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RESEARCH ARTICLE

Improving the fruit quality of Sichuan-pepper (*Zanthoxylum bungeanum* Maxim.) ‘Dahongpao’ by optimized GA_3 treatments

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ABSTRACT

To improve fruit quality, application of plant growth regulators as gibberellic acid (GA_3) has emerged as a significant cultivation and management method. In the study, different concentrations of GA_3 solutions (25, 50, 75, and 100 mg L^{-1}) were sprayed on Sichuan-pepper (*Zanthoxylum bungeanum* Maxim.; Rutaceae) ‘Dahongpao’ trees at fruit setting and enlargement stages. Spraying 75 mg L^{-1} GA_3 was most effective at both stages: vertical diameter, transverse diameter, hundred-grain weight, and number of oil sacs on the peel increased by 12%, 5%, 12%, and 42%, respectively, at fruit setting stage; and the aforementioned indexes increased by 14%, 11%, 12%, and 35%, respectively at fruit enlargement stage. Spraying 100 mg L^{-1} GA_3 at fruit setting stage, the content of total volatile oil, pungent compounds, non-volatile ether extracts, and total flavonoids significantly increased by 28%, 6%, 10%, and 3%, respectively; spraying 75 mg L^{-1} GA_3 at fruit enlargement stage, the indexes increased by 33%, 27%, 13%, and 11%, respectively. The comprehensive evaluation results based on Technique for Order Preference by Similarity to an Ideal Solution (TOPSIS) indicated that GA_3 treatment in a concentration of 75 mg L^{-1} could improve the quality of ‘Dahongpao’ fruits, thereby providing novel insights for the cultivation and management of such plant. Although spraying GA_3 may change fruit quality of *Z. bungeanum*, influencing mechanism of GA_3 treatments on the external and internal quality of *Z. bungeanum* fruits remains unclear.

Key words: ‘Dahongpao’, external quality, gibberellin, internal quality, Sichuan-pepper, TOPSIS, *Zanthoxylum bungeanum*.

INTRODUCTION

Improving fruit quality with scientific and effective methods has emerged as a trending research topic along with the increasing cultivation of commercial forests for harvesting fruits. Fruit quality can be evaluated using several indexes related to external quality (such as size, color, and weight of fruits), in addition to several indexes related to internal quality (such as nutrients and compounds that produce special flavors) (Hao et al., 2003; Krauss et al., 2006). At present, fertilization, growth regulators, and effective management and maintenance are the main cultivation methods for improving fruit quality (Yang et al., 2022; Peng et al., 2023).

As previously identified, spraying Ca fertilizers contributes to such physical properties as hardness, weight, length, and nutrients of fruits of *Ziziphus jujuba* Mill., *Cyanococcus*, and *Actinidia chinensis* Planch. (Lobos et al., 2021; Sotiropoulos et al., 2021). Additionally, spraying tryptophan and glycine on *Malus domestica* trees can significantly improve diameter, quality, and chemical characteristics of fruits (Mosa et al., 2021). According to prior research on the influence of growth regulators on fruit quality, different growth regulators can exert positive impacts on plants through single or combined spraying. Sridhara et al. (2021) confirmed that spraying 0.12 g brassinolide (BR) solution could significantly improve the yield and quality of *Solanum lycopersicum*. In shaping plant morphological characteristics and enhancing population photosynthesis, Gong et al. (2021) revealed that EDAH (1.5 mL L^{-1})+DA-6 (1 mL L^{-1}) treatment in a certain concentration could improve the yield of summer maize (*Zea mays* L.) Ali et al. (2022) sprayed the 24-epibrassinolide (EBL) solution in a certain concentration on strawberry (*Fragaria* ×*ananassa* Duchesne ex Rozier) plants, finding that EBL treatment could not only improve the vegetative growth of such fruits but also improve the yield and quality. Spraying plant growth regulators on *Punica granatum* (Drogoudi and Pantelidis, 2022), *Prunus* (Time et al., 2021), *Vitis* (Li et al., 2022), *Prunus armeniaca* (Roussos et al., 2021) and other commercial crops has also been found to significantly improve fruit yield and quality. Hence, the application of plant growth regulators has emerged as a significant method for improving fruit quality.

As one of the six major plant growth hormones, gibberellin is a tetracyclic diterpenoid. The regulation of gibberellins is involved in the whole process of plant growth and development, including seed germination, plant growth, flowering regulation, and fruit development and maturation (Camara et al., 2018). In a previous study, spraying gibberellins on *P. granatum* trees at the growing stage was found to improve the diameter, weight, yield, and quality of fruits (Hosein-Beigi et al., 2019). Spraying gibberellins on *P. armeniaca* trees could also contribute to the vegetative growth, yield, and quality of fruits (Mohamed, 2019). Further, the application of gibberellins to *Vitis* trees also increased the fruit setting rate, promoted fruit enlargement, and improved fruit nutrients (Song et al., 2023). As such, spraying gibberellin has become a major method for improving the external and internal quality of fruits in commercial forest crops.

With its long cultivation history, Sichuan-pepper (*Zanthoxylum bungeanum* Maxim.) is a commercial forest plant that can be processed into spice, oil, medicine, and other products, which originated in China, and has been widely distributed across the Asian continent. As a significant cultivar of *Z. bungeanum*, ‘Dahongpao’ is characterized by large fruit, thick oil sac, and rich pungent compounds, and has become the main cultivated of *Z. bungeanum* (Yang, 2008). At present, fertilization measures have been the major methods for increasing the fruit yield and quality of *Z. bungeanum* in most existing studies (Zhang, 2020). Notably, research is scarce on the improvement of the internal and external quality of *Z. bungeanum* fruits by hormones, and *N*-(2-chloro-4-pyridyl)-*N*-phenylurea (CPPU) and 6-benzylaminopurine (6-BA) have only been explored in a small number of studies (Zhang, 2020). The focus of most studies on the application of gibberellins in *Z. bungeanum* has been on promoting seed germination (Sun et al., 2017; 2019). However, there have not been reports on utilizing gibberellins to improve the external and internal quality of *Z. bungeanum*. Based on previous findings that spraying gibberellic acid (GA_3) on other fruit trees could improve fruit quality, different concentrations of GA_3 solution were sprayed on ‘Dahongpao’ at the fruit setting and enlargement stages in this study. We aim to identify the optimal treatment measures that can improve fruit quality through the systematic analysis of the influence of such plant growth regulators on the external and internal quality of fruits. The findings of the present study can provide a theoretical basis and technical support for the subsequent rational use of GA_3 to improve the quality and yield of *Z. bungeanum*.

