Correlation of Jasmonic Acid Signaling Pathway-Related Gene Expression with Rubber Yield in Rubber Trees (Postprint)

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Abstract

Tapping-promoted natural rubber synthesis in rubber trees is closely associated with the activation of the jasmonic acid signaling pathway in laticifer cells. The correlation between gene expression levels at key nodes of this pathway and dry rubber yield remains unclear. This study employed qPCR technique to analyze the expression of nine genes related to key nodes of the jasmonic acid signaling pathway in laticifer cells from five rubber tree Wickham germplasm and five 1981' IRRDB germplasm under tapping conditions. The results demonstrated that the dry rubber yield per tapping of most Wickham germplasm was significantly higher than that of 1981' IRRDB germplasm. Among the nine genes, except for HbMYC4 and HbMYCS, the remaining seven genes exhibited significantly higher expression levels in most rubber tree Wickham germplasm compared to 1981' IRRDB germplasm, with HbMYC3 showing particularly good expression differentiation and high correlation with dry rubber yield, thus representing a promising molecular marker for rubber tree yield breeding.

Full Text

Preamble

Correlation between the Expression Level of Genes Related to Jasmonate Signaling and Rubber Yield

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Abstract

Tapping-enhanced rubber biosynthesis is closely related to the activation of jasmonate signaling in laticifer cells of rubber tree. The relationship between the expression level of the genes involved in jasmonate signaling and dry rubber yield remains not elucidated. In the present study, the expression of 9 genes related to jasmonate signaling was analyzed by qPCR in the laticifer cells of 5 Wickham germplasms and 5 1981' IRRDB germplasms upon tapping with S/2 d/3 tapping system. The rubber yield per tapping of most Wickham germplasms was significantly higher than that of 1981' IRRDB germplasms. Except for HbMYC4 and HbMYC5, the expression level of the other seven genes in most of Wickham germplasms was significantly higher than that of 1981' IRRDB germplasms. It was noted that the expression of HbMYC3 was highly difference and closely related to the rubber yield, which may be used as a candidate marker for rubber yield-breeding of rubber tree.

Keywords: *Hevea brasiliensis*, tapping, rubber biosynthesis, jasmonate signaling, HbCOI1, HbJAZs, HbMYCs, gene expression

Introduction

Jasmonic acid is a key signaling molecule in plant stress responses (Qi et al, 2011), and the core components of its signaling pathway are COI1, JAZ, and MYC (Chini et al, 2009). In the absence of JA-Ile (the bioactive form of jasmonic acid) (Fonseca et al, 2009), JAZ proteins interact with MYC2 to inhibit MYC2-mediated transcriptional activation of jasmonate-responsive genes. For example, in *Arabidopsis*, AtJAZ1 and AtJAZ3 proteins affect glucosinolate synthesis by inhibiting the transcription factors AtMYC2, AtMYC3, and AtMYC4 (Schweizer et al, 2013). These proteins also interact with WD-Repeat/bHLH/MYB complexes to suppress anthocyanin synthesis (Qi et al, 2011). However, when JA-Ile is present, JAZ proteins bind to COI1 and are subsequently degraded via the 26S proteasome, enabling MYC2 to activate jasmonate-responsive genes (Donnell et al, 1996; Chini et al, 2007; Qi et al, 2011). Rubber biosynthesis in laticifer cells of rubber tree represents a typical plant isoprenoid metabolism. Recent studies have shown that jasmonate content in laticifer cells of tapped trees is significantly higher than in untapped trees, and that tapping-enhanced natural rubber biosynthesis is closely associated with activation of the jasmonate signaling pathway in laticifer cells (Deng et al, 2018). Nevertheless, the correlation between expression of key genes in the jasmonate signaling pathway and rubber yield remains unclear. This study aims to identify molecular markers associated with yield by analyzing the relationship between expression of key jasmonate signaling genes and rubber production, which holds important practical value for rubber tree breeding given its long breeding cycle.

