Transcriptome Analysis Reveals Regulatory Framework for Salt and Drought Tolerance in *Hibiscus hamabo* Siebold & Zuccarini

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Key messages

Hibiscus hamabo Siebold & Zuccarini (*H. hamabo*) is tolerant to salt and drought conditions, but the molecular mechanisms that underlie this stress tolerance remain unclear. In this study, the transcriptome of *H. hamabo* roots was investigated under NaCl or PEG treatment. A total of 20,513 and 27,516 significantly changed known genes at 6 h and 24 h, respectively, were detected be-tween the salt or drought treatments and the control libraries. Among these, there were 3,845 and 7,430 overlapping genes under the two stresses at 6 h and 24 h, respectively. Based on the analysis of enriched KEGG pathways and clustering of expression patterns, the DEGs that were continuously up- or down-regulated under both salt and drought treatments were mainly enriched in MAPK signaling pathway, transcription factors, transporters and other pathways. The transcriptome expression profiles of *H. hamabo* provide a genetic resource for identifying common regulatory factors involved in responses to different abiotic stresses. In addition, the identified factors may be useful to developing genetic breeding strategies for the Malvaceae.

Materials and Methods

➢ **Sampling**

The roots of the seedlings exposed to the PEG (PEG 6000) or NaCl (200 mM NaCl) treatments for 0, 6, and 24 h were sampled.

➢ **Illumina Sequencing for Transcriptome Analysis**

The library preparations were sequenced on an Illumina HiSeq 2500 system.

➢ **Bioinformatic Analysis of RNA-Seq Data**

Principal component analysis (PCA) was performed using the “principal” function in R software (v. 3.0.1).

Gene expression pattern analysis was performed using Short Time-series Expression Miner software (STEM) on the OmicShare tools platform.

The functional analysis of the DEGs was performed using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

➢ **Quantitative Real-Time PCR (RT-qPCR)**

The relative gene expression level was calculated using the $2^{-\Delta\Delta Ct}$ method.

Result

➢ **Summary of *H. hamabo* Sequences**

Sample	Clean Reads Pairs	Clean Base (bp)	Length	Q20 (%)	Q30 (%)	GC (%)
CK_1	33,187,452	9,956,235,600	150;150	98.1;97.6	94.5;93.1	48.7;48.8
CK_2	41,835,177	12,550,553,100	150;150	98.1;97.4	94.5;92.4	48.1;48.1
CK_3	29,667,431	8,900,229,300	150;150	98.0;97.6	94.3;93.0	46.1;46.2
P6_1	27,041,273	8,112,381,900	150;150	97.6;96.7	93.0;90.9	45.2;45.3
P6_2	28,359,168	8,507,750,400	150;150	97.7;96.9	93.3;91.3	46.9;46.9
P6_3	27,797,544	8,339,263,200	150;150	97.7;96.9	93.3;91.4	48.0;48.0
P24_1	30,032,491	9,009,747,300	150;150	97.7;96.7	93.5;90.9	46.6;46.6
P24_2	28,182,367	8,454,710,100	150;150	97.6;96.8	93.2;91.2	46.4;46.4
P24_3	28,148,479	8,444,543,700	150;150	97.5;96.7	92.9;90.8	45.3;45.4
CL6_1	29,023,247	8,706,974,100	150;150	97.7;97.3	96.3;95.6	49.4;49.4
CL6_2	34,393,028	10,317,908,400	150;150	97.4;95.8	96.0;93.6	47.7;47.8
CL6_3	27,607,372	8,282,211,600	150;150	97.7;97.1	96.3;95.4	46.4;46.4
CL24_1	28,320,012	8,496,003,600	150;150	97.5;96.4	96.1;94.4	46.8;46.9
CL24_2	33,286,710	9,986,013,000	150;150	97.8;97.2	96.5;95.5	48.2;48.2
CL24_3	37,889,245	11,366,773,500	150;150	97.7;96.8	96.4;95.0	48.6;48.6

Note: The numbers 1–3 after CK, P6, P24, CL6 and CL24 identify the three independent biological replicates for the control, salt treatment for 6 and 24 h and drought treatment for 6 and 24 h, respectively.

