雌雄异型异熟胡桃楸性别分化关键 miRNA 的鉴定和分析

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摘要：胡桃楸是雌雄同株异型异熟植物，分为雌先型和雄先型两种类型，其在东北地区是良好的经济树种和生态树种。miRNA 是一类内源性真核非编码 RNA，在植物体内对其靶基因有沉默作用。由 miRNA 介导胡桃楸性别分化和雌雄异型异熟机制尚不清楚。本研究利用高通量测序技术对胡桃楸雌雄先型的雌雄花芽进行 MicroRNA 测序分析，构建了 12 个 sRNA 文库，共鉴定了 310 个已知的和 278 个未知的 miRNA，对 miRNA 的长度、家族等信息进行统计。使用 Deseq 软件包筛选出 129 个显著差异表达的 miRNA。对这些差异表达的 miRNA 层次聚类。使用 PSRobots 预测了 638 个 miRNA 靶基因，并对这些靶基因进行 GO 和 KEGG 富集分析，这些靶基因在生殖、花发育和植物激素信号传导路径显著富集，推测 miR159c-3p, miR156d-3p, miR172d, miR171b, miR164a, miR167a, miR319a, miR399a、miR396t 可能是影响胡桃楸性别分化的关键基因，其中 miR164a、miR167c 和 miR171c-5p 可能是胡桃楸雌雄异型异熟分化的关键基因。将这些关键 miRNA 和其靶基因共同建立了一个预测调控网络。随机选择 9 个差异显著的 miRNA 进行实时荧光定量 PCR 检测，并结合转录组测序结果进行联合分析，建立了一个差异 miRNA-差异 mRNA 的初级共表达调控网络。进一步发掘了这些 miRNA 在胡桃楸性别分化中起到了重要的作用，揭示了 miRNA 与一些成花相关的重要转录因子之间的调控作用。推测 miRNA 主要以调控胡桃楸靶基因的表达水平，进而调控其激素分泌而影响性别分化，并且形成了雌雄异型异熟这种由雌雄同株和雌雄异株之间的中间过度形态。该研究为最终发现胡桃楸性别分化和雌雄异型异熟的分子机制奠定了研究基础。

Identification and analysis of key miRNA for sex differention in hermaphroditic Juglans mandshurica Maxim

Abstract: Juglans mandshurica Maxim is a monoecious heterozygous heterozygous plant, divided into female-first and male-first types, which is a good economic and ecological tree species in Northeast China. miRNA are a class of endogenous eukaryotic non-coding RNAs, which have a silencing effect on their target genes in the plant. The mechanism of miRNA-mediated sex differentiation and dioecious heterozygosity in J.mandshurica is not clear. In this study, microRNA sequencing analysis was performed on male and female flower buds of the male and female-predominant types of J.mandshurica by using high-throughput sequencing technology. 12 sRNA libraries were constructed, and a total of 310 known and 278 unknown miRNAs were identified, and the length, family and other information of the miRNAs were counted. 129 significantly differentially expressed miRNAs were screened using the Deseq package. these differentially expressed miRNAs were hierarchically clustered. 638 miRNA target genes were predicted using PSRobots, and these target genes were analysed by GO and KEGG enrichment. these target genes were significantly enriched in reproductive, floral developmental and phytohormone signalling pathways, and it was speculated that miR159c-3p, miR156d-3p, miR172d, miR171b, miR164a, miR167a , miR319a, miR399a, miR396t may be the key genes affecting sex differentiation in J.mandshurica. miR164a, miR167c and miR171c-5p may be the key genes for the differentiation of J.mandshurica into male and female heterozygotes and heterozygotes. A predictive regulatory network was established by combining these key miRNAs and their target genes. Nine miRNAs with significant differences were randomly selected for real-time fluorescence quantitative PCR detection and jointly analysed with transcriptome sequencing results to establish a differential miRNA-different mRNA primary co-expression regulatory network. These miRNAs were further uncovered to play an

important role in sex differentiation in J.mandshurica, revealing the regulatory roles between miRNAs and some important transcription factors related to flower formation. It is hypothesised that miRNAs mainly affect sex differentiation by regulating the expression levels of target genes and hence hormone secretion in J.mandshurica, and form the intermediate over-expression morphology between monoecious and dioecious plants, such as dioecious and dioecious heterozygosity. This study lays the foundation for the discovery of the molecular mechanism of sex differentiation and dioecious heterosis in J.mandshurica.