MATERIALS AND METHODS

Experimental site and materials

The experiment was performed in Xinyu Planting and Production Park, Xingji Town (32°58' N, 113°86' E), Pingqiao District, Xinyang City, Henan Province, China. At the experimental site, there is an annual average sunshine period of 1900-2100 h, and the annual average temperature is 15.3-15.8 °C. Moreover, there is a long frost-free period (with an average frost-free period of 220-230 d) and abundant rainfall (with an average annual rainfall of 993-1294 mm). Located in a transitional area from the subtropical zone to the warm temperate zone, the site features a marked monsoon climate. In the present study, ‘Dahongpao’ trees grown for 8 yr were selected as the experimental materials, with a row spacing of 2 m × 3 m in the East-West direction. From the third year of age, the management of ‘harvesting instead of cutting’ and the measures of returning branches and leaves after harvest are adopted every year, and weeding and pest control are carried out according to conventional management measures.

Gibberellic acid (GA_3) treatments

After the Sichuan-pepper (*Zanthoxylum bungeanum* Maxim.) 'Dahongpao' trees entered the fruit setting stage (FSS) (9 May 2021) and fruit enlargement stage (FES) (5 June 2021), those with essentially the same growth potential were selected as the experimental stock plants. The experiment was performed based on the single plant plot and random block treatment, being repeated three times. Specifically, the GA_3 solutions were sprayed on the fruit-bearing branches of stock plants from the south. In total, there were nine treatment patterns in the experiment, including 25 (T1), 50 (T2), 75 (T3), and 100 mg L^{-1} GA_3 (T4) in the FSS treatment group; 25 (T5), 50 (T6), 75 (T7), and 100 mg L^{-1} GA_3 (T8) in the FES group; and the control group (CK) with the stock plants sprayed with clear water. Different treatment solutions were sprayed on the stock trees with a sprayer until water dripped on the ground from the trees. All the trees were labelled, and the fruits without mechanical damage were selected for subsequent experimental analysis after fruit ripening. The GA_3 powder with a purity of 99% was commercially obtained from MoreBetter Biotechnology (Hangzhou, China).

Fruit morphology and hundred-grain weight

The ventral suture of 'Dahongpao' fruits was taken as the vertical diameter, and that perpendicular to the vertical diameter was taken as the transverse diameter. The two diameters (mm) were measured with a digital vernier caliper (VC 5150S, Victory Instruments, Xi'an, China). During measurement, 20 fruits were selected repeatedly from one stock tree in each treatment pattern to take the average value.

A certain number of 'Dahongpao' fruits were randomly selected at each replicate from each treatment pattern, and the stems and impurities of dried fruits were removed. Subsequently, 100 fruits were randomly selected by means of a quartering method, and the hundred-grain weight was weighed using an electronic balance (FB124L, Shunyu Hengping Scientific Instrument, Shanghai, China).

Number and microstructure of oil sacs on the peel

A stereoscopic microscope (SZMN45TR-B4, Kwong Kuk, China) was used to observe and measure the number of oil sacs on the peel. Specifically, 20 fruits were selected repeatedly from one stock tree in each treatment pattern to take the average value.

Firstly, for microstructure of oil sacs on the peel, the 'Dahongpao' fruits were cut with a blade and sprayed with metal materials by means of the SBC-12 ion sputtering instrument, before being observed and photographed under an EM-30 Plus scanning electron microscope (COXEM, Daejeon, Korea).

Total volatile oil and pungent compounds (amides)

The total volatile oil content was measured by means of steam distillation (Nazem et al., 2019). Firstly, 30 g dried fruit powder of 'Dahongpao' and 300 mL ultrapure water were poured into a 500 mL flask, which was heated until boiling. The heating was continued for more than 4 h until no oil flowed out. The value could then be observed after the oil interface remained stable. The total volatile oil content was expressed by the oil volume (mL g^{-1}).

In the present study, the content of pungent compounds in 'Dahongpao' fruits was measured by means of high-performance liquid chromatography (HPLC) (Xu, 2020). Firstly, the fruit samples were crushed and sieved with a 0.3 mm sieve, and then transferred to a conical flask. Subsequently, methanol was added to the conical flask according to the material-liquid ratio of 1:10 (g mL^{-1}). Next, the extraction was conducted with ultrasonic assistance at 50 °C for 30 min. After, the extracts were centrifuged at 3000 g min^{-1} for 5 min, and the supernatant was filtered through a 0.22 μm membrane to obtain the sample solution. The analysis was then carried out by HPLC, detection conditions: Chromatography was performed on a Welchrom C18 column with HPLC (LC-210, Qiuzuo Scientific Instrument, Shanghai, China), the separation was carried out with a gradient elution using methanol-water (50%-75% methanol) as the mobile phase with an elution time of 50 min, a flow rate of 1.00 mL min^{-1}, a column temperature of 30 °C and a sample volume of 10.00 µL at a maximum UV absorption of 268 nm.

