



## Article

# The Effects of Bagging on Color Change and Chemical Composition in 'Jinyan' Kiwifruit (*Actinidia chinensis*)

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**Abstract:** To explore the effect of bagging on the nutritional quality and color of kiwifruit (*Actinidia* spp.), the fruits of yellow-fleshed kiwifruit cultivars were analyzed after bagging treatment. Bagging treatment promoted the degreening of mesocarp and increased brightness. Bagging significantly reduced the accumulation of dry matter, titratable acids, starch, sucrose, fructose, and glucose during kiwifruit development. Additionally, bagging significantly reduced the accumulation of chlorophyll and carotenoids during development, whereas after debagging, the chlorophyll and carotenoid contents were significantly increased. Gene expression analysis showed that during most of the fruit development periods, the chlorophyll biosynthesis genes *AcRCBS*, *AcGLUTR*, and *AcCHLG*, and degradation genes *AcCBB*, *AcPAO*, *AcPPH*, *AcCLH*, and *AcSGR* had significantly lower expression levels in bagged fruit. Bagging also inhibited the expression of carotenoid metabolism genes, especially *AcSGR* and *AcLCYB*, which may play a key role in the process of fruit development during bagging by decreasing the accumulation of chlorophyll and carotenoids in kiwifruit. Additionally, bagging significantly reduced the content of AsA. The expression of the AsA biosynthesis genes *AcPMI2*, *AcGPP2*, and *AcGADH* in bagged fruit was significantly lower than in the control, indicating that these may be the key genes responsible for the difference in the accumulation of AsA after bagging.

**Keywords:** bagging; chlorophyll; carotenoids; AsA; gene expression



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## 1. Introduction

Bagging is an important cultivation method for improving the appearance of fruits, effectively avoiding fruit surface pollution, and reducing contamination with residues of pesticides, which are currently used in diverse fruits, such as apples [1], peaches [2], grapes [3], and kiwifruit (*Actinidia* spp.) [4]. Bagging can regulate fruit development by altering the fruit microenvironment, such as light quality and temperature, to affect fruit appearance and flavor factors [5].

Fruit color is an important component of fruit quality that mainly depends on chlorophyll, carotenoids, and anthocyanins [6]. In many fruits, such as peppers [7], carrots [8] and oranges [9], the yellow color of the pericarp is caused by carotenoids. During the development of yellow-fleshed kiwifruit, chlorophyll is degraded, causing the green color to fade and the carotenoid color to be revealed [6]. The metabolic pathways of chlorophyll and carotenoid metabolism in kiwifruit have been studied. The metabolism of chlorophyll mainly depends on the catalysis of enzymes such as ribulose-1,5-bisphosphatecarboxylase (RBCS), glutamyl tRNA reductase (GLUTR), chlorophyll synthase (CHLG), chlorophyll b

reductase (CBR), pheophorbide a oxygenase (PAO), chlorophyllase (CLH), and pheophytin pheophorbide hydrolase (PPH) [10,11]. The yellowness of kiwifruit results from the degradation of chlorophylls under the control of STAY-GREEN (SGR) [6]. The metabolism of carotenoids mainly depends on the catalysis of enzymes such as phytoene synthase (PSY), phytoene desaturase (PDS), carotenoid isomerase (CRTISO), zetacarotene desaturase (ZDS), lycopene  $\epsilon$ -cyclase (LCYE), lycopene  $\beta$ -cyclase (LCYB), cytochrome P450  $\beta$ -ring carotenoid hydroxylase (CYP97A), cytochrome P450  $\epsilon$ -ring carotenoid hydroxylase (CYP97C),  $\beta$ -carotene hydroxylase (CHYB), zeaxanthin epoxidase (ZEP), violaxanthin de-epoxidase (VDE), carotenoid cleavage dioxygenases (CCD), and 9-cis-epoxycarotenoid dioxygenase (NCED) [12]. Studies have shown that bagging affects the color of fruits. Bagging can significantly reduce the content of chlorophyll and carotenoids in kiwifruits [4]. However, the mechanism by which bagging affects chlorophyll and carotenoid metabolism in kiwifruits remains unclear.

Ascorbic acid (AsA), also known as vitamin C, is essential for normal human growth and development [13]. However, as the human body cannot synthesize AsA, it can only be obtained from the diet, and fresh fruit is one of the most important sources of AsA [13]. Research on the biosynthetic pathways of AsA in plants has made great progress. Four possible pathways for the biosynthesis of AsA in plants have been proposed [14,15], among which the L-galactose pathway is considered to play a dominant role, with the other pathways being complementary to AsA biosynthesis [16]. AsA biosynthesis mainly depends on the catalysis of some enzymes, such as glucose-6-phosphate isomerase (PGI), phosphomannose isomerase (PMI), phosphomannomutase (PMM), GDP-mannose pyrophosphorylase (GMP), GDP-mannose-3,5-epimerase (GME), GDP-L-galactose-1-phosphorylase (GGP), L-galactose-1-phosphatase (GPP), L-galactose dehydrogenase (GalDH), and L-galactono-1,4-lactone dehydrogenase (GalLDH) [17]. AsA biosynthesis requires light, and the process would be blocked by shading, resulting from bagging or exposure to strong light [18].

