Representative temperature responses in *Cnidium officinale* leaves by higher temperature treatment

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INTRODUCTION

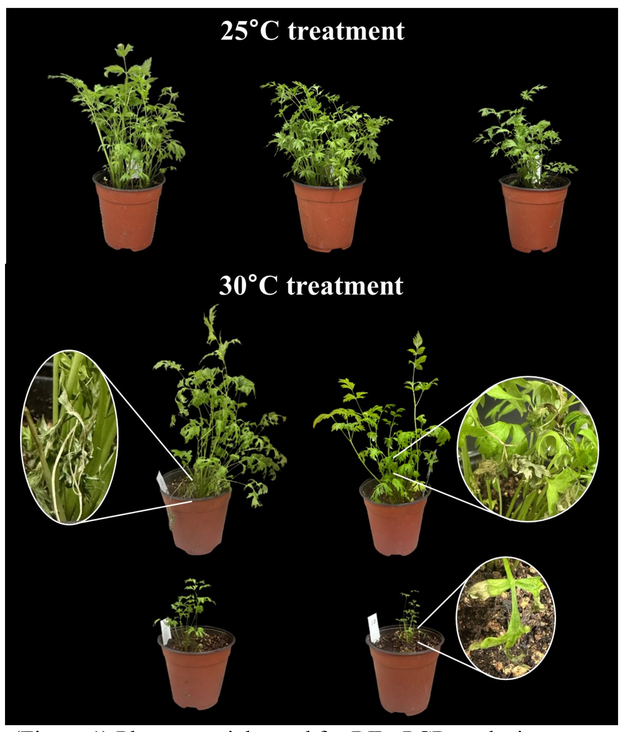
Traditional medicinal plant *Cnidium officinale* (CO) has low resistance to environmental factors such as temperature, making its suitable temperature range for cultivation quite narrow. Due to climate change, production and quality of CO could be significantly affected, making it increasingly difficult to ensure a stable supply. Therefore, it is necessary to molecularly understand the mechanism by which CO responds to higher temperatures. In this study, we analyzed the representative temperature response to establish foundational data for tracking physiological changes induced by higher temperatures.

MATERIAL & METHOD

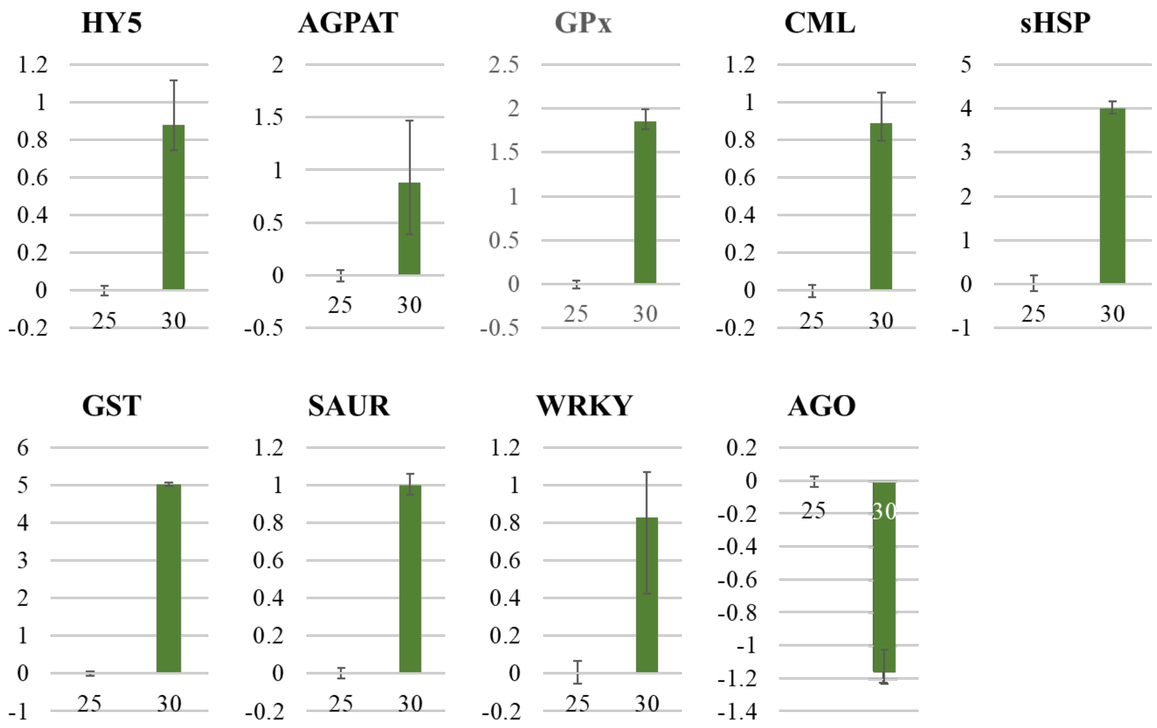
In this study, we utilized the rhizome of CO, obtained from the National Institute of Horticultural Science. After approximately one month of growth under the conditions of 25°C and at 16/8h(light/dark) photoperiod condition, we initiated the high temperature treatment. The high temperature treatment started at 25°C and increased gradually by 1°C every two days until reaching 30°C. Total RNA from the leaves of CO was extracted using Trizol Reagent(Invitrogen, USA), and reverse transcription polymerase chain reaction(RT-PCR) was performed using AccuPower® CycleScript™ RT Master Mix (Bioneer, Korea), qPCR was conducted using AccuPower® 2X GreenStar™ qPCR Master Mix(Bioneer, Korea).