Non-volatile ether extracts

The content of non-volatile ether extracts was analyzed by means of the anhydrous ether (HG3-1002) extraction method (Xiang et al., 2016). The filtration paper cylinder containing samples was plugged with absorbent cotton and then put into a Soxhlet extractor. The cylinder was then heated in a water bath for 18 h. Subsequently, the solvent was recovered and evaporated in a water bath after treatment, before being heated in an oven at 110 ℃ for 1 h, cooled in a dryer, and weighed (accurate to 0.001 g). The heating, cooling, and weighing were repeated numerous times until the mass difference obtained from two successive times was less than 0.002 g. The calculation formula is presented as follows:

$$X = (m_2 - m_1) \times \frac{100}{m_0} \times \frac{100}{100 - H} \qquad (1)$$

where, X represents the content of non-volatile ether extracts (%); m_2 is the mass of the receiving bottle and non-volatile ether extracts (g); m_1 is the mass of the receiving bottle (g); m_0 is the sample mass (g); H is the moisture content of samples (%).

Total flavonoids

For measuring the content of total flavonoids in the fruits, the aluminum nitrate-sodium nitrite-sodium hydroxide ($Al(NO_3)_3$-$NaNO_2$-NaOH) colorimetric method was adopted (Qing et al., 2019). Firstly, 2.0 g dried fruit powder were weighed and extracted with 60 mL 60% ethanol solvent at 30 ℃ (power: 150 W) for 1 h, which was then filtered to obtain the sample solution. Subsequently, 20 mL sample solution was collected and mixed thoroughly with 1.0 mL 5% $NaNO_2$ solution. The mixed sample remained stable for 6 min. Next, 1.0 mL 10% $Al(NO_3)_3$ solution was added and shaken thoroughly, followed by stable placement for 6 min. Finally, 10 mL 10% NaOH solution was added and shaken thoroughly to develop color for 15 min. After stabilization, the solution was scanned under a UV-Vis spectrophotometer at 200-700 nm wavelength. The extraction rate of flavonoids could be calculated by the following formula:

$$\text{Flavonoid extraction rate} = \text{Total flavonoids in the extract (mg)/Raw materials (g)} \qquad (2)$$

Data processing

SPSS Statistics 24 (IBM, Armonk, New York, USA) was used to analyze the significance of the Waller-Duncan test difference in one-way ANOVA (α = 0.05), while Origin 2021 (Origin Lab, Northampton, Massachusetts, USA) was used to plot relevant diagrams.

Comprehensive scoring of indexes based on TOPSIS

Technique for order preference by similarity to an ideal solution (TOPSIS) was used to evaluate the effects of different GA_3 treatments (Chen et al., 2016).

The specific steps are elucidated as follows: A decision matrix was established, which can be denoted as follows,

$$Z = \begin{bmatrix} & C_1 & C_2 & \cdots & C_n \\ M_1 & Z_{11} & Z_{12} & \cdots & Z_{1n} \\ M_2 & Z_{21} & Z_{22} & \cdots & Z_{2n} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ M_m & Z_{m1} & Z_{m2} & Z_{m3} & Z_{mn} \end{bmatrix} \qquad (3)$$

where Z represents the initial evaluation matrix; Z_{ij} represents the initial value of the j^{th} index in the i^{th} treatment; i = 1, 2, ..., m, m represents the number of treatments to be evaluated; and j = 1, 2, …, n, n represents the number of test indexes.

The treatment matrix could be normalized as follows:

$$V_{ij} = \frac{Z_{ij}}{\sqrt{\sum_{i=1}^{m} Z_{ij}^2}} \qquad (4)$$

where V_{ij} is the standardized value of the jth index in the ith treatment.

The positive ideal solution (V_j^+) and the negative ideal solution (V_j^-) could be calculated as follows:

$$V_j^+ = \max\left(V_{1j}, V_{2j}, \cdots, V_{ij}\right), V_j^- = \min\left(V_{1j}, V_{2j}, \cdots V_{ij}\right) \qquad (5)$$

The distance from each index to the positive and negative ideal solutions could be determined as follows:

$$D_i^+ = \sqrt{\sum_{j=1}^{n}\left(V_{ij} - V_j^+\right)^2}, D_i^- = \sqrt{\sum_{j=1}^{n}\left(V_{ij} - V_j^-\right)^2} \qquad (6)$$

The proximity degree (C_i) between each evaluation object and the positive ideal solution could be calculated as follows:

$$C_i = \frac{D_i^-}{D_i^+ + D_i^-} \qquad (7)$$

RESULTS

Influence of GA_3 on the fruit shape

The vertical and transverse diameters of ‘Dahongpao’ fruits are significant indexes for measuring fruit size. Compared with the CK group, the vertical diameter of fruits after GA_3 treatments at 50, 75, and 100 mg L^{-1} at fruit setting stage (FSS), which increased by 7%, 12%, and 7%, respectively ($P < 0.05$; Figure 1A). The transverse diameter after 75 and 100 mg L^{-1} GA_3 at the FSS, which increased by 5% and 4%, respectively ($P < 0.05$; Figure 1B).

Compared with the CK group, the vertical diameter of ‘Dahongpao’ fruits after 25, 50, and 75 mg L^{-1} GA_3 at the fruit enlargement stage (FES), which increased by 8%, 11%, and 14%, respectively ($P < 0.05$). The transverse diameter after GA_3 treatments at 50 and 75 mg L^{-1} at FES, which increased by 7% and 11%, respectively ($P < 0.05$) (Figure 1C). An observation can be made that GA_3 treatments with a certain concentration can increase the fruit size at the FSS and FES (Figure 1A). However, the same concentration of GA_3 sprayed during FES is more effective in enhancing the vertical diameter and transverse diameter of ‘Dahongpao’ than FSS, and the mechanism is not yet clear, but it is recommended that GA_3 should be sprayed during FES.

