Influences of Shading on Ascorbic Acid Biosynthesis of Blackcurrant (Ribes nigrum L.)

Huixin Gang †, Danni Zhang †, Xiaojuan Sun, Junwei Huo * and Dong Qin *

Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (Northeast Region), Ministry of Agriculture and Rural Affairs, College of Horticulture & Landscape Architecture, Northeast Agricultural University, Harbin 150030, China; ganghx@neau.edu.cn (H.G.); zhangdanni@163.com (D.Z.); sunxiaojian@163.com (X.S.)
* Correspondence: huojunwei@neau.edu.cn (J.H.); dongq9876@126.com (D.Q.)
† These authors contributed equally to this work.

Abstract: Cultivation conditions may greatly affect fruit quality, especially in the accumulation of functional metabolites. Blackcurrant fruits (Ribes nigrum L.) have high ascorbic acid (AsA) concentrations. The purpose of the current study was to investigate the influence of different shading treatments (full sunlight, and 40% and 60% sunlight) on the fruits’ maturity, and on the levels of fruit firmness, soluble solid, AsA, and enzyme activity involved in AsA biosynthesis and recycling in two blackcurrant (Ribes nigrum) cultivars, ‘Heifeng’ and ‘Adelinia’. Shading conditions of 40% and 60% sunlight delayed fruit ripening and increased fruit firmness in both ‘Adelinia’ and ‘Heifeng’. Soluble solids in ‘Adelinia’ were markedly reduced by shading compared with ‘Heifeng’. Compared with full sunlight, the AsA content was significantly decreased in the ripe fruits under the 40% and 60% shading treatments. Additionally, the AsA content was decreased during the fruit development process under the 60% shading treatment, which was associated with the reduced activity of the enzymes monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione oxidoreductase (GR), ascorbate peroxidase (APX), and L-galactose dehydrogenase (GalDH) involved in the biosynthesis and recycling pathway of AsA. The correlation analysis results showed that the activity of MDHAR, DHAR, GR, APX, and GalDH was significantly positively correlated with AsA concentrations during the 60% shading treatment in ‘Adelinia’ and ‘Heifeng’ fruits, suggesting that AsA biosynthesis and recycling were affected and the two cultivars have similar mechanisms to deal with shading. Our results not only provide a better understanding of the regulation mechanism of AsA accumulation under shading, but also provide a theoretical basis for taking effective cultivation measures aimed at the improvement of AsA levels in blackcurrant fruits.

Keywords: shading; blackcurrant; ascorbic acid; enzyme activity
obtain AsA through fruits and vegetables [7]. Therefore, it is essential to clarify the factors that affect the AsA content in plants, and this will help effectively increase the AsA content in harvestable plant organs.

AsA is synthesized from GDP-D-mannose via GDP-L-galactose, L-galactose, and L-galactono-1,4-lactone as intermediates [8] in apple, kiwi, tomato, and citrus fruits [9,10]. Similarly, Hancock et al. [11] reported that the ASA in blackcurrants is also synthesized through the L-galactose pathway. The AsA level in plants is maintained by an effective balance of the biosynthesis, regeneration, and degradation pathways. The regeneration pathway was described by Noctor and Foyer [12] as the ascorbic acid glutathione system (AsA-GSH). Monodehydroascorbic acid (MDHA) could be reduced to AsA by monodehydroascorbate reductase (MDHAR) or generate AsA and dehydroascorbic acid (DHA) through non-enzymatic disproportionation. Additionally, most DHA could be reduced to AsA using glutathione (GSH) as a reducing substrate by dehydroascorbate reductase (DHAR). GSH could be regenerated from oxidized glutathione dimer (GSSG) by NADPH-dependent glutathione oxidoreductase (GR).

The AsA content in plants could be influenced by environmental factors. Walker et al. [13] revealed that growing season, location, air temperature, and solar radiation have significant effects on AsA accumulation in blackcurrant fruits. In addition, Sari et al. [14] found that the leaves under high light intensity had higher AsA content than those under shading. The negative effects of shading on AsA accumulation have also been described in apple peel [15,16], grape berries [17], kiwifruit [18], and tomato [19,20]. The AsA level in apple fruits exposed to sunlight was significantly higher than those on the shaded side [21]. This was mainly due to two reasons: Firstly, the glucose was synthesized through photosynthesis in leaves under light [22]. AsA could be synthesized from glucose and the AsA level was reported to be positively associated with the glucose and sucrose level [23,24]. Secondly, the large amount of \( \text{H}_2\text{O}_2 \) generated in the PSI system under light could be removed by the redox reaction process of AsA [25,26]. However, the regulation mechanism of AsA accumulation in blackcurrant under shading is still unknown.

