果 树 学 报 2021，38（9）：1423-1434
Journal of Fruit Science

DOI:10.13925/j.cnki.gsxb.20200553

金星无核与其短节间突变体紫金早生的节间长度差异机制初探

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摘 要：【目的】探索金星无核短节间突变体紫金早生节间长度短缩的形成机制。**【方法】**以金星无核（JXWH）及其短节间突变体紫金早生（ZJZS）为研究材料，借助扫描电镜、LC-MS/MS和高通量测序分析两种材料在细胞形态、内源激素含量和miRNA上的差异。调查外源激素处理（JA及其合成抑制剂DIECA）下突变体节间长度的变化。**【结果】**紫金早生节间长度和节间细胞数量显著低于金星无核；紫金早生中JA、JA-ILE和SA含量显著提高，而ICA和ICAld含量显著降低；小RNA测序结果发现2种材料中共有21个差异miRNA，GO注释分析发现它们主要参与细胞分裂、茉莉酸代谢、蛋白代谢、环境应答和器官发育等过程。外源DIECA处理后，紫金早生节间伸长，外源JA处理抑制了紫金早生节间长度的伸长。**【结论】**紫金早生短节间突变性状与茉莉酸信号通路的调节有关，茉莉酸可能受miR319的调控。

关键词：葡萄；突变体；节间长度；miRNA；茉莉酸

中图分类号：S663.1 **文献标志码：**A **文章编号：**1009-9980(2021)09-1423-12

Preliminary analysis on the mechanism leading to difference in internode length between Venus Seedless grapevine and its shorter internode mutant Zijinzaosheng

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Abstract:【Objective】Grape industry is labor-intensive, especially in the management of new shoots. In practice, grape with shorter internode length has such unique advantages as labor saving and simplification in canopy management. Although the dwarfing mechanisms in many fruit trees have been studied, little has been studied in grapevine. The mechanism of short internode in grapevine remains unclear. Mutant is ideal materials for study the genetic mechanism of traits. However, short internode mutants of grapevine are scarce. A grape mutant named Zijinzaosheng with short internode was discovered from the progeny of Venus Seedless grapevines after treatment with colchicine. A previous study showed that the chromosome ploidy of Zijinzaosheng was the same as that of Venus Seedless. The DNA sequences had little difference between Zijinzaosheng and Venus Seedless, and the genetic background of the two materials is highly consistent. The short internode trait of Zijinzaosheng was stable in multi-plot trials for several years. The fruit of Zijinzaosheng was not sensitive to exogenous GA_3. We speculated that the mutation mechanism of Zijinzaosheng was different from that of Point Meunier. In order to elucidate the mechanism and regulation of grapevine internode, comparative analyses on internode length, cell morphology of stem, endogenous hormone contents and small RNA sequencing were conducted in the study. Meanwhile, the influence of exogenous hormones on the internode length in Zijinzaosheng was

收稿日期：2020-12-24 **接受日期：**2021-05-20

基金项目：江苏省农业科技自主创新资金[CX（20）2022]；财政部和农业农村部：国家现代农业产业技术体系资助

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also evaluated.【Methods】7-year-old trees of Venus Seedless and Zijinzaosheng were used as the experimental materials. They were planted in Lishui plant science station of Jiangsu Academy of Agricultural Sciences, a core demonstration vineyard of Nanjing Experimental Station of China Agriculture Research System for Grape Industry. All the plant materials were maintained under rain-shelter. The study was conducted in 2019, and shoot tips were collected for high throughput sequencing; the young leaves at the shoot tip were sampled to determine the endogenous hormones. Cell morphology were observed under a scanning electron microscopy, and endogenous hormone contents analyzed with an LC-MS/MS. High throughput sequencing was used to analyze the different miRNA. The study of exogenous hormone treatments was conducted in 2020. Shoot tips and shoot internodes were treated with 0.25 $mmol \cdot L^{-1}$ GA_3, 0.25 $mmol \cdot L^{-1}$ Jasmonic acid or 0.5 $mmol \cdot L^{-1}$ DIECA (biosynthesis inhibitor of Jasmonic acid), respectively. The treatment with ddH_2O was used as control. The internode length was measured after exogenous hormone treatments.【Results】The results showed that the internode length of one-year hard shoots of Zijinzaosheng was lower (47%) than that of Venus Seedless. The cell number in the stem of Zijinzaosheng was smaller than that of Venus Seedless. There was no significant difference in cell size between the two cultivars. Jasmonic acid (JA), jasmonoyl-L-Isoleucine (JA-ILE) and salicylic acid (SA) in Zijinzaoheng were significantly higher, while indole-3-carboxylic acid (ICA) and indole-3-carboxaldehyde (ICAld) were significantly lower. The contents of gibberellins (GA_{15}, GA_{19} and GA_3), cytokinins (IP, cZ and tZ), auxin (IAA, ME-IAA) and ABA were not significant different between the two cultivars. Some gibberellins (GA_1, GA_4, GA_7, GA_9, GA_{20}, GA_{24} and GA_{53}), auxin (IBA), jasmonates (H2JA, MEJA) and cytokinin (DZ) were not detected in either of the two cultivars. Small RNA sequencing results showed that 21 differentially expressed miRNA genes were detected, including 7 known miRNAs (miR156f, miR535a, miR535b, miR535c, miR3635-5p, miR319b and miR319f) and 14 novel miRNAs (miR48, miR28, miR33, miR68, miR55, miR81, miR70, miR78, miR165, miR162, miR152, miR58, miR108 and miR45). 556 target genes were predicted. GO annotation analysis revealed that these genes were involved in cell division, protein metabolism, environmental responses and organ development, etc. Two internode growth regulation miRNAs, miR319b and miR319f, were selected. The candidate target gene included TCP, which is involved in jasmonic acid metabolism and mitotic G2 phase. Compared with the control, the internode length of Zijinzaosheng was decreased by exogenous jasmonic acid while increased by DIECA. The difference in the internode length between the control and GA_3 treatment was not significant.【Conclusion】The internode length of Zijinzaosheng was sensitive to exogenous jasmonic acid, and can be increased by exogenous DIECA treatment. The study suggests that jasmonic acid signaling pathway participates in the formation of short internode length in Zijinzaosheng. MiR319b/f may play a role in the grapevine internode length development. The study provides a new direction for further elucidation of the regulatory mechanism in grapevine internode growth and supplies material resource for breeding varieties with short internodes.

