

# 柑橘直立枝和斜生枝增粗生长 及相关基因的比较分析

韦 欣,柳东海,韩 晗,罗 银,陈 欢,刘永忠\*

(华中农业大学园艺林学学院·园艺植物生物学教育部重点实验室,武汉 430070)

**摘要:**【目的】探究柑橘直立枝和斜生枝的粗度差异,阐释导致差异的原因,为调控柑橘枝梢增粗奠定基础。【方法】以枳砧3年生的红心柚和南丰蜜橘的秋梢直立枝(枝梢垂直于地面水平线)和斜生枝(枝梢与地面水平线呈45°~60°)为材料,比较粗度、长度、枝梢解剖结构和枝梢内增粗生长相关基因的相对表达量。【结果】红心柚、南丰蜜橘的直立枝粗度均显著高于斜生枝,解剖分析发现直立枝的形成层细胞层数显著多于斜生枝,且直立枝的木质部横截面积较大,髓细胞较大;34个增粗生长相关基因的定量PCR分析表明,2个品种的直立枝中SND1、WOX4、PXY和MYB46-1的相对表达量均显著高于斜生枝,APL、BRXL2、BES1、HAM4和NAC3的相对表达量则显著低于斜生枝。【结论】柑橘直立枝粗度显著高于斜生枝,与直立枝的形成层层数增加和木质部增厚有关;形成层增加相关基因WOX4、PXY和木质部增厚相关基因SND1的高表达可能是柑橘直立枝梢增粗显著高于斜生枝的重要原因。

**关键词:**柑橘;直立枝;斜生枝;增粗生长;形成层

中图分类号:S666

文献标志码:A

文章编号:1009-9980(2022)04-0509-09

## Comparative analysis of secondary growth and its related genes between erect and oblique branches of *Citrus*

WEI Xin, LIU Donghai, HAN Han, LUO Yin, CHEN Huan, LIU Yongzhong\*

(College of Horticulture & Forestry Sciences, Huazhong Agricultural University/Key Laboratory of Horticultural Plant Biology, Ministry of Education, Wuhan 430070, Hubei, China)

**Abstract:**【Objective】*Citrus* is an important fruit tree in south China. Because the fruit-bearing shoots of branch are soft, high-yielding trees often require supporting poles to avoid fruit squeezing or branch breaking and to ensure fruit quality and tree health. However, erection of poles needs much labor and it is difficult to finish such work in the current era with labor shortages and aging. Promoting the secondary growth of branches and simplifying tree structure play an important role in removing erection of poles, reducing labor cost and increasing orchard revenue. The present study is to explore the difference in secondary growth between erect and oblique branches of citrus, explain the reasons behind the difference, and lay a foundation for regulating the secondary growth of citrus shoots.【Methods】Three-year-old red pomelo (*Citrus grandis* ‘Hongxinyou’) and Nanfeng tangerine (*C. reticulata* ‘Kinokuni’) were used as materials to compare the thickness, length, anatomical structure, and expression of genes related to secondary growth between erect (perpendicular to the ground) and oblique (the shoot is at a 45–60 degree angle to the ground level) shoots.【Results】The thickness of the erect shoots of Nanfeng tangerine and red pomelo was significantly higher than that of the oblique shoots. Moreover, the length of the erect shoots of the two varieties was slightly longer than that of the oblique shoots, but the difference was not significant. The anatomical analysis showed that the primary phloem cell size was larger, the

收稿日期:2021-08-30 接受日期:2021-12-16

基金项目:国家重点研发计划项目(2020YFD1000100);财政部和农业农村部:国家现代农业产业技术体系专项

作者简介:韦欣,女,硕士,研究方向:分子生理与品质形成。Tel:15927114092,E-mail:1572508393@qq.com

\*通信作者 Author for correspondence. Tel:15347103401,E-mail:liuyongzhong@mail.hzau.edu.cn

cambium width was significantly larger, and the xylem width was significantly lower in the upper part of erect shoots than in that of the oblique shoots, while in the lower part of shoots, the width of cambium and xylem of the erect shoots was significantly higher than that of oblique shoots. The expression levels of 34 genes related to secondary growth were compared. Of the four genes related to xylem secondary growth, *KNAT1* and *STM* in the erect shoots of Red pomelo were significantly more active than those in the oblique shoots, but there was no significant difference between them in Nanfeng tangerine; moreover in both varieties, the expression level of *SND1* in the erect shoots was significantly higher than that in the oblique shoots, but the expression level of *BOP1/2* was not significantly different between the two types of shoots. The expression levels of *APL*, *BRX*, *BRXL2*, *NEN1/2-1*, *NEN4* and *BAM3* related to phloem secondary growth were significantly lower and the expression levels of *BRXL4* and *NAC45/86* were significantly higher in red pomelo the erect shoots than in the oblique shoots; in Nanfeng tangerine erect shoots, the expression levels of *APL*, *BRXL2*, *BRXL4*, *NAC45/86* and *NEN1/2-1* were significantly lower and the expression levels of *NEN4*, *OPS* and *BAM3* were significantly higher than those of the oblique shoots. As for the 15 genes related to secondary growth in cambium, the expression levels of *WOX4*, *WOXI4*, *MYB46-1*, *MYB46-2*, *PXY*, *AF1* and *NST1* were significantly higher and the expression levels of *BES1*, *CLE8*, *HAM4* and *NAC3* were significantly lower the erect shoots than in the oblique shoots in red pomelo; in the erect shoots of Nanfeng tangerine, the expression levels of *WOX4*, *CLE8*, *MYB46-1*, and *PXY* were significantly higher and the expression levels of *BES1*, *WOXI4*, *HAM4*, *KNAT6*, *MYB46-2*, *MYB59*, and *SD1* were significantly lower than in the oblique shoots. Finally, the expression levels of two genes (*ARR11* and *ARR12*) related to cytokinin synthesis and three genes (*ARF5*, *ATHB8* and *PIN1*) related to auxin transport were also compared between the two types of shoots in both varieties. The expression level of *ARR12* in Nanfeng tangerine was significantly higher in the erect shoots than in the oblique shoots; the expression level of *ARF5* in red pomelo was significantly higher and the expression level of *PIN1* was significantly lower in the erect shoots than in the oblique shoots; moreover, the expression level of *ATHB8* was significantly lower in erect shoots in both varieties. 【Conclusion】The difference in cambium activity and xylem thickness was the main reason for the difference in shoot thickening in citrus. Specially, *PXY* and *WOX4* played a key role in the maintenance of cambium activity. The expression level of the two genes may be the key reason to determine the speed of shoot secondary growth. On the other hand, the high expression of *SND1* in erect branches of citrus may be the important reason for their thicker (wider) xylem than in the oblique branches. Namely, the high expression of cambium-related genes, *WOX4* and *PXY*, and xylem-related gene *SND1* may contribute to the higher shoot thickening growth of erect shoots. The function of such genes needs to be further verified.

