Zinc Uptake and Shoot Partitioning Between Zinc Efficient and Inefficient *Exacum* Genotypes

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ABSTRACT. Interspecific hybrids of *exacum* (*Exacum* L.) display variable responses to zinc nutrition. Our research compared two genotypes with contrasting zinc efficiency phenotypes in terms of root cation exchange capacity (CEC), whole plant ⁶⁵Zn uptake, and the effects of Cu²⁺ and Mg²⁺ on ⁶⁵Zn uptake and partitioning to shoot tissues. Results show that the zinc efficient and inefficient genotypes had significantly different root CEC [27.2 and 16.9 cmol(+)·kg⁻¹ root dry weight (DW), respectively] and whole plant ⁶⁵Zn uptake rates (0.048 and 0.026 μmol·h⁻¹·g⁻¹ DW, respectively). In equimolar concentrations to Zn²⁺, Cu²⁺ reduced Zn²⁺ uptake by approximately 50% in both genotypes while supplemental Mg²⁺ enhanced Zn²⁺ uptake. In addition, Mg²⁺ facilitated a larger proportion of absorbed ⁶⁵Zn to the upper shoot of the efficient genotype. We conclude zinc is absorbed through a specific Zn²⁺/Cu²⁺ transporter and that zinc efficiency in *exacum* is based on a combination of apoplastic and symplastic traits. In addition, a secondary Mg²⁺ × Zn²⁺ interaction may contribute to the zinc efficiency phenotype.

Exacum (Gentianaceae) contains ≈65 species (Klackenberg, 1985) that are hypothesized to comprise a polyploid series of species (Darlington and Wylie, 1955). Of these species, only *E. affine* L. has gained economic importance as an ornamental crop with this species' commercial history dating back to 1904 (Riseman, 2005). However, descriptions of other *exacum* species indicate the presence of valuable horticultural traits that include large flowers (>2.5 cm) that range in color from light blue to dark violet, diverse growth habits (upright to procumbent), and attractive glossy green foliage. Based on these descriptions, several species native to Sri Lanka were collected and formed the basis of an interspecific breeding program designed to develop this germplasm for horticultural use.

During production of the interspecific populations, a significant number of genotypes developed Zn²⁺ deficiency symptoms severe enough to cause concern for commercial production while other genotypes remained healthy and symptom free. We categorized our germplasm into efficient and inefficient genotypes based on both the phenotypic expression of classic Zn²⁺ deficiency symptoms (i.e., chlorotic and stunted growth due to shortening of internodes leading to "rosetting" and a drastic decrease in leaf size) and overall biomass production (i.e., Zn²⁺ inefficient genotypes produced approximately one-third the biomass of Zn²⁺ efficient genotypes) when grown under identical conditions. Nutrient analyses identified Zn²⁺ as the only element that differed significantly between these phenotypes with the efficient and inefficient genotypes containing 35 and 8 μg·g⁻¹ DW shoot tissue, respectively (Riseman, 1997). In addition, the zinc

deficiency phenotype was correlated with specific interspecific pedigrees so we concluded the presence of a significant genetic component originating from the native species used in the primary interspecific hybridizations (Riseman, 1997). The species used in these hybridizations displayed significant variation in morphology, cytology, and isozyme profiles (Sumanasinghe, 1986) but were never observed with Zn²⁺ deficiency symptoms in their native habitats (Riseman, 1997). In addition to these plant-based characters, these Sri Lankan species have been associated with distinct edaphic conditions found in their native habitats (Riseman, 1997). Significant differences were found among soil samples for many edaphic variables including Zn²⁺ content (0.28 to 2.91 kg·ha⁻¹), Cu²⁺ content (0.18 to 6.50 kg·ha⁻¹), Mg²⁺ content (0.33 to 5.04 kg·ha⁻¹), Cu²⁺:Zn²⁺ ratio (0.5 to 6.0), and Mg²⁺:Zn²⁺ ratio (0.28 to 4.5) (Riseman, 1997). There exists the possibility these disparate edaphic conditions acted as a selection pressure in the evolution of distinct taxa. It has been shown that variation in edaphic conditions can affect the evolution and development of ecotypes with genetically distinct nutrient uptake and translocation mechanisms (Rajakaruna et al., 2003).