Kiwifruit is rich in nutrients (e.g., AsA, dietary fiber, carotenoids, flavonoids, phenolics, and minerals), has a regular shape and a pleasant flavor, making it popular among consumers worldwide [19]. During the growth and development of kiwifruit, it is highly susceptible to infection by the pathogenic microorganisms present in the environment, resulting in a decline of fruit quality [20]. Therefore, bagging, which reduces exposure to pathogens, is an important kiwifruit-cultivation measure that helps to preserve the appearance of fruits, reduce pesticide pollution, and improve the commerciality of fruits [21,22]. However, while bagging improves the appearance of the fruit, it also has an adverse effect on its quality [23]. In this study, yellow-fleshed kiwifruit (*A. chinensis*) was used to determine the effects of bagging and debagging on the external and internal quality of the fruit, as well as transcript levels of key genes involved in chlorophyll and carotenoid metabolism, to lay a foundation for analyzing the effects of light on the molecular mechanism of chlorophyll and carotenoid metabolism in kiwifruit.

## 2. Materials and Methods

### 2.1. Plant Materials and Treatments

The experiment was conducted in a commercial orchard (287° N, 115.38° E) in Fengxin County, Jiangxi Province, China. The 6-year-old 'Jinyan' kiwifruit (*A. chinensis* Planch.) grafted onto a rootstock of *A. arguta* (Siebold et Zucc.) Planch. ex Miq. was selected as the research subjects. Eighteen vines in the full bearing period were selected for bagging treatments. The vines were put into three treatment groups with six trees in each. The following treatments were carried out: (1) no bagging (control); (2) single fruit bagging performed 30 days after anthesis, followed by debagging at 90 days after bagging; (3) single fruit bagging performed 30 days after anthesis until harvesting. About 30 fruitlets from each vine (a total of 540 fruits) were bagged, and 10 fruits from each vine (a total of 180 fruits) were debagged at 90 days after bagging. The bags were 3 mm-thick double-layer opaque paper bags with black inside and yellow outside, and the specification was 16 cm (length)  $\times$  12 cm (width) (Guokang, Pingdu, China). Each treatment group was sampled at 0, 30, 60, 90, 105,

120, and 135 days after bagging, and two fruits from four directions (east, west, north, and south) and three biological replicates for a total of 24 fruits were collected and mixed per each treatment plot. All fruits were taken to the laboratory without delay, and the pulp after was frozen with liquid nitrogen for three biological replicates after measuring the color difference and stored at  $-80\text{ }^{\circ}\text{C}$ .

## 2.2. Color, Starch, and Dry-Matter Determination

A portable colorimeter CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan) was used to detect the color differences. For epicarp color, the fruits from sampling point were randomly selected and tested at four locations around the equatorial plane of the fruits. For mesocarp color, fruits from sampling point were cut horizontally to determine the chroma of fruits at the inner equator. Eight fruits were determined each time and three biological replicates were performed. The  $a^*$ ,  $b^*$ , and  $L^*$  values were recorded, with  $a^*$  and  $b^*$  representing the chromaticity components ( $a^*$ , negative to positive corresponding to a shift from green to red;  $b^*$ , negative to positive corresponding to a shift from blue to yellow, respectively) and  $L^*$  representing the brightness of the color (negative to positive corresponding to a shift from dark to light).

Total starch content in 0.1 g of frozen samples based on absorbance at 620 nm was measured using a starch-content kit (No. A148-1-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Starch content was measured in three biological replicates and expressed as  $\text{mg g}^{-1}$  fresh weight (FW).

Dry-matter content was determined using the drying method. After measuring the color difference, 1/4 pulp was taken from each of the 8 fruits and mixed together, and repeated 3 times. The samples were dried in an oven at  $105\text{ }^{\circ}\text{C}$  until a constant weight was achieved. Dry-matter content (%) = fruit dry weight (g)/fruit fresh weight (g)  $\times$  100, with measurements repeated three times.

## 2.3. Measurement of Chlorophyll, Total Carotenoid, and AsA Content

Approximately 1.0 g of kiwifruit pulp was used for extraction by grinding to a fine powder with liquid nitrogen and then transferring the powder to a 10 mL centrifuge tube. The powder was mixed with 5.0 mL of ice-cold 80% acetone containing 0.1% butylated hydroxytoluene (BHT). The mixture was incubated in the dark for 60 min until the pulp turned white. The mixture was then centrifuged at  $12,000\times g$  for 10 min at  $4\text{ }^{\circ}\text{C}$ , and the supernatant was collected for use.

Chlorophyll content was measured using 80% acetone solution as the blank control for zeroing, and the absorbance of the extracted solution was then determined by colorimetry at wavelengths of 663 nm and 646 nm in a 1 cm light diameter cuvette using a spectrophotometer (Thermo Scientific GENESYS 40, Waltham, MA, USA). Chlorophyll content was calculated as follows: Chlorophyll a =  $(12.21 A_{663} - 2.81 A_{646}) \times V / (W \times 1000)$ ; Chlorophyll b =  $(20.13 A_{646} - 5.03 A_{663}) \times V / (W \times 1000)$ ; Total chlorophyll = Chlorophyll a + Chlorophyll b. The values of  $A_{663}$  and  $A_{646}$  represent absorbance at 663 nm and 646 nm, respectively, and V and W indicate the total volume of the extract (mL) and the fresh weight of the sample (g). The chlorophyll content was expressed as  $\text{mg g}^{-1}$ .