Key words: Grapevine; Mutant; Internode length; miRNA; Jasmonic acid

葡萄是一种藤本蔓生果树，萌芽力强、生长量大。夏季新梢修剪需要投入大量劳动力。生产者发现短节间葡萄品种整形修剪频率低，具有省工轻简化的栽培优势，然而人们对葡萄节间长度的形成机制却知之甚少。

节间长度是一个重要的农艺性状，主要由细胞数量和大小所决定[1]。研究发现许多基因家族和转录因子通过植物激素作用于细胞分裂和细胞分化，进而调节植物的节间长度[2-4]。目前，已证实参与调控节间长度的激素包括赤霉素、油菜素内脂、生长素、独脚金内脂和茉莉酸等[5]。可见，植物激素是调控植物株型的重要媒介。

microRNA是一类长度为18~24 nt的非编码低分子RNA[6]，在生物体内主要起转录后水平调控的作用。miRNA作为上层调控因子，标靶了许多植物激素信号分子[7]，通过影响激素在植物体内的代谢、分布和感应，成为影响植物激素应答的关键调控因子[8]。研究者首次在拟南芥*hyl1*基因敲除突变体中发现了miRNA与激素的关联[9]。miRNA-靶基因模块能直接影响合成激素的结构基因，还能调控激素信号转导因子间接影响激素的合成。例如，OsmiR159d靶向*OsGAMYBL2*调节*BU1*（BR信号转导的基因），OsmiR159d-*OsGAMYBL2*模块也能参与GA生物合成中2个基因*CPS1*和*GA3ox2*的表达[10]。miRNA通过植物激素调控植物生长发育与器官的形态建成。miR390-*TAS3*模块通过*ARF*的作用调节植物体内的生长素水平，参与植物叶、花和果实等多个器官的发育[11]。上述研究表明，miRNA是研究激素调控植物生长发育机制的有效切入点。

曾有报道酿酒葡萄黑比诺DELLA蛋白-VvGAI1基因发生单碱基突变造成葡萄节间缩短、植株矮化[12]。此后尚未见有关葡萄节间长度突变体的报道。吴伟民等[13]通过化学诱变的方法获得了金星无核葡萄的短节间突变体紫金早生，为葡萄节间长度性状研究提供了有价值的材料。前期研究证实金星无核与紫金早生遗传背景一致[14]，经过多年多点种植，其短节间突变性状稳定。为此，笔者在本研究中以金星无核与其短节间突变体紫金早生为研究对象，分析两者节间细胞形态、内源激素含量和小RNA的差异，将差异内源激素和差异小RNA进行联合分析，从表观遗传学角度发掘紫金早生短节间性状形成的分子机制，为开展葡萄节间长度发育机制研究提供有价值的参考。

1　材料和方法

1.1　植物材料及处理

以江苏省农业科学院溧水植物科学基地内种植的金星无核和紫金早生为试验材料，于2019年取当年生新梢，选取顶梢部位（去除叶片）用于miRNA测序，顶梢周围未展开叶片组织用于植物激素检测[15]。将采集样品立即置于液氮中，带回实验室置于-80℃超低温冰箱保存备用。同时采集新鲜茎段，将其立即置于FAA固定液中，带回实验室用于组织学分析。

外源激素处理：外源处理试验于2020年春季进行。以4年生紫金早生为试验材料，分别以赤霉素（GA_3，0.25 mmol·L^{-1}）、茉莉素（JA，0.25 mmol·L^{-1}）和二乙基二硫代氨基甲酸钠（DIECA，0.5 mmol·L^{-1}）喷施紫金早生的梢尖和茎，以梢尖滴水为度，以ddH_2O处理为对照。JA试验浓度的设定参照张培安等[16]的研究，赤霉素浓度设定参照王壮伟等[17]的研究。处理15 d后对紫金早生节间长度进行统计。每个处理选取3个植株，每株葡萄选定2个枝条用于节间长度测量。

1.2　方法

1.2.1　*茎段组织形态学与长度测量*　将新鲜茎段组织在固定液中固定2 h后，使用0.1 mol·L^{-1}磷酸缓冲液PB（pH=7.4）漂洗3次。然后使用0.1 mol·L^{-1}磷酸缓冲液PB（pH=7.4）配制的1%（φ）锇酸室温避光固定1~2 h。利用不同体积分数乙醇（30%-50%-70%-80%-90%-95%-100%-100%）进行梯度脱水，将样本置于干燥仪内进行干燥。干燥后，将样本紧贴于导电碳膜双面胶上放入离子溅射仪样品台上进行喷金30 s左右。利用扫描电镜（HITACHI，SU8100）进行观察采图。利用Pannoramic Viewer软件对采图进行分析，在40×条件下，选取相同单位面积视野，统计细胞数量，同时利用该软件的测量功能，测定细胞宽度和长度。每个指标随机选取3个视野进行分析。

