the girdling and defoliation significantly decreased the fructose and sucrose levels in the pedicel 1–2 days and 1–4 days after treatment, respectively, with decreasesof nearly 25% for both (Figure 2H,I).

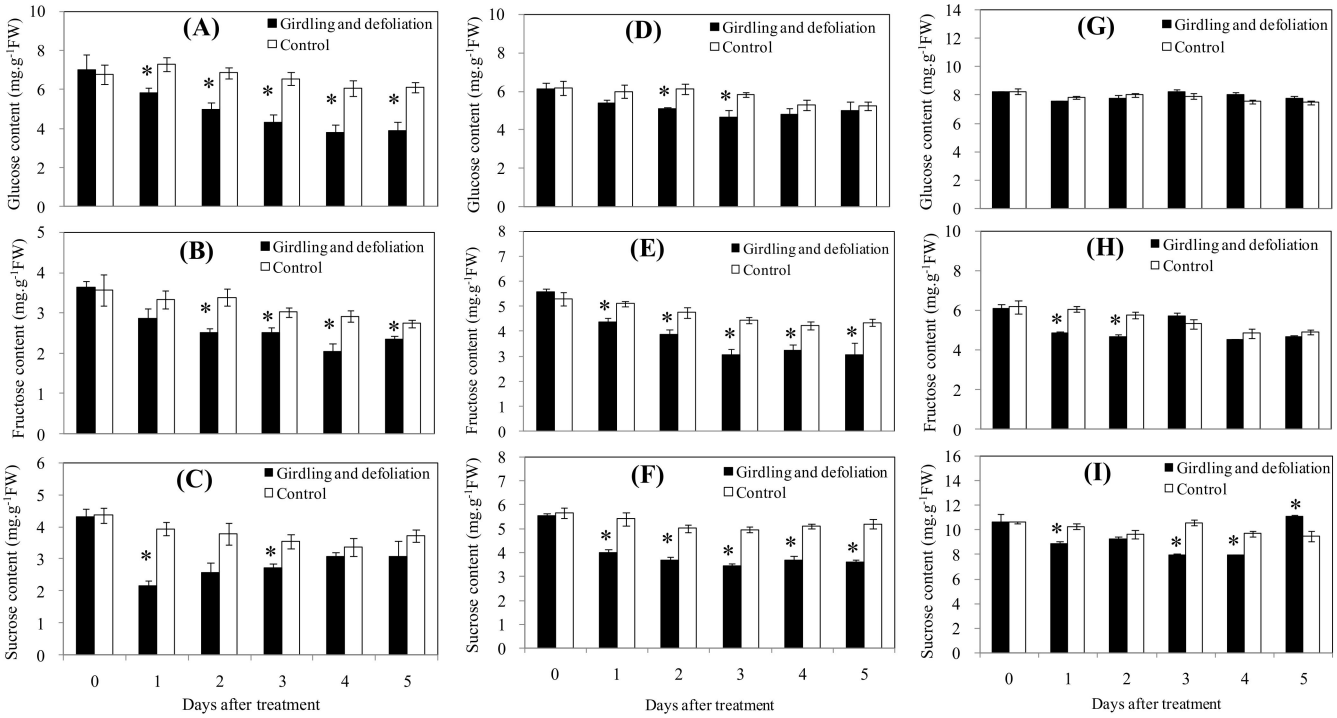

Figure 2. Effect of girdling and defoliation treatment on the contents of sugar composition in the husk (**A**–**C**), seed (**D**–**F**), and pedicel (**G**–**I**) of macadamia. The asterisks indicate significant differences between the treatment and control based on *t*-test ($p < 0.05$).

3.3. Effect of Starvation Stress on the Endogenous Hormonesin Fruit Tissues and Pedicel

The girdling and defoliation treatment did not significantly influence any of the tested hormones in the husk, except for IAA, which was significantly decreased 2 to 3 days after treatment (Figure 3A–D). In the seed, the ZR levels in the period from 3 to 4 days after treatment were significantly reduced by the girdling and defoliation compared with the control, as well as the GA_3 on day 2, whereas the ABA level was significantly increased on day 4 after treatment (Figure 3E–H). In the pedicel after girdling and defoliation treatment, the IAA content was generally augmented relative to that of the control, and the increase was significant 2 to 3 days after treatment and on day 5 (Figure 3I). On the contrary, the ZR and GA_3 contents in the pedicel were reducedby girdling and defoliation, and the contents of ZR on days 3 and 5 after treatment were significantly lower than those in the control, as well as the GA_3 on day 4 after treatment (Figure 3J,K). The ABA level in the pedicel showed a significant decrease on day 1 after girdling and defoliation treatment and then displayed a significant increase 3 to 5 days after treatment (Figure 3L).