Influence of GA_3 on the hundred-grain weight

In the present study (Figure 2), compared with the CK group (1.19 g), the hundred-grain weight of ‘Dahongpao’ fruits after at 50 and 75 mg L^{-1} GA_3 treatments at the FSS, which increased by 11% and 12%, respectively ($P < 0.05$). The hundred-grain weight after 50 and 75 mg L^{-1} GA_3 treatments at FES, which increased by 10% and 12%, respectively ($P < 0.05$). However, 25 and 100 mg L^{-1} GA_3 at the FSS and FES had nonsignificant influence on the hundred-grain weight of ‘Dahongpao’ fruits.

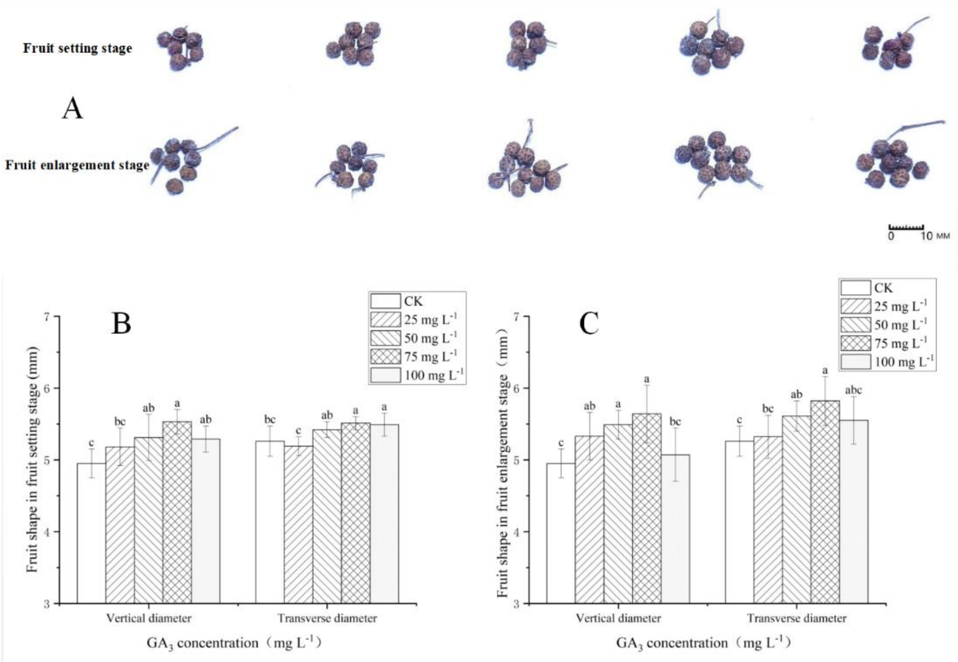

Figure 1. Influence of GA$_3$ treatments at different concentrations and two different stages on the 'Dahongpao' Sichuan-pepper fruit size. Data are shown as the mean ± standard and derived from three replicates (n = 3). Bars indicate standard errors. Different letters indicate significant difference at the 5% significance level according to Waller-Duncan's multiple range tests.

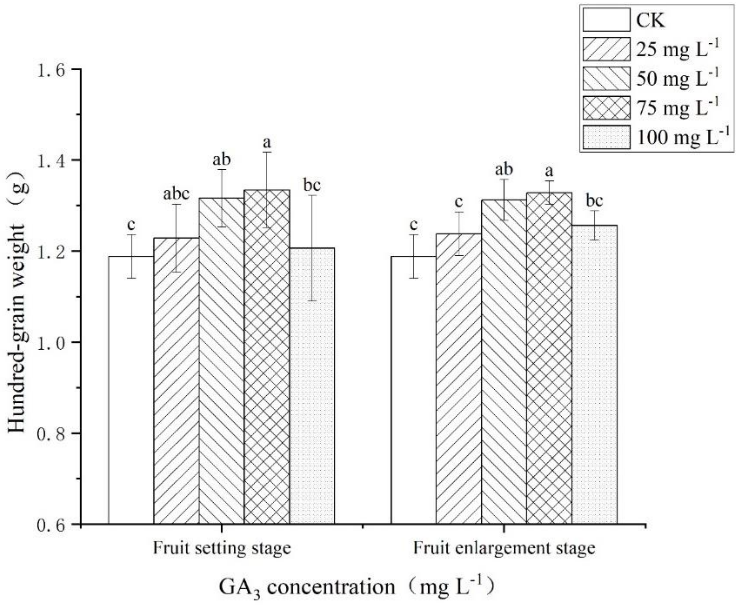

Figure 2. The hundred-grain weight of 'Dahongpao' Sichuan-pepper fruits after GA$_3$ treatments at different concentrations and two different stages. Data are shown as the mean ± standard and derived from three replicates (n = 3). Bars indicate standard errors. Different letters indicate significant difference at the 5% significance level according to Waller-Duncan's multiple range tests.

Influence of GA$_3$ on the oil sacs on the peel

In a woody oil crop, a higher number of oil sacs on the peel indicates a higher oil content. In the present study, spraying exogenous GA$_3$ solutions in a certain concentration at the FSS could significantly increase the number of oil sacs on the peel (Figure 3). Spraying solutions of 50, 75, and 100 mg L^{-1} GA$_3$ at the FSS increased the number of oil sacs on the peel by 18%, 42%, and 36%, respectively ($P < 0.05$). Spraying 75 and 100 mg L^{-1} GA$_3$ at the FES increased the number of oil sacs on the peel by 35% and 26%, respectively ($P < 0.05$).

