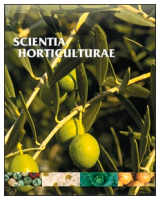
Scientia Horticulturae 336 (2024) 113410

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



Research Paper

# A high-efficiency method for simultaneous quantitation of bioactive gibberellins in *Litchi chinensis* using UHPLC-QQQ-MS/MS

Xingling Su [a,b,c], Jiakun Zheng [a,b,c], Yanwei Hao [a,b,c], Rui Xia [a,b,c,*], Jing Xu [a,b,c,*]

[a] State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Horticulture, South China Agricultural University, Guangdong Guangzhou 510642, China
[b] Guangdong Laboratory for Lingnan Modern Agriculture, South China Agricultural University, Guangdong Guangzhou 510642 China
[c] Key Laboratory of Biology and Germplasm Enhancement of Horticultural Crops in South China, Ministry of Agriculture and Rural Affair, South China Agricultural University, Guangdong Guangzhou 510642, China

ARTICLE INFO

*Keywords:*
Lychee
Bioactive GAs
Pre-treatment produces
UHPLC-QQQ-MS/MS

ABSTRACT

Gibberellins (GAs) are a group of phytohormones that have profound and diverse effects on plant growth and development. Within this group, bioactive GAs, including GA1, GA3, GA4, and GA7, play a particularly significant role in regulating these processes. Lychee (*Litchi chinensis* Sonn.) is a tropical fruit tree native to southern China. Optimization of rapid and precise methods for analyzing bioactive GAs in lychee is crucial to enhance our understanding of the underlying physiological mechanisms of fruit and flower development and to explore its potential industrial applications in this fruit tree. In this study, we developed a high-efficiency method for simultaneous quantitation of bioactive GAs in lychee using ultra-high performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry (UHPLC-QQQ-MS/MS). Ultimately, this method was effectively utilized to quantify the bioactive GAs in various tissues of lychee, including fruits, seeds, and flowers. The developed method exhibited satisfactory recoveries and precision, as well as short chromatographic run time and low limits of detection and quantitation of bioactive GAs in lychee. This protocol provides a valuable method for the characterization of physiological roles and molecular mechanism of GAs in lychee.

## 1. Introduction

Gibberellins (GAs) are a diverse group of phytohormones comprising a wide range of diterpenoid compounds (Hedden, 2020). Initially, GAs were discovered from the pathogenic fungus *Gibberella fujikuroi*, which is responsible for causing the highly prevalent disease known as "bakanae" in rice (*Oryza sativa* L. subspecies Japonica) (Yabuta, 1938). Currently, more than 130 GAs have been identified from fungi, bacteria, and plants. These GAs have been systematically designated as $GA_1$ to GAn, based on the chronological order of their discovery and their structural properties (Macmillan and Takahashi, 1968). The chemical structure of GAs can be divided into two categories: $C_{19}$-GAs and $C_{20}$-GAs (Fig. 1A) (Salazar-Cerezo et al., 2018). The majority of GAs are inactive, with only a small portion exhibiting physiological activity. $GA_1$, $GA_3$, $GA_4$, and $GA_7$ are the predominant bioactive GAs found in plants, which are all $C_{19}$-GA and all contain hydroxyl at carbon 3 and carboxylic group at carbon 6 (Fig. 1B) (Yamaguchi, 2008). Bioactive GAs serve as vital regulators in numerous biological processes related to plant growth and development. They play a crucial role in multiple facets of fruit tree development, including floral bud differentiation, inflorescence development, flowering, fruit set, fruit growth, and the establishment of plant morphology. GAs have been applied in fruit tree production. Specifically, the treatment of grape clusters with exogenous $GA_3$ during the later stages of flowering has been proven to be highly successful in inducing the formation of seedless fruits and promoting fruit enlargement in various grape varieties (Wang et al., 2017). Additionally, the exogenous application of GAs during the flowering stage of pear trees can lead to embryonic abortion, thereby inducing parthenocarpy (Ayele et al., 2006). The role of GAs in regulating the flower and fruit development in fruit trees is well established.

Lychee (*Litchi chinensis* Sonn.) is a significant tropical fruit tree species worldwide, and it possesses a valuable fruit characterized by an edible portion known as an aril (Hu et al., 2022). The lychee undergoes a series of complex growth and development processes from flowering to fruit maturation. In recent years, notable advancements have been achieved in understanding the physiological and molecular mechanisms

* Corresponding authors.
*E-mail addresses:* rxia@scau.edu.cn (R. Xia), jxu@scau.edu.cn (J. Xu).

https://doi.org/10.1016/j.scienta.2024.113410
Received 11 March 2024; Received in revised form 31 May 2024; Accepted 10 June 2024
Available online 14 June 2024

pertaining to flowering and fruit development in lychee (Ding et al., 2015; Li et al., 2019; Xia et al., 2012; Zhang et al., 2023). It was found that GAs play a crucial role in the development of flowers and fruits in lychee. Li et al. (2005) reported that the levels of GAs are closely associated with seed abortion in lychee. In addition, $GA_3$ treatment was found to be a viable alternative to the manual inflorescence pruning in 'Yu Her Pau' lychee (Chen et al., 2014). Recent research has demonstrated that miR482/2118-*TASL-GID1* pathway plays a role in seed development in lychee, and this regulatory module is subject to a feedback regulation by GA signaling (Zhang et al., 2023). Nonetheless, we still know very little about the underlying physiological and molecular mechanisms associated with GAs in lychee due to the inherent constraints imposed by research methods employed thus far. The functionality of GAs is determined by the spatial and temporal distribution as well as the concentration level of bioactive GAs within the plant. Therefore, accurate quantification of GAs in lychee tissues is a prerequisite for comprehending the underlying GA-mediated mechanisms and effectively improving practical applications. To date, there have been no reports on the simultaneous quantitation of bioactive GAs in lychee. The limited investigation in this field can be primarily attributed to the low abundance of GAs (Hao et al., 2015), the existence of multiple GA types with closely similar chemical structures (Mander, 1992), and the susceptibility of GAs to fluctuations in environmental conditions (Urbanová et al., 2013). Furthermore, lychee tissues contain a diverse array of complex compounds, as the fruits are replete with sugars and organic acids, the pericarp are abundant in anthocyanins, and the seeds are rich in phenolic substances. Therefore, the selection and decontamination of sample pre-treatment for GAs extraction are of utmost importance, and the use of detection methods and instruments with ultra-high sensitivity is required.

In general, the determination method for phytohormones can be primarily classified into two sequential steps: sample pre-treatment and instrumental analysis. Efficient pre-treatment processes can successfully concentrate target compounds and effectively eliminate interference from other compounds. In the past decade, the liquid-liquid extraction (LLE) technique has been extensively used for the pre-treatment of phytohormones. The underlying principle of the LLE technique lies in the use of highly polar solvents to augment the dissolution and extraction of phytohormones from the plant tissues, operating on the basis of solubility similarity (Wu et al., 2014). Typically, the process of LLE involves a series of steps, including homogenization, extraction, purification, concentration, and reconstitution (Du et al., 2012). In addition, there have been significant advancements and refinements in analytical instrumentation in recent years. One notable technique is the hyphenation of liquid chromatography with mass spectrometry (LC-MS), which combines the separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry, resulting in high sensitivity and selectivity in the analysis of various compounds (Jiang et al., 2020). Indeed, this analytical instrument is highly appropriate for conducting the analysis and identification of compounds characterized by low concentrations and similar structures within complex compounds. For these specific applications, the commonly employed technique for quantitation is ultra-high performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry (UHPLC-QQQ-MS/MS). During sample analysis, the use of ultra-high performance liquid chromatography (UHPLC) enables the delivery of the sample into the chromatographic column by employing mobile phases, thereby facilitating the separation of the sample in the temporal dimension. Following the separation process, the isolated sample undergoes ionization through an ion source, after which it is introduced into a triple quadrupole mass analyzer. (Birkemeyer et al., 2003). By utilizing the response signal generated by specific precursor and fragment ions, the triple quadrupole mass analyzer facilitates the re-separation of the sample, ultimately enabling accurate quantitative analysis of the compound.

In this study, a high-sensitivity method utilizing UHPLC-QQQ-MS/MS was developed for the simultaneous quantitation of bioactive GAs, specifically $GA_1$, $GA_3$, $GA_4$, and $GA_7$, from a mere 300 mg of lychee sample. To achieve this, several pre-treatment steps and detection parameters were systematically compared and optimized by referring to the published literatures. Ultimately, by employing optimized pre-treatment methods and incorporating internal standards, we successfully accomplished accurate quantitative analysis of bioactive GAs in different lychee tissues.

