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Proteome Changes Reveal the Protective Roles of Exogenous Citric Acid (CA) in Mitigating Cadmium (Cd) Stress in *Brassica napus* L.

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[Introduction]

Cadmium (Cd) is a toxic substance and a significant environmental threat because it tends to build up in plants from the soil and can then be transferred into the food chain. The purpose of this study was to investigate the proteome changes in the leaves of Brassica seedlings in response to CA-mediated alleviation of Cd toxicity. Citric acid (CA), an organic chelator, is essential for reducing the oxidative stress brought on by cadmium (Cd) stress. Due to organic chelator, CA approach is an environmentally friendly and highly demandable approach. Therefore, the objectives of the study were to explore the Cd toxicity mitigation through CA using proteomic approach.

[Materials and Methods]

Healthy seeds of *B. napus* L. were sterilized and placed in petri dishes containing two layers of filter papers and germinated in controlled conditions. Following germination, the morphologically uniform seedlings were transferred to plastic pots and hydroponically grown for 7-days containing Hoagland solution. After one weeks of transplanting, uniform plants were treated with $CdCl_2$ and Citric Acid as T1: Control, T2: Cd (30 μM), T3: Cd (30 μM) + CA (1.0 mM), and T4: CA (1.0 mM) with three replications. The control plants were free from $CdCl_2$ and CA.

[Results and Discussion]

The research was focused on studying the effects of revealing Brassica plants to $CdCl_2$. The metal ion, Cd affected growth parameters and caused morpho-physiological alterations. *Brassica napus* seedlings exposed to $CdCl_2$ for 7 days did not show any leaf chlorosis or withering symptoms. However, Cd stress significantly affects the plant growth characters and plants become yellowish in CA treated plants. Under Cd stress, H_2O content increased by 29.55 % in the treated seedlings with cadmium (30 μM) in 4 weeks harvested seedlings as compared to control. Addition of CA along with Cd significantly decreased H_2O_2 contents in leaves of plants as compared to respective Cd treated plants without CA addition. In leaves, Pro content was increased by 70% in Cd-stressed seedlings (Cd 30 μM) compared with control. On the other hand, exogenous CA application noticeably decreased the elevated level of Pro in contrast to the stress treatments without CA. A total of 4947 proteins were found in the differentially treated leaves using the label-free proteome technique, and 476 of those proteins showed differential expression between the treatment groups where 316 were up regulated and 160 were down regulated. These DAPs may offer important insights on how Brassica seedlings adjust to Cd stress, according to the increased abundance of proteins involved in stress and defense. Taken together, the results open the door for additional in-depth field research to permit thorough explanation of the molecular pathways underlying Cd stress responses and the potential function of CA in reversing the detrimental effects of Cd toxicity in *B. napus.*

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