The plant materials used for DEG analysis were collected from CO leaves, which cultivate at temperatures elevated by 1°C, 3°C, and 5°C compared to the air temperature. For the DEG analysis, quantile normalization was applied to the read counts to minimize the potential skewness of the data due to differing library sizes(Zhang, 2020). Following quantile normalization, the analysis of differentially expressed genes was conducted.

RESULTS AND CONCLUSION



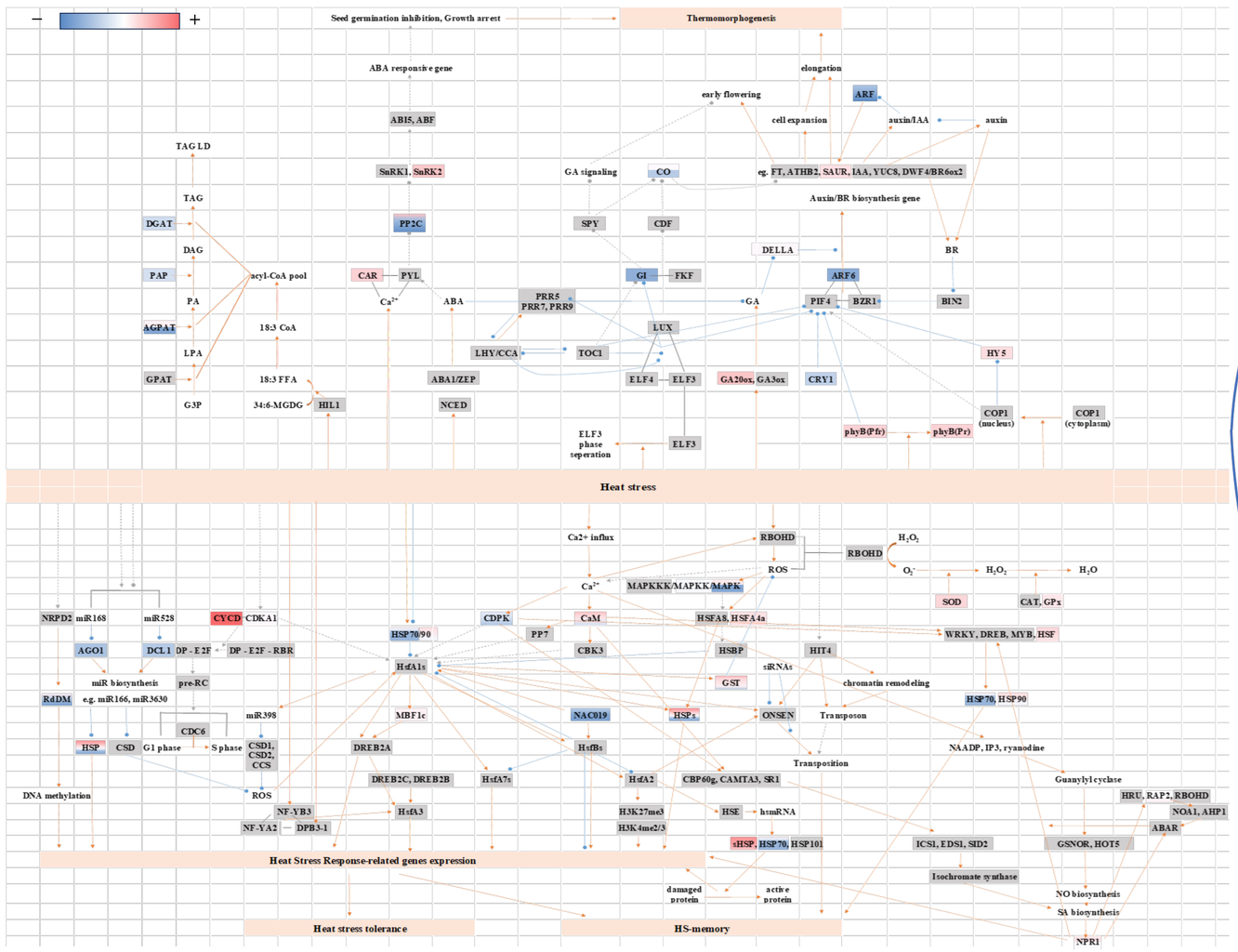
(Figure 1) Plant materials used for RT-qPCR analysis



