

Effect of bleeding to *Actinidia arguta* (Sieb. & Zucc) Planch. ex Miq. on plant growth, physiological characteristics and fruit quality

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Abstract

Bleeding, as particularly serious phenomenon in *A. arguta*, has an important effect on plant growth and development. In the study, to study the effects of bleeding on the growth and development of leaves and fruits after the occurrence of bleeding, we chose as *Actinidia arguta* (Sieb. & Zucc) Planch. ex Miq. Results revealed that bleeding damaged the leaf structure. The physiological indexes of plants after bleeding were detected and analyzed. The relative electrical conductivity and malondialdehyde (MDA) of leaves increased in treatment. Nitro blue tetrazolium chloride (NBT) and 3,3-diaminobenzidine (DAB) staining showed that reactive oxygen species (ROS) accumulation was obvious in leaves after bleeding. The chlorophyll content, *Tr* (transpiration rate), *Ph* (net photosynthetic rate), *Gi* (intercellular CO₂ concentration) and *Gs* (stomatal conductance) of plants were also decreased. The pulp and the seed water content decreased after the damage. The fruit vitamin C, soluble sugar content and soluble solids content (SSC) decreased after the damage, but the titratable acid content had no significant difference. Therefore, the bleeding will affect the physiological indexes of *A. arguta*, and our study lay a theoretical basis for understanding the physiological changes of *A. arguta* after bleeding.

Introduction

Actinidia arguta Sieb. & Zucc. Planch. ex Miq., widely distributed East Asia including to China, Korea and Japan, has gradually developed into the second largest *A. arguta* species in the world [1, 2]. It has excellent characteristics such as cold tolerance, delicious fruit, and immense health benefits [3, 4]. Additionally, its edible and distinctive flavor fruit has high content of vitamin C, abundant nutritious substances and medicinal usage, and was considered as one of the most nutritious fruits [5–7]. For its edible peel, the bright fruit color, the majority of consumers love this fruit, people are aware of the health benefits associated with consuming *A. arguta* [8, 9]. And it also has many horticultural advantages over the common *A. arguta*, due to high frost resistance [10]. However, many plants have the phenomenon of bleeding.

Bleeding in plant is a very common phenomenon. In general, bleeding is described by guttation or wound from leaves, stems and roots for xylem pressure. However, the mechanism of the effects of bleeding on plant still remain largely unknown [11]. Bleeding can occur at any season and under any environmental regardless plant age and the position of the wounded branch. In general, the length and position of branches also directly relate to the intensity of bleeding [12]. Therefore, the factors affecting the bleeding include season, species, temperature and soil temperature [13–15].

Recent studies have also suggested that bleeding can lead to decay of bud burst, flowering set and flower bud formation [16]. In addition, bleeding can lead to nutrient loss and induced plant bleeding sap canker disease [17, 18]. However, bleeding sap contains a large number of beneficial substances, so bleeding sap is widely used as medical product [19, 20]. A lot of plant species can secretion bleeding sap after bleeding. For example, a study has reported that grape tree bleeding sap can be used as a medical health product. Luffa bleeding sap can improve the antioxidant capacity of mice. Bitter melon bleeding sap can

improve the liver and kidney function and immune function of diabetic mice [21–25]. Thus, the study on the bleeding sap mechanism can provide useful information for keep fresh and promote plant growth.

A. arguta is also a woody plant species which can exhibit the phenomenon of bleeding due to root pressure [26]. Generally, *A. arguta* stem can show bleeding after cutting or when temperature changes. After a short time, a lot of xylem sap can bleeding (more than $100 \text{ mL} \cdot \text{h}^{-1}$) [27]. For rich nutrient in xylem sap, the loss of these sap may lead to damage of the growth and fruitfulness. In other word, bleeding often occur for commercial *A. arguta* at any time from to early spring to late spring. Due to the large pith core of the branches of *A. arguta*, it is easy to produce freezing damage [28]. The cold in late spring mainly occurs in spring, when the temperature rises obviously and then drops sharply, resulting in freezing damage to *A. arguta*. At this time, *A. arguta* has germination, so the freezing damage is more serious [29]. When the temperature drops to the unbearable range of plants, it may hinder the growth of plants and even lead to the death of plants [30, 31]. The frost damage was severe and the epidermis was broken when the plant was 1 m away from the ground, then the liquid flowed out during the bleeding period [32].

The bleeding in *A. arguta* is a serious problem when environment changes and will be resulted in dramatic yield losses and deterioration of fruit quality. However, the study of bleeding in *A. arguta* is few. For this purpose, we designed the current experiment to study the physiology variation and structure of one-year-old bleeding *A. arguta* shoots in other to examine the influence of bleeding on the fruit quality. The results revealed the changes of phenotype and physiological indexes of bleeding in *A. arguta* and their response to bleeding. These results will provide a theoretical basis for studying the breeding and molecular mechanism of *A. arguta*.

Materials and methods

Plant Materials and Growing Conditions

The leaves and fruits of *A. arguta* were collected from annual branch "Kuily" variety from *A. arguta* Base of Horticulture Branch of Heilongjiang academy of Agricultural Sciences. Three marked 1-year-old shoots on different branches of 10 to 12 randomly selected *A. arguta* were treated with pruned. The non-pruned trees were used as control, and the artificial pruned trees were used as treatment. The leaves and fruits of the trees were collected and stored at -80°C immediately. The following tests were repeated three replications per treatment (Fig. S1).

Histological analysis of leaf

Make paraffin sections of leaves by referring to Liu et al. method with minor modifications [33]. The leaves were fixed immediately in FAA (10% formaldehyde (37%), 5% acetic acid, 50% ethanol) for 24 hours, and then they were placed in to a lidded glass bottle containing 70% tert-butanol-glycerol mixture (1:1, v/v) at 50°C for 2 days. Following this, samples were immersed in 50% paraffin solution (tert-

butanol: paraffin (Leica) = 1:1, v/v) for 6 h at 56°C. Then the samples were embedded to 75% paraffin solution (tert-butanol: paraffin = 1:3, v/v) and polymerized for 6 h at 58°C. Finally, pour the paraffin into the steel embedding die (21 mm×21 mm×5 mm) and adjust the position of the material. The paraffin blocks were processed at -20°C for 2 min after cooling at room temperature. Subsequently, the embedded samples were sectioned (10 µm thick) by slicer (Leica RM2245). The cut sample was attached to a positively charged adhesive slide (Seitai, 188105W). The slides were put in a 45°C baking machine for 50 min. Then Safranin O (0.5% (w/v)) staining was performed for 9 h at room temperature and imaged using an Olympus BX51 microscope.

