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ZINC UPTAKE AND SHOOT PARTITIONING BETWEEN ZINC EFFICIENT AND INEFFICIENT *EXACUM* GENOTYPES

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Abstract

Interspecific hybrids of *Exacum* display differential susceptibility to zinc deficiency. Our research compared two genotypes with contrasting zinc deficiency phenotypes in terms of root CEC, whole plant Zn uptake, and the effects of Cu^{+2} and Mg^{+2} ions on Zn uptake and partitioning to shoot tissues. Results show that the Zn efficient and inefficient genotypes had significantly different root CEC (27.2 and 16.9 meq 100 g^{-1} root dry weight, respectively). Significant differences in whole plant zinc uptake rates were present with the efficient genotype absorbing 0.048 μmoles $Zn \bullet h^{-1} \bullet g^{-1}$ dry weight while the inefficient genotype absorbed only 0.026 μmoles $Zn \bullet h^{-1} \bullet g^{-1}$ dry weight. In equimolar concentrations Cu^{+2} reduced Zn uptake by approximately 50% in both genotypes while supplemental Mg^{+2} enhanced Zn uptake. In addition, Mg^{+2} facilitated a larger proportion of absorbed Zn to the upper shoot of the efficient genotype. We conclude Zn is absorbed through a specific Zn/Cu transporter and that Zn efficiency in *Exacum* is based on a combination of apoplastic and symplastic traits. In addition, a secondary Mg x Zn interaction may contribute to the zinc efficiency phenotype.

Abbreviations: CEC- Cation Exchange Capacity; IBA- Indole-3-Butyric Acid; PAR- Photosynthetically Active Radiation; CPM- Counts Per Minute;

Introduction

The genus *Exacum* L. (Gentianaceae or the gentian family) contains approximately 65 species (Klackenberg, 1985) that are hypothesized to comprise a polyploid series (Riseman, in press). Of these species, nine are native to Sri Lanka and display significant variation in morphology, cytology, and isozyme profiles (Sumanasinghe, 1986). In addition, the Sri Lankan taxa 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 zinc content (0.28 to 2.91 kg ha^{-1}), copper content (0.18 to 6.50 kg ha^{-1}), magnesium content (0.33 to 5.04 kg ha^{-1}), Cu:Zn ratio (0.5 to 6.0) and Mg:Zn ratio (0.28 to 4.5) (Riseman, 1997). Variation in edaphic conditions has been shown to affect the evolution and development of ecotypes with genetically distinct ion uptake and translocation mechanisms (Rajakaruna et al., 2003).

In 1983 and 1985, five of the nine species native to Sri Lanka were collected for botanical and horticultural research. Once collected, these plants formed the basis of an interspecific breeding program. However, during cultivation of the interspecific populations, significant differences in zinc deficiency symptoms were observed among genotypes suggesting a genetic basis for this trait (Riseman, 1997). Rengel (2001) outlines several generalizations relating to genotypic differences in nutrient efficiency: 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 zinc efficiency. These include differences in nutrient uptake kinetics where individual genotypes differ in their ability to transport zinc into the symplasm (Bowen, 1987; Graham and Rengel, 1993); differences in root cation exchange capacity where genotypes possess different apoplastic binding affinities for zinc that may provide a resource or pool of zinc available for uptake mechanisms to access (Sakal et al., 1988); differences in translocation rates where absorbed zinc 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 zinc (Amber and Brown, 1969; Jolley and Brown, 1991; Brown and McDaniel, 1978; Parker et al., 1992; Pedler et al, 2004).

Research in our program demonstrated that zinc efficiency in interspecific *Exacum* germplasm is primarily root-based (Riseman, 1997) and highly correlated with Zn uptake per cm root length (Riseman and Craig, 2000). However, additional root-based traits have been associated with Zn efficiency in *Exacum.* This research is intended to compare Zn efficient and inefficient genotypes with respect to 1) root CEC; 2) whole plant Zn uptake rates; 3) the competitive effects of Cu^{+2} and Mg^{+2} ions on Zn uptake rates; and 4) the effects of Cu^{+2} and Mg^{+2} ions on Zn 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 10^{th} generation hybrids arising from directed hybridizations among five species: *E. macranthum* Arn., *E. pedunculatatum* L., *E. pallidum* Trimen., *E. trinervium* (L.) Druce, and *E. trinervium* ssp. *ritigalensis* (Willis) Cramer. The Zn efficient (92-49-5) and inefficient (92-39-4) genotypes were selected based on expression of contrasting Zn efficiency phenotypes with respect to development of Zn deficiency when grown under glasshouse conditions.

