Postprint: Effects of Climatic Factors on Siraitia grosvenorii Quality and Its Molecular Regulatory Mechanism

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Date: 2024-02-07T00:00:00+00:00

Abstract

To investigate the effects of climatic factors on the quality of Siraitia grosvenorii pollinated in different seasons and their underlying molecular mechanisms, this study employed the main cultivar 'Qingpiguo' with fruits pollinated in summer and autumn as experimental materials. Using methods including monitoring of climatic factors at different developmental stages, measurement of morphological parameters, metabolic analysis of mogrosides, and qRT-PCR-based gene expression analysis, statistical comparisons were conducted on differences in habitat climatic factors, quality traits, and gene expression between the two groups. The results demonstrated that, compared with summer-pollinated fruits, autumn-pollinated fruits exhibited: (1) significantly lower average habitat temperature and effective accumulated temperature after 35 days, significantly higher diurnal temperature variation before 65 days, with differences in average temperature and effective accumulated temperature being more pronounced than those in diurnal temperature variation, while light intensity and air humidity remained similar; (2) increased transverse diameter, longitudinal diameter, and single fruit weight, though these differences were not statistically significant; (3) delayed synthesis and accumulation rates of mogroside V and 11-O-mogroside V by approximately 10 days, with both compounds showing significant reductions in content from 55 days onward, decreasing by 40.66% and 46.07% respectively at maturity; (4) poor consistency in the coordinated expression of mogroside V synthase genes at 55 days, fewer up-regulated genes and smaller fold changes, and down-regulated expression of the glucosyltransferase gene SgUGT94-289-3 responsible for the final synthetic step. In summary, the morphological size of summer- and autumn-pollinated S. grosvenorii was not affected by climatic factors, whereas mogroside V quality was significantly influenced by temperature, which may cause differences in mogroside V quality between the two groups by regulating the consistency and level of coordinated

expression of mogroside V synthase genes. These findings provide a theoretical basis for high-quality cultivation and genetic breeding of S. grosvenorii.

Full Text

Effects of Climatic Factors on the Fruit Quality of *Siraitia grosvenorii* and Its Molecular Regulation Mechanism

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Abstract

This study investigated the impact of climatic factors on the quality of *Siraitia grosvenorii* fruits pollinated in different seasons and the underlying molecular mechanisms. Using the main cultivar "Qingpiguo," we compared summer- and autumn-pollinated fruits through continuous monitoring of climatic factors during development, morphological measurements, mogroside metabolite detection, and qRT-PCR analysis of gene expression. Statistical analysis revealed significant differences in habitat climate factors, quality traits, and gene expression between the two groups. Compared to summer-pollinated fruits, autumn-pollinated fruits showed: (1) significantly lower average temperature and effective accumulated temperature after 35 days, with increased diurnal temperature variation before 65 days, though differences in temperature were more pronounced than those in diurnal variation; light intensity and air humidity remained similar; (2) increased transverse diameter, longitudinal diameter, and single fruit weight, though these differences were not statistically significant; (3) delayed synthesis and accumulation of mogroside V and 11-O-mogroside V by approximately 10 days, with contents decreasing significantly from 55 days onward, dropping by 40.66% and 46.07% respectively at maturity; and (4) poor consistency in coordinated expression of mogroside V biosynthetic genes at 55 days, with fewer upregulated genes and lower expression levels, particularly for the glucosyltransferase gene *SgUGT94-289-3* responsible for the final biosynthetic step, which was downregulated in all cases. These results indicate that while fruit morphology and size were not affected by climatic factors, mogroside V quality was significantly influenced by temperature, which likely modulates the consistency and level of coordinated expression among mogroside V biosyn-

thetic genes, thereby causing quality differences between summer- and autumn-pollinated fruits. This study provides a theoretical basis for high-quality cultivation and genetic breeding of *S. grosvenorii*.

Keywords: different seasons, *Siraitia grosvenorii* quality, climatic factors, fruit size, mogroside V content, gene expression

Introduction

Siraitia grosvenorii (Swingle) C. Jeffrey ex A.M. Lu & Zhi Y. Zhang, a member of the Cucurbitaceae family, is both a medicinal and natural sweetener plant. Its dried fruits are renowned traditional Chinese medicinal materials with multiple therapeutic effects, including heat-clearing, cough relief, anti-inflammatory, anti-asthmatic, phlegm reduction, lung moistening, bowel regulation, hypoglycemic, lipid-lowering, antioxidant, anti-cancer, and anti-fibrotic properties. The fruits contain various high-intensity sweet triterpenoid saponins, with mogroside V being the primary active component. The sweetness intensity of mogrosides III, VI, siamenoside I, V, 11-O-mogroside V, and isomogroside V are 195, 300, 465, 378, 68, and 500 times that of sucrose, respectively. Due to their low caloric content and safety, these sweeteners are suitable for consumption by diabetic, hypertensive, and obese populations and have been extensively extracted for commercial use as natural sweeteners. In recent years, increasing market demand and economic benefits have made *S. grosvenorii* cultivation a pillar industry for rural revitalization, with cultivation areas rapidly expanding from the traditional production zones to surrounding regions.

