Effect of zinc and manganese supply on the activities of superoxide dismutase and carbonic anhydrase in Medicago truncatula wild type and raz mutant plants

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Abstract

The novel raz (requires additional zinc) mutant of Medicago truncatula can accumulate high Zn concentrations in all tissues when compared to wild type plants, yet still develops Zn deficiency symptoms, suggesting that total Zn in tissues may not be physiologically available. The objectives of this study were first to determine whether there are differences in the biochemical Zn availability between wild type and raz mutant plants based on the activity of carbonic anhydrase and superoxide dismutase and second to assess the suitability of using the activities of these two Zn-requiring enzymes as indicators of utilizable Zn in a wide range of Zn tissue concentrations. In leaf extracts, CA and total SOD activities as well as the distribution of the tSOD activity among the different isoforms were similar in both genotypes. In roots, there were no significant differences observed in total SOD activities between genotypes; however, CuZnSOD activities were lower in raz than in wild type plants when grown at high Zn concentrations. Based on these results, availability of Zn in raz leaves does not seem to be altered; however, in roots of the raz mutant, Zn availability is restricted or limited in comparison to the wild type plants, especially when raz roots accumulate high levels of Zn. With increasing total Zn tissue concentrations, CA activity increased linearly and then reached a plateau in both leaves and roots, whereas CuZnSOD also increased linearly in leaves but no significant correlation was found in roots, suggesting that CA is a better indicator of tissue Zn status.

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Keywords: Superoxide dismutase; Carbonic anhydrase; Zinc; Manganese; Medicago truncatula; raz mutant

1. Introduction

Metal ions such as Zn$^{2+}$ and Mn$^{2+}$ are essential nutrients for plants. Zinc has been identified as a component of over 300 enzymes in plants, is required for the maintenance of membrane integrity, is involved in protein synthesis, DNA and RNA stability [1,2] and in carbohydrate, nucleic acid and lipid metabolism [3]. Manganese plays an important role in photosynthesis, carbohydrate metabolism and in fatty acid and carotenoid synthesis [3]. However, when accumulated in excess in plant tissues, these metals cause alterations in various vital growth processes such as photosynthesis and photosynthetic electron transport [4,5], biosynthesis of chlorophyll [6], and cell membrane integrity [7,8]. Therefore, plants must carefully regulate both metal acquisition from the soil and the cellular partitioning of metals in order to prevent excess accumulation while obtaining adequate intake.

Tissue metal concentrations have been widely used to evaluate the metal nutritional status of plants; however, it has been observed, under several circumstances, that these two are not always well correlated. When plants are grown under severe Zn-limiting conditions, they may accumulate sufficient Zn and yet develop Zn-deficiency stress [9]. Also,
it has been reported that Zn-inefficient genotypes exhibiting deficiency symptoms may accumulate as much Zn as Zn-efficient genotypes [10,11]. Moreover, the novel raz (requires additional zinc) mutant of Medicago truncatula can accumulate high Zn concentrations in all tissues when compared to wild type plants, yet still develops Zn-deficiency symptoms, suggesting that total Zn in tissues may not be physiologically available [12]. In such situations, biochemical tests, such as measurements of the activity of metal-containing enzymes, may serve as better measures of physiologically available metals. Because metal deficiencies often result in decreases in the activity of metal-requiring enzymes, and these enzymes have specific compartmental locations, we believed that an analysis of the activities of certain metal-containing enzymes would help establish if the raz mutant is functionally deficient in Zn (and/or Mn) [13,14].

Superoxide dismutases (SODs) are key enzymes in the plant’s defense against oxidative damage [15,16]. SODs are classified into three types based on their metal cofactor: those that contain Mn (MnSOD), Fe (FeSOD) or Zn and Cu (CuZnSOD). In general, MnSOD is located in the mitochondria, FeSOD in the chloroplast and peroxisomes, and CuZnSOD in the chloroplast and in the cytosol [16]. Measurements of these isoforms of SOD are useful for assessing the micronutrient status of plants, and have been used in the past to study deficiencies of Zn [17–20], Fe [21,22], Mn [23–25] and Cu [26].

Another potential zinc-containing enzyme that may be used to estimate physiological Zn availability is carbonic anhydrase (CA). Carbonic anhydrase catalyzes the hydration of CO₂ and is located in the cytosol, root plastids and chloroplasts. A decrease in leaf CA activity in plants grown under Zn deficient conditions has been previously described in tomato [27], citrus [28], spinach [29], and wheat [11], although little is known about CA activities in plants grown at high Zn concentrations.