Plant Culture

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., NJ), and placed in plastic cell packs filled with 0.03 mm sized silica sand. Cuttings were placed under intermittent mist for approximately 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/14 C day/night temperatures and an average irradiance level of 800 $\mu mol \bullet s^{-1} \bullet m^{-2}$ PAR (Photosynthetically Active Radiation). Plants were irrigated to container capacity daily with the following solution (Jonson et al., 1957): KNO_3 (6mM), $Ca(NO_3)_2$ (6mM), $NH_4H_2PO_4$ (2mM), $MgSO_4$ (225µM), KCl (50 µM), H_3BO_3 (25 µM), $MnSO_4$ (2 µM), $CuSO_4$ (0.5 µM), $(NH_4)_6Mo_7$ (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 foliar spray to prevent zinc stress development and to allow for equal biomass production between genotypes. All experimental units were without symptoms of any nutrient stress and were selected based on comparable biomass (+/- 1% total fresh weight) and tissue nutrient content. 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 2 minutes. 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 hours. Root CEC is expressed as meq 100 g^{-1} dry tissue.

Zn Desorption, Uptake, and Tissue Partitioning Experiments

Preliminary trials were conducted to empirically determine an adequate desorption period following 6 hours of exposure to ^{65}Zn labeled uptake solution. Plants were removed from the uptake solution (described 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 minutes 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 desorption period of 15 minutes was used for all subsequent experiments.

Upon initiation of the ^{65}Zn uptake experiments, plants were moved from the greenhouse 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 (6mM), $Ca(NO_3)_2$ (6mM), $NH_4H_2PO_4$ (2mM), $MgSO_4$ (225µM), KCl (50 µM), H_3BO_3 (25 µM), $MnSO_4$ (2 µM), $CuSO_4$ (0.5 µM), $(NH_4)_6Mo_7O_{24}$ (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 hrs. 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 hours. Containers were placed in a water bath to maintain uptake solution temperature at 30 C. Irradiance levels of 450 $\mu mol \bullet s^{-1} \bullet m^{-2}$ PAR were produced by one 1000w metal halide lamp positioned 1 m above the plants.

Following the second 24 h pretreatment, the $CaCl_2$ + $ZnCl_2$ solution was replaced with the uptake solution. Composition of the uptake solution was identical to the second pretreatment solution except for the addition of 370 kBq ^{65}Zn (10 µCi) supplied as the chloride salt (Amersham Corp., Arlington Heights, IL).

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 hrs. 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 2nd 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 concentration (2 µM) 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 Company, Inc. Downers Grove, IL). 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 minutes 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 CPM/μmol Zn based on the initial Zn concentration of 2 μmol 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 Inc. Berkeley, CA) 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 ten replicates were utilized in each experiment. Analysis of variance, mean separation and paired t-tests of means were performed by Systat for the Macintosh version 5.2 (Systat, Inc. Evanston, IL). For the ion competition studies, data are presented as both absolute values and as a percentage of control. For zinc uptake and partitioning comparisons, plant tissues were grouped as described above.

Results

Root CEC

Average root CEC values for the Zn efficient and inefficient genotypes were 27.2 and 16.9 meq 100 g^{-1} root dry weight, respectively (Fig 1) and that this was a significant difference ($p<0.001$). This difference is presumed to have affected the amount of Zn released from the root tissues following 15 minutes of desorption. Following desorption, the two genotypes released significantly different amounts of Zn, with the efficient genotype releasing 0.25 μmoles Zn•g^{-1} dry weight root while the inefficient genotype released only 0.16 μmoles Zn (data not shown).

Zinc Uptake and Tissue Partitioning

After six hours of exposure to the uptake solution, whole plant Zn uptake rates were significantly different ($p<0.05$) between the efficient genotype (188.2 pmoles Zn•h^{-1}•g^{-1} dry weight) and inefficient genotype (94.8 pmoles Zn•h^{-1}•g^{-1} dry weight) (calculated from Table 1). In addition, significant differences were observed for Zn allocation rates (i.e. sum of Zn in all non-root tissues divided by the uptake period and tissue dry weight) between the genotypes. Zinc allocation rates were 6.0 pmoles and 3.9 pmoles Zn•h^{-1}•g^{-1} dry weight, for the efficient and inefficient genotypes, respectively (calculated from Table 1)

For whole plant zinc uptake, analysis of variance indicated significant ($p<0.001$) genotype, tissue category, and time effects (data not shown). However, at one-hour exposure, there were no significant genotypic differences in Zn uptake within any individual tissue category although the Zn efficient genotype contained higher mean Zn concentrations than the inefficient genotype for each tissue category (Table 1). After six hours exposure, the Zn efficient genotype continued to have higher concentrations of Zn with significantly higher concentrations in both the upper shoot and root categories (Table 1).