Total carotenoid content was determined based on the absorbance of the extract at a wavelength of 450 nm. Total carotenoid content was calculated as follows: Carotenoid =  $A_{450} \times 1000 \times V / (W \times 250)$ . The value of  $A_{450}$  represents absorbance at 450 nm, and V and W indicate the total volume of the extract (mL) and the fresh weight of the sample (g), respectively. The carotenoid content was expressed as  $\text{mg g}^{-1}$ .

The AsA content was measured following the methods described in a previous report, with some modifications [24]. Approximately 1.0 g of kiwifruit pulp was ground to a fine powder with liquid nitrogen and then transferred to a 50 mL centrifuge tube. The powder was mixed with 20.0 mL of a 0.05% (*w/v*) oxalic-acid solution. The mixture was incubated for 10 min and centrifuged at  $10000\times g$  for 15 min. The supernatant was then collected. The supernatant was diluted with 0.05% (*w/v*) oxalic-acid solution to obtain a 0.5 mg/mL

extract for the determination of AsA. The AsA content of kiwifruit was determined by high-performance liquid chromatography (HPLC) (Agilent HPLC 1100, Agilent, Palo Alto, CA, USA) using a C18 column (250 mm × 4.6 mm, 5 μm, Waters). The mobile phase consisted of 0.05% (*w/v*) oxalic-acid aqueous solution and methanol (98:2), the column temperature was 25 °C, and the flow rate was 0.7 mL/min. L-Ascorbic acid (SV8120, Solarbio, Beijing, China) was used as the standard. The AsA content was identified by comparing the retention times of the peaks with those of the external standard, and quantification was performed using standard calibration curves.

#### 2.4. Gene Expression Analysis

Approximately 0.5 g of kiwifruit pulp was ground to a fine powder with liquid nitrogen. Total RNA was extracted according to the manual of the Quick-RNA isolation kit (Huayueyang, Beijing, China). The concentration of the extracted RNA was determined using a BioDrop spectrophotometer (Bio-Rad, Hercules, CA, USA). Extracted RNA (1 μg) was reverse-transcribed into cDNA using the Hifair® II 1st-strand cDNA synthesis kit (Yeasen, Shanghai, China). Quantitative real-time polymerase chain-reaction (qRT-PCR) analysis was performed using the TB Green Master Mix (RR420A, Takara, Dalian, China) on a CFX96 Touch Real-time PCR instrument (Bio-Rad, Hercules, CA, USA). Each reaction was carried out in triplicate using a reaction volume of 20 μL containing 2.0 μL of cDNA, 0.3 μL of each gene-specific primer (1 μM, Table S1), 10 μL of TB Green Premix Ex Taq, and 7.4 μL of sterile distilled water. The reaction conditions were as follows: 95 °C for 1 min, followed by 40 cycles of 15 s at 95 °C and 25 s at 63 °C. Product accumulation was determined using fluorescence measurement during a 55–95 °C melting-curve analysis. The relative expression of genes was normalized to *actin* and calculated using the  $2^{-\Delta\Delta Ct}$  method [24].

#### 2.5. Statistical Analysis

Each assay was performed in triplicate to ensure the validity of the experimental data and to reduce variance. All results are presented as the mean ± standard error (SE). Data were analyzed and drawn using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA). The differences in mean between individual treatments were analyzed by one-way ANOVA, followed by paired-samples *t*-test using significance thresholds of  $p < 0.05$  or  $p < 0.01$ .

### 3. Results

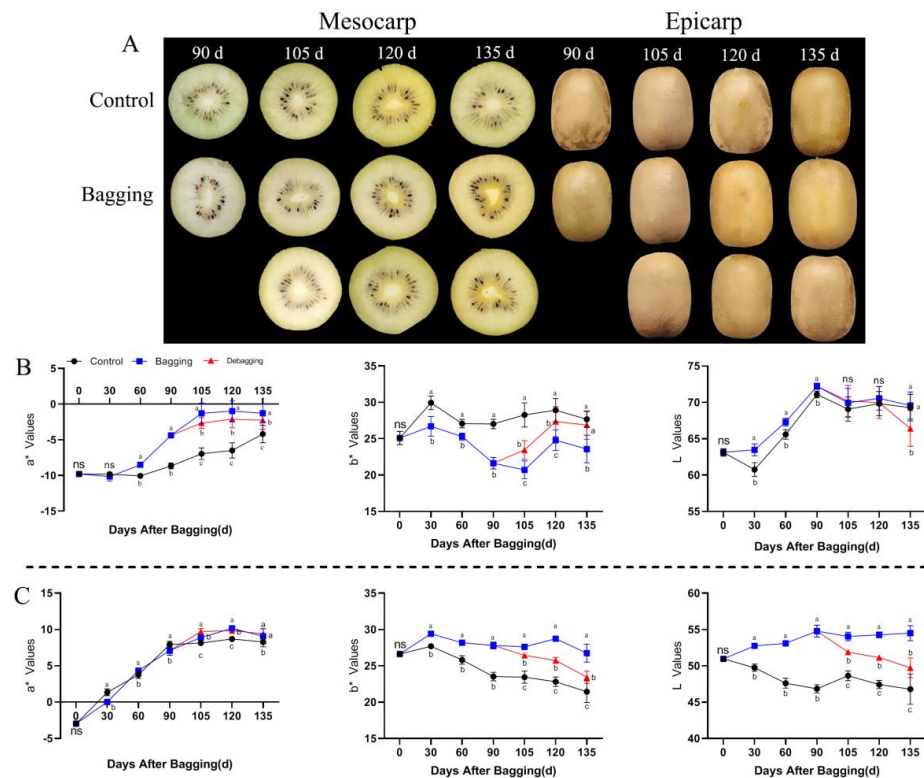
#### 3.1. Effect of Bagging on the Color of Kiwifruit