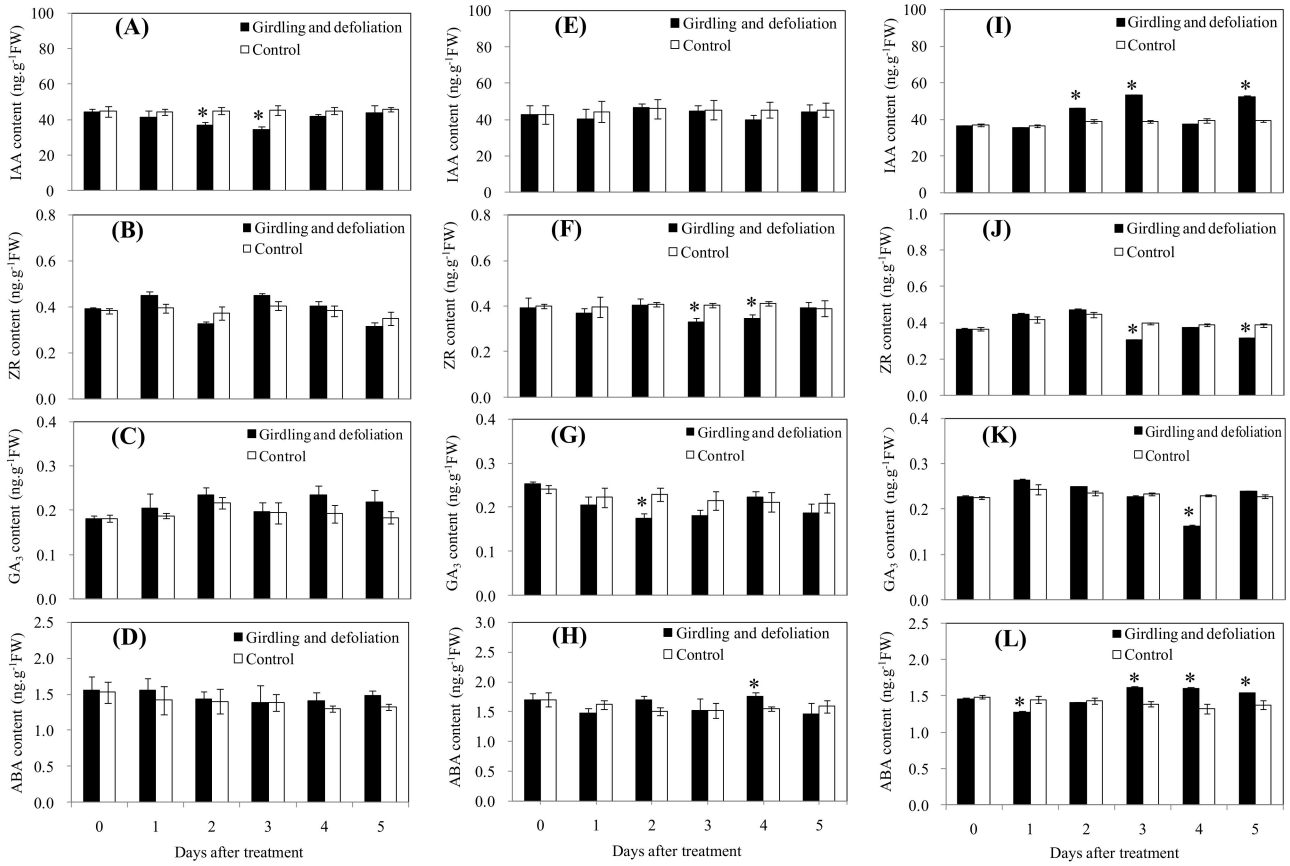

Figure 3. Effect of the girdling and defoliation treatment on the contents of endogenous hormones in the husk (**A**–**D**), seed (**E**–**H**), and pedicel (**I**–**L**) of macadamia. The asterisks indicate significant differences between the treatment and control based on *t*-test ($p < 0.05$).

3.4. Effect of Starvation Stress on the Gene Expression in Fruit Tissues and Pedicel

All 15 tested genes (*NI*, *HXK2*, *TPS*, *TPP*, *ACO2*, *ACS*, *NCED1.1*, *AAO*, *BG*, *GH3.9*, *Aux/IAA22*, *HD-ZIP12*, *bZIP124*, *AP2/ERF*, and *ABI5*) involved in sugar and hormone metabolism and its signaling in the husk (Figure 4) and the pedicel (Figure 5) were significantly affected by girdling and defoliation treatment. In the husk after girdling and defoliation treatment (Figure 4), the expression of *NI*, *HXK2*, *TPS*, and *TPP* genes related to sugar metabolism and signaling was significantly increased relative to that of the control, and the increase in *NI* was significant 4 to 5 days after treatment, as well as *HXK2*, *TPS*, and *TPP* on days 2 to 5. The transcription of genes involved in ethylene biosynthesis (*ACO2* and *ACS*) and ABA metabolism (*NCED1.1*, *AAO*, and *BG*), were significantly enhanced the girdling and defoliation treatment in the husk 1 to 5 days after treatment, except for *BG*, which decreased significantly in the period 2 to 5 days after treatment. Additionally, the *GH3.9* and *Aux/IAA22* genes responding to IAA showed significant downregulated expression in the husk 3 to 5 days after the girdling and defoliation treatment, whereas the expression of *Aux/IAA22* on day 1 after treatment was significantly upregulated. On the contrary, the transcription factors (*HD-ZIP12*, *bZIP124*, *AP2/ERF*, and *ABI5*) responding to ABA were significantly strengthened in the husk by the girdling and defoliation treatment, and the reinforcement in *HD-ZIP12* on days 2 to 5, *AP2/ERF* on days 1 to 2, *bZIP124* on days 3 to 4, and *ABI5* on days 1 to 3 was significant.

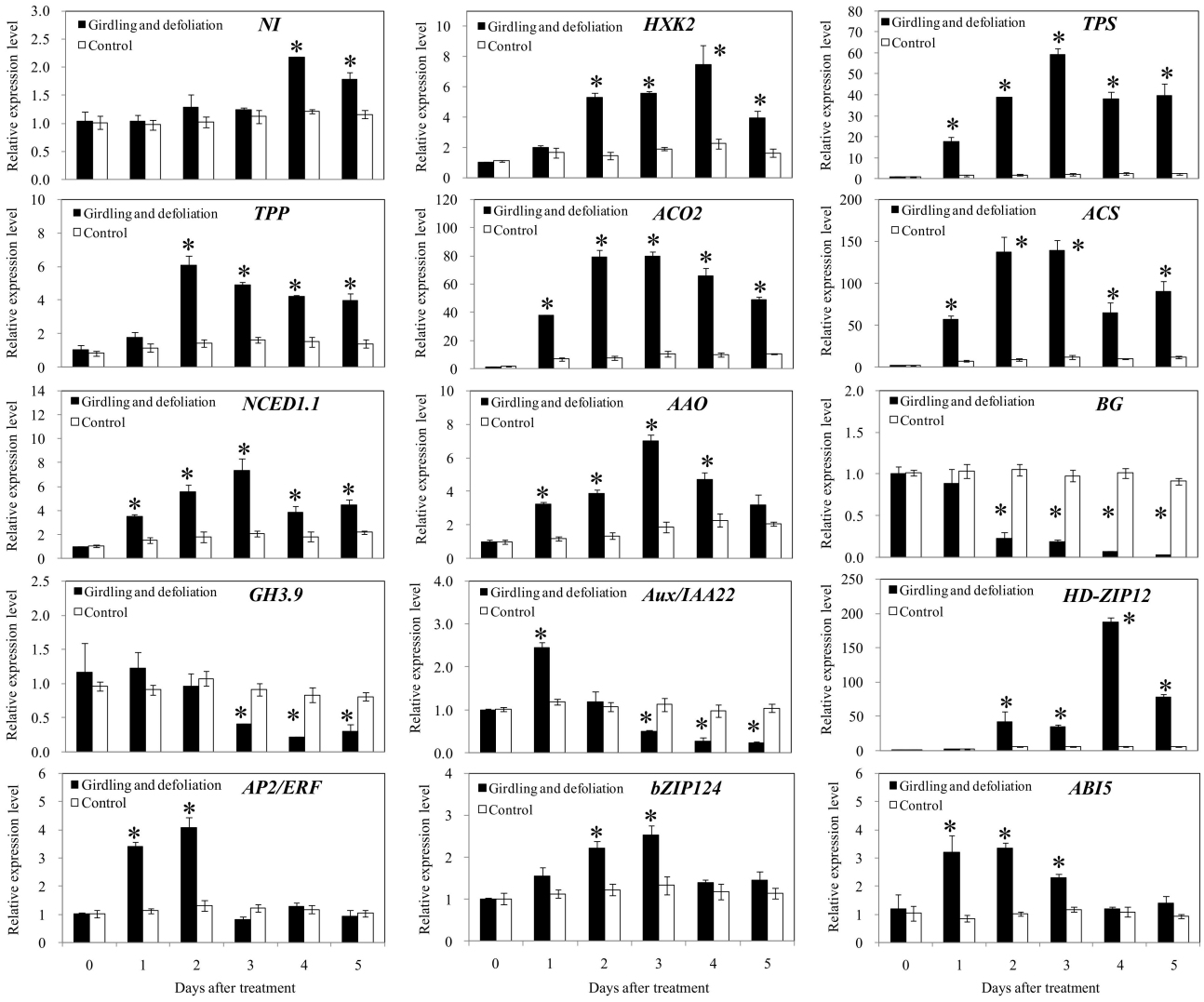

Figure 4. Effect of girdling and defoliation treatment on the gene expressions in the husk of macadamia. The asterisks indicate significant differences between the treatment and control based on *t*-test ($p < 0.05$).