In evaluating nutrient efficiencies, Rengel (2001) outlines several generalizations relating to genotypic differences: 1) efficiency mechanisms vary among crop species and genotypes, 2) more than one mechanism is often responsible for efficiency in a particular genotype, 3) increased efficiency of one genotype in comparison to the other is due to the involvement of additional mechanisms not present (or expressed) in the less efficient genotype, and 4) expression of more than one efficiency mechanism is likely to result in an additive effect. In addition, the mechanisms of nutrient efficiency may operate at various levels of plant organization (i.e., molecular, physiological, structural, or developmental). Many individual processes have been cited in the literature as possible sources of Zn²⁺ efficiency. These include differences in nutrient uptake kinetics where individual genotypes differ in their ability to transport Zn²⁺ into the symplasm (Bowen, 1987; Graham and Rengel, 1993); differences in root cation exchange capacity where genotypes possess different apoplastic binding

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affinities for Zn^{+2} that may provide a resource or pool of Zn^{+2} available for uptake mechanisms to access (Sakal et al., 1988); differences in translocation rates where absorbed Zn^{+2} is differentially translocated within the plant (Graham and Rengel, 1993); and differences in the effects of competing ions where elemental interactions positively or negatively affect the uptake or translocation of Zn^{+2} (Amber and Brown, 1969; Brown and McDaniel, 1978; Jolley and Brown, 1991; Parker et al., 1992; Pedler et al., 2004).

This research was conducted to identify factors which contribute to zinc efficiency and were suitable for use in germplasm evaluations designed to identify zinc efficient genotypes. In addition, we feel this information helps to elucidate mechanisms that may have affected the evolution of exacum taxa. Previously, zinc efficiency in our germplasm was determined to be primarily root-based (Riseman, 1997) and highly correlated with Zn^{+2} uptake per unit of root length (Riseman and Craig, 2000). However, additional root-based traits have been associated with Zn^{+2} efficiency. In this research, we compared Zn^{+2} efficient and inefficient genotypes with respect to 1) root cation exchange capacity (CEC); 2) whole plant Zn^{+2} uptake rates; 3) the competitive effects of Cu^{+2} and Mg^{+2} on Zn^{+2} uptake rates; and 4) the effects of Cu^{+2} and Mg^{+2} on Zn^{+2} partitioning to shoot tissues.

Materials and Methods

PLANT MATERIAL. Two interspecific hybrids of exacum, derived from species native to Sri Lanka, were used. These genotypes were 10th generation hybrids arising from directed hybridizations among five species: *E. macranthum* Arn., *E. pedunculatum* L., *E. pallidum* Trimen., *E. trinervium* (L.) Druce, and *E. trinervium* ssp. *ritigalensis* (Willis) Cramer. The Zn^{+2} efficient (92-49-5) and inefficient (92-39-4) genotypes were selected based on the predictable expression of contrasting Zn^{+2} efficiency phenotypes with respect to development of Zn^{+2} deficiency when grown under common glasshouse conditions.