The objectives of this study were first to infer whether there are differences in the Zn availability between Medicago truncatula wild type and raz mutant plants based on the activities of carbonic anhydrase and superoxide dismutase, and secondly to assess the suitability of the activities of these two Zn-requiring enzymes as indicators of functional Zn availability in a wide range of Zn tissue concentrations.

2. Materials and methods

2.1. Plant growth

Two genotypes of Medicago truncatula were used in this study: a wild type parental line (A17, derived from cultivar Jemalong), and a mutant from the same parental line (raz; [12]). Seeds of the raz mutant used in these experiments were progeny from self-crosses of the M₄ generation; details on the isolation of the mutant are provided in Ellis et al. [12]. Plants were grown in a controlled environment chamber with a photosynthetic flux density of 350 μmol m⁻² s⁻¹, and a 16-h, 20 °C/8-h, 15 °C day/night regime. Seeds were scarified by nicking the seed coat; they were then imbibed overnight in distilled water and germinated on filter paper in darkness for four days. Plants were grown hydroponically (45–60 plants) for 10 days in 211 l of nutrient solution (standard solution) containing: 0.6 mM K₂SO₃, 0.5 mM Ca(NO₃)₂, 1.0 mM NH₄NO₃, 0.3 mM KH₂PO₄, 0.2 mM MgSO₄, 25 μM CaCl₂, 25 μM H₃BO₃, 2 μM MnSO₄, 1 μM ZnSO₄, 0.5 μM CuSO₄, 0.5 μM H₂MoO₄, 0.1 μM NiSO₄, and 5 μM Fe(III)-EDDHA. All hydroponic solutions were buffered by the addition of 1 mM MES, pH 5.5. After 10 days, plants were transferred to 4.5 l containers (four plants per container) filled with various treatment solutions and were grown under these conditions for 3 weeks. The treatment solutions were the same as described above, except with the Zn or Mn concentrations altered as indicated throughout the paper. Solutions were changed weekly.

2.2. Mineral analysis

At harvest, individual plants were divided into roots, stems, and leaves. Roots were washed in deionized water to remove any remaining nutrient solution. Tissue was dried to constant weight in a forced air oven at 60 °C. Tissue was wet digested using concentrated HNO₃ and HClO₄ as previously described [30]. Mineral concentration was determined using an atomic absorption spectrophotometer (Model 2100, Perkin-Elmer, Stafford, Texas). Tomato leaf powder (SRM 1573a; National Institute of Science and Technology, Gaithersburg, MD, USA) was used as a standard.

2.3. Enzyme activities and protein concentrations

Extracts for measuring CA (EC 4.2.1.1) were made by grinding 400 mg FW of root or leaf material in 3 ml of 100 mM Tris, 10 mM mercaptoethanol and 1 mM EDTA, pH 8.3. The extract was stirred for 15 min at room temperature, centrifuged at 7000 × g for 10 min and the supernatant was stored on ice until assayed. The Wilbur–Anderson [31] electrometric method was used to assay CA activity. One milliliter of extract was added to 3 ml of 25 mM veronal buffer (barbitone, 5-5-diethyl barbituric acid), pH 8.2. Four milliliters of CO₂-saturated water were added and the time taken for the pH to change from 8.2 to 7.0 was measured. Blanks were run using 1 ml of extract buffer.

Extracts for measuring SOD activity (EC 1.15.1.1) were made by grinding 400 mg FW of roots or leaves in 4 ml (leaves) or 2 ml (roots) of 50 mM phosphate buffer, 0.1 mM EDTA, 1% PVP, 1% Triton X-100, 1 mM PMSF, pH 7.8. The crude homogenate was centrifuged at 10,000 × g for 15 min and SOD activity was immediately assayed. SOD activity was measured by monitoring the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT)
according to the method of Giannopolitis and Ries [32] and Beyer and Fridovich [33]. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm. Different isoforms of SOD were identified by using 3 mM KCN and/or 5 mM H2O2 as inhibitors, as described in Yu and Rengel [25].

Protein concentrations in the extracts were measured using the Bio-Rad Detergent Compatible Protein Assay Kit (Bio-Rad, Hercules, CA) according to the manufacturer's instructions.

