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Mechanism of Arsenic Transport in *Brassica juncea*

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Introduction

Arsenic (As) is a non-essential element and food chain contaminant that can be present in the edible tissues of crop species, in some cases at concentrations that exceed safe limits for human consumption. Because of the prevalence of these elements as contaminants in plant foods, there are clear detrimental outcomes for agricultural productivity, food safety, and human health (Wash et al., 1977).

Arsenic occurs naturally in the environment and is associated with ore containing metals such as copper and lead. It mainly enters the environment during smelting and mining of these ores, use of As containing pesticides, herbicides, wood preservatives and additives in feed (ATSDR, 2007).

In the United States, As in contaminated soils ranges from 13 to as high as 2553 mg/Kg while As concentrations in uncontaminated soils can be as high as 80mg/kg.

The largest source of arsenic is food and of these the predominant source of arsenic is seafood followed by rice and rice cereal. However, for seafood, most of the arsenic is in the less toxic organic form while the more toxic inorganic form is found in plant foods. In the United States, the dietary intake of As is estimated to range from 1 to 20 mg per day from grains, which is supposed to be a significant contributor of dietary inorganic arsenic (toxic form) (ATSDR, 2007).

Plants can accumulate arsenic in edible tissues such as the leaves, fruits and seeds. However, plants vary considerably in their ability to accumulate As. In a market based survey of 40 commodities, highest inorganic arsenic was found in raw rice followed by flour, grape juice and cooked spinach (Meharg et al., 2009).

Fig 1: *Brassica juncea* growing in hydroponics

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Materials and Methods

Brassica juncea (Indian mustard) seeds were obtained from the USDA-ARS Plant Introduction Center at Iowa State University. surface sterilized in 1.0% sodium hypochlorite for 10 minutes and were sown in a 1:1 moist perlite/vermiculite mixture. After germination, seedlings were transferred to 4 L pots containing a modified Johnson's nutrient solution with the following composition: 6.0 mM KNO_3, 4.0 mM $Ca(NO_3)_2$, 0.1 mM $NH_4H_2PO_4$, 1.0 mM $MgSO_4$, 50 mM KCl, 12.5 mM H_3BO_3, 1.0 mM $MnSO_4$, 1.0 mM $ZnSO_4$, 0.5 mM $CuSO_4$, 0.1 mM H_2MoO4, 0.1 mM $NiSO_4$ and 10 mM Fe-EDDHA [N,N'-ethylenediamine-di(O-hydroxyphenylacetic acid)] as Fe source and all the solutions were buffered with MES [2-(morpholino)ethanesulfonic acid] at a pH of 5.5. The solutions were continuously aerated and replaced weekly.

Growth conditions were achieved in the greenhouse (natural lighting plus supplemental lighting using metal halide lamps with a 15-h day and 9-h night photoperiod, 25 ± 3 °C day/23 ± 3 °C night). Once the plants reached the four to five leaf stage, they were treated with one of following concentrations of sodium arsenate (NaH_2AsO_4): 0, 2 and 5μM.

Visual symptoms, such as chlorosis, and growth were noted throughout the experiment. Relative chlorophyll content (RCC) was recorded weekly to ensure proper growth and development of the plants. RCC was obtained using the SPAD 502 Plus chlorophyll meter.

All plants were harvested during the flowering stage. The tissues from all the plants were separated into the following parts: roots, stems, leaves, and flowers. All samples were oven dried at 60°C to constant mass and weighed. Tissues were then ground and subsamples (~0.25 g) were digested using nitric acid and hydrogen peroxide. The tissue extracts will be analyzed for As using a SpectrAA 220FS atomic absorption spectroscopy (Varian, Walnut Creek, CA) in flame and the furnace mode.

Figure 2: Aim 600 digestion system used for digesting *Brassica juncea* tissues

References

•ATSDR (2007) Arsenic.

•Meharg et al. (2009) Environ. Sci. Technol. 43(5): 1612-7.

• Wash, L, M, et al. (1977) Environ. Health Perspect. 19, 67-71.

Results and Conclusions

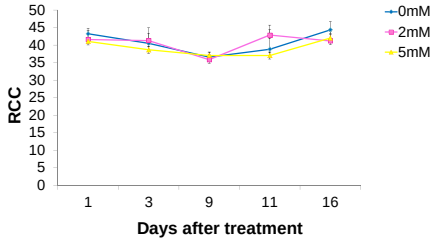

Fig 3: Relative Chlorophyll Content (RCC) of *Brassica juncea* treated with 0, 2 and 5 μM arsenic. Values represent mean ± s.d

No significant differences were observed between the different treatments for all the weeks. This indicates that the As treatments were not high enough to produce any toxicity to the plants in terms of RCC.

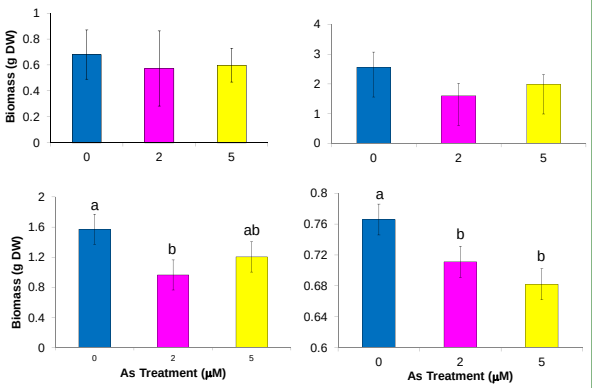

Fig 5: Tissue biomass (g DW) of a) roots b) stems c) leaves and d) flowers of *Brassica juncea* plants grown in different concentrations of As. Values represent mean ± s.d

Although there were some differences in leaves and flowers biomass, there seems to be no significant effect of As treatments on the tissue biomass indicating that As concentrations were not high enough to cause any toxicity to the plants.

Tissue As concentrations will be analyzed and partitioning will be assessed to understand the transport and accumulation of arsenate into the different tissues of *Brassica juncea*.