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# Uptake, Translocation, and Remobilization of Zinc Absorbed at Different Growth Stages by Rice Genotypes of Different Zn Densities

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Zinc (Zn) is an essential micronutrient for humans, and increasing Zn density in rice (*Oryza sativa* L.) grains is important for improving human nutrition. The characteristics of Zn translocation and remobilization were investigated in high Zn density genotype IR68144, in comparison with the low Zn density genotype IR64. Stable isotope tracer $^{68}$Zn was supplied at various growth stages, either to the roots in nutrient solution or to the flag leaves to investigate the contribution of $^{68}$Zn absorbed at different growth stages to grain accumulation and the remobilization ability of $^{68}$Zn within plants. Significant differences in $^{68}$Zn allocation were observed between the two rice genotypes. Much higher $^{68}$Zn concentrations were found in grains, stems, and leaves of IR68144 than in IR64, but higher $^{68}$Zn was found in roots of IR64. More than half of the Zn accumulated in the grains was remobilized before anthesis, accounting for 63 and 52% of the total Zn uptake for IR68144 and IR64, respectively. Without supply of external Zn, at vegetative or reproductive stages, more $^{68}$Zn was retranslocated from "old tissues" to "new tissues" in IR68144 than in IR64. Retranslocation of $^{68}$Zn from flag leaves to grains was twice as high in the former when $^{68}$Zn was applied to the flag leaves during booting or anthesis. These results indicate that Zn density in rice grains is closely associated with the ability to translocate Zn from old tissues to new tissues at both early and late growth stages and with phloem remobilization of Zn from leaves and stems to grains.

**KEYWORDS: Zinc; stable isotope labeling; allocation; remobilization; translocation**

## INTRODUCTION

Zinc (Zn) is an essential micronutrient for all organisms (*1*). It is required as a cofactor in over 300 enzymes and plays critical roles in many proteins. Its deficiency results in extensive damage in plants and humans (*2*−*4*). Reliance on cereal-based diets may induce Zn deficiency related health problems in humans, such as impairments in physical development, immune system, and brain function (*5*). Approximately one-fifth of the world's population is estimated to be at risk of inadequate zinc intake (*6*), which generally occurs in regions with Zn-deficient soils. Rice (*Oryza sativa* L.) is the dominant staple food for more than half of the world's population (*7*), but a poor source of essential micronutrients such as Fe and Zn. Several strategies have been suggested to enhance Zn concentrations in grains, among which plant breeding (e.g., genetic biofortification) appears to be the most sustainable and cost-effective one (*5*).

The accumulation of microelements (e.g., Zn and Fe) varies genetically among the grains of different cereal crops (*8*, *9*). Different cultivars of polished rice, for example, contain very different Zn concentrations (*10*, *11*). The mechanisms of Zn transfer from soil to rice grains, however, are not fully understood. Although the physiological basis of differences in Zn uptake efficiency is well studied (*12*−*15*), little is known about the translocation and redistribution of Zn after it enters the transpiration stream, particularly from vegetative plant tissues to grains. In most cases, Zn accumulation in rice grains is not only related to root uptake but also depends on internal redistribution and remobilization of stored Zn within the plants (*16*). Redistribution of Zn within rice plants largely occurs through transport in the xylem, transfer from the xylem to the phloem, and retranslocation in the phloem. Xylem transport is simply directed from roots to shoots in the transpiration stream, whereas phloem transport from old to new leaves is more selective (*17*). In wheat, Zn can be readily transported in the phloem (from old leaves to young tissues), indicating that phloem mobility of Zn is important for Zn allocation to the grains (*18*, *19*). Zn unloading in rice phloem is different from wheat (*20*). Phloem transport of Zn from leaves was not found to be as important as xylem transport of Zn from root for grain filling in rice (*16*). However, the physiology of Zn transport and allocation in rice plants is still unclear.