节间长度由当年生新梢的所有节间长度之和除以节间数计算得到。

1.2.2　*内源植物激素测定*　样品中内源植物激素委托迈维代谢生物公司测定。具体流程如下：用研磨机（MM400，Retsch）将样品研磨至粉末状，称取50 mg样本，加适量内标，利用提取液（甲醇、水和甲酸混合液）进行提取，提取液经过浓缩、复溶和过滤后进行LC-MS/MS分析。采用MetWare（http://www.metware.cn/）的AB Sciex QTRAP 6500 LC-MS/MS检测平台对赤霉素（GA_1、GA_3、GA_4、GA_7、GA_9、GA_{15}、GA_{19}、GA_{20}、GA_{24}和GA_{53}）、茉莉素（MEJA，JA，H2JA和JA-ILE）、细胞分裂素（IP、tZ、cZ和DZ）、生长素（IAA、IBA、ME-IAA、ICAld和ICA）、水杨酸和脱落酸进行定性和定量分析。

1.2.3　*小RNA测序分析*　采用试剂盒提取RNA后，检测RNA浓度和纯度，连接5’和3’接头后进行反转录，经PCR扩增后构建cDNA文库。小RNA测序委托百迈克（北京）生物科技有限公司完成。利用

高通量Illumina HiSeq平台，获取原始序列经质控处理后进而获得长度为18~30 nt的Clean Reads，用于下游生物信息学分析。

1.2.4 miRNA鉴定与差异分析 将比对到参考基因组的reads与miRBase（v22）数据库中的已知miRNA的成熟序列及其上游2 nt与下游5 nt的范围进行比对，最多允许1个错配，这样鉴定到的reads被认为是鉴定到的已知miRNA。对于未鉴定到已知miRNA的序列，利用miRDeep2[18]软件进行新miRNA的预测。在差异表达miRNA选取中，使用$|\log_2(FC)|\geqslant 0.58$，FDR≤0.05作为筛选标准。

1.2.5 miRNA靶基因预测与功能分析 根据已知miRNA和新预测的miRNA与对应物种的基因序列信息，用TargetFinder软件[19]进行靶基因预测，参照葡萄参考基因组和NCBI对靶基因进行注释，通过GOseq R包对差异表达基因进行GO（Gene Ontology）功能分析。

1.2.6 数据分析 使用SPSS 20.0进行分析，多个处理采用Duncan's test，2个处理采用独立样本*T*检验。

2 结果与分析

2.1 节间长度表现

紫金早生是金星无核的短节间突变体。调查表明紫金早生1年生硬枝节间长度约为金星无核的47%，显著低于金星无核（图1）。新梢茎段组织形态学观察结果显示，紫金早生茎段内细胞数量明显少于金星无核（图2-A，D），2种材料在细胞长度和宽度上无明显差异（图2-B，C）。

2.2 内源植物激素含量

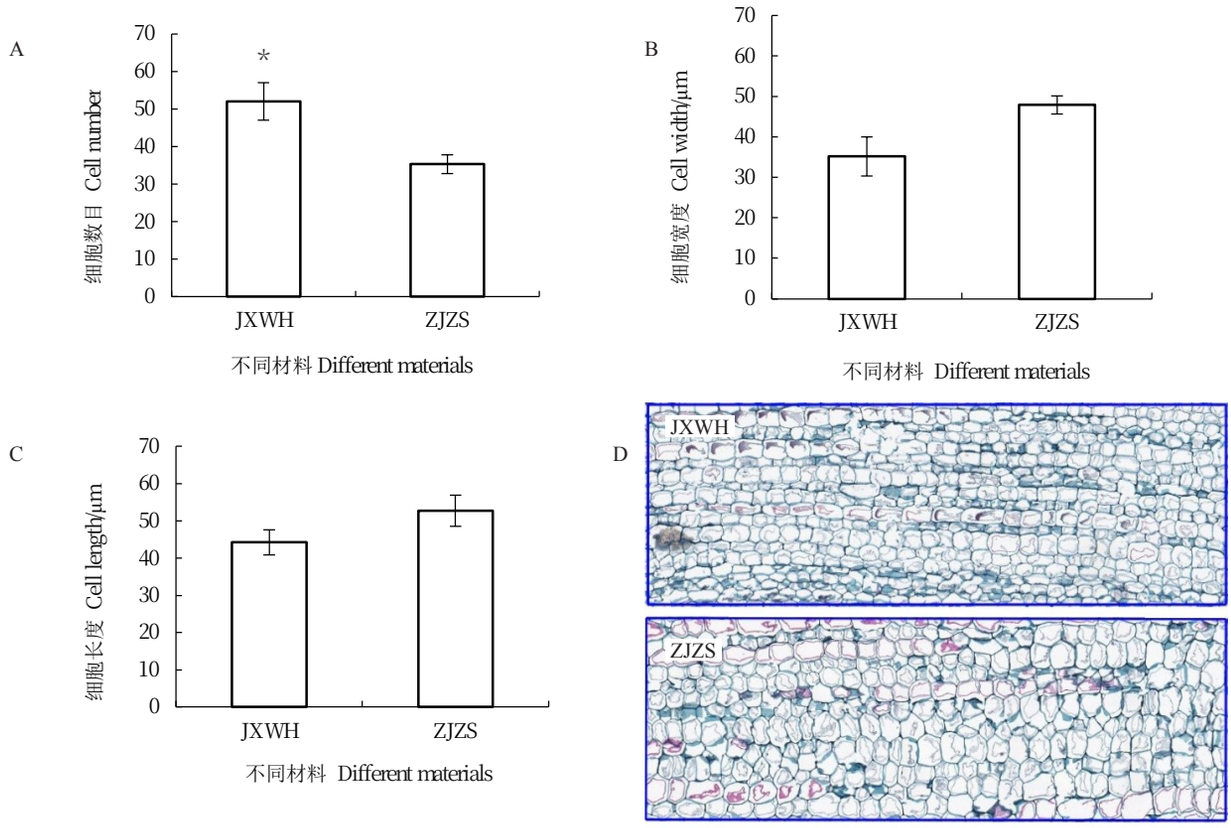
激素含量测定结果（图3）表明，紫金早生顶梢周围未展开幼叶中JA、JA-ILE和SA含量显著高于金星无核，而ICA和ICAld含量显著低于金星无核。2种材料中的赤霉素（GA_{15}、GA_{19}、GA_3）、细胞分裂素（IP、cZ、tZ）、生长素（IAA、ME-IAA）和ABA含量均无明显差异。由于含量低于检测线，赤霉素（GA_1、GA_4、GA_7、GA_9、GA_{20}、GA_{24}和GA_{53}）、生长素（IBA）、茉莉素（H2JA、MEJA）和细胞分裂素（DZ）在