However, the relatively low content of sweet glycosides in fruits has led to high raw material costs for sweetener extraction, representing a major bottleneck for industry development. This limitation is primarily associated with current cultivars and also correlates with fruit maturation batches. *S. grosvenorii* requires artificial pollination to set fruit, with the pollination period concentrated from early July to late August. Fruits pollinated in July (summer) and August (autumn) typically mature in two distinct batches. Climatic variations across different production regions and growing seasons directly affect fruit maturation and both internal and external quality. In coffee, high temperatures accelerate fruit maturation but compromise quality. In grapes, high temperatures increase maturity and acidity, while low temperatures enhance the accumulation of vitamin C, polyphenols, and anthocyanins. High average temperature and humidity favor sugar accumulation. In blackcurrant, high temperatures increase terpenoid volatile accumulation, whereas low temperatures enhance fruit diameter and organic acid accumulation that determine flavor. In cucumber, C6 aldehyde content correlates positively with light intensity, while C9 aldehyde content correlates negatively with relative humidity. In blackberry, early-season fruits exhibit higher single fruit weight and organic acid content but lower anthocyanin and soluble solids compared to late-season fruits.

Processing enterprises believe that early-maturing *S. grosvenorii* fruits have inferior quality due to high habitat temperatures, increasing raw material costs, while late-maturing fruits, produced under lower temperatures and greater diurnal temperature variation, offer superior quality and reduced costs. Consequently, there is a clear preference for purchasing late-season fruits. Since breeding new high-glycoside cultivars requires long cycles, investigating the specific quality differences between fruits pollinated in different seasons and their responses to habitat climatic factors can guide the selection of suitable cultivation areas and optimization of high-yield, high-quality cultivation techniques. This approach can reduce quality variation among mature fruits, lower sweetener extraction costs, and help alleviate the industry bottleneck.

Commercial *S. grosvenorii* fruits are round or oblong, with pricing based on transverse diameter grades, and mogroside V content serves as the primary internal quality indicator. Previous studies on external quality have shown that fruit transverse diameter, longitudinal diameter, and single fruit weight increase rapidly within 25 days after pollination and remain essentially unchanged after 30 days. Research on internal quality has revealed that high-sweetness mogrosides are gradually converted from tasteless or bitter low-glycosides. Before 30 days, fruits primarily contain mogrosides I and IIE; between 30-50 days, mogroside IIE decreases and is converted to mogrosides III, IV, and V; during 50-70 days, mogroside V accumulates rapidly approaching maximum content while mogrosides IIE and III decrease and disappear; and at 70-85 days maturity, the fruit mainly contains sweet mogroside V.

The synthesis and accumulation of low-glycosides are controlled by the expression of *SgSQE*, *SgCDS*, *SgEPH1-3*, *SgCYP102801*, and *SgUGT85-269-1,4* genes in young fruits, while their conversion to mogroside V is regulated by *SgUGT94-289-1,2,3* genes during mid-to-late fruit development. However, the diurnal expression patterns of these genes in mogroside V biosynthesis remain unclear. Bai et al. (2009a,b) analyzed historical meteorological data and growth survey records to assess climate factor effects on *S. grosvenorii* growth across different production regions, showing that mountainous areas had higher large fruit rates (78%) compared to paddy fields with higher temperatures, stronger light, and lower humidity (73%). The main production zones with annual average temperatures of 17-18°C, July-August mean temperatures of 26.8-27.1°C, sunshine duration of 1400 hours, and air humidity of 80% produced fruits with higher total sugar content than promotion areas with higher average temperature, sunshine duration, and lower humidity. They also classified optimal, suitable, and unsuitable cultivation zones based on climate effects on flowering, fruit set, and fruit size. When *S. grosvenorii* was introduced to India outside the traditional production zone, mogroside V content was low and the rapid accumulation period was delayed to 70-80 days. However, these survey results require experimental verification, and the individual effects of temperature, light, and humidity on fruit morphology, total sugar content, and mogroside V content remain unclear. Moreover, the suitability zones classified without using mogroside V content as a criterion need refinement. Additionally, no studies have reported the effects of

different growing seasons' climatic factors on *S. grosvenorii* internal and external quality, necessitating research on how seasonal climate factors induce gene expression to affect fruit quality.