ESTABLISHMENT OF TEST PLANTS. In all experiments, analytical grade salts and deionized water were used for stock and treatment solutions. All glassware and containers were acid washed and thoroughly rinsed with deionized water. Asexually propagated cuttings were used for this research. Terminal cuttings were harvested from vegetative stock plants, bases treated with 1% IBA (Hormodin #1; MSD-AGVET, Division of Merck and Co., Rahway, N.J.), and placed in plastic cell packs filled with 0.03-mm-sized silica sand. Cuttings were placed under intermittent mist for ≈ 4 weeks for rooting, transplanted individually into 10-cm plastic containers containing 0.03-mm-sized silica sand, and transferred to a glasshouse maintained at 18 °C day/14 °C night temperatures and an average irradiance level of 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetically active radiation (PAR). Plants were overhead irrigated daily to container capacity (plus 10%) with the following solution (Jonson et al., 1957): KNO_3 (6 mM), $\text{Ca}(\text{NO}_3)_2$ (6 mM), $\text{NH}_4\text{H}_2\text{PO}_4$ (2 mM), MgSO_4 (225 μM), KCl (50 μM), H_3BO_3 (25 μM), MnSO_4 (2 μM), CuSO_4 (0.5 μM), $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ (0.5 μM), FeSO_4 (2 μM), ZnSO_4 (2 μM). At biweekly intervals, a 2- μM ZnSO_4 solution was applied to "runoff" as a prophylactic foliar spray on both genotypes to prevent Zn^{+2} stress development and to allow for equal biomass production. When produced with the ZnSO_4 foliar supplement, both genotypes maintained equal concentrations of shoot Zn^{+2} averaging, 28 $\mu\text{g}\cdot\text{g}^{-1}$ DW. All experimental units were without symptoms of any nutrient stress and were selected based on comparable total biomass averaging 108 g (± 4

g) fresh weight. Average age of experimental units was 8 weeks from time of transfer to greenhouse.

ROOT CEC EXPERIMENTS. Root CEC was measured according to the method of Helmy and Elgabaly (1958). Five-gram fresh weight root samples were acidified through five rinses of 0.1 N HCL. Samples were then rinsed five times in deionized water followed by back titration to pH 8 with a 0.04 N KOH solution. Titration time was approximately 2 min. Extra care was paid to titration time to equalize the effect of carbonic acid formation by CO_2 diffusion into the solution. After titration, tissues were dried at 80 °C in a forced air oven for 24 h. Root CEC is expressed as $\text{cmol}(+)\cdot\text{kg}^{-1}$ dry tissue.

Zn^{+2} DESORPTION, UPTAKE, AND TISSUE PARTITIONING EXPERIMENTS. Preliminary trials were conducted to empirically determine an adequate desorption period following 6 h of exposure to ^{65}Zn -labeled uptake solution. Plants were removed from the uptake solution (see ^{65}Zn uptake solution section below), blotted free of surface solution and placed in ice cold desorption solution consisting of 0.5 mM CaCl_2 . The plant roots and solution were agitated by gentle manual manipulation. Desorption solutions were sampled at 1, 2, 3, 4, 5, 7, 10, 12, 15, 20, and 25 min after root contact. At each sample interval, a 0.5-mL aliquot was collected and analyzed for radioactivity. A final desorption period was determined when no additional radioactivity was recorded in three consecutive aliquots. This time period was used for all subsequent trials and for both genotypes. Based on these trials, a 15-min desorption period was used for all subsequent experiments.

Upon initiation of the ^{65}Zn uptake experiments, plants were moved from the glasshouse to the laboratory where the roots were washed free of sand with deionized water. Roots were easily removed from the sand without any perceivable damage. Plants were then placed into 1-L plastic containers with holes drilled through the lid to provide support for the shoot while allowing the roots to be immersed in the uptake solution. Two pretreatment solutions were used consecutively. The first solution consisted of full-strength solution (Jonson et al., 1957) composed of the following salts and concentrations: KNO_3 (6 mM), $\text{Ca}(\text{NO}_3)_2$ (6 mM), $\text{NH}_4\text{H}_2\text{PO}_4$ (2 mM), MgSO_4 (225 μM), KCl (50 μM), H_3BO_3 (25 μM), MnSO_4 (2 μM), CuSO_4 (0.5 μM), $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ (0.5 μM), FeSO_4 (2 μM), ZnSO_4 (2 μM). All solutions were constantly aerated. Plants remained exposed to the first pretreatment solution for 24 h. The second solution consisted of only CaCl_2 (0.5 mM) and ZnCl_2 (2 μM). Plants remained exposed to the second pretreatment solution for an additional 24 h. All pretreatments and uptake experiments were used a water bath to maintain solution temperatures at 30 °C. Irradiance levels of 450 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR were produced by one 1000-W metal halide lamp positioned 1 m above the plants.