A. 1年生硬枝节间长度；B. 田间生长状况；C. 1年生硬枝。*表示在 $p<0.05$ 差异显著。下同。

A. Internode length of one year hard branch; B. Phenotypes of two grapevines in the field; C: One year hard branch. * indicate significant difference at $p<0.05$. The same below.

图1 紫金早生（ZJZS）和金星无核（JXWH）的生长特性

Fig. 1 Growth characteristic of Zijinzaosheng and Venus Seedless



A. 细胞数目（40×）；B. 细胞宽度；C. 细胞长度；D. 扫描电镜形态（5×）。

A. Cell numbers (40×); B. Cell width; C. Cell length; D. Scanning electron microscope (5×).

图2　紫金早生（ZJZS）和金星无核（JXWH）茎段细胞形态（纵切）

Fig. 2　Cell morphology of Zijinzaosheng and Venus Seedless stem

2种材料中均未检测到。

2.3　外源激素处理结果

以植物内源激素含量的测定结果为依据，对紫金早生进行外源激素处理。由图4可知，与对照（CK）相比，外源GA_3处理后，紫金早生的节间长度未发生明显改变。外源JA处理后，紫金早生的节间长度为2.74 cm，明显低于对照（3.17 cm），而外源DIECA处理后，紫金早生的节间长度伸长至3.62 cm，显著高于对照。

2.4　小RNA测序结果及差异miRNA分析

Illumina测序获得原始序列经质量控制处理后，Q30比例超过96%，符合后续数据分析要求（表1）。金星无核与紫金早生中分别鉴定到已知miRNA数量为101和98个，鉴定到新miRNA数量为177和178个（图5-A），2种材料中鉴定到的miRNA数量差异不显著。金星无核与紫金早生中差异表达miRNA数量较少，上调表达miRNA为8个，下调表达miRNA为13个（图5-B）。

对筛选出的差异表达miRNA进行层次聚类分析，如图6所示，21个差异miRNA聚成3大类。与金星无核相比，novel_miR48、novel_miR28、novel_miR33、novel_miR152、novel_miR58、novel_miR108、novel_miR145和vvi-miR3635-5p上调表达，其余miRNA下调表达。GO注释（表2）发现，Group Ⅰ类靶基因功能主要集中在酯类代谢作用，生物过程多与逆境响应有关。Group Ⅱ类靶基因功能包括能量代谢和蛋白代谢等方面（表3）。Group Ⅲ类靶基因分子功能与谷氨酸代谢相关，细胞组分涉及茉莉酸代谢途径等；参与的生物过程包括器官发育与形态建成、有丝分裂等（表4）。

通过分析筛选，逆境响应相关miRNA为miR156f，其靶基因为泛素羟基末端水解酶（VIT_00s0199g00030、VIT_00s0407g00030）。筛选出参与调控茉莉酸miRNA为miR319b和miR319f，其靶基因有TCP转录因子（VIT_10s0003g00870）、转录因子TCP2-like（VIT_10s0003g03910）。筛选出可能参

