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# Cadmium and zinc partitioning and accumulation during grain filling in two near isogenic lines of durum wheat



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# ABSTRACT

Plants can readily accumulate cadmium (Cd), transferring this element to edible leaves, fruits, and seeds. Rice and wheat are among the top crop sources of Cd. Toxic heavy metals like Cd have chemical properties similar to essential micronutrients such as zinc (Zn) and are generally transported in plants by the same transporters as those essential micronutrients. Unfortunately our knowledge of Cd translocation and accumulation in seeds is still unclear. We conducted a partitioning study to assess both the whole plant Cd distribution and accumulation and potential source-sink remobilization during grain filling period in two near-isogenic lines of durum wheat that differ in root to shoot translocation and grain Cd content. We also assessed the role of Zn fertilization in Cd translocation and accumulation in the grains. There was no remobilization of Cd in both lines during grain filling. Although majority of Cd partitioned to the roots in both lines, root to shoot translocation of Cd differed in both lines. In contrast, there were no significant differences in Zn partitioning between the lines and remobilization was observed in different tissues. Although there was some remobilization of Zn, the main source of Cd and Zn is continued uptake and translocation to sources during grain fill.

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# 1. Introduction

Since the majority of the world's population relies on cereal crops such as rice for their caloric intake, most biofortification efforts focus on improvement of seed nutritional density and quality. There is a simultaneous need to reduce the accumulation of toxic metal analogs (e.g., Cd) of essential micronutrients (e.g., Zn) in seeds. Cadmium is a contaminant that threatens biodiversity, agricultural productivity, food safety, and human health. In the United States, the largest source of Cd exposure in non-smoking adults and children is through the diet ([Agency for Toxic](#page--1-0) [Substances and Disease Registry \(ATSDR\), 2008](#page--1-0)). It is estimated that in the United States, the average person consumes about 30  $\mu$ g of Cd per day in food, with the largest contribution from grain cereal products, potatoes and other vegetables, of which  $1-3 \mu$ g is absorbed via the gastrointestinal tract [\(Clemens et al., 2013;](#page--1-0) [Schwartz and Reis, 2000](#page--1-0)). This has led federal agencies to set strict limit on the concentration of Cd in foods, generally

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0.1 mg  $kg^{-1}$  DW for crops such as cereals.

Studies suggest an important role of vegetative tissues in supplying micronutrients or heavy metal analogs to the seeds during the seed fill stage of plant development ([Chan et al., 2007;](#page--1-0) [Kashiwagi et al., 2009](#page--1-0)). However, other studies [\(Cakmak et al.,](#page--1-0) [2000a; Harris and Taylor, 2001; Herren and Feller, 1997; Jiang](#page--1-0) [et al., 2007; Sankaran and Ebbs, 2008; Waters and Grusak, 2008\)](#page--1-0) have demonstrated that during seed fill there is a critical window where uptake of micronutrients and heavy metal analogs by the roots represents a significant if not the predominant source of micronutrients ultimately transported to seeds. In a recent study, Harris and Taylor ([Harris and Taylor, 2013](#page--1-0)) concluded that the two near isogenic durum wheat lines absorbed and transported Cd throughout the grain filling period suggesting absence of remobilization and a direct pathway of Cd transport from root to grain via the stem. Such results support a growing belief ([Grusak et al., 1999\)](#page--1-0) that when the roots of plants undergoing seed fill are provided with a continuous supply of an element, xylem transport of that element to the shoots provides a supply that is rapidly mobilized to the seeds via the phloem.