Measurements of physiological trait

The leaves of *A. arguta* were collected at different growth stages (initial flowering stage, blooming stage, fruiting stage and fruit maturity stage). The leaf length, leaf width, petiole length, petiole width, leaf area and longest leaf of the plants were statistically analyzed according to the method reported by Li et al. and then photographed [34]. And the relative water content (RWC) was measured according to the method of the Huang et al. [35].

Determination of electrolyte conductivity was made using the method described by Han et al. [36]. The water was added 5 mL into the test tube, and recorded as E0 by the plant leaves conductivity meter (DDS-A). The samples were added 0.1 mL into the above test tube, the shock was performed at 180 rpm for 1 h, and the conductivity was recorded as E1. Then it was placed in a boiling water bath for 15 min, and after cooling the conductivity was recorded as E2. Electrolyte conductivity was calculated with the formula electrolyte conductivity (µS/cm) = $E1 - E0 / E2 - E0$.

Determination of malondialdehyde (MDA) content according to the method of Yang et al. [37] with minor modifications. The 0.3 mL samples were added with 2 mL 0.5% TBA, and boiled in a water bath for 15 min. After cooling, the samples were centrifugated at 12000 rpm for 10 min. The OD₄₅₀, OD₅₃₂ and OD₆₀₀ were determined with supernatant solution.

The photosynthesis and chlorophyll content of leaves was measured by the method of Huang et al. [35]. *Tr* (transpiration rate), *Ph* (net photosynthetic rate), *C_i* (intercellular CO₂ concentration) and *G_s* (stomatal conductance) of the *A. arguta* leaves were measured from 9:00–11:00 a.m. using a Li-6400 portable photosynthetic system (LI-COR Inc). Leaf portions measuring 6 cm² (3 cm in length, 2 cm in width) were placed in the instrument under white light (1,000 µmol mol⁻² s⁻¹). Five plants were chosen from each group and each plant was measured 5 times. The fifth fully expanded leaves of each plant were selected for chlorophyll determination. One leaf was taken from each plant, and 0.2 g of fresh tissue leaves were weighed and placed in 10 mL 80% acetone solution for 10 h in the dark to determine OD₄₇₀, OD₆₄₅, and OD₆₆₃.

Investigation of ROS (Reactive Oxygen Species)

Histochemical staining of O_2^- and H_2O_2 was performed using nitro blue tetrazolium chloride (NBT) and 3,3-diaminobenzidine (DAB). The leaves of *A. arguta* at different treatment stages were immersed in 0.5 $mg \cdot mL^{-1}$ NBT solution for 24 h and 0.5 $mg \cdot mL^{-1}$ DAB solution for 24 h, respectively. The stained leaves were rinsed with 80% ethanol, 70°C for 15 min, and then bleached in a boiling water bath with bleaching solution for 10 min before the leaves were photographed [35].

Fruits quality analysis

Five fruits were randomly selected and each fruit was weighed. Fruits slices were cut 5 g and dried in a constant temperature drying oven at 105°C to constant weight, repeated three times. The flesh is the peeled tissue of the whole fruit. The seeds include all the mature and immature seeds in the fruit. Five *A. arguta* were randomly selected from each group and divided into 2 groups. The fresh weight of whole fruit, the fresh weight of pulp and the fresh weight of seeds were successively weighed.

Vitamin C (V_C) were measured using the methods described earlier [34]. The 100-grain weight, pulp water content, seed water content, soluble solids content (SSC) according to the method of Yang et al. [37]. The content of soluble sugar was were performed as described by anthrone colorimetric method [38]. The titratable acid was determined in accordance with the protocol described by Yang et al. [37]. Three fruits were taken from each treatment, and the results were repeated three times.

Statistical Analysis

The data were recorded and calculated by Microsoft® Excel® 2021MSO. The data were subject to one-way analysis of variance (ANOVA) and statistics were performed by SPSS 19.0 and Duncan's new complex range method. We designated a P value less than 0.05 as a significant difference ($p < 0.05$). All figures were constructed using GraphPad Prism 6 and Adobe Illustrator CC2018.

Results

The influence of bleeding on the anatomical structure of leaf

In this study, we observed leaf structure including collenchyma, palisade tissue, sponge tissue, and so on (Fig. 1). At initial flowering stage, there no differences in leaves anatomical structure were detected among control and treatment (Fig. 1A, E). At blooming stage, treatment displayed significantly increased numbers of collenchyma relative to control (Fig. 1B, F). And control presented significantly increased than treatment for thickness of xylem and phloem. At fruiting stage, control showed increased thickness of xylem and regular arrangement of vein compared to treatment (Fig. 1C, G). At fruit maturity stage, treatment showed the incomplete and damaged structure of xylem relative to control (Fig. 1D, H). However, the xylem and mesophyll integrity of treatment was relatively poor. In addition, control had more

collenchyma and parenchyma cells than treatment. In this study, the bleeding obviously damaged the leaf structure and affected the leaf development in different periods.

Effect of bleeding on leaf morphology and growth parameters

There were significantly different in leaf length, leaf width, longest leaf, petiole length, petiole width and leaf area between control and treatment at different growth periods. The values of these parameters in treatment were lower than those in control. The leaves of control and treatment were significantly different at any periods (Fig. 2B-G). The size of the leaves in treatment was significantly lower than that in control. Compared with the shape of the leaves in treatment and control, the leaves in treatment were more deformity than that in control. With the extension of the growth time, the color of the leaves in control was darker than treatment (Fig. 2A). In addition, the number of veins in control was also significantly higher than that in treatment. At the initial flowering stage, there was no significant difference in leaf length and leaf area. While at the blooming stage, fruiting stage, and fruit maturity stage, the length and leaf area of treatment were smaller than those of control (Fig. 2B, G).

Effect of bleeding on relative electrical conductivity, relative water content and malondialdehyde (MDA) content of leaves

Relative electric conductivity in treatment showed a trend of increasing from initial flowering stage to fruit maturity stage (Fig. 3A). Treatment was slightly higher than control, indicating that the membrane of leaf tissue was damaged. The maximum value of relative electric conductivity appeared at fruiting stage, and the minimum value appeared at fruit maturity stage. Furthermore, the MDA contents of the leaves increased first and then decreased at different phenological periods (Fig. 3B). At the initial flowering stage, blooming stage, fruiting stage and fruit maturity stage, MDA content in leaves of treatment was higher than that of control. At the fruiting stage, MDA content in leaves of treatment was significantly different from control, and the maximum MDA content in leaves appeared which was $10.36 \mu\text{mol}\cdot\text{g}^{-1} \text{FW}$ and $14.94 \mu\text{mol}\cdot\text{g}^{-1} \text{FW}$, respectively. The relative water content increased first and then decreased (Fig. 3C). At the fruiting stage, the relative water content reached the maximum value. In conclusion, the bleeding can decrease the water content, increase the relative electric conductivity and MDA content, and at the fruiting stage, the damage to leaves was the greatest.