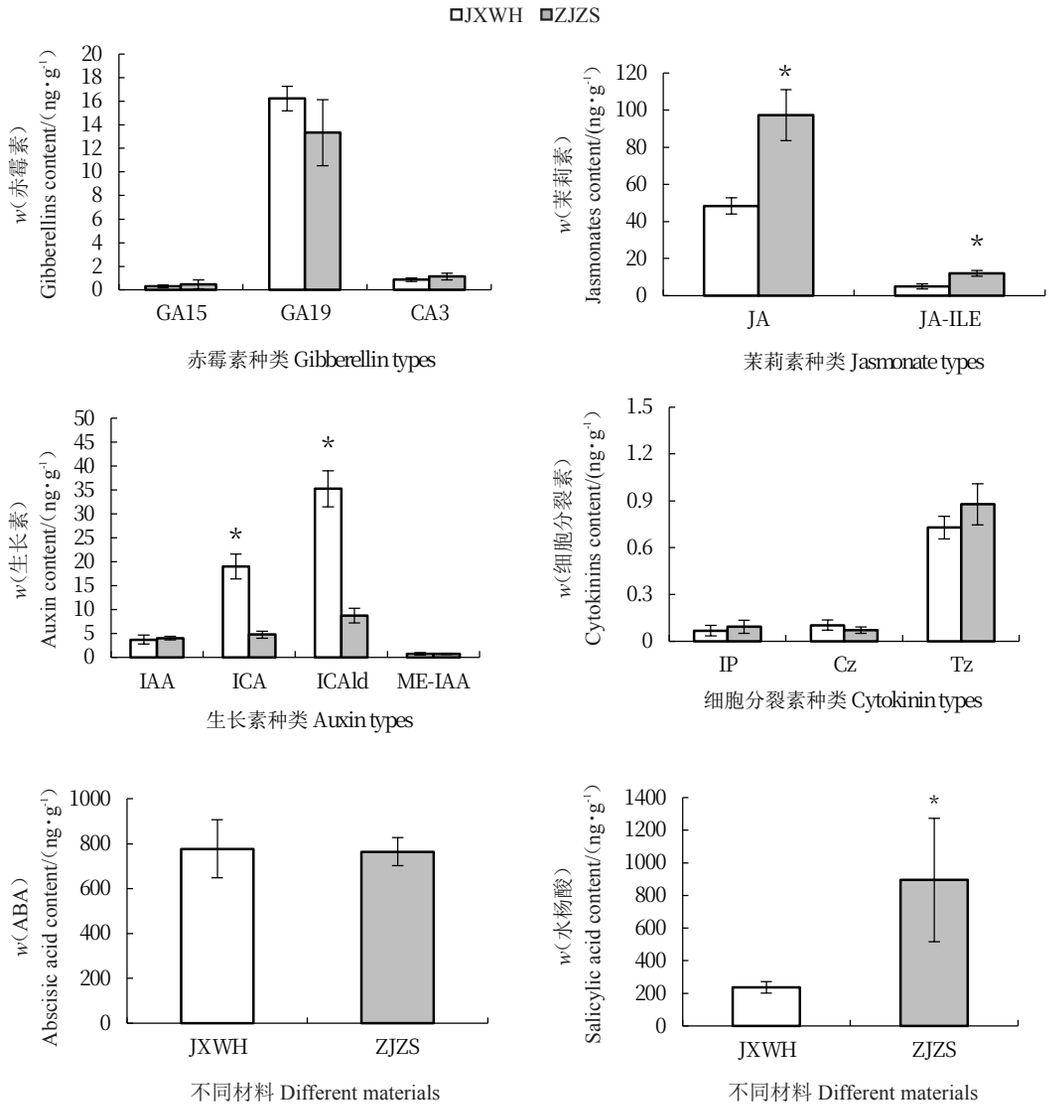
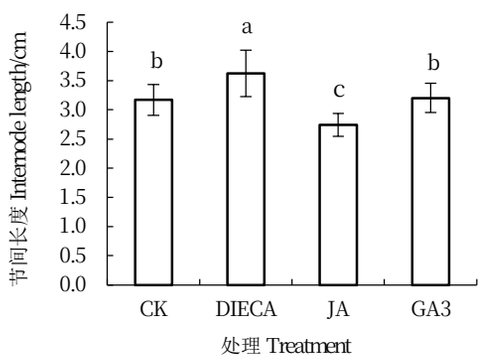


图 3 紫金早生(ZJZS)和金星无核(JXWH)内源植物激素含量比较

Fig. 3 Comparisons of endogenus hormones content between Zijinzaosheng and Venus Seedless



不同小写字母表示在 $p < 0.05$ 差异显著。

Different small letters indicate significant difference at $p < 0.05$.

图 4 外源激素对紫金早生(ZJZS)节间长度的影响

Fig. 4 Effect of exogenous hormone treatments on Zijinzaosheng internode growth

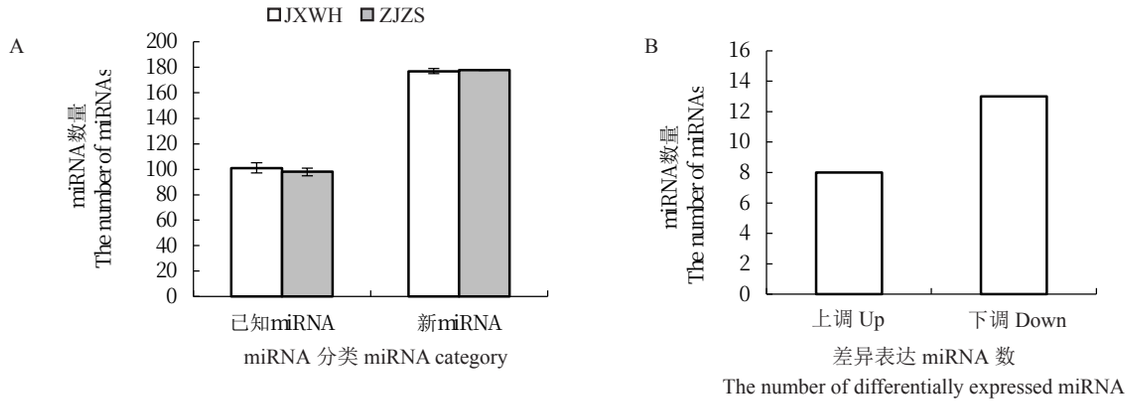
表 1 数据产出质量控制

Table 1 List of data quality control

样本 Sample	测序原始数据 Raw reads	低质量序列 Low quality reads	纯净序列 Clean reads	Q30 百分比 Q30 percentage/%
JXWH1	24 750 775	0	0	96.40
JXWH2	25 650 868	0	0	96.23
JXWH3	23 274 241	0	0	96.25
ZJZS1	27 556 381	0	0	96.40
ZJZS2	24 325 484	0	0	96.44
ZJZS3	26 811 348	0	0	96.41

注:低质量序列 . 质量值低于 30 的碱基所占比例超过 20%的 reads;纯净序列 . 质量值大于或等于 30 的碱基的 Reads 数;Q30 百分比 . 质量值大于 30 的比例。

Note: Low quality reads. The reads ratio of quality score < 30 was more than 20%; Clean reads. The reads numbers of quality score ≥ 30; Q30. The ratio of quality score > 30.