During the growth and development of the yellow-fleshed kiwifruit ('Jinyan'), the color of the mesocarp and epicarp differed between the bagging and non-bagging treatment groups (Figure 1A). The value of  $a^*$  showed an upward trend during fruit development, indicating that the greenness of the fruit decreased gradually with fruit growth and development. Bagging affected the  $a^*$  value during the nondevelopmental period, especially in the mesocarp. The  $a^*$  value was significantly increased by bagging, and the  $a^*$  value decreased after debugging, indicating that bagging affects the green appearance of the mesocarp. During the entire fruit development period, bagging decreased the  $b^*$  value of mesocarp but increased the  $b^*$  value of the epicarp, indicating that bagging reduced and increased the yellow appearance of the mesocarp and epicarp, respectively. Bagging increased the L value of the mesocarp and epicarp, indicating that the brightness of the fruit improved in response to bagging (Figure 1B,C).

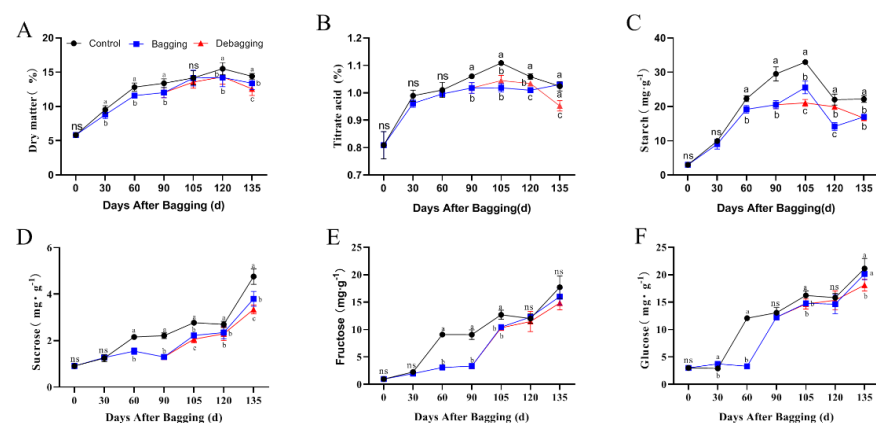
#### 3.2. Effect of Bagging on the Internal Quality of Kiwifruit

Bagging affected the internal quality of fruits. Bagging significantly reduced the accumulation of dry matter, titratable acids, starch, sucrose, fructose, and glucose during kiwifruit development, and there was no significant increase in these substances after

debagging (Figure 2). These results indicated that bagging negatively affects the internal quality of kiwifruit.



**Figure 1.** Effects of bagging on the color change of kiwifruit during development. (A) Appearance of mesocarp and epicarp with three treatments during kiwifruit development. (B,C) Changes in  $a^*$ ,  $b^*$ , and L values of different treatments in mesocarp (B) and epicarp (C) during kiwifruit development. Each assay was performed in triplicate to ensure the validity of the experimental data and to reduce the variance. All results are presented as mean  $\pm$  standard error (SE). Bars with the same letter are not significantly different at  $p > 0.05$  among three treatments, according to paired-samples  $t$ -test, ‘ns’ means not significant.

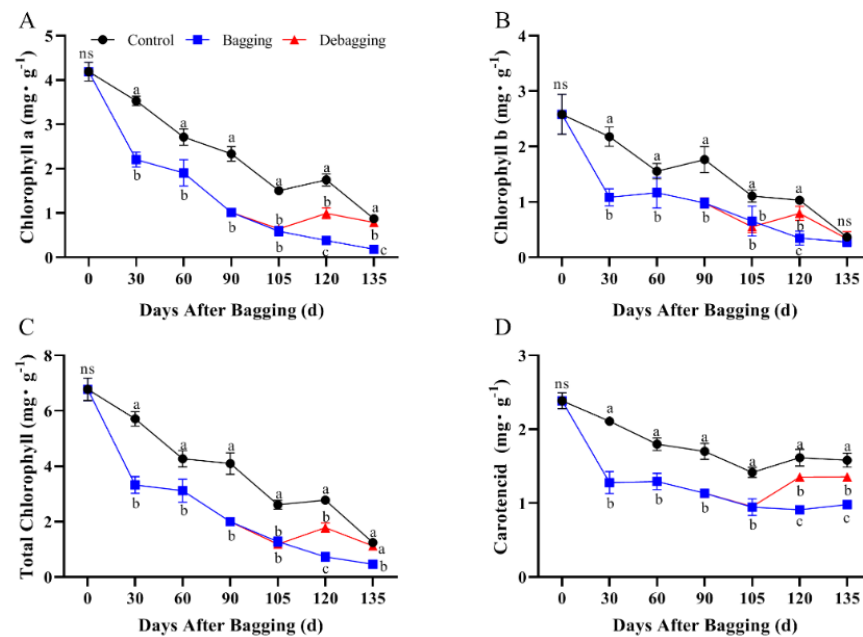


**Figure 2.** Effects of bagging on dry matter (A), titratable acids (B), starch (C), sucrose (D), fructose (E), and glucose (F) content during kiwifruit development. Each assay was performed in triplicate to ensure the validity of the experimental data and to reduce the variance. All results are presented as mean  $\pm$  standard error (SE). Bars with the same letter are not significantly different at  $p > 0.05$  among three treatments, according to paired-samples  $t$ -test, ‘ns’ means not significant.



