Quantitative Proteomic Revealed Metabolic Changes of Jasmonic Acid in Regulating the Response of *Malus baccata* (L.) Borkh. Roots to Low Root-Zone Temperature

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Abstract

Malus baccata (L.) Borkh. is widely used as an apple rootstock in northern China. Previous studies have shown that jasmonic acid (JA) can alleviate the oxidative damage in *M. baccata* roots under low root-zone temperature (LT), yet the regulatory mechanism is unclear. To uncover the mechanism of JA in regulating the response of roots to LT, the differential expression of proteins was profiled using TMT combined with MRM. The quantitative data were then confirmed using MRM, thereby proving that the TMT results were reliable. The results can provide theoretical reference for the regulation of apple roots under low-temperature stress.

Experimental Design

The experiment was conducted in an artificial climate room with a day/night temperature regime of 18/8 ° C and a photoperiod of 14h light/10h dark. The low root-zone temperature (LT) treatment involved maintaining the roots of experimental plants at 5℃. Plant roots maintained under the same room temperature conditions, but without soil cooling, were used as controls. Jasmonic acid (JA) was dissolved in 1 mL of 100% ethanol, and diluted with distilled water to a stock concentration of 150 μM. In the JA treatment group, a group of plants (15 seedlings) was watered with 150 μM JA for 12 h and then exposed to LT (5 ℃) for 24 h.

Results

Of the 4979 proteins quantified, 403 differentially expressed proteins (DEPs) responded to the LT treatment and 264 DEPs responded to the JA+ LT treatment (Fig. 1).

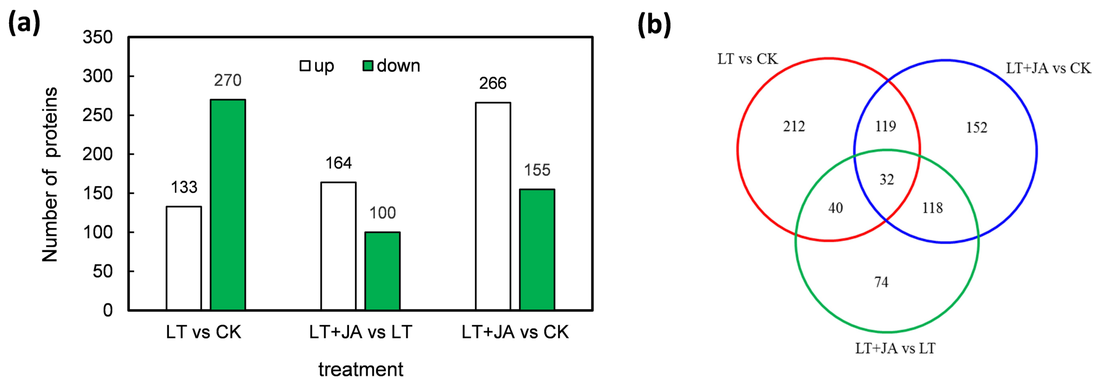

Fig. 1 Functional classifications of differentially regulated proteins. a The numbers of upregulated and downregulated proteins; b a venn diagram of differentially regulated proteins. CK, control treatment; LT, low root-zone temperature (5 ℃); LT+JA, 5 ℃ +JA

Most of the DEPs were involved in processes of endoplasmic reticulum protein processing, the ribosome, amino acid biosynthesis, plant–pathogen interaction, carbon metabolic, etc. Furthermore, the application of JA resulted in the regulation of these metabolic pathways on a different level (Table 1).

Table. 1 KEGG pathway of the most differentially expressed proteins

Pathway	number of proteins			Pathway ID
	LT vs CK	LT+JA vs LT	LT+JA vs CK	
Protein processing in endoplasmic reticulum	24	14	8	ko04141
Ribosome	21	6	8	ko03010
Biosynthesis of amino acids	21	12	20	ko01230
Plant–pathogen interaction	20	10	11	ko04626
Carbon metabolism	20	12	17	ko01200
Phenylpropanoid biosynthesis	13	8	17	ko00940
Glutathione metabolism	12	5	13	ko00480
Flavonoid biosynthesis	11	4	10	ko00941
Starch and sucrose metabolism	11	6	7	ko00500
Endocytosis	11	8	10	ko04144
Spliceosome	10	7	6	ko03040
Glycolysis / Gluconeogenesis	10	5	10	ko00010
Amino sugar and nucleotide sugar metabolism	6	6	10	ko00520
alpha-Linolenic acid metabolism	3	7	9	ko00592

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The DEPs were further analyzed by hierarchical cluster. It was found that protein processing in the endoplasmic reticulum, alpha-linolenic acid metabolism, and secondary metabolites play key roles in the regulation of JA in M. baccata roots exposed to LT.