In the pedicel after girdling and defoliation treatment (Figure 5), the expression of *HXK2*, *TPS*, *ACO2*, *ACS*, and *HD-ZIP12* genes was significantly increased in the period from day 1 to day 5, but *BG* and *Aux/IAA22* did the opposite. Similarly, the *NCED1.1* expression 2 to 5 days after treatment was significantly strengthened relative to that of the control, whereas *GH3.9* did contrary. Furthermore, the transcription levels of the *TPP*, *AAO*, and *bZIP124* genes were reinforced 1 to 4 days after treatment compared with the control. In addition, the expression patterns of the *NI*, *AP/ERF*, and *ABI5* genes were similar after the girdling and defoliation treatment, increasing initially and decreasing later. The expression levels of *NI* on day 3 and those of *AP/ERF* and *ABI5* on day 1 after treatment in the treated pedicel were significantly higher than those in the control, whereas *ABI5* expression was downregulated on day 5 after treatment, as well as *NI* and *AP/ERF* expression in the period 4 to 5 days and 2 to 5 days after treatment, respectively.

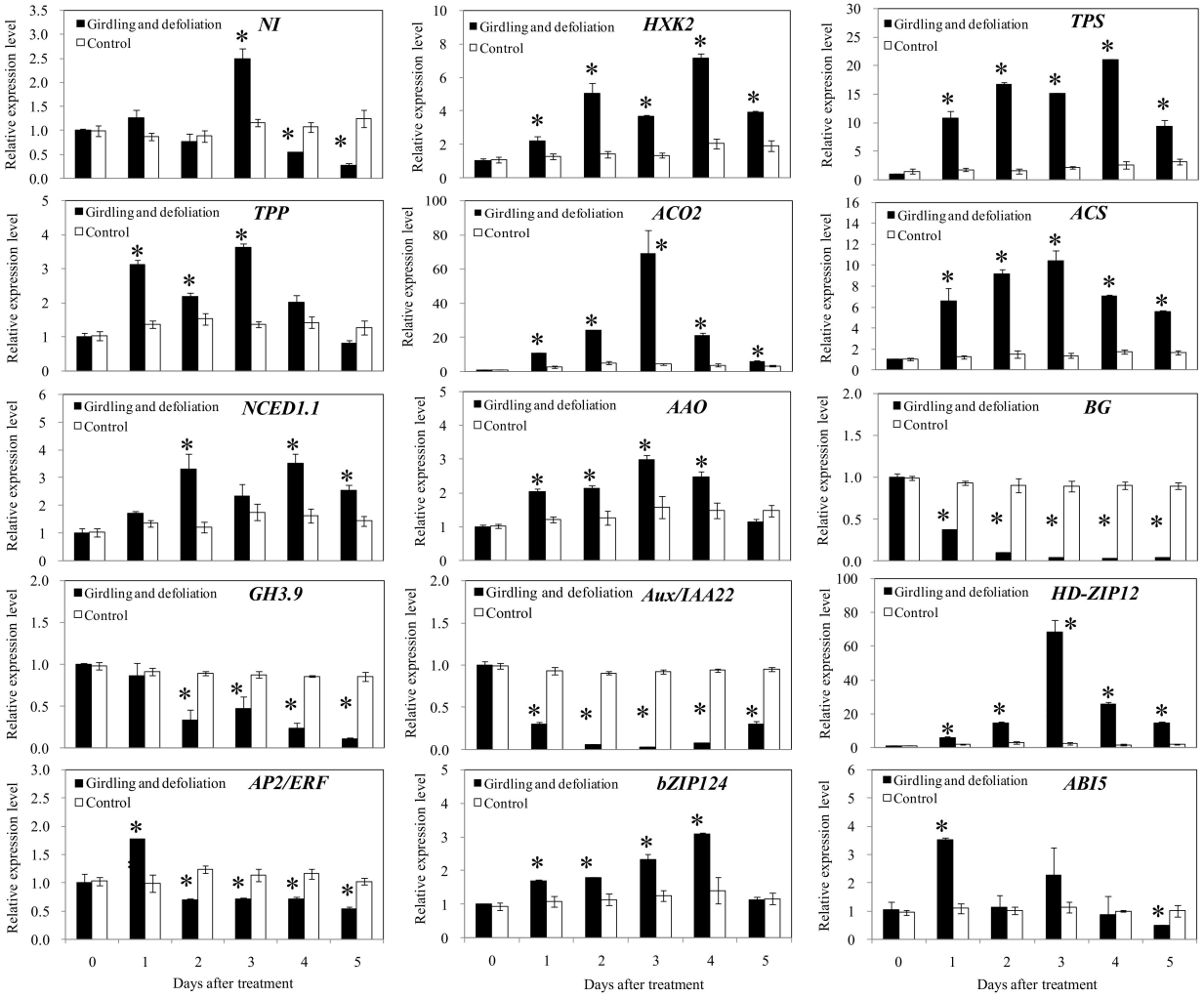

Figure 5. Effect of girdling and defoliation treatment on the gene expressions in the pedicel of macadamia. The asterisks indicate significant differences between the treatment and control based on *t*-test ($p < 0.05$).

4. Discussion

4.1. Effects of Sugar on Fruit Abscission

Fruit development is well known to be initiated by the endogenous hormones in ovary after pollination and fertilization and then sustained by an adequate supply of carbohydrates. As a powerful heterotrophic organ, the developmental fruit depends mainly on photosynthesis from the leaf to meet its growth needs. Soluble sugar, such as sucrose and glucose, is the main product of photosynthesis in the leaf and is transported to the fruit for its development and coping with abiotic stresses [28]. Treatment that reduces or blocks photosynthate supply, such as tree shading [14–16], defoliation [17], and pedicel-girdling [11], led to an imbalance of carbohydrate supply and demand and resulted in a severe fruit drop. Through the girdling and defoliation treatment of the bearing shoots of macadamia, the fruits were induced to fall off rapidly by starvation stress due to the interruption of carbon nutrient supply, which is consistent with a report on longan [18,19].

In the period of fruit development, the soluble sugar content in young fruits, especially the levels of glucose and sucrose, determined the possibility of fruit shedding [14,17]. Stopar et al. [14] reported that tree shading led to a rapid decrease in glucose concentration in young apple fruit that was easy to shed. Defoliation caused a far more significant decrease in glucose than that in sucrose in the abscised citrus fruitlet [17]. Starvation stress induced by the girdling plus defoliation treatment gave rise to a serious reduction in the soluble

sugar in longan fruit, with a much larger decrease in glucose than that in fructose [12,18]. Similarly, the contents of sucrose, glucose, and fructose in the husk and seed of macadamia, together with the sucrose and fructose in the pedicel, were significantly decreased after the girdling and defoliation treatment in this study, and all these sugars were greatly reduced before severe fruit shedding, which indicates that the drastic decrease in the available carbohydrates in the fruit and pedicel might be an initial event of starvation stress that initiated fruit abscission [19]. Furthermore, the decline in these sugars in the husk was larger than that in the seed, which confirms the previous report that the husk might be a vital site where signals for fruit shedding in response to sugar starvation are generated in macadamia fruit [20]. Fruit shedding was closely related to the formation of an abscission layer in the pedicel, and the AZ was the most sensitive tissue to stress [29]. Sucrose content in the pedicel of citrus was greatly reduced by pedicel girdling, and the sugar content might be a signal regulating fruit abscission through AZ [11]. Our study also showed that the sucrose and fructose levels in the pedicel were significantly reduced by the girdling and defoliation treatment, which suggests that sugar starvation might accelerate to form the abscission layer by inducing a burst of reactive oxygen species [18], implying that the AZ probably sensed sugar starvation and triggered the signal inducing fruit drop [16,19].