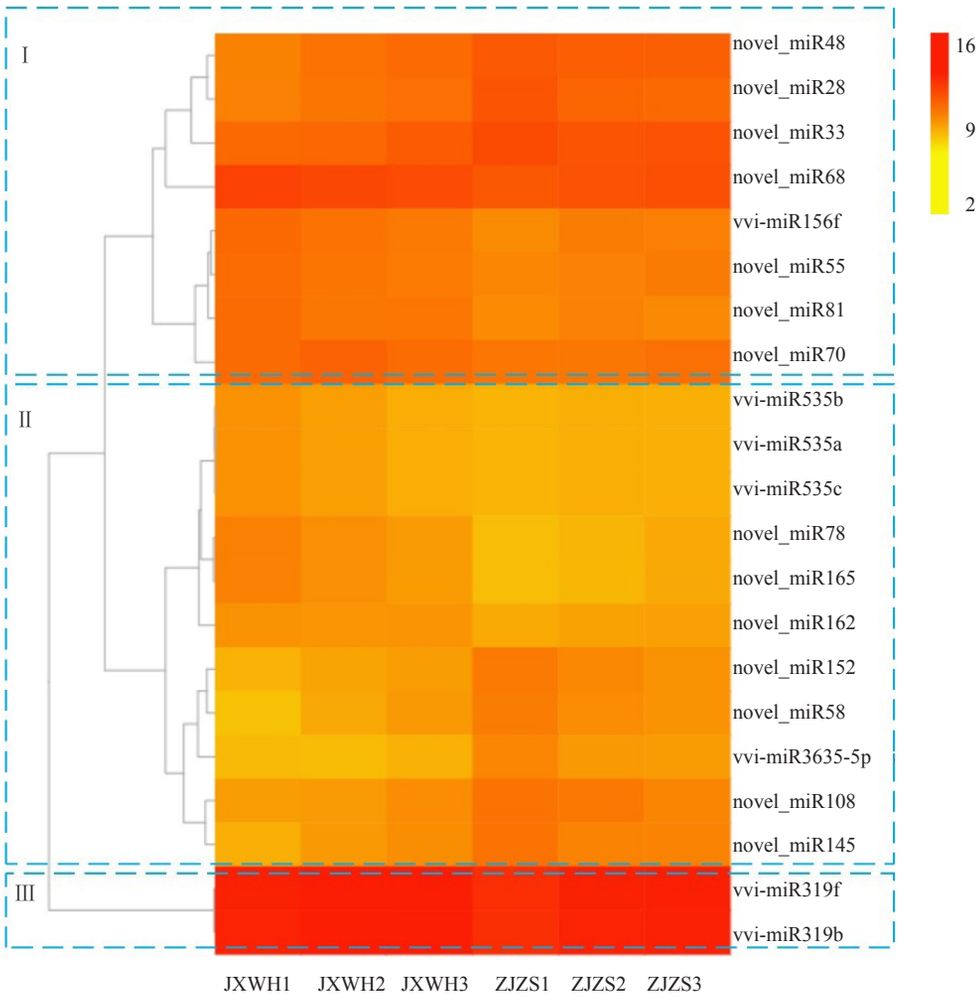


A. 梢尖中 miRNA 鉴定；B. 差异表达 miRNA 数量。

A. The number of miRNA in shoot tip; B. The number of differential expression miRNA.

图 5　紫金早生（ZJZS）和金星无核（JXWH）miRNA 鉴定统计

Fig. 5　The summary of identification of miRNAs in Zijinzaosheng and Venus seedless



列代表不同样品，行代表不同 miRNAs，以 $\log_{10}$(TPM)值进行聚类。

Columns and rows represent sample and miRNA, respectively. The value of $\log_{10}$(TPM) was used for cluster.

图 6　紫金早生（ZJZS）和金星无核（JXWH）梢尖中差异表达 miRNA 聚类分析

Fig. 6　Clustering analysis of differentially expressied miRNA in shoot tip between Zijinzaosheng and Venus Seedless

表 2 紫金早生(ZJZS)和金星无核(JXWH)差异表达Ⅰ类 miRNA 的 GO 注释

Table 2 GO annotation of the predicted target genes of differentially expressed Group Ⅰ miRNA between Zijinzaosheng and Venus Seedless