Trace element interactions can influence the extent to which metals and micronutrients are taken up and transported to plant tissues. Toxic trace heavy metals such as Cd have chemical

properties similar to essential micronutrients such as Zn and are generally transported in plants by the same transporters as those essential micronutrients. Some studies on Cd and Zn interactions in plants have reported that Zn competitively inhibits Cd movement and accumulation ([Harris and Taylor, 2001; Grant et al., 1998; Hart](#page--1-0) [et al., 2002](#page--1-0)) while some others have reported that the interaction between Cd and Zn is non-competitive [\(Salt et al., 1997\)](#page--1-0). Hart et al. ([Hart et al., 2006](#page--1-0)) while studying the interaction of Zn on Cd accumulation in two wheat isolines differing in grain Cd concentration, showed that there was no difference in Zn partitioning between the two isolines but Zn reduced Cd uptake in the roots and the shoot concentration in both the lines. Previous studies have shown that rice more so than other crop plants can accumulate much higher proportions of Cd to Zn in grains even when grown in soils with much higher proportions of Zn to Cd [\(Chaney et al., 2001;](#page--1-0) [Simmons et al., 2003](#page--1-0)). Although the nature of the  $Cd - Zn$  interaction is not fully understood, previous research has shown that significant accumulation of Zn occurred in the rice grain  $4-14$  days after flowering resulting in high Cd:Zn ratios [\(Simmons et al., 2003;](#page--1-0) [Kitagishi, 1981](#page--1-0)). Previous research has established that rice has been an important source of Cd due to the relatively low levels of Zn, Fe and Ca in the grains [\(Chaney et al., 2001; Reeves and Chaney,](#page--1-0) [2002](#page--1-0)). Studies with different genotypes and NILs in wheat, rice and other species were done where only selected tissues were harvested, or grains were harvested only at one time point. If they were multiple sampling points, Cd-Zn interaction was not examined. The main goal of our study was to understand how Zn interacts or interferes with Cd distribution within the plants especially during the grain filling stage so that we can enhance the Zn:Cd ratios by biofortification. To provide a better understanding of Cd accumulation and interactions with Zn during grain filling, two near isogenic lines of durum wheat differing in grain Cd were compared in the presence of either regular or Zn sufficient conditions to understand how it altered Cd partitioning and accumulation during grain filling.

#### 2. Materials and methods

Seeds of two durum wheat (Triticum turgidum v. durum) W9262-339A-L, and W9262-339A-H near isogenic lines that differ in grain Cd accumulation were obtained from USDA GRIN. These lines differ 2.5 fold in grain Cd concentrations but are otherwise similar. The low Cd line retained more Cd in the roots and translocated less Cd to the shoots than the high Cd lines ([Clarke et al.,](#page--1-0) [1997](#page--1-0)). Seeds were surface sterilized and imbibed overnight and then germinated on filter paper. Germinated seedlings were then transferred to 4L pots containing complete nutrient solution. The nutrient solution was comprised of 2 mM KNO<sub>3</sub>, 1 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 1 mM KH<sub>2</sub>PO<sub>4</sub>, 1 mM MgSO<sub>4</sub>, 25 µM CaCl<sub>2</sub>, 25 µMH<sub>3</sub>BO<sub>3</sub>,  $0.5 \mu$ M H<sub>2</sub>MoO<sub>4</sub>, 0.1  $\mu$ M NiSO<sub>4</sub>, 2  $\mu$ M MnSO<sub>4</sub>, 0.5  $\mu$ M CuSO<sub>4</sub>, and iron will be supplied as 20  $\mu$ M Fe(III) HEDTA (N-(2-hydroxymethy)ethylenediaminetriacetic acid), and 2 mM 2-(N-morpholino)ethanesulfonic acid (MES; Sigma Chemical, St. Louis, MO, USA) to buffer the solution to pH 6.0. Solutions were aerated continuously and replaced every 10 days. All pots were placed in the greenhouse for the duration of the experiments. Growth conditions were achieved in the greenhouse (natural lighting plus supplemental lighting using metal halide lamps [1000 W] with a 15-h day and 9-h night photoperiod, 25  $\S$  3\_C day/23  $\S$  3\_C night).