**Key words:** *Citrus*; Erect shoots; Oblique shoots; Secondary growth; Cambium

柑橘是我国南方重要果树,在其果实成熟过程中对丰产树进行立杆拉枝,可以避免果实挤压和枝干折断,确保果实品质和树体健康。但是,立杆拉枝需要大量劳动投入。在当前从业劳动力不足和老龄化现象日渐严重的情况下,降低劳动投入对实现柑橘种植降本增效非常必要。促进枝干增粗、简化树体结构有助于立杆拉枝的逐步取消。但是,柑橘枝梢增粗机制及调控措施的相关研究严重滞后。

目前,植物增粗生长的研究主要集中在杨树<sup>[1-5]</sup>和拟南芥<sup>[6-13]</sup>等模式植物上。植物增粗生长主要受维管形成层的驱动。维管形成层在茎中呈闭环状态并且具有持续分裂的能力,通过平周分裂和切向分裂不断向内外产生次生木质部和次生韧皮部<sup>[9,14-16]</sup>。维管组织的不断积累是双子叶和裸子植物的茎不断增粗的重要原因<sup>[12,17-19]</sup>。此外,维管形成层的分子调控途径的相关研究亦有报道。拟南芥根部维管形成

层的研究表明,形成层的活动受 *WOX4*、*WOX14*、*MYB87*、*KNAT1* 和 *PTL* 等基因的转录因子构建而成的网络调控<sup>[4,7,11,17,20]</sup>;杨树茎部的转录组学分析表明,MYB 家族和 NAC 家族包括 *MYB59*、*MYB46*、*NAC2*、*NAC3*、*NST1*、*AF1* 等基因,均参与杨树茎部次生生长的调控<sup>[1-2,13]</sup>。此外,植物激素是调控植物生长发育的重要因素,对维管形成层的活动起到了关键作用<sup>[3,5,10,21-23]</sup>。例如,生长素响应基因 *ARF5* 能够通过激活 *ATHB8* 来诱导生长素转运载体基因 *PINI* 的表达,从而在木本植物次生生长中发挥作用<sup>[5,10]</sup>;细胞分裂素能够引起萝卜根中形成层相关转录因子的差异表达,进而影响形成层细胞的增殖活性<sup>[22]</sup>;*ARR11* 和 *ARR12* 是属于磷酸化介导的细胞分裂素信号途径中结合 DNA 的转录因子,其中 *ARR12* 能够影响生长素向内运输<sup>[23]</sup>,而细胞分裂素可以通过阻断生长素的梯度重塑进而影响杨树形成层的形成<sup>[3]</sup>。以上报道表明植物增粗生长受到木质部、韧皮部、形成层以及激素代谢相关基因的表达调控,在柑橘枝梢增粗过程中是否如此,目前并不清楚。

笔者在本试验中以红心柚和南丰蜜橘为材料,从解剖和分子 2 个层面比较直立枝和斜生枝的增粗差异,以期明确柑橘直立枝梢比斜生枝梢增粗快的原因,为后续柑橘枝梢增粗的进一步研究、生产上轻简化树形培育奠定基础。

## 1 材料和方法

### 1.1 试验材料

2020 年秋季(2020-09-08)随机选择枳砧红心柚 (*Citrus grandis* ‘Hongxinyou’)、枳砧南丰蜜橘 (*C. reticulata* ‘Kinokuni’) 直立枝(垂直于地面水平线)和斜生枝(与地面水平线呈 45°~60°)发育良好、老熟程度基本一致的秋梢各 30 根,分别用游标卡尺和卷尺测定枝梢(基部)的粗度和长度,然后各随机剪取 10 根枝梢,去掉叶片放入冰盒中带回实验室。

### 1.2 目的基因挖掘和引物设计

根据拟南芥和杨树中与次生生长相关的基因登录号<sup>[2,9]</sup>,利用同源比对法分别在 Phytozome (<http://www.phytozome.net>) 甜橙、克里曼丁基因组数据库和华中农业大学甜橙基因组数据库<sup>[24]</sup> (<http://citrus.hzau.edu.cn/orange/>) 中筛选出柑橘中次生生长相关基因的序列。筛选的标准为有相同的注释名称和 *e-value* ≤ e<sup>-30</sup>。将查找到的同一类基因利用 BioEdit 软

件进行序列比对分析<sup>[25]</sup>,确定目的基因及其序列,采用 Primer 5.0 进行定量引物设计(表 1)。

### 1.3 石蜡切片

随机剪取 5 根枝梢上部(距枝梢顶端 5 cm)和下部(距枝梢基部 5 cm)2~3 mm 的圆柱形茎段,迅速保存于福尔马林醋酸乙醇(formalin-acetic acid-alcohol, FAA)固定液中,采用微波快速石蜡切片法<sup>[26]</sup>进行切片和解剖结构观察。

### 1.4 总 RNA 提取和 cDNA 合成

随机选择直立枝和斜生枝各 3 根,分别剪碎混匀后迅速用液氮处理。枝梢总 RNA 提取采用艾德莱 TRIPure Reagent(Aidlab, 北京)提取剂和生工植物柱式 RNA 提取试剂盒。参照 TransScript One-step gDNA Removal and cDNA Synthesis SuperMix 试剂盒(北京全式金生物技术)中的方法,将 1 μg 高质量总 RNA 合成 cDNA。

### 1.5 实时荧光定量 PCR 分析

实时荧光定量 PCR(quantitative real-time PCR, qRT-PCR)分析采用 Power SYBR Green PCR Master Mix 试剂盒(翊圣公司),反应体系为:0.5 μL cDNA、5.0 μL Mix、0.2 μL 正向引物、0.2 μL 反向引物、4.1 μL ddH<sub>2</sub>O, 每个样品进行 4 次重复。采用 QuantStudioTM 6Flex Real-time PCR System,程序反应为:准备阶段,50 °C 2 min, 95 °C 5 min;扩增阶段,95 °C 15 s, 60 °C 15 s, 72 °C 30 s, 40 个循环。参照 2<sup>-ΔΔCt</sup> 法<sup>[27]</sup>计算基因的相对表达量。

### 1.6 数据分析

试验数据利用 SPSS V20、采用最小显著性差异法(least significant difference, LSD)进行差异性显著分析,*p* < 0.05 认定为处理之间差异显著。