## 2. Materials and methods

### 2.1. Plant materials

'Huaizhi' fruits (94 days after flowering, 94 DAF), male and female flowers, and leaves were obtained from the germplasm orchard of South China Agricultural University. The lychee fruits was carefully dissected into its constituent parts, including the pericarp, flesh and seeds. Furthermore, the anthers and aborted ovaries were meticulously separated from the male flowers. To acquire soaked germinating seeds, a subset of seeds was subsequently immersed in water for different durations of 6 h (h), 12 h, and 24 h, respectively. To obtain sprouted seeds and seedlings, an additional set of seeds were placed in soil under normal environment to facilitate germination. All samples were promptly submerged in liquid nitrogen after removal and subsequently stored at −80℃ for subsequent analyses. Before use the plant materials were ground to a fine powder.

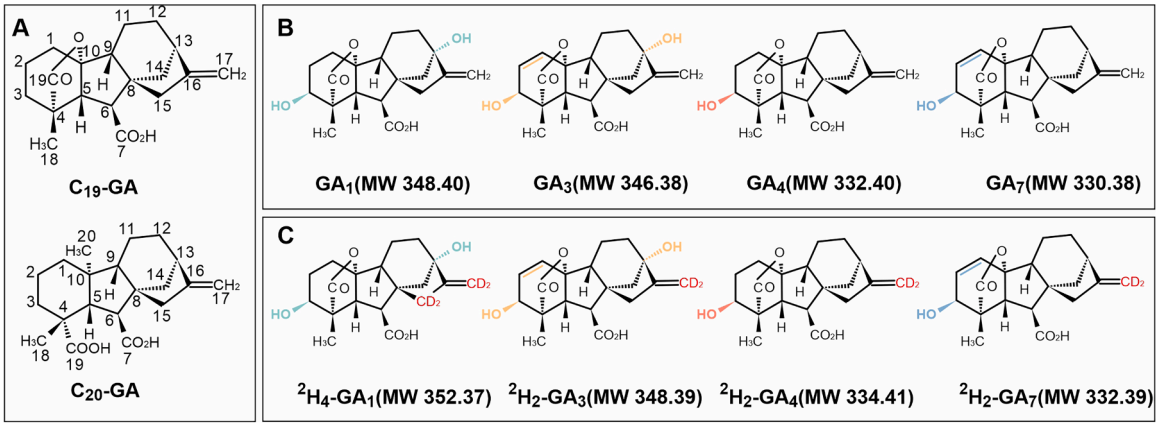



**Fig. 1.** Chemical structures and MW values of the bioactive GAs. (A) Skeleton of $C_{19}$-GA and $C_{20}$-GA. (B) Structures and MW of the bioactive GAs. (C) Structures and MW of the internal standard of bioactive GAs. Distinct colors were employed to emphasize the discernible differences within the same subgraph.

### 2.2. Chemicals and reagents

Gibberellin standards ($GA_1$, $GA_3$, $GA_4$, and $GA_7$) and their corresponding isotope-labeled internal standards ($^2H_4$-$GA_1$, $^2H_2$-$GA_4$, and $^2H_2$-$GA_7$) were all purchased from OlChemIm (Olomouc, Czech Republic). LC/MS grade methanol, acetonitrile, and formic acid were procured from Thermo Fisher Scientific (Waltham, MA, USA). Analytically pure grade isopropanol, hydrochloric acid, dichloromethane and acetic acid were obtained from Guangzhou Chemical Works (Guangzhou, China). Distilled water was acquired from Watsons.

### 2.3. Sample pre-treatments methods

To enhance the extraction efficiency of GAs from lychee tissues, the effectiveness of three pre-treatment methods was evaluated, employing soaked lychee seeds as the experimental material. Firstly, seeds were frozen in liquid nitrogen and subsequently comminuted into a fine powder using a cryogenic grinding mill (LuKa, Guangzhou, China). Furthermore, each internal standard was introduced to the extractant, resulting in a final concentration of 20 ng·mL$^{-1}$ for the internal standard. The extraction method's viability was assessed based on the retention time and response value of the ion pair.

The sample pre-treatment processes were optimized, based on the protocol of Pan et al. (2010). Initially, Firstly, lychee samples (300 mg) were accurately weighed and then transferred to separate screw-cap tubes with a capacity of 10 mL. Subsequently, a volume of 3 mL of a mixture composed of Isopropanol/$H_2O$/HCl (2/1/0.002, v/v/v) was added to each individual tube. Secondly, the tubes were placed on a shaker operating at a velocity of 1500 rotations per minute (rpm.) for 2 h at 4 °C in the dark. Thirdly, 3 mL of dichloromethane was added to each tube, and the agitation was continued for an additional 2 h at 4 °C. Subsequently, the mixed extract was allowed to undergo settling at 4℃ for a duration of 30 min (min) until the mixture visibly stratified into three distinct layers. Following this, the lower phase was collected and concentrated using a nitrogen evaporator under a nitrogen flow (not completely dried). Finally, the samples are redissolved in 0.2 mL 80 % methanol. To prevent blockage of the chromatographic column. the redissolved solution should be filtered using a 0.22 μm polytetrafluoroethylene membrane filter (JINTENG, Tianjin, China). Finally, the extracts were analyzed by UHPLC-QQQ-MS/MS.

In this study, we also examined two additional pre-treatment methods, as described in the studies by Li et al. (2017) and Chiwocha et al. (2003), involving the use of MeOH/$H_2O$/Formic acid (75/20/5, v/v/v) and Isopropanol/Acetic acid (99/1, v/v) as extraction reagents, respectively.

### 2.4. Conditions of UHPLC-QQQ-MS/MS

All analyses in this study were carried out using an ACQUITY UPLC™ H—Class (Waters, Milford, MA, USA) coupled with a Xevo® TQD MS triple-stage quadrupole mass spectrometer (Waters MS Technologies, Manchester, UK).

Bioactive GAs are chromatographically separate with ACQUITY UPLC BEH C18 column (1 × 50 mm, 1.7 μm). The column is maintained at 40℃ with a flow rate of 0.4 mL·min$^{-1}$. The injection volume for the sample is 2 uL. The mobile phase consisted of acetonitrile (A) and Watson's distilled water with 0.1 % formic acid (B). The optimized gradient elution processes are shown in Table 1. The retention times for each bioactive GAs and their respective isotope-labeled internal standards are listed in Table 2.

The mass spectrometer was operated in multiple reaction monitoring (MRM) with negative electrospray ionization (ESI-). The ESI source parameters used in this study were as follows: source temperature 150℃, capillary voltage 2.00 kV, desolvation temperature 500℃, desolvation gas flow 1000 L·Hr$^{-1}$, collision energy 15–30 V, cone 30–40 V, cone gas flow 50 L·hr$^{-1}$. Peristaltic pump injection method was implemented with a concentration of 300 ng·mL$^{-1}$ for each standard. In this work, the MS scan method was employed to identify the optimal tuning parameters for the precursor ion, followed by the daughter scan method to identify the fragment ions and their corresponding mass spectrometry tuning parameters. The daughter scan from *m/z* 100 to 350 was performed in ESI-. The collision energy and cone voltage were adjusted to optimize the signal intensity of the fragment ion signal. The specific MRM parameters for each GA standard and the corresponding isotope-labeled internal standards are shown in Table 2.

**Table 1**
The UHPLC gradient elution procedure used for the separation of bioactive GAs.

| Time (min) | Flow (mL·min$^{-1}$) | A (%) | B (%) |
|---|---|---|---|
| Initial | 0.400 | 10.0 | 90.0 |
| 4.00 | 0.400 | 90.0 | 10.0 |
| 5.00 | 0.400 | 90.0 | 10.0 |
| 5.10 | 0.400 | 10.0 | 90.0 |
| 7.00 | 0.400 | 10.0 | 90.0 |

UHPLC: Ultra-high Performance Liquid Chromatography; A: acetonitrile; B: distilled water with 0.1 % formic acid.

**Table 2**
Optimized MS/MS conditions for each of the bioactive GAs and internal standard.

| Analyte | Precursor and product ion (*m/z*) | Collision energy (V) | Cone (V) | Dwell time (s) | Retention time (min) |
|---|---|---|---|---|---|
| $GA_1$ | 347.0 > 259.2*/273.1 | 20 | 40 | 0.007 | 1.87 |
| $GA_3$ | 345.1 > 143.2*/239.1 | 25 | 30 | 0.007 | 1.85 |
| $GA_4$ | 331.1 > 213.2*/243.1 | 30 | 30 | 0.007 | 3.03 |
| $GA_7$ | 329.1 > 223.2*/241.1 | 20 | 40 | 0.007 | 2.99 |
| $^2H_4$-$GA_1$ | 351.3 > 263.4 | 20 | 30 | 0.005 | 1.85 |
| $^2H_2$-$GA_4$ | 333.1 > 215.2 | 15 | 30 | 0.007 | 3.02 |
| $^2H_2$-$GA_7$ | 331.3 > 225.2 | 15 | 35 | 0.007 | 3.00 |

*Refer to the confirmation transition.