A completely randomized factorial design was used to study the Cd and Zn interactions at different stages during grain filling. The Cd and Zn exposures during experiments spanned the period of plant even though the experiments focused on the period from anthesis until reproductive maturity. The exposure regime was selected because it emulates field grown conditions. The Cd treatment chosen for the studies (0.5  $\mu$ M Cd<sup>2+</sup>) represents agriculturally relevant, sub toxic concentrations ([Hart et al., 2006](#page--1-0)). Zinc, being a micronutrient was supplied for all the plants at  $2 \mu M$  or  $10 \mu$ M concentrations depending on the treatments. The treatments were designated Cd0.5Zn2 and Cd0.5Zn10 for 0.5 µM Cd,  $2 \mu$ M Zn and 0.5  $\mu$ M Cd, 10  $\mu$ M Zn treatments respectively. The study uses multiple time point sampling (0, 7, 14, 28 and 42 days after anthesis (DAA) designated here as T1, T2, T3, T4, and T5 respectively), focusing the efforts on understanding the physiology and regulatory processes that occur during grain filling. As flower heads began to emerge, all plants were monitored carefully to ensure they were harvested at a uniform developmental stage. Therefore plants designated 0 DAA, were harvested on the first day of the emergence of flowering heads and the rest of the stages were harvested at the designated number of days after the first date of flowering. Hence, the replicate plants within a treatment were not necessarily harvested on the same day. Studies have shown that high nutrient concentrations common in plants grown in solution culture induces high rates of tillering in cereal crops [\(Harris and](#page--1-0) [Taylor, 2013; Hart et al., 2006\)](#page--1-0). The plants produced variable number of tillers in the different treatments and all the tillers were used as part of the study. These tillers were collected and pooled for harvest. Each treatment was replicated at least 4 times while a few of them due to loss of plants had as few as 2 replicates.

### 2.1. Measurements

At harvest, plants were separated into roots, stems, lower leaves, flag leaves, peduncle, rachis, florets, and grains. For the first three time point, florets, rachis and developing grains were combined. Leaves included lamina and sheath. Roots were rinsed thoroughly with deionized water and blotted dry. All the tissue samples were dried at  $60 °C$  to constant mass and weighed. Al tissues were then ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ). For each tissue, 0.25 g of dried tissue sample were digested using 4 mL of concentrated nitric acid and 2 mL of hydrogen peroxide at temperatures up to 200 $\degree$ C and then taken to dryness. Digests were resuspended in 1 mL 2 M HNO<sub>3</sub> and, after 1 h, were brought to 10 mL with deionized water. The acids used were trace metal grade (Fisher Scientific, Pittsburgh, Pennsylvania, USA) and the water was deionized via a MilliQ system (Millipore, Billerica, Massachusetts, USA) ([http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/](http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3050b.pdf) [3050b.pdf\)](http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3050b.pdf). Samples were analyzed for Cd and Zn using a spectrAA to calculate mineral concentrations, as previously described ([Farnham et al., 2011](#page--1-0)). Cadmium sulfate was used as positive controls and blanks were also used for every run of the digestion and analysis. Cadmium sulfate was used to make the standard solutions for the Cd treatment of the plants. Mineral content for each tissue was calculated by multiplying each tissue's average mineral concentration by the average total tissue weight at a given time point.

Cadmium and zinc concentrations and content were first analyzed using a two-way analysis of variance (ANOVA) in JMP SAS (SAS Institute, USA). This was followed by pairwise testing between the high and low lines at each harvest with Holm-Bonferroni correction with significance at  $p \leq 0.05$ .

## 3. Results

#### 3.1. Biomass accumulation

Total biomass production was variable between both lines (L and H) across the different time points. The low Cd lines (L) had higher biomass production when compared to the high Cd lines (H). Biomass values showed great variability in roots, stems, lower leaves, flag leaves, peduncle, rachis and florets, and grains ranging Download English Version:

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