Stable isotope ratios are routinely used in studying the biogeochemical cycles of light elements in the environment. In human Zn metabolism, double (or multiple) Zn tracer techniques are often used (*21*). In contrast, there are few studies on plant Zn

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metabolism using stable Zn tracer techniques. There is little fractionation of Zn in different plant tissues. The maximum range of fractionation is just 0.52‰ per amu (*22*, *23*). Thus, we can use the stable isotope $^{68}Zn$ as a tracer to study Zn transport in rice plants. In the present study, we used $^{68}Zn$ to study the characteristics of Zn translocation and remobilization in a high Zn density rice genotype in comparison with a low Zn density genotype. The objectives are (1) to determine the contribution of $^{68}Zn$ absorbed at different growth stages to grain accumulation, (2) to compare the remobilization ability of $^{68}Zn$ from old tissues to new tissues at vegetative and reproductive stages between the two genotypes, and (3) to investigate the phloem transport ability of $^{68}Zn$ supplied on leaves at the late growth stage.

## MATERIALS AND METHODS

**Plant Culture.** Two rice genotypes, one high Zn density genotype (IR68144) and one low Zn density genotype (IR64), were used for this study. It has been reported that Zn in the unpolished rice grains of IR68144 was about 37.0 mg $kg^{-1}$, whereas much lower Zn (25.5 mg $kg^{-1}$) was recorded in IR64 (*24*). The seeds of both genotypes were supplied courtesy of Dr. Glenn from the International Rice Research Institute (Manila, The Philippines). The preculture condition was performed according to the method given in ref *25*. Pregerminated seeds were precultured in deionized water (resistivity ≥ 18.2 MOhm $cm^{-2}$) containing 0.02 mM $CaSO_4$ for 5 days and then grown in nutrient solution with the same composition as described in ref *26* (in mM): 1.5 $NH_4NO_3$, 1.0 $CaCl_2$, 1.6 $MgSO_4$, 1.0 $K_2SO_4$, 0.3 $KH_2PO_4$; and ($\mu$M) 2.0 $H_3BO_3$, 5.0 $MnSO_4$, 1.0 $ZnSO_4$, 0.2 $CuSO_4$, and 0.05 $(NH_4)_6Mo_7O_{24}$. Iron was supplied as $Na_2FeEDTA$ (ethylenediaminetetraacetic acid) at 20 $\mu$M. Rice seedlings after 13 days of growth were transplanted into a 2.5 L black plastic container, which was covered with a polystyrol plate with seven evenly spaced holes (2 cm in diameter). The nutrient solutions were replaced every 4 days. The solution pH was adjusted to 5.5 ± 0.1 every other day with either NaOH or HCl. Plants were grown in a growth chamber under a photo flux density of 400 $\mu$mol $m^{-2}$ $s^{-1}$, a light/dark period of 16/8 h, day/night temperatures of 30/25 °C, and day/night relative humidities of 75/85%.

For the different experiments described below, 1.0 $\mu$M $ZnSO_4$ in nutrient solution was replaced by 1.0 $\mu$M $^{68}ZnSO_4$ for stable isotope tracing during treatments. The nutrient solutions containing 1.0 $\mu$M $^{68}ZnSO_4$ were also replaced every 4 days. $^{68}Zn$-enriched isotope was purchased as solid powder of $^{68}ZnO$ from Cambridge Isotope Laboratory. The isotope abundances were 98.60% $^{68}Zn$, 0.44% $^{64}Zn$, 0.39% $^{66}Zn$, 0.54% $^{67}Zn$, and 0.03% $^{70}Zn$. The solution of $^{68}ZnSO_4$ was prepared as follows: 21.0 mg of $^{68}ZnO$ (powder) was dissolved in 5 mL of 1.0 M $H_2SO_4$, gently stirred for 48 h until $^{68}ZnO$ was completely dissolved. The solution was then diluted by using deionized water and solution pH adjusted to 5.0 by adding 1.0 M NaOH. The solution was then transferred to a 250 mL volumetric flask and added with deionized water to make the volume up to 250 mL. The final concentration of $^{68}ZnSO_4$ in the solution was 1.0 mM. The abundances of Zn isotope in nonenriched $ZnSO_4$ were 18.75% $^{68}Zn$, 48.63% $^{64}Zn$, 27.90% $^{66}Zn$, 4.10% $^{67}Zn$, and 0.62% $^{70}Zn$.