### 2.5. Validation of the method

Stock solutions of bioactive GAs and isotope-labeled internal standard were dissolved in methanol at a concentration of 10 μg·mL$^{-1}$, and then stored at −20℃. These stock solutions can be subsequently diluted with 80 % methanol to obtain different working solutions.

To construct the calibration curves, a series of working standard solutions at concentration of 0, 25, 50, 100, 125, 200, 250, and 500 ng·mL$^{-1}$ were prepared by diluting the corresponding GA stock solutions with 80 % methanol. An equal volume of the corresponding internal standard solution was added to each working solution to achieve an internal standard concentration of 20 ng·mL$^{-1}$. The analytical approach described above was utilized to sequentially determine the peak areas of the GA standard solution (designated as S1) and the internal standard solution (designated as S2) for each working solution. Subsequently, a standard curve was constructed by plotting the concentration (C) on the x-axis and the ratio of S1 to S2 on the y-axis. The linear regression equation and correlation coefficient ($r^2$) were obtained to characterize the linearity of the curve. Linearity was considered acceptable when $r^2$ values were higher than 95 %. This entire process was implemented using MassLynx Software 4.1.

The recovery of the pre-treatment methods was determined through the determination of the standard recovery. A standard solution of GAs was added to the extraction solvent to achieve a final concentration of

50 ng·mL$^{-1}$. GAs were extracted from lychee seeds using both the extraction solvent containing a standard solution of 50 ng·mL$^{-1}$ and the extraction solvent without the standard solution, and GAs content was subsequently determined. The recovery rate (R) was calculated using the formula $R = (C_2 - C_1) / 50\ ng{\cdot}mL^{-1} \times 100\ \%$, where $C_2$ represents the measured concentration with the standard solution and $C_1$ represents the measured concentration without the standard solution. The range of recovery rate was within 80–120 %, demonstrating a high recovery and a low loss of the pre-treatment process in this method.

The matrix effects (ME) in various lychee tissues were compared the peak area of equimolar IS in the presence and absence of matrix components. The detected peak area of IS was recorded as $S_a$. The peak area of the same amount of IS dissolved in blank solvent was recorded as $S_b$. The matrix effect was calculated as $S_a/S_b$.

The relative standard deviation (RSD) of the internal standard peak area reflects the precision of the method. To assess the precision of the method, the internal standard solution at a concentration of 20 ng·mL$^{-1}$ was subsequently injected and analyzed five times. The RSD of the internal standard peak area can be calculated using the formula RSD = (Standard Deviation / Average) × 100 %. An RSD value below 20 % indicates high precision of the detection method.

In this study, the limit of detection (LOD) and the limit of quantification (LOQ) were determined by evaluating the injected quantity of GA, using a signal-to-noise ratio of 3:1 for LOD and a signal-to-noise ratio of 10:1 for LOQ.

### 2.6. Detection of bioactive GAs content

In order to accurately quantify the concentration of GAs in the lychee samples, we employed the internal standard method for GAs quantitative analysis. In principle, The chemical structure of the internal standard should closely resemble that of the component to be measured in order to ensure accurate measurements (Fig. 1B and C). In the absence of $^2H_2$-$GA_3$, $^2H_4$-$GA_1$ can be regarded as an appropriate internal standard for the quantitative analysis of $GA_3$, as it exhibits the highest degree of structural similarity to $GA_3$ (Fig. 1C). Therefore, we chose to utilize $^2H_4$-$GA_1$ for the simultaneous quantification of both $GA_1$ and $GA_3$. The formula for calculating the contents of GA is as follows:

GA (FW, ng·$g^{-1}$) = CV/W. In the formula: C refers to the concentration of GA obtained from the corresponding internal standard calibration curve (ng·mL$^{-1}$). V refers to the volume of the sample being measured (mL), while W represents the mass of the pre-treatment sample (g).

### 2.7. Data analysis and graphing

Data acquisition and instrument control were conducted using MassLynx software version 4.1. Group comparisons were carried out using Student's *t* tests in Microsoft Excel 2019. Two-way analysis of variance (ANOVA) was performed using GraphPad Prism version 9.0. Graphical representation was generated using CorelDRAW 2020 and Origin 2021.

## 3. Results

### 3.1. Establishment and optimization of UHPLC-QQQ-MS/MS conditions

Accurate quantitative analysis of GAs highly relies on the selection of appropriate UHPLC-QQQ-MS/MS parameters. To obtain the optimal mass spectrometry parameters for GAs, we performed mass spectrometry tuning parameter setting on the GAs standard and their isotope-labeled internal standard. The secondary mass spectrum presents the highest response values for the confirmation transition of GA standards and their isotope-labeled internal standards (Fig. 2). The mass spectrometry detection parameters for each analyte can be found in Table 2. Consequently, the selection of confirmation transition for $GA_1$ was 347.0

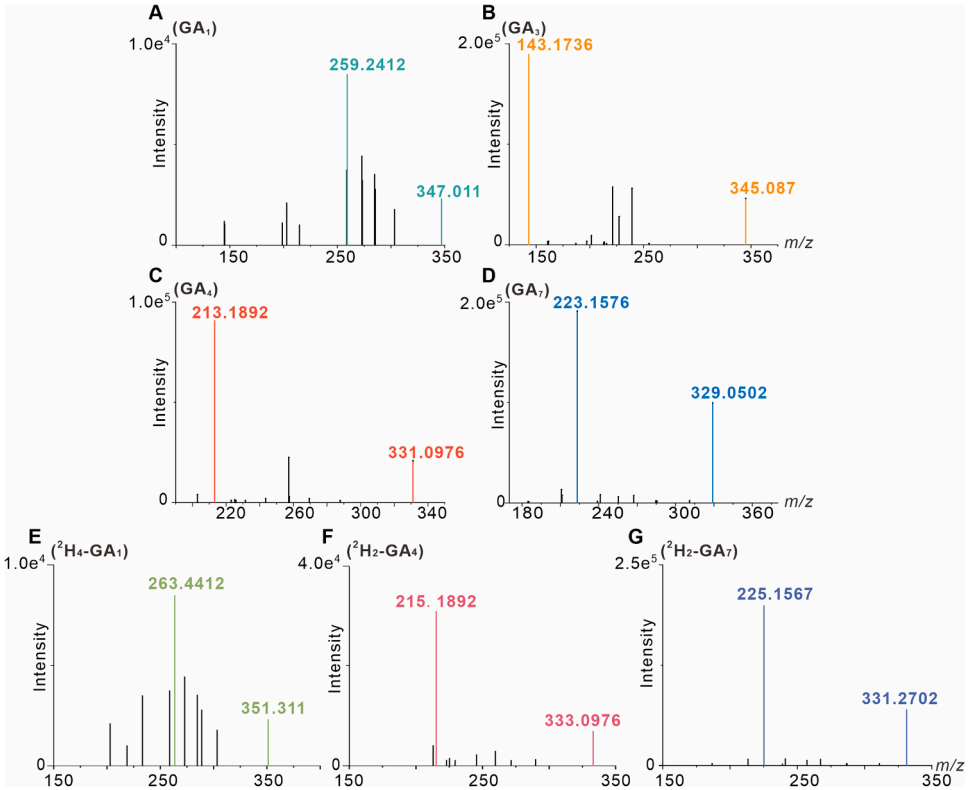



**Fig. 2.** Secondary mass spectra of $GA_1$ (A), $GA_3$ (B), $GA_4$ (C), $GA_7$ (D), $^2H_4$-$GA_1$ (E), $^2H_2$-$GA_4$ (F) and $^2H_2$-$GA_7$ (G) under negative electrospray ionization. The colored segments depicted in the figures represent confirmation transition of different analytes.

> 259.2, for $GA_3$ was 345.1 > 143.2, for $GA_4$ was 331.1 > 213.2, for $GA_7$ was 329.1 > 223.2, for $^2H_4$-$GA_1$ was 351.3 > 263.4, for $^2H_2$-$GA_4$ was 333.1 > 215.2 and for $^2H_2$-$GA_7$ was 331.3 > 225.2 (Fig. 2 and Table 2). The selection of the confirmation transition is largely consistent with previous studies (Cao et al., 2016; Manzi et al., 2015; Pan et al., 2010; Xin et al., 2020), with slight deviations possibly attributed to variations in the sensitivity of the machine equipment.