Effects of bleeding current on chlorophyll content and photosynthetic parameters of leaves

From initial flowering stage to fruiting stage, the concentration of chlorophyll a and chlorophyll b in the leaves of treatment markedly remained lower level than those of control. However, the high concentration of chlorophyll a and chlorophyll b was found in the leaves of treatment compared to control (Fig. 4A, B). The ratio of chlorophyll a/b showed decreased value in treatment at initial flowering stage, fruiting stage and fruit maturity stage but increased in treatment at blooming stage compared to control (Fig. 4C). The

concentration of carotenoid decreased at initial flowering stage, blooming stage and fruiting stage, and increased at fruit maturity stage (Fig. 4D).

To further confirm that bleeding is responsible for changes of photosynthetic parameters, the values of P_n , $\bar{C}_i$, G_s and T_r were determined between control and treatment (Fig. 5). The values of P_n , $\bar{C}_i$, G_s and T_r in control were higher than those in treatment from initial flowering stage to fruiting stage. However, at fruit maturity stage the values of P_n and T_r in control were lower than that in treatment, and the bleeding on *A. arguta* did not produce a significant effect on G_s .

Effects of bleeding on ROS Accumulates in Leaves of *A. arguta*

Total H_2O_2 and $O_2^{\cdot-}$ levels of leaves were examined in control and treatment. It is clearly shown in Fig. 6, the NBT staining of control was lighter than that of treatment, and the NBT staining was the deepest at the fruiting stage and $O_2^{\cdot-}$ accumulation was enhanced in treatment when compared with control from initial flowering stage to fruit maturity stage. DAB staining results showed that H_2O_2 contents were significantly lower in control relative to treatment under all growth stages.

Effects of bleeding on fruit morphology, growth parameters and fruit quality

As shown in Fig. 7A, the fruit of control had high maturity, full seeds and more seeds. In treatment, the fruit maturity was low, the phenomenon of late ripening occurred, and the seed development was incomplete and the number was small. In addition, the fruit also appeared slight deformity phenomenon. Thus, bleeding can lead to delayed fruit development, incomplete seed development, fruit deformity and other phenomenon. There was significant difference between control and treatment in terms of 100-grain weight, single fruit weight, seed water content and fruit fresh water content (Fig. 7B, C). Treatment had the lower 100-grain weight (0.26 g), single fruit weight (8.07 g), seeds water content (23.19%) and pulp water content (26.18%).

In addition, treatment also showed decrease in V_C content, soluble sugar content, titratable acid content and soluble sugar content (SSC). The content of V_C in control was about twice that in treatment. The soluble sugar content in control was higher than that in treatment ($5.8207 \text{ mg}\cdot\text{g}^{-1}$ in control), and the soluble sugar content in treatment was $4.0377 \text{ mg}\cdot\text{g}^{-1}$. There was no significant difference in fruit titratable acid content between control and treatment, and the SSC of control was slightly higher than that of treatment, but the difference was small (Fig. 8B). It shows that there was a significant difference in fruit SSC between control and treatment. The SSC in control and treatment were 17.6% and 12.3%, respectively. In conclusion, the bleeding has a great effect on fruit quality of *A. arguta*. The contents of V_C , soluble sugar, SSC in treatment is lower than those in control, but no significant difference in titratable acid content.

Discussion

It is well established that the medicinal value of plant bleeding sap is high [25]. However, the bleeding is easy to induce the fruit canker during the process of production. For instance, in European beech, the production of phytophthora increased the damage of bleeding to the tree body [11]. In the pear trees also found this kind of phenomenon that bleeding canker is a devastating disease [39]. Therefore, during the process of collecting bleeding sap, tree body should be avoided to be seriously harmed by bleeding, and proper methods should be adopted to obtain high-purity bleeding sap [40]. In our study, the collection device based on the bottle of celine was used (Fig. S1), which had good sealing and reduced the infection of pathogenic bacteria.

Although the bleeding sap has many benefits, bleeding can cause damage to *A. arguta*. In this study, it was found that bleeding could cause leaf deformities, increased collenchyma tissues, damaged xylem and phloem integrity, and reduced leaf area of *A. arguta* (Fig. 1, 2). Furthermore, the flowering period was delayed and shorter, the number of fruits decreased and the immaturity rate increased. Accordingly, for this stress, the cell membrane first suffers functional damage and structural damage, leading to the extravasation of electrolytes in the cell, which makes the increase of the relative electrical conductivity [41, 42]. Therefore, the damage degree of the cell membrane and the tolerance of plants can be detected according to the changed level of relative electrical conductivity [43]. At different phenological stages of plants, the relative electrical conductivity of leaves in treatment was slightly higher than that in control, indicating that the tissue membrane of the leaf is slightly damaged after bleeding. Besides, relative water content is a reliable indicator of plant water deficit and can be used to detect plant response to stress [44]. In this study, the relative water content of leaves decreased after bleeding. Furthermore, soluble sugars can play the role of osmotic regulator, which is conducive to the maintenance of cell structure and function under stress, and promote the accumulation of proline to increase membrane stability [45, 46]. Through the determination of leaf physiological indexes, it was also found that the content of MDA increased from the initial flowering stage to the fruiting stage (Fig. 3), while in the fruit maturity stage, most of physiological indexes decreased, indicating that bleeding would stimulate plants to produce stress response, which is similar to the research results of Lu et al. [47]. In our study, the consistent trend of changes in MDA and other osmotic regulatory substances may be caused by the serious injury and loss of nutrients in plants, and may also be related to the response of plants to adversity stress. Bleeding would stimulate the stress response of plants. In this regard, this finding is consistent with a previous report in grape leaves. For example, stress-related osmotic regulatory substances and stress products showed an upward trend, and the activity of protective enzymes also increased to a certain extent [16]. Thus, we propose that the bleeding phenomenon will have a negative effect on plant growth.

In addition, the regulation of photosynthesis in response to environmental stimuli is a crucial process in plant adaptation to stress [48, 49]. Photosynthesis is the basis of plant growth and development and an important factor of productivity, as an important reference index in the field of plant cultivation, resistance to stress and breeding. *Ph*, *Cl*, *Gs*, and *Tr* are important indicators reflecting the photosynthetic capacity of plant leaves and chlorophyll is the main pigment for photosynthesis in plants [50–52].

