Effects on Plant Hormone levels at Different Growth Stages in Diverse Grape Organs with Root Restriction

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Abstract

Endogenous plant hormones play important roles in germination, blossom, senescence, abscission of plants by a series of signal transduction and molecular regulation. The purpose of this research was to investigate the influence of root restriction (RR) cultivation on plant hormones variation tendency at different growth stages in diverse organs or tissues, ‘Muscat Hamburg’ (Vitis ‘Muscat of Alexandria’ × Vitis ‘Trollinger’) grapevine was used as test material. High Performance Liquid Chromatography (HPLC) was used to quantify hormone levels, aiming to investigate the influence of root restriction on the formation and transportation of plant hormones. The results revealed that RR treatment increased abscisic acid, salicylic acid, zeatin riboside, N6-(delta 2-isopentenyl)-adenine nucleoside concentrations, while reduced auxin, 3-indolepropionic acid, 3-indolebutyric acid, gibberellin A$_3$, zeatin, N6-(delta 2-Isopentenyl)-adenine, kinetin, jasmonic acid and methyl jasmonate concentrations. To sum up, our results suggested that RR treatment could initiate stress responses via up-regulating abscisic acid and salicylic acid contents while down-regulating auxin and kinetin contents, resulting in the changes of fruit appearance and improvement of berry quality.

Introduction

Grape (Vitis vinifera L.), an important economic fruit variety widely cultivated around the world, could be produced for both fresh eating and wine making. With the development of berries, several changes of phenotypes take place including fruit size, soluble sugar content, anthocyanin content as well as stresses and diseases resistance$^{1-5}$. Such phenotypic alterations are regulated by endogenous plant hormones including auxin (IAA), cytokinin (CTK), gibberellin (GA), abscisic acid (ABA), ethylene (ETH), jasmonic acid (JA) and salicylic acid (SA)$^{6-18}$. Previous studies focusing on the signaling pathways and anabolism of endogenous hormones have revealed the transport mechanism successfully. For example, it had been found that $AUX/IAA$ and $ARF$ proteins could mediate both vascular bundle development and organogenesis by regulating the formation of IAA$^7$, it had also been demonstrated that the ubiquitin-proteasome pathway was closely related to the IAA signaling$^19$, and the degradation of this kind of protein was extremely vital for the fission of plant cells$^{20}$. In several studies of ABA biosynthesis and signal transduction, it has been widely acknowledged that ABA synthesis is regulated by $FDA$ modifications$^{21,25}$, while carotenoids might also mediate its synthesis in higher plants$^{22}$. In addition, previous studies focused on the CTKs biosynthesis and degradation have revealed that $STM$ could regulate cytokinin accumulation$^{23}$ and $KNOXI$ could also activate cytokinin responses to cell fission$^{24}$. Moreover, the anabolic pathways and transport mechanisms of other plant hormones in recent studies are more distinct$^{26-32}$.

Current studies have proved that the level of endogenous hormones in plants was not only affected by the climate$^{33}$, but also by the cultivation environment and irrigation regime$^{34,43}$. For examples, researchers found the ABA and ethylene signal components were differently accumulated in two distinct climates by analysing the transcriptional expression patterns of carotenoid metabolism in ‘Cabernet
Sauvignon’ (*Vitis vinifera* L.) grapes\(^{35}\), further study also revealed that regulated deficit irrigation (RDI) could significantly improve ABA levels in ‘*Cabernet Sauvignon*’ (*Vitis vinifera* L.) wine grapes while reducing IAA levels\(^{36}\). However, researches on the effect of other cultivation patterns on endogenous hormone contents in grapevine are not clear now.