Therefore, this study investigated the effects of temperature, light, and humidity on the internal and external quality of *S. grosvenorii* fruits pollinated in different seasons and the underlying molecular regulatory mechanisms. Using summer- and autumn-pollinated fruits, we monitored habitat climate factors, fruit morphology, mogroside V synthesis and accumulation, and gene expression changes. Through statistical analysis of differences in average temperature, diurnal temperature variation, effective accumulated temperature, light intensity, air humidity, fruit transverse diameter, longitudinal diameter, single fruit weight, mogroside content, and gene expression levels at different developmental stages, we aimed to address: (1) what quality differences exist between summer- and autumn-pollinated fruits; (2) which climatic factors affect these quality differences; and (3) how climate factors regulate gene expression to cause quality differences. The results will guide selection of suitable cultivation areas, development of high-yield and high-quality cultivation techniques, improve *S. grosvenorii* quality, alleviate the industry bottleneck of high raw material costs for mogroside V sweetener extraction, and provide a theoretical basis for future breeding of high-glycoside cultivars.

Materials and Methods

1.1 Plant Materials

The main cultivar "Qingpiguo"was used as experimental material. Seedlings were transplanted on March 20, 2022, and cultivated using conventional methods at Shuangdong Village, Longsheng Town, Longsheng County, Guilin City, Guangxi Zhuang Autonomous Region (110°2 57 E, 25°46 6 N). The experimental site is located in a subtropical monsoon climate zone at 251 m altitude, with an annual average temperature of 18.1°C, extreme maximum temperature of 39.5°C, extreme minimum temperature of -4.8°C, annual rainfall of 1,500-2,400 mm, and frost-free period of 314 days.

1.2 Equipment and Reagents

1.2.1 Equipment HPLC 1260 (Agilent, USA), 4000 QTRAP® LC-MS/MS (AB Sciex, Canada), Poroshell 120 SB-C18 column (Agilent, USA), 99-Vertical -86°C ultra-low temperature freezer (Thermo, USA), Veriti 96-Well Thermal Cycler PCR instrument (Thermo, USA), Light Cycler 480 II real-time PCR instrument (Roche, Switzerland), 220R high-speed refrigerated centrifuge (MIKRO, Germany), MX2301A temperature-humidity recorder (ONSET, USA), MX2202 temperature-light recorder (ONSET, USA), four-purpose digital electronic caliper (Guilin Measuring Tool Company), and JJ500 electronic balance (Changshu Shuangjie Test Instrument Factory).

1.2.2 Reagents Standard compounds including mogroside IA (MIA), mogroside IE (MIE), mogroside IIE (MIIE), mogroside IIIE (MIIIE), mogroside III (MIII), mogroside IVA (MIVA), siamenoside I (MSI), mogroside V (MV), and 11-O-mogroside V (11-O-MV) were purchased from Chengdu Mansite Biotechnology Co., Ltd. HPLC-grade formic acid, methanol, acetonitrile, and hexane were obtained from Emerson Fisher (USA). The CW0581M ultra-pure RNA extraction kit was purchased from Beijing Kangweishiji Biotechnology Co., Ltd. DL2000 Marker, 10×Glycerol DNA Loading Buffer, R232 reverse transcription kit, and Q711 real-time PCR kit were obtained from Nanjing Novizan Biotechnology Co., Ltd. Primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. Analytical-grade chloroform, anhydrous ethanol, and agarose were purchased from Guangxi Foland Biotechnology Co., Ltd.

1.3 Experimental Design and Measurements

1.3.1 Experimental Design During the full-bloom stage, 300 uniformly developed female flowers were randomly selected for pollination and date-tagged. Fruits pollinated and tagged on July 16, 2022, were designated as summer-pollinated fruits, while those pollinated on August 19, 2022, were designated as autumn-pollinated fruits. Fruit transverse diameter, longitudinal diameter, and single fruit weight were measured at pollination day (0 d) and at 5, 15, 25, 35, 45, 55, 65, 75, 85, and 95 days after pollination, with ten fruits measured per time point. Additionally, at 5, 35, 55, 75, 85, and 95 days after pollination, three well-developed summer- and autumn-pollinated fruits were randomly collected. Fruit flesh was excised, wrapped in labeled aluminum foil, frozen in liquid nitrogen for 30 min, and stored at -80°C for mogroside content determination, with three biological replicates per sample. Furthermore, during the rapid accumulation period of mogroside V at 55 days after pollination, three well-developed summer- and autumn-pollinated fruits were randomly collected at 14:00, 18:00, 02:00, 06:00, and 09:00. Fruit flesh samples were collected and stored using the same method for RT-qPCR analysis of mogroside V biosynthetic gene expression levels.