作用 Function	描述 Term	差异基因数量 Differentially expressed gene number	背景基因数量 Background gene number	*P* 值 *P*-value
分子功能 Molecular function	Long-chain-alcohol O-fatty-acyltransferase activity	3	4	0.000 169 362
	DNA binding	18	212	0.000 292 881
	Diacylglycerol O-acyltransferase activity	3	6	0.000 803 918
	UDP-glucuronate 4-epimerase activity	2	2	0.001 251 696
生物过程 Biologyical process	Translation initiation factor activity	4	15	0.001 505 401
	Regulation of vegetative phase change	4	4	0.000 000 854
	Regulation of timing of transition from vegetative to reproductive phase	5	17	0.000 115 910
	Glycerolipid biosynthetic process	3	5	0.000 274 034
	Regulation of transcription, DNA-templated	17	234	0.000 550 709
	Single-organism developmental process	9	80	0.000 638 386
	Translational initiation	4	15	0.000 898 307
	Protein secretion	3	7	0.000 916 859
	Monosaccharide metabolic process	2	2	0.000 949 283
	Wybutosine biosynthetic process	2	2	0.000 949 283
	Response to gamma radiation	4	19	0.002 321 192
	Regulation of response to salt stress	2	3	0.002 790 510
	Nucleotide-sugar metabolic process	2	3	0.002 790 510
	Cellular response to calcium ion	2	3	0.002 790 510
	Male meiosis	2	3	0.002 790 510
	Microgametogenesis	3	10	0.002 938 677

表 3 紫金早生(ZJZS)和金星无核(JXWH)差异表达Ⅱ类 miRNA 的 GO 注释

Table 3 GO annotation of the predicted target genes of differentially expressed Group Ⅱ miRNA between Zijinzaosheng and Venus Seedless

作用 Function	描述 Term	差异基因数量 Differentially expressed gene number	背景基因数量 Background gene number	*P*值 *P*-value
分子功能 Molecular function	Amine transmembrane transporter activity	3	4	0.004 110 93
	Acidic amino acid transmembrane transporter activity	3	4	0.004 110 93
	Chlorophyll catabolite transmembrane transporter activity	4	8	0.005 704 88
	Glutathione S-conjugate-exporting ATPase activity	4	8	0.005 704 88
细胞组分 Cellular component	Integral component of membrane	47	245	1.156 6E-05
	Trans-Golgi network	12	40	0.000 529 52
	Endosome	11	42	0.003 060 39
	Chloroplast envelope	25	137	0.003 364 25
	Mitochondrial inner membrane	4	8	0.005 940 38
生物过程 Biologyical process	Histone acetylation	5	8	0.000 464 68
	Response to high light intensity	12	45	0.001 318 14
	Response to hydrogen peroxide	11	40	0.001 591 58
	Transmembrane transport	14	63	0.003 545 36
	Regulation of vegetative phase change	3	4	0.003 909 75
	Protein maturation	3	4	0.003 909 75
	Protein modification by small protein removal	3	4	0.003 909 75
	Protein folding	11	45	0.004 395 15
	Organic substance catabolic process	5	13	0.006 937 49
	Divalent metal ion transport	4	9	0.008 879 93
	Regulation of seed growth	4	9	0.008 879 93

表4　紫金早生（ZJZS）和金星无核（JXWH）差异表达Ⅲ类miRNA的GO注释

Table 4　GO annotation of the predicted target genes of differentially expressed Group Ⅲ omiRNA between Zijinzaosheng and Venus Seedless

作用 Function	描述 Term	差异基因数量 Differentially expressed gene number	背景基因数量 Background gene number	*P*值 *P*-value
分子功能 Molecular function	NADPH-hemoprotein reductase activity	2	3	0.000 130 777
	Oxidoreductase activity, acting on NAD(P)H	2	3	0.000 130 777
	Protein binding	10	465	0.000 180 826
	Intracellular ligand-gated ion channel activity	2	4	0.000 260 519
	Glutamate receptor activity	2	4	0.000 260 519
	Ionotropic glutamate receptor activity	2	4	0.000 260 519
	Extracellular-glutamate-gated ion channel activity	2	4	0.000 260 519
	Sequence-specific DNA binding transcription factor activity	6	168	0.000 519 597
细胞组分 Cellular component	Nucleus	13	713	0.000 002 383
生物过程 Biologyical process	Jasmonic acid metabolic process	4	16	0.000 001 299
	Ovule development	5	68	0.000 026 614
	Positive regulation of development, heterochronic	2	3	0.000 095 626
	Ionotropic glutamate receptor signaling pathway	2	4	0.000 190 609
	Petal morphogenesis	2	6	0.000 473 332
	Positive regulation of transcription, DNA-templated	5	138	0.000 788 890
	Embryo development ending in seed dormancy	5	140	0.000 843 104
	Root hair cell development	2	8	0.000 877 635
	Cellular calcium ion homeostasis	2	9	0.001 124 604
	Response to ionizing radiation	2	10	0.001 401 041
	Mitotic G2 phase	2	10	0.001 401 041

与细胞周期调控miRNA为miR319b和miR319f，其靶基因分别为VIT_14s0006g03070和VIT_19s0014g03110，均为FAD结合域。

3　讨　论

3.1　植物激素对节间长度的影响

诸多研究证实植物激素参与节间生长发育过程，是该农艺性状形成的终端信号分子。赤霉素（GA）作为一类重要的植物激素，在植物节间长度的形成中发挥了重要作用，GA信号通路中，DELLA蛋白的累积是导致GA无法发挥正常作用，引起植物节间长度变短的主要原因[20-22]。然而，本研究中金星无核和紫金早生梢尖周围未展开叶片中GA含量差异不显著，同时外源GA_3处理紫金早生后，节间长度无明显变化，说明紫金早生对GA不敏感。