## 2 结果与分析

### 2.1 生长比较

柑橘直立枝垂直地面,一般生长势较旺,而斜生枝与地面形成一定角度,一般缓慢向上生长(图 1-A)。分别测定红心柚、南丰蜜橘的秋梢直立枝和斜生枝长度和粗度,发现红心柚、南丰蜜橘的直立枝的粗度均显著大于斜生枝,2 个品种的直立枝的长度略长于斜生枝,但是差异不显著(图 1-B~C)。

### 2.2 解剖结构比较

进一步分析红心柚直立枝和斜生枝解剖结构,发现上部分茎中以髓部为主,髓细胞较大,未形成明

表1 柑橘增粗相关基因及其引物

Table 1 Secondary growth-related genes in citrus and their primer sequences

归类 Category	基因名 Gene name	序列ID Sequence ID	引物序列 Primer sequence (5'-3')	
			正向引物 Forward primer	反向引物 Reverse primer
木质部中基因 Genes in xylem	<i>KNAT1</i>	Cs3g16460	AGCCAAGATCATCTCCA	TCGTAATAAGCCTCCATAA
	<i>STM</i>	Cs2g02590	TAATGGCTCATCCTCACT	GATCTTGACCGATACAGC
	<i>SND1</i>	Cs5g01350	AATCGGATTGAGAAAGAC	CATTACTGAAGTGGAGGTT
	<i>BOP1/2</i>	Orange1.1t00760	CTACTGCTTCCCCTATCAA	ACAACCTCGTTCACCACA
韧皮部中基因 Genes in phloem	<i>APL</i>	Orange1.1t03247	GCTTACGCTTACCAACCT	TTGCATCCTAACCGCATC
	<i>BRX</i>	Cs3g07250	CGGCAAGCTCTAACACT	CGTATCTCGGACCACCTC
	<i>BRXL2</i>	Cs1g17720	CCAATACACTGGCAGGTC	TAGTAGGCGTCCAATCA
	<i>BRXL4</i>	Orange1.1t00156	AAAGATGGTGGGCTGAAA	TTGGACGATACAAGTTACGAG
形成层中基因 Genes in cambium	<i>NAC45/86</i>	Orange1.1t03242	GGCACCTGTAGGATTACC	CTGAACCCATTGGATAT
	<i>NEN1/2-1</i>	Cs5g11010	CTCCAACCCCTACTCCACTC	CTCGCACAATCAAACCTCC
	<i>NEN1/2-2</i>	Orange1.1t03999	GCTGTAACGGCATCACTC	CTTATTCTCGCACAATCAA
	<i>NEN4</i>	Cs1g01750	AGTCGGAGCAATCGTAG	ATATTATGCCCTGCCAC
激素相关基因 Phytohormone-related genes	<i>OPS</i>	Cs6g07730	GCAAATGGGAATAGCAAA	CGCGTCAAGTAGAACCT
	<i>BAM3</i>	Cs2g12040	TCCGCAGTCTGGTCTATC	GAACCGTTGAACCGTTG
	<i>BES1</i>	Cs3g24470	GAGGAAGACGGCACCAC	CTCGGAAAGGATGAAGATAAAG
	<i>WOX4</i>	Cs3g23280	GATAGGGATACTGGAGATGC	CAATGGCTAACGACAGAC
	<i>WOXI4</i>	Cs1g26550	ACTTACGCAACCTCCCTC	TCCACCCATCTTCCTGT
	<i>CLE8</i>	Cs7g12750	ACCACCAAGCAATACTATCC	TCAATCTCCGCACTTCCA
	<i>HAM4</i>	Cs1g23790	GTCGTGCCGTGTTAG	CCGCAGCAGATAGGTTTC
	<i>KNAT6</i>	Cs7g08990	CCGATGAGTTACTATCTGCT	CTCCTACCTCTGGCAAT
	<i>MYB46-1</i>	Cs2g30230	AGCCACGCAACAGCAAT	ATCCAGCGAAGGCGACAG
	<i>MYB46-2</i>	Cs7g01800	GAAAGGCAATAACAACAA	AAAGGAAAGGAGGAAATG
	<i>MYB59</i>	Orange1.1t00911	CTCTTCGTCCTCTAACTACTC	TTGCCATCCTGTTCTATA
	<i>PXY</i>	Cs9g14980	AACAGCATTGGATTGGGTG	GCTTCGGCTTCGCTTCCT
	<i>SD1</i>	Cs9g07450	GGGACAGCCACATTAGCC	CATTCTGCCCTCCTGC
	<i>NAC2</i>	Cs7g21830	TTGCCTCGGAGTTCCCTAC	ATTCTTGCCAACCGACCA
	<i>AF1</i>	Cs3g19890	TGAGTTACAGTTACCAACAGG	CTTGAACCGTTGGATACTT
	<i>NAC3</i>	Cs1g06760	GTCTTGCAGGCTTCATT	TGATTCCCTAACCCCCACTC
	<i>NST1</i>	Orange1.1t00561	GAGCTACTGCTGCTGGTT	ATTTCATTGGGATTGTCG
	<i>ARR11</i>	Cs7g10020	CATTGCCAGGACCACATC	AAAGACGACCCAACCTCCC
	<i>ARR12</i>	Cs7g06180	ACCACAAGTCAGGCAATC	TCCATCTCAAGTCCCACA
	<i>ARF5</i>	Cs3g25860	TGGCACATCTCGAGTAA	ATCGCAGAGCATAGTTCA
	<i>ATHB8</i>	Cs4g19310	ATGAGGGATGGTCTATGTT	AGAAGTATTGCTGGAGGC
	<i>PIN1</i>	orange1.1t00089	GTGCCAGGTTGCTTATCT	ATCTCAGCGTCTGTTCA