1.3.2 Measurement Indices (1) Environmental Factor Monitoring
Before pollination and tagging, temperature-humidity and temperature-light recorders were placed 20 cm above the cultivation 棚 in the center of the experimental field, programmed to record temperature, air humidity, and light intensity every 30 minutes.

(2) Fruit Size Measurement
Transverse diameter was measured at the maximum width of the fruit middle using a digital electronic caliper. Longitudinal diameter was measured from the fruit stalk to the fruit navel using the same caliper. Single fruit weight was measured using an electronic balance with 0.01 g precision.

(3) Mogroside Content Determination
Flesh samples stored at -80°C were transferred to liquid nitrogen, then rapidly

ground into uniform powder in a liquid nitrogen-pre-cooled mortar. Mogroside IA, IE, IIE, III, IIIE, IVA, siamenoside I, mogroside V, and 11-O-mogroside V contents were extracted and determined using the HPLC-MS/MS method described by Qiao et al. (2019).

(4) Mogroside Biosynthetic Gene Expression Analysis

Total RNA was extracted from 55-day summer- and autumn-pollinated fruit samples using the CW0581M ultra-pure RNA extraction kit. Complementary DNA (cDNA) was synthesized from total RNA using the R232 kit via a two-step reverse transcription procedure. The 20 l reaction system contained 4 l of 4×gDNA wiper Mix and 1 g template RNA, brought to 16 l with RNase-free ddH_2O, mixed thoroughly, and incubated at 42°C for 2 min. Subsequently, 4 l of 5×Hi Script III qRT Super Mix was added to reach a final volume of 20 l, mixed, and incubated at 37°C for 15 min and 85°C for 5 sec. Using the reverse-transcribed cDNA as template and *SgUBQ* as the reference gene, RT-qPCR was performed with gene-specific primers listed in Table 1 to determine expression levels of mogroside biosynthetic genes in 55-day summer- and autumn-pollinated fruits. The reaction system contained 10 l SYBR Green I, 1 l each of forward and reverse primers, 2 l template, and RNase-free ddH_2O to a final volume of 10 l. The thermal cycling program was: 95°C pre-denaturation for 30 sec, followed by 40 cycles of 95°C for 10 sec and 60°C for 30 sec. The melting curve program was: 95°C for 15 sec, 60°C for 60 sec, 95°C for 15 sec, and 37°C for 30 sec.

Table 1 RT-qPCR primers

Gene name	Forward primer (5’ -3’)	Reverse primer (5’ -3’)
SgHMGR	TAGGCTCCAAAGTATCCG	CAGTTTACAGCAGCAGGTT
SgSQS	CTGAGACACCCAGATGACT	GAGGGCTCGCAGAACAAGA
SgSQE	GCTTCGACCATCAACACATTG	TTCCTCCAAGGCTCAAGTAATC
SgCDS	GTTGGGTTGAAGATCCCTACTC	CCACAACTGGCTCCCATTAT
SgCAS	CAAATACAACATGCTCACCT	AGCCCTTCTTAGAGTGCC
SgEPH1	CCGATGATACCGAAAGAGAAGG	GAACTTGCCGGCGAAATAATC
SgEPH2	GTGACCCACAAGCTCCATATT	CGGCGTAAACGTCGATATCTT
SgEPH3	CTTGGGATCGAGAAGGTGTTT	CACCAGCGCCTTGATTCTAT
SgCYP102801	GACGATGTCTACGAAGCGATAC	GTGGGTGACATTGAAGGAAGA
SgUGT85-269-1	CCGATTGAAGTAGCGGAAGAA	CCTCAACGAGCTTCGGTATAAA
SgUGT85-269-4	CTCAATCCTCCGGTCTCATTC	GGGTCCAACGGTGTAGATTT
SgUGT94-289-1	CTGTTAACCTCGACGCCATTA	GCTGATCAGGAGAAGATGGAAG
SgUGT94-289-2	CCAAACACGGACCTACCTATT	GACCAACCGTCAGTGTAGTT
SgUGT94-289-3	CAGAGAAGATGTGCGGAAGAA	TCAGCGACCATCTCATCAAAC
SgUBQ	ATAAAAGACCCAGCACCACATTC	CCCTTGCCGACTACAACATCC