**Contribution of $^{68}Zn$ Absorbed at Different Growth Stages to Grain Accumulation.** The entire rice growth period was divided into four stages: seedling (I), tillering (II), heading and anthesis (III), and grain filling (IV). The precultured plants were treated until harvest with 1.0 $\mu$M $^{68}ZnSO_4$ starting at the beginning of the (a) tillering stage (after eighth leaf stage), (b) heading stage (the emergence of first rice ear), or (c) grain filling stage (15 days after anthesis). Each of these three treatments was replicated three times. At harvest, the plants were divided into grains, hulls, leaves, stems, and roots for determination of $^{68}Zn$ and total Zn concentrations by means of inductively coupled plasma mass spectrometer (ICP-MS) using an Agilent 7500a instrument (Agilent Technologies, Palo Alto, CA) and ICP-AES (Shimadzu ICPS-7510). On the basis of the uptake of $^{68}Zn$ and total Zn, the accumulations of Zn in the different plant parts during various growth stages were calculated as

$$Zn(I) = Zn_{(whole)} - Zn_{(a)};\ Zn(II) = Zn_{(a)} - Zn_{(b)}$$

$$Zn(III) = Zn_{(b)} - Zn_{(c)};\ Zn(IV) = Zn_{(c)}$$

$Zn_{(whole)}$ refers to the total Zn content of a specified plant part at harvest; $Zn_{(a)}$, $Zn_{(b)}$, and $Zn_{(c)}$ refer to newly accumulated Zn in the respective plant parts after supplying 1.0 $\mu$M $^{68}ZnSO_4$ from tillering stage, heading stage, and grain filling stage until harvest, respectively.

**Remobilization of $^{68}Zn$ within Plant at Vegetative Stage.** Plants of both genotypes were precultured in normal nutrient solution before the fifth leaf stage and then treated with 1.0 $\mu$M $^{68}ZnSO_4$ for 10 days until tillering stage (45 days after transferring to nutrient solution). Thereafter, plants were transferred into normal nutrient solution without Zn supply. After time intervals of 0, 7, 14, and 21 days, plants were harvested and divided into leaves, tillers, stems, and roots for the determination of $^{68}Zn$ and total Zn concentrations. Leaves were numbered by leaf sequence from plant base to top. Each treatment was replicated three times.

**Remobilization of $^{68}Zn$ within Plant at Reproductive Stage.** Plants were precultured in normal nutrient solution until tillering stage and then treated with 1.0 $\mu$M $^{68}ZnSO_4$ for 30 days until first ears started to emerge (heading stage, 75 days after transferring to nutrient solution). Thereafter, the procedure was analogous as in the preceding experiments: Plants were transferred into normal nutrient solution without Zn supply. After time intervals of 0, 7, 14, and 21 days, plants were harvested and divided into whole grains (with hulls), flag leaves, nonflag leaves, stems, and roots for the determination of $^{68}Zn$ and total Zn concentrations. Each treatment was replicated three times.

**Reutilization of $^{68}Zn$ Applied onto Flag Leaf.** Plants of both genotypes were grown in normal nutrient solution until either booting or anthesis stage. Before $^{68}Zn$ treatment, the flag leaves were washed thoroughly with deionized water. Then each flag leaf was treated with 5 mL of 1.0 mM $^{68}ZnSO_4$ solution (pH 5) containing 0.01% Tween 80. A small Zn-free cotton bud was soaked in the $^{68}ZnSO_4$ solution and then used to gently daub the flag leaf from the stalk to the abaxial face of the leaf. Each flag leaf was daubed five times with a single cotton bud at each application. Each application lasted for about 5 min and took place three times per day at 4 h intervals during daylight. All 5.0 mL of solution was used at the end of the treatments. Three plants were treated as one replication, and each treatment had three replications. The foliar application of $^{68}Zn$ was terminated 10 days before harvest. At harvest the plants were divided into grains, hulls, flag leaves, nonflag leaves, stems, and roots for determination of $^{68}Zn$ and total Zn concentrations.