Effects of Cu^{+2} and Mg^{+2} on Zinc Uptake and Shoot Partitioning

Copper ions reduced overall Zn uptake while Mg^{+2} facilitated uptake, regardless of genotype or exposure time. After six hours, Zn uptake in the presence of Cu^{+2} was significantly reduced in the efficient genotype from 1129.2 to 745.3 pmoles while in the inefficient genotype, Zn uptake was significantly reduced from 568.9 to 312.9 pmoles (Fig. 2A). On average, Cu^{+2} reduced root Zn uptake compared to the control by 64% and 51% for the Zn efficient and inefficient genotypes, respectively (Fig. 2B).

Magnesium enhanced Zn uptake in both genotypes at both time intervals. After six hours, Zn uptake in the presence of Mg^{+2} was significantly increased in the efficient genotype from 1129.2 to 1309.9 pmoles while in the inefficient genotype, Zn uptake was significantly increased from 568.9 to 676.9 pmoles (Fig. 2A). These increases were 116% and 119%, over the controls for the efficient and inefficient genotypes, respectively (Fig. 2B).

After six hours, Zn 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 efficient genotype, the lower stem tissue contained less than 60% of the shoot Zn regardless of competing ion (Fig. 3). In addition, Mg^{+2} altered Zn distribution within the plant by facilitating movement from the lower stem to the upper shoot category (i.e. reducing the percentage of allocated Zn 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 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 while the upper shoot accounted for approximately 4%, regardless of competing ion. In addition, Cu^{+2} appeared to significantly reduce allocation out 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 zinc efficient and inefficient genotypes of *Exacum*. The zinc efficient genotype possessed greater root CEC, greater zinc uptake, greater zinc partitioning to the upper shoot and greater ability to absorb and translocate zinc in the presence of competing ions. We believe these differences are the basis for the zinc 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: 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 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 zinc efficiency in *Exacum*.

In reviewing specific traits, the zinc efficient genotype was found to have significantly higher root CEC and greater overall zinc uptake. As in soil, root CEC is 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. Root CEC has been implicated in zinc efficiency but no specific mechanism has been confirmed (Sakal et al., 1988). However, it is hypothesized that root CEC binding acts as a reservoir for ions within the root from which nutrient uptake mechanisms access (Sakal et al., 1988). In addition, 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 zinc uptake, apoplastic CEC may selectively positioning greater amounts of zinc near the 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 zinc uptake, Mg^{+2} and Cu^{+2} ions were used as competitive inhibitors. In both genotypes, Mg^{+2} enhanced zinc uptake while Cu^{+2} inhibited uptake. Two models, each with empirical support, have been proposed for zinc uptake: 1) a general divalent cation channel and 2) a specific zinc/copper 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 zinc uptake, indicating a common uptake mechanism. Kochian (1993) found that Zn^{+2}, as well as Cu^{+2}, Mn^{+2} and Mg^{+2}, were 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 Zn/Cu transporters. Schmid et al. (1965) demonstrated that Cu^{+2} strongly inhibited Zn^{+2} uptake while Mn^{+2} had no effect. Bowen (1987) reported 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. Inadequate methods that do not account for influx/efflux rates, uptake of ions from complex solutions (e.g. solutions with multiple ions), and electrostatic affinities of ions present in low concentrations, all 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 zinc 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 Zn/Cu specific transporter. Data from this

research support the presence of a specific Zn/Cu transporter. However, no attempt was made to characterize or evaluate the presence of multiple uptake mechanisms or ionic affinities of transporters.