1.4 Statistical Analysis

Experimental data were organized and statistically analyzed using Excel 2016. For the periods 0-5 d, 5-15 d, 15-25 d, 25-35 d, 35-45 d, 45-55 d, 55-65 d, 65-75 d, and 75-85 d, average temperature, light intensity, and air humidity were calculated as mean values of all recorded data. Effective accumulated temperature was calculated as the sum of differences between average temperatures above 15°C and the 15°C baseline. Diurnal temperature variation was calculated as the difference between daytime and nighttime average temperatures. Relative expression levels of mogroside V biosynthetic genes at each time point were calculated using the 2^(-ΔΔCt) method. Significant differences in transverse diameter, longitudinal diameter, single fruit weight, mogroside V and its precursor/derivative contents between summer- and autumn-pollinated fruits, as well as differences in expression of mogroside V biosynthetic genes between 14:00 and other time points, were analyzed using t-tests.

Results

2.1 Comparison of Climatic Factors in Fruit Habitats

As shown in Figure 1 [Figure 1: see original paper] and Figure 2 [Figure 2: see original paper], the average temperature for summer-pollinated fruits remained relatively stable at approximately 27.79°C, and effective accumulated temperature remained stable at approximately 118.50°C after an initial lower period of 0-5 days. In contrast, autumn-pollinated fruits exhibited greater variation, with average temperature ranging between 15-28°C and effective accumulated temperature between 18-130°C, both generally lower than summer-pollinated fruits. However, the average temperature and effective accumulated temperature were similar between the two groups before 35 days, after which autumn-pollinated fruits were significantly lower, decreasing to approximately 20°C and 60°C respectively, and further declining to 16.80°C and 18.03°C during 76-85 days.

Diurnal temperature variation for summer-pollinated fruits was 3.75°C during 0-5 days, stable at approximately 9.43°C during 6-45 days, increased to approximately 13.01°C during 46-75 days, and decreased markedly to 7.41°C during 76-85 days. For autumn-pollinated fruits, diurnal variation was stable at approximately 11.97°C during 0-45 days, increased to 16.97°C during 46-55 days, decreased to 11.95°C after 65 days, and sharply reduced to 2.13°C during 76-85 days. Autumn-pollinated fruits maintained diurnal temperature variations above 10°C before 65 days, exceeding those of summer-pollinated fruits (which remained mostly below 10°C), and only fell below summer-pollinated fruits after 65 days. These results demonstrate significant temperature differences between the habitats of summer- and autumn-pollinated fruits.

As illustrated in Figure 3 [Figure 3: see original paper], light intensity for summer-pollinated fruits increased from 13,480.40 lx during 0-5 days to

22,582.37 lx during 6-15 days, then gradually decreased to the lowest value of 12,843.09 lx during 76-85 days. Autumn-pollinated fruits showed a continuous decreasing trend from 0-85 days. Except for 0-5 days when light intensity was 49.57% higher than summer-pollinated fruits (20,162.95 lx), all other periods were lower, with significant reductions of 28.71% and 26.53% during 66-75 d and 76-85 d respectively, and smaller differences of 7.52-19.88% during other periods. These results indicate that overall average light intensity was similar between the two groups.

As shown in Figure 4 [Figure 4: see original paper], air humidity for summer-pollinated fruits was 88% during 0-5 days, decreased to 74.91% during 6-15 days, and remained relatively stable from 16-85 days. For autumn-pollinated fruits, air humidity decreased significantly by 24.32% during 46-55 days and increased by 21.11% during 76-85 days compared to summer-pollinated fruits, with other periods showing minor differences of 3.88-16.45%. These results demonstrate that overall air humidity was similar between the habitats of summer- and autumn-pollinated fruits.

2.2 Comparison of Fruit Size

As shown in Figures 5 [Figure 5: see original paper]-7, transverse diameter, longitudinal diameter, and single fruit weight of autumn-pollinated fruits were all higher than those of summer-pollinated fruits, with both groups increasing gradually during 0-35 days. The period of 0-25 days represented rapid growth, after which these parameters remained essentially unchanged. Only longitudinal diameter and single fruit weight at 15 days showed significant differences, with no significant differences observed at other time points. These results indicate that fruit size differences between summer- and autumn-pollinated fruits were not significant.

Error bars represent standard deviation. Lowercase letters indicate significant differences between summer- and autumn-pollinated fruits ($P < 0.05$). The same applies below.