Sugar was the main metabolic signal that regulated gene expression [30], and sucrose hydrolysis played a major regulatory role in generating sugar signals [31]. In the metabolic sink, fruit, sucrose, and its catabolic products acted as the primary sugar signals [32], and glucose was the most important signal molecule [31]. During citrus fruit shedding caused by the vigorous growth of summer shoot, the increased activity of NI accelerated sucrose consumption in fruit, which was considered to be one of the causes of fruit abscission [22]. The genes encoding NI in the AZ of apple pedicel showed a strengthened expression in the period of fruit abscission induced by tree shading, which resulted in a decrease in sucrose content in the AZ [16]. Similarly to the results reported in longan [19], our study showed that the *NI* expression in the husk and pedicel of macadamia was greatly enhanced when the girdling and defoliation treatment induced severe fruit abscission, which indicates that NI played an important role in the regulation of fruit shedding by participating in sucrose metabolism and then generating a sugar signal to regulate the sink power of fruit [33,34]. Consistent with the report in longan [12], the upregulation of *NI* expression occurred significantly later than the decrease in sucrose content after the girdling and defoliation treatment, which might be a stress response of fruit to the rapid sucrose deficiency in order to increase its sink power.

Carbohydrate starvation stress contributed to the production of sugar signals [25]. HXK, as a bifunctional enzyme, can not only catalyze glucose phosphorylation but also sense the cellular glucose level as a glucose-sensing protein and then trigger a glucose signal [35]. In this study, the *HXK2* expression in the husk and pedicel of macadamia was considerably enhanced by the girdling and defoliation treatment, which confirms the reports of Zhu et al. [16], who suggested that the increased *HXK* expression in the pericarp and AZ might activate the HXK-dependent glucose signaling pathway during apple fruit abscission induced by sugar starvation. O'Hara et al. [7] reported that a trehalose signaling pathway in plants was also performed to cope with various stresses through the messenger trehalose-6-phosphate (T6P) that was produced from glucose via the catalyzation of HXK and sensed by TPS and TPP. Under starvation stress, the content of T6P was decreased in fruit [36,37]. T6P might be a key component of the sucrose signaling network, acting as both a signal sensing the sucrose availability and a negative feedback regulator of sucrose level [38,39]. In contrast to the studies on apple [16] and longan [19], under starvation stress, the expression of *TPS* and *TPP* genes in the husk and pedicel was significantly upregulated after girdling and defoliation in the present study, which implies that more T6P andtrehalose might be generated via the catalyzation of TPS and TPP to respond to the shortage of sucrose rather than to participate in the sucrose signaling involved in fruit drop.

4.2. Effects of Endogenous Hormones on Fruit Abscission

Ethylene and ABA are well-known to be the main hormones and signal molecules regulating plant organ abscission [11,40]. It has been proven that ACS and ACO are the key enzymes involved in ethylene synthesis, as well as NCED and AAO for ABA synthesis. In this study, the levels of *ACO2* and *ACS* transcripts in the husk and the pedicel increased significantly during macadamia fruit drop caused by girdling and defoliation treatment, coinciding with the reports on litchi [26,41], which implied that more ethylene might be synthesized to trigger abscission. Similarly, the expressions of the *NCED1.1* and *AAO* genes in the husk and pedicel were also greatly enhanced by the girdling and defoliation, which is consistent with a study on apple fruit abscission induced by shading [16]. However, the *BG* gene encoding a *β*-glucoesterase that is responsible for dissociating the conjugated ABA into free ABA was sharply downregulated in the husk and pedicel to repress the dissociation metabolism of ABA, which is similar to the result reported by Yang et al. [19] on longan. Thus, the increased ABA in the macadamia pedicel after girdling and defoliation might be mainly attributed to the activation of the ABA synthesis pathway under starvation stress, resulting in the rapid formation of an abscission layer and then fruit shedding in combination with ethylene. However, in the husk, a slight increase in ABA was probably not caused by the greatly enhanced expression of *NCED1.1* and *AAO* genes, suggesting that these genes might play an important role in the developmental regulation of macadamia husk [19,27].

The interplay among the IAA, GA, and CTK is necessary for fruit set and fruit growth. Among these three phytohormones, GA and CTK usually function as the positive regulators in fruit set and development [6,42], and IAA has a pivotal regulating role in fruit retention [43,44]. Mitigated fruit drop after girdling litchi trees was ascribed to increased GA_3 and ZR contents in the panicles [45]. For young macadamia fruit, the increased IAA, GA_3, and ZR levels achieved by the applications of main-branch girdling [21] and synthetic cytokinin CPPU [20] were beneficial for fruit retention. In this study, a reduced content of GA_3 and ZR in the seed and pedicel and of IAA in the husk and seed during severe fruit abscission induced by girdling and defoliation indicated that the positive effect of sugar starvation on increasing macadamia fruitlet drop was related to the decreased IAA, GA_3, and ZR levels. Shinozaki et al. [46] found that IAA- and CTK-induced fruit set was associated with the repression of ethylene biosynthesis. Similar to a reported in litchi [43], considerably reduced IAA in the husk of young macadamia fruit might activate *ACO2* and *ACS* expressions and then synthesize ethylene to induce fruit abscission under conditions of sugar shortage resulting from girdling and defoliation treatment. It is well-known that the endogenous balance of auxin and ethylene in AZ affects abscission, and a basipetal IAA flux through AZ is essential for fruit retention by inhibiting ethylene biosynthesis as a result of the decreased sensitivity of AZ to ethylene [44,47]. Application of exogenous IAA decreased the AZ cell sensitivity to ethylene and inhibited pedicel abscission by improving carbohydrate availability [43,44,48,49]. In this study, the *ACO2* and *ACS* expression in the pedicel was significantly upregulated after girdling and defoliation and prior to the increase in IAA content, which did not support the conclusions mentioned above. Studies on peach [50] and tomato [51] showed that IAA had some crosstalk with ethylene during fruit ripening because the production of ethylene was concomitant with an increase in IAA, and auxin-signaling components were upregulated by ethylene. Thus, the prior increase in ethylene biosynthesis gene expression might enhance IAA synthesis by improving ethylene production during macadamia pedicel abscission induced by starvation stress. In addition, Yang et al. [18] reported that the cell senescence and death in longan pedicel evoked by a burst of reactive oxygen specieswas apparent from 3 days after girdling and defoliation treatment. In macadamia, the IAA content in the pedicel increased from 2 days after treatment, which suggests that programmed cell death caused by carbohydrate stress might result in the blocking of IAA exportation from the pedicel, leading to IAA accumulation. Further studies are needed to clarify the mechanism of IAA regulation of macadamia fruitlet abscission.