Shading affects fruit yield and quality, and the impact is closely associated with plant species and environmental conditions. In croton, the photosynthetic intensity was decreased, while plant height and leaf area were increased under 70% shading treatment [27]. Olives (Olea europaea) grown under 50% shading significantly increased the plant height and the number of leaves compared with those under full sunlight [14]. Seed yield and nutritional quality parameters were significantly improved in the slow growth stage and mature stage of oilseed peony trees treated with shading [28]. Qiao et al. [29] reported that shading intensity was positively correlated with the protein content, wet gluten content, dough development time, and dough stability time of wheat, but negatively correlated with the softening degree.

The fruits of blackcurrant are rich in AsA, which was higher than in apple, grape, kiwifruit, tomato, etc. [15–21]. Although the AsA content in these fruit has been reported, the regulation mechanism of AsA accumulation in blackcurrant fruits, resulting from the changes in enzyme activity in AsA biosynthesis and recycling, remains unknown. Blackcurrant is a small shrub and is usually shaded by tall trees. Thus, it is essential to clarify the regulation mechanism of AsA accumulation in blackcurrant under shading. In this work, we used two blackcurrant cultivars, ‘Heifeng’ and ‘Adelinia’, with different levels of AsA content in the fruits as materials. ‘Heifeng’ is a common blackcurrant cultivar with the characteristics of high yield but a low AsA content (144.79 ± 15.09 mg/100 g FW). ‘Adelinia’ is a cold-resistant blackcurrant cultivar with large fruit and a high AsA content (274.15 ± 9.49 mg/100 g FW) [30,31]. To further understand the regulation mechanism of AsA accumulation in blackcurrant under shading, we systematically studied the AsA levels, enzyme activity, and AsA recycling in blackcurrant fruits under different light conditions. Our results provide a better understanding of the regulation mechanism of AsA accumulation and the theoretical basis for improving AsA levels in blackcurrant fruits.
2. Materials and Methods

2.1. Plant Materials

Ten-year-old ‘Adelinia’ (High AsA) and ‘Heifeng’ (Low AsA) plants were chosen for the study. The plants were grown in a chernozem soil with spacing of 1.0 m × 2.0 m in the blackcurrant germplasm resource garden at Northeast Agricultural University (Harbin, China, 44°04’ N, 125°42’ E). They were well watered under natural growing conditions using routine management.

2.2. Experimental Treatments

Nine blackcurrant trees, with each cultivar a similar size, were divided into three groups. Group 1 (T1) and group 2 (T2) were treated with about 40% and 60% shading rates, respectively. Group 3 (CK) was grown under full sunlight. All the trees were treated from April 25 and each treatment consisted of 3 replications. Shade nets were installed approximately 30 cm above the crown of each tree to provide shading treatment. The shade nets were cleaned daily to remove fouling organisms and debris. Fresh fruit without diseases and insect pests were collected every ten days from the young fruit stage to the ripe stage. The control group was collected 5 times while the treatment groups were collected 6 times as a result of delaying of the maturity period after shading in this experiment. The collected samples were immediately frozen in liquid nitrogen and stored at −80 °C for further experiments.

2.3. Measurement of Fruit Quality

Thirty fruits were randomly selected for measurement. The soluble solid content of the fruits was determined using a digital refractometer BD-Z55. The firmness of the fruits was determined using a durometer GY-4.

2.4. Measurement of Components and Enzyme Activity in AsA Biosynthesis and Metabolism

The AsA, DHA, GSH, and GSSG contents were determined, according to the manufacturers’ protocols, using AsA, DHA, GSH, and GSSG detection kits (Solarbio, Beijing, China). The activity of DHAR, MDHAR, GR, and ascorbate peroxidase (APX) was determined using DHAR, MDHAR, GR, and APX activity detection kits (Solarbio, Beijing, China). The total AsA (T-AsA) content and total GSH (T-GSH) content were calculated as follows:

\[ C_{T-AsA} = C_{AsA} + C_{DHA} \]  
\[ C_{T-GSH} = C_{GSH} + C_{GSSG} \]

3. Results

3.1. Effects of Shading on Maturity of Blackcurrant Fruits

Shading conditions delayed the ripening of blackcurrant fruit in both ‘Adelinia’ and ‘Heifeng’. The trees were treated from 25 April, and the young fruit stages were recorded on 20 May in both shading treatments and the control. However, the fruit expanding stage was delayed by 10 days compared with the control group. Since then, the half-veraison stage, veraison fruit stage, and ripe fruit stage were all delayed by about 10 days under the 40% and 60% shading treatments (Table S1).

