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University, Lanzhou, China**Pathways related to ROS production, clearance and signal transduction during cold response in *Brassica napus* L. with strong cold resistance****Background:**

Brassica napus L. has been considered an important cash crop and oil crop. However, it is difficult to popularise and apply *B. napus* in north China because of its weak cold resistance.

Objective:

To clarify the mechanism of cold stress on gene regulation and signal transduction in *B. napus*.

Methods:

We performed the transcriptome sequencing and gene expression, Go and KEGG pathways profiling was analysed under the natural (25°C) and cold (4°C) conditions of cold tolerant 16VHNTS309 and cold sensitive Tianyou 2238 of *B. napus* seedlings. Subsequently, more complete genomic annotation was obtained by sequencing.

Results:

A total of 6127 and 8531 differentially expressed genes (DEG) were identified in *B. napus* of 16VHNTS309 and Tianyou 2238, respectively. Analysis of the expression patterns of 23 differentially expressed genes (DEGs) by quantitative real-time PCR (qRT-PCR) confirmed the accuracy of the RNA-Seq results. Under cold stress, 58 pathways in strong cold-resistant *B. napus* 16VHNTS309 showed significant changes (qValue <0.05), while 9 pathways in weak cold-resistant *B. napus* Tianyou 2238 showed significant changes (qValue <0.05). This indicated that *B. napus* has a complex and ingenious network regulation system, which could coordinate various problems encountered in the process of plant growth and development to a certain extent. After 48h of cold stress treatment, several genes related to reactive oxygen species (ROS) clearance, such as antioxidant VB6, sulphur metabolism, peroxisome, and phagosome, were significantly up-regulated in *B. napus* 16VHNTS309, suggesting that *B. napus* 16VHNTS309 has a strong ROS clearance ability.

Conclusions:

There were significant expressions of several genes in the signal pathway of Ca²⁺, MAPK and transcription factors related to ROS, which further suggested that the varieties with strong cold resistance had complex signal regulation mechanism. A comprehensive analysis of stomatal cell results, physiological parameters of ROS, ABA and H₂S, and transcriptomic data showed that appropriate ROS interacted with ABA and H₂S to regulate stomatal closure in *B. napus* 16VHNTS309 under the scavenging action of antioxidant enzymes.