4.3. Crosstalk between Sugar and Hormones under Starvation Stress

Carbohydrates and hormones participate in a complex signal transduction system that finely regulates fruit abscission [9,16,17]. Sugar starvation signal transduction might induce changes in auxin, ABA, and ethylene signaling [26]. It has been reported that auxin signaling is a prerequisite for organ shedding [52]. *Aux/IAA* and *GH3*, which acted as the early auxin responsive genes related to auxin signal transduction, were notable factors in the initiation of the fruitlet abscission process [49]. Abebie et al. [53] reported that the *Aux/IAA* expression levels in AZ were negatively correlated with floret abscission in Cestrum cut flowers. Meir et al. [54] found that the transcript levels of *Mj-Aux/IAA1* and *Mj-Aux/IAA2* were downregulated in the AZ of *Mirabilis jalapa* by removing IAA sources. The downregulation of *Aux/IAA* expression following IAA depletion might mediate auxin regulation of ethylene sensitivity in the tomato flower AZ [48]. Likewise, the *GH3* genes encoding IAA-conjugating enzymes also acted as negative feedback regulators in auxin signaling by reducing free auxin levels [55]. In agreement with the report of Li et al. [26] on litchi, our work also found that the girdling and defoliation treatment significantly reduced the transcript levels of *Aux/IAA22* and *GH3.9* in the husk and pedicel during the massive abscission of young fruits, which indicates that the *Aux/IAA22* and *GH3.9* genes were involved in the fruitlet shedding of macadamia under starvation stress. The downregulation of *Aux/IAA22* and *GH3.9* in the husk occurred later than the decline in IAA level, as well as the increase in *ACO2* and *ACS* expression, suggesting that auxin depletion in the husk led to the inhibition of these two early auxin-responsive genes in response to the rapid synthesis of ethylene [16,49] and supporting the finding that auxin and ethylene signaling crosstalk is mediated by *Aux/IAA* and *GH3* genes [48,50]. However, prior to the accumulation of IAA, the expression level of *Aux/IAA22* was decreased in the pedicel, whereas that of *ACO2* and *ACS* was upregulated, which indicates that auxin signaling in the pedicel was impaired to render the AZ sensitive to ethylene before the remarkable increase in IAA. Additionally, the increased IAA in the pedicel could specifically induce the expression of *ACO2* and *ACS* genes and promote ethylene production [56,57], rather than motivate the transcription of early auxin-responsive genes. This seemingly contradicts the finding that IAA decreased AZ sensitivity to ethylene largely by suppressing the gene expression of ethylene biosynthesis [48,54], but supports the contention that the auxin depressing or stimulating ethylene biosynthesis at least partly depends on plant species, organ, and developmental stage, showing its different regulatory mechanisms and biological functions [44].

In plants, crosslinking between sugar and hormone signaling pathways plays an important role in balancing carbohydrate availability [58]. ABA is well-known to be implicated in nutrient stress [16,17]. In response to carbohydrate starvation, ABA rises rapidly in fruit, functioning as a sensor of the intensity of sugar shortage during fruit drop [11,17,26]. Cheng et al. [59] reported that the induction of the ABA biosynthesis and its signal transduction gene expression by glucose was the key mechanism of glucose signaling, and the low concentration of glucose greatly promoted *NCED* and *AAO* expression, as well as ABA accumulation. Cho et al. [60] revealed that the crosstalk between glucose and ABA signals was mediated by HXK. Consistent with the result reported in longan [19], our study showed that *HXK2*, *NCED1.1*, and *AAO* expressions were significantly increased in both the husk and the pedicel after the girdling and defoliation, which suggests that *HXK2* might act as a signaling gene that senses sugar starvation and then mediates the regulation of ABA synthesis, also implying that the crosstalk between sugar and ABA signals might be involved in macadamia fruit abscission induced by starvation stress.

It has been proposed that abiotic stress-responsive transcription factors, such as *AP2/ERF*, *HD-ZIP*, *bZIP*, and *ABI5*, are involved in the stress signal perception and subsequent signal transduction through ABA-dependent and ABA-independent pathways [61,62]. During the shading induced abscission of litchi fruitlet, the transcript abundance of *AP2/ERF* increased in the fruits [15], which is supported by our finding that girdling and defoliation greatly upregulated *AP2/ERF* expression in both the husk and pedicel prior to massive fruit drop.

However, during the period of massive macadamia fruitlet abscission, the expression of *AP2/ERF* in the pedicel was remarkably depressed by the girdling and defoliation treatment, which is in agreement with a report by Li et al. [26] on litchi. Additionally, the change in *AP2/ERF* expression in the pedicel was almost opposed to ABA in this study, suggesting that *AP2/ERF* might participate in the ABA signaling pathway that regulates fruit abscission. Several studieshave reported that the *bZIP* and *HD-ZIP* transcription factors are involved in fruit abscission [26,44,63]. The *bZIP* in plants has been revealed to participate in hormone and glucose signaling processes [64]. Joo et al. [65] reported that *OsbZIP12* is a positive regulator of ABA signaling and involved the crosstalk between ABA and sugar signals in rice under stress. Likewise, *ABI5*, as a kind of bZIP transcription factor, was induced by ABA and involved in ABA signaling andstress response [62]. In this study, the expression of *bZIP124* and *ABI5* was upregulated in both the husk and the pedicel by girdling and defoliation treatment, indicating that the transcriptional changes of *bZIP124* and *ABI5* genes induced by sugar starvation signaling might mediate the crosstalk between ABA and sugar signaling. HD-Zip TFs are a large class of plant TFs that widely participates in the regulation of different growth and developmental processes. Studies on tomato flowers [48], apple fruitlets [16,66], and olive mature fruit [67] showed that HD-Zip genes encoded the differentially expressed TFs that might have central roles in the shedding of organs. Li et al. [63] found a putative HD-Zip gene (*LcHB2*) that displayed the greatly increased transcript abundance during litchi fruitlet shedding under carbohydrate stress. Here, the significantly augmented expression of *HD-ZIP12* in the husk and pedicel was probably associated with the abscission of macadamia young fruits induced by sugar starvation. Taken together, the results suggest that the young fruit abscission of macadamia ispotentially controlled by the regulation of ABA-responsive transcription factors (*AP2/ERF*, *HD-ZIP*, *bZIP*, and *ABI5*) under sugar starvation conditions.

5. Conclusions

The objective of this work was to reveal the changes in carbohydrate and hormone levels in the fruit and pedicel, as well as the expressions of genes involved in fruit abscission, during macadamia fruitlet shedding induced by girdling and defoliation treatment. In summary, girdling and defoliation imposed starvation stress in both the fruit and pedicel by decreasing the carbohydrate contentand then caused alterations in the level of hormones (IAA, GA_3, ZR, and ABA) and the expression of genes involving sugar metabolism and signaling, biosynthesis of ethylene and ABA, and signal transduction of IAA and ABA. Macadamia fruitlet abscission induced by carbohydrate starvation might be associated with the complicated crosstalk between sugar, IAA, and ABA signaling. Our study provides a basis to further elucidate the regulatory mechanism of macadamia young fruit abscission under carbohydrate stress.