^{65}Zn UPTAKE SOLUTION. Following the second 24-h pretreatment, the CaCl_2 + ZnCl_2 solution was replaced with the uptake solution. Composition of the uptake solution was CaCl_2 (0.5 mM) and ZnCl_2 (2 μM) with the addition of 370 kBq ^{65}Zn (10 μCi) supplied as the chloride salt (Amersham Corp., Arlington Heights, Ill.).

At harvest, roots were blotted with paper towels to remove surface solution, desorbed for 15 min, partitioned by tissue category and dried at 80 °C for 48 h. Roots were cut into 10-mm segments and mixed to form homogenous subsamples. Shoots were partitioned into individual leaf pairs and internode sections. After drying, all samples were weighed and measured for radioactivity.

COMPETITION EXPERIMENTS. For ion competition experiments, a single radioactive CaCl_2 + ZnCl_2 solution, as described above,

was made and divided into two equal aliquots. The first aliquot remained unchanged and served as the control while the second aliquot was supplemented with either Mg^{+2} or Cu^{+2} . This arrangement allowed for a common specific activity to be used within each replication. Both Mg^{+2} and Cu^{+2} were used in equimolar concentrations to the Zn^{+2} concentration ($2\ \mu M$) and evaluated individually in separate trials. Magnesium chloride and copper chloride were used as the source of the competing ions. Three complete replications were conducted for each ion. Plant tissues were divided into the following categories prior to analyses: “upper shoot,” meaning all tissue distal to and including youngest fully expanded leaf pair; “mid shoot,” meaning all tissue basal to upper shoot to the second oldest leaf pair; “lower shoot,” meaning all tissue basal to the mid shoot to the oldest leaf pair; “lower stem,” meaning all tissue basal to the oldest leaf pair; and “roots,” meaning all root tissue.

^{65}Zn radioactivity was measured on a Gamma Scintillation Spectrometer (model 5230; Packard Instrumentation Co., Downers Grove, Ill.). The detection parameters were set at a lower limit of 450 MeV with a window of 210 MeV. All samples were counted for a minimum of either 20 min or 10,000 counts. Background radiation was accounted for by bracketing each sample with blanks and subtracting the average between them from the embedded sample. Specific activity was determined by counting an initial aliquot collected from the uptake solution prior to exposure to the plants and then converted to counts per minute (CPM)/ $\mu mol\ Zn^{+2}$ based on the initial Zn^{+2} concentration of $2\ \mu mol\cdot L^{-1}$. Individual specific activity calculations were determined for each block and utilized in only that block.

EXPERIMENTAL DESIGN AND STATISTICS. For root CEC experiments, five plants of each genotype were utilized as replicates. The roots of each plant were partitioned into three groups and considered subsamples. Statistics were performed by StatView SE⁺ Graphics (Abacus Concepts, Berkeley, Calif.) statistical software.

For ^{65}Zn uptake and ion competition experiments, two plants of each genotype were placed in a single container; each container served as a replicate in the statistical analyses. A total of 10 replicates were utilized in each experiment. Statistical procedures (i.e., analysis of variance, mean separation and paired *t* tests of means) were performed with Systat for the Macintosh version 5.2 (Systat, Evanston, Ill.). For the ion competition studies, data are presented as both absolute values and as a percentage of control. For Zn^{+2} uptake and partitioning comparisons, plant tissues were grouped as described above.

Results

Root CEC. The efficient genotype had significantly greater root CEC than the inefficient genotype (Fig. 1). This difference is presumed to have affected the amount of Zn^{+2} released from the root tissues following 15 min of desorption. Following desorption, the two genotypes released significantly different amounts of Zn^{+2} , with the efficient genotype releasing $0.25\ \mu mol\cdot g^{-1}\ DW$ root while the inefficient genotype released only $0.16\ \mu mol\ Zn^{+2}$.