The mobile phase is a critical factor influencing liquid chromatography as it has a substantial influence on not only the separation of analytes and retention time, but also on ionization (Chambers et al., 2007). To optimize the elution efficiency of GAs, we investigated the influence of different mobile phases on the separation of bioactive GAs standard. Regarding the choice of organic phase, our findings demonstrated that acetonitrile exhibited superior advantages compared to methanol in terms of separating the four bioactive GAs (Fig. 3A). Moreover, the pH of mobile phase can be effectively manipulated to enhance the ionization of acid phytohormones by introducing volatile acids, such as formic acid, into the aqueous mobile phase (Blackwell et al., 1997). We conducted a further comparison to evaluate the impact of formic acid concentration ranging from 0.05 to 0.2 % on the separation of GAs. The results demonstrated that the addition of a 0.1 % formic acid solution into the aqueous mobile phase resulting in the highest peak area for all bioactive GAs (Fig. 3B), which is consistent with the results reported by Pan et al. (2010). In summary, the combination of acetonitrile (mobile phase A) and a 0.1 % aqueous formic acid solution (mobile phase B) was chosen for the separation of bioactive GAs.

After establishing the parameters for UHPLC-QQQ-MS/MS, we performed detection on the bioactive GA standards and their corresponding isotope-labeled internal standards. The resulting chromatogram exhibited smooth pyramidal shapes for each GA, effectively separating the bioactive GAs with stable retention times (Fig. 3C and D). The retention times of $GA_1$, $GA_3$, $GA_4$, $GA_7$, $^2H_4$-$GA_1$, $^2H_2$-$GA_4$, and $^2H_2$-$GA_7$ were determined to be 1.87, 1.85, 3.03, 2.99, 1.85, 3.02, and 3.00 min, respectively (Fig. 3C, D and Table 2). Primarily, it has been demonstrated that the established UHPLC-QQQ-MS/MS method is highly suitable for the detection of GAs.

### 3.2. Selection of pre-treatment procedures

The determination of GAs can be divided into sample pre-treatment and UHPLC-QQQ-MS/MS detection. Empirically, it has been observed that sample pre-treatment plays a more critical role in influencing the quality of analytical results (Chen et al., 2008; Xin et al., 2020). The LLE method was utilized in this study to extract GAs from lychee tissues. Therefore, the careful selection and appropriate utilization of extraction solvents are essential in achieving successful extraction of GAs. Based on the principle of similarity and solubility, the combination of alcohols and organic acid aqueous solutions has been widely utilized for the extraction of GAs (Bieleski, 1964). Thus, we conducted a comparative analysis of various published extraction solvents used for GA extracting. Notably, Isopropanol/$H_2O$/HCl (2/1/0.002, v/v/v) (Pan et al., 2010), Isopropanol/Acetic acid (99/1, v/v) (Chiwocha et al., 2003) and MeOH/$H_2O$/Formic acid (75/20/5, v/v/v) (Li et al., 2017) were found to be effective in extracting GAs from lychee samples. To assess the extraction efficiency of the three extraction solvents, lychee mature seeds (94 DAF), soaked germinating seeds, sprouted seeds, and lychee seedlings were selected as the testing materials (Fig. 4A), as seeds and tender tissues of plant are rich in GAs (Hedden and Thomas, 2012).

It has been demonstrated that Isopropanol/$H_2O$/HCl (2/1/0.002) and Isopropanol/Acetic acid (99/1) can effectively extract all bioactive GAs from mature lychee seeds soaked in water for 12 h and 24 h (Fig. 4B and C). In contrast, the use of MeOH/$H_2O$/Formic acid (75/20/5) was found to be ineffective in the extraction of all bioactive GAs in the examined lychee samples (Fig. 4D). Moreover, the extraction efficiency of bioactive GAs in soaked seed using Isopropanol/$H_2O$/HCl (2/1/0.002) was higher than that using Isopropanol/Acetic acid (99/1). For example, when extracting GAs from seeds soaked in water for 12 h, the extraction efficiency of the four bioactive GAs was increased by 439.3 %, 269.7 %, 25.1 % and 37.6 %, respectively (Supplemental table S1). In conclusion, these results indicated that the employment of Isopropanol/

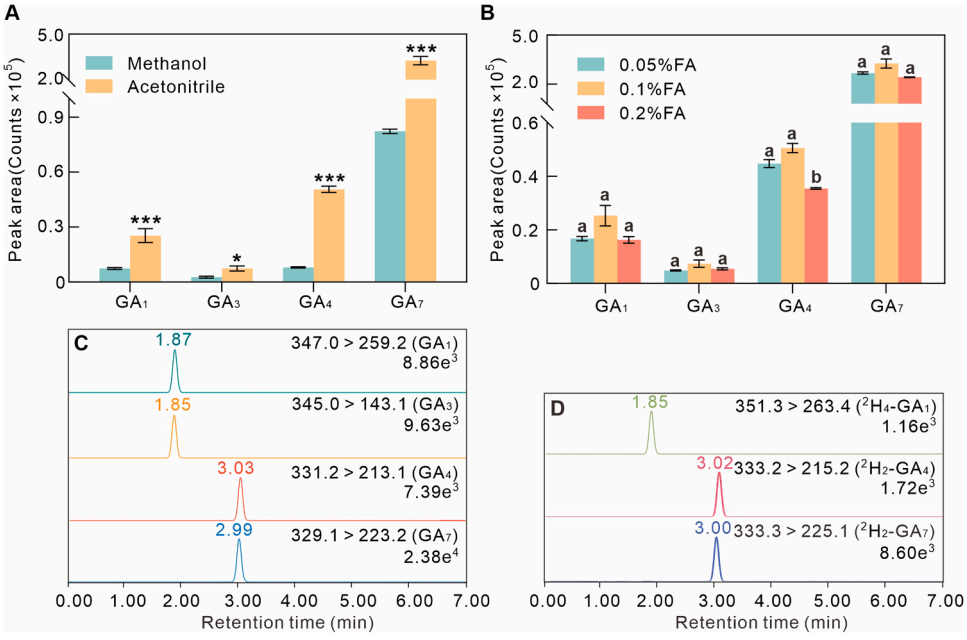



**Fig. 3. Comparative analysis of the effectiveness of different mobile phases and the chromatograms of GAs.** (A) Comparative analysis of the effectiveness of the organic phase. Asterisks indicate the significance of differences between methanol and acetonitrile for the peak area in Student's *t* tests (ns, no significance; *, $0.01 < P \leq 0.05$; ***, $P \leq 0.001$, $n = 3$). (B) Comparative analysis of the effectiveness of various concentrations of formic acid employed in the aqueous phase. Different letters indicate significant differences between different formic acid concentrations for the peak area in a two-way ANOVA test. FA: formic acid. Each bar represents the mean of three biological replicates with SE. (C) The chromatograms of bioactive GA standards. (D) The chromatograms of isotope-labeled internal standards.