Author Contributions: Conceptualization, methodology, and validation, P.X. and W.Y.; formal analysis and investigation, P.X.; data curation and writing, W.Y.; supervision, project administration, and funding acquisition, W.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the National Natural Science Foundation of China (32060652 and 31572082), the Science and Technology Research Project of Education Department of Jiangxi Province (GJJ190847), and the key research and development project of Jiangxi Province (20212BBF63019).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: All the authors acknowledge Wenhua Zhu (Guizhou Institute of Subtropics Crops) for his assistance in obtaining the experimental materials.

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

Abbreviations

The following abbreviations are used in this manuscript:

AAO	Abscisic aldehyde oxidase
ABA	Abscisic acid
ABI	Abscisic acid insensitive 5
ACO	1-Aminocyclopropane-1-carboxylate oxidase
ACS	1-Aminocyclopropane-1-carboxylate synthase
AP2/ERF	APETALA2/Ethylene Responsive Factor
Aux/IAA	Auxin/indole-3-acetic acid protein
bZIP	Basic leucine zipper
CTK	Cytokinin
CPPU	*N*-(2-Chloro-4-pyridyl)-*N*′-phenylurea
ELISA	Enzyme-linked immune sorbent assay
GA	Gibberellic acid
GH3	Gretchen Hagen 3
HD-ZIP	Homeodomain-leucine zipper
HPLC	High-performance liquid chromatography
HXK	Hexokinase
IAA	Indole-3-acetic acid
NCED	9-cis-epoxycarotenoid dioxygenase
NI	Neutral invertase
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
TPP	Trehalose-6-phosphate phosphatase
TPS	Trehalose-6-phosphate synthase
ZR	Zeatin-riboside

References

1. Ito, P.J. Effect of style removal on fruit set in *Macadamia*. *HortScience* **1980**, *15*, 520–521.
2. Trueman, S.J.; Turnbull, C.G.N. Fruit set, abscission and dry matter accumulation on girdled branches of *Macadamia*. *Ann. Bot.* **1994**, *74*, 667–674. [CrossRef]
3. Taylor, J.E.; Whitelaw, C.A. Signals in abscission. *New Phytol.* **2001**, *151*, 323–329. [CrossRef]
4. Lakso, A.N.; Robinson, T.L.; Greene, D.W. Integration of environment, physiology and fruit abscission via carbon balance modeling-implications for understanding growth regulator responses. *Acta Hortic.* **2006**, *727*, 321–326. [CrossRef]
5. Picken, A.J.F. A review of pollination and fruit set in the tomato (*Lycopersicon esculentum* Mill.). *J. Hortic. Sci.* **2015**, *59*, 1–13. [CrossRef]
6. McAtee, P.; Karim, S.; Schaffer, R.J.; David, K. A dynamic interplay between phytohormones is required for fruit development, maturation, and ripening. *Front. Plant Sci.* **2013**, *4*, 79. [CrossRef]
7. O'Hara, L.E.; Paul, M.J.; Wingler, A. How do sugars regulate plant growth and development? New insight into the role of trehalose-6-phosphate. *Mol. Plant* **2013**, *6*, 261–274. [CrossRef]
8. Lordan, J.; Reginato, G.H.; Lakso, A.N.; Francescatto, P.; Robinson, T.L. Natural fruitlet abscission as related to apple tree carbon balance estimated with the MaluSim model. *Sci. Hortic.* **2019**, *247*, 296–309. [CrossRef]
9. Zhao, M.; Li, J. Molecular events involved in fruitlet abscission in litchi. *Plants* **2020**, *9*, 151. [CrossRef]
10. Iglesias, D.J.; Tadeo, F.R.; Primo-Millo, E.; Talon, M. Fruit set dependence on carbohydrate availability in citrus trees. *Tree Physiol.* **2003**, *23*, 199–204. [CrossRef]
11. Iglesias, D.J.; Tadeo, F.R.; Primo-Millo, E.; Talon, M. Carbohydrate and ethylene levels related to fruitlet drop through abscission zone A in citrus. *Trees* **2006**, *20*, 348–355. [CrossRef]
12. Yang, Z.; Li, M.; Zhang, X.; Yu, Y.; Wang, H.; Huang, X. Effects of starvation stress on fruit abscission and sugar metabolism in longan. *J. Fruit Sci.* **2011**, *28*, 428–432.
13. Hagemann, M.H.; Winterhagen, P.; Roemer, M.G.; Hegele, M.; Wünsche, J.N. Proposed physiological mechanism of mango fruitlet abscission. *Acta Hortic.* **2016**, *1119*, 73–80. [CrossRef]
14. Stopar, M.; Resnik, M.; Pongrac, V.Z. Non-structural carbohydrate status and CO_2 exchange rate of apple fruitlets at the time of abscission influenced by shade, NAA or BA. *Sci. Hortic.* **2001**, *87*, 65–76. [CrossRef]
15. Li, C.; Wang, Y.; Huang, X.; Li, J.; Wang, H.; Li, J. *De novo* assembly and characterization of fruit transcriptome in *Litchi chinensis Sonn* and analysis of differentially regulated genes in fruit in response to shading. *BMC Genom.* **2013**, *14*, 552. [CrossRef]
16. Zhu, H.; Dardick, C.D.; Beers, E.P.; Callanhan, A.M.; Xia, R.; Yuan, R. Transcriptomics of shading-induced and NAA-induced abscission in apple (*Malus domestica*) reveals a shared pathway involving reduced photosynthesis, alterations in carbohydrate transport and signaling and hormone crosstalk. *BMC Plant Biol.* **2011**, *11*, 138–157. [CrossRef]