Carotenoids have a strong antioxidant effect, and treatment carotenoids were significantly lower than control, indicating that the antioxidant capacity of plants was reduced by bleeding treatment [53]. And the chlorophyll A/B of leaves in treatment was lower than that in control (Fig. 4), showing that the cell membrane of plant leaves was damaged by bleeding and the ion exchange ability was weakened [54–56]. Several studies demonstrated that photosynthetic pigment content and photosynthase content influence the photosynthetic rate to a certain extent, which is the similar results observed in our results [57, 58]. Hou et al also suggested that photosynthetic rate is positively correlated with the relative content of chlorophyll, and closely related to P_n , T_r and C_s . P_n can reflect the T_r and C_s to a certain extent [59]. P_n was the same with the overall trend that the contents of chlorophyll a, chlorophyll b and carotenoid were lower than that in control. It indicated that P_n and chlorophyll content were positively correlated before and after the bleeding. In this study, it may be due to leaf deformities after the bleeding, which affected the chloroplast function.

Increased production of reactive oxygen species (ROS) is a common consequence of exposure to some abiotic stresses. This results from excitation of the light reactions of photosynthesis [48]. Above we prove that stress can inhibit the photosynthetic process. Moreover, excessive accumulation of ROS can cause damage to plants, adversity can lead to oxidative bursts that increase ROS levels and cause cell damage [35]. After bleeding, from initial flowering stage to fruit maturity stage, the accumulation of $O_2^{\cdot-}$ in treatment was enhanced compared with control. All growth stages H_2O_2 accumulation was increased in treatment based on DAB staining results, indicating that the bleeding can increase ROS levels in *A. arguta* leaves (Fig. 6).

A. arguta is not only rich in nutrients, but also has high medicinal value [60]. In our study, it was found that the water content of pulp decreased, and the water content of seeds differed greatly (Fig. 7). The contents of Vitamin C (V_C), soluble sugar and SSC in the fruit decreased. The SSC is also an important factor that unnoticeably sways consumer's preferences, and V_C is one of the important vitamins to maintain the normal physiological function of the body, which is mainly taken from fruits and the *A. arguta* is rich in V_C [61]. So bleeding has a great influence on the quality of fruit.

A. arguta has high vitality and strong climbing ability, it can be used for shading in summer and has landscape and ecological value [62]. Its fruit is a nutritious edible health fruit, but the fruit has problems such as short harvesting time and poor storage resistance, so improving its storage and preservation technology will become an important trend of future research and development [63]. *A. arguta* is rich in wild resources. In recent years, the research and development of new varieties of *A. arguta* has been strengthened. However, the development process of *A. arguta* industry is slow, and the speed of new varieties research should be accelerated [64]. In this study, we only analyzed the influence of bleeding of *A. arguta* "Kuili" variety, other varieties still need to be studied, and the development and utilization of "Kuili" bleeding sap is still worthy of further study.

Conclusion

Outcomes of current study revealed that bleeding significantly negative impact *A. arguta* growth, photosynthetic and chlorophyll content, etc. Furthermore, bleeding increased the relative electrical conductivity MDA and ROS production. The pulp and the seed water content and the fruit vitamin C, soluble sugar content and soluble solids content decreased after the damage. Therefore, this study provides a theoretical basis for the physiological changes of *A. arguta* after bleeding, and laid the foundation for the research of *A. arguta* breeding and long-term field studies should be executed in order to gain insights into underlying mechanisms.

Declarations

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Contributions

All authors conceived and designed the experiments, performed the experiments, collection and analyzing the data, wrote the paper and revising of manuscript. All authors read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate

All experimental studies on plants were complied with relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