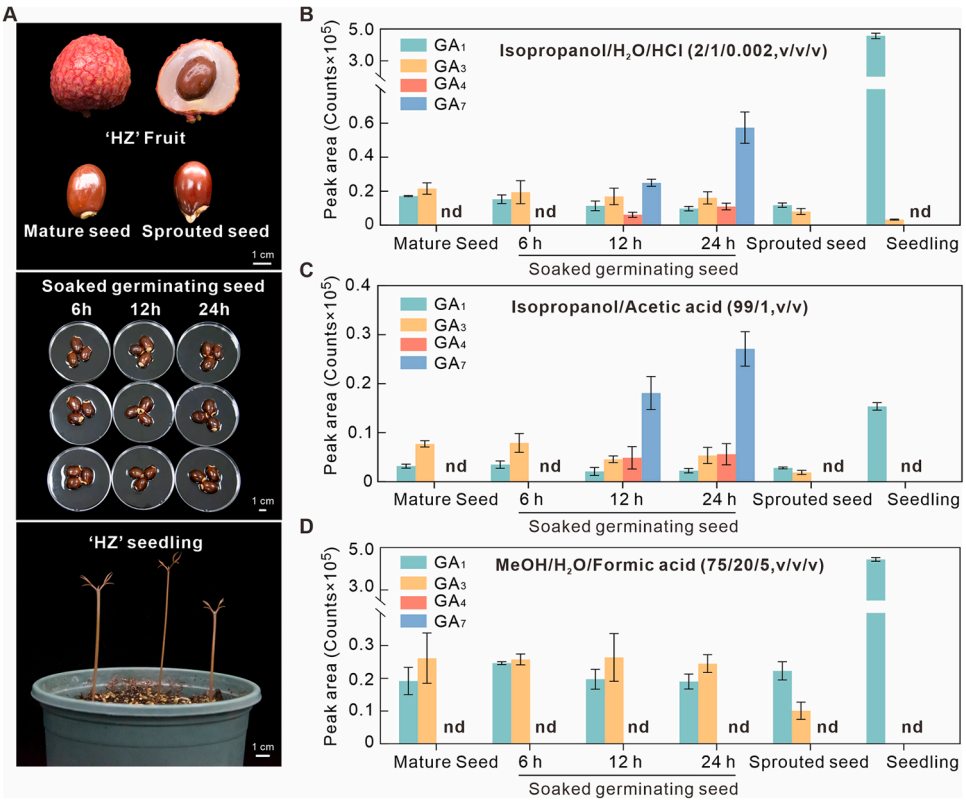



**Fig. 4. Comparative analysis of the effectiveness of various extraction solvents in extracting bioactive GAs from lychee tissues.** (A) The materials used for extracting bioactive GAs, including mature seeds, sprouted and soaked germinating seeds, as well as seedlings. 'HZ': the lychee cultivar 'Huaizhi'. Mature seed: seed harvested 94 days after flowering (DAF). 6 h, 12 h, and 24 h: mature seed soaking in water for 6 h, 12 h and 24 h. (B-D) The peak area of bioactive GAs extracted from different lychee samples using different extraction solvents: Isopropanol/$H_2O$/HCl (2/1/0.002, v/v/v) (B), Isopropanol/Acetic acid (99/1, v/v) (C) and MeOH/$H_2O$/Formic acid (75/20/5) (D). nd, not detected. Each bar represents the mean of three biological replicates with SE.

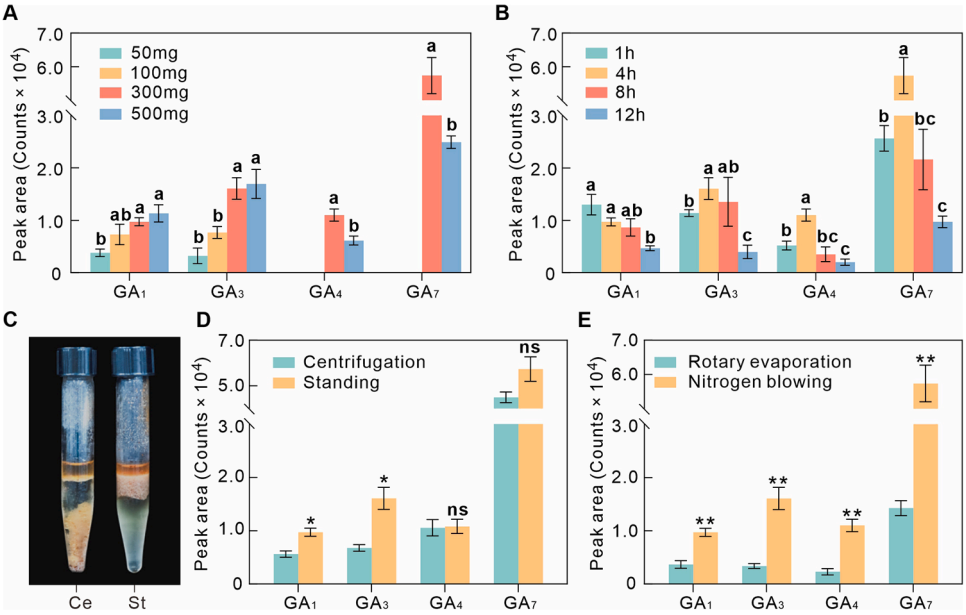



**Fig. 5. Optimization of pre-treatment produces using Isopropanol/$H_2O$/HCl (2/1/0.002) extraction.** (A) Comparison of sample weight for pre-treatment. (B) Comparison of sample extraction time for pre-treatment. Different letters indicate significant differences between different treats for the peak area in a two-way ANOVA test, $n = 3$. (C) Details of the appearance after sample extraction. Ce: Centrifugation, St: Standing. (D) Comparative analysis of the influence of centrifugation and standing for GAs extraction. (E) Comparative analysis of the influence of the concentration methods for GAs extraction. Asterisks represented significant differences between different treatments for the same traits at the same P level in *t*-tests. *: $0.01 < P \leq 0.05$; **: $0.001 < P \leq 0.01$; ns: not significant at 0.05 level. nd, not detected. Each bar represents the mean of three biological replicates with SE.

$H_2O$/HCl (2/1/0.002) could result in a notable improvement in the extraction efficiency of all bioactive GAs presented from lychee seeds, as compared to Isopropanol/Acetic acid (99/1) and MeOH/$H_2O$/Formic acid (75/20/5).

### 3.3. Further optimization of pre-treatment produces

To further improve the efficacy of GAs extraction from lychee samples, we optimized several pre-treatment steps that are closely linked to the extraction efficiency. Initially, the selection of an appropriate sample weight is essential for the extraction of target compounds. Insufficient sample weight may result in inadequate recovery of the bioactive GAs, while excessive sample weight can increase matrix complexity and subsequently reduce the sensitivity of UHPLC-QQQ-MS/MS detection. Therefore, a comparative analysis was performed to evaluate the impact of different sample weights on the extraction efficiency of bioactive GAs from soaked lychee seeds. It was found that sample weights of 50 mg and 100 mg were limited to extract only $GA_1$ and $GA_3$, while with sample weights of 300 mg and 500 mg all the bioactive GAs were successfully detected (Fig. 5A). In addition, it was noteworthy that the extraction of $GA_4$ and $GA_7$ in the 300 mg sample weight was even more significant in comparison to the 500 mg sample (Fig. 5A). Besides, the matrix effect values of the samples at 500 mg are consistently below 50 % (Supplemental Fig.1), it indicating had effect on the mass spectrometry responses of GAs. Consequently, it is advisable to employ a 300 mg sample for the extraction of bioactive GAs from lychee samples.

In this study, Isopropanol/$H_2O$/HCl (2/1/0.002) and dichloromethane were used for sample extraction and purification, respectively. The time of sample extraction and purification is also an important factor that influences the enrichment of bioactive GAs. To examine the impact of varying extraction and purification times on the efficiency of bioactive GAs extraction, a comparative analysis was performed. The results indicated that a 2-hour extraction and 2-hour purification resulted in a higher content of GAs, such as $GA_3$, $GA_4$ and $GA_7$ (Fig. 5B). Therefore, a 4-hour duration was optimal to extract bioactive GAs from lychee samples.

Furthermore, Pan et al. (2010) has reported that upon the addition of dichloromethane, followed by high-speed centrifugation, the resulting mixture would undergo phase separation, resulting in the formation of three distinct layers. The bottom layer would consist of sedimented tissue fragments, the top layer would contain the pigment, and the intermediate layer would contain the desired target extraction liquid (Fig. 5C). Nevertheless, we observed that the target extraction liquid obtained following centrifugation of the pre-treatment mixture still exhibited the presence of residual tissue fragments, impeding the attainment of a pure bottom layer extract (Fig. 5C). By introducing a standing phase into the protocol rather than centrifugation, the mixture could be unequivocally segregated into three distinct layers, thereby yielding a substantially purer bottom layer extraction liquid (Fig. 5C). Additionally, this modified methodology led to a higher content of GAs (Fig. 5D). Therefore, the replacement of centrifugation with standing is expected to facilitate the purification of the GAs extraction solution, resulting in a higher content of GAs extraction.

In the final step of GAs extraction, it is necessary to concentrate the purified extraction solution in order to reduce its volume and increase the concentration of bioactive GAs. The appropriate concentration method can effectively reduce the loss of the bioactive GAs. At present, nitrogen blowing and rotary evaporation are two commonly utilized concentration methods in the pre-treatment process of phytohormones extraction. In the extraction of bioactive GAs from lychee seeds, we found that the nitrogen blowing method yielded significantly higher enrichment of bioactive GAs compared to rotary evaporation (Fig. 5E). Hence, the nitrogen blowing was selected as the preferred method for concentrating the GAs extraction solution.