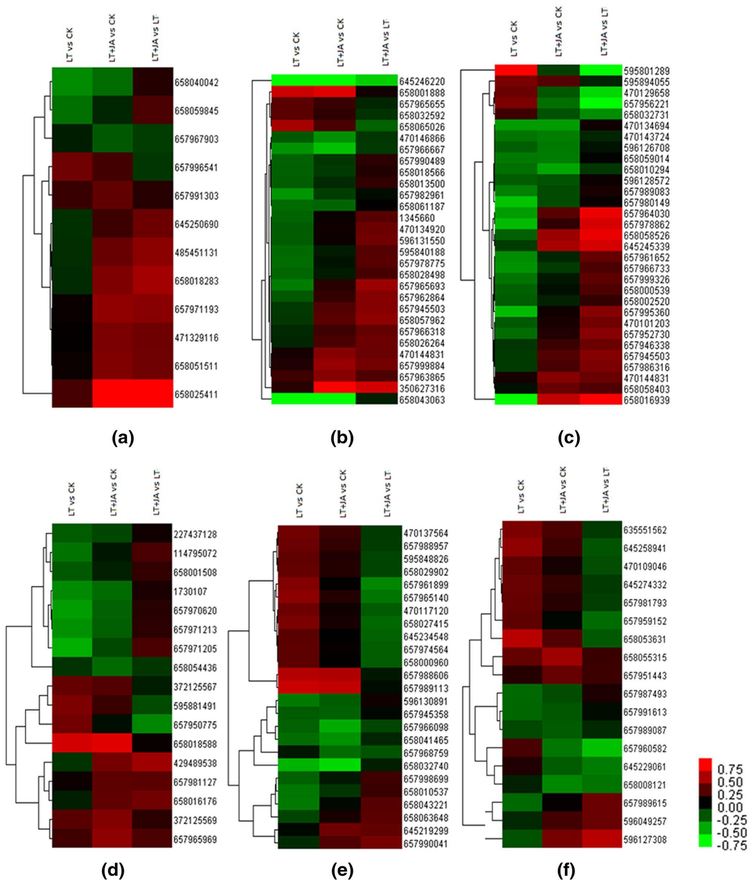

Fig. 2 Hierarchical clustering analysis of the relative abundance profiles for *Malus baccata* (L.) Borkh. roots in response to LT and LT +JA treatment with different functions. a: Proteins involved in alpha-linolenic acid metabolism; b: Proteins related to plant–pathogen interaction; c: proteins related to protein processing in the endoplasmic reticulum; d proteins related to flavonoid biosynthesis; e: proteins related to the ribosome; and f: proteins involved in starch and sucrose metabolism.

The quantitative data were then confirmed using MRM (Fig 3), thereby proving that the TMT results were reliable.

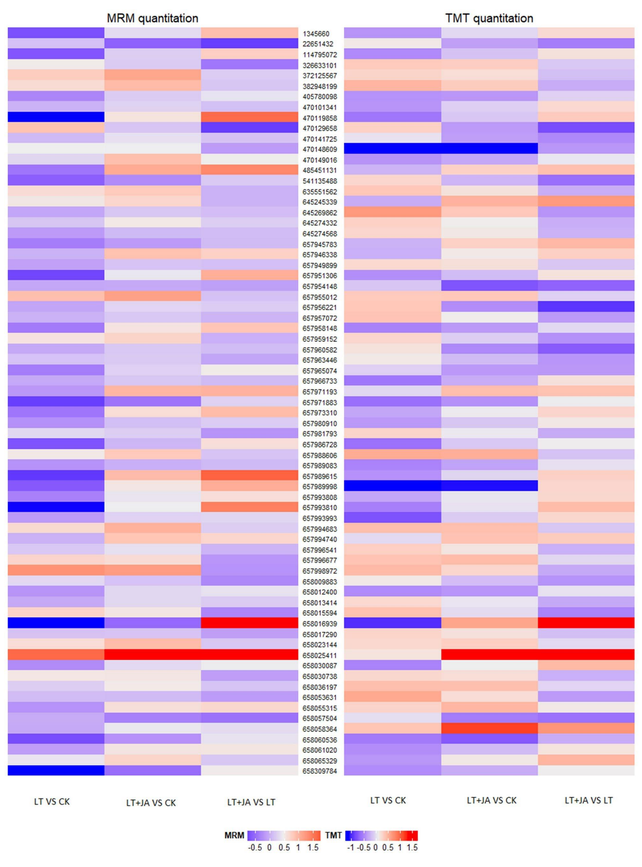

Fig. 3 Heat map showing the changes in abundance of 71 differentially expressed proteins in response to the different treatments, as measured using multiple reactions monitoring (MRM; left panel) and tandem mass tag (TMT) labeling (right panel).

Conclusions