3. Results

3.1. Mineral composition

Zn concentrations in leaves and roots from wild type plants ranged from 14 to 539 μg g⁻¹ DW⁻¹ and from 18 to 5445 μg g⁻¹ DW⁻¹, respectively, in response to an altered Zn supply in the nutrient solution (from 0 to 5 μM) (Tables 1 and 2). In general, mutant plants showed higher Zn concentrations than wild type plants, in both leaves and roots, in most nutritional regimes studied (Tables 1 and 2); ranz tissue concentrations ranged from 27 to 657 μg g⁻¹ DW⁻¹ and from 60 to 15972 μg g⁻¹ DW⁻¹ in leaves and roots, respectively. For Mn, the ranz mutant also showed higher concentrations than wild type plants following growth at most nutrient levels. Increasing Mn or Zn supply in the nutrient solution from 0 to 5 μM resulted in higher Mn concentrations in roots and leaves of both genotypes (Tables 1 and 2). Across both genotypes, the achieved ranges of Mn concentrations were 25–2089 μg g⁻¹ DW⁻¹ and 10–8605 μg g⁻¹ DW⁻¹ for leaves and roots, respectively. While Zn and Mn concentrations varied according to the growth regime, only minor to moderate changes were seen in concentrations of Fe and Cu (Tables 1 and 2). It should be kept in mind that Fe and Cu levels were maintained constant in the nutrient solutions. Leaf Fe concentration in both genotypes was approximately 100 μg g⁻¹ DW⁻¹ in most of the nutrient regimes studied (Table 1). For Cu, leaf concentration in wild type plants ranged from 23 to 37 μg g⁻¹ DW⁻¹ and no significant changes were found when Zn or Mn supply was increased (Table 1). However, leaf Cu concentrations in the mutant were higher when compared to wild type leaves in most of the regimes studied, with Cu concentrations increasing significantly in response to higher Zn or Mn supply in the nutrient solution (Table 1). In roots of wild type plants, Fe and Cu concentrations ranged between 160–330 and 130–390 μg g⁻¹ DW⁻¹, respectively, and did not change significantly in response to the metal supply. For ranz roots, Fe and Cu concentrations were generally higher than in wild type plants and concentrations increased when the Zn or Mn supply in the nutrient solution was increased.

3.2. Carbonic anhydrase activity

Carbonic anhydrase activity in leaf extracts from wild type plants varied three-fold (from 5.2 to 14.7 units CA mg protein⁻¹ s⁻¹) when the Zn supply in the nutrient solution was increased from 0 to 5 μM (Fig. 1A). Leaf

![Fig. 1. Carbonic anhydrase activity (units CA mg protein⁻¹ s⁻¹) in leaves (A) and roots (B) of wild type (white bars) and ranz (black bars) plants grown under different Zn and Mn supply to the nutrient solution. Data are means ± S.D. of at least five replicates.](image)

Table 1

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<th>Growth conditions</th>
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<td>ranz</td>
<td>WT</td>
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<td>90 ± 5</td>
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<td>25 ± 4</td>
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<td>657 ± 37</td>
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<td>115 ± 9</td>
<td>614 ± 104</td>
<td>1020 ± 224</td>
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</tbody>
</table>

Data are mean ± S.D. of four replicates.
protein concentrations for wild type plants averaged 40.4 ± 4.8 mg g⁻¹ FW⁻¹ across all growth conditions. In *raz* leaf extracts, a similar three-fold variation in CA activity (from 5.1 to 14.5 units CA mg protein⁻¹ s⁻¹) was observed when the Zn supply was increased from 0 to 3 μM, but just a two-fold variation was measured (from 5.1 to 10.1 units CA mg protein⁻¹ s⁻¹) for plants grown with 0 μM versus 5 μM Zn. Leaf protein concentrations for *raz* plants averaged 49.3 ± 11.4 mg g⁻¹ FW⁻¹ across all growth conditions. CA activity in both genotypes did not change when the Mn supply was increased up to 5 μM, whereas in *raz* root extracts a similar three-fold increase (from 1.33 to 5.1 units CA mg protein⁻¹ s⁻¹) was already measured when Zn supply in the nutrient solution increased from 0 to 1 μM (Fig. 1A).