### 3.4. Method validation

In this work, the applicability of the detection method was assessed through linear relationship evaluation, while the sensitivity of the method was evaluated through the limit of detection (LOD) and the limit of quantitation (LOQ) (Xin et al., 2020). The GAs standard solutions in the concentration range of 0–500 ng·mL$^{-1}$ were analyzed, and the calibration curves resulting from three separate injections exhibited excellent linearity. The results presented in Table 3 indicated that the correlation coefficient ($r^2$) values ranged from 0.996 to 0.999. Moreover, the limit of detection (LOD) ranged from 0.035 to 0.143 pg·$g^{-1}$, and the limit of quantification (LOQ) ranged from 0.116 to 0.472 pg·$g^{-1}$ (Table 3). Therefore, when the content of GA in 1 g of lychee sample exceeds the range of 0.116 to 0.472 pg, all bioactive GAs can be quantitatively detected. Additionally, the peak area precision of GAs is from 3.58 % to 10.06 % (Table 3). In summary, the optimized method exhibits exceptional sensitivity and accuracy, allowing for the detection of bioactive GAs at remarkably low concentrations in lychee samples. To assess the efficiency of the pre-treatment method for GAs extraction, lychee samples were extracted using an extraction solvent containing a standard solution of each GA at a concentration of 50 ng·mL$^{-1}$. The recoveries of all bioactive GAs consistently exceeded 85 % (Table 3), thus confirming the high effectiveness of the pre-treatment method. As is shown in Fig. 7B, matrix effects ranged from 70 % to 100 %, suggesting that impurity residues in the eluent did not notably impact the MS responses of phytohormone compounds. Thus, the present method exhibits effective purification capabilities.

### 3.5. Application of the analytical method to lychee samples

As previously mentioned, a comprehensive and efficient analytical method was developed for the extraction and detection of GAs from lychee tissues. The method involved optimizing the extraction procedures for bioactive GAs in lychee samples and determining the parameters of UHPLC-QQQ-MS/MS for detecting GA contents (Fig. 6A). We firstly utilized this method to analyze the GAs extract from lychee seeds. The respective chromatographic peaks for each GA were observed in the extraction solution, showing consistent retention times with GA standards (Figs. 6D, E; 3C and 3D). These results validated the effectiveness of this optimized method in extraction and detection of GAs in lychee samples.

We further employed this method in GAs measurement for lychee tissues besides seeds, such as young and mature leaves, pericarp, flesh, as well as female and male flowers, anthers, and aborted ovaries (Fig. 7A). It demonstrates that the average matrix effects ranged from 70 % to 100 % (Fig. 7B), implying that the presence of impurity residues in the eluent of various lychee tissues had no significant effect on the mass spectrometry responses of GAs. As depicted in Fig. 7C, the analysis revealed the simultaneous detection of four bioactive GAs in seeds, with $GA_1$ exhibiting the highest concentration. Only $GA_1$ was detected in the leaves, with higher levels observed in young leaves compared to mature leaves. Similarly, only a little $GA_1$ was detected in flesh and pericarp. $GA_1$, $GA_4$, and $GA_7$ were found in lychee female flowers, male flower anthers, and its aborted ovaries. Additionally, both $GA_1$ and $GA_3$ were quantified in the entire lychee fruit. On the whole, the presence of bioactive GAs was detected in various lychee tissues, indicating the suitability of this method in the accurate quantification GAs in lychee tissues.

## 4. Discussion

GA is one of the most important phytohormones in plants. The determination of GAs content in plants is tremendously crucial for studying the mechanisms of plant growth and development and providing scientific basis for agricultural production. Hence, it is crucial to explore accurate, sensitive, and widely applicable method for GAs

**Table 3**
The linearity, correlation coefficient, precisions, LODs, LOQs, recovery of bioactive GAs.

| Analyte | Linear Range ($ng{\cdot}mL^{-1}$) | Linear Equation | Correlation Coefficient ($r^2$) | LOD ($pg{\cdot}g^{-1}$) | LOQ ($pg{\cdot}g^{-1}$) | Precisions (%, $n = 5$) | Recovery (%, $n = 3$) |
|---|---|---|---|---|---|---|---|
| $GA_1$ | 0–500 | $y = 4.528x-2.807$ | 0.998 | 0.035 | 0.116 | 10.06 | 88.98±7.8 |
| $GA_3$ | 0–500 | $y = 1.521x-26.56$ | 0.999 | 0.143 | 0.472 | 3.68 | 85.67±3.5 |
| $GA_4$ | 0–500 | $y = 15.60x+108.5$ | 0.996 | 0.080 | 0.265 | 4.71 | 93.35±1.7 |
| $GA_7$ | 0–500 | $y = 97.65x+111.4$ | 0.999 | 0.044 | 0.145 | 3.58 | 89.77±0.9 |

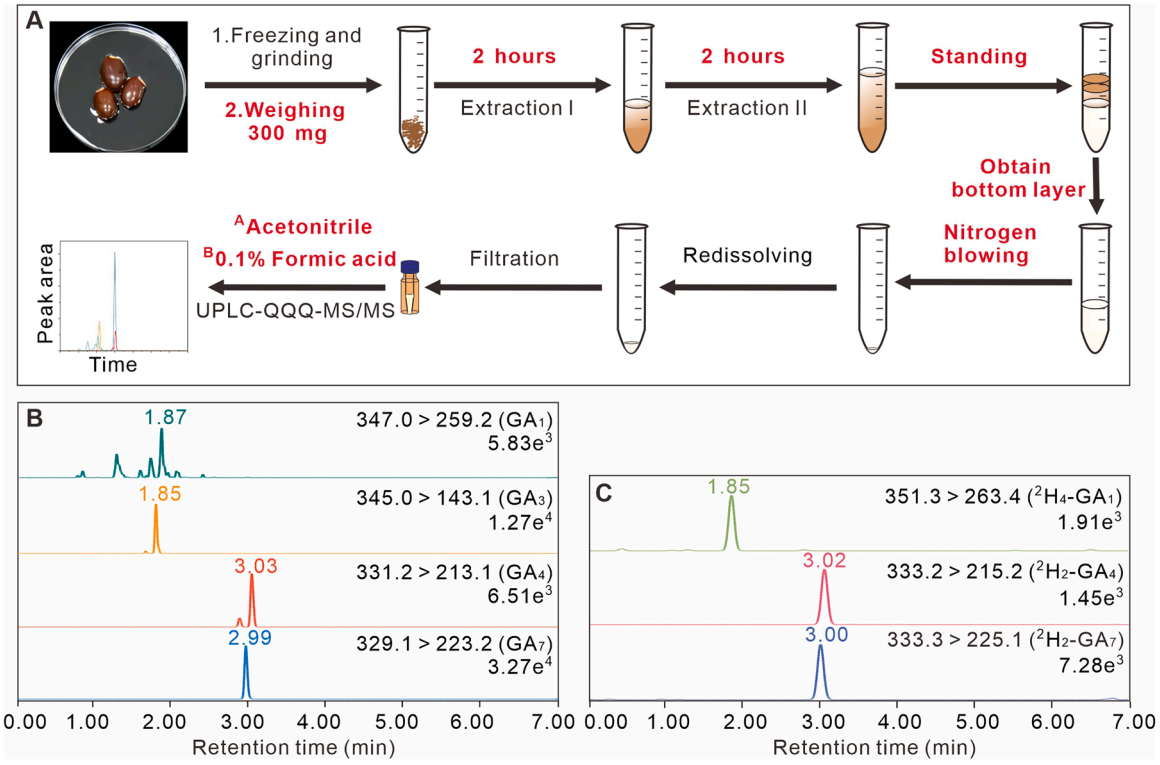



**Fig. 6. Analytical method and its application in analyzing GAs.** (A) Scheme for the developed pre-treatment produces for extracting bioactive GAs from lychee sample. Extraction I: Isopropanol/$H_2O$/HCl (2/1/0.002, v/v/v) with 20 ng•$mL^{-1}$ isotope-labeled internal standard; Extraction II: dichloromethane. The red fonts indicate the optimized conditions applied specifically to lychee samples in comparison to the original method. (B) The chromatograms of bioactive GAs detected in 300 mg 'HZ' seeds. (C) The chromatograms of isotope-labeled internal standards added in 300 mg 'HZ' seeds.

extraction and detection. In this study, we compared several published methods for GAs extraction, and the optimized the mass spectrometry parameters, the chromatographic conditions and the extraction processes. A liquid chromatography-tandem mass spectrometry method was successfully developed for the simultaneous quantification of bioactive GAs in lychee samples, exhibiting high efficiency in terms of sensitivity, selectivity, and accuracy. Compared to the complex pre-treatment methods and lengthy detection time of molecular imprinting technology (Zhang et al., 2012), solid-phase extraction (Li et al., 2016), and chemical derivatization (Xin et al., 2020), the optimized method in this work offers a simple pre-treatment produce and rapid analysis. After undergoing a 4-hour extraction and purification process, followed by a 1-hour concentration and resuspension procedure, the samples can be utilized directly for analysis. The separation and identification of four bioactive GAs in each sample could be completed within 7 min (Table 1), significantly improving the time efficiency compared to other detection methods that require 20–30 min of analysis time (Li et al. 2017; Chiwocha et al. 2003). Additionally, a mere 300 mg of lychee samples is sufficient for the quantification of GAs in small organs such as embryos, anthers, and ovaries, thereby minimizing the sample requirement (Figs. 5A and 7B). The simple pre-treatment method demonstrated high efficacy in the extraction of GAs, yielding recovery rates exceeding 85 % for each GA (Table 4). Furthermore, the proposed detection method utilized the internal standard approach for the quantitative analysis of different GAs in lychee samples. This methodology ensures that the extracted GAs and internal standards coelute during the entire analytical process, exhibiting nearly identical retention times upon entering the chromatographic column. As a result, any potential loss or error incurred during the extraction and quantification steps is effectively corrected, leading to improved accuracy in the measured outcomes, thereby providing accurate results that reflect the actual content of GAs in lychee samples (Gómez-Cadenas et al., 2002).

