Glutathione peroxidases act as important antioxidant regulators in response of mulberry to drought stress

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Background

Mulberry (*Morus alba* L.) is an economically important food crop for the domesticated silkworm, *Bombyx mori* in China and some other Asian countries for more than 5000 years (http://khartasia-crcc.mnhn.fr/en/content_en/morus-alba-l). However, little is known about molecular mechanism underlying mulberry response to environmental stresses., In this study, isobaric tags for relative and absolute quantification (iTRAQ)-based quantitative proteomics combined with physiological analysis and gene function validation was used to elucidate the changes of proteome in mulberry.

Methods

One-year-old seedlings of mulberry (Neo-Ichinose, Japan) were used as materials. For drought stress, mulberry seedlings were treated by withholding water for 11 days. Contents of malondialdehyde, free proline, soluble sugar, soluble proteins, total chlorophyll and relative water content (RWC) were determined to evaluate physiological responses of mulberry seedling after drought stress. The iTRAQ-

based proteomics were performed using a customer service by Jingjie PTM-Biolabs Co., Ltd (Hangzhou, China). The MS data were analyzed using Mascot Server (http://www. matrixscience.com/), followed by database searching and bioinformatics analysis. The relative quantitation of the proteins was divided into two categories. Quantitative ratio (drought/control) over 1.3 was considered up-regulation, whereas quantitative ratio of less than 1/1.3 (0.77) was considered down-regulation (One-sample *t*-test, $P < 0.05$). To elucidate the functions of mulberry GPX genes in *Arabidopsis* response to drought stress, transgenic lines (OE-MaGPX1, OE-MaGPX2, OE-MaGPX3, OE-MaGPX4, OE-MaGPX5 and OE-MaGPX6) were constructed and subjected to drought stress, then we investigated the activities of antioxidant system and ROS accumulation in the OE transgenic plants.

Results (up to 4 figures and tables can be included)

Compared with that of control, the mulberry seedlings displayed a drought stress phenotype after 11 days of withholding water. The physiological data indicated that drought stress significantly repressed mulberry seedling growth and development through reducing water transport, photosynthesis and carbohydrate metabolism. A total of 2908 proteins in mulberry leaves were quantified using iTRAQ-labeled LC-MS/MS, of which 265 differentially expressed proteins (DEPs) were identified. Notably, the proteomic profiles associated with cellular redox and antioxidant system were extensively changed by drought stress (**Fig.1**). Some thiol-dependent antioxidant enzymes especially the glutathione peroxidase (GPX) proteins, acting as scavenger of reactive oxygen species (ROS), were extensively up-regulated, whereas another important ROS scavenger, ascorbate peroxidase (APX) was significantly decreased under drought stress. Given that mulberry genome encodes six GPX isoforms, five of them were significantly induced by drought stress, indicating a significant role of GPX in response to drought (**Fig.2**). The proteomic data was further confirmed by analyses of gene expressions and antioxidant enzymes activities. These results demonstrated that GPX rather than APX for ROS-scavenging

modulates mulberry response to drought stress. Furthermore, the overexpression of mulberry MaGPX3 and MaGPX5 in Arabidopsis led to comprehensive enhancement of antioxidant system and ROS-scavenging capacity and drought tolerance in the transgenic plants (**Fig.3**).

Conclusion

Taken together, these results imply that mulberry GPX genes can enhance plant tolerance to drought stress through activating the antioxidant system for ROS scavenging.

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References (No more than 15 references)