2.3 Comparison of Fruit Mogroside Content

As shown in Figures 8 [Figure 8: see original paper] and 9 [Figure 9: see original paper], both fruit groups contained mogrosides IA, IE, IIE, and III before 35 days, with no significant differences in content, consistent with the minimal differences in habitat temperature during this period. At 55 days, both groups still contained mogrosides IA, IE, and IIE, and like the 75+ day period, also contained mogrosides IIIE, III, IVA, siamenoside I, mogroside V, and 11-O-mogroside V. However, starting at 55 days, all mogrosides except IIE showed significant differences between groups, coinciding with the emergence of significant differences in average temperature and effective accumulated temperature. Specifically, contents of mogrosides IIIE, III, IVA, and siamenoside I were significantly higher in summer-pollinated fruits at 55 days, but the opposite pattern

was observed thereafter. Mogroside V and 11-O-mogroside V contents were significantly higher in summer-pollinated fruits from 55 days onward.

Regarding accumulation patterns from 5-85 days, summer-pollinated fruits showed increased contents of mogrosides IIE, IIIE, and III during 5-35 days, while mogrosides IVA, siamenoside I, mogroside V, and 11-O-mogroside V were undetectable. During 35-55 days, mogroside IIE content decreased, mogrosides IIIE and III continued to increase, and mogrosides IVA, siamenoside I, mogroside V, and 11-O-mogroside V appeared. During 55-75 days, contents of mogrosides IIE, IIIE, III, and IVA decreased rapidly, while siamenoside I, mogroside V, and 11-O-mogroside V increased rapidly. During 75-85 days, mogrosides IIE, IIIE, III, and IVA essentially disappeared, while siamenoside I, mogroside V, and 11-O-mogroside V showed no significant differences.

In contrast, autumn-pollinated fruits exhibited continued increase in mogroside IVA content during 55-75 days, with decrease only beginning during 75-85 days at a much smaller magnitude than summer-pollinated fruits. At 85 days, autumn-pollinated fruits still contained substantial amounts of mogrosides III and IVA, which only disappeared at 95 days. Mogroside V content ceased to increase significantly after 95 days, while siamenoside I and 11-O-mogroside V contents continued to increase significantly. These results demonstrate that significant differences in mogroside V and other saponins between summer- and autumn-pollinated fruits began at 55 days, with autumn-pollinated fruits showing approximately 10 days slower accumulation of mogroside V and 11-O-mogroside V, and significantly lower contents at maturity (reduced by 40.66% and 46.07%, respectively).

Q5D: 5-day autumn-pollinated fruit; X5D: 5-day summer-pollinated fruit; Q35D: 35-day autumn-pollinated fruit; X35D: 35-day summer-pollinated fruit; Q55D: 55-day autumn-pollinated fruit; X55D: 55-day summer-pollinated fruit; Q75D: 75-day autumn-pollinated fruit; X75D: 75-day summer-pollinated fruit; Q85D: 85-day autumn-pollinated fruit; X85D: 85-day summer-pollinated fruit. The same applies below.

2.4 Comparison of Mogroside V Biosynthetic Gene Expression

As shown in Figure 10 [Figure 10: see original paper], compared to 14:00 (the time of highest temperature at 48.15°C) in summer-pollinated fruits, genes *SgSQS*, *SgEPH3*, *SgCYP102801*, *SgUGT85-269-1*, *SgUGT85-269-4*, *SgUGT94-289-1*, *SgUGT94-289-2*, and *SgUGT94-289-3* were all upregulated from 18:00 to 09:00 the next day when temperatures ranged from 19.51-28.30°C. Among these, *SgEPH3*, *SgCYP102801*, *SgUGT85-269-1*, *SgUGT85-269-4*, *SgUGT94-289-1*, *SgUGT94-289-2*, and *SgUGT94-289-3* showed significant or extremely significant upregulation of 1-463 fold starting from 02:00. Genes *SgHMGR*, *SgSQE*, and *SgEPH2* were downregulated at 18:00 but upregulated from 02:00 onward. Genes *SgCAS* and *SgEPH1* were downregulated at all time points, while *SgCDS* showed no clear expression pattern. *SgCAS* encodes an enzyme