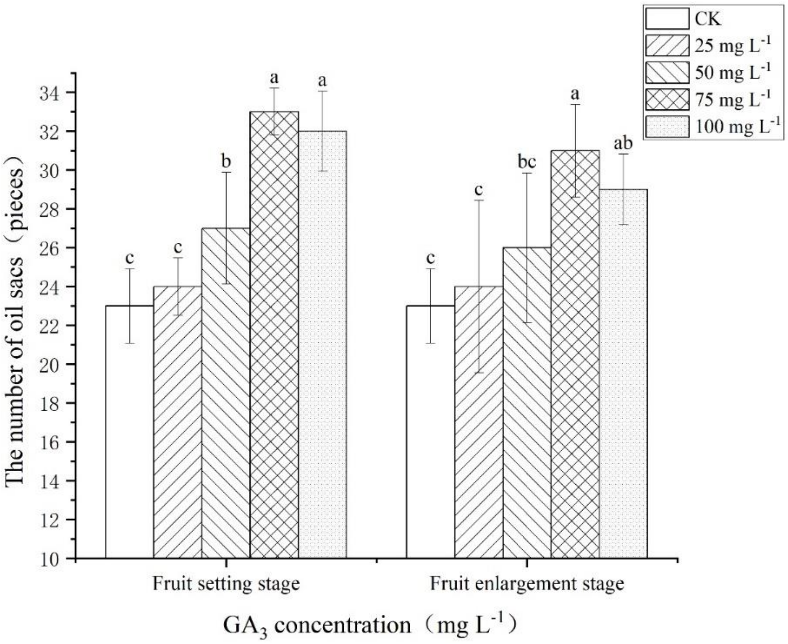

Figure 3. The number of oil sacs on the peel of 'Dahongpao' Sichuan-pepper fruits after GA$_3$ treatments at different concentrations and two different stages. Data are shown as the mean ± standard and derived from three replicates ($n = 3$). Bars indicate standard errors. Different letters indicate significant difference at the 5% significance level according to Waller-Duncan's multiple range tests.

The size of single oil sacs of treated with different concentrations of GA$_3$ was observed by means of scanning electron microscope. Compared with the CK group, spraying an appropriate concentration of GA$_3$ could significantly increase the size of the oil sacs (Figure 4). With the increase in the GA$_3$ concentration, the oil sac thickness of 'Dahongpao' fruits exhibited an increasing trend (Figure 5). The diameter of the oil sac on the peel increased by 13.2%, 26.3%, 48.3% and 70%, respectively, after 25, 50, 75, and 100 mg L^{-1} GA$_3$ at the FSS. Meanwhile, the diameter of the oil sac increased by 10.3%, 30%, 85.5% and 49.9%, respectively, after GA$_3$ treatments in the aforementioned concentrations at the FES. Compared with the oil sac thickness in the CK group, the maximum oil sac thickness after 100 mg L^{-1} GA$_3$ treatments increased by 125.9%, thereby exhibiting significant effects on thickness increases.

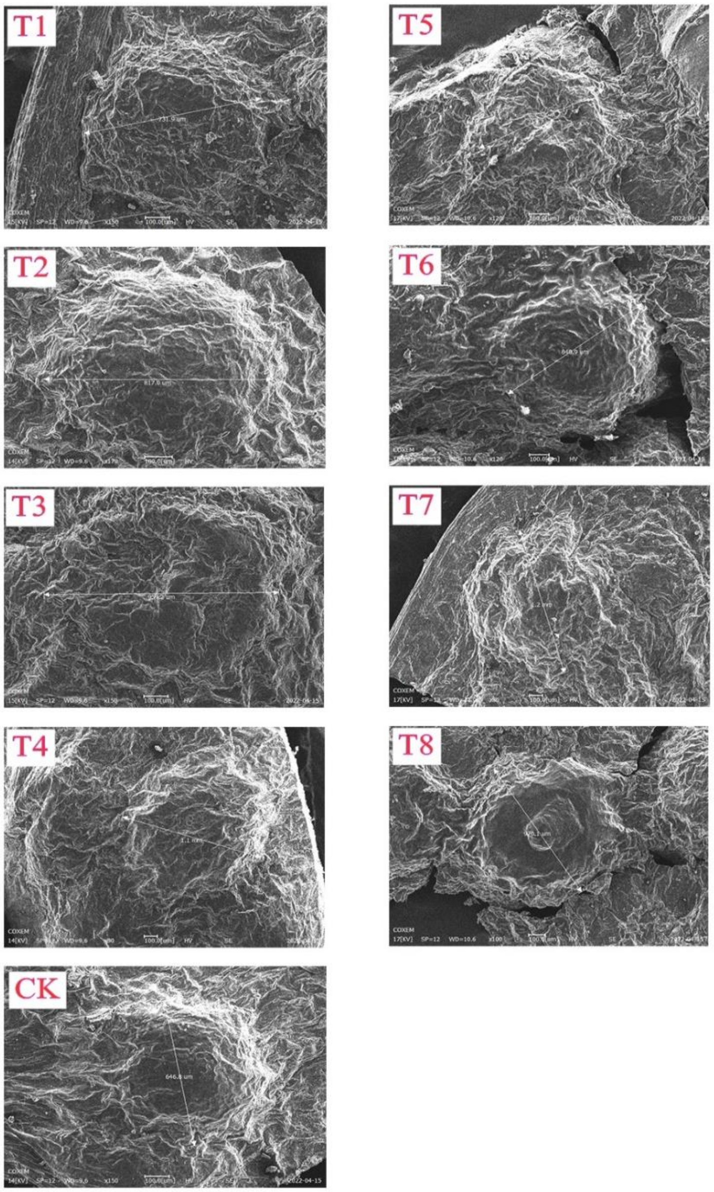

Figure 4. The size of a single oil sac on the peel of ‘Dahongpao’ Sichuan-pepper fruits after GA_3 treatments at different concentrations under electron microscope scanning. T1: 25 mg L^{-1} GA_3; T2: 50 mg L^{-1} GA_3; T3: 75 mg L^{-1} GA_3; T4: 100 mg L^{-1} GA_3 at the fruit setting stage treatment group; T5: 25 mg L^{-1} GA_3, T6: 50 mg L^{-1} GA_3, T7: 75 mg L^{-1} GA_3, T8: 100 mg L^{-1} GA_3 at the fruit enlargement stage treatment group; CK: control group.