3.2. Effects of Shading on Firmness and Soluble Solid Level in Blackcurrant Fruits

The firmness of the ripe fruits was enhanced under both the 40% and 60% shading treatments (Table 1). The firmness of the ripe ‘Adelinia’ fruits under the treatment of 40% and 60% shading were about 1.97 and 2.36 times higher than that of the control fruit, respectively. The firmness of the ripe ‘Heifeng’ fruits under the treatment of 40% and 60% shading were 1.70 and 2.21 times higher than that of the control fruits, respectively. The firmness of the ‘Heifeng’ fruits was slightly higher than that of the ‘Adelinia’ fruits.
Table 1. Influence of shading on the firmness and soluble solid content of ripe fruits.

| Cultivar | Treatment | Firmness (kg/cm² × 105 Pa) | Soluble Solid (%) |
|----------|-----------|-----------------------------|-------------------|
|          |           |                             |                   |
| Adelinia | CK        | 2.44 ± 0.36 c               | 18.9 ± 0.46 a     |
|          | T1        | 4.81 ± 0.45 b               | 17.8 ± 0.15 b     |
|          | T2        | 5.77 ± 0.29 a               | 17.5 ± 0.33 b     |
| Heifeng  | CK        | 2.93 ± 0.59 c               | 17.4 ± 0.12 a     |
|          | T1        | 4.99 ± 0.32 b               | 17.3 ± 0.26 a     |
|          | T2        | 6.48 ± 0.16 a               | 17.4 ± 0.19 a     |

Note: The different letters a, b, c represent significant differences (p < 0.05, Student’s t-test). CK: fruits were grown under full sunlight; T1: fruits were treated with about 40% shading rates; T2: fruits were treated with about 60% shading rates.

The effects of shading on soluble solid content were different between cultivars. The soluble solid content in the ripe fruits of ‘Adelinia’ without the shading treatment (18.9%) was higher than that of ‘Heifeng’ (17.4%). The shading treatment of 40% and 60% both reduced the soluble solid content in the ripe fruits of ‘Adelinia’. However, there was no significant difference in ‘Heifeng’ under 40% and 60% shading, compared with CK. As for ‘Heifeng’, our results show that soluble solid content was not affected by shading (Table 1).

3.3. Effects of Shading on AsA, DHA and T-AsA Levels in Blackcurrant Fruits

The AsA, T-AsA, and DHA contents in fruits were measured during fruit growth under different shading treatments. As shown in Figure 1, the AsA, T-AsA, and DHA contents showed a decreasing trend during fruit ripening under different shading treatments in both ‘Adelinia’ and ‘Heifeng’. AsA had the same tendency as T-ASA in the two cultivars. The highest AsA and T-AsA concentrations were both observed at the young fruit stage. Compared with CK, a huge decline in T-AsA and AsA contents was found in the 60% shading treatment in both the ‘Adelinia’ and ‘Heifeng’ fruits, while the T-AsA and AsA contents under 40% shading showed no significant differences in the young fruit stage and decreased in the ripe fruit stage.

The T-AsA and AsA contents in the ‘Adelinia’ fruits without shading were, on average, 1.64 and 2.03 times higher than those under 60% shading at the same fruit developmental stage, respectively. The T-AsA and AsA contents in the ‘Heifeng’ fruits without shading were, on average, 2.47 and 2.88 times higher than those under 60% shading at the same fruit developmental stage. Remarkably, the largest differences were observed at the fully ripe stage, and the T-AsA and AsA concentrations were 2.59 and 2.83 times higher in ‘Adelinia’ fruits treated with 60% shading than those of CK, respectively. Additionally, on average, they were 2.47 and 2.88 times higher in ‘Heifeng’ under 60% shading than those of CK.