Zn^{+2} UPTAKE AND TISSUE PARTITIONING. After 1 h, there were no significant genotype differences in ^{65}Zn concentration within any tissue category (Table 1). However, in each category, the zinc efficient genotype contained a higher mean ^{65}Zn concentration than the inefficient genotype. After 6 h of exposure to the uptake solution, whole-plant Zn^{+2} uptake rates were significantly greater in the efficient genotype ($188.2\ pmol\cdot h^{-1}\cdot g^{-1}\ DW$) than

Fig. 1. Root cation exchange capacity (CEC) for efficient and inefficient genotypes of exacum. Mean of 15 replicates; error bars = SE; means of efficient and inefficient genotypes significant at $P < 0.001$.

Table 1. Radiolabeled zinc uptake in efficient and inefficient exacum genotypes for each tissue category at 1 h and 6 h after exposure to solution [mean (SE) of 10 replicates].

Tissue category ^z	Zinc uptake (pmol·g ⁻¹ dry wt)			
	1 h ^y		6 h ^y	
	Efficient genotype	Inefficient genotype	Efficient genotype	Inefficient genotype
Upper shoot	0.5 (0.04) a	0.2 (0.05) a	4.0 (0.1) a	0.9 (0.4) b
Mid shoot	0.2 (0.06) a	0.1 (0.04) a	0.6 (0.1) a	0.5 (0.2) a
Lower shoot	0.5 (0.09) a	0.4 (0.03) a	0.6 (0.1) a	0.5 (0.1) a
Lower stem	26.0 (9) a	24.0 (5) a	31.0 (8) a	22.0 (3) a
Roots	374.0 (90) a	298.0 (50) a	1093.0 (280) a	545.0 (90) b

^zTissue categories: upper shoot = all tissue distal to and including youngest fully expanded leaf pair; mid shoot = all tissue basal to upper shoot to the second oldest leaf pair; lower shoot = all tissue basal to the mid shoot to the oldest leaf pair; lower stem = all tissue basal to the oldest leaf pair; and roots = all root tissue.

^y*t* statistics performed between genotypes within an exposure time and within tissue category; means followed by a common letter are not different ($P \leq 0.05$) by Fisher's protected least significant difference.

in the inefficient genotype ($94.8\ pmol\cdot h^{-1}\cdot g^{-1}\ DW$) (calculated from Table 1, $P < 0.05$). In addition, significant differences were observed for Zn^{+2} allocation rates (i.e., sum of Zn^{+2} in all non-root tissues divided by the uptake period and total shoot dry weight) between the genotypes where the Zn^{+2} efficient and inefficient genotypes translocated 6.0 and $3.9\ pmol\cdot h^{-1}\cdot g^{-1}\ DW$, respectively (calculated from Table 1)

EFFECTS OF Cu^{+2} AND Mg^{+2} ON Zn^{+2} UPTAKE AND SHOOT PARTITIONING. As compared to the inefficient genotype, the efficient genotype absorbed significantly greater amounts of Zn^{+2} regardless of the competing ion (Fig. 2A). In general, Cu^{+2} reduced overall Zn^{+2} uptake while Mg^{+2} facilitated uptake, regardless of genotype or exposure time. After 6 h, Zn^{+2} uptake in the presence of Cu^{+2} was significantly reduced in the efficient genotype from 1129.2 to $745.3\ pmol$ while in the inefficient genotype, Zn^{+2} uptake was significantly reduced from 568.9 to $312.9\ pmol$ (Fig. 2A). On average, Cu^{+2} reduced root Zn^{+2} uptake compared to the control by 64% and 51% for the Zn^{+2} efficient and inefficient genotypes, respectively (Fig. 2B).

