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Morpho-physiological and Proteome Analysis of Citric acid Induced Phytoremediation under Copper Stress in *Brassica napus* L.

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[Introduction]

Phytoremediation, one of popular way, has low cost and environment friendly nature as compared with chemical ways. Various hyperaccumulator plants can perform phytoremediation, especially *Brassica*. Among *Brassicaceae* canola has advantages such as big canopy, short period of growth and heavy metal tolerance. Citric acid (CA) is one of organic chelating which can enhance phytoremediation and has more biodegradability and environment friendly nature against chemical chelating. Copper (Cu) is essential nutrient but excess Cu may cause chlorosis and root is restrained. In this study, we investigate the potential role of CA increasing phytoremediation of Cu and effect of CA induced phytoremediation of *brassica napus* L. on physiological, biochemical and protein changes under Cu stress.

[Materials and Methods]

The seeds (Jungmo 7001) collected from Mokpo Korea were used. The seeds were germinated in growth chamber at 25~23°C for 3 days. Weak seedlings were discarded, others were grown for 4 days. The seeds were washed by distilled water (DW). After 4 days, the uniformed seedlings were transferred into a box containing Hoagland solution. The solution was changed every 3 days. After two weeks, the various concentration of Cu and CA were treated on plants for 7 days. The plants were exposed to various treatments of CuSO₄ and CA as Cu (25 μM), Cu (50 μM), CA (1.0 mM), CA (1.0 mM) + Cu (25 μM), and CA (1.0 mM) + Cu (50 μM). After treatment, the plants were harvested and measured physiological, biochemical and proteome analysis.

[Results and Discussion]

Plants growth characteristics were inhibited by Cu stress. Plant height and fresh and dry weight of leaves and roots were inhibited. The maximum reduction of growth was Cu 50 μM whereas CA induced in Cu-contaminated one was ameliorated Cu toxicity promoting plants growth. Chlorophyll contents was also declined significantly, and the maximum reduction was Cu 50 μM, CA induced in Cu-contaminated one was ameliorated reduction too. These results revealed that Cu stress caused plants growth reduction and chlorophyll contents, but CA application can ameliorate the toxicity of Cu. Proline accumulation was increased by exposing Cu stress but especially it was increased in CA application. The highest one was CA 1.0mM + Cu 50 μM treated, and the lowest one was none treated. Superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) contents were increased in CA 1.0mM + Cu 50 μM treated. These findings suggest that application of CA may increase the biochemical accumulation so that plant can be more tolerant to heavy metal stress. Using the gel-free proteome approach, a total of 1473 proteins were identified in *B. napus* of these, 67 proteins were identified in Brassica species, whereas 36 proteins identified from *B. napus* that were classified as differentially abundant (DAPs). Among the 36 proteins, a total of 21 proteins were upregulated and 15 proteins were downregulated Cu stress and CA application treated.

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