that competes for the upstream precursor epoxysqualene to synthesize steroidal saponins, diverting flux from the mogroside pathway. *SgCDS* and *SgEPH1* encode enzymes that catalyze synthesis of the upstream precursor cucurbitadienol in young fruits. These results indicate that, except for *SgCAS*, *SgCDS*, and *SgEPH1*, mogroside V biosynthetic genes in summer-pollinated fruits showed substantial upregulation from 18:00 to 09:00 the next day, with excellent coordinated expression consistency. Particularly, downstream glucosyltransferase genes *SgUGT85-269-1*, *SgUGT85-269-4*, *SgUGT94-289-1*, *SgUGT94-289-2*, and *SgUGT94-289-3*, which catalyze the conversion of mogrosides IIE, III, and IVA to high-glycosides like mogroside V, were highly expressed. The cooler temperature environment from afternoon to next morning was more favorable for biosynthetic gene expression and mogroside V accumulation, consistent with *S. grosvenorii*' s preference for cool conditions, suggesting this may be the optimal daily accumulation period.

14, 18 represent 14:00 and 18:00 in the afternoon; 2, 6, 9 represent 02:00, 06:00, and 09:00 the next morning. indicates significant difference ($P < 0.05$), ** indicates extremely significant difference ($P < 0.01$). The same applies below.*

As shown in Figure 11 [Figure 11: see original paper], compared to 14:00 (43.26°C) in autumn-pollinated fruits, genes *SgCAS*, *SgEPH2*, *SgEPH3*, *SgUGT85-269-1*, and *SgUGT94-289-2* were upregulated from 18:00 to 09:00 the next day when temperatures ranged from 16.36-31.37°C with rapid high-low temperature transitions. Only *SgCAS* and *SgEPH3* showed significant or extremely significant upregulation from 02:00. *SgCDS* was upregulated from 18:00 to 06:00 but downregulated at 09:00. Genes *SgHMGR*, *SgSQE*, *SgEPH1*, and *SgCYP102801* were upregulated from 18:00 to 02:00 but downregulated from 06:00 to 09:00. *SgUGT94-289-1* was downregulated at 18:00 but upregulated from 02:00 to 09:00. *SgUGT94-289-3* was downregulated at all time points, with more than 2-fold downregulation from 02:00. Genes *SgSQS* and *SgUGT85-269-4* showed no clear expression patterns. Except for *SgCAS* (4-fold upregulation at 02:00) and *SgUGT94-289-2* (8-fold upregulation at 09:00), upregulated genes only reached 1-2 fold increases at few time points. These results indicate that autumn-pollinated fruits showed fewer upregulated genes with smaller magnitude and poor coordinated expression consistency from 18:00 to 09:00. The period from 18:00 to 02:00 (19.32-26.47°C) represented cool temperatures similar to summer-pollinated fruits, with relatively more upregulated genes, suggesting this may be the main accumulation period for autumn-pollinated fruits. However, from 02:00 (approximately 17°C) to 09:00 (31.37°C), the rapid temperature increase resulted in fewer upregulated genes and substantial downregulation of many genes, particularly the glucosyltransferase *SgUGT94-289-3*. Both low (around 17°C) and high temperature conditions were unfavorable for biosynthetic gene expression and mogroside V accumulation.

Discussion

Fruit quality traits such as transverse diameter, longitudinal diameter, single fruit weight, and ginsenoside content in grape, peach, goji berry, and tomato are substantially affected by temperature, light, and air humidity. Since average light intensity and air humidity were similar between summer- and autumn-pollinated *S. grosvenorii* fruits, these factors likely had minimal impact on quality differences. Although diurnal temperature variation was higher in autumn-pollinated fruits before 55 days, no significant differences were observed in fruit dimensions, weight, or mogroside content. After 55 days, when diurnal variation became similar between groups, mogroside content differences became significant, suggesting diurnal temperature variation also had limited impact. In contrast, differences in average temperature and effective accumulated temperature were more pronounced than other climatic factors and aligned temporally with the emergence of mogroside content differences. Therefore, temperature appears to be the primary climatic factor affecting internal quality, with average temperature and effective accumulated temperature being most relevant.

The growth curves of both fruit groups were consistent with previous reports, with no significant differences in transverse diameter, longitudinal diameter, or single fruit weight, indicating that external quality was not significantly affected by habitat climatic factors. This may be because the critical fruit expansion period before 35 days had similar average temperature and effective accumulated temperature between groups. Previous studies showed that mogroside V synthesis and accumulation increased when habitat temperature decreased from 29.54°C to approximately 25°C during 30-70 days after pollination, while postharvest storage temperature decreases inhibited mogroside V accumulation, with almost complete cessation below 15°C. Compared to summer-pollinated fruits, the significantly lower average temperature (down to 20°C) and effective accumulated temperature (below 60°C) after 35 days in autumn-pollinated fruits delayed the glycosylation reactions converting mogrosides III, IV, and siamenoside I to mogroside V and 11-O-mogroside V by approximately 10 days, resulting in significant content differences. This demonstrates that reduced temperature in autumn-pollinated fruits inhibited mogroside V synthesis and accumulation by affecting glycosylation reactions, consistent with previous postharvest studies. While low temperature typically promotes triterpenoid saponin accumulation in cucumber cucurbitacin and ginsenosides, it inhibits mogroside V accumulation, contrary to common perception, suggesting different regulatory mechanisms that warrant further investigation.