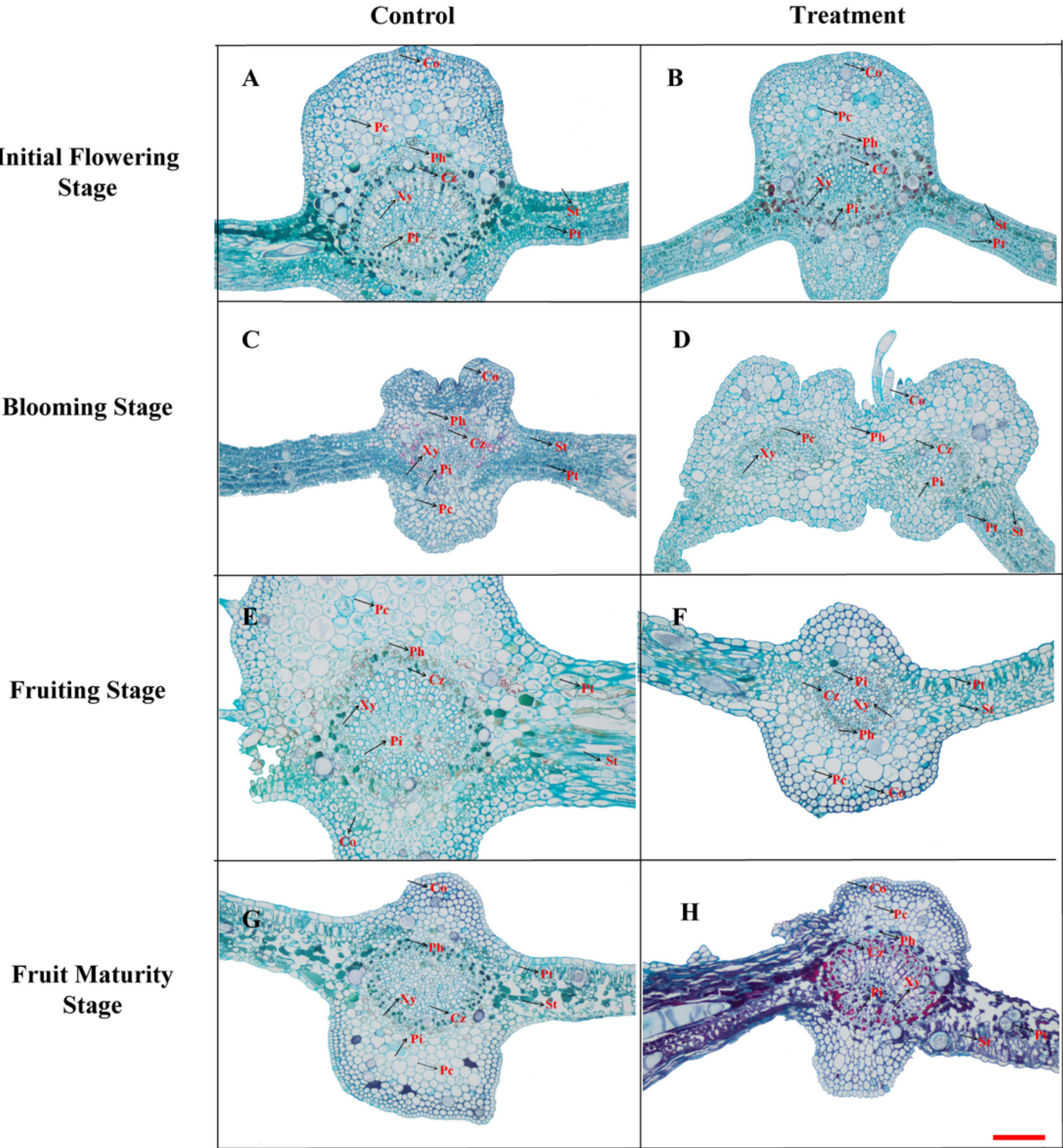


Figure 1

The anatomical structure of main veins of leaves

Note: A-D: Control group; E-H: Treatment group; Xy: Xylem; Pc: Parenchyma cell; CZ: Cambium zone; Ph: Phloem; Pi: Pith; Co: Collenchyma; Pt: palisade tissue; St: Sponge tissue; Magnification: 100 times; Bar = 100 μm

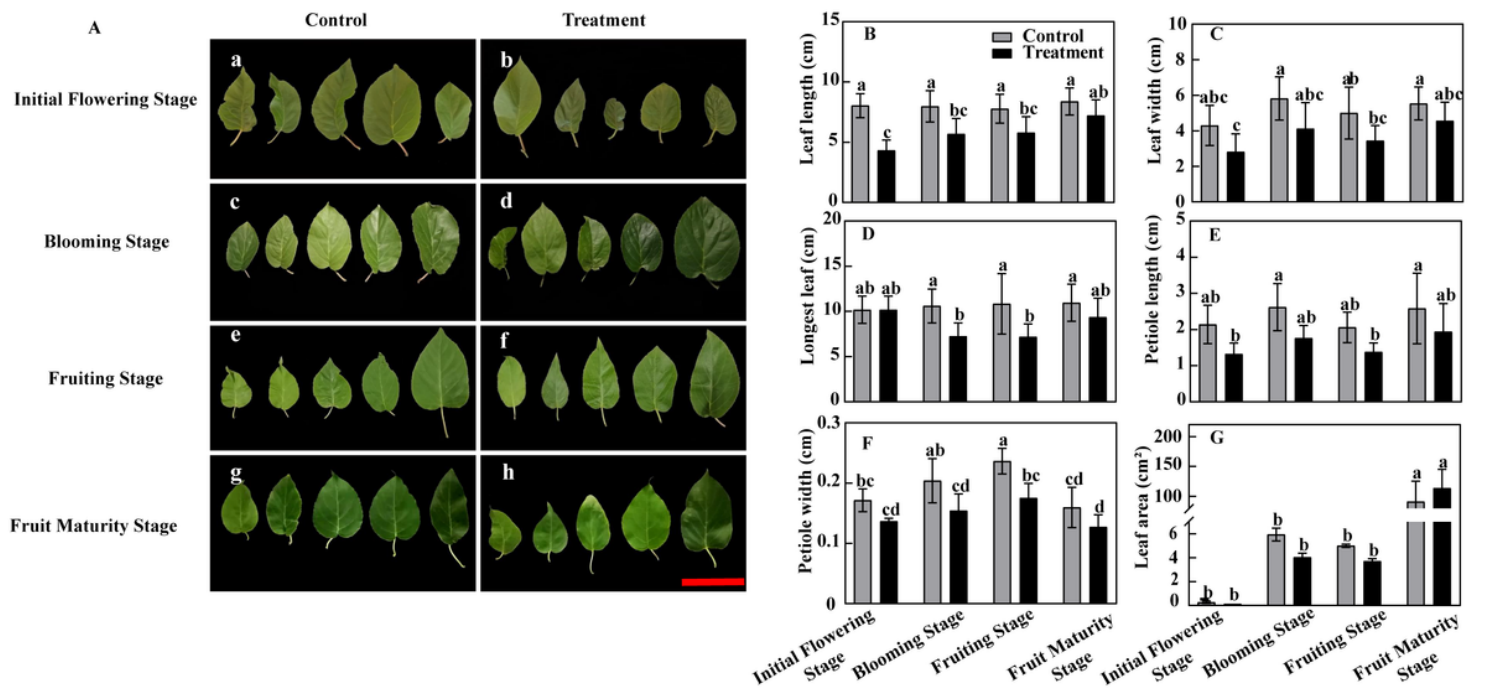


Figure 2

The effects of bleeding on leaves morphology and growth parameters in different phenological stages

Note: A: a, c, e, g: Control group; b, d, f, h: Treatment group B: Leaf length; C: Leaf width; D: Petiole long; E: Petiole width; F: The longest leaf; G: Leaf area; Bar = 10 cm; Different letters indicate significant differences ($P < 0.05$).

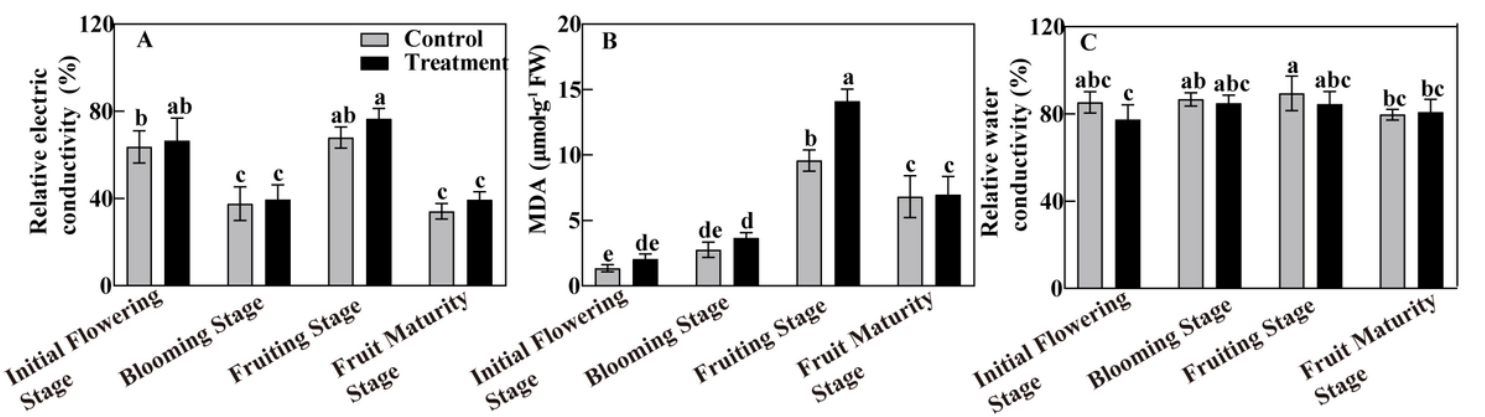


Figure 3

The effects of bleeding treatment on relative conductivity (A), relative water content (B) and malondialdehyde (MDA) content (C) of leaves at different phenological stages

Note: A: Relative conductivity; B: Relative moisture content; C: Malondialdehyde; Different letters indicate significant differences ($P < 0.05$).