Root restriction (RR) is a widely used cultivation pattern which restrict the root system in a closed container in order to control the growth of roots, ultimately leading to the accumulation of sugar in berries and anthocyanin in pericarp\(^ {37}\). In southern China, because of the hot and humid climate, the grape berries always attain a very low level of sweetness, but the root restriction cultivation technique breaks this limitation successfully. Previous studies have demonstrated that RR treatment could shorten shoot length\(^ {38}\), increase total soluble solid content\(^ {39}\), regulate source-base balance as well as improve stress resistance response\(^ {40}\). It has been widely acknowledged that RR treatment could influence the formation and transportation of plant hormones, but the variation tendency during different stages in different organs are still poor to understand. A thorough research in exploring the variation tendencies of endogenous hormones including IAA, IBA, IPA, CTKs, GA, ABA, JA, MeJA was performed, and the results will better understand the action mechanism and effect with RR treatment. Therefore, a total of eight physiological parameters of ‘*Muscat Hamburg*’ (*Vitis ‘Muscat of Alexandria’ × Vitis ‘Trollinger’*) grape were measured, thirteen endogenous hormone concentrations during four growth stages (\(\ddag\): Budbreak, \(\ddagger\): Blossom, \(\mathbb{C}\): Veraison, \(\mathbb{D}\): Maturity) in different organs (bud, root, berry, flower, stem, leaf) were analyzed by HPLC, aiming to investigate the impact of RR treatment on the concentrations of endogenous hormones, ultimately exploring the relationship between root restriction and changes in plant endogenous hormone concentrations.

**Results**

**Analysis of different physiological parameters.**

The variation tendencies of physiological parameters with RR treatment and control group during veraison were shown in the Fig. 1, Total eight physiological parameters with RR treatment and the control group were quantified. Berry weights with RR treatment were significantly higher than with the control group in DAF 35 and 44 stages. The berry longitudinal diameters with RR treatment were significantly higher than with the control group in DAF 23 stage as well as the parameter of transverse diameter in DAF 38 stage. Shoot lengths and diameters had the same variation trends, which displayed that with RR treatment would be lower than with the control group in DAF 7, 13, 19, 25 stages. To further investigate other physiological parameters, TSS, photosynthetic rate and stomatal conductance were also detected. Figure 1 well reported that there existed extremely significant differences in TSS between RR treatment and control group in DAF 57, 67, 77, 87, 90, 100, 110 stages. Meanwhile, the photosynthetic rates with the RR treatment were significantly lower than in the control group at 6:00, 8:00, 12:00, 16:00 in a day, while the stomatal conductance with the RR treatment were significantly higher than in the control group at 6:00, 8:00, 10:00, 12:00, 14:00, 16:00 in a day.
Analysis of different endogenous plant hormone contents.

Throughout the growth and developmental period of grapevine, different types of plant hormones will undergo a serious of changes, leading to the huge differences in variation tendencies of their contents. According to the previous study, the concentrations of endogenous plant hormones could be significantly developed with RR treatment. To gained more insight into the variation tendencies of different types of hormones in growth and development of grapevine under RR treatment, 13 different endogenous plant hormones were analyzed in different grapevine organs and tissues during different developmental stages. As the results shown in Fig. 2 (A), the concentrations of ABA with RR treatment were significantly higher than with the control group during the stages of budbreak in root, of blossom in root, stem, and leaf, of veraison in berry, stem, and leaf, of maturity in berry, stem, and leaf. Adversely, the concentrations of IAA were significantly lower under RR treatment than the control group during the stages of budbreak in root and bud, of blossom in flower, leaf, of veraison in root, berry, stem and leaf, of maturity in root, berry and stem. Interestingly, IAA concentrations during the stages of blossom in root were significantly higher with RR treatment compared with in control group as Fig. 2 (D) demonstrated. IBA and IPA are both important precursors in IAA biosynthetic pathways which means their levels could have direct impact on IAA concentration. As shown in the Fig. 2 (F), IBA concentrations roughly had the same variation tendency with IAA levels, its content was signicantly lower with RR treatment than in control group in most organs and tissues during the whole developmental period. In addition, IPA concentrations shown in the Fig. 2 (E) had revealed that its concentrations under the RR treatment during the stages of budbreak in root, of blossom in stem, of maturity in berry and leaf were significantly higher than with the control group, while in the control group during the stages of budbreak in bud, of blossom in root and flower, of veraison in berry and stem, of maturity in stem were significantly higher compared with the RR treatment group. As shown in the Fig. 2 (B), the concentrations of GA3 had the same variation tendency with IAA either. During stages of budbreak in bud, of blossom in stem and leaf, of veraison in root, berry, stem and leaf, of maturity in root, stem, GA3 concentrations were significantly higher in control group than with RR treatment, while during budbreak in root and maturity in berry, GA3 concentrations were significantly higher with RR treatment than in control group. SA has the same function in increasing grapevine stress resistance similarly with ABA. As shown in the Fig. 2 (C), SA concentrations were significantly higher with RR treatment during all growth stages in all organs compared with the control group. Interestingly, the variation tendency of SA concentrations was opposite to the variation tendency of ABA concentrations, indicating these two plant hormones might play a role at different stages of grapevine development. JA also has impact on increasing grapevine resistance to diseases, RR treatment could significantly develop JA concentration compared with control treatment. As shown in the Fig. 2 (G), JA concentrations in the RR treatment group and the control group showed opposite results. During the stages of budbreak in root, of blossom in flower, of veraison in root and of maturity in root and berry, its concentrations with RR treatment were significantly lower than with control treatment, while other developmental periods in different organs showed the inverse results, as the previous studies indicated RR treatment could significantly develop the JA concentrations along with the increasing ability of disease resistance. MeJA as an organic compound, is widely generated in plants and plays important role in the induction of
Chemical defenses and improvement of stress resistance. As shown in the Fig. 2 (H), the change trend of MeJA concentrations showed the partial same variation tendency with JA. MeJA concentrations with the treatment of RR were significantly lower than with the control group in different organs during different developmental stages, except in the stages of budbreak in bud, of veraison in root and of maturity in berry.