Magnesium enhanced Zn^{+2} uptake in both genotypes at both time intervals. After 6 h, Zn^{+2} uptake in the presence of Mg^{+2} was

Fig. 2. Effect of Cu^{+2} and Mg^{+2} ions on whole plant Zn uptake of efficient and inefficient genotypes of exacum; (A) whole plant concentration and (B) proportion of control. Mean of 10 replicates; error bars = SE; ^{65}Zn = CaCl_2 (0.5 mM) and ZnCl_2 (2 μM) with the addition of 370 kBq ^{65}Zn ; ^{65}Zn + Cu^{+2} = ^{65}Zn treatment solution with the addition of 2 μM CuCl_2 ; ^{65}Zn + Mg^{+2} = ^{65}Zn treatment solution with the addition of 2 μM MgCl_2 .

significantly increased in the efficient genotype from 1129.2 to 1309.9 pmol while in the inefficient genotype, Zn^{+2} uptake was significantly increased from 568.9 to 676.9 pmol (Fig. 2A). These increases were 116% and 119%, over the controls for the efficient and inefficient genotypes, respectively (Fig. 2B).

After 6 h, Zn^{+2} partitioning to shoot tissue categories significantly differed between the two genotypes with the addition of either Cu^{+2} or Mg^{+2} to the uptake solution. In the Zn^{+2} efficient genotype, the lower stem tissue contained <60% of the shoot Zn^{+2} regardless of competing ion (Fig. 3). In addition, Mg^{+2} altered Zn^{+2} distribution within the plant by facilitating movement from the lower stem to the upper shoot category (i.e., reducing the percentage of allocated Zn^{+2} in lower stem from 50% to 20%) while both the mid shoot and lower shoot categories were unchanged. Copper appeared to have a less pronounced effect than Mg^{+2} in that more Zn^{+2} was present in the upper than the mid shoot categories while the percentage in the lower stem remained approximately equal. In the inefficient genotype, neither Mg^{+2} nor Cu^{+2} induced the same responses observed in the efficient genotype. Overall, the lower stem category contained over 60% of the shoot Zn^{+2} while the upper shoot accounted for $\approx 4\%$, regardless of competing ion. In addition, Cu^{+2} appeared to significantly reduce allocation out

Fig. 3. Effect of Cu^{+2} and Mg^{+2} as competing ions on shoot zinc allocation in zinc efficient and inefficient genotypes of exacum after 6 h of exposure to uptake solution. Upper shoot = all tissue distal to and including youngest fully expanded leaf pair; mid shoot = all tissue basal to upper shoot to the second oldest leaf pair; lower shoot = all tissue basal to the mid shoot to the oldest leaf pair; lower stem = all tissue basal to the oldest leaf pair; Zn = CaCl_2 (0.5 mM) and ZnCl_2 (2 μM) with the addition of 370 kBq ^{65}Zn ; Zn + Cu^{+2} = Zn treatment solution with the addition of 2 μM CuCl_2 ; Zn + Mg^{+2} = Zn treatment solution with the addition of 2 μM MgCl_2 .

of the lower stem to any other tissue category while Mg^{+2} had no overall effect on distribution.

Discussion

Our results have identified several significant differences between Zn^{+2} efficient and inefficient genotypes of exacum. The Zn^{+2} efficient genotype possessed greater root CEC, greater Zn^{+2} uptake, greater Zn^{+2} partitioning to the upper shoot and greater ability to absorb and translocate Zn^{+2} in the presence of competing ions. We believe these differences are the basis for the Zn^{+2} efficient phenotype previously observed in common glasshouse experiments (Riseman, 1997). The presence of multiple traits associated with a nutrient efficiency is not surprising based on Rengel's (2001) generalizations for genotypic differences in nutrient efficiency already mentioned. In addition to these generalizations, mechanisms associated with nutrient efficiency may operate at various levels of plant organization (i.e., molecular, physiological, structural, or developmental). Our results indicate multiple traits, across different levels of plant organization are associated with Zn^{+2} efficiency in exacum.