Materials and Methods

Plant Materials

The experimental trees consisted of 5 Wickham germplasms and 5 1981' IRRDB germplasms of 10-year-old *Hevea brasiliensis*. The five Wickham germplasms were artificially bred cultivars: PR107, RRIM600, Reken 628, Reken 525, and Reken 523. The five 1981' IRRDB germplasms were unselected wild germplasms with accession numbers RO/CM/10 44/160, MT/IT/13 29/8, RO/C/8 24/104, RO/I/103 107, and RO/CM/10 44/454. All experimental trees were planted at the experimental farm of the Chinese Academy of Tropical Agricultural Sciences. DNase was purchased from Tiangen. The RevertAidTM H Minus First Strand cDNA Synthesis Kit was obtained from Ferments. SYBR® Premix Ex TaqTM II (2×) (Tli RNaseH Plus) for qPCR was purchased from TaKaRa (Dalian, Japan). Other biochemical reagents were either imported or domestic analytical grade. Primers were synthesized by Invitrogen.

1.2.1 Material Treatment

For each germplasm, three trees were selected. On the tenth tapping under normal production conditions (S/2D d3: half spiral, upward cut, every three days), latex flowing during the first 10 minutes was collected. Equal volumes from the three trees per germplasm were pooled for total RNA extraction from latex.

1.2.2 Dry Rubber Yield Measurement

Dry rubber yield was determined following the method described by Zeng et al (2006).

1.2.3 Total RNA Extraction and cDNA Synthesis

Total RNA extraction from latex was performed according to the method of Zeng et al (2003). First-strand cDNA synthesis was carried out following the kit protocol: 1 g of total RNA was reverse-transcribed to synthesize first-strand cDNA, which was diluted 10-fold and used as template for qPCR analysis.

1.2.4 Gene Expression Analysis

1.2.4.1 qPCR Reaction The qPCR reaction system (20 L) contained 10 L SYBR® Premix Ex TaqTM II (2×), 0.4 L forward primer, 0.4 L reverse primer (primer concentration 10 mol · L ^{1}, final concentration 0.2 mol · L ^{1} each), 1 L cDNA template, and 8.2 L ddH O. Reactions were performed in LightCycler® Capillaries (20 µl, Roche) on a LightCycler Real-Time PCR system (Roche Diagnostics) following the manufacturer' s instructions. The qPCR program consisted of 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 20 s, and 72 °C for 20 s. After 40 cycles, melting curve analysis was performed (60–95 °C, 0.2 °C·s ¹), and the system was cooled to 40 °C. Each sample was run in two technical replicates with Cq standard deviation controlled within 0.2. Cq values were collected using LightCycler Software 4.05.

1.2.4.2 qPCR Primer Selection Primers for qPCR were designed based on full-length cDNA sequences from GenBank. Standard curves were generated using 10-fold serial dilutions of cDNA to determine primer amplification efficiency; only primer pairs with efficiency above 85% were selected. Primer specificity was assessed by melting curve analysis, and only those producing a single peak were used. qPCR products were verified by sequencing. The primers used in this study are listed in Table 1 .

1.2.4.3 Gene Expression Calculation Gene expression values (Q) were calculated using the formula Q = 2^(ΔCq) = 2^(min Cq - Sample Cq). The relative expression value (E) of target genes was determined using Hb18S as the reference gene according to E = Q_target / Q_reference. The fold difference in relative gene expression between samples (Bénédicte et al, 2002; Silvia et al, 2012; Yuki et al, 2015) was calculated as F = E_A / E_B to directly reflect expression differences between sample A and sample B.

1.3 Data Processing

Graphs were generated using Excel 2003. Multiple comparisons were performed using Duncan' s test in SPSS: different uppercase letters indicate extremely significant differences between groups ($P < 0.01$), different lowercase letters indicate significant differences ($P < 0.05$), and identical lowercase letters indicate no significant difference ($P > 0.05$). Pairwise comparisons were conducted using Excel TTEST (Array 1, Array 2, Tails 1, Type 1): $P < 0.01$ indicates extremely significant differences, and $P < 0.05$ indicates significant differences.