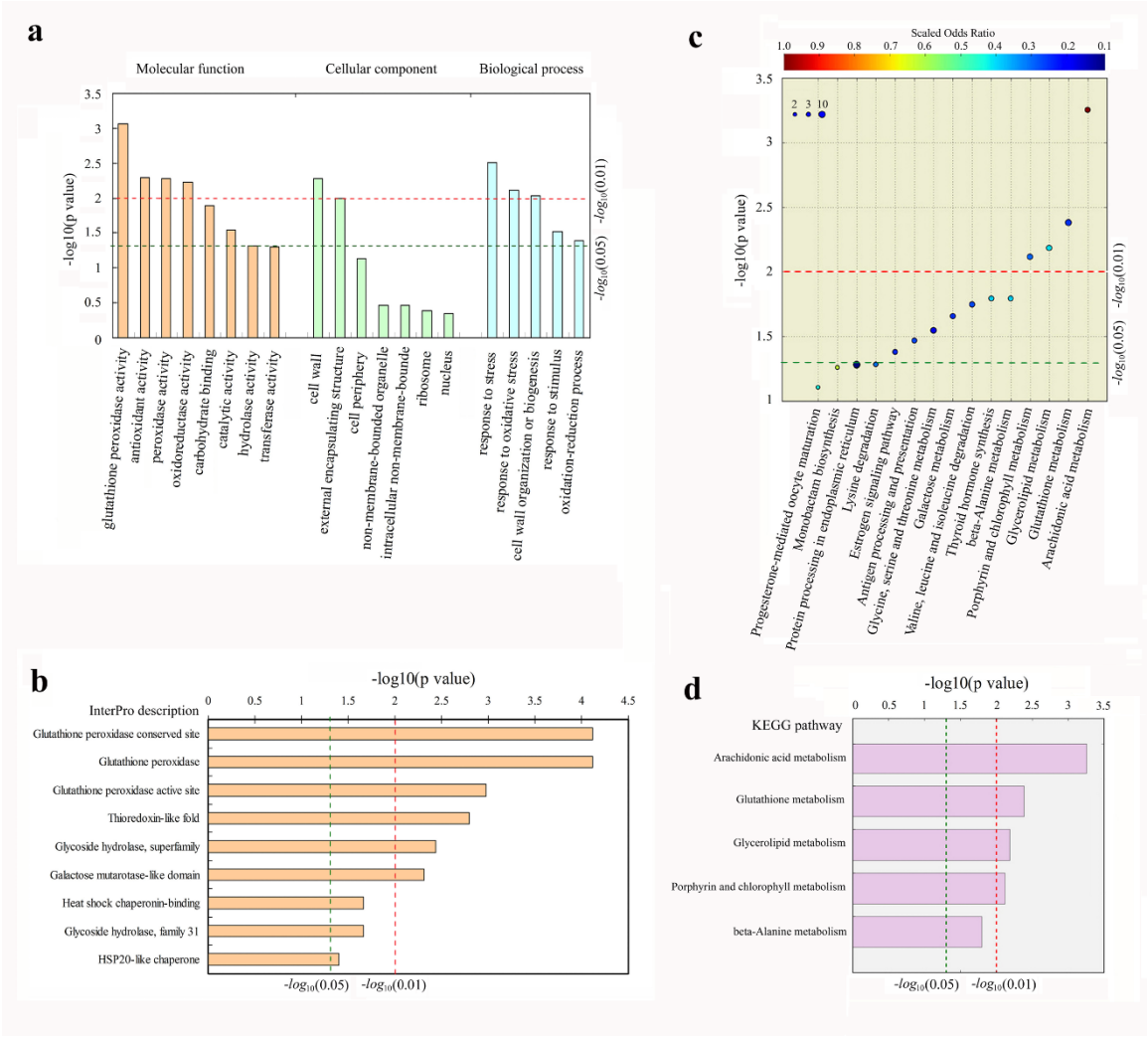

Fig.1 Functional enrichment analyses of the DEPs from iTRAQ-based quantitative proteomics. (a) GO enrichment; (b) InterPro protein domain enrichment; (c) KEGG pathway enrichment; (d) The top 5 most enriched KEGG pathways. Fisher's exact tests were used to calculate the significance. The $P < 0.05$ represents significant differences while $P < 0.05$ represents very significant differences.

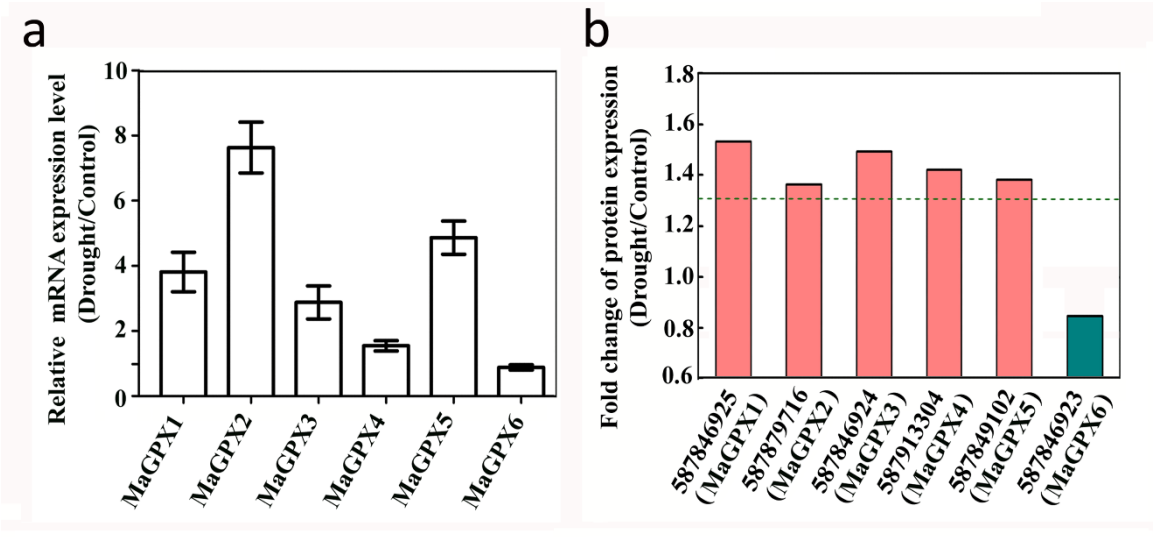

Fig.2 The expression levels of MaGPX genes and proteins in mulberry under drought stress. **(a)** Relative mRNA levels of *MaGPX1*, *MaGPX2*, *MaGPX3*, *MaGPX4*, *MaGPX5*, and *MaGPX6* in the leaves of mulberry seedlings. The data represents the ratio between drought-treated sample and control (no drought). The qRT-PCR was performed with three biological replicates each with three technical replicates. **(b)** Fold change of protein levels of MaGPX1, MaGPX2, MaGPX3, MaGPX4, MaGPX5, and MaGPX6 in mulberry under drought stress. The data are selected from iTRAQ-based quantitative proteomics.