17. Gómez-Cadenas, A.; Mehouachi, J.; Tadeo, F.R.; Primo-Millo, E.; Talon, M. Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus. *Planta* **2000**, *210*, 636–643. [CrossRef]
18. Yang, Z.; Zhong, X.; Fan, Y.; Wang, H.; Li, J.; Huang, X. Burst of reactive oxygen species in pedicel-mediated fruit abscission after carbohydrate supply was cut off in longan (*Dimocarpus longan*). *Front. Plant Sci.* **2015**, *6*, 360. [CrossRef]
19. Yang, W.; Zeng, L.; Xiao, Q.; Shi, S. Changes of fruit abscission and carbohydrate, ABA and related genes expression in the pericarp and fruit abscission zone of longan under starvation stress. *Acta Hortic. Sin.* **2021**, *48*, 1457–1469.
20. Zeng, H.; Yang, W.; Lu, C.; Lin, W.; Zou, M.; Zhang, H.; Wan, J.; Huang, X. Effect of CPPU on carbohydrate and endogenous hormone levels in young macadamia fruit. *PLoS ONE* **2016**, *11*, e0158705. [CrossRef]
21. Yang, W.H.; Lu, C.Z.; Chen, W.; Xu, H.Y. Reduction of early fruit abscission by main-branch-girdling in macadamia is related to the favorable status of carbohydrates and endogenous hormones. *Hortscience* **2022**, *57*, 40–47. [CrossRef]
22. Huang, Y.J.; Ma, P.Q.; Wu, W.; Chen, J.Z.; Li, J.; Tang, X.L.; Liu, X.Y. Effects of summer shoot growth on sugar metabolism and abscission of fruitlet in 'Shatangju' mandarin. *Acta Hortic. Sin.* **2013**, *40*, 1869–1876.
23. Guo, C.M.; Yang, B.; Mubareke, A.; Che, Y.; Xu, J.; Gong, P.; Xu, Y.; Liao, K. The sucrose metabolism dynamics in different tissues of almond during the physiological fruit drop and its relations with fruit drop. *Plant Physiol. J.* **2020**, *56*, 317–326.
24. Zhou, C.; Lakso, A.N.; Robinson, T.L.; Gan, S. Isolation and characterization of genes associated with shade-induced apple abscission. *Mol. Genet. Genom.* **2008**, *280*, 83–92. [CrossRef]
25. Botton, A.; Eccher, G.; Forcato, C.; Ferrarini, A.; Begheldo, M.; Zermiani, M.; Moscatello, S.; Battistelli, A.; Velasco, R.; Ruperti, B.; et al. Signalling pathways mediating the induction of apple fruitlet abscission. *Plant Physiol.* **2011**, *155*, 185–208. [CrossRef]
26. Li, C.; Wang, Y.; Huang, X.; Li, J.; Wang, H.; Li, J. An improved fruit transcriptome and the identification of the candidate genes involved in fruit abscission induced by carbohydrate stress in litchi. *Front. Plant Sci.* **2015**, *6*, 439. [CrossRef]
27. Eccher, G.; Botton, A.; Dimauro, M.; Boschetti, A.; Ruperti, B.; Ramina, A. Early induction of apple fruitlet abscission is characterized by an increase of both isoprene emission and abscisic acid content. *Plant Physiol.* **2013**, *161*, 112–120.
28. Han, J.X.; Zheng, H.; Zhang, Q.; Zhong, C. Research advances in the metabolism and regulation of carbohydrate in fruit trees. *Plant Sci. J.* **2020**, *38*, 143–149.
29. Li, C.; Wang, Y.; Ying, P.; Ma, W.; Li, J. Genome-wide digital transcript analysis of putative fruitlet abscission related genes regulated by ethephon in litchi. *Front. Plant Sci.* **2015**, *6*, 502. [CrossRef]
30. Price, J.; Laxmi, A.; StMartin, S.K.; Jang, J.C. Global transcription profiling reveals multiple sugar signal transduction mechanisms in Arabidopsis. *Plant Cell* **2004**, *16*, 2128–2150. [CrossRef]
31. Rolland, F.; Baena-Gonzalez, E.; Sheen, J. Sugar sensing and signaling in plants: Conserved and novel mechanisms. *Annu. Rev. Plant Biol.* **2006**, *57*, 675–709. [CrossRef]
32. Roitsch, T.; Gonzalez, M.C. Function and regulation of plant invertases: Sweet sensations. *Trends Plant Sci.* **2004**, *9*, 606–613. [CrossRef]
33. Ruan, Y.L.; Jin, Y.; Yang, Y.J.; Li, G.J.; Boyer, J.S. Sugar input, metabolism, and signaling mediated by invertase: Roles in development, yield potential, and response to drought and heat. *Mol. Plant* **2010**, *3*, 942–955. [CrossRef]
34. Bihmidine, S.; Hunter, C.T.; Johns, C.E.; Koch, K.E.; Braun, D.M. Regulation of assimilate import into sink organs: Update on molecular drivers of sink strength. *Front. Plant Sci.* **2013**, *4*, 1–14. [CrossRef]
35. Cho, J.I.; Ryoo, N.; Eom, J.S.; Lee, D.W.; Kim, H.B.; Jeong, S.W.; Lee, Y.H.; Kwon, Y.K.; Cho, M.H.; Bhoo, S.H.; et al. Role of the rice hexokinases OsHXK5 and OsHXK6 as glucose sensors. *Plant Physiol.* **2009**, *149*, 745–759. [CrossRef]
36. Cho, Y.H.; Hong, J.W.; Kim, E.C.; Yoo, S.D. Regulatory functions of SnRK1 in stress responsive gene expression and in plant growth and development. *Plant Physiol.* **2012**, *158*, 1955–1964. [CrossRef]
37. Liu, Y.H.; Offler, C.E.; Ruan, Y.L. Regulation of fruit and seed response to heat and drought by sugars as nutrients and signals. *Front. Plant Sci.* **2013**, *4*, 282. [CrossRef]
38. Paul, M.J.; Primavesi, L.F.; Jhurreea, D.; Zhang, Y. Trehalose metabolism and signaling. *Annu. Rev. Plant Biol.* **2008**, *59*, 417–441. [CrossRef]
39. Yadav, U.P.; Lvakov, A.; Feil, R.; Duan, G.Y.; Walther, D.; Giavalisco, P.; Piques, M.; Carillo, P.; Hubberten, H.M.; Stitt, M.; et al. The sucrose-trehalose 6-phosphate (Tre6P) nexus: Specificity and mechanisms of sucrose signalling by Tre6P. *J. Exp. Bot.* **2014**, *65*, 1051–1068. [CrossRef]
40. Wilmowicz, E.; Frankowski, K.; Kućko, A.; Świdziński, M.; de Dios, A.J.; Nowakowska, A.; Kopcewicz, J. The influence of abscisic acid on the ethylene biosynthesis pathway in the functioning of the flower abscission zone in *Lupinus luteus*. *J. Plant Growth Regul.* **2016**, *206*, 49–58. [CrossRef]
41. Wu, J.Y.; Li, C.Q.; Li, J.G. Isolation of *ACS1* gene and the relationship between its expression and fruitlet abscission in litchi. *J. Fruit Sci.* **2017**, *34*, 817–827.
42. Iglesias, D.J.; Cercos, M.; Colmenero-Flore, J.M.; Naranjo, M.A.; Rios, G.; Carrera, E.; Ruiz-Rivero, O.; Lliso, I.; Morillon, R.; Tadeo, F.R.; et al. Physiology of citrus fruiting. *Braz. J. Plant Physiol.* **2007**, *19*, 333–362. [CrossRef]
43. Kuang, J.F.; Wu, J.Y.; Zhong, H.Y.; Li, C.Q.; Chen, J.Y.; Lu, W.J.; Li, J.G. Carbohydrate stress affecting fruitlet abscission and expression of genes related to auxin signal transduction pathway in litchi. *Int. J. Mol. Sci.* **2012**, *13*, 16084–16103. [CrossRef]
44. Xie, R.; Ge, T.; Zhang, J.; Pan, X.; Ma, Y.; Yi, S.; Zheng, Y. The molecular events of IAA inhibiting citrus fruitlet abscission revealed by digital gene expression profiling. *Plant Physiol. Biochem.* **2018**, *130*, 192–204. [CrossRef] [PubMed]