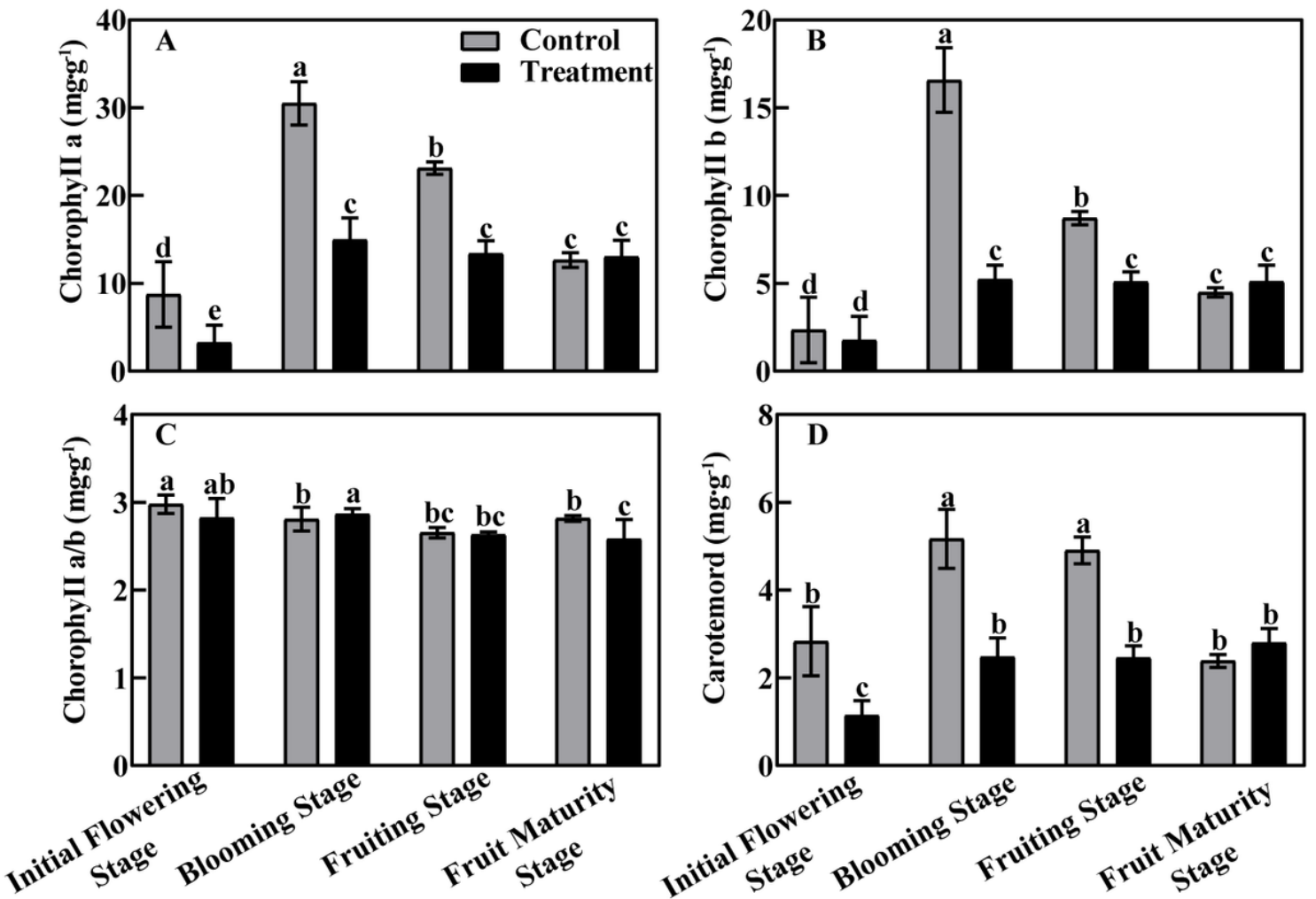


Figure 4

The effect of bleeding treatment on chlorophyll at different phenological periods

Note: A: Chlorophyll a; b: Chlorophyll b; C: Chlorophyll a/b; D: Carotenoids; Different letters indicate significant differences ($P < 0.05$).