### 3.3. Effect of Bagging on the Accumulation of Chlorophyll and Carotenoids

To determine the effect of bagging on color change in kiwifruit, the chlorophyll and carotenoid contents were determined. The contents of chlorophyll a, chlorophyll b, and total chlorophyll showed a decreasing trend during the fruit development period, whereas carotenoid content decreased during early development and remained stable in the late-development stage. Bagging significantly reduced the accumulation of chlorophyll and carotenoids during development, whereas after debagging, the chlorophyll and carotenoid contents increased significantly (Figure 3). These results indicated that bagging has a significant effect on chlorophyll and carotenoid metabolism in kiwifruit.



**Figure 3.** Effects of bagging on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and carotenoid (D) content during kiwifruit development. Each assay was performed in triplicate to ensure the validity of the experimental data and to reduce the variance. All results are presented as mean  $\pm$  standard error (SE). Bars with the same letter are not significantly different at  $p > 0.05$  among three treatments, according to paired-samples  $t$ -test, 'ns' means not significant.

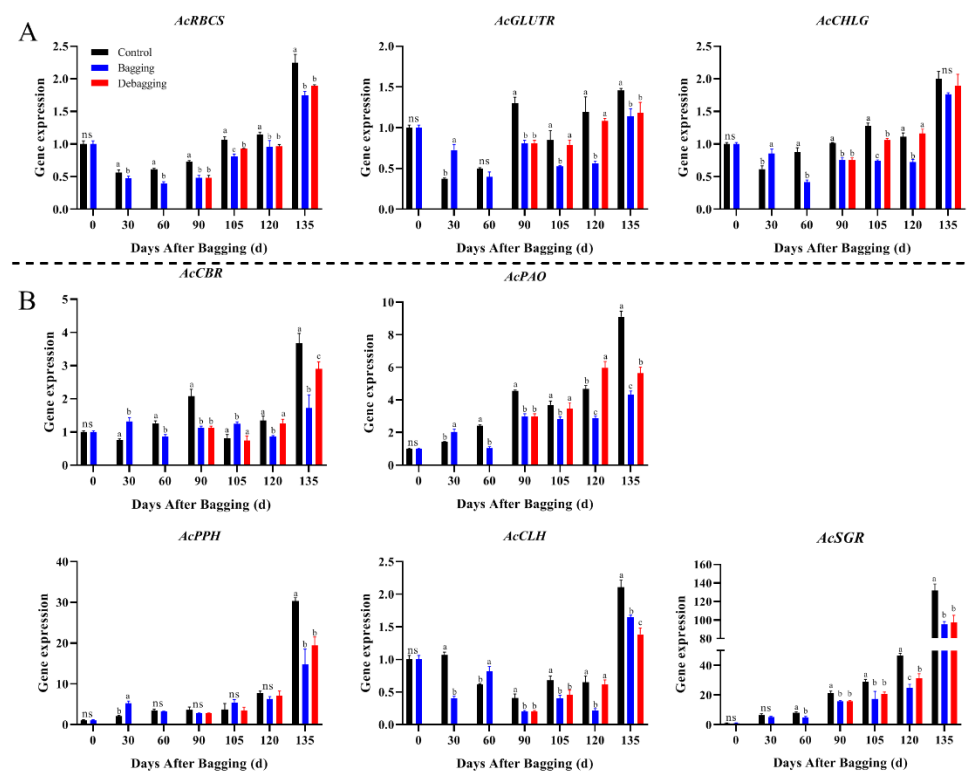
### 3.4. Effects of Bagging on the Expression of Genes Associated with Chlorophyll and Carotenoids Metabolism-Related Genes in Kiwifruit

To explore the reasons for the difference in chlorophyll and carotenoid contents after bagging, the expression levels of genes involved in chlorophyll and carotenoid metabolism during fruit development were investigated by qRT-PCR. The expression of chlorophyll metabolism-related genes showed an upward trend during fruit development. During most of the development period, the chlorophyll biosynthesis genes *AcRCBS*, *AcGLUTR*, and *AcCHLG*, and degradation genes *AcCBR*, *AcPAO*, *AcPPH*, *AcCLH*, and *AcSGR* had significantly lower expression levels in bagged fruit, while the expression of these genes increased after debagging (Figure 4). The results indicate that the reason for chlorosis during the fruit development period of yellow-fleshed kiwifruit is not due to the reduction of chlorophyll synthesis, but due to the degradation of chlorophyll. Regarding carotenoid metabolism, nine biosynthesis genes and one degradation gene were investigated during fruit development. The expression of *AcPSY*, *AcPDS*, *AcCRTISO*, and *AcZEP* were stable during the fruit development period, but the expression of *AcPSY* and *AcZEP* increased significantly at the fruit ripening stage. The expression of the biosynthesis genes *AcLCYE*, *AcCYP97A*, *AcCHYB2*, and *AcVDE* were decreased during fruit development, while the expression of *AcPSY* and *AcZEP* increased significantly at the fruit ripening stage. Bagging inhibited the expression of carotenoid metabolism genes, whereas debagging promoted the

