

基于转录组和代谢组的尖萼耧斗菜花色变异机制研究

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摘 要: 耧斗菜属植物是我国东北地区重要的乡土植物资源, 其中尖萼耧斗菜 (*Aquilegia oxysepala*) 萼片深红色, 株型优美, 覆盖效果好, 适应性强, 在北方地区作为景观绿化材料应用前景广阔。黄花尖萼耧斗菜 (*A. oxysepala* f. *pallidiflora*) 萼片白色, 是适应环境而产生的进化结果。花色直接影响植物传粉、结实及后代的数量和质量, 花色变异反映了植物对环境变化的适应, 因此两种耧斗菜是研究花色进化和花青素生物合成的理想材料。【目的】揭示尖萼耧斗菜和黄花尖萼耧斗菜出现花色变异的物质基础和分子机制, 为耧斗菜属资源的繁育和保护奠定基础。【方法】以尖萼耧斗菜和黄花尖萼耧斗菜为研究对象, 利用英国皇家园艺比色卡进行色彩评价, 采用盐酸乙醇法、硝酸铝显色法和丙酮提取法分别测定总花青素、总黄酮、总叶绿素和类胡萝卜素含量; 利用第二代 Illumina 测序平台构建转录组数据库; 通过超高效液相色谱串联质谱 (UPLC-MS/MS) 进行广靶代谢物质检测; 通过联合分析和 WGCNA 挖掘调控花青素的转录因子。

【结果】花青素和总黄酮是两种尖萼耧斗菜萼片色素的主要类别; 本试验共获得 14120 个差异基因, 1159 种代谢物, KEGG 富集表明苯丙氨酸合成途径 (ath00940) 和类黄酮合成途径 (ath00941) 均有基因和代谢物被显著富集; 根据 KEGG 注释锚定 15 个花青素合成功能基因和 17 种代谢物; 尖萼耧斗菜萼片主要色素物质为 Cyanidin-3-O-(6"-O-malonyl) glucoside, Delphinidin-3-O-(3",6"-O-dimalonyl) glucoside 和 Cyanidin-3-O-(3",6"-O-dimalonyl) glucoside; 黄花尖萼耧斗菜主要的色素物质为: Apigenin-8-C-Glucoside, Apigenin-6-C-glucoside 和 Kaempferol-3-O-galactoside; 与尖萼耧斗菜相比, 黄花尖萼耧斗菜的花青素合成功能基因表达量整体下调, 加之 *F3'5'H* 和 *FLS* 的竞争, 促进底物流向黄酮和黄酮醇合成分支, 是两种耧斗菜花色出现变异的原因; 通过联合分析和 WGCNA, 确定调控功能基因的 WRKY (gene-AQUCO_02700161v1), C2H2 (gene-AQUCO_01000437v1) 和 bHLH (gene-AQUCO_05400130v1) 3 个转录因子, 在尖萼耧斗菜花发育过程中显著上调, 而在黄花尖萼耧斗菜中表达量无明显变化, 推测它们在两种耧斗菜的花色变异过程中发挥重要作用。【结论】本研究阐述了尖萼耧斗菜花色变异的分子机理, 为尖萼耧斗菜花色生态适应性进化研究提供新思路, 同时为尖萼耧斗菜资源的深入研究和利用奠定基础。

关键词: 尖萼耧斗菜; 花青素; 转录组; 代谢组

Transcriptome and metabolome profiling the mechanism of sepals color variation in *Aquilegia oxysepala*

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Abstract: *Aquilegia* is an important domestic plant resource in the northeast of China, among them, *A. oxysepala* is good landscape material with great potential for its dark red sepals, elegant shape, intensive coverage and well adaption. *A. oxysepala* f. *pallidiflora* is the result of evolutionary adaptation to the environment with white sepals. Floral color directly affects quantity and quality of plant pollination, fruiting and offspring populations, and variation reflects plant adaptation to environments. Therefore, both *A. oxysepala* and *A. oxysepala* f. *pallidiflora* are appropriate for floral color evolution and anthocyanin biosynthesis researches. 【Objective】 This research aimed to reveal the material basis and molecular mechanisms of floral color variation between *A. oxysepala* and *A.*

oxysepala f. *pallidiflora*, which provided a basis for the breeding and conservation of *Aquilegia*. **【Methods】** *A. oxysepala* and *A. oxysepala* f. *pallidiflora* were used as materials. Sepals color evaluation was conducted by comparing with Royal Horticultural Society Color Chart (RHSCC). Total anthocyanin, flavonoids, chlorophyll and carotenoids contents were measured by ethanol-HCl, aluminum nitrate colorimetric and acetone methods. Transcriptome was constructed using the second-generation Illumina sequencing platform; broad-target metabolites were detected by ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS); transcription factors that regulated anthocyanin were identified by co-analysis and WGCNA. **【Results】** Anthocyanin and total flavonoids were the main pigments in two *Aquilegia* species. Total of 14120 differential expressed genes and 1159 metabolites were identified. Genes and metabolites were both enriched on Phenylpropanoid biosynthesis (ath00940) and Flavonoid biosynthesis (ath00941) pathways. 15 functional genes and 17 metabolites were annotated according to KEGG. The main pigments of *A. oxysepala* were Cyanidin-3-O-(6"-O-malonyl) glucoside, Delphinidin-3-O-(3",6"-O-dimalonyl) glucoside and Cyanidin-3-O-(3",6"-O-dimalonyl) glucoside, while Apigenin-8-C-Glucoside, Apigenin-6-C-glucoside and Kaempferol-3-O-galactoside for *A. oxysepala* f. *pallidiflora*. Compared with *A. oxysepala*, anthocyanin biosynthesis functional genes down-regulated in *A. oxysepala* f. *pallidiflora* with competition from *F3'5'H* and *FLS*, the substrate flowed to flavonoids and flavonol biosynthesis pathways, which was the reason for the sepals color variation. According to co-joint and WGCNA, 3 transcription factors that regulated anthocyanin biosynthesis functional genes: WRKY (gene-AQUCO_02700161v1), C2H2 (gene-AQUCO_01000437v1) and bHLH (gene-AQUCO_05400130v1) were identified. They significantly up-regulated during floral development in *A. oxysepala*, however, no changes occurred in *A. oxysepala* f. *pallidiflora*, which could play an important role in floral color variation in *A. oxysepala*. **【Conclusion】** This research illustrated the molecular mechanism of floral color variation between *A. oxysepala* and *A. oxysepala* f. *pallidiflora*, which provided a new insight for evolution of ecological adaptations study in the floral color, while laid the foundation for the in-depth study and utilization of *A. oxysepala*.

Key words: *A. oxysepala*; anthocyanin; transcriptome; metabolism