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Citric Acid-mediated Regulation of Oxidative Stress in *Brassica napus* L. under Copper Stress using Proteomics

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[Introduction]

Copper (Cu), as an essential micronutrient for plants, plays key roles in the citric acid (CA) cycle, pyruvate metabolism, and cell wall metabolism. However, excess Cu induces phytotoxicity, leading to growth inhibition, stunting, leaf chlorosis. Understanding of plants responses to heavy metal stress might help to develop effective detoxification measures. Although the tolerance to Cu stress has been studied extensively various way like genetic level, candidate genes associated with heavy metal detoxification or tolerance response have been identified, the underlying mechanisms remain unclear.

[Materials and Methods]

Seeds of *B. napus* were surface sterilized, placed in petri dishes containing two layers of filter papers moistened with de-ionized water. After 28-days old seedlings were exposed to different concentrations of Cu (25 and 50 μ M CuSO₄) with CA (1.0 mM) and without CA application. After 7 days of Cu-treatment, the seedling leaves were harvested and morpho-physiological, and the proteome analysis were performed using gel-free proteome techniques.

[Results and Discussion]

This study aimed to examine the proteome changes of *B. napus* L. under Cu stress and CA application for 7 days. The fresh and dry weights of Cu + CA treated seedlings were higher than those of the Cu treated. The CA enhanced the activities of antioxidant enzymes as compared with Cu treated seedling leaves. Using the gel-free proteome approach, a total of 2004 proteins were identified in *B. napus*. However, a total of 552 common proteins were identified of which 426 differentially abundant proteins were identified. The gene ontology analysis showed that the proteins with increased abundance were mainly associated with energy metabolism, detoxification and stress defense and protein metabolism, whereas the proteins related to the cell growth/division, and photosynthesis were downregulated. Protein-protein interaction analyses highlighted an energy metabolism centered sub-network that synergistically responded to Cu stress. Overall, this study provided new insights into the molecular mechanisms of plant response to Cu at the protein level in Brassica.

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