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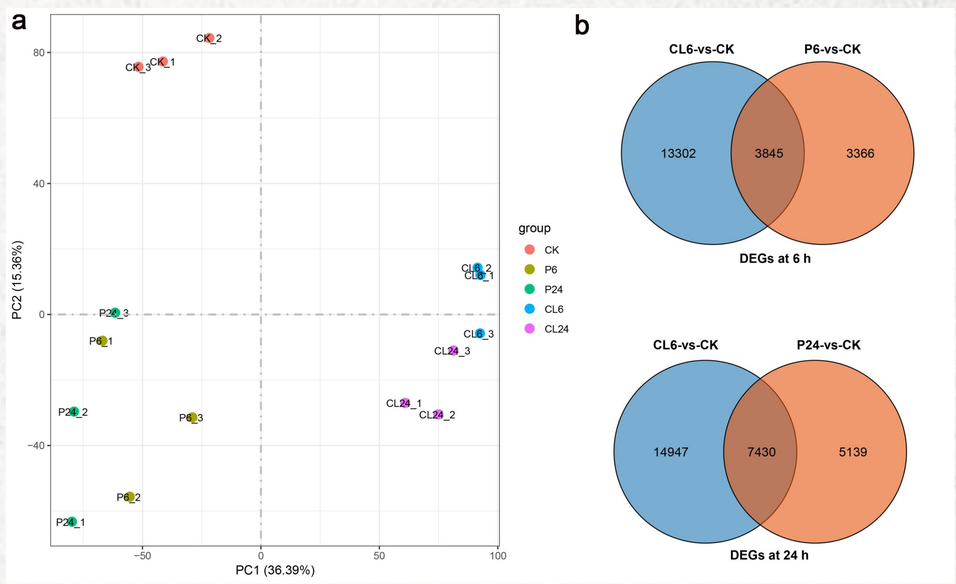
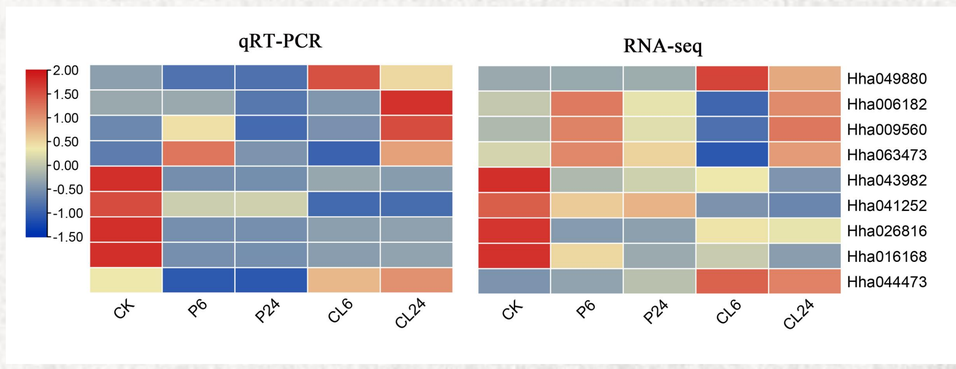
Roles: formal analysis, investigation, writing-original draft preparation, writing-review & editing.

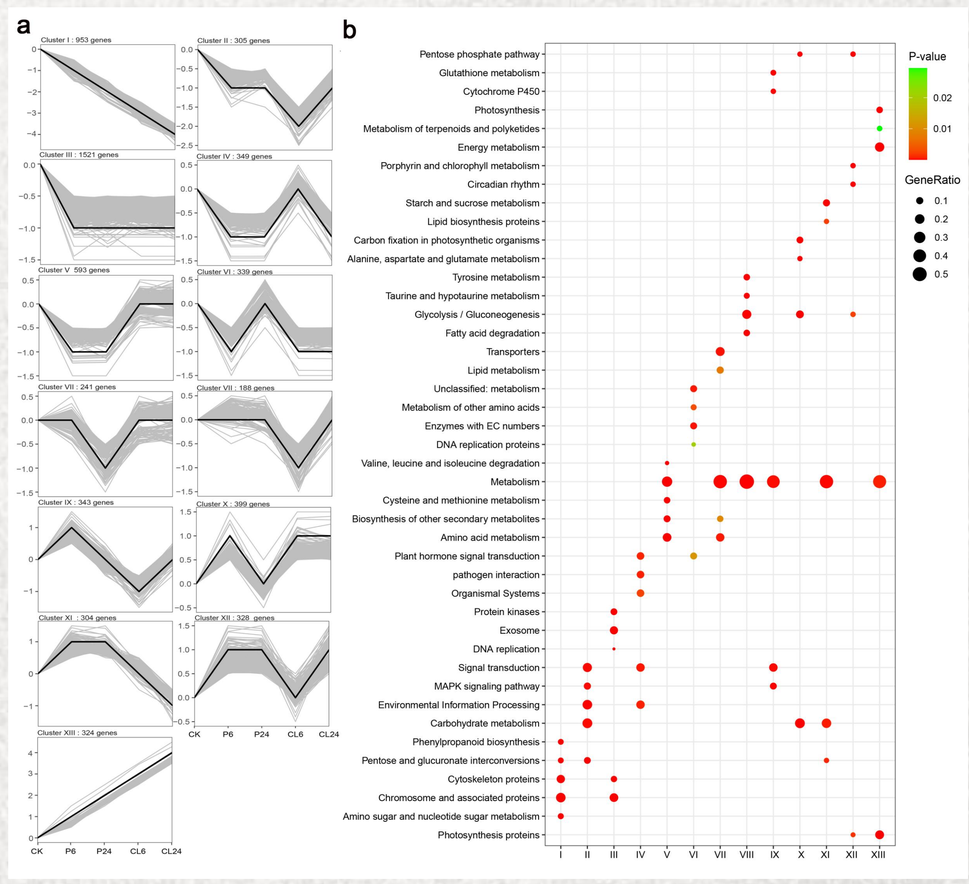
➢ **Global Comparisons of Salt- and Drought-Treated Transcriptomes**



➢ **Validation of Gene Expression Levels with RT-qPCR Analysis**



➢ **Time Course RNA-seq Analysis in Response to Salt and Drought**



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