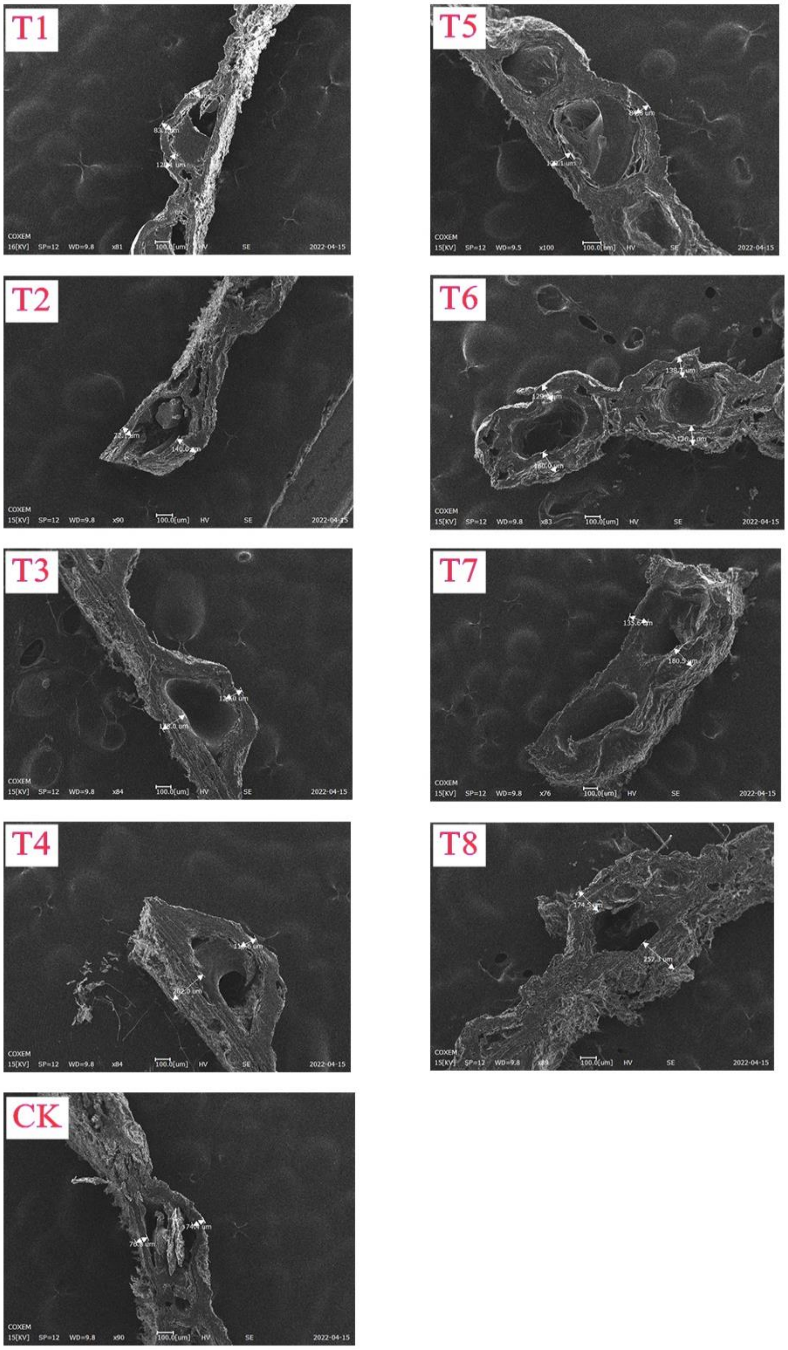

Figure 5. The thickness of a single oil sac on the peel ‘Dahongpao’ Sichuan-pepper fruits after GA_3 treatments at different concentrations under electron microscope scanning. T1: 25 mg L^{-1} GA_3; T2: 50 mg L^{-1} GA_3; T3: 75 mg L^{-1} GA_3; T4: 100 mg L^{-1} GA_3 at the fruit setting stage treatment group; T5: 25 mg L^{-1} GA_3, T6: 50 mg L^{-1} GA_3, T7: 75 mg L^{-1} GA_3, T8: 100 mg L^{-1} GA_3 at the fruit enlargement stage treatment group; CK: control group.

Influence of GA_3 on the internal quality

Compared with the CK group, the total volatile oil content after 75 and 100 mg L^{-1} GA_3 treatments at the FSS, which increased by 33% and 29%, respectively. The total volatile oil content after 50, 75, and 100 mg L^{-1} GA_3 treatments at the FES, which increased by 10%, 33%, and 43%, respectively (Figures 6A and 6B).