**Sample Preparation and Digestion.** All plant samples were washed with deionized water to remove superficial nutrient solution. Roots and flag leaves were submerged for 10 min in a 1.0 L bath containing 1.0 mM $LaCl_3$ and 0.05 mM $CaCl_2$ to remove apoplastic Zn (following the method given in ref *27*). Oven-dried samples were milled using an MM301 (Retsch, Germany) with agate ball and internal wall. Samples of 0.1 g (accuracy 0.1 mg) of dried flour were digested using a hot block system (LabTech ED36, Germany) with 4.0 mL of nitric acid ($HNO_3$, reagent grade) and 1.0 mL of hydrogen peroxide ($H_2O_2$, analytical reagent, Beijing Chemical Works, China). All samples were digested and analyzed in triplicates.

**Determination of $^{68}Zn$ Tracer Concentration in Rice Plants by ICP-MS.** Total Zn concentration ($Zn_{tot}$) and final ratio of $^{68}Zn/^{66}Zn$ ($R_{fin}$) in rice plants were obtained directly by analysis of ICP-AES (Shimadzu ICPS-7510) and ICP-MS (Agilent 7500A), respectively. The ultimate goal of total Zn and Zn isotopic analysis was to determine the allocation of newly accumulated Zn in various parts of the rice plants during the tracing periods. Concentrations of the newly accumulated Zn in different plant parts ($Zn_{acc}$; mg $kg^{-1}$ of DW) were calculated from total Zn concentrations after exposure in the respective plant parts ($Zn_{tot}$; mg $kg^{-1}$ of DW), the final $^{68}Zn/^{66}Zn$ ratio in the respective parts ($R_{fin}$), and the relative fractions of $^{68}Zn$ and $^{66}Zn$ in both exposure media ($f_{68\text{-enr}}$ and $f_{66\text{-enr}}$) and in respective parts of control rice plants ($f_{68\text{-nat}}$ and $f_{66\text{-nat}}$), according to eq 1

$$R_{fin} = \frac{(Zn_{acc} \times f_{68\text{-enr}}) + f_{68\text{-nat}}(Zn_{tot} - Zn_{acc})}{(Zn_{acc} \times f_{66\text{-enr}}) + f_{66\text{-nat}}(Zn_{tot} - Zn_{acc})} \quad (1)$$

which solved for $Zn_{acc}$ yields

$$Zn_{acc} = \frac{Zn_{tot}[f_{68\text{-nat}} - (R_{fin} \times f_{66\text{-nat}})]}{R_{fin}(f_{66\text{-enr}} - f_{66\text{-nat}}) + f_{68\text{-nat}} - f_{68\text{-enr}}} \quad (2)$$

$f_{68\text{-nat}}$ and $f_{66\text{-nat}}$ are the natural abundances of $^{68}Zn$ and $^{66}Zn$ in normal nutrient solution (18.75 and 27.90%, respectively); $f_{68\text{-enr}}$ and $f_{66\text{-enr}}$ represent the abundances of $^{68}Zn$ and $^{66}Zn$ in $^{68}Zn$-enriched $ZnSO_4$ bought from Cambridge Isotope Laboratory (98.60 and 0.39%, respectively). Please note that all data of $^{68}Zn$ in the tables and figures of this paper refer