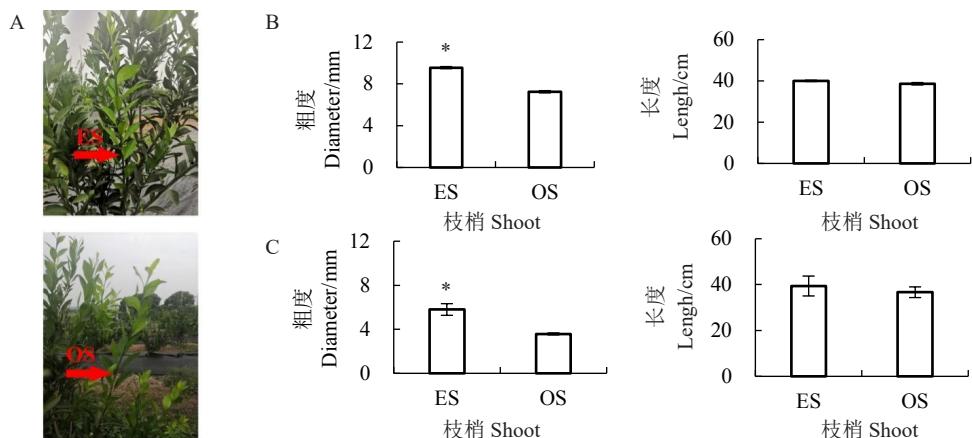
显的髓射线,初生木质部初步形成,形成层细胞排列整齐;其中直立枝的初生韧皮部细胞要大于斜生枝、形成层宽度显著高于斜生枝、木质部宽度显著低于斜生枝(图2-A)。此外,枝梢下部茎中主要以次生木质部为主,髓射线明显凸现,导管细胞数量增加,形成层细胞呈扁平长方形、排列紧密,直立枝形成层和木质部的宽度均显著高于斜生枝(图2-B)。

进一步分析南丰蜜橘直立枝和斜生枝解剖结构,发现枝梢上部的髓部及其细胞较大,形成层的细胞呈扁平状,排列比较规整;其中直立枝韧皮部的细

胞数量多于斜生枝,但是暂未形成明显的次生木质部,而斜生枝则已经形成次生木质部,并出现导管细胞(图2-C)。此外,直立枝上部形成层和木质部宽度均显著高于斜生枝,枝梢下部均以木质部为主,髓射线明显形成;直立枝的导管细胞数量少但细胞较大,形成层细胞数量多且比斜生枝排列紧密;直立枝下部的形成层和木质部宽度均显著高于斜生枝(图2-D)。

### 2.3 木质部内增粗相关基因表达比较

通过分析4个木质部内增粗相关基因的表达发

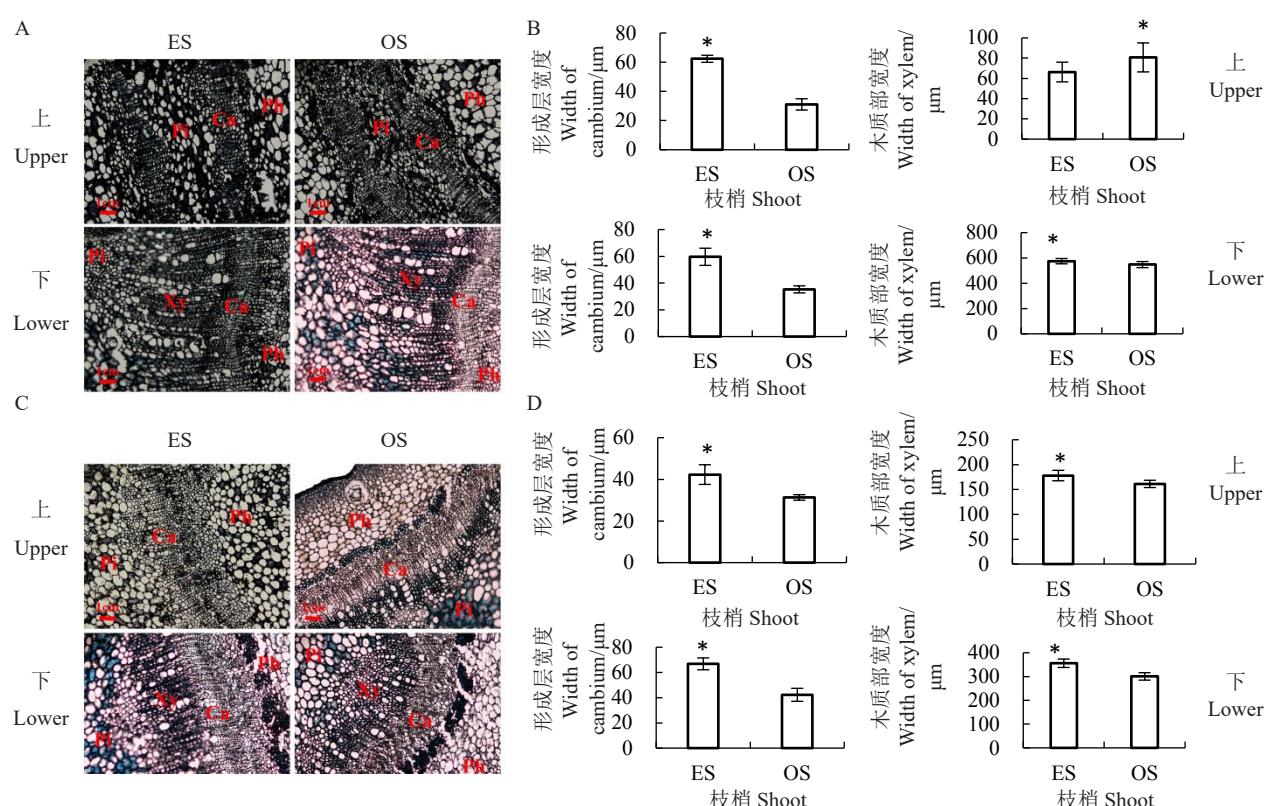


A. 直立枝和斜生枝生长状态; B. 红心柚秋梢直立枝和斜生枝粗度和长度; C. 南丰蜜橘秋梢直立枝和斜生枝粗度和长度。ES、OS 分别代表直立枝、斜生枝。\*表示直立枝和斜生枝粗度和长度的差异达到显著差异水平( $p < 0.05$ )。下同。

A. Growth status of erect and oblique shoots; B. The length of erect and oblique shoots of hongxinyou; C. The length of erect and oblique shoots of Nanfeng tangerine. ES and OS represent erect shoots and oblique shoots, respectively. \* represents significant difference of the diameter and length between erect shoots and oblique shoots at  $p < 0.05$  by  $t$ -test. The same below.

图 1 红心柚、南丰蜜橘直立枝和斜生枝生长差异比较

Fig. 1 Comparison of the shoot growth between the erect and the oblique shoots of red pomelo (*C. grandis* ‘Hongxinyou’) and Nanfeng tangerine (*C. reticulata* ‘Kinokuni’)



A. 红心柚枝梢上下部横切结构; B. 红心柚枝梢上下部形成层和木质部的宽度; C. 南丰蜜橘枝梢上下部横切结构; D. 南丰蜜橘枝梢上下部形成层和木质部的宽度。Pi. 髓; Xy. 木质部; Ca. 形成层; Ph. 韧皮部。

A. The upper and lower transverse sections of Hongxinyou erect and oblique shoots; B. The width of cambium and xylem in upper and lower shoots of Hongxinyou; C. The upper and lower transverse sections of erect and oblique shoots of Nanfeng tangerine; D. The width of cambium and xylem in upper and lower shoots of Nanfeng tangerine. Pi, Xy, Ca, and Ph refer to pith, xylem, cambium, and phloem, respectively.