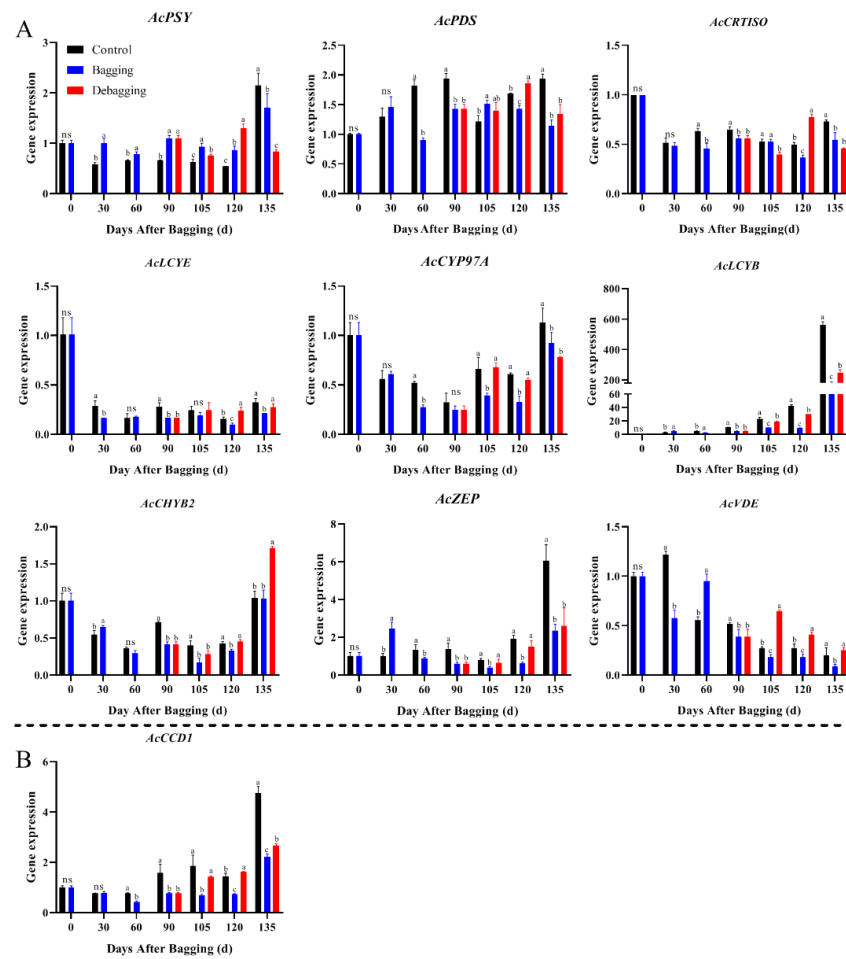
expression to a certain extent (Figure 5). Notably, AcLCYB was highly expressed during late fruit development and was significantly affected by shade bagging (Figure 5A), indicating that it may play an important role in carotenoid accumulation during fruit development.

### 3.5. Effect of Bagging on the AsA Content and the Expression of AsA Biosynthesis Genes

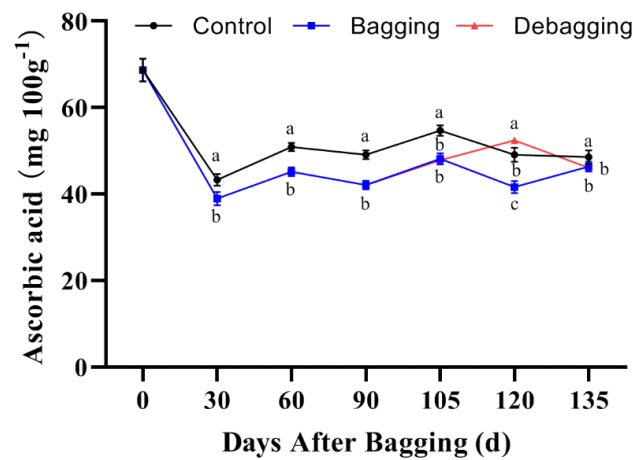
The AsA content of fruits at different developmental stages was assayed using HPLC. The results showed that bagging significantly inhibits the accumulation of AsA; however, the AsA content increased significantly after debagging following bagging for 120 days (Figure 6). L-galactose pathway is considered to play a dominant role of AsA biosynthesis in kiwifruit. To explore the molecular mechanisms through which bagging affects AsA accumulation, we determined the expression of genes related to AsA biosynthesis. The expression of AcPMM, AcGMP1, AcGMP4, AcGME1, AcGME2, AcGGP1, AcGPP1, AcGPP2, and AcGALDH showed high levels at the early stage of fruit development (0 days after bagging). The expression of AcPMI2, AcGPP2, and AcGALDH in bagged fruit was significantly lower than that in the control, indicating that AcPMI2, AcGPP2, and AcGALDH may be the key genes responsible for the difference in AsA accumulation after bagging (Figure 7).