The proportion of DHA present in the T-AsA pool is an indication of the degree of oxidative stress being experienced by the tissue. In the ‘Adelinia’ fruits, the DHA content decreased by 10.4% and 35.5% in mature fruits treated with 40% and 60% shading, indicating either a decreased level of oxidative stress or an improved capacity for AsA recycling. In addition, the change in DHA content in ‘Heifeng’ fruit was affected more by shading treatment than in ‘Adelinia’. The DHA content in ‘Heifeng’ decreased by 24.4% and 77.1% in mature fruits treated with 40% and 60% shading. DHA content decreased rapidly under shading treatments in ‘Heifeng’. Interestingly, the DHA content in ‘Adelinia’ fruits decreased rapidly at the young fruit stage, but slowly at the expansion stage when treated with shading.
Figure 1. Influence of shading on Adelinia AsA (a), Heifeng AsA (b), Adelinia DHA (c), Heifeng DHA (d), Adelinia T-AsA (e), Heifeng T-AsA (f) contents in ‘Adelinia’ and ‘Heifeng’ fruits during fruit growth and development. Square: Heifeng/Adelinia control under natural light; round: Heifeng/Adelinia 40% shading; point: Heifeng/Adelinia 60% shading. The standard bars indicated standard deviation. Values shown by different letters for each stage are significantly different at p < 0.05 (Student’s t–test). AsA: ascorbic acid; DHA: dehydroascorbic acid; T-AsA: total ascorbic acid.

3.4. Effects of Shading on GSH, GSSG, and T-GSH Levels in Blackcurrant Fruits

The contents of T-GSH, GSH, and GSSG in the ripe blackcurrant fruits of ‘Adelinia’, and the contents of T-GSH and GSH in ‘Heifeng’ were markedly decreased under the 60% shading treatment. Compared with the control, the highest T-GSH and GSH contents were detected in the fruits treated with 40% shading. However, the 40% shading treatment had little effect on the GSSG, GSH, and T-GSH contents in the fruits of ‘Adelinia’ and ‘Heifeng’ (Figure 2).

The T-GSH, GSH, and GSSG contents were also varied during fruit ripening (Figure 2). However, the T-GSH content had the same tendency as the GSH content, and the difference between the T-GSH and GSH contents was no more than 0.1 μmol/g·FW in ‘Heifeng’ during fruit ripening for the same treatment. The lowest GSH in ‘Adelinia’ (0.12 μmol/g·FW) and ‘Heifeng’ (0.18 μmol/g·FW) and the lowest T-GSH content in ‘Adelinia’ (0.22 μmol/g·FW) and ‘Heifeng’ (0.24 μmol/g·FW) were both observed in the young fruits. In ‘Adelinia’, the peak values of the GSH and T-GSH contents were observed in the ripe fruit, which were 4.03 and 2.79 times higher than in the young fruits, respectively. In ‘Heifeng’, the peak values of the GSH and T-GSH contents were observed in the veraison fruits, which were 4.42 and 3.61 times higher than in the young fruits, respectively. The GSSG content in ‘Adelinia’ was not affected by shading since there was no significant variation compared with the control. Additionally, the GSSG content in the ripe fruit with three treatments was 0.09 μmol/g·FW, 0.10 μmol/g·FW, and 0.08 μmol/g·FW, respectively.