Cytokinins (CTKs) play an important role in the growth and development of plants whole lives. In order to further explore the effect of RR treatment on the variation tendencies of this types of plant hormones in grapevine, five types of CTKs including ZT, ZR, iP, iPR and KT were analyzed by HPLC. As shown in the Fig. 3 (A), ZT concentrations with RR treatment were significantly lower than in the control treatment during the stages of budbreak in root, of blossom in flower and leaf, of veraison in berry and leaf, of maturity in berry. Adversely, different results were shown during four developmental stages in root as well as during the maturity in leaf: ZT concentrations with RR treatment were higher than with the control group. ZR is an important precursor in ZT biosynthesis pathway, as shown in the Fig. 3 (B), its concentrations under RR treatment were significantly higher than in the control group during the stages of budbreak in root and bud, of blossom in root, flower and stem, of veraison in berry and stem, of maturity in root, berry and leaf. iP and iPR are also important CTKs playing a vital role in developing plant growing and cells division, as shown in the Fig. 3 (C) and Fig. 3 (D), the concentrations of iP with RR treatment were significantly lower than in the control group during the stages of budbreak in bud, of veraison in berry, stem and leaf, of maturity in root and berry. In different organs at other stages showed different results, displaying its concentrations with RR treatment were significantly higher than in the control group. The concentrations of iPR had similar variation tendency with iP, indicating that iPR would have synergistic effect with iP. During the stages of budbreak in root and bud, of blossom in root, flower, stem and leaf, of veraison in root, berry and leaf, of maturity in leaf, its concentrations were significantly higher with RR treatment than with control treatment. Interestingly, during other stages in different organs the concentrations were significantly decreased under RR treatment. KT, as the first types of cytokinin isolated from maize plan, could significantly improve the growth of cells. Figure 3 (E) revealed that KT concentrations were significantly lower with the RR treatment than the control group during the stages of budbreak in root and bud, of blossom in root, flower, stem and leaf, of veraison in berry and stem, of maturity in root and leaf, while at other stages in different organs showed inverse results.

**Correlation analysis of plant hormones**

Figure 4 displayed the correlation of different plant hormone contents during different developmental stages in different organs, we discovered that KT, IBA and iPR were positively correlated with other plant hormones, and IAA and ABA showed a strong negative correlation with SA. Above results suggested that there existed synergistic and antagonistic effects between plant hormones, which jointly regulated plant growth and development, maturation and senescence. In conclusion, at different stages of grape development and ripening, the contents of various plant hormones were constantly changing in different organs due to different physiological effect. The interaction of various plant hormones mediated the
generation of other nutrients, and eventually led to the change of grape phenotype and the improvement of grape quality.

**Discussion**

Plant hormones play important role in the whole growth and development of different plants. Through many years of exploration, it has been identified five main kinds of endogenous plant hormone including IAA, GA, ETH, CTK, and ABA. Nowadays, more than 20 types of endogenous plant hormones had been extracted from different organs and tissues in plants along with the revelation of their physiological functions and actional mechanism. With the development and progress of scientific technology, hormonal biosynthesis and hormonal transduction pathways have been clarified.