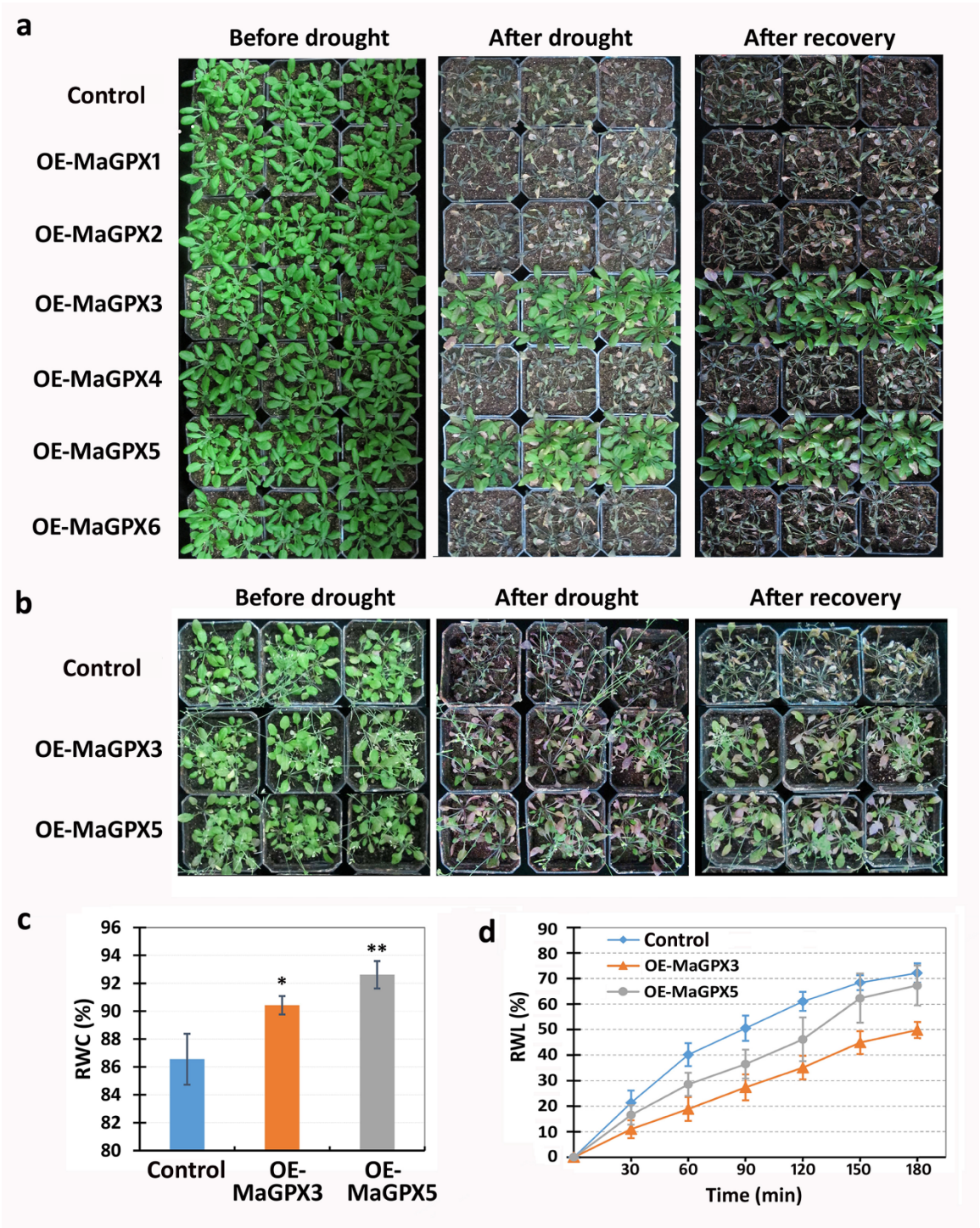

Fig.3 Overexpression of *MaGPX3* and *MaGPX5* enhanced the drought tolerance in transgenic *Arabidopsis*. (**a**) Drought tolerance of transgenic *Arabidopsis* overexpressing mulberry GPX genes (OE-MaGPX1, OE-MaGPX2, OE-MaGPX3, OE-MaGPX4, OE-MaGPX5 and OE-MaGPX6) at the seedling stage. Phenotypes of the control and OE transgenic lines before drought (3-week-old plants), after withholding water for 14 days, and after recovery for 5 days are shown, respectively. Bar = 4cm.