The concentrations of T-GSH, GSH, and GSSG differed between the two cultivars. The result showed that the GSH and T-GSH contents in the fruits of ‘Heifeng’ were higher than those in ‘Adelinia’. However, the GSSG content in ‘Adelinia’ fruits was obviously higher than ‘Heifeng’ during the entire growth stage of the fruits.
Similarly, DHAR activity in 'Heifeng' increased at 40%–60% shading was 1.97 and 2.82 times greater than in the control, and 12%, and 4% in young fruit. APX activity in mature 'Heifeng' treated with 40% shading was 8% in young fruit. Dramatic gaps in APX, MDHAR, and GR activity in 'Adelinia' were both significantly reduced with 40%–60% shading in 'Adelinia' and 40% shading in 'Heifeng' (Figure 3). DHAR activity with the 40–60% shading treatment in mature fruits were significantly decreased, and DHAR and APX activity with the 40% shading treatment in mature fruits were significantly increased. DHAR activity in mature 'Adelinia' with 40%–60% shading was 1.97 and 2.82 times greater than in the control, and MDHAR activity with 40% shading was 2.73 times greater. As for APX and GR activity, they both significantly reduced with 40%–60% shading in 'Adelinia' and 40% shading in 'Heifeng' (Figure 4). GR activity in mature 'Adelinia' treated with 40%–60% shading was 12%, and 4% in young fruit. APX activity in mature 'Heifeng' treated with 40% shading was 8% in young fruit. Dramatic gaps in APX, MDHAR, and GR activity in 'Adelinia' and 'Heifeng' were observed in the young fruit, while DHAR activity was observed in the mature fruit. Interestingly, APX activity specifically increased at the swelling stage in 'Heifeng' while decreased in 'Adelinia'. Similarly, DHAR activity in 'Heifeng' increased at color transfer, while 'Adelinia' declined as well. GR activity in the young fruit of 'Adelinia' treated with 40%–60% shading did not show a significant decrease, at 0.044 μmol/L·g·FW and 0.042 μmol/g·FW, respectively. However, whether under exposure to sun or treatment with shading, APX and GR activity in the fruits of 'Adelinia' was higher than that of 'Heifeng'; the maximum activity gaps were up to 1.14 and 2 times those of the same development stage.

**Figure 2.** Influence of light on Adelinia GSH (a), Heifeng GSH (b), Adelinia GSSG (c), Heifeng GSSG (d), Adelinia T-GSH (e), and Heifeng T-GSH (f) contents during fruit growth and development of blackcurrant fruits. Square: Heifeng/Adelinia control under natural light; round: Heifeng/Adelinia 40% shading; point: Heifeng/Adelinia 60% shading. Values shown by different letters for each stage are significantly different at \( p < 0.05 \) (Student’s t-test). GSH: glutathione; GSSG: oxidized glutathione; T-GSH: total glutathione.

### 3.5. Effects of Shading on APX, DHAR, MDHAR, and GR Enzyme Activity in Blackcurrant Fruits

Compared with the control, the activity of MDHAR and GR in the fruits under the 40% and 60% shading treatments was slightly decreased, and DHAR and APX activity was significantly decreased (Figure 3). DHAR activity with the 40–60% shading treatment and MDHAR activity with the 40% shading treatment in mature fruits were significantly reduced, and became stronger as the shading intensity increased. DHAR activity in mature ‘Adelinia’ with 40%–60% shading was 1.97 and 2.82 times greater than in the control, and MDHAR activity with 40% shading was 2.73 times greater. As for APX and GR activity, they both significantly reduced with 40%–60% shading in ‘Adelinia’ and 40% shading in ‘Heifeng’ (Figure 4). GR activity in mature ‘Adelinia’ treated with 40%–60% shading was 12%, and 4% in young fruit. APX activity in mature ‘Heifeng’ treated with 40% shading was 8% in young fruit. Dramatic gaps in APX, MDHAR, and GR activity in ‘Adelinia’ and ‘Heifeng’ were observed in the young fruit, while DHAR activity was observed in the mature fruit.
Figure 3. Influence of light on Adelinia DHAR (a), Heifeng DHAR (b), Adelinia MDHAR (c), Heifeng MDHAR (d), Adelinia GR (e) and Heifeng GR (f) activity during fruit growth and development of blackcurrant fruits. Square: Heifeng/Adelinia control under natural light; round: Heifeng/Adelinia, 40% shading; point: Heifeng/Adelinia, 60% shading. Values shown by different letters for each stage are significantly different at $p < 0.05$ (Student’s $t$-test). DHAR: dehydroascorbate reductase; MDHAR: dehydroascorbic reductase; GR: glutathione oxidoreductase.

Figure 4. Influence of light on Adelinia APX (a) and Heifeng APX (b) activity during fruit growth and development of blackcurrant fruits. Square: Heifeng/Adelinia control under natural light; round: Heifeng/Adelinia, 40% shading; point: Heifeng/Adelinia, 60% shading. Values shown by different letters for each stage are significantly different at $p < 0.05$ (Student’s $t$-test). APX: ascorbate peroxidase.
The DHAR and MDHAR activity was highest at the fruit expanding stage and lowest at the ripe fruit stage in ‘Adelinia’ and ‘Heifeng’. In ‘Adelinia’, the MDHAR and DHAR activity in the ripe fruits was decreased by 85.82% and 57.31% compared to those in the expanding fruits. In ‘Heifeng’, the MDHAR and DHAR activity in the ripe fruits was decreased by 90.89% and 42.58% compared to those in the expanding fruits. The highest GR and APX activity was observed at the young fruit stage both in ‘Adelinia’ and ‘Heifeng’.