MYB, bHLH, and AP2/ERF transcription factors interact in plant temperature stress responses and secondary metabolite accumulation. For example, MYB-bHLH-WD40 ternary complexes activate anthocyanin synthesis, while high temperature-induced HY5 degradation and MYBL expression inhibit anthocyanin accumulation. bHLH transcription factors CsBi and CsBt respond to high (>30°C) or low (<13°C) temperature stress to synthesize cucurbitacin C, causing cucumber bitterness. TcERF15 and TcERF12 positively and negatively

regulate taxol biosynthesis, respectively. These transcription factors regulate secondary metabolite accumulation by controlling biosynthetic gene expression. Apple MdMYB88 directly regulates glucosyltransferase MdUGT83L3 to accumulate anthocyanins and flavonoids under low temperature stress. Low temperature downregulates papaya linalool synthase gene *LIS* expression, inhibiting terpenoid aroma accumulation. Plant secondary metabolite accumulation typically requires coordinated expression of biosynthetic genes. Low temperature induces coordinated expression of ginsenoside biosynthetic genes to promote accumulation. Other cucurbit species cannot accumulate mogroside V due to lack of coordinated expression of homologous biosynthetic genes. MYB, bHLH92a, and ERF transcription factors can bind to promoters of mogroside V biosynthetic genes to regulate their expression. Compared to summer-pollinated fruits, autumn-pollinated fruits at 55 days showed fewer upregulated biosynthetic genes with lower expression levels and poor coordinated expression consistency, particularly for glucosyltransferase genes responsible for glycosylation reactions, with *SgUGT94-289-3* showing substantial downregulation. These results suggest that habitat temperature may cause quality differences between summer- and autumn-pollinated fruits by regulating the consistency and level of coordinated expression of mogroside V biosynthetic genes (especially glucosyltransferases) through MYB, bHLH, and AP2/ERF transcription factors.

Although 17-25°C is considered optimal for plant cellular function and productivity, the suitable temperature for secondary metabolite accumulation varies by species and compound. Both high and low temperatures can promote or inhibit secondary metabolite accumulation. In summer-pollinated fruits, temperatures of 19.51-28.30°C supported high coordinated expression consistency and levels of mogroside V biosynthetic genes, resulting in high mogroside V content. In contrast, autumn-pollinated fruits exposed to prolonged low temperature (~17°C) followed by abrupt increase to high temperature (31.37°C) showed low coordinated expression consistency and levels, leading to poor mogroside V quality. This suggests that mogroside V accumulation has an optimal temperature range of 20-28°C, with temperatures above 31°C or below 17°C being unfavorable.

S. grosvenorii has strict habitat requirements, with mogroside V quality in different seasonal pollinations being severely affected by temperature. Cultivation is concentrated in a narrow region of northern Guangxi and neighboring provinces, with northern Hunan and southern Guangxi being unsuitable for cultivation, and only northern Guangxi representing the genuine producing area. Habitat temperature may be a crucial factor determining this geo-authenticity. In recent years, due to market demand, cultivation has expanded to high-latitude and high-altitude regions with less sunshine and lower temperatures, such as Hunan and Sichuan provinces. To obtain high-quality fruits and alleviate the industry bottleneck of high raw material costs for mogroside V sweetener extraction, cultivation region and site selection must consider temperature effects on fruit quality. Measures such as cultivating large seedlings and using short-growth-period cultivars can adjust pollination and fruit-set timing to ensure mogroside V accumulation occurs within the optimal temperature range, thereby prevent-

ing quality reduction across different cultivation regions and pollination batches and maximizing fruit quality.

Conclusion

Fruit morphology and size of summer- and autumn-pollinated *S. grosvenorii* were not significantly affected by climatic factors, whereas mogroside V quality was significantly influenced by temperature. The primary climatic factor, temperature, caused differences in mogroside V quality between the two groups by regulating the consistency and level of coordinated expression among mogroside V biosynthetic genes.

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