The Zn^{+2} efficient genotype was found to have significantly higher root CEC and greater overall Zn^{+2} uptake. As in soil, root CEC is a measure of the ability to electrostatically bind cations to negative surface charges. In roots, this is often called apoplastic binding and is comprised of charges on both the cell wall and exterior plasma membrane. These charges often arise from ligands containing negatively charged organic functional groups such as the carboxyl residues of pectins (Ostateck-Boczynski et al., 1995). Apoplastic CEC can influence cation selectivity and ion competition for membrane transporters. This is because diverse cations have different affinities for apoplastic exchange sites, and because the distribution of one ionic species around a charged surface will effect the distribution of other cationic species through repulsion of like charges (Shomer et al., 2003). In relation to the observed differences in Zn^{+2} uptake, apoplastic CEC may selectively position greater amounts of Zn^{+2} near transporters thereby

resulting in greater uptake by efficient genotypes. However, other possibilities exist and include differences in transporter affinities, kinetics, number, or transporter gene expression/regulation.

To evaluate whether specific ions affected Zn^{+2} uptake, Mg^{+2} and Cu^{+2} were used in equimolar concentrations to Zn^{+2} as competitive inhibitors. In both genotypes, Mg^{+2} enhanced Zn^{+2} uptake while Cu^{+2} inhibited uptake. Two models, each with empirical support, have been proposed for Zn^{+2} uptake: 1) a general divalent cation channel and 2) a specific $\text{Zn}^{+2}/\text{Cu}^{+2}$ transporter. In support of the general divalent cation model, Chaudhry and Loneragan (1972) demonstrated that all of the alkaline earth cations (Ca^{+2} , Mg^{+2} , Sr^{+2} and Ba^{+2}) inhibited Zn^{+2} uptake in wheat (*Triticum aestivum* L.), indicating a common uptake mechanism. Kochian (1993), in summarizing zinc uptake research, proposed that Zn^{+2} , as well as Cu^{+2} , Mn^{+2} and Mg^{+2} , is probably absorbed through a common divalent cation channel and not by specific ion transporters. In opposition to this model, there is research that supports the existence of specific $\text{Zn}^{+2}/\text{Cu}^{+2}$ transporters. Schmid et al. (1965) demonstrated using barley (*Hordeum vulgare* L.), that Cu^{+2} strongly inhibited Zn^{+2} uptake while Mn^{+2} had no effect. Bowen (1987), using rice (*Oryza sativa* L.) and tomato (*Lycopersicon esculentum* Mill.), showed that Cu^{+2} and Zn^{+2} mutually and competitively inhibited uptake of each other and suggested both micronutrients were absorbed through the same uptake mechanism or carrier site. These apparently conflicting models may, in reality, both be correct. Often, inadequate experimental methods, that do not account for uptake/release rates, uptake of ions from complex solutions (e.g., solutions with multiple ions), or electrostatic affinities of ions present in low concentrations, inhibit the accurate characterization of ion specific transporters. Therefore, much of the published micronutrient uptake research must be viewed with care. However, research that attempted to account for these issues has identified both high and low affinity transporters within a single plant species (Reid et al., 1996). They state that the low affinity transporter had broad substrate specificity while they made no comment on the substrate specificity for the high affinity transporter. These two Zn^{+2} uptake mechanisms may individually represent the two proposed models where the low affinity transporter is analogous to the general divalent cation channel and the high affinity transporter is analogous to the $\text{Zn}^{+2}/\text{Cu}^{+2}$ specific transporter. Data from this research, using the relatively low concentration of $2 \mu\text{M}$ Zn, support the presence of a high-affinity transporter specific to Zn^{+2} and Cu^{+2} . However, future research should evaluate the presence of multiple uptake mechanisms and ionic affinities of transporters in this germplasm.