The cause for increased zinc 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 blocked, Zn^{+2} transport through the apoplast was facilitated. Another possibility is that the experimental plants were in the initial stages of magnesium deficiency due to exposure to a simple $CaCl_2$ + $ZnCl_2$ solution prior to 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 zinc 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 zinc tolerant genotype of *Silene vulgaris* displayed higher Zn^{+2} transport rates than the sensitive genotype and that transport required Mg-ATP. If our experimental plants did have an induced magnesium 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 Zn x Mg interaction, we accept the possibility that a tissue-specific Zn^{+2} transporter, that requires Mg^{+2}, may be present in *Exacum* and that the genotypic differences may relate to a breakdown of interacting mechanisms that results in the inefficient phenotype.

Genomic analyses of a broad range of angisperms have revealed several gene families involved in the transport of divalent cations (Mäser et al., 2001). Unfortunately, the range of ion specificities and cellular localization for most plant Zn^{+2} transporters remains unknown because the required physiological studies have lagged gene discovery. Reports do indicate the presence of at least one family of transporters relatively specific for Cu^{+2} and Zn^{+2} uptake (Shingles et al., 2004); however, the precise metal substrates of these transporters may vary according to the metal ion composition in the environment (Grotz et al., 1998; Pence et al., 2000; Moreau et al., 2002). These gene families may represent transporters with different ion specificities or localized to different cellular regions. There is general agreement that ion uptake into cortical cells and ion loading into the xylem are independently regulated processes. This separate regulation offers the plant the opportunity to control both the selectivity and rate of ion transport to the shoot; a possible action site for feedback regulation based on shoot demand (Marchner, 1995). For example, one family of Zn^{+2} transporter genes display distinct expression patterns within *Arabidopsis* (Grotz et al. 1998) and may function in tissue-specific roles (i.e. initial absorption vs. xylem uploading) (Mäser, et al., 2001). Ionic selectivity is particularly important for plant Zn^{+2} transporters because in soils that contain nutritionally imbalanced ratios of divalent cations, Zn^{+2} uptake may be inhibited while undesired metals may be absorbed to toxic levels. The *Exacum* germplasm used in this research originated from diverse edaphic conditions, including unusual nutrient

concentrations and ratios (Riseman, 1997). Under these conditions, species-specific and/or edaphic condition-specific zinc transport mechanisms may have evolved to ensure absorption of ions in proper proportions.

Zinc efficiency in *Exacum* is a composite trait involving both apoplastic (i.e. CEC) and symplastic (i.e. zinc transporters, Mg 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 zinc 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 zinc 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 zinc 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 Zn^{+2} x 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 zinc efficiency and has proposed a mechanism for their development and evolution. However, several important questions were raised that require additional research. Are multiple zinc 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 zinc uptake and translocation in the presence of micromolar concentrations of magnesium? And finally, is zinc efficiency in *Exacum* the result of several interacting mechanisms that only operate effectively when present in their original arrangement?

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Table 1. Zinc uptake (pmole Zn g^{-1} dry wt) at one hour and six hours after exposure to radiolabeled solution; Mean of 10 replicates (SE).

Tissue Category	1 Hour[Z]		6 Hour[Z]	
	Efficient Genotype	Inefficient Genotype	Efficient Genotype	Inefficient Genotype
Upper Shoot	0.5 (.04) [a]	0.2 (.05) [a]	4.0 (.1) [a]	0.9 (.4) [b]
Mid Shoot	0.2 (.06) [a]	0.1 (.04) [a]	0.6 (.1) [a]	0.5 (.2) [a]
Lower Shoot	0.5 (.09) [a]	0.4 (.03) [a]	0.6 (.05) [a]	0.5 (.09) [a]
Lower Stem	26.0 (9.0) [a]	24.0 (5.0) [a]	31.0 (8.0) [a]	22.0 (3.0) [a]
Roots	374.0 (90.0) [a]	298.0 (50.0) [a]	1093.0 (280.0) [a]	545.0 (90.0) [b]

[Z] 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 LSD.

Figure 1. Root cation exchange capacity for efficient and inefficient genotypes of *Exacum*; Mean of 15 replicates; Error bars = SE.