图 2 红心柚、南丰蜜橘直立和斜生枝的横切结构

Fig. 2 The upper and lower transverse sections of erect and oblique shoots of red pomelo (*C. grandis* ‘Hongxinyou’) and Nanfeng tangerine (*C. reticulata* ‘Kinokuni’)

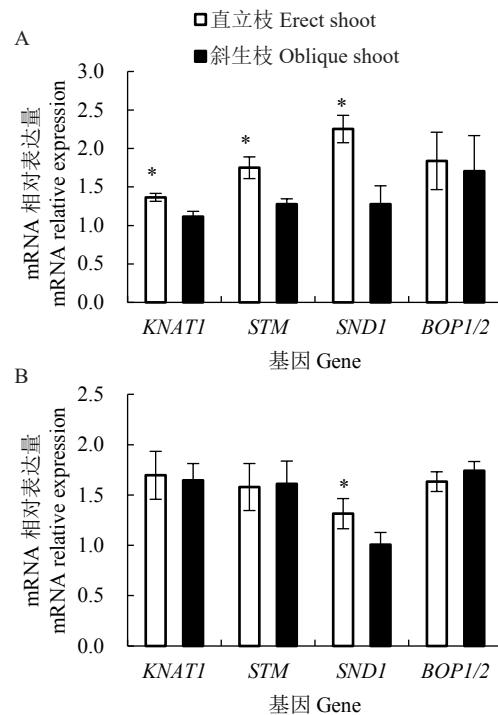
现(图3),红心柚中 $KNAT1$ 和 $STM$ 在直立枝中的相对表达量显著高于斜生枝,但是在南丰蜜橘中的相对表达量无显著差异;2个品种直立枝中 $SND1$ 的相对表达量均显著高于斜生枝,而 $BOP1/2$ 的相对表达量在直立枝和斜生枝间无显著差异。

#### 2.4 韧皮部内增粗相关基因表达比较

通过分析10个韧皮部内增粗相关基因的表达(图4),发现红心柚直立枝中 $APL$ 、 $BRX$ 、 $BRXL2$ 、 $NEN1/2-1$ 、 $NEN4$ 和 $BAM3$ 的相对表达量均显著低于斜生枝,而 $BRXL4$ 和 $NAC45/86$ 的相对表达量显著高于斜生枝, $NEN1/2-2$ 和 $OPS$ 则无显著差异;在南丰蜜橘中,直立枝中的 $APL$ 、 $BRXL2$ 、 $BRXL4$ 、 $NAC45/86$ 和 $NEN1/2-1$ 的相对表达量显著低于斜生枝,而 $NEN4$ 、 $OPS$ 和 $BAM3$ 的相对表达量显著高于斜生枝, $NEN1/2-2$ 和 $BRX$ 的相对表达量则无显著差异。综合来看,2个品种直立枝中的 $APL$ 和 $BRXL2$ 的相对表达量均显著低于斜生枝。

#### 2.5 形成层内增粗相关基因表达比较

通过分析15个在形成层内增粗相关基因的相对表达量(图5),发现红心柚直立枝中的 $WOX4$ 、 $WOX14$ 、 $MYB46-1$ 、 $MYB46-2$ 、 $PXY$ 、 $AF1$ 和 $NST1$ 的相对表达量显著高于斜生枝,而 $BES1$ 、 $CLE8$ 、 $HAM4$ 和 $NAC3$ 的相对表达量则显著低于斜生枝, $KNAT6$ 、 $MYB59$ 、 $SD1$ 和 $NAC2$ 的表达量无显著差异;南丰蜜



A. 红心柚; B. 南丰蜜橘。数据表示4个重复的平均值±标准误。下同。

A and B represent Honxinyou and Nanfeng tangerine, respectively. Values are means of four replications±SE. The same below.

图3 红心柚、南丰蜜橘木质部中增粗相关基因的相对表达量比较分析

Fig. 3 Comparative analysis of the expression of thickening-related genes in xylem of red pomelo (*C. grandis* ‘Honxinyou’) and Nanfeng tangerine (*C. reticulata* ‘Kinokuni’)

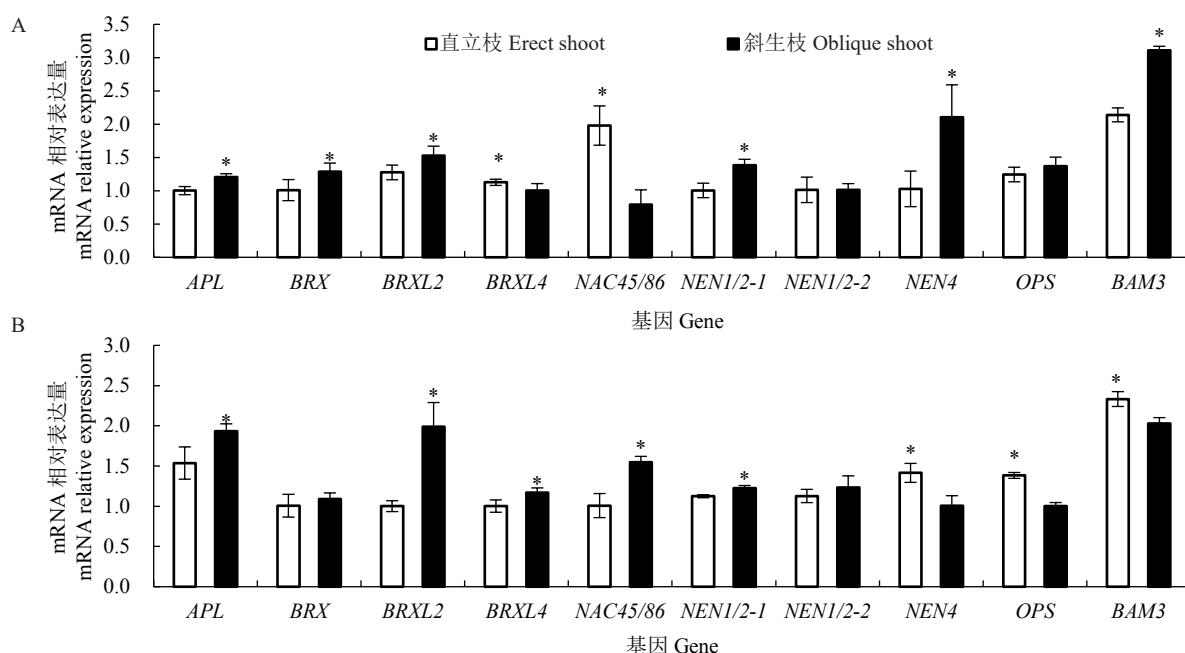


图4 红心柚、南丰蜜橘韧皮部中增粗相关基因的表达量比较分析

Fig. 4 Comparative analysis of the expression of thickening-related genes in phloem of red pomelo (*C. grandis* ‘Honxinyou’) and Nanfeng tangerine (*C. reticulata* ‘Kinokuni’)