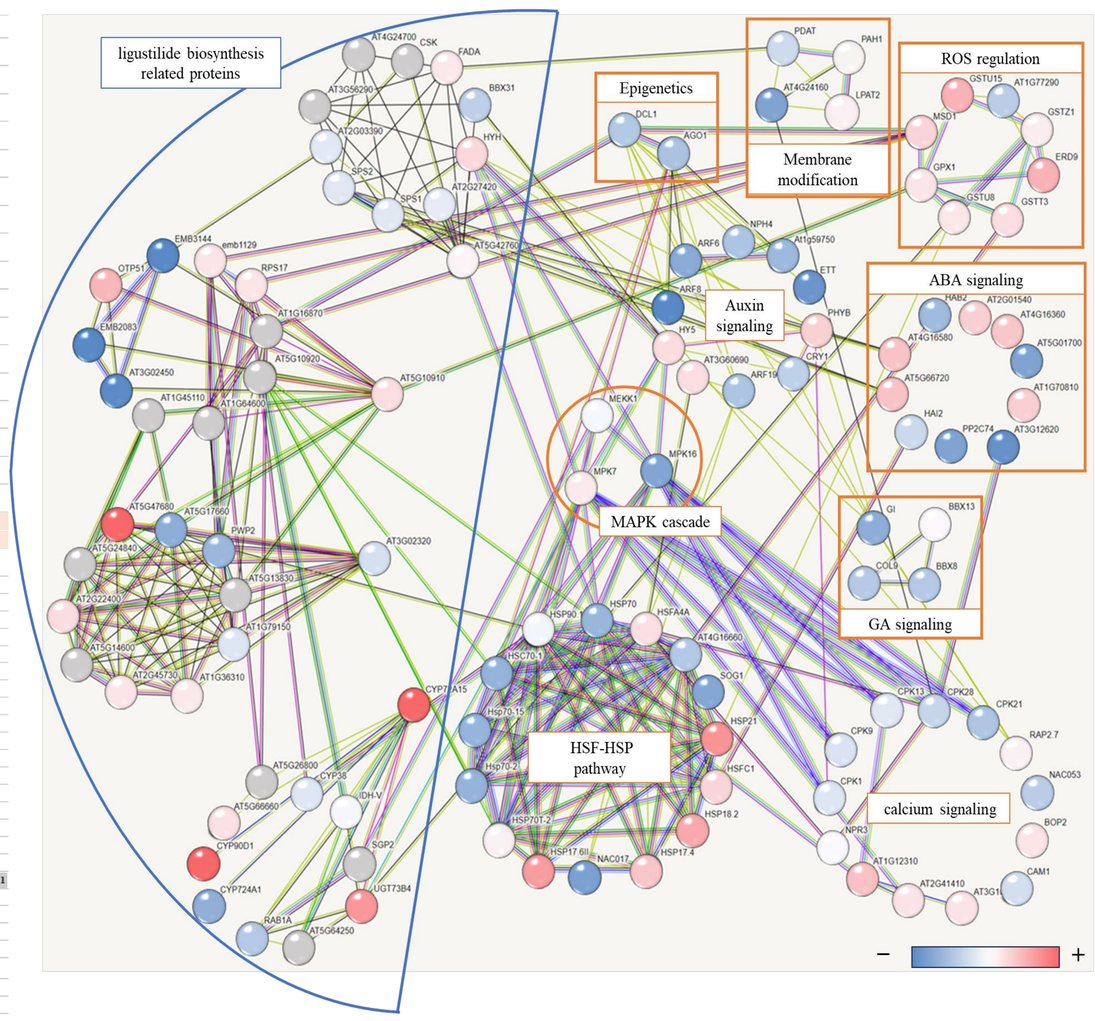
(Figure 2) Expression patterns of CO(*Cnidium officinale*) representative heat-related gene by quantitative RT-PCR. We used CO leaves growing at different elevated temperatures up to 5°C compared to the air temperature(Fig. 1).