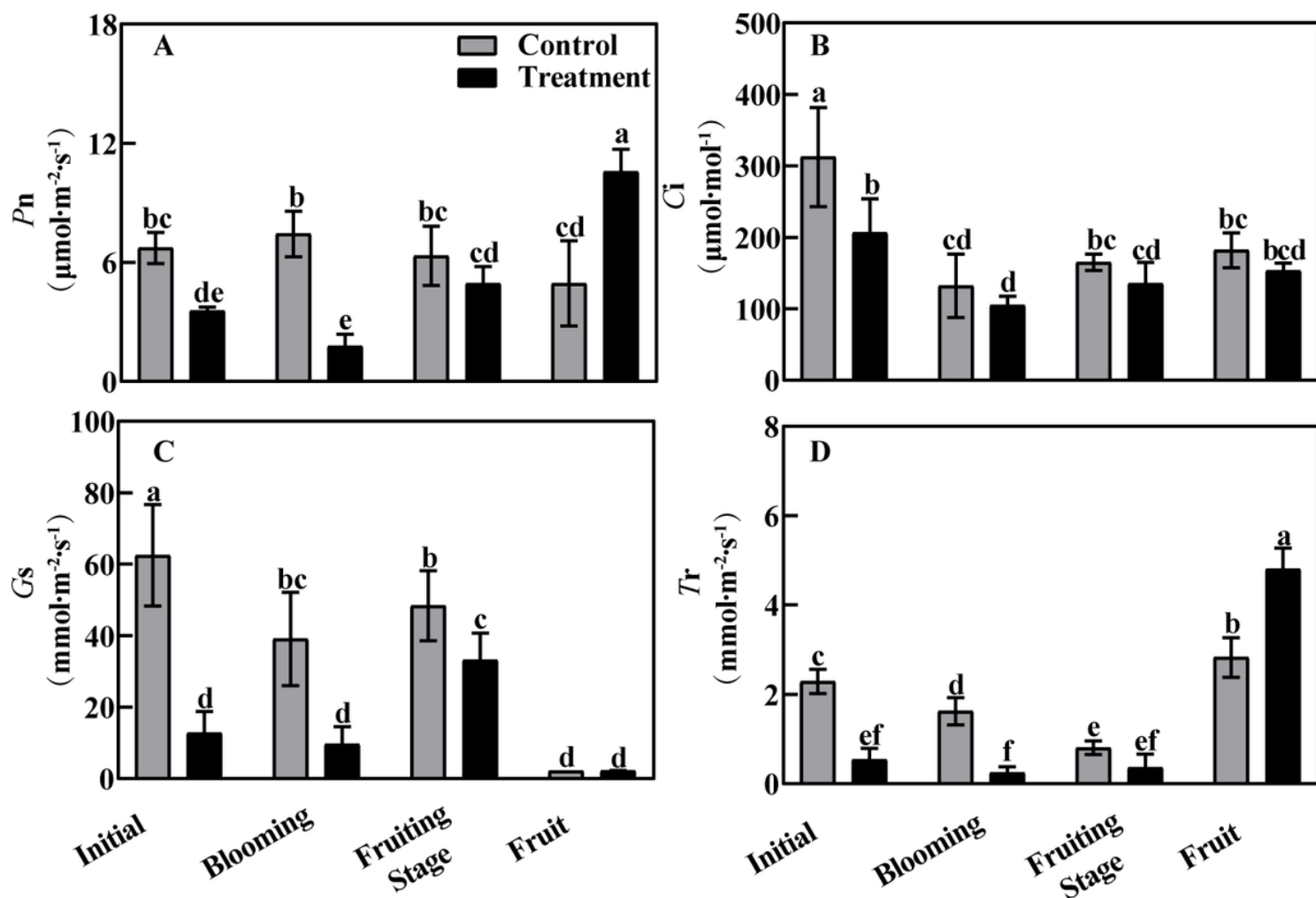


Figure 5

The effect of bleeding treatment on photosynthetic parameters of leaves in different phenological periods.

Note: A: The net photosynthetic rate (P_n); B: Intercellular CO_2 concentration (C_i); C: Stomatal conductance (G_s); D: Transpiration rate (T_r); Different letters indicate significant differences ($P < 0.05$).

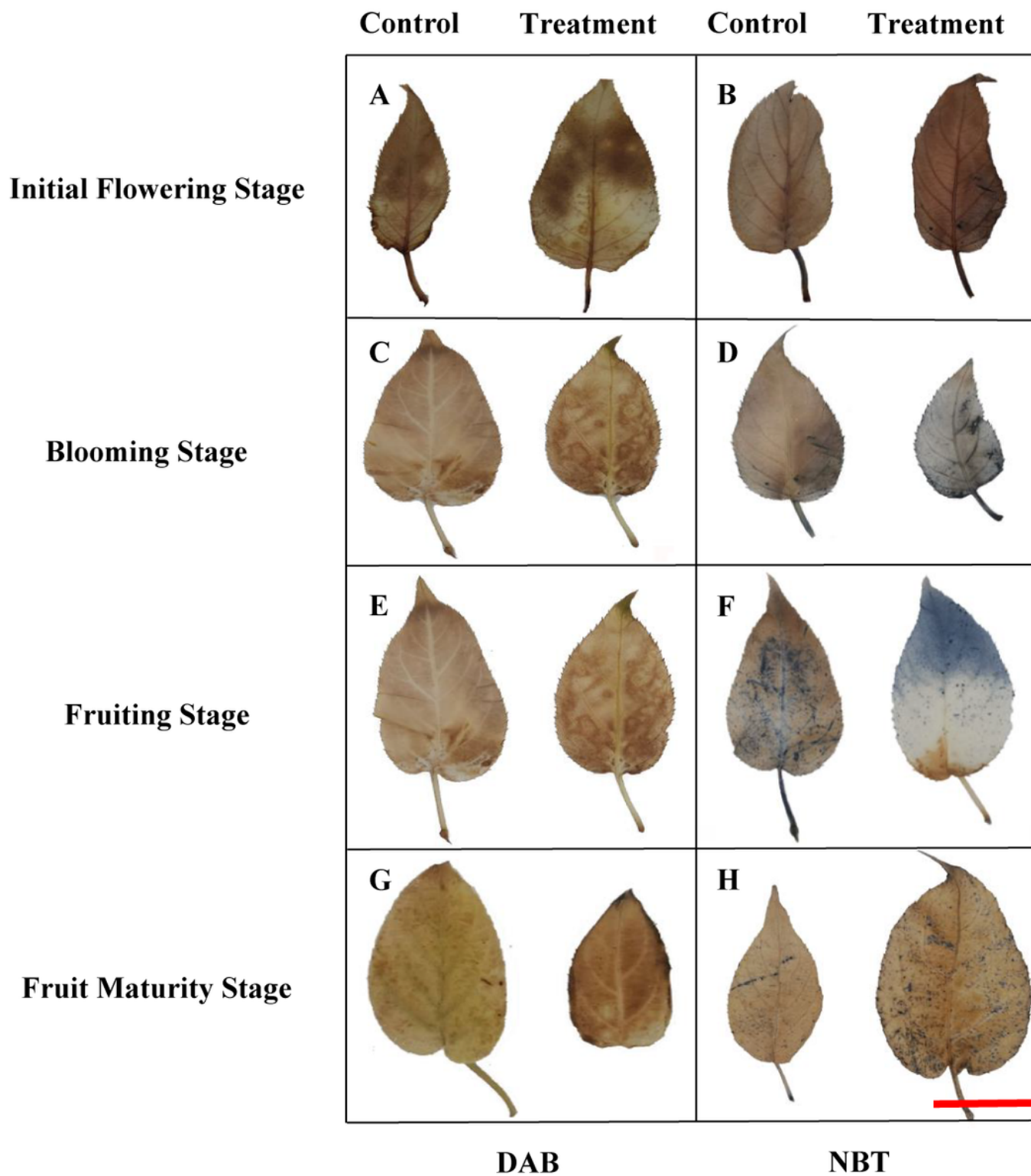


Figure 6

The effect of bleeding on DAB and NBT staining of leaves

Note: A, C, E, G: DAB staining; B, D, F, H: NBT staining; Bar = 5 cm.