CA activity in root extracts from wild type plants increased approximately three-fold (from 1.33 to 4.33 units CA mg protein⁻¹ s⁻¹) when Zn supply in the nutrient solution increased from 0 to 3 μM, whereas in *raz* root extracts a similar three-fold increase (from 1.80 to 5.1 units CA mg protein⁻¹ s⁻¹) was already measured when Zn supply to the nutrient solution increased from 0 to 1 μM (Fig. 1B). Further increases of Zn supply in the nutrient solution did not change CA activity in wild type or *raz* root extracts. Increasing the Mn supply to the nutrient solution from 0 to 5 μM had no significant effect on CA activity of wild type or *raz* root extracts (Fig. 1B). Root protein concentrations (mg g⁻¹ FW⁻¹) averaged 6.0 ± 1.8 and 8.6 ± 2.5 across all growth conditions in wild type and *raz* plants, respectively.

3.3. Superoxide dismutase activity

Total SOD activity (tSOD) in wild type leaf extracts increased 1.4-fold, from 6.7 to 9.20 units SOD mg protein⁻¹, when the Zn supply in the nutrient solution was increased from 0 to 3 μM but increased only 1.1-fold (from 6.7 to 7.3 units SOD mg protein⁻¹) when the Zn supply increased up to 5 μM (Fig. 2A). In *raz* leaf extracts, total SOD activity was similar or slightly higher to the activity found in wild type leaf extracts from plants grown in similar conditions and followed a similar pattern when the Zn supply in the nutrient solution was modified (1.9- and 1.5-fold increases at the 3 and 5 μM Zn regimes, respectively, when compared to the 0 μM Zn regime) (Fig. 2A). Leaf protein concentrations for wild type and *raz* plants were as noted above.

The increases observed in leaf tSOD activity in both genotypes when the Zn supply increased from 0 to 3 μM were associated with 1.4- and 1.9-fold increases in CuZnSOD activities in wild type and *raz* plants, respectively (Fig. 2A). Leaf MnSOD activity showed a similar pattern to tSOD activity in both genotypes, increasing 1.6- and 1.5-fold in wild type and *raz* leaves, respectively, when the Zn supply increased to 3 μM and 1.5- and 1.2-fold when the Zn supply increased to 5 μM (Fig. 2A). Leaf FeSOD activity was only detected in wild type plants, with the highest activities observed in plants grown in Zn- or Mn-deficient conditions (Fig. 2A).

Increasing the Mn supply in the nutrient solution from 0 to 5 μM increased tSOD activity 1.5- and 1.3-fold in wild type and *raz* leaf extracts, respectively (Fig. 2A). These increases were associated with 1.9- and 1.6-fold increases in the CuZnSOD activity in wild type and *raz* leaf extracts,
respectively. Surprisingly, MnSOD activities did not change much with increased Mn supply to the nutrient solution (Fig. 2A).

Total SOD activity in root extracts of both genotypes did not change appreciably in response to an increased Zn supply in the nutrient solution with 2 mM Mn (Fig. 2B). Root extracts from raz plants presented a tSOD activity similar or slightly higher to that found in wild type root extracts in most of the regimes assayed (Fig. 2B). However, the activity of CuZnSOD in wild type root extracts increased 2.3-fold (from 5.0 to 11.3 units SOD mg protein−1) and decreased 1.4-fold (from 7.0 to 4.9 units SOD mg protein−1) in raz root extracts when the Zn supply in the nutrient solution was increased from 0 to 5 µM with 2 µM Mn (Fig. 2B). This decrease in CuZnSOD activity in raz roots was accompanied by a three-fold increase (from 1.2 to 3.5 units SOD mg protein−1) in the FeSOD activity (Fig. 2B). Leaf protein concentrations for wild type and raz plants were as noted above.

MnSOD activity in wild type root extracts decreased from 7.5 to 2.1 units SOD mg protein−1 when the Zn supply was increased from 0 to 3 µM (Fig. 2B). In root extracts from raz plants, MnSOD activity was higher than in wild type plants in all the growth regimes, and did not change with the Zn supply in the nutrient solution (Fig. 2B). Thus, the contribution of MnSOD activity to the total SOD activity in raz root extracts (approximately 50%) was higher than in wild type root extracts (approximately 25%).