**Figure 4.** Effect of bagging on the expression levels of chlorophyll synthesis (A) and cleavage (B) related genes during kiwifruit development. Each assay was performed in triplicate to ensure the validity of the experimental data and to reduce the variance. All results are presented as mean  $\pm$  standard error (SE). Bars with the same letter are not significantly different at  $p > 0.05$  among three treatments, according to paired-samples  $t$ -test, 'ns' means not significant.

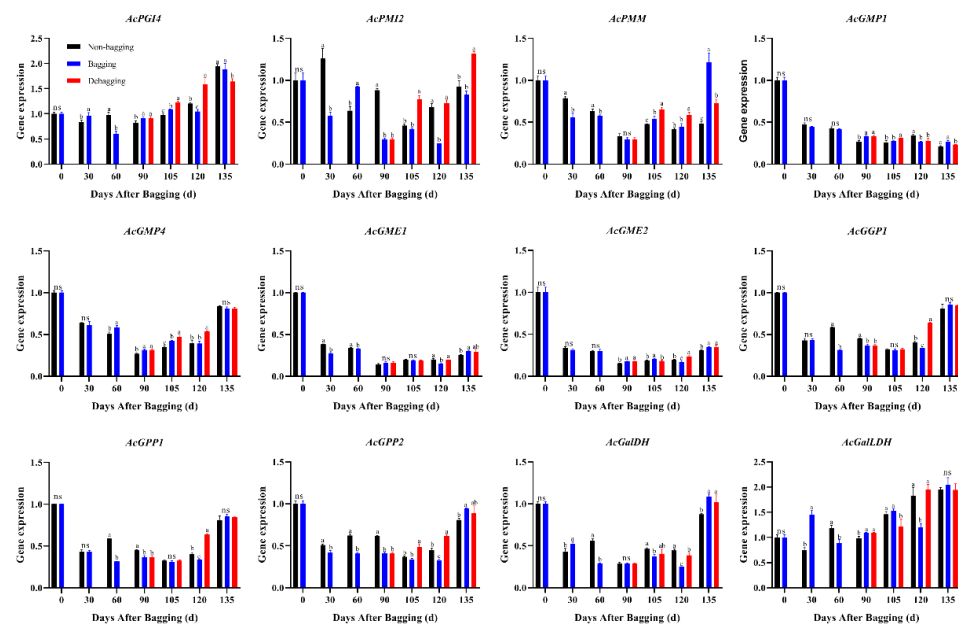


**Figure 5.** Effect of bagging on the expression levels of carotenoid synthesis (A) and cleavage (B) related genes during kiwifruit development. Each assay was performed in triplicate to ensure the validity of the experimental data and to reduce the variance. All results are presented as mean  $\pm$  standard error (SE). Bars with the same letter are not significantly different at  $p > 0.05$  among three treatments, according to paired-samples  $t$ -test, ‘ns’ means not significant.



**Figure 6.** Effects of bagging on AsA content during kiwifruit development. Each assay was performed in triplicate to ensure the validity of the experimental data and to reduce the variance. All results are presented as mean  $\pm$  standard error (SE). Bars with the same letter are not significantly different at  $p > 0.05$  among three treatments, according to paired-samples  $t$ -test.





**Figure 7.** Effect of bagging on the expression levels of AsA synthesis-related genes during kiwifruit development. Each assay was performed in triplicate to ensure the validity of the experimental data and to reduce the variance. All results are presented as mean  $\pm$  standard error (SE). Bars with the same letter are not significantly different at  $p > 0.05$  among three treatments, according to paired-samples  $t$ -test, 'ns' means not significant.

#### 4. Discussion

Fruit bagging is a common practice used in the production of peach [2], apple [1], pear [25], and grapefruit [3], which has multiple effects on internal and external quality of fruit. Bagging can change the growth environment of fruits, and paper bags commonly used in production have a particularly obvious impact on light. Light can affect fruit coloring, and the effect of light on different fruit tissues varies [21]. The amount of light absorbed by different kiwifruit tissues is inconsistent; the light exposure of the inner axial placenta is only 1/30 of that of the surface [26]. In this study, we found that bagging had different effects on the color of the epicarp and mesocarp. Bagging mainly affected the color of the mesocarp and the brightness in the epicarp, indicating that bagging can improve the brightness of the fruit surface and enhance the appearance of the inner fruit. Bagging followed by debagging produces fruit with a bright red color, which is more appealing to consumers [27]. In our study, debagging improves the brightness of the fruit surface, which is consistent with previous research [28].

Although bagging improves fruit's appearance quality, it also has some negative effects on fruit quality [27]. Studies on kiwifruit showed that bagging reduced the soluble solids of 'Jinkui' (*A. deliciosa*) and 'Jinyan' (*A. chinensis*) [29], but had no effect on the soluble solids, dry matter, soluble sugars, and titratable acids of *A. eriantha* [4]. In this study, bagging reduced the accumulation of dry matter, titratable acid, and starch during fruit development, which is consistent with previous findings [29]. Fructose, glucose, and sucrose are the main components of soluble sugars in fruits [30]. The proportion of soluble sugar components constantly changes with the development and maturity of the fruit. The content of fructose and glucose in mature kiwifruit is the highest, followed by sucrose [31]. Studies on pears have shown that bagging fruits reduces sugar content and the activities of enzymes related to sucrose metabolism [32]. The reason why bagging reduces the edible quality of fruits may be that after bagging, fruits are in shading condition for a long time, and photosynthesis of fruit skins is weakened or almost absent, which reduces the assimilates of fruits. Meanwhile, respiration in bags needs to consume certain assimilates [28]. Soluble sugar content plays an important role in fruit development and

ripening. Sucrose treatment can promote the accumulation of anthocyanin in strawberry fruit, thereby accelerating fruit ripening [33]. Fruit ripening was significantly delayed in low-sugar-content orange mutant [34]. Exogenous sucrose treatment accelerates color change of postharvest tomato fruit [35]. In this study, the contents of sucrose, fructose, and glucose gradually increased as the fruits matured, but the sugar content of bagged fruits decreased compared to that in the control. This implies that the changes of soluble sugar content and color are correlated during the development and ripening of kiwifruit.