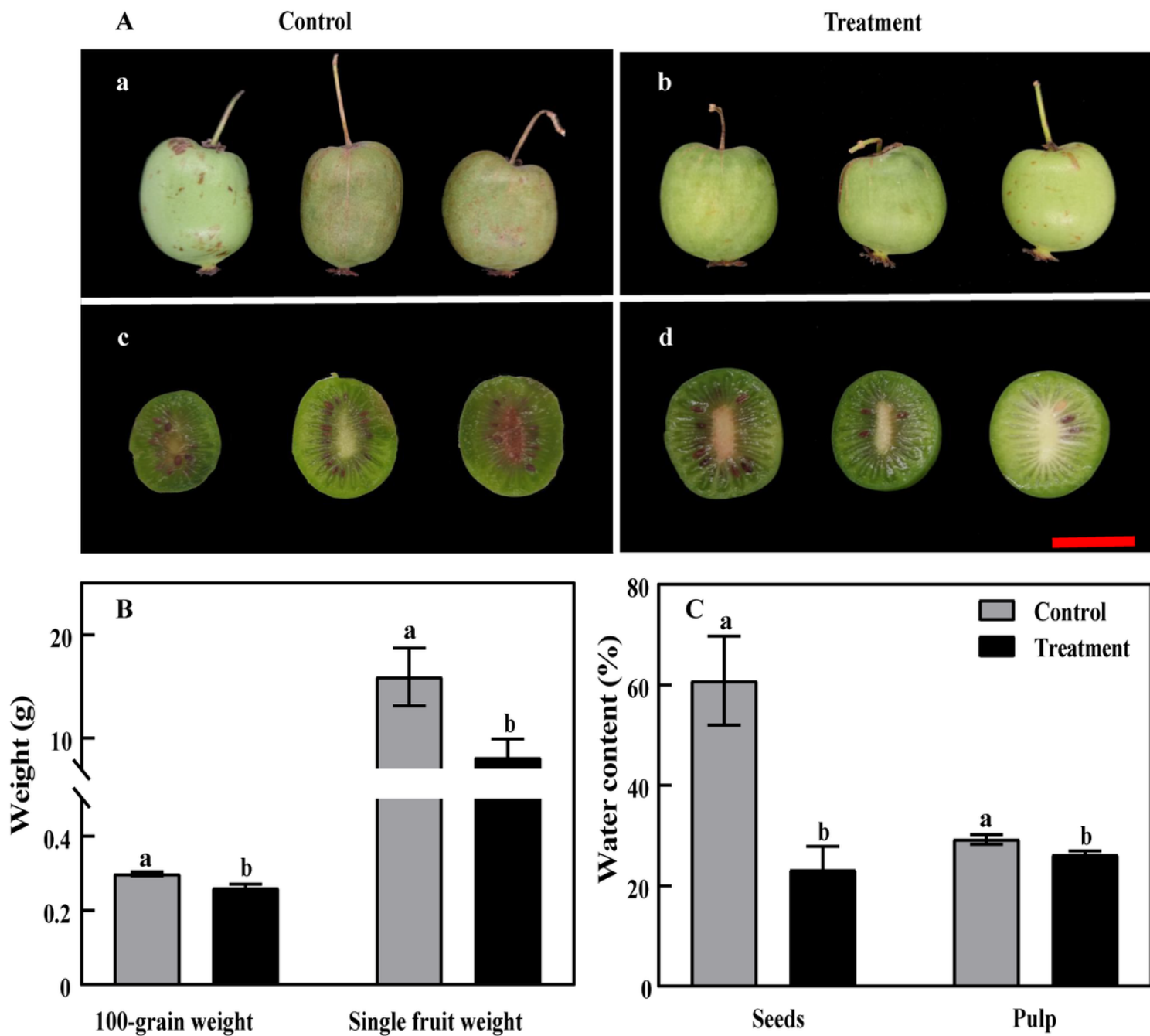


Figure 7

The fruit phenotypic image and phenotypic index during fruit ripening under bleeding condition

Note: A: (a, c): Control group; (b, d): Treatment group; B: The 100-grain weight and single fruit weight; C: Seed water content and pulp water content; Bar = 5 cm; Different letters indicate significant differences ($P < 0.05$).

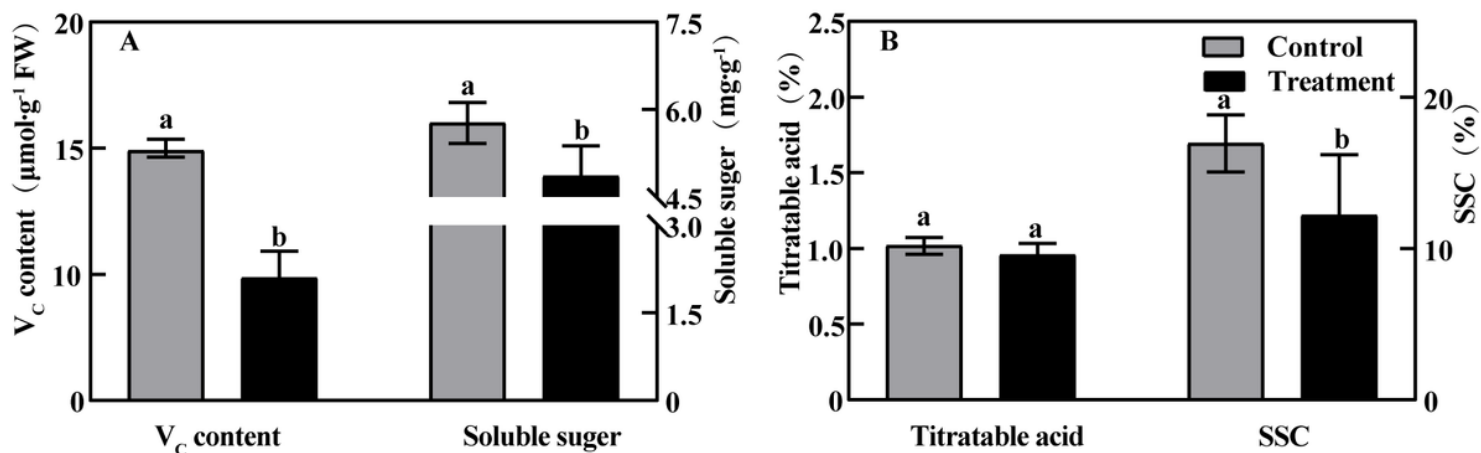


Figure 8

The effects of bleeding treatment on fruit quality

Note: A: Vitamin C; B: Soluble sugar; C: Titratable acid; D: Soluble solids content (SSC); Different letters indicate significant differences ($P < 0.05$).

Supplementary Files

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