3.6. Effects of Shading on GalDH Activity in Blackcurrant Fruits

The L-galactose dehydrogenase (GalDH) activity was highest in the young fruits and lowest in the veraison fruits, and the GalDH activity in the young fruits was 1.94 and 2.19 times higher than that in the veraison fruits of ‘Adelinia’ and ‘Heifeng’, respectively.

The shading treatments decreased the GalDH activity in the fruits of both ‘Adelinia’ and ‘Heifeng’ during blackcurrant fruit development and ripening, and the GalDH activity was decreased more under 60% shading than the 40% shading treatment. The largest influence on GalDH activity by shading treatment was observed in the ripe fruits in both ‘Adelinia’ and ‘Heifeng’. Compared with the control, the GalDH activity in the ripe fruits of ‘Adelinia’ treated with 40% and 60% shading was decreased by 10.81% and 88.99%, respectively. Additionally, the GalDH activity in the ripe fruits of ‘Heifeng’ treated with 40% and 60% shading was decreased by 13.43% and 86.24 compared with the control (Figure 5). In addition, the GalDH activity declined more in the ripe fruits than in the veraison fruits under the 60% shading treatment, while GalDH activity showed an increasing trend in the ripe fruit under full sunlight and the 40% shading treatment.

In addition, the GalDH activity declined more in the ripe fruits than in the veraison fruits under the 60% shading treatment, while GalDH activity showed an increasing trend in the ripe fruit under full sunlight and the 40% shading treatment.

![Figure 5. Influence of light on Adelinia GalDH (a) and Heifeng GalDH (b) activity during fruit growth and development of blackcurrant fruits. Square: Heifeng/Adelinia control under natural light; round: Heifeng/Adelinia, 40% shading; point: Heifeng/Adelinia, 60% shading. Values shown by different letters for each stage are significantly different at p < 0.05 (Student’s t-test). GalDH: L-galactose dehydrogenase.](image-url)
3.7. The Correlation between AsA Content and Enzyme Activity

The changes in AsA concentrations during the shading process were partially reflected in the changes in enzyme activity (Table 2). The activity of MDHAR, DHAR, GR, APX, and GalDH was significantly positively correlated with AsA concentrations during the 60% shading treatment in the ‘Adelinia’ and ‘Heifeng’ fruits, suggesting that the two cultivars have similar mechanisms to deal with shading. The recycling and biosynthesis processes of AsA were both affected by shading treatment, and the changes in these enzyme activities led to the differences in AsA content in the ripe fruits. The activity of MDHAR, DHAR, and GR was significantly positively correlated with AsA concentration during the 40% shading treatment in the ‘Adelinia’ and ‘Heifeng’ fruits, suggesting that the recycling process of AsA played a more important role than the biosynthesis process of AsA under the 40% shading treatment. As expected, there was a negative correlation between the GSH content and the activity of MDHAR, DHAR, GR, APX and GalDH in the blackcurrant fruit of the two cultivars. Most of these enzyme activities were significantly negatively correlated with GSH content under the 40% and 60% shading treatments in the ‘Heifeng’ fruits, but not in the ‘Adelinia’ fruits. Additionally, these differences might lead to the different patterns of GSH content during the fruits’ ripening and shading treatments.

Table 2. Correlation analysis of AsA content, GSH content, and enzyme activity in blackcurrant.

