Unveiling the complexity of the litchi transcriptome and pericarp browning by single-molecule long-read sequencing

Yijie Zhou, Zhongsuzhi Chen, Meiying He, Huijun Gao, Hong Zhu, Ze Yun, Hongxia Qu, Yueming Jiang

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Abstract

Litchi is a perennial fruit crop with a highly heterozygous genome and complex transcripts. Although fruit senescence and pericarp browning have been extensively studied, the underlying regulatory mechanisms are still poorly understood. In this study, we used long-read sequencing technology in combination with RNA-seq analysis to investigate the diversity and complexity of the litchi transcriptome, as well as differential express of transcripts during litchi fruit storage. We obtained a reference transcriptome with 50,808 unique full-length isoforms, including 41,290 coding sequences (CDS), 1658 transcription factors, 22,100 simple sequence repeats, 2434 long noncoding RNAs, and 151 alternative splicing (AS) events. In addition, 41,290 isoforms had transmembrane helical structure, 33,579 isoforms contained Pfam protein domains, 14,348 isoforms contained SMART protein domains, 4180 isoforms had signal-peptide structure, 19,183 isoforms contained glycosylation sites, and 30,436 isoforms contained furin protease cleavage sites. Using this transcriptome, 1272 isoforms were found to be differentially expressed in litchi fruit pericarp during postharvest storage. The postharvest storage could be divided into a 'senescence onset' stage and a subsequent 'browning' stage according to RNA-seq data. During the 'senescence onset' stage, the expression of isoforms related to senescence and stress response was significantly up-regulated. During the 'browning' stage, the expression of isoforms related to cell wall degradation, oxidation, and disease response was significantly up-regulated. In addition, qPCR analysis showed that the expression changes of 30 isoforms during the postharvest storage of both 'Huaizhi' and 'Guiwei' fruit were consistent with the RNA-seq results, indicating high reliability of our results and inferences.