

## 多组学整合分析揭示热响应微 RNA 参与调控高温胁迫下棉花 CMS-D2 恢复系雄性育性稳定性

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**摘要:** 哈克尼西棉细胞质雄性不育(CMS-D2)胞质是目前市场上推广种植的棉花“三系”杂交种的主要胞质来源。然而,棉花 CMS-D2 恢复系花药和花粉发育易受夏季持续高温胁迫影响,这严重阻碍了“三系”杂交种在生产上大规模应用。本研究选用 1 套同核异质近等基因恢复系 NH(耐高温)和 SH(以前命名为 ZBR,敏高温),通过对河南安阳和江西九江环境下 2 个材料成熟花粉的小 RNA、转录组、降解组和代谢组数据进行整合分析,探究高温胁迫下微 RNA(miRNA)在调控雄性育性稳定性中的作用。本研究共鉴定出 211 个已知的和 248 个新的 miRNA,其中 159 个是差异表达的 miRNA (DEM)。此外,还鉴定了 39 个 miRNA 簇(PmCs),包含 45 个 DEM;在极端高温胁迫下,大多数高表达的 miRNA,尤其是 4 个 MIR482 和 6 个 MIR6300 家族 miRNA 在 SH 中被显著诱导。miRNA 簇 PmC28 位于 *Rf<sub>1</sub>* 基因的精细定位区间,包含 2 个 DEM,即 *gra-miR482\_L-2R+2* 和 *gma-miR2118a-3p\_R+1\_1ss18TG*。转录组测序鉴定了 6 281 个差异表达基因,其中热激蛋白(HSP)相关基因,如 *HSP70*、*HSP22*、*HSP18.5-C*、*HSP18.2* 和 *HSP17.3-B* 在高温胁迫下的 SH 中均显著下调表达。通过整合多组学数据,还构建了 1 个参与调控高温胁迫下花粉发育的“miRNA-mRNA-gene-KEGG”复杂分子网络。该网络包含 35 对微 RNA/靶基因,其中 *mtr-miR167a\_R+1*、*tcc-miR167c* 和 *ghr-miR390a*、*tcc-miR396c\_L-1* 和 *ghr-MIR169b-p3\_1ss6AG* 可分别通过影响负责生长素信号转导的 ARF8、抗坏血酸和醛酸盐代谢以及糖和脂质代谢与转运途径来调控花粉育性稳定性。进一步结合代谢组数据,发现高温诱导的茉莉酸信号可以激活下游生长素合成相关基因的表达,并引起生长素的过度积累;随之级联的生长素信号转导,最终导致花粉败育。本研究结果为热响应 miRNA 如何在高温胁迫下调控棉花 CMS-D2 恢复系的花粉育性稳定性提供了新见解,同时对生产上解决 CMS-D2 恢复系花药发育对高温胁迫敏感的育种瓶颈具有重要实践意义。

**关键词:** CMS-D2 恢复系;雄性育性稳定性;miRNA 簇;高温胁迫;降解组;植物激素;信号转导

### Integrated multi-omics analysis reveals heat-responsive microRNAs participate in the regulation of male fertility stability in cotton CMS-D2 restorer line under high-temperature stress

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**Abstract:** Cytoplasmic male sterility (CMS) conditioned by *Gossypium harknessii* cytoplasm (CMS-D2) is

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the main source of cytoplasm for the "three-line" hybrid cotton varieties currently being promoted and planted in the market. However, the anther and pollen development of cotton CMS-D2 restorer line is susceptible to continuous high-temperature (HT) stress in summer, which seriously hinders the large-scale application of "three-line" hybrids in production. Here, integrated small RNA, transcriptome, degradome, and metabolome data analysis was performed to explore the roles of microRNA (miRNA) in regulating male fertility stability in mature pollens of isonuclear alloplasmic near-isogenic restorer lines NH (HT-tolerant) and SH (Previously named ZBR, HT-sensitive) under HT stress at Anyang, Henan and Jiujiang, Jiangxi environments. A total of 211 known and 248 novel miRNA were identified, of which 159 were differentially expressed miRNA (DEM). Additionally, 45 DEM in 39 miRNA clusters (PmCs) were also identified, and most highly expressed miRNA were significantly induced in SH under extreme HT, especially four MIR482 and six MIR6300 family miRNA. PmC28 was located in the fine-mapped interval of the *Rf<sub>1</sub>* gene and contained two DEM, *gra-miR482\_L-2R+2* and *gma-miR2118a-3p\_R+1\_1ss18TG*. Transcriptome sequencing identified 6 281 differentially expressed genes, of which heat shock protein (HSP)-related genes, such as *HSP70*, *HSP22*, *HSP18.5-C*, *HSP18.2* and *HSP17.3-B*, presented significantly reduced expression levels in SH under HT stress. Through integrating multi-omics data, we constructed a comprehensive molecular network of miRNA-mRNA-gene-KEGG containing 35 pairs of miRNA/target genes involved in the regulation of pollen development in response to HT, among which the *mtr-miR167a\_R+1*, *tcc-miR167c*, and *ghr-miR390a*, *tcc-miR396c\_L-1* and *ghr-MIR169b-p3\_1ss6AG* regulated the pollen fertility stability by influencing *ARF8* responsible for the auxin signal transduction, ascorbate and aldarate metabolism, and the sugar and lipid metabolism and transport pathways, respectively. Further combination with metabolome data revealed that HT-induced jasmonic acid signaling could activate the expression of downstream auxin synthesis-related genes and cause excessive auxin accumulation, followed by a cascade of auxin signal transduction, ultimately resulting in pollen abortion. The results obtained in this study provide a new understanding of how heat-responsive miRNA regulate the pollen fertility stability of cotton CMS-D2 restorer line under HT stress, and has important practical significance for solving the breeding bottleneck in which the anther development of CMS-D2 restorer line is sensitive to HT stress in production.

**Keywords:** CMS-D2 restorer line; male fertility stability; miRNA clusters; high-temperature stress; degradome; plant hormone; signal transduction