GA play a crucial role in regulating the development of flowers and fruits in fruit trees. Up to date, a strong association has been observed between the GA pathway and the developmental processes of lychee fruit (Huang, 2001; Qiu et al., 1998; Ma et al., 2018; Zhang et al., 2023). Previous studies have proposed specific methods for detecting GAs in certain fruit trees, such as citrus (Manzi et al., 2015) and apple (Zhang et al., 2010). However, to date, there is no reported method available for detection of GAs in lychee. This knowledge gap presents a significant challenge in understanding and harnessing the potential benefits of GAs application in lychee cultivation. In this study, a high-efficiency method for detecting bioactive GAs applicable to various tissues of lychee, was established. A precise quantification of various bioactive GAs was achieved by combining liquid-phase extraction with UHPLC-QQQ-MS/MS in a low sample input. Similar to the findings reported by MacMillan

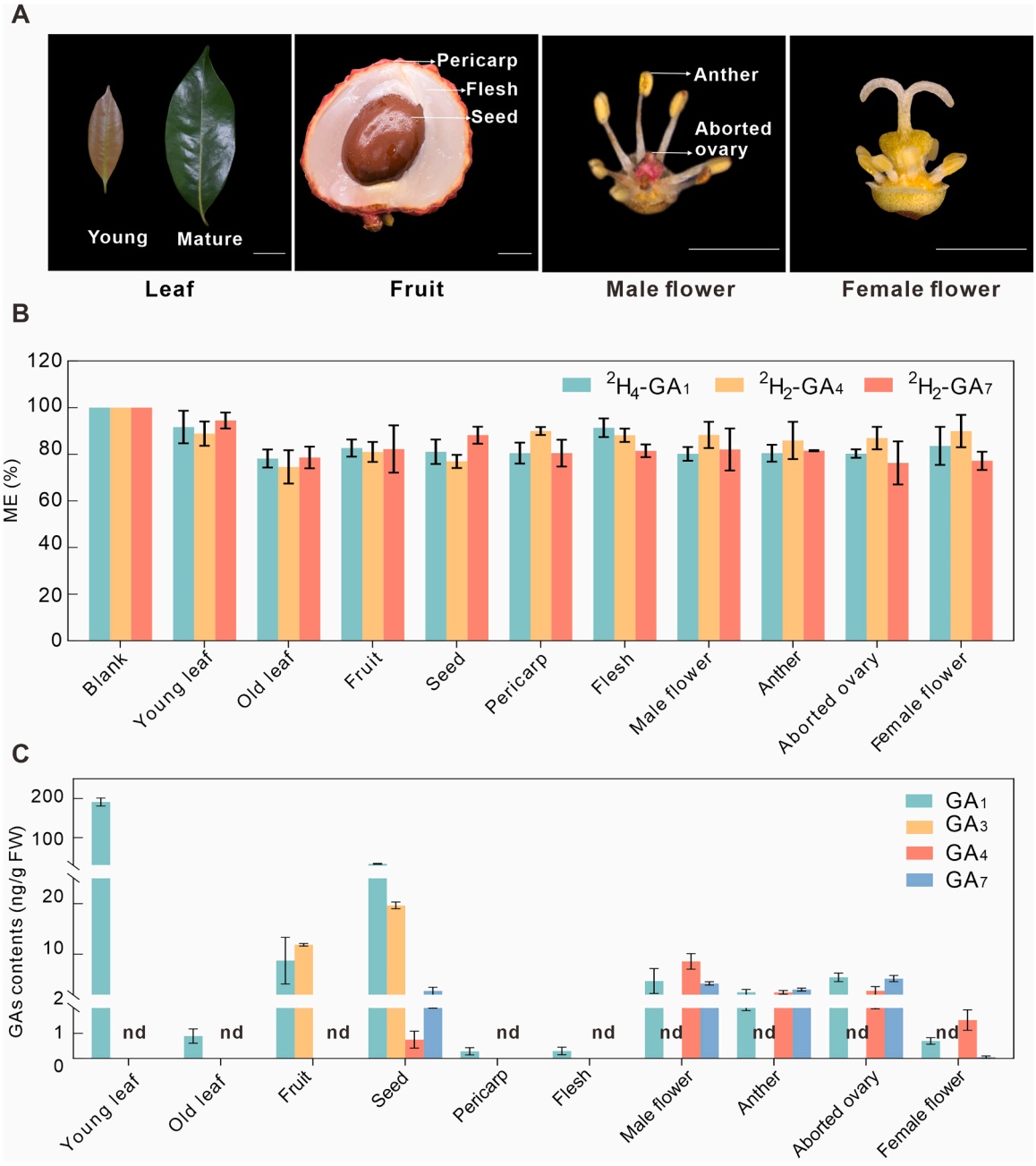



**Fig. 7. Quantification of bioactive GAs in various lychee tissues.** (A) Lychee tissues for analysis. (B) The evaluation of matrix effects in various lychee tissues. ME: Matrix effects. Blank: IS dissolved in 80 % methanol. (C) The content of bioactive GAs in various lychee tissues. nd, not detected. Each bar represents the mean of three biological replicates with SE.

(2001), our study revealed that $GA_3$ was exclusively detected in fruits, and seeds, whereas $GA_1$ was primarily accumulated in leaves, fruits and seeds. Interestingly, $GA_4$ and $GA_7$ were primarily quantified in flowers (Fig. 7B). Furthermore, $GA_3$ was not detected in lychee flowers and leaves (Fig. 7B), which is consistent with the findings in citrus reported by Manzi et al. (2015). These results demonstrated that $GA_1$ and $GA_3$ may play an important role in the regulation of lychee fruit development, while $GA_4$ and $GA_7$ may be vital for the flower development.

## 5. Conclusions

All in all, the optimized GA determination method in this study offers advantages, such as simplicity, high sensitivity, good selectivity, short analysis time, and high accuracy and precision. The establishment of the method for GA detection contributes to the clarification of the spatio-temporal distribution and content of bioactive GAs during lychee growth and development, and facilitates the application of GAs in lychee production as well as the elucidation of the mechanisms underlying GA regulation of lychee flower and fruit development.

## CRediT authorship contribution statement

**Xingling Su:** Writing – original draft, Methodology, Formal analysis, Data curation. **Jiakun Zheng:** Writing – review & editing, Supervision. **Yanwei Hao:** Writing – review & editing. **Rui Xia:** Writing – review & editing, Funding acquisition, Conceptualization. **Jing Xu:** Writing – review & editing, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgments

This research is supported by the National Natural Science Foundation of China (#32072547 and #31872063), and the China Agricultural Research System (CARS-32–09).

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2024.113410.