| Cultivars | MDHAR µmol/g FW | DHAR µmol/g FW | GR µmol/g FW | APX µmol/g FW | GalDH µmol/g FW |
|-----------|-----------------|----------------|-------------|---------------|-----------------|
| AsA µmol/L·g FW | Adelinia CK | 0.772 | 0.928 * | 0.965 ** | 0.657 | 0.590 |
| | Adelinia T1 | 0.833 * | 0.983 ** | 0.962 ** | 0.703 | 0.559 |
| | Adelinia T2 | 0.969 ** | 0.987 ** | 0.995 ** | 0.985 ** | 0.990 ** |
| | Heifeng CK | 0.894 * | 0.532 | 0.938 * | 0.877 | 0.872 |
| | Heifeng T1 | 0.950 ** | 0.856 * | 0.816 * | 0.813 * | 0.922 ** |
| | Heifeng T2 | 0.942 ** | 0.941 ** | 0.925 ** | 0.891 * | 0.963 ** |
| GSH µmol/L·g FW | Adelinia CK | −0.950 * | −0.606 | −0.847 | −0.820 | −0.817 |
| | Adelinia T1 | −0.894 * | −0.847 * | −0.759 | −0.596 | −0.541 |
| | Adelinia T2 | −0.641 | −0.797 | −0.785 | −0.726 | −0.770 |
| | Heifeng CK | −0.673 | −0.236 | −0.845 | −0.758 | −0.913 * |
| | Heifeng T1 | −0.883 * | −0.831 * | −0.782 | −0.879 * | −0.994 ** |
| | Heifeng T2 | −0.899 * | −0.906 * | −0.869 * | −0.846 * | −0.906 * |

* and ** represent significant differences at the 0.05 and 0.01 levels, respectively (Pearson test). CK: fruits were grown under full sunlight; T1: fruits were treated with about 40% shading rates; T2: fruits were treated with about 60% shading rates. AsA: ascorbic acid; GSH: glutathione.

4. Discussion

AsA levels in plant cells vary among species and even between genotypes of a given species, and they are highly correlated with developmental processes [11]. In the present study, the AsA levels in blackcurrant fruits were found to be highest during the young fruit stage, and declined progressively during fruit development and ripening. These results indicate that AsA accumulation occurred mainly in young blackcurrant fruits, which is similar to kiwifruit [32]. We also observed that the activity of enzymes, including APX, MDHAR, DHAR, and GR, in fruits before the expansion stage were high. This suggests that the immature fruits had a stronger capability for AsA recycling and biosynthesis than mature fruits, which is consistent with strawberry [33], and sweet berry [34]. AsA was involved in cell division and enlargement [7] and, thus, the young fruits had a higher AsA concentration and biosynthesis ability than the mature fruits. AsA concentration decreased as the fruit matured. This is presumably due to the slowing of AsA biosynthesis and dilution with cell expansion. Moreover, The AsA accumulation pattern varies during fruit ripening in different plant species, suggesting that differential and specific regulation mechanisms may operate for the AsA concentration in different genotypes.

AsA in plants could alter their defense potential against reactive oxygen species, and protect plant cells from abiotic stress such as wounding, ozone, high salinity, drought,
extreme temperatures, and irradiation stress [35,36]. AsA regulates the antioxidant defense system by scavenging ROS and controlling cellular redox potential, finally inducing tolerance to stress conditions [37]. Light is an indispensable key factor for plant growth and development. Light intensity has an impact not only on plant photosynthesis, but also on the antioxidant capacity of plants [38]. Under high-light-intensity stress, the increased light-driven production of ROS in photosynthesis leads to an increased AsA level in the leaf and fruit [14]. Meanwhile AsA levels have been found to decrease under low light conditions, as the synthesis of AsA could be influenced via photosynthetic electron transport through light [39]. Here, we investigated two blackcurrant varieties with large differences in AsA content under different shading conditions (0, 40%, and 60%). The results show that the AsA, DHA, and T-AsA contents were decreased during fruit development in both cultivars (Figure 1). It was noticeable that the 60% shading treatment markedly reduced AsA accumulation in the fruits (Figure 1). Similarly, AsA-related enzyme activity was also affected by shading. AsA translocation from source-to-sink tissues has also been demonstrated in a range of plants, including Arabidopsis, Medicago sativa [40], and Solanum tuberosum [41]. In the present study, the decrease in T-AsA and AsA contents in sink fruits might be influenced by the shading of the source tissues. AsA biosynthesis in situ is considered to be the primary mechanism of AsA accumulation in most plants, including peach [42], kiwifruit [8], apple [43], and strawberry [44]. GaLDH is a key enzyme in the biosynthetic pathway of l-ascorbate (AsA) in plants. Mieda et al. [45] reported that GaLDH gene expression and GaLDH enzyme activity in spinach leaves were significantly decreased under shading compared with the control. Consistent with this, we found that changes in GaLDH activity showed a similar trend, with changes in AsA content, and GaLDH activity showed an extremely significant correlation ($p < 0.01$) with AsA content under the 60% shading treatment (Figure 5). Thus, we speculated that the reduced GaLDH activity in blackcurrant fruits led to the decrease in AsA content under shading.