When plants were grown at either 0 or 5 µM Mn, tSOD activities in root extracts were higher than in plants grown in standard conditions (2 µM Mn) in both genotypes (Fig. 2B). In Mn deficient plants, this higher tSOD activity was associated with a 1.8-fold increase in CuZnSOD activity in wild type roots and with 1.8- and seven-fold increases in MnSOD and FeSOD activities, respectively, in raz roots (Fig. 2B). In plants grown at 5 µM Mn, the higher tSOD activity was associated with higher MnSOD activities in both genotypes (Fig. 2B).

4. Discussion

In the present study we have used a novel mutant of Medicago truncatula (raz) that has the capacity to exhibit increased concentrations of Zn, Mn and Cu in all tissues and Fe in roots compared to wild type plants [12]. The two major reasons for using this mutant were first, to study the possibility that in the mutant, Zn might be accumulated in the tissues as an inactive or unavailable form, thus causing the Zn-deficient like phenotype [12]. Secondly, the high tissue metal concentration measured in the mutant roots and leaves allowed us to use a wide range of Zn tissue concentrations to assess the suitability of various enzymes as indicators of Zn status in the plant. Total SOD activities in leaf extracts as well as the distribution of the activities among the different isoforms were similar in wild type and raz plants under all the conditions assayed. Accordingly, CA activities were similar or higher in raz than in wild type leaves in most of the conditions assayed. These data may indicate that apparently no alterations exist in the biochemical Zn availability in leaves from the mutant plants. In roots, there were no significant differences observed in total SOD activities between wild type and raz plants when the Zn supply to the nutrient solution was increased; however, the contribution of SOD isoforms was found to differ between the genotypes. CuZnSOD activities were lower in raz than in wild type roots when plants were grown at high Zn concentrations (3 and 5 µM Zn) although, Zn concentrations were much higher in raz than in wild type roots from plants grown under the same conditions (Table 2). These results suggest that the biochemical availability of Zn in roots of the mutant might be restricted or limited in comparison to the wild type plants, especially when raz roots accumulate higher levels of Zn. Interestingly, this is not the case in wild type roots, as elevated Zn concentrations (5445 µg g−1 DW−1; growth with 5 µM Zn and 2 µM Mn) does not lead to a decline in CuZnSOD activity. Apparently, excess Zn may be partitioned differently in root cells of wild type and raz plants. However, it also should be noted that roots were washed only with water at the time of harvest, and some of the excess Zn could have been localized to the apoplast.

Parallel to the lower CuZnSOD activity, raz roots demonstrated higher FeSOD and MnSOD activities than wild type roots and, concomitantly higher Fe and Mn concentrations were found in raz roots. These micronutrient-induced changes in activities of SOD in raz roots may reflect both a response of plants to increased free radical production in a specific subcellular compartment triggered by the high metal concentrations, and/or an altered micronutrient availability (a decrease in utilisable Zn and/or an increase in utilisable Fe and Mn). Surprisingly, CA activities were similar in wild type and raz roots when plants were grown under different Zn supplies indicating that Zn availability for CA activity was not altered in raz roots. The CA results imply that Zn would be available in raz roots and the fact that CuZnSOD activity in these roots did not increase with increased Zn levels in the tissues was related to possibly both a higher Mn and Fe availability, and to higher demands on FeSOD and/or MnSOD activity in the subcellular compartments where these isoforms are located. Also, it should be kept in mind that copper is needed for the activity of CuZnSOD. As occurs with Zn, our results demonstrated higher Cu concentrations in roots of mutant plants when compared to wild type plants. It has been suggested that CuZnSOD may be relatively more sensitive to Zn than to Cu status [19,26]. This is likely due to the different roles that Cu and Zn play in CuZnSOD activity; Cu is catalytic, and required for enzyme action, whereas Zn is structural and required for enzyme stability [26]. Nevertheless, the changes observed in CuZnSOD activity in roots of mutant plants could reflect a change in the availability of other metals (not just Zn or Cu).
Under Zn-limiting conditions, CuZnSOD activity in wild type roots and leaves was lower than in standard conditions although total SOD did not change much. These decreases were associated with an increase in MnSOD activity in roots and in FeSOD in leaves. It has been previously suggested that a limitation in the concentration of a micronutrient can depress its corresponding isoform activity but apparently there is a compensatory mechanism involving the induction of other isoforms, in order to keep an adequate level of SOD for the cell protection against deleterious effects of superoxide radicals. So far, this compensatory mechanism has been described between MnSOD and CuZnSOD in leaves of pea [23] and tobacco plants grown under limiting Mn levels [23,25,29], between MnSOD and FeSOD in Fe-deficient pea leaves [22] and between CuZnSOD and FeSOD in pea leaves under Cu-limiting conditions [13]. Our data suggest that this compensatory mechanism can occur between any of the isoforms depending on the tissue as well as on the availability of the metal cofactors. Also, it appears that this mechanism not only occurs in metal-limiting conditions but also when the ratios between the different micronutrients are altered internally, as shown in wild type roots when increased Zn concentrations are supplied to the nutrient solution (Table 2). The shift between the activities of the different isoforms could take place as a result of interactions occurring between micronutrients within the plant that can produce synergistic and antagonistic effects in the plant nutrient status. If, as a consequence of metal interactions, the availability to the plant cell of either Mn, Zn or Cu is altered, the situation could trigger a cellular response by inducing another enzyme whose prosthetic metal is available, in order to maintain an adequate level of SOD activity. However, it must be kept in mind that these micronutrient-induced changes in activities of SOD may also reflect a response of the plant to increased free radical production in the compartment where they are located.