IAA is one of the first plant hormones isolated from living plant tissues, it plays extremely significant role in the elongation of cells and plants as well as the prevention of flowers and berries dropping. In terms of IAA concentration, our results revealed that RR treatment could significantly decreased its concentrations in most stages and organs, indicating that RR treatment could inhibit the growth of grapevine, which led to grapevine phenotypes changed including shorter stem length or smaller berry vertical and horizontal diameter, these finding was consistent with previous studies implying that RR treatment could reduce IAA concentration in grapevine. Thus, we deduced that RR treatment could retard the growth of grapevine by reducing secretion of IAA, resulting in the dwarf of grapevine and the shorten of new tips. ABA is another widely studied plant hormone which has been demonstrated to play important role in accelerating abscission of leaves and berries, dormancy of buds, formation of stress resistance, regulation of sucrose transporter expression, formation of anthocyanin, regulation of stomatal opening. Our results implied that RR treatment could significantly develop its concentrations during most growth stages especially in berries and leaves consistently with the previous studies. We deduced that RR treatment could develop ABA content and correspondingly develop grapevine stress resistance. Earlier studies focused on physiological responses implied GAs could significantly promote elongation of stems and germination of seeds as well as acceleration of blossom. It could be implied in our results that RR treatment could significantly reduce GA concentrations during most growth stages in different organs accordantly with previous studies. The results also confirmed that GA might generate during the veraison stage and indirectly promoted grapevine growth and berry enlargement. We also found GA concentrations were higher in root and leaf than other organs, directly confirming the biosynthesis sites of GA might mainly concentrated in root or leaf. SA, a phenolic derivative, have been confirmed to play important role in plant growth, thermogenesis, flower induction as well as stress and disease resistance. Our results implied that RR treatment could significantly increase SA contents during all stages especially in root and berry, which were consistent with previous studies. We deduced that RR treatment could increase SA concentration to improve grapevine's ability of resisting stresses and diseases. JA, an endogenous plant hormone found in higher plants, could regulat stomatal opening, rubisco biosynthesis, glucose transportation and disease resistance formation. Our results showed that
the variation tendency of MeJA contents in different organs during different developmental stages were partial consistent with JA contents, uncovering that MeJA might have a synergistic effect with JA in growth regulation as well as stress resistance improvement of plants. It had been sufficiently revealed that CTKs could promotes cell division, control cell differentiation and regulated vascular cambium development. Through measuring 5 types of CTKs, our results revealed that during different stages CTKs concentration showed significantly differences. ZT concentrations with RR treatment were significantly lower than the control group in berry and flower while higher in root and leaf. Then we deduced that ZT would be generated or be transported into root and leaf more. ZR concentrations showed completely different results, indicating RR treatment could significantly increase its contents. Based on the results, we deduced that ZR and ZT had antagonistic effects and played different roles in regulating grape growth and development. Meanwhile, iP concentrations with RR treatment were significantly higher than with the control treatment during the stages of blossom in all organs and of maturity in stem and leaf. We deduced that RR treatment could regulate iP transporting direction and lead to variation of distribution location as well as different content. Interestingly, iPR concentrations showed the same variation tendency as iP concentrations, it could be inferred that iP played synergistic role in regulating grapevine growth with iPR. Our results also revealed that KT concentrations under RR treatment were significantly decreased especially during blossom and maturity in leaf, stem and root. Therefore, we deduced that RR treatment could shorten new-tip length and slow grapevine growth by reducing KT concentration.

Root restriction, as a used cultivation technique in agriculture, could significantly influence endogenous plant hormones levels during the whole cultivation period. The present results showed that a closely relationship between the hormones was existed, we deduced that under the root restriction treatment, the contents of hormones that promoted plant growth including IAA, GA₃, CTKs, JA as well as MeJA showed the mostly consistent trends in the whole developmental stages in different organs revealing that they might have synergistic effect in promoting plant elongation and berry ripening. On the contrary, the concentrations of SA and ABA were significantly enhanced with the RR treatment along with the improvement of stress resistance. Therefore, we deduced that plant hormones such as SA and ABA that enhanced plant stress resistance would respond to root restriction, resulting in the increase of their contents. Each hormone was bound to pass through a series of signal transduction and crosstalk affecting the expression of genes on their synthetic pathway, finally resulting in differences in hormone contents. Our studies aimed to investigate the variation tendency of endogenous plant hormones during four stages (budbreak, blossom, veraison, maturity) in different organs (bud, root, berry, flower, stem, leaf) between RR treatment and the control treatment. Finally, based on the obtained results, we concluded that RR treatment could indeed extremely influence the concentration of several plant hormones.