In addition to the biosynthesis of AsA, AsA recycling also plays an important role in the regulation of AsA concentration in tissues. Previously, numerous works of research have demonstrated that over-expressing enzymes involved in the recycling of oxidized AsA, such as DHAR [46] and MDHAR [47], could enhance AsA content. Qin et al. [48] reported that the overexpression of StDHAR1 and StDHAR2 enhanced the accumulation of AsA in potato tubers and green organs, respectively. Similarly, AsA regeneration in kiwi fruit was dependent on DHAR and MDHAR activity as well [49]. In Arabidopsis, leaves grown under low light conditions contained low activity of DHAR and MDHAR compared with those grown in high light conditions [50]. APX plays an important role in AsA metabolism, which uses AsA as the electron donor. GR genes encode enzyme that catalyze GSH generation in the AsA recycling pathway. Similarly, Wang et al. observed that APX and GR activity was continuously decreased in cauliflower (Brassica oleracea) with an increase in shading [51]. In apple, the fruits in the sun-exposed side contained higher activity of AsA–GSH cycle enzymes and a higher content of AsA compared with the shaded side [21]. Additionally, similar results were observed in the present study. The activity of AsA–GSH cycle enzymes (MDHAR, DHAR, GR, APX) was significantly reduced in blackcurrant fruits, with changes in AsA levels under the 40% and 60% shading treatments for 40 days (Figures 3 and 4). The results indicate that the capacity for recycling AsA from its oxidized forms was affected by light exposure in fruits, and the recycling systems are crucial to maintaining the cellular AsA pool in the reduced state. In addition, DHAR and MDHAR activity in ‘Heifeng’ was higher than in ‘Adelinia’ under shading, indicating that ‘Heifeng’ had higher capability for AsA recycling under shading. Thus, the AsA synthesis pathway might provide greater contributions than regeneration to maintain AsA content under shading. A simplified graphic illustration of the processes that regulate AsA levels is shown in Figure 6.
The changes in light conditions could have impacts on plant growth and development. Here, we found that shading treatments could delay blackcurrant ripening and reduce fruit firmness compared with the control. Similar results were reported by by Cangi et al. [52], whereby a black shading net providing a 50% treatment delayed harvest by 13 days compared with the control. This may be owing to the low sugar content in fruits under shading, and the down-regulation of genes related to AsA biosynthesis and metabolism such as APX and DHAR [53]. Moreover, soluble solids in ‘Adelinia’ showed relatively low content when treated with shading compared with the control (Table 1), which might ameliorate the fruit flavor. However, ‘Heifeng’ contained similar soluble solids compared with the control fruit. This was similar to the results in apples. Light intensity is known for playing an important role in the soluble solid accumulation of various horticultural fruit crops [54], and shading may delay maturity by lowering the soluble solid content [55]. The different phenotypes and contents observed in ‘Adelinia’ and ‘Heifeng’ might be due to the different genotypes between the two cultivars. A similar result was observed in the total phenolic and anthocyanin contents of five blackcurrant varieties treated with shading [56]. Moreover, ‘Heifeng’ may be more adaptable than ‘Adelinia’ to low light levels. When plants are cultivated in an environmental condition that differs from their native habitat, their ability to grow and develop will mainly depend on their capacity to adapt to such changes at the level of photosynthesis [57]. Di et al. [58] reported that the antioxidant properties of
different genotypes of wheat varied in response to environmental fluctuations. Similarly, Franc found that the ‘Sajonia’ variety displayed more efficiency in using light for fixing biomass than ‘Cayutüe’ and ‘Tirol’ in response to low light conditions in murta [59].

5. Conclusions

In this study, Two blackcurrant varieties ‘Adelinia’ and ‘Heifeng’ were treated with 40%, 60% and full sunlight conditions. The results showed that shading conditions delayed fruit ripening and increased fruit firmness in both ‘Adelinia’ and ‘Heifeng’. AsA and T-AsA contents in the two blackcurrant varieties were decreased during fruit development and under 60% shading treatment, which was associated with the reduced activity of the MDHAR, DHAR, GR, APX, and GalDH. Although differential and specific regulation mechanisms may operate for the AsA concentration in different genotypes, they have similar mechanisms to deal with shading.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f13071127/s1, Table S1: Effect of shading on the fruiting phenology of ‘Adelinia’ and ‘Heifeng’.

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