橘直立枝中的 *WOX4*、*CLE8*、*MYB46-1* 和 *PXY* 的相对表达量显著高于斜生枝, 而 *BES1*、*WOXI4*、*HAM4*、*KNAT6*、*MYB46-2*、*MYB59*、*SD1*、*NAC2*、*AF1* 和 *NAC3* 的相对表达量则显著低于斜生枝, *NST1* 在

直立枝和斜生枝间的相对表达量无显著差异。综合来看, 2个品种直立枝中 *WOX4*、*PXY* 和 *MYB46-1* 的相对表达量均显著高于斜生枝, 而 *BSE1*、*HAM4* 和 *NAC3* 的相对表达量均显著低于斜生枝。

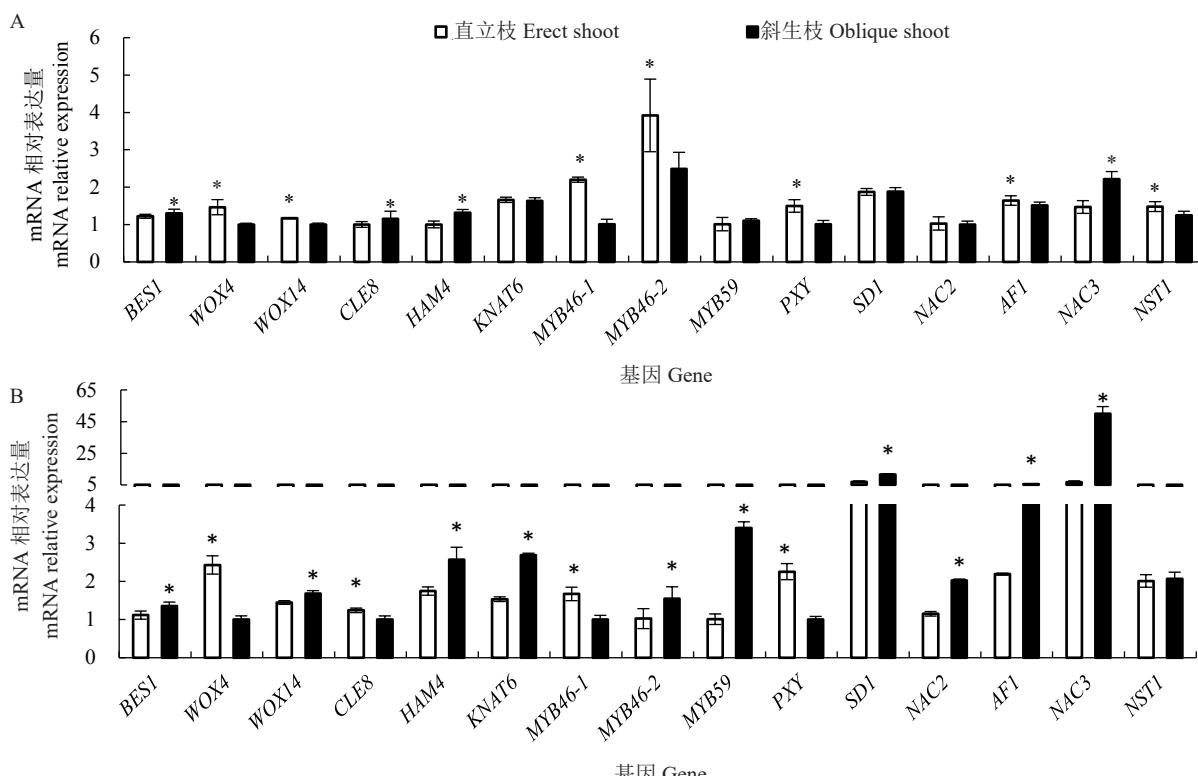


图 5 红心柚、南丰蜜橘形成层中增粗相关基因的表达量比较分析

Fig. 5 Comparative analysis of the expression of thickening-related genes in cambium of red pomelo (*C. grandis* 'Hongxinyou') and Nanfeng tangerine (*C. reticulata* 'Kinokuni')

## 2.6 增粗相关的激素基因表达比较

通过比较细胞分裂素合成基因(*ARR11* 和 *ARR12*)以及生长素响应运输相关基因(*ARF5*、*ATHB8*、*PIN1*)的相对表达量(图6), 发现红心柚的直立枝和斜生枝中 *ARR11* 和 *ARR12* 的相对表达量

无显著差异, *ARF5*在直立枝中的相对表达量显著高于斜生枝, 而 *ATHB8* 和 *PIN1* 的相对表达量显著低于斜生枝; 南丰蜜橘直立枝中 *ARR11* 的相对表达量显著低于斜生枝, 而 *ARR12* 的相对表达量则显著高于斜生枝, *ARF5* 和 *ATHB8* 在直立枝中的相对表达

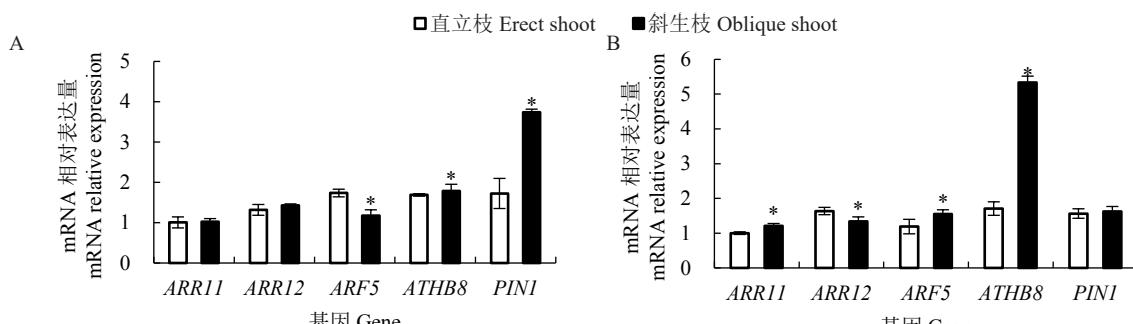


图 6 红心柚、南丰蜜橘枝梢中细胞分裂素合成和生长素运输相关基因的表达量比较分析

Fig. 6 Comparative analysis of the expression of cytokinin synthesis and auxin transport related genes in shoots of red pomelo (*C. grandis* 'Hongxinyou') and Nanfeng tangerine (*C. reticulata* 'Kinokuni')