The cause for increased Zn^{+2} uptake and shoot transport in the presence of Mg^{+2} is unclear. It may be due to apoplastic binding affinities that may have otherwise been available to bind Zn^{+2} . With these binding sites occupied, Zn^{+2} transport through the apoplast was facilitated. Another possibility is that the experimental plants were in the initial stages of Mg^{+2} deficiency due to exposure to a simple $\text{CaCl}_2 + \text{ZnCl}_2$ solution for 24 h prior to the uptake experiments. With the addition of Mg^{+2} to the uptake solution, the deficiency was alleviated. In evaluating the effects of Mg^{+2} on Zn^{+2} toxicity, micromolar concentrations of Mg^{+2} were found to alleviate Zn^{+2} toxicity while simultaneously facilitating higher tissue Zn^{+2} concentrations (Pedler et al. 2004). The authors concluded that the protective effect of Mg^{+2} was not due to diminished Zn^{+2} uptake or translocation but rather, Mg^{+2} was involved in some type of internal detoxification or sequestration mechanism. However, working with isolated vesicles from tonoplasts, Verkleij et al. (1998) found that a Zn^{+2} -tolerant

genotype of *Silene vulgaris* (Moench.) Garcke displayed higher Zn^{+2} transport rates than the sensitive genotype and that transport required Mg-ATP. If our experimental plants did have an induced Mg^{+2} deficiency, the addition of Mg^{+2} to the uptake solution may have replenished the biologically available Mg^{+2} pool required for normal Zn^{+2} uptake. Although no clear explanation or mechanism has been determined to explain this $\text{Zn}^{+2} \times \text{Mg}^{+2}$ interaction, we accept the possibility that a tissue-specific Mg^{+2} requiring Zn^{+2} transporter may be present in *Exacum*.

Zn efficiency in *exacum* is a composite trait involving both apoplastic (i.e., CEC) and symplastic (i.e., Zn^{+2} transporters, Mg^{+2} requirement) traits that are most likely under independent regulation. When we consider the polyploid nature of the original taxa combined with distinct edaphic conditions of their native habitats, the opportunity for gene diversification is great. Gene diversification for micronutrient uptake can be a relatively simple process in which a single amino acid replacement can significantly alter substrate specificity (Rogers et al., 2000). In their native habitats, no *Exacum* taxon was observed with Zn^{+2} deficiency symptoms despite the large range in divalent cation concentrations and ratios (Riseman, 1997). Therefore, we propose that Sri Lankan *exacum* have experienced edaphic selection where taxa have evolved unique genes, regulators, or linkage groups that allow for adequate Zn^{+2} uptake and transport under disparate nutrient conditions. With subsequent interspecific hybridization, these genes, regulators, or linkage groups have been rearranged and mismatched to the point where Zn^{+2} uptake mechanisms are less effective. These conclusions are consistent with Rengel's generalizations (2001) concerning genotypic differences in nutrient efficiency in that 1) we identified more than one mechanism associated with the efficiency phenotype (i.e., root CEC, Zn^{+2} uptake, and $\text{Zn}^{+2} \times \text{Mg}^{+2}$ interaction), 2) our genotypic differences were based on mechanisms not present (or expressed) in the less efficient genotype (i.e., lower root CEC, reduced Zn^{+2} uptake in inefficient genotype), and 3) mechanisms identified in *exacum* operate at various levels of plant organization (i.e., genetic and physiological differences between genotypes).

This research has presented information on various physiological traits associated with Zn^{+2} efficiency and has proposed a mechanism for their development and evolution. With this information, we are better able to breed and select genotypes suitable for commercial production because we now have specific evaluation and selection criteria linked to the zinc efficiency phenotype. However, several important questions were raised that require additional research. Are multiple Zn^{+2} uptake mechanisms present in *exacum* that are analogous to the low and high affinity transporters identified in other organisms? What is the physiological basis for enhanced Zn^{+2} uptake and translocation in the presence of micromolar concentrations of Mg^{+2} ? And finally, is Zn^{+2} efficiency in *exacum* the result of several interacting mechanisms that only operate effectively when present in their original arrangement?

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