In conclusion, root restriction could significantly influence the contents of endogenous plant hormones. The results provided data support for rapid detection and quantitative analysis of endogenous plant hormones in grape under root restriction cultivation. While, further researches on mechanisms of plant
hormone interaction and regulation of key genes and transcription factors in plant hormone biosynthesis pathway with root restriction treatment need to be implemented.

Materials And Methods

Materials Collected

This experiment was established during the year of 2019 to 2020 in the experimental greenhouse located in Shanghai Jiao Tong University, Shanghai, China (31°11′N, 121°29′W), using five years old ‘Muscat Hamburg’ (Vitis ‘Muscat of Alexandria’ × Vitis ‘Trollinger’). The grapevines applying with RR treatment were planted in a spacing of 1.5 m × 2.0 m in north–south oriented rows in a 40 cm deep cultivation soil, while the grapevines cultivated in the control group were only placed in 40 cm deep cultivation soil, the comparison of the two cultivation patterns was shown in Fig. 5. Both two groups were applied to the same water and fertilizer condition. This experiment respectively collected buds, roots, flowers, berries, stems and leaves from seven grapevines in four different developmental stages including the budbreak stage (Ⅰ), the blossom stage (Ⅱ), the veraison stage (Ⅲ) and the maturity stage (Ⅳ). According to the collecting stages, twenty-eight grape clusters without visible pests and diseases were randomly picked from seven ‘Muscat Hamburg’ grapevines and immediately transported back to laboratory within 2 hours. Flowers of blossom stage (Ⅱ), fruits of veraison (Ⅲ) and maturity stages (Ⅳ), buds of budbreak stage (Ⅰ) as well as stems and leaves of blossom (Ⅱ), veraison (Ⅲ) and maturity (Ⅳ) stages without damage were selected to be prepared for physiological parameters measurements, and then immediately grinded by nitrogen and frozen in -80 °C refrigerator for further studies. The specific technical route was shown in Fig. 6.

Physiological Parameters Measurement

Different physiological parameters, such as berry weight, longitudinal and transverse diameter, shoot length and diameter, total soluble solid (TSS), photosynthetic rate and stomatal conductance were measured during the veraison of ‘Muscat Hamburg’ (Vitis ‘Muscat of Alexandria’ × Vitis ‘Trollinger’). Twenty shoots were randomly picked from each RR treatment group and the control group on fourteen grapevines with the measurement of shoot lengths and diameters using tapeline in the specific sampling times (shown as DAF). In the same time, twenty berries without damage from each group on fourteen grapevines also were selected to prepare for the next experiments. Approximately 50 grape berries without pedicels and seeds were squeezed into grape juice for further researches. Berry weights were measured by analytical balance (Sartorius, German), longitudinal and transverse diameters were measured by vernier caliper (Mitutoyo, TKY, Japan), total soluble solid (TSS) was measured by refractometer (OWELL, Hangchow, CHN), net photosynthetic rate and stomatal conductance were quantified by CIRAS-3 portable photosynthetic fluorescence measurement system (Hanshatech, CA, USA) on August 15, 2019. All tests were implemented for more than three technical duplicates.