巨飞燕[23]研究证实IAA能够促进棉花果枝节间的伸长，并且生长素促进细胞伸长和扩增的过程多与GA和BR信号通路相关。笔者在本研究中发现金星无核中ICA和ICAld含量显著高于紫金早生，而IAA含量差异不显著。ICA也是植物体内生长素存在的主要形式之一。杨永岗等[24]研究证实高含量ICA能够抑制西瓜分生侧枝，然而目前未见ICA与植物节间长度具有直接相关性的报道。水杨酸是植物应对逆境胁迫的重要信号分子。研究表明，NaCl胁迫下，外施水杨酸能够促进植物的生长[25]，但是水杨酸对植物生长的影响存在低促高抑的剂量效应[26]，本研究2种材料中水杨酸含量确存在显著差异，但其差异剂量对葡萄节间长度的影响尚未可知。差异miRNA的GO注释分析发现，在21个差异miRNA中，只注释到茉莉酸信号通路相关的miRNA。因此，生长素和水杨酸可能参与调控了紫金早生短节间性状的形成，但在本文中未检测到参与生长素和水杨酸信号通路的miRNA。

茉莉素是细胞生长抑制因子，其抑制作用多与有丝分裂相关[27-28]。MeJA以依赖COI1的方式延迟从有丝分裂周期向内核复制周期的转变，导致细胞处于S期之前的G1期，细胞数量降低[29]。茎段细胞形态学分析表明，紫金早生茎段中细胞数量明显低于金星无核，而细胞长度和宽度并无差异（图2）。笔者认为紫金早生短节间表型主要是由茎段中细胞数量减少引起的，其细胞数量的减少可能与内源茉莉酸含量的升高有关。

茉莉素是植物中一类非常重要的脂类激素，被认为是植物的抗性激素[30]。通常植物细胞质中的

JA-Ile（JA主要的活体形式）含量较低，遇外界刺激后，JA的含量会增加并迅速转化成JA-Ile，促使植物产生一系列防御反应。例如盐分和干旱胁迫均能明显提升拟南芥和番茄内源JA水平[31]。机械损伤也会诱导茉莉素分泌，抑制植株生长[32]。前期以秋水仙素处理金星无核获得了其短节间突变体紫金早生，2种材料倍性一致，未出现加倍，推测可能是机械损伤和秋水仙素的双重胁迫激发了JA信号通路，抑制了细胞有丝分裂，降低了细胞数量，导致其节间长度变短。

有研究报道JA对植物株型有调控作用。Hong等[33]研究发现茉莉酸信号通路调控因子微蛋白LNJ（LITTLE NINJA）通过JA信号通路调节作物的株型。JA对细胞的抑制作用分为直接和间接两种模式。JA能够直接作用于细胞的分裂和扩张活动，影响节间的伸长。例如，水稻JA受体缺陷或JA失活均会促进水稻节间长度伸长和植株高度的增加[34-35]。JA也能调控其他生长激素的水平，间接影响细胞生长，从而抑制植物的生长发育。过表达*AP2*使大麦对GA反应不敏感，JA信号表达增强，从而抑制了节间的伸长[36]。虽然紫金早生对外源GA_3不敏感，JA对紫金早生节间长度的调控是否与GA或其他生长激素有关值得进一步探索。

3.2 miRNA调控植物节间长度的作用机制

随着测序技术的发展，研究者发现许多miRNA作为上层调控因子，调控了植物的节间长度和株高。例如水稻子代系*L1710*中6个miRNAs（osa-miR164c、osa-miR164d、osa-miR164e、osa-miR169k、osa-miR1863a和osa-miR1861b）靶向*OsNAC2*参与了节间的伸长[37]。在果树中也有许多miRNA调控节间长度的报道。miRNA167、miRNA396和miR159通过调控GA的表达抑制细胞分裂降低了苹果的节间长度[38]。miR171-*SCL6*/*SCL22*模块通过对IAA的响应调节梨新梢的发育[39]。苹果（*Malus*×*domestica*）同源四倍体内miR390-*MdTAS3*-*MdARF3*模块的调控引起植株矮化[40]。

本研究在金星无核和紫金早生中共筛选到21个差异miRNA，GO注释分析显示仅有miR319参与植物激素（茉莉酸）代谢。miR319是第一个通过正向遗传突变筛选方法被发现的植物miRNA，已有研究表明miR319对水稻[41]、甘蓝型油菜[42]和小麦[43]等作物的株高具有调控作用，关于miR319在果树株型中的作用还未见报道。miR319主要靶向负调控Ⅱ类TCP转录因子基因家族[44-45]，对JA信号通路具有调控作用。Schommer等[46]发现miR319靶向TCP4调节*LIPOXYGENASE2*（*LOX2*）的表达，从而直接影响JA的生物合成。在番茄中，过表达*miR319b*使转基因植株中的JA含量升高[47]，而过表达*miR319a*使转基因植株中的JA含量降低[48]。上述研究表明miR319参与了植物的株型调控，其作用机制取决于下游靶基因的作用，具体作用通路还需探索。葡萄miR319能够靶向TCP家族（*VvTCPs* 2、4、8、10和13）[49]，但目前未见葡萄miR319-TCP模块参与茉莉酸合成代谢的报道。综上，笔者推测紫金早生与金星无核中的JA含量差异可能是受miR319的调控，有关葡萄miR319调控JA信号通路的作用机制有待进一步研究。

4 结 论

紫金早生的节间长度和茎段中的细胞数量显著低于金星无核，内源JA含量明显高于金星无核，同时紫金早生的节间长度对外源JA及其生物合成抑制剂处理存在响应，说明紫金早生的短节间突变性状与JA信号通路的调节有关。进一步分析表明miR319可能介导JA信号通路参与了紫金早生短节间性状的形成。

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