Zn concentrations in tissues are not always satisfactory indicators for the diagnosis of Zn status in plants [34]. Several reports suggest that only a proportion of total measurable Zn is physiologically available [35]; for example, Zn may be bound to cell walls [36] or chelated by organic ligands [37], and thus become physiologically inactivated in tissues. In order to study the suitability of the two Zn-containing enzymes, CA and CuZnSOD, as indicators of plant Zn status, the correlations between the measured activities and the total metal concentrations in both roots and leaves from plants grown in the different nutritional regimes were calculated (Figs. 3 and 4). The best correlation was found between CA activity and total Zn concentration; CA activity increased linearly with increased total Zn tissue concentration exhibiting a regression coefficient of 0.798 and a plateau when leaf Zn concentrations exceeded 200 µg Zn g⁻¹ DW⁻¹ (Fig. 4A). By comparison, CA activity increased with Zn leaf concentrations up to 550 µg Zn g⁻¹ DW⁻¹. In roots, no significant correlation tissue concentration exhibiting a regression coefficient of 0.798 and a plateau when leaf Zn concentrations exceeded 200 µg Zn g⁻¹ DW⁻¹ (Fig. 4A). By comparison, CA activity increased with Zn leaf concentrations up to 550 µg Zn g⁻¹ DW⁻¹. In roots, no significant correlation

![Fig. 3. Carbonic anhydrase activity (units CA mg protein⁻¹ s⁻¹) in leaves (A) and roots (B) of wild type (○) and raz (●) plants vs. Zn concentration in leaves and roots from plants grown under the same conditions. Data are means ± S.D. of at least five replicates.](image)

![Fig. 4. CuZnSOD activity (units SOD mg protein⁻¹) in leaves (A) and roots (B) of wild type (○) and raz (●) plants vs. Zn concentration in leaves and roots from plants grown under the same conditions. Data are means ± S.D. of at least five replicates.](image)
between CuZnSOD activity and total Zn tissue concentration was found (Fig. 4B). This lack of correlation is mainly associated to the fact that CuZnSOD activity in mutant root extracts was similar or even lower to that measured in wild type roots in most of the regimes assayed although Zn tissue concentrations for these same roots were higher in raz than in wild type roots (Fig. 2, Table 2). These results, as well as the fact that CA is not related to the oxidative stress defense mechanism, indicate that CA activity is a better indicator of Zn status for plants since it saturated only with high Zn tissue concentrations, that are rarely reached in most plants even when grown at high Zn supply.

Since one of the SOD isoforms is a Mn-containing enzyme, the suitability for the use of the activity of this isoform to assess Mn biochemical utilization was also analyzed in our experiments. No correlations were found between MnSOD activity and Mn tissue concentrations in leaves or roots (Fig. 5). These results indicate that MnSOD is not a good marker for total Mn concentration in plants.

In conclusion, based on our results, the availability of Zn in raz leaves does not seem to be altered; however, in roots of the raz mutant, Zn availability is restricted or limited in comparison to wild type plants, especially when raz roots accumulate high levels of Zn. Furthermore, our data demonstrate that CA activity is well correlated to total Zn tissue concentrations, and could be a useful indicator of Zn status over a wide range of Zn concentrations (i.e., not just Zn deficiency). The results also underline the importance of mutants which exhibit altered metal homeostasis as useful tools to study metal physiology in plants.

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