IAA, ABA, SA, IPA, IBA, GA₃, JA and MeJA Quantification
For the quantification of IAA, ABA, SA, IPA, IBA, GA₃, JA and MeJA, the experiment was performed according to the previous methods with a little adjustment[2]: Firstly, 0.1 g of sample was dissolved in the 1 ml Mod-Bielesk solution (Methanol: Formic acid: ddH₂O = 15:1:4), then placed in -20 °C for a night, next mixed liquor was centrifuged using high speed centrifuge (CENCE, CHN) under the condition (4 °C, 13000 r/min) for 20 min, and then supernatant was collected and extracted by 0.5 ml Mod-Bielesk, next shocked about 5 min for 2 times and centrifuged again (4 °C, 13000 r/min) to collect liquid supernatant. Then solution should be extracted by CNWBOND HC-C18 SPE Cartridge (CNW, German) and filter liquor was evaporated by rotary stem evaporator (RE, SHH, CHN) at 40 °C, then promptly redissolved by 5ml of 1 M formic acid. Next, solution should be extracted by Poly-Sery MCX SPE Cartridge (CNW, German) and then 5 ml of 1 M formic acid was added to wash the column (W1), finally eluted by 5 ml methanol (W2) and redissolved in 0.5 ml extraction solution (Methanol: Isopropanol: Acetic Acid = 20:79:1) after eluant was condensed by rotary stem evaporator at 40 °C (RE, SHH, CHN). Etractions containing IAA, ABA, SA, IPA, IBA, GA₃, JA and MeJA were prepared for analyzation by HPLC. Standard substances of IAA, ABA, SA, IPA, IBA, GA₃, JA and MeJA (Anpel, SHH, CHN) dissolved in the same extraction solution (Methanol: Isopropanol: Acetic Acid = 20:79:1) with different concentrations were also prepared for analyzation by HPLC.

Chromatographic separation was performed in the LC3000 Semi-preparation Isocratic HPLC System (CXTH, BJ, CHN) by a Capecell PAK C18 column (4.6 mm × 50 mm, 1.8 µm) with mobile phase A (0.01% formic acid-methanol) and mobile phase B (0.01% formic acid-water), with an injection volume of 0.5 µl and the flow rate of 0.35 ml/min (IAA, IPA, IBA and GA₃), 0.8 ml/min (ABA), 1.5 ml/min (SA, JA and MeJA). The gradient program was as follows: 0–4 min, 20% A; 4–8min, 20% A -50% A; 8–20 min, 50% A-80% A; 20–22 min, 80% A; 22–22.2 min, 80% A-20% A; UV wavelength was set to 254 nm and temperature of chromatographic column was adjusted to 40 °C. All reagents (CNW, German) used for HPLC were chromatographic grade, being filtered through 0.22 µm membrane and ultrasonic treated (200Hz, 30min) for more than twice.

**ZT, ZR, iP, iPR and KT Quantification**

After the Poly-Sery MCX SPE Cartridge (CNW, German) had been eluted by methanol, the column should be washed again by volume of 5 ml 0.35 M ammonia (E1) and then eluted by the volume of 5 ml 0.35 M ammonia in 60% methanol (E2), finally filter liquor should be condensed by rotary stem evaporator (RE, SHH, CHN) at 40 °C and then redissolved with 0.5 ml of 5% acetonitrile. Etractions containing ZT, ZR, iP, iPR and KT were prepared for analyzing by HPLC. Standard substances of ZT, ZR, iP, iPR and KT (Anpel, SHH, CHN) dissolved in the 5% acetonitrile with different concentrations were also ready for quantification in HPLC.

Chromatographic separation was performed in the LC3000 Semi-preparation Isocratic HPLC System (CXTH, BJ, CHN) by a Capecell PAK C18 column (4.6 mm × 50 mm, 1.8 µm) with mobile phase A (0.06% acetic acid water) and mobile phase B (0.06% acetic acid-methanol), then an injection volume of 0.5 µl and the flow rate of 0.5 ml/min should be set in HPLC program. The gradient program was set as follows:
0–8 min, 99% A-55% A; 8–14 min, 55% A-30% A; 14–16 min, 30% A-1% A; 16–24 min, 1% A-99% A; UV wavelength was 254 nm and the temperature of chromatographic column was 40 °C. All reagents (CNW, German) used for HPLC were chromatographic grade, being filtered through 0.22 µm membrane and ultrasonic treated (200Hz, 30min) for more than twice. The information of each hormone eluted by HPLC was shown in the Table 1.

Statistical Analysis.