Most fleshy fruits are green during the early stage of development. With the maturation of the fruits, the contents of various components in the fruits change significantly. Chlorophyll and carotenoids are the main pigments involved in the development of yellow-fleshed fruits [6]. During fruit development and ripening, chlorophyll is degraded, and carotenoids are synthesized and accumulated [11]. Both green-fleshed kiwifruit and yellow-fleshed kiwifruit are green in color when immature, and their carotenoid content is stable [36]. As the fruit matures, chlorophyll in the mesocarp of yellow-fleshed kiwifruit is degraded into colorless metabolites, and the mesocarp color changes to yellow as carotenoids become dominant. However, in green-fleshed kiwifruit, the degree of chlorophyll degradation is far less than that in yellow-fleshed kiwifruit, and therefore green-fleshed kiwifruit contains higher chlorophyll at maturity and the mesocarp remains green in color [36,37]. In conclusion, the main factor contributing to the yellow color of kiwifruit mesocarp is the degradation of chlorophyll rather than carotenoid synthesis. In this study, chlorophyll content decreased during fruit development and ripening, and bagging treatment accelerated the rate of chlorophyll degradation. Carotenoid content was relatively stable in the later stages of development, and bagging reduced the accumulation of carotenoids. Notably, chlorophyll and carotenoid contents increased after debagging, indicating that light plays an important role in chlorophyll and carotenoid metabolism.

Recent studies on chlorophyll and carotenoid metabolism have also been reported. The metabolism of chlorophyll and carotenoids in plants is a dynamic process involving synthesis and degradation [11]. Chlorophyll degradation was shown to result in chlorosis of the mesocarp, in response to the combined effect of downregulated chlorophyll-synthesis genes and upregulated chlorophyll-degradation genes [6]. In this study, with the development of kiwifruit, the expression of chlorophyll-synthesis genes was slightly upregulated, whereas its degradation genes were significantly upregulated. Although bagging treatment inhibited the expression of chlorophyll-synthesis and -degradation genes, the upregulation trend of chlorophyll-degradation genes remained unchanged, which may be the reason for chlorosis during the development of 'jinyan' kiwifruit. Studies have shown that SGR proteins play a key role in the degradation of plant chlorophyll [38]. Chlorophyll degradation in kiwifruit is regulated by SGR proteins [10]. The chlorophyll content and expression of chlorophyll-metabolism genes in green and yellow kiwifruit during development showed that SGR was positively correlated with chlorophyll degradation [6]. In this study, it was also found that the upregulation of *AcSGR* expression during fruit development was much higher than that of other chlorophyll-degradation genes, which once again proved that chlorophyll degradation in kiwifruit is regulated by the SGR gene. Similar to chlorophyll metabolism, carotenoid synthesis and degradation are also regulated by the associated genes. In this study, bagging reduced the expression of most genes related to carotenoid metabolism, which is consistent with previous research results of studies on peaches [39]. Previous studies on kiwifruit have shown that  $\beta$ -carotene is the main carotenoid in kiwifruit [40], and its content is positively correlated with the expression of the *LCYB* gene [40], indicating that *LCYB* may play an important role in the process by which light affects the accumulation of carotenoids in kiwifruit.

The effect of light on AsA content has been confirmed in many plants. A bagging study on kiwifruit showed that bagging reduces the AsA content of *A. deliciosa*, *A. chinensis*, and *A. eriantha* [4]. The results of the present study also support this conclusion. The L-galactose pathway is the dominant biosynthesis pathway of AsA in plants, and all structural genes in this pathway have been identified [16]. Analysis of the promoter sequences of these

structural genes revealed that light-responsive elements were distributed on most gene promoters and their activity could be induced by light [18]. Studies on kiwifruit showed that only *GallDH*, *GalUR*, and *DHAR* expression were affected by bagging, while a study on 'Jinyan' (*A. chinensis*) found that only the expression of *AcPMI2*, *AcGPP2*, and *AcGalDH* could be significantly suppressed by darkness, indicating that the synthesis of AsA in kiwifruit is regulated by multiple structural genes.

## 5. Conclusions

The results of this study show that bagging reduces the fruit quality and decreases the accumulation of chlorophyll and carotenoids in kiwifruit. The content of chlorophyll, carotenoids, and AsA simultaneously not change and increase after debagging. Analysis of gene expression showed that *AcSGR* and *AcLCYB* were the key factors behind the decrease in chlorophyll and carotenoid content after bagging. This study is a preliminary attempt to investigate the effect of bagging on chlorophyll, carotenoids, and AsA in *A. chinensis*, and further studies are required to explore the specific mechanisms.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae8060478/s1>, Table S1: Primers for gene expression.

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