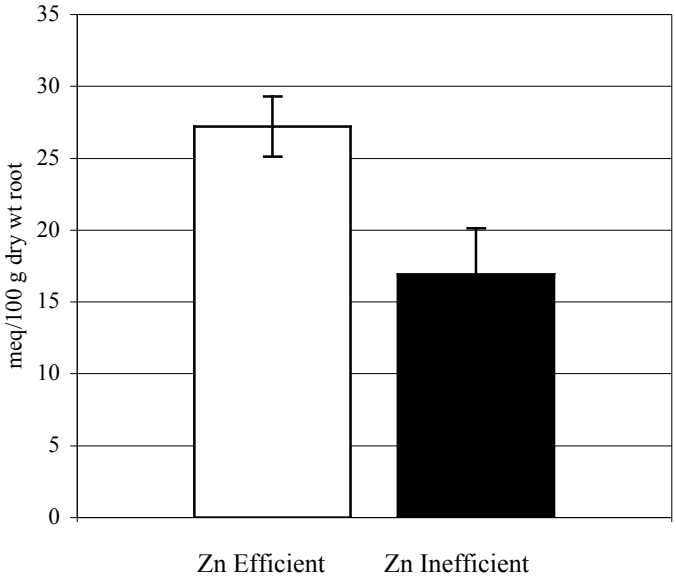

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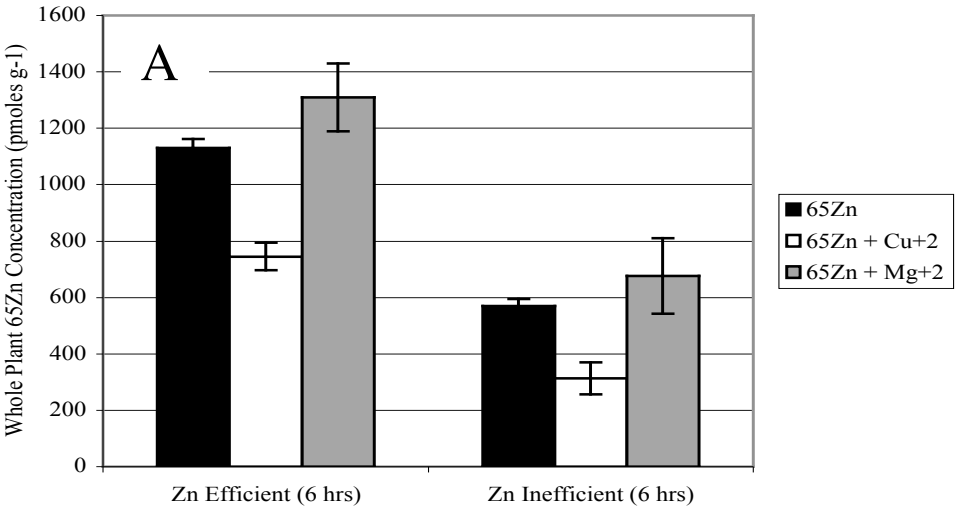
Figure 2. Effect of Cu+2 and Mg+2 ions on whole plant Zn uptake of efficient and inefficient genotypes; (A) pmoles•g^{-1} and (B) % of control; Mean of 10 replicates; Error bars = SE.



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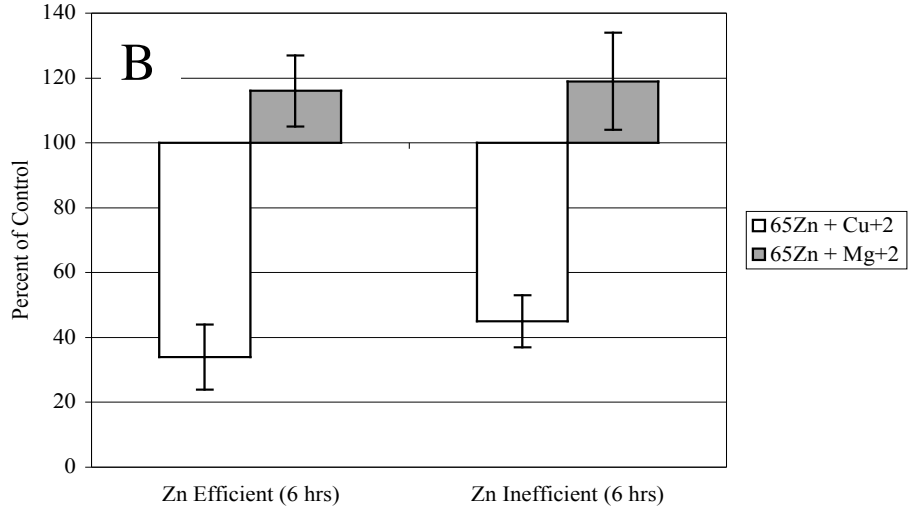

Figure 3. Shoot allocation of Zn at 6 hours as affected by Cu+2 and Mg+2 competition in Zn efficient and inefficient *Exacum* genotypes.