

# Transcriptome Analysis of Isolated Microspores in *Brassica Napus* Under Heat Shock Treatment

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## Abstract

Microspore embryogenesis is one of the most important approaches for generating pure lines in crop genetic breeding and molecular biology studies. Stress treatment plays a critical role in inducing microspore embryogenesis. In *Brassica napus*, high-temperature heat shock at 32 °C can effectively reprogram microspores, redirecting them from gametophytic development toward a sporophytic pathway. This study used the highly embryogenic *B. napus* line "X02" as donor parent, isolated microspores were subjected to 32 °C heat shock treatment, and samples were collected at 0day(E0), 1day(E1), and 2day (E2) for transcriptome analysis, aiming to identify genes associated with early embryogenesis under heat shock. Pairwise comparisons of gene expression levels across the three heat shock durations (E0 vs. E1, E0 vs. E2, and E1 vs. E2) identified 3,652, 4,785, and 1,359 differentially expressed genes (DEGs), respectively. GO enrichment analysis indicated that the DEGs under heat shock were significantly enriched in several cellular components, including the apoplast, vacuole, and cytoskeleton. At the molecular function level, these DEGs showed notable enrichment in antioxidant activity, peroxidase activity, and pectinesterase activity. Furthermore, the biological processes most associated with these DEGs included cell wall organization, pectin catabolic processes, oxidative stress response, and auxin-activated signaling pathways. KEGG pathway analysis indicated that the DEGs were enriched in ascorbate and aldarate metabolism, MAPK signaling pathway-plant, starch and sucrose metabolism, and zeatin biosynthesis. Further analysis of genes related to ascorbate and aldarate metabolism showed that, compared to 0day, microspores subjected to 1- or 2-day heat shock exhibited significant up-regulation in the expression of aldehyde dehydrogenase (NAD<sup>+</sup>), L-ascorbate oxidase, and UDP-glucuronate 4-epimerase genes.

## Keywords

Microspore Embryogenesis, Heat Shock, Transcriptome Analysis