Results

2.1 Comparison of Dry Rubber Yield per Tapping among Rubber Tree Germplasms

Significant differences in dry rubber yield per tapping were observed among different rubber tree germplasms (Figure 1 [Figure 1: see original paper]). Reken 523 showed the highest yield, while RO/C/8 24/104 had the lowest. Overall, the dry rubber yield of most Wickham germplasms was significantly higher than that of 1981' IRRDB germplasms. RO/I/103/107 exhibited the highest yield among 1981' IRRDB germplasms, reaching only the level of PR107 (the lowest among Wickham germplasms) and remaining significantly lower than other Wickham germplasms. The remaining four 1981' IRRDB germplasms showed extremely significant differences compared to Wickham germplasms, with no significant differences among themselves.

Fold changes in dry rubber yield between Wickham and 1981' IRRDB germplasms (Table 2) revealed that Wickham germplasms produced 0.81-17.39 times more yield than 1981' IRRDB germplasms, with an average of 6.81-fold difference. Notable variation existed among germplasms, with a coefficient of variation of 63.66%.

2.2 Gene Expression Analysis

2.2.1 qPCR Primer Validation Using 10-fold serial dilutions of cDNA to generate standard curves, the amplification efficiency of target gene primers ranged from 87% to 100% (Figure 2 [Figure 2: see original paper]: A). Melting curve analysis showed that all gene primers produced single peaks, indicating specific amplification products. No-template controls (NTC) showed no amplification, confirming the absence of contamination (Figure 2: B). Although minimal amplification was observed for 18S in NTC (Cp = 31.1 $\pm$ 2.2), the Cq value was far lower than that of samples (9.5 $\pm$ 0.3), thus not affecting quantification accuracy.

2.2.2 Expression Analysis of Jasmonate Signaling Genes among Rubber Tree Germplasms qPCR analysis revealed that HbCOI1 (Figure 3 [Figure 3: see original paper]: A) and HbMYC2 (3: F) reached Wickham expression levels in only one (20%) 1981' IRRDB germplasm. HbJAZ2 (Figure 3: C) and HbJAZ3 (Figure 3: D) were expressed at 1981' IRRDB levels in only one (20%) Wickham germplasm. HbJAZ1 (Figure 3: B) showed low expression in two (40%) Wickham germplasms comparable to 1981' IRRDB levels. HbMYC4 (Figure 3: H) and HbMYC5 (Figure 3: I) had similar expression levels between germplasm groups in over 80% of cases. Notably, HbMYC1 (Figure 3: E) and HbMYC3 (Figure 3: G) were expressed at significantly higher levels in all Wickham germplasms compared to 1981' IRRDB germplasms. Statistical analysis confirmed that HbMYC1 and HbMYC3 expression was extremely significantly higher in the Wickham group, while HbCOI1, HbJAZ1, HbJAZ2, HbJAZ3, and HbMYC2 were significantly higher in Wickham germplasms. In contrast, HbMYC4 and HbMYC5 showed no significant differences between groups (Figure 3: J).

Fold changes in gene expression between Wickham and 1981'IRRDB germplasms (Table 2) identified HbJAZ2, HbJAZ3, HbMYC1, and HbMYC3 as dominantly expressed genes, with fold changes of 5.30, 8.96, 5.09, and 27.79, respectively. However, expression fold changes varied considerably among germplasms for each gene, with coefficients of variation of 47.66% (HbCOI1), 63.01% (HbJAZ1), 72.60% (HbJAZ2), 76.41% (HbJAZ3), 32.83% (HbMYC1), 48.45% (HbMYC2), 45.77% (HbMYC3), 81.17% (HbMYC4), and 58.16% (HbMYC5). Notably, except for Reken 628 vs. RO/I/103 107 (8.74-fold, 0.04%), HbMYC3 showed over

10-fold higher expression in all five Wickham germplasms compared to 1981' IRRDB germplasms (96%).

Statistical analysis demonstrated that the average expression of HbCOI1, HbJAZ1, HbJAZ2, HbJAZ3, HbMYC1, HbMYC2, and HbMYC3 was significantly higher in Wickham germplasms, with 80% reaching extremely significant levels (Figure 4 [Figure 4: see original paper]).