量显著低于斜生枝, *PIN1*的相对表达量在直立枝和斜生枝间没有显著差异。

### 3 讨 论

维管形成层是不断进行细胞分裂的侧分生组织<sup>[15-16]</sup>。维管形成层的活动能够影响植物茎的增粗生长<sup>[12,18-19]</sup>,有效增加花序茎和下胚轴的径向直径<sup>[11-12]</sup>。前人研究发现,形成层组织可以不断向内外分化成木质部和韧皮部组织<sup>[6,15,18]</sup>,并且杨树中木质部的产出速率明显高于韧皮部,从而木质部不断积累形成更多有用的木材<sup>[1,4]</sup>。本研究中,无论是红心柚还是南丰蜜橘的直立枝粗度均显著高于斜生枝,其形成层和木质部的横截面宽度也均显著高于斜生枝,说明柑橘枝梢增粗的快慢与形成层的分裂和木质部增粗快慢有密切关系。

维管形成层属于多向分化的次生分生组织<sup>[15-17]</sup>,受激素、基因、激酶和多肽等因子的调控<sup>[5,8,21]</sup>,其中以PXY-WOX4调控途径研究最为广泛。*WOX4*属于WOX转录因子家族成员,是*CLE41/44*相关信号通路的关键靶点,并且位于PXY途径下游<sup>[7]</sup>。PXY是导管分子分化的抑制因子(tracheary element differentiation inhibitory factors, TDIF)受体,属于富含亮氨酸的受体激酶,主要在形成层中表达,同时还参与了细胞分裂素的信号传导<sup>[28-29]</sup>。TDIF-PXY调节下游*WOX4*以维持原形成层和形成层细胞的分裂活性<sup>[8]</sup>。在杨树中,*PtPXY*和*PtWOX4*共同调节杨树茎的次生生长,TDIF-PXY-WOX4形成固定保守的途径,调节着植物维管组织的活性<sup>[4]</sup>。同样在拟南芥中,经过RNA干扰处理后,*WOX4*的低表达强烈抑制形成层的细胞分裂<sup>[20]</sup>。在本研究中,红心柚和南丰蜜橘直立枝的粗度显著高于斜生枝,而且直立枝中*WOX4*和*PXY*的相对表达量显著高于斜生枝,说明*PXY*和*WOX4*的高表达是柑橘直立枝梢增粗生长高于斜生枝的重要原因。

另外,前人研究发现*SND1*是参与木质部纤维次生壁合成的主要激活因子,在茎的束间纤维中特异性表达,其高表达是促进植物增粗生长过程中木质部纤维和导管细胞次生壁加厚的重要原因,而*SND1*的低表达会导致纤维次生壁厚度明显下降<sup>[13]</sup>。本研究解剖发现,2个柑橘品种直立枝的木质部厚(宽)度显著高于斜生枝,而直立枝中*SND1*的相对表达量也显著高于斜生枝,说明*SND1*的高表

达可能是直立枝的木质部纤维和导管细胞次生壁厚度显著高于斜生枝的重要原因。

植物增粗生长同样受到内源激素的调控,木本植物中的生长素-*ARF5-ATHB8-PIN1*正反馈调节环在次生生长过程中起调控作用<sup>[5,10]</sup>。*ARR11*和*ARR12*在细胞分裂素转导途径中起正调控作用<sup>[3,22-23,29]</sup>。本研究中,生长素和细胞分裂素相关基因在红心柚和南丰蜜橘直立枝和斜生枝中的表达并没有表现出一致的规律,说明其可能不是决定柑橘直立枝和斜生枝粗度差异的重要因素。

### 4 结 论

柑橘直立枝粗度显著高于斜生枝,并且与直立枝的形成层数增加和木质部增厚有关。其中,*PXY*、*WOX4*这2个基因的表达量高低可能是决定柑橘枝梢增粗生长快慢的关键因素,而*SND1*高表达可能是柑橘直立枝比斜生枝木质部更厚(宽)的重要原因。相关基因的功能有待进一步验证。

### 参考文献 References:

- [1] BOSSINGER G, SPOKEVICIUS A V. Sector analysis reveals patterns of cambium differentiation in poplar stems[J]. Journal of Experimental Botany, 2018, 69(18):4339-4348.
- [2] CHAO Q, GAO Z F, ZHANG D, ZHAO B G, DONG F Q, FU C X, LIU L J, WANG B C. The developmental dynamics of the *Populus* stem transcriptome[J]. Plant Biotechnology Journal, 2019, 17(1):206-219.
- [3] CHEN J J, WANG L Y, IMMANEN J, NIEMINEN K, SPICER R, HELARIUTTA Y, ZHANG J, HE X Q. Differential regulation of auxin and cytokinin during the secondary vascular tissue regeneration in *Populus* trees[J]. New Phytologist, 2019, 224(1): 188-201.
- [4] KUCUKOGLU M, NILSSON J, ZHENG B, CHAABOUNI S, NILSSON O. WUSCHEL-RELATED HOMEOBOX4 (*WOX4*)-like genes regulate cambial cell division activity and secondary growth in *Populus* trees[J]. New Phytologist, 2017, 215(2): 642-657.
- [5] ZHU Y Y, SONG D L, XU P, SUN J Y, LI L G. A HD-ZIP III gene, *PtrHB4*, is required for interfascicular cambium development in *Populus*[J]. Plant Biotechnology Journal, 2018, 16(3): 808-817.
- [6] CHAFFEY N, CHOLEWA E, REGAN S, SUNDBERG B. Secondary xylem development in *Arabidopsis*: A model for wood formation[J]. Physiologia Plantarum, 2002, 114(4):594-600.
- [7] ETCHELLS J P, PROVOST C M, MISHRA L, TURNER S R. *WOX4* and *WOX14* act downstream of the *PXY* receptor kinase to regulate plant vascular proliferation independently of any role in vascular organisation[J]. Development, 2013, 140(10): 2224-