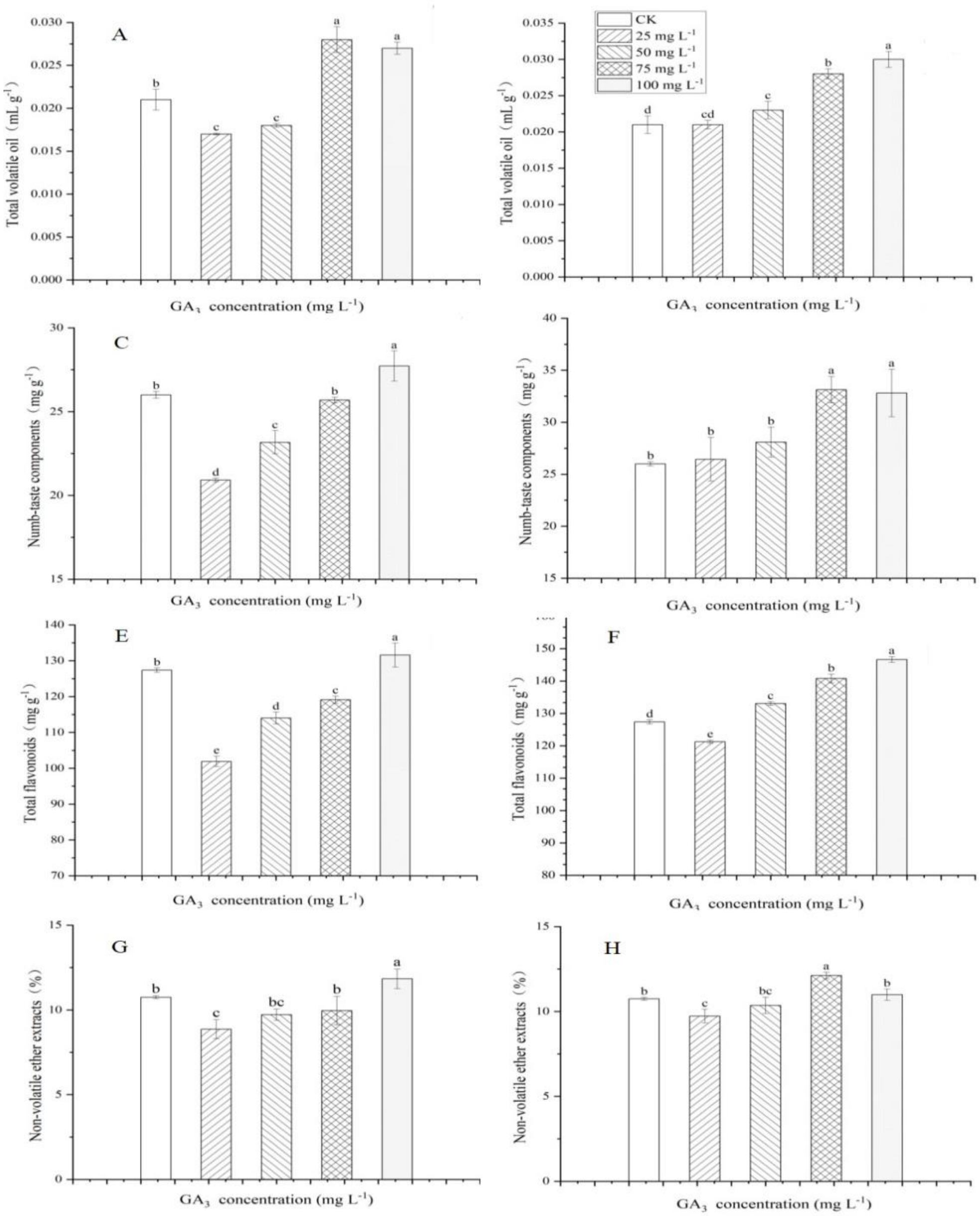

Figure 6. Influence of GA_3 treatments at different concentrations and at fruit setting (FSS) and fruit enlargement stages (FES) on the internal quality of 'Dahongpao' Sichuan-pepper fruits. (A-B) Influence of GA_3 treatments at FSS and FES on the total volatile oil; (C-D) GA_3 treatments at FSS and FES on the pungent compound content; (E-F) GA_3 treatments at FSS and FES on the total flavonoid content; (G-F) GA_3 treatments at FSS and FES on the non-volatile ether extract content. Data are shown as the mean ± standard and derived from three replicates (n = 3). Bars indicate standard errors. Different letters indicate significant difference at the 5% significance level according to Waller-Duncan's multiple range tests.

This study found that after 100 mg L^{-1} GA_3 solution was sprayed at the FSS, the content of pungent compounds increased by 6% compared with the CK group. After GA_3 solutions in the concentrations of 75 and 100 mg L^{-1} were sprayed at the FES, the content of pungent compounds increased by 27% and 26%, respectively, compared with the CK group (Figures 6C and 6D).

After 100 mg L^{-1} GA_3 solution was sprayed at the FSS, the total flavonoid content increased by 3% compared with the CK group. After GA_3 solutions at 50, 75, and 100 mg L^{-1} were sprayed at the FES, the content of total flavonoids increased by 4%, 11%, and 15%, compared with the CK group, respectively (Figures 6E and 6F).

Spraying 100 mg L^{-1} GA_3 solution at FSS significantly increased the content of non-volatile ether extracts, being 10% higher than that of the CK group. while spraying 75 mg L^{-1} GA_3 solution at FES also significantly increased the content of non-volatile ether extracts, being 13% higher than that of the CK group (Figures 6G and 6H).

Comprehensive evaluation for improving fruit quality of ‘Dahongpao’ by GA_3 treatments based on TOPSIS

In the present study, data were normalized based on the evaluation indexes measured for the external and internal quality of ‘Dahongpao’ fruits. Moreover, the relative proximity value C of different treatments was obtained by means of TOPSIS (Table 1). The influence of different concentrations of GA_3 on the fruit quality could be ranked from high to low as follows: 75 mg L^{-1}(FES) > 100 mg L^{-1}(FES) > 100 mg L^{-1}(FSS) > 75 mg L^{-1}(FSS) > 50 mg L^{-1}(FES) > CK > 25 mg L^{-1}(FES) > 50 mg L^{-1}(FSS) >25 mg L^{-1}(FSS). The results show that GA_3 treatment in a concentration of 75 mg L^{-1} at the FES could achieve the optimal improvement effect.

Table 1. Comprehensive evaluation and ranking of GA_3 treatments based on TOPSIS. CK: Control group; T1: 25 mg L^{-1} GA_3; T2: 50 mg L^{-1} GA_3; T3: 75 mg L^{-1} GA_3; T4: 100 mg L^{-1} GA_3 at the fruit setting stage treatment group; T5: 25 mg L^{-1} GA_3, T6: 50 mg L^{-1} GA_3, T7: 75 mg L^{-1} GA_3, T8: 100 mg L^{-1} GA_3 at the fruit enlargement stage treatment group.