Discussion and Conclusion

Tapping promotes natural rubber synthesis in rubber trees, and rubber regeneration between two successive tappings involves induced expression regulation of rubber synthesis enzyme genes (Deng et al, 2018; Zhao, 2011). Tapping-enhanced rubber biosynthesis is closely associated with activation of the jasmonate signaling pathway in laticifer cells (Deng et al, 2018). The core components of jasmonate signal transduction are COI1, JAZ, and MYC (Chini et al, 2009). Previous studies have shown that HbMYC1 and HbMYC3 expression levels correlate positively with dry rubber yield in rubber tree germplasms (Lu, 2010; He, 2013). Therefore, investigating expression differences of the "HbCOI1-HbJAZs-HbMYCs" module among germplasms with varying rubber yields enables screening for yield-related molecular markers.

Genes showing greater than 2-fold variation are considered differentially expressed (Bénédicte et al, 2002). The expression levels of HbCOI1, HbJAZ1, HbJAZ2, HbJAZ3, HbMYC1, HbMYC2, and HbMYC3 in high-yield germplasms were more than 2-fold higher than in low-yield germplasms, indicating positive correlation between expression of these seven genes and dry rubber yield. Among them, HbMYC3 showed the greatest expression difference and highest correlation with dry rubber yield, making it a promising molecular marker for rubber tree yield breeding. Future studies will expand the population size to validate this hypothesis.

Among the five HbMYC family members, HbMYC1, HbMYC2, and HbMYC3 are specifically expressed in latex, whereas HbMYC4 and HbMYC5 are predominantly expressed in flowers (Zhao, 2011). The lack of correlation between HbMYC4 and HbMYC5 expression levels and dry rubber yield suggests that HbMYC1, HbMYC2, and HbMYC3 are the primary members involved in rubber synthesis regulation. Notably, HbMYC5 expression in RO/C/8 24/104 was extremely significantly higher than in other germplasms (4.34–9.67-fold, averaging 6.96-fold) with a low coefficient of variation (29.15%). Since HbMYC5 function in rubber trees remains unclear, RO/C/8 24/104 may serve as excellent material for functional studies of HbMYC5.

Interactions among "HbCOI1-HbJAZs-HbMYCs" and between family members (Zhao, 2011; Liu, 2011; He, 2013; Bao, 2014; Xiao, 2015; Wang, 2016; Yao, 2016) suggest their synergistic roles in regulating rubber biosynthesis. Although individual genes in some low-yield germplasms reached expression levels comparable to high-yield germplasms, the overall average expression of the seven yield-related genes in high-yield germplasms was significantly—and in 80% of cases extremely significantly—higher than in low-yield germplasms, further confirming their synergistic effects.

Three main factors influence rubber yield: the capacity for secondary laticifer differentiation in phloem (Lu, 2010; Chen et al, 2016), rubber regeneration capacity between tappings (Deng et al, 2018), and latex flow capacity (Wang et al, 2016; Cai, 2011; Li et al, 2015). This study obtained valuable results regarding the regulation of rubber regeneration capacity. Under non-stimulated tapping, PR107 shows short latex flow duration, which can be significantly extended by ethephon stimulation (Wang et al, 2016; Cai, 2011) with increased latex flow (Li et al, 2015), suggesting that latex flow capacity may be a primary factor limiting PR107 yield. The high expression levels of all seven yield-related genes in PR107 indicate strong rubber regeneration capacity, while its relatively low dry rubber yield (without stimulation) further supports this hypothesis. RO/I/103/107 showed the highest yield among low-yield germplasms, reaching PR107 levels, yet six of the seven yield-related genes were expressed at low levels, with only HbMYC2 expression comparable to PR107. This suggests weak rubber regeneration capacity but certain laticifer differentiation and/or latex flow capacities, while also demonstrating that single genes contribute little to rubber yield and confirming the synergistic (synergistic enhancement) effects of these genes in regulating rubber regeneration. Investigating the contribution rates of these three factors to dry rubber yield across different rubber tree germplasms represents a meaningful research direction.

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Note: Figure translations are in progress. See original paper for figures.