When the plants are under heat stress, pathways such as Ca^{2+} signaling, MAPK cascade, ROS (reactive oxygen species) regulation, hormone signaling, HSF(heat shock factor)-HSP(heat shock protein) pathway, membrane modification, cell cycle regulation, epigenetic mechanism act in combination for responding to stress.

Changes in expression of key genes in the high-temperature stress response mechanism were tracked with qRT-PCR. HY5, AGPAT, GPx, CML, sHSP, GST, SAUR, and WRKY genes were up-regulated and AGO was down-regulated(Fig. 2).



(Figure 3) Outline of representative projected responses in plants under heat stress condition. Arrows denote activation or up-regulation, while lines with circle ends indicate inhibition or down-regulation. Dashed gray lines represent the predicted pathways. As the temperature increased, genes with increased expression are represented in red, while down-regulated genes are represented in blue.



(Figure 4) STRING protein-protein interaction network of heat response proteins and ligustilide biosynthesis related proteins in CO. By using the contig sequences of CO included in high temperature stress response mechanism. as query, the proteins with homologous sequences in Arabidopsis was found. They were used as inputs. As the temperature increased, genes with increased expression are represented in red, while down-regulated genes are represented in blue.

We observed alternations in an expression of genes involved in those reactions in the CO transcript database (cobi.knu.ac.kr/projects/herbalplant/). Under higher temperature conditions, most of the genes related to ROS regulation were upregulated. In contrast, the majority of genes associated with auxin and gibberellin signal transduction, as well as miRNA synthesis were downregulated. Taking into account the function of genes and their expression patterns, we predicted changes in the mechanism of the heat stress response. In CO, as the temperature rises, it is predicted that ROS scavenging processes and ABA signaling become fortified and gibberellin signal transduction, miRNA(microRNA) biosynthesis, and TAG(triacylglycerol) biosynthesis processes are inhibited. The mechanisms of heat stress responses are linked to the biosynthesis of ligustilide, a key active ingredient in CO, and it is predicted that changes in ligustilide content will occur due to the heat stress pathway as temperatures rise.

REFERENCE

Zhao, Yaxing, Limsoon Wong, and Wilson Wen Bin Goh. "How to do quantile normalization correctly for gene expression data analyses." Scientific reports 10.1 (2020): 15534.

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