Results were analyzed using at least three independent replicates including the mean ± standard error (SE) by data processing system SPSS 16.0 statistical software package (IBM, Armonk, NY, USA). The method of Duncan's multiple range test (ANOVA) was used to analyze the differences of data significance at the level of P < 0.05. Figures were drawn by GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA) and Visio 2020 (Microsoft, SEA, USA).
| Compounds                  | Retention time (min) | Peak area | Molecular formula | Calibration Curves |
|---------------------------|----------------------|-----------|-------------------|-------------------|
| Abscisic acid             | 12.286               | 7573628   | C_{15}H_{20}O_{4} | Y = 120550X-6162.4 |
| Auxin                     | 20.46                | 13852722  | C_{10}H_{9}NO_{2} | Y = 35324X + 109746 |
| Gibberellin A3            | 17.781               | 2309297   | C_{19}H_{22}O_{6} | Y = 29314X + 38787 |
| Salicylic acid            | 10.033               | 175455    | C_{7}H_{6}O_{3}   |                   |
| 3-Indolepropionic acid    | 22.059               | 54120     | C_{11}H_{10}NO_{2} | Y = 36831X-18773  |
| 3-Indolebutyric acid      | 23.623               | 88712     | C_{12}H_{13}NO_{2} | Y = 36604X + 59323 |
| Jasmonic acid             | 19.245               | 1418457   | C_{12}H_{18}O_{3} | Y = 17424X-265.8  |
| Methyl jasmonate          | 24.089               | 1602      | C_{13}H_{20}O_{3} | Y = 23484X-5404.8 |
| Zeatin                    | 17.248               | 89642     | C_{10}H_{13}NO_{5} | Y = 113736X + 16113 |
| Zeatin riboside           | 19.688               | 116127    | C_{15}H_{21}N_{5}O_{5} | Y = 78039X + 35944 |
| N6-(Delta 2-Isopentenyl)-adenine | 22.539           | 6058588   | C_{10}H_{13}N_{5}   | Y = 16766X + 2689.6 |
| N6-(Delta 2-Isopentenyl)-adenine nucleoside | 14.268             | 89391     | C_{15}H_{21}N_{5}O_{4} | Y = 1440.7X + 10175 |
| Kinetin                   | 17.824               | 72057     | C_{10}H_{9}N_{5}O   | Y = 22046X + 11778 |

**Declarations**

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Author information

J.J.L. performed experiments including HPLC detection, data analyzation, figures drawing and manuscript writing. D.M.L. collected the samples and performed the physiological parameters measurements. B.Y.L. provided the experimental methods and ideas. R.Q.W. collected and grinded the samples. Y.X.Y., G.H.L., C.M., W.P.X., L.W. and L.P.Z. reviewed the manuscript. S.P.W. and X.Y.L designed the experiments, reviewed the manuscript and supervised this study.

Ethics declarations

The authors declare no competing interests. Experimental and field studies of plants (cultivated or wild), including the collection of plant material, have been conducted in compliance with relevant institutional, national and international guidelines and legislation.

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**Figures**
**Figure 1**

The variation tendencies of physiological parameters with RR treatment and control in veraison. (A) Berry weight, (B) transverse diameter, (C) longitudinal diameter, (D) shoot length, (E) shoot diameter, (F) total soluble solid (TSS), (G) photosynthetic rate and (H) stomatal conductance. Data from the control group were presented as hollow black circles and data from the RR treatment were presented as solid red circles.

**Figure 2**

The variation tendencies of endogenous plant hormone contents (ABA, GA3, SA, IAA, IPA, IBA, JA, MeJA) during four developmental stages (I: Budbreak; II: Blossom; III: Veraison; IV: Maturity) in six different organs (bud, root, flower, berry, stem and leaf) with RR treatment (black) and the control (grey). * shows the significant differences with Duncan-test (p < 0.05, n=3).
Figure 3

The variation tendencies of different endogenous plant hormone contents (ZT, ZR, iP, iPR, KT) during four developmental stages (Ⅰ: Budbreak; Ⅱ: Blossom; Ⅲ: Veraison; Ⅳ: Maturity) in six different organs (bud, root, flower, berry, stem and leaf) with RR treatment (black) and control (grey). * shows the significant differences with Duncan-test (p < 0.05, n=3).
Figure 4

Heatmap of plant hormone concentrations in ‘Muscat Hamburg’ grape during developmental stages. A good correlation between hormones is represented by red while a poor correlation between hormones is represented by blue.
Figure 5

Root restriction cultivation and open cultivation patterns. The open cultivation mode was shown in the right, while the root restriction cultivation mode was shown in the left.
Figure 6

Technical route of this experiment.