- 2234.
- [8] HIRAKAWA Y, KONDO Y, FUKUDA H. TDIF Peptide signaling regulates vascular stem cell proliferation via the WOX4 homeobox gene in *Arabidopsis*[J]. *Plant Cell*, 2010, 22(8): 2618-2629.
- [9] LEHMANN F, HARDTKE C S. Secondary growth of the *Arabidopsis* hypocotyl-vascular development in 4 dimensions[J]. *Current Opinion in Plant Biology*, 2016, 29: 9-15.
- [10] MÜLLER C J, VALDÉS A E, WANG G D, RAMACHANDRAN P, BESTE L, UDDEMBERG D, CARLSBECKER A. PHABULOSA mediates an auxin signaling loop to regulate vascular patterning in *Arabidopsis*[J]. *Plant Physiology*, 2015, 170 (2): 956-970.
- [11] ZHANG J, ESWARAN G, SERRA J A, KUCUKOGLU M, XIANG J L, YANG W B, ELO A, NIEMINEN K, DAMÉN T, YUN Y J, LEE J H, RAGNI L, REUILLE P B, EAHERT S, LEE J Y, MÄHÖNEN P, HELARIUTTA Y. Transcriptional regulatory framework for vascular cambium development in *Arabidopsis* roots[J]. *Nature Plants*, 2019, 5(10): 1033-1042.
- [12] ZHAO C S, CRAIG J C, PETZOLD H E, DICKEMAN A W, BEERS E. The xylem and phloem transcriptomes from secondary tissues of the *Arabidopsis* root-hypocotyl[J]. *Plant Physiology*, 2005, 138(2): 803-818.
- [13] ZHONG R Q, DEMURA T, YE Z H. SND1, a *NAC* domain transcription factor, is a key regulator of secondary wall synthesis in fibers of *Arabidopsis*[J]. *Plant Cell*, 2006, 18(11): 3158-3170.
- [14] 潘国清.植物茎构造剖析[J].现代农业科技,2009(11): 108-109.  
PAN Guoqing. Anatomy of plant stem structure[J]. *Modern Agricultural Sciences and Technology*, 2009(11): 108-109.
- [15] 李正理.维管形成层的活动及其增生方式[J].生物学通报,1983,18(4):7-9.  
LI Zhengli. The activity of vascular cambium and its proliferation mode[J]. *Bulletin of Biology*, 1983, 18(4): 7-9.
- [16] JOUANNET V, BRACKMANN K, GREB T. (Pro)cambium formation and proliferation: Two sides of the same coin?[J]. *Current Opinion in Plant Biology*, 2015, 23: 54-60.
- [17] DE RYBEL B, MÄHÖNEN A P, HELARIUTTA Y, WEIJERS D. Plant vascular development: From early specification to differentiation[J]. *Nature Reviews Molecular Cell Biology*, 2016, 17(1): 30-40.
- [18] SHI D, LEBOVKA I, LÓPEZ-SALMERÓN V, SANCHEZ P, GREB T. Bifacial cambium stem cells generate xylem and phloem during radial plant growth[J]. *Development*, 2019, 146(1): 171355.
- [19] SPICER R, GROOVER A. Evolution of development of vascular cambia and secondary growth[J]. *New Phytologist*, 2010, 186 (3): 577-592.
- [20] JI J B, STRABLE J, SHIMIZU R, KOENIG D, SINHA N. *WOX4* promotes procambial development[J]. *Plant Physiology*, 2009, 152(3): 1346-1356.
- [21] SORCE C, GIOVANNELLI A, SEBASTIANI L, ANFODILLO T. Hormonal signals involved in the regulation of cambial activity, xylogenesis and vessel patterning in trees[J]. *Plant Cell Reports*, 2013, 32(6): 885-898.
- [22] JANG G, LEE J H, RASTOGI K, PARK S, OH S H, LEE J Y. Cytokinin-dependent secondary growth determines root biomass in radish (*Raphanus sativus* L.) [J]. *Journal of Experimental Botany*, 2015, 66(15): 4607-4619.
- [23] ZHANG W J, SWARUP R, BENNETT M, SCHALLER G E, KIEBER J J. Cytokinin induces cell division in the quiescent center of the *Arabidopsis* root apical meristem[J]. *Current Biology*, 2013, 23(20): 1979-1989.
- [24] XU Q, CHEN L L, RUAN X A, CHEN D J, ZHU A D, CHEN C L, BERTRAND D, JIAO W B, HAO B H, LYON M P, CHEN J J, GAO S, XING F, LAN H, CHANG J W, GE X H, LEI Y, HU Q, MIAO Y, WANG L, XIAO S, BISWAS M K, ZENG W, GUO F, GAO H, YANG X, XU X W, CHENG Y J, XU J, LIU J H, LUO O J, TANG Z, GUO W W, KUANG H H, ZHANG H Y, ROOSE M L, NAGARAJAN N, DENG X X, RUAN Y. The draft genome of sweet orange (*Citrus sinensis*)[J]. *Nature Genetics*, 2013, 45(1): 59-66.
- [25] THOMPSON J D, GIBSON T J, PLEWNIAK F, JEANMOUGIN F, HIGGINS D G. The CLUSTAL\_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools[J]. *Nucleic Acids Research*, 1997, 25(24): 4876-4882.
- [26] 魏欣悦,苏源,杨静,刘林,李成云.微波快速石蜡切片法观察水稻叶片组织[J].云南农业大学学报,2011,26(4):454-457.  
WEI Xinyue, SU Yuan, YANG Jing, LIU Lin, LI Chengyun. Microwave paraffin section in rice leaf tissue[J]. *Journal of Yunnan Agricultural University*, 2011, 26(4): 454-457.
- [27] LIVAK K J, SCHMITTGEN T D. Analysis of relative gene expression data using real-time quantitative PCR and the  $2^{-\Delta\Delta C_t}$  method[J]. *Methods*, 2001, 25(4): 402-408.
- [28] HIRAKAWA Y, SHINOHARA H, KONDO Y, INOUE A, NAKANOMYO I, OGAWA M, SAWA S, OHASHI-ITO K, MATSUBAYASHI Y, FUKUDA H. Non-cell-autonomous control of vascular stem cell fate by a *CLE* peptide/receptor system[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2008, 105(39): 15208-15213.
- [29] HAN S, CHO H, NOH J, QI J Y, JUNG H J, NAM H, LEE S, HWANG D, GREB T, HWANG I. *BIL1*-mediated MP phosphorylation integrates *PXY* and cytokinin signalling in secondary growth[J]. *Nature Plants*, 2018, 4(8): 605-614.