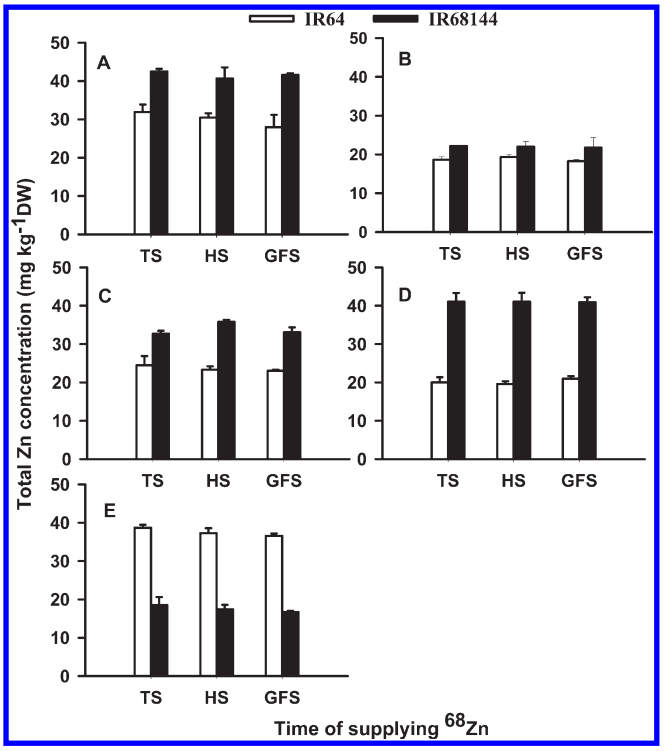


**Figure 1.** Effect of $^{68}ZnSO_4$ treatment on the total concentration of Zn in (**A**) grains, (**B**) hulls, (**C**) leaves, (**D**) stems, and (**E**) roots of two rice genotypes, IR68144 (high Zn density) and IR64 (low Zn density), at harvest. Rice plants were supplied with $^{68}ZnSO_4$ from tillering stage (TS), heading stage (HS), and grain filling stage (GFS) until ripeness, with the concentration of 1.0 $\mu$M $^{68}ZnSO_4$. Values shown represent means (bars) and standard errors (error bars) calculated from three replicate plants ($n$ = 3).

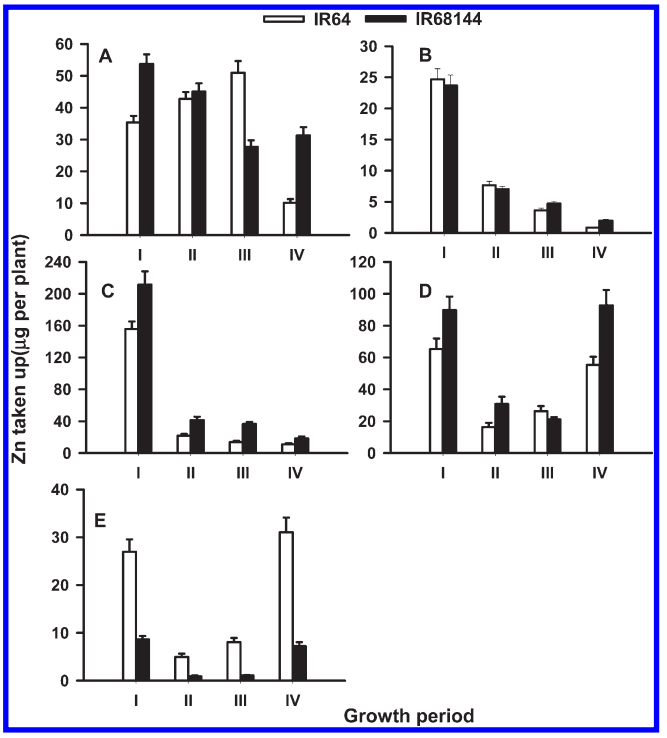


**Figure 2.** Distribution of Zn ($\mu$g per plant) taken up during subsequent growth stages in plants of the two genotypes, IR68144 (high Zn density) and IR64 (low Zn density): (**A**) grains, (**B**) hulls, (**C**) leaves, (**D**) stems, and (**E**) roots. The entire growth period was divided into four stages: (I) seedling, (II) tillering, (III) heading + anthesis, and (IV) grain filling. Values shown represent means (bars) and standard errors (error bars) calculated from three replicate plants ($n$ = 3) in each case.

to $Zn_{acc}$ only. They do not include the $^{68}Zn$ accumulated with "normal Zn" ($Zn_{tot} - Zn_{acc}$).