## References

Ayele, B.T., Ozga, J.A., Kurepin, L.V., Reinecke, D.M., 2006. Developmental and embryo axis regulation of gibberellin biosynthesis during germination and young seedling growth of pea. Plant Physiol. 142, 1267–1281.
Bieleski, R.L., 1964. The problem of halting enzyme action when extracting plant tissues. Anal. Biochem. 9, 431–442.
Birkemeyer, C., Kolasa, A., Kopka, J., 2003. Comprehensive chemical derivatization for gas chromatography–mass spectrometry-based multi-targeted profiling of the major phytohormones. J. Chromatogr. A 993, 89–102.
Blackwell, J.A., Stringham, R.W., Weckwerth, J.D., 1997. Effect of mobile phase additives in packed-column subcritical and supercritical fluid chromatography. Anal. Chem. 69, 409–415.
Chambers, E., Wagrowski-Diehl, D.M., Lu, Z., Mazzeo, J.R., 2007. Systematic and comprehensive strategy for reducing matrix effects in LC/MS/MS analyses. J. Chromatogr. B 852, 22–34.
Cao, Y., Sun, H., Mou, X., Zhang, P., Lin, Y., Zhu, W., Chen, X., 2016. Profiling of phytohormones and their major metabolites in rice using binary solid-phase extraction and liquid chromatography-triple quadrupole mass spectrometry. J. Chromatogr. A 1451, 67–74.
Chen, A., Lee, L., Roan, F., Chen, Z., 2014. Effects of $GA_3$ application on the inflorescence and yield of 'Yu Her Pau' litchi. Sci. Hortic. 171, 45–50.
Chen, Y., Guo, Z., Wang, X., Qiu, C., 2008. Sample preparation. J. Chromatogr. A 1184, 191–219.
Chiwocha, S.D.S., Abrams, S.R., Ambrose, S.J., Cutler, A.J., Loewen, M., Ross, A.R.S., Kermode, A.R., 2003. A method for profiling classes of plant hormones and their metabolites using liquid chromatography-electrospray ionization tandem mass spectrometry: an analysis of hormone regulation of thermodormancy of lettuce (*Lactuca sativa* L.) seeds. Plant J. 35, 405–417.
Ding, F., Zhang, S., Chen, H., Su, Z., Zhang, R., Xiao, Q., Li, H., 2015. Promoter difference of LcFT1 is a leading cause of natural variation of flowering timing in different litchi cultivars (*Litchi chinensis* Sonn. Plant Sci. 241, 128–137.
Du, F., Ruan, G., Liu, H., 2012. Analytical methods for tracing plant hormones. Anal. Bioanal. Chem. 403, 55–74.
Gómez-Cadenas, A., Pozo, O.J., García-Augustín, P., Sancho, J.V., 2002. Direct analysis of abscisic acid in crude plant extracts by liquid chromatography-electrospray/tandem mass spectrometry: determination of abscisic acid in plant extracts. Phytochem. Anal. 13, 228–234.
Hao, Y.-H., Zhang, Z., Wang, L., Liu, C., Lei, A.-W., Yuan, B.-F., Feng, Y.-Q., 2015. Stable isotope labeling assisted liquid chromatography–electrospray tandem mass spectrometry for quantitative analysis of endogenous gibberellins. Talanta 144, 341–348.
Hedden, P., 2020. The current status of research on gibberellin biosynthesis. Plant Cell Physiol. 61, 1832–1849.
Hedden, P., Thomas, S.G., 2012. Gibberellin biosynthesis and its regulation. Biochem. J. 444, 11–25.
Hu, G., Feng, J., Xiang, X., Wang, J., Salojärvi, J., Liu, C., Wu, Z., Zhang, J., Liang, X., Jiang, Z., Liu, W., Ou, L., Li, Jiawei, Fan, G., Mai, Y., Chen, C., Zhang, X., Zheng, J., Zhang, Y., Peng, H., Yao, L., Wai, C.M., Luo, X., Fu, J., Tang, H., Lan, T., Lai, B., Sun, J., Wei, Y., Li, H., Chen, J., Huang, X., Yan, Q., Liu, X., McHale, L.K., Rolling, W., Guyot, R., Sankoff, D., Zheng, C., Albert, V.A., Ming, R., Chen, H., Xia, R., Li, J., 2022. Two divergent haplotypes from a highly heterozygous lychee genome suggest independent domestication events for early and late-maturing cultivars. Nature Genetic 54, 73–83.
Huang, H., 2001. Towards a better insight into the development of the arillate fruit of litchi and longan. Acta Hortic. 558, 185–192.
Jiang, C., Dai, J., Liu, X., 2020. Chromatography alone and coupled with mass spectrometry for the determination of trace GAs in complex matrices: a review[J]. Food Sci. 41 (13), 249–259.
Li, C., Ma, X., Huang, X., Wang, H., Wu, H., Zhao, M., Li, J., 2019. Involvement of HD-ZIP I transcription factors LcHB2 and LcHB3 in fruitlet abscission by promoting transcription of genes related to the biosynthesis of ethylene and ABA in litchi. Tree Physiol. 39 (9), 1600–1613.
Li, D., Guo, Z., Chen, Y., 2016. Direct derivatization and quantitation of ultra-trace GAs in sub-milligram fresh plant organs. Mol. Plant 9, 175–177.
Li, D., Guo, Z., Liu, C., Li, J., Xu, W., Chen, Y., 2017. Quantification of near-attomole GAs in floral organs dissected from a single Arabidopsis thaliana flower. Plant J. 91, 547–557.
Li, J., Zhou, B., 2005. Comparison on fruit development and changes in endogenous hormone contents in pericarp between large- and aborted-seeded litchi (*Litchi chinensis* Sonn. cv. Guiwei) [J]. Plant Physiol. J. 41 (5), 587–590.
Ma, W., Chen, C., Liu, Y., Zeng, M., Meyers, B.C., Li, J., Xia, R., 2018. Coupling of microRNA-directed phased small interfering RNA generation from long noncoding genes with alternative splicing and alternative polyadenylation in small RNA-mediated gene silencing. New Phytologist 217, 1535–1550.
MacMillan, J., 2001. Occurrence of GAs in vascular plants, fungi, and bacteria. J. Plant Growth Regul. 20, 387–442.
Macmillan, J., Takahashi, N., 1968. Proposed procedure for the allocation of trivial names to the GAs. Nature 217, 170–171.
Mander, L.N., 1992. The chemistry of GAs: an overview. Chem. Rev. 92, 573–612.
Manzi, M., Gómez-Cadenas, A., Arbona, V., 2015. Rapid and reproducible determination of active GAs in citrus tissues by UPLC/ESI-MS/MS. Plant Physiol. Biochem. 94, 1–9.
Pan, X., Welti, R., Wang, X., 2010. Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography–mass spectrometry. Nature Protocol 5, 986–992.
Qiu, Y., Xiang, X., Wang, B., Zhang, Z., Yuan, P., 1998. Endogenous hormone balance in three types of litchi fruit and their fruit set mechanism. J. Fruit Sci. 15, 39–43.
Salazar-Cerezo, S., Martínez-Montiel, N., García-Sánchez, J., Pérez-y-Terrón, R., Martínez-Contreras, R.D., 2018. Gibberellin biosynthesis and metabolism: a convergent route for plants, fungi and bacteria. Microbiol. Res. 208, 85–98.
Urbanová, T., Tarkowská, D., Novák, O., Hedden, P., Strnad, M., 2013. Analysis of GAs as free acids by ultra performance liquid chromatography–tandem mass spectrometry. Talanta 112, 85–94.
Wu, Q., Wang, L., Wu, D., Duan, C., Guan, Y., 2014. Recent advances in sample preparation methods of plant hormones. Chin. J. Chromatogr. 32 (4), 319–329.
Wang, X., Zhao, M., Wu, W., Korir, N.K., Qian, Y., Wang, Z., 2017. Comparative transcriptome analysis of berry-sizing effects of gibberellin ($GA_3$) on seedless *Vitis vinifera* L. Genes Genomics 39, 493–507.
Xia, R., Li, C., Lu, W., Du, J., Wang, Z., Li, J., 2012. 3-Hydroxy-3-methylglutaryl coenzyme A reductase 1 (HMG1) is highly associated with the cell division during the early stage of fruit development which determines the final fruit size in *Litchi chinensis*. Gene 498, 28–35.
Xin, P., Guo, Q., Li, B., Cheng, S., Yan, J., Chu, J., 2020. A tailored high-efficiency sample pretreatment method for simultaneous quantification of 10 classes of known endogenous phytohormones. Plant Commun. 1, 100047.
Yabuta, T., 1938. On the crystal of gibberellin, a substance to promote plant growth. J. Agric. Food Chem. Soc 14, 1526.
Yamaguchi, S., 2008. Gibberellin metabolism and its regulation. Annu Rev. Plant Biol. 59, 225–251.
Zhang, H., Wu, Y., Lu, Y., Zhang, W., Jiang, J., Feng, N., Xie, W., 2010. Determination of gibberellin, abscisic, carbaryl, paclobutrazol and uniconazole in apples by ultra performance liquid chromatography-tandem mass spectrometry. Sci. Technol. Food Ind. 31 (10), 383–385, 2010.
Zhang, Y., Zeng, Z., Hu, H., Zhao, M., Chen, C., Ma, X., Li, G., Li, J., Liu, Y., Hao, Y., Xu, J., Xia, R., 2023. MicroRNA482/2118 is lineage-specifically involved in gibberellin signalling via the regulation of GID1 expression by targeting noncoding *PHAS* genes and subsequently instigated phasiRNAs. Plant Biotechnol. J. 14226.
Zhang, Z., Tan, W., Hu, Y., Li, G., Zan, S., 2012. Microwave synthesis of gibberellin acid 3 magnetic molecularly imprinted polymer beads for the trace analysis of gibberellin acids in plant samples by liquid chromatography-mass spectrometry detection. Analyst 137, 968–977.