45. Zhou, C.; He, Z.; Yang, B.; Li, G.; Yao, L. Effect of girdling on litchi foliar nutrient and development of flower and fruit. *Guangdong Agric. Sci.* **2018**, *45*, 34–42.
46. Shinozaki, Y.; Hao, S.; Kojima, M.; Sakakibara, H.; Ozeki-Iida, Y.; Zheng, Y.; Fei, Z.; Zhong, S.; Giovannoni, J.J.; Rose, J.K.; et al. Ethylene suppresses tomato (*Solanum lycopersicum*) fruit set through modification of gibberellin metabolism. *Plant J.* **2015**, *83*, 237–251. [CrossRef] [PubMed]
47. Else, M.A.; Stankiewicz-Davies, A.P.; Crisp, C.M.; Atkinson, C.J. The role of polar auxin transport through pedicels of *Prunus avium* L. in relation to fruit development and retention. *J. Exp. Bot.* **2004**, *55*, 2099–2109. [CrossRef]
48. Meir, S.; Philosoph-Hadas, S.; Sundaresan, S.; Selvaraj, K.S.V.; Burd, S.; Ophir, R.; Kochanek, B.; Reid, M.S.; Jiang, C.Z.; Lers, A. Microarray analysis of the abscission-related transcriptome in the tomato flower abscission zone in response to auxin depletion. *PlantPhysiol.* **2010**, *154*, 1929–1956. [CrossRef]
49. Xie, R.; Pang, S.; Ma, Y.; Deng, L.; He, S.; Yi, S.; Lv, Q.; Zheng, Y. The *ARF*, *AUX/IAA* and *GH3* gene families in citrus: Genome-wide identification and expression analysis during fruitlet drop from abscission zone A. *Mol. Genet. Genom.* **2015**, *290*, 2089–2105. [CrossRef]
50. Trainotti, L.; Tadiello, A.; Casadoro, G. The involvement of auxin in the ripening of climacteric fruits comes of age: The hormone plays a role of its own and has an intense interplay with ethylene in ripening peaches. *J. Exp. Bot.* **2007**, *58*, 3299–3308. [CrossRef]
51. Jones, B.; Frasse, P.; Olmos, E.; Zegzouti, H.; Li, Z.G.; Latche, A.; Pech, J.C.; Bouzayen, M. Down-regulation of DR12, an auxin-response-factor homolog, in the tomato results in a pleiotropic phenotype including dark green and blotchy ripening fruit. *Plant J.* **2002**, *32*, 603–613. [CrossRef] [PubMed]
52. Basu, M.M.; González-Carranza, Z.H.; Azam-Ali, S.; Tang, S.; Shahid, A.A.; Roberts, J.A. The manipulation of auxin in the abscission zone cells of Arabidopsis flowers reveals that indoleacetic acid signaling is a prerequisite for organ shedding. *Plant Physiol.* **2013**, *162*, 96–106. [CrossRef] [PubMed]
53. Abebie, B.; Lers, A.; Philosoph-Hadas, S.; Goren, R.; Riov, J.; Meir, S. Differential effects of NAA and 2,4-D in reducing floret abscission in Cestrum (*Cestrum elegans*) cut flowers are associated with their differential activation of AUX/IAA homologous genes. *Ann. Bot.* **2008**, *101*, 249–259. [CrossRef] [PubMed]
54. Meir, S.; Hunter, D.A.; Chen, J.; Halaly, V.; Reid, M.S. Molecular changes occurring during acquisition of abscission competence following auxin depletion in *Mirabilis jalapa*. *Plant Physiol.* **2006**, *141*, 1604–1616. [CrossRef] [PubMed]
55. Staswick, P.E.; Serban, B.; Rowe, M.; Tiryaki, I.; Maldonado, M.T.; Maldonado, M.C.; Suza, W. Characterization of an Arabidopsis enzyme family that conjugates amino acids to indole-3-aceticacid. *Plant Cell* **2005**, *17*, 616–627. [CrossRef] [PubMed]
56. Bangerth, F. Dominance among fruits/sinks and the search for a correlative signal. *Physiol. Plant.* **1989**, *76*, 608–614. [CrossRef]
57. Abel, S.; Nguyen, M.D.; Chow, W.; Theologis, A. *ASC4*, a primary indoleacetic acid-responsive gene encoding 1-Aminocyclopropane-1-carboxylate synthase in *Arabidopsis thaliana*. *J. Biol. Chem.* **1995**, *270*, 19093–19099. [CrossRef]
58. Solfanclli, C.; Poggi, A. Sucrose-specific induction of the anthocyaninbiosyntheticpathway in Arabidopsis. *Plant Physiol.* **2006**, *140*, 637–646. [CrossRef]
59. Cheng, W.H.; Endo, A.; Zhou, L.; Penney, J.; Chen, H.C.; Arroyo, A.; Leon, P.; Nambara, E.; Asami, T.; Seo, M.; et al. A unique short-chain dehydrogenase/reductase in Arabidopsis glucose signaling and abscisic acid biosynthesis and functions. *Plant Cell* **2002**, *14*, 2723–2743. [CrossRef]
60. Cho, Y.H.; Sheen, J.; Yoo, S.D. Low glucose uncouples hexokinase1-dependent sugar signaling from stress and defense hormone abscisic acid and C_2H_4 responses in Arabidopsis. *Plant Physiol.* **2010**, *152*, 1180–1182. [CrossRef]
61. Yamaguchi, S.K.; Shinozaki, K. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Ann. Rev. Plant Physiol.* **2006**, *57*, 781–803.
62. Zhao, W.; Guan, C.; Feng, J.; Liang, Y.; Zhan, N.; Zuo, J.; Ren, B. The Arabidopsis CROWDED NUCLEI genes regulate seed germination by modulating degradation of ABI5 protein. *J. Integr. Plant Biol.* **2016**, *58*, 669–678. [CrossRef]
63. Li, C.; Zhao, M.; Ma, X.; Wen, Z.; Ying, P.; Peng, M.; Ning, X.; Xia, R.; Wu, H.; Li, J. The HD-Zip transcription factor LcHB2 regulates litchi fruit abscission through the activation of two cellulase genes. *J. Exp. Bot.* **2019**, *70*, 5189–5203. [CrossRef]
64. Kang, S.G.; Price, J.; Lin, P.C.; Hong, J.C.; Jang, J.C. The arabidopsis bZIP1 transcription factor is involved in sugar signaling, protein networking and DNA binding. *Mol. Plant* **2010**, *3*, 361–373.
65. Joo, J.; Lee, Y.H.; Song, S.I. Overexpression of thericebasic leucine zipper transcription factorOsbZIP12confers drought tolerance toriceand makes seedlings hypersensitive to ABA. *Plant Biotechnol. Rep.* **2014**, *8*, 431–441. [CrossRef]
66. Heo, S.; Hwang, J.H.; Jun, J.H.; Lee, H.J. Abscission-related genes revealed by RNA-Seq analysis using self-abscising apple (*Malus* × *domestica*). *J. Pomol. Hortic. Sci.* **2016**, *91*, 271–278. [CrossRef]
67. Gil-Amado, J.A.; Gomez-Jimenez, M.C. Transcriptome analysis of mature fruit abscission control in olive. *Plant Cell Physiol.* **2013**, *54*, 244–269. [CrossRef]