**Statistical Analysis.** All data were statistically analyzed using SPSS (version 12.0). Differences between treatments were determined by the least significant difference ($p \leq 0.05$) from the analysis of variance (ANOVA). Differences between two genotypes were tested by a paired $t$ test ($p \leq 0.05$ or $p \leq 0.01$). The figures were made using the software SigmaPlot 10.0.

## RESULTS

**Distribution of Zn Supplied at Different Growth Stages.** No significant variation of total tissue Zn concentrations was noted in response to the different $^{68}ZnSO_4$ supply times (**Figure 1**), indicating that the plants had no preference in absorbing different Zn isotopes. Significantly higher concentrations of total Zn were observed in the grains, hulls, stems, and leaves of the high Zn density genotype IR68144 than in the low Zn density genotype IR64 (**Figure 1A−D**). The opposite was noted for roots (**Figure 1E**).

In comparison with the low Zn density genotype IR64, IR68144 accumulated more Zn in the grains and stems during periods I and IV, but less Zn during period III (**Figures 2A,D**). In all periods, IR68144 accumulated more Zn in the leaves (**Figure 2C**), but less in the roots (**Figure 2E**) than IR64. In both genotypes, the Zn taken up during growth period I was mainly (> 50%) allocated to leaves and the Zn taken up during period IV mainly (> 50%) to the stems (**Figure 3**). Large percentages of Zn taken up in period II or III were allocated to the grains in both IR64 (48%) and IR68144 (30%). In all growth periods, the percentages of $^{68}Zn$ allocated to the roots of the low Zn density genotype IR64 were 3−7 times higher than in the high Zn density genotype IR68144 (**Figure 3**). In contrast, the high Zn density genotype allocated a greater percentage of Zn into leaves

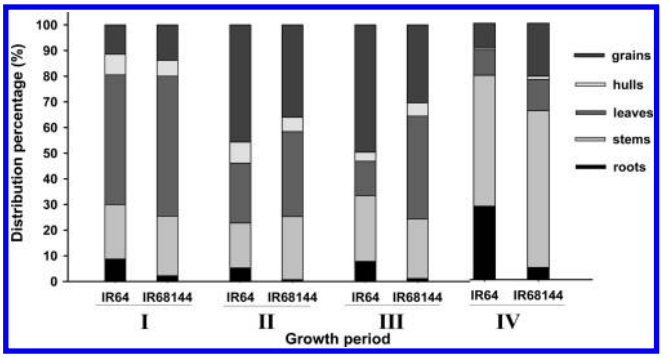


**Figure 3.** Distribution of Zn (in %) taken up during subsequent growth stages in plants of the two genotypes, IR68144 (high Zn density) and IR64 (low Zn density). From top to bottom: grains, hulls, leaves, stems, and roots. The entire growth period was divided into four stages: (I) seedling, (II) tillering, (III) heading + anthesis, and (IV) grain filling. Values shown represent means from three replicate plants ($n$ = 3).

and stems than the low Zn density genotype (**Figure 3**). After anthesis, a higher percentage of Zn (almost 2 times more) was allocated to the grains in IR68144 than in IR64 (**Figure 3**).

**Contribution of Zn Absorbed at Different Growth Stages to Grain Accumulation.** Large differences between the two rice genotypes were observed in the contribution of Zn taken up at different growth stages to grain Zn (**Figure 4A**). For instance, $^{68}Zn$ absorbed after grain filling (period IV) contributed about 20% to total grain Zn in IR68144, but only 7% in the low Zn density genotype (**Figure 4A**). For the low Zn density genotype, around 35% of Zn in grains was taken up during period III (heading and