**Exploration of mechanism underlying the lipid alterations in the yellowing leaves of ‘HAES344’ macadamia**

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**Abstract:** Macadamia variety ‘HAES344’ (*Macadamia integrifolia*) is a high-value, evergreen nut tree, and grown commercially in the tropical and subtropical regions around the world for its premium edible kernel. However, the shoots of ‘HAES344’ are prone to generate the etiolated leaves under the continuous high temperature in summer, which eventually resulting in a decrease of yield. Lipids are the important subcellular components and participate in a variety of physiological processes. However, the profile and the metabolic mechanism of lipids during leaf yellowing of‘HAES344’remain unclear. In this study, three 8-year-old ‘HAES344’ macadamia trees with similar canopy sizes, grown in Zhanjiang, China, were selected and performed using a single tree as the experimental block, and the newly mature leaves on June 1 (green leaf), 20 (moderately yellow leaf with etiolation area between 50% and 70%) and 28 (severely yellow leaf with etiolation area nearly 100%) were collected and used for analyzing the lipid composition and the transcript expression of related genes in lipid metabolism. Widely targeted metabolomics analysis showed that a total of 20 differential accumulated lipids, including 5 free fatty acids, 8 glycerol esters, 5 lysoPC and 2 lysoPE, were identified, and all these lipids were significantly down-regulated in the yellowing leaves. Transcriptomic analysis revealed 108 differential expressed genes related to lipid metabolism, including 22 up-regulated and 69 down-regulated genes in the severely etiolated leaves compared to the green leaves. Of these 108 genes, a largest proportion of genes involved in the cutin, suberine and wax biosynthesis, followed by the linolenic acid metabolism, fatty acid degradation, and glycerolipid metabolism, while the least genes participated in the sphingolipid metabolism. Through the qRT-PCR analysisof 20 key functional genes, the relative expression levels of *SDP1*, *ACX1*, *S-ACP-DES6*, *FATB1*, *LACS4*, *accD*, and *ALDH3F1* were found to be gradually increased during leaf yellowing, while those of *LOX1.5*, *LACS1*, *LACS2*, *BCCP2*, *KCS12*, *LDH3H1*, *DGAT2*, *GPAT8*, and *GDPD2* decreased. Additionally, the changes of the *LOX2*, *LOX1.6* and *ADH* expression showed a pattern of increasing initially and decreasing later, while the *OLE1* did an opposite. With the leaves turned yellow, the transcription levels of *LOX1.5*, *LACS1*, *KCS12*, *DGAT2* and *GPAT8* were significantly decreased, while those of *ACX1*, *S-ACP-DES6*, *FATB1*, *accD*, and *ALDH3F1* remarkably increased. Compared with the green leaves, the relative expression levels of *SDP1*, *LACS4*, *LOX2*, *LOX1.6* and *ADH* were significantly enhanced in the moderately and/or the severely etiolated leaves, while those of *OLE1*, *LACS2*, *BCCP2*, *LDH3H1*, and *GDPD2* markedly reduced only in the moderately or the severely yellow leaves. These results of the reduced lipids and the differentially expressed genes showed the distinctive characteristics of lipid metabolism during leaf yellowing, which lays a foundation for further exploration of the molecular mechanisms of macadamia leaf yellowing under heat stress.

**Keywords:** Macadamia; Leaf yellowing; Lipid metabolism; Lipid composition; Fatty acids

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