EFFECT OF SELENIUM AND MOLYBDENUM ON GROWTH, CADMIUM TRANSLOCATION, AND POLLEN GRAINS DEVELOPMENT OF *BRASSICA NAPUS* UNDER CADMIUM STRESS.

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Abstract

Environmental pollution by heavy metals has become a severe problem worldwide. Soil is becoming increasingly contaminated with heavy metals due to large-scale technological development and industrialization. Cadmium (Cd) is the third major contaminant of greatest hazard to the environment, and it is considered as the only heavy metal that poses health risks for both human and animal at the plant tissue concentrations that are generally not phytotoxic. Cadmium affects plant growth negatively, and its toxicity can be observed at both morphological and physiological levels. To cope with the cadmium toxicity as a health issue, several approaches could be implemented. Essential and beneficial plant nutrients were proved to have the ability to

mitigate Cd toxicity in plants and control Cd accumulation particularly in edible parts. In most plants, fruit formation necessitates effective pollination, and the understanding of pollen biology, including pollen morphology, viability and pollen tube growth, is required to improve the plant productivity. Pollen is extremely sensitive to heavy metal pollutants; however, less attention has been paid to the protection of this vital part under heavy metal stress.

Though Se is well known to mitigate Cd toxicity in many plants, there is no available data about the possible extension of its role to the reproductive organs in plants. Additionally, the role of Mo to lessen Cd toxicity hasn't been investigated. Here, we have studied the mitigation effect of Mo and Se on Cd-stressed *Brassica napus.* The fertilizers were applied using three different methods including foliar spray and soil addition in pot experiments, as well as addition into hydroponic culture. Further, we have included two cultivars of *B. napus,* with different accumulation ability of Cd, and used several concentrations of both Se and Mo. The study analyzed the response of Cd-contaminated plants from the aspects of gene expression to plant growth, biomass change, photosynthesis, nutrient concentration, antioxidant enzymes, and pollen morphological changes by different application methods of Se and Mo such as foliar spray, soil application and hydroponic culture.

Firstly, a pot experiment was designed to investigate the effect of foliar application of Se (1.0 mg/L) and Mo (0.3 mg/L) either alone or in combination on their absorption, translocation, and their impact on Cd uptake and its further distribution in *B. napus* tissues, as well as the influence of these fertilizers on the pollen grains morphology, viability, and germination rate under Cd stress. Foliar application of either Se or Mo could counteract Cd toxicity and increase the plant biomass, while their combined foliar application has no significant promotional effect on plant root and stem, but reduces the seeds' weight by 10–11%. Se and Mo have decreased the accumulated Cd in seeds by 6.8% and 9.7%, respectively. Microscopic studies, SEM, and pollen viability tests demonstrated that pollen grains could be negatively affected by Cd, thus disturbing the plant fertility. Se and Mo foliar application could reduce the toxic symptoms in pollen grains when any of them was sprayed alone on plants. In an *in vitro* pollen germination test, 500 μM Cd stress could strongly inhibit the pollen germination rate to less than 2.5%, however, when Se (10 μM) or Mo (1.0 μM) was added to the germination medium, the rate increased, reaching 66.2% and 39.4%, respectively. At the molecular level, Se and Mo could greatly affect the expression levels of some genes related to Cd uptake by roots (*IRT1*), Cd transport (*HMA2* and *HMA4*), Cd sequestration in plant vacuoles (*HMA3*), as well as *PCS1*, which finally control Cd distribution in plant tissues in a consistent fashion with its distribution at the physiological level.

In the second experiment, we have investigated the effect of soil application of Se and Mo on *B. napus* under Cd stress, considering two different levels of Cd (1.0 and 10 mg kg^{-1} soil), and two cultivars of *B. napus* (L351; with high Cd translocation rate from root to shoot (L351), and L338; with low translocation of Cd from root to shoot). Plant growth, photosynthesis rate, macro- or micro-nutrients, and pollen grains were negatively affected by Cd contamination in *B. napus* at the used Cd doses. Soil application of Mo or Se could lessen the negative effects caused by Cd stress. In comparison to foliar spray, joint soil application of Mo and Se mostly enhanced the plant growth, either in the presence or the absence of Cd. In a 1.0 mg kg^{-1} Cd-contaminated soil, as a low contamination level, L351 cultivar accumulated around 1.0 mg kg^{-1} in seeds which is beyond the allowed maximum levels. An antagonistic relationship between Mo and Cd was observed, where Cd at used levels (both Cd1.0 and Cd10) lessened Mo concentration in all plant tissues. In contrast, a synergetic relationship between Mo and Se, caused significant enhancement of Mo and Se in all plant tissues. The application of Cd as well as Mo and Se fertilizers could affect the anther structure and pollen grains morphology. Finally, Mo and Se fertilizers are found to be beneficial at the used dosages for *B. napus* either in non- or Cd-contaminated soil.

Finally, the application of Se and Mo was studied in hydroponic culture, where various concentrations (0.1, 1.0, and 2.0 µM) of Se or/and Mo were employed on *B. napus* under 10 µM Cd stress. *B. napus* shows visual symptom of growth retardation under Cd-stress, which varies, to some extent, between the two cultivars. Symptoms included plant height, number of leaves, plant biomass, root morphology, chlorophyll content, and photosynthesis rate, *etc*. Se added at a concentration of 1.0-2.0 µM could counter the growth inhibition caused by Cd, to the level of the control experiment. The two cultivars disclosed significant variation in their response to the added Se in the presence of Cd stress, demonstrating that the threshold for the beneficial Se might vary not only based on the used dosage of Se but also on the plant species and even cultivar. High concentration of Mo had also positive effects on the plant growth under Cd stress, however, low concentration revealed the contrary effects. Additionally, the optimum concentration when either Se or Mo are applied alone, might not be beneficial to plants when both are used together and thus amounts added should be optimized independently for combined application, which might vary between plant cultivars. The positive effects of Se and Mo on *B. napus* growth under Cd stress could be explained based on their role in improving photosynthesis process, chlorophyll content, antioxidant enzymes activities, and manipulating either Cd uptake, translocation, or storage into old leaves. While low Se and Mo concentrations have constructive effects on pollen grains, and their application limits the abnormalities observed in pollen grains. High concentrations of both fertilizers have substantially increased the abnormal pollen grains which are mainly observed as burst and fully opened pollen grains. Energy Dispersive X-ray

spectroscopy (EDX) analysis have been used to explain the negative effect of Cd on pollen grain growth and formation. EDX results demonstrated that Cd contamination greatly disturb the elemental composition of the pollen grains, and significantly decreases its content of several essential elements such as P, Ca, Na, and S. Additionally, Mo and Se, though their positive effect on pollen growth under Cd stress, didn’t affect the elemental composition of the pollen. This might propose that the role of Mo and Se to alleviate the toxic symptoms of Cd on pollen grains, is not mainly *via* recovering the elemental structure of pollen, but it might be through decreasing Cd reaching to the pollen at the pollination stage.

Key words: Heavy metal stress; *Brassica napus*, Cadmium; Selenium; Molybdenum; Pollen grains.