GA_3 treatments	Positive ideal solution distance D+	Negative ideal solution distance D-	Relative proximity C	Rank
CK	0.216	0.125	0.367	6
T1	0.310	0.022	0.065	9
T2	0.248	0.084	0.253	8
T3	0.142	0.213	0.600	4
T4	0.102	0.231	0.693	3
T5	0.214	0.113	0.345	7
T6	0.158	0.169	0.517	5
T7	0.044	0.284	0.866	1
T8	0.075	0.283	0.791	2

DISCUSSION

Gibberellin is a growth regulator widely used in agricultural production with the ability to significantly promote the division and elongation of plant cells (Gao and Fu, 2018). Being mainly involved in enhancing the activity of α-amylase, total amylase, and sucrose invertase, gibberellin can increase the soluble sugar content in the cytoplasm, enlarge the osmotic pressure, and promote external water to enter cells, thereby having significant involvement in cell enlargement and elongation (Yuda et al., 1984). The size, shape and hundred-grain weight of fruits are the main indexes for evaluating the external quality of fruits. Spraying gibberellins at the fruit setting (FSS) and fruit enlargements stages (FES) can significantly improve fruit quality. As confirmed by Yang et al. (2020), spraying gibberellins on *Cyanococcus* plants can significantly increase the vertical and transverse diameters of fruits. Habibi et al. (2021) revealed that GA_3 treatment in a certain concentration can promote the length and diameter of *Citrus sinensis* fruits. In the present study, GA_3 treatment in an appropriate concentration could also contribute to the fruit size of 'Dahongpao'.

The volatile oil content in Sichuan-pepper fruits reflects the aroma degree thereof. The pungent compounds were mainly composed of amides, which can reflect the pungent degree of the Sichuan-pepper fruits. As a group of secondary metabolites, flavonoids can fulfil anti-aging and blood sugar lowering functions, while also mitigating cardiovascular and cerebrovascular diseases. The non-volatile ether extract is also a significant reference index in the quality grade of Sichuan-pepper fruits. All these substances are important reflections of the internal qualities of Sichuan-pepper. The present findings show that spraying a certain concentration of GA_3 solutions could improve the external and internal quality of 'Dahongpao' fruits. In particular, the best result is achieved by spraying GA_3 at a concentration of 75 mg L^{-1}. However, the internal molecular mechanism of the improvement effect remains to be clarified. According to prior research (Cai, 2017), after exogenous GA_3 was sprayed on *Vitis* trees, the transcription balance between endogenous hormone synthesis and response genes in fruits was broken, which affected the synthesis, decomposition, regulation, and signal transduction of endogenous hormones in plants. As such, the expression of genes related to cell wall modifying enzymes and cell osmotic pressure regulation were affected, which ultimately resulted in the relaxation of fruit cell walls and cell expansion. Numerous xyloglucan endotransglycosylase transferase (XET) genes were up-regulated at the first rapid growth stage of *Vitis* trees, but the expression of *XET* genes exhibited a downward trend at the subsequent growth and development stages (Schlosser et al., 2008).

Cai (2017) found that GA_3 treatments significantly up-regulated the expression of *XET* genes in *Vitis* fruits, which may be the internal cause leading to the expansion of such fruits. Thus, the influence of exogenous GA_3 treatments on the fruit quality of *Z. bungeanum* may also involve the response to the endogenous hormone regulation pathway. Despite such findings, further investigations are required to identify whether there is any correlation with the high expression of *XET* genes. Hence, the regulation mechanism of exogenous hormone GA_3 on the external quality of *Z. bungeanum* fruits may be investigated from the perspectives of the endogenous hormone balance pathway and the mechanisms related to signal transduction. Moreover, DELLA proteins have been confirmed to participate in hormone signal transduction and are a significant factor in the process (Zhao et al., 2017). The main function of DELLAs is to inhibit plant growth, while gibberellins can achieve cell enlargement by specifically destroying the inhibition of DELLAs (Achard and Genschik, 2009).

There are three main members in the DELLA protein gene family in *Vitis* fruits, including VvGAII (VIT_201s0011g05260), VvRGA (VIT_214s0066g00640), and VvSLRI (VIT_211s0016g04630) (Zhang et al., 2018). The functional analysis of the DELLA protein gene family in *Z. bungeanum* has not been reported. Hence, the members of the DELLA protein gene family in *Z. bungeanum* can be further identified, and the response mechanism to exogenous GA_3 can also be verified.

CONCLUSIONS

The results demonstrate that an appropriate concentration of gibberellic acid (GA_3) treatments can increase the vertical and transverse diameters, hundred-grain weight, and the number of oil sacs on the peel of 'Dahongpao' Sichuan-pepper fruits at fruit setting and fruit enlargement stages (FES). In addition, GA_3 treatments in a proper concentration can also significantly improve internal quality of 'Dahongpao' fruits, such as the contents of the total volatile oil, pungent compounds, non-volatile ether extracts, and total flavonoids. The TOPSIS-based comprehensive evaluation results indicate that spraying the GA_3 solution in a concentration of 75 mg L^{-1} at FES can achieve the optimal effect in terms of improving the fruit quality of 'Dahongpao'. Such findings can provide novel insights and research directions for improving the external and internal quality of 'Dahongpao' fruits.

Author contribution

Conceptualization: Y.F., Y.W., Z.W., J.L. Methodology: Y.F., Y.W., Z.W., J.L. Validation: Y.F., Y.W., Z.W., J.L. Formal analysis: Y.F., Y.W., M.L., Y.L., Q.H. Data curation: Y.F., Y.W., M.L., Y.L., Q.H. Writing-original draft preparation: Y.F., Y.W., M.L., Y.L., Q.H. Writing-review and Editing: Y.F., M.L., Y.L. Supervision: H.W., D.Z. Project administration: H.W. Funding acquisition: H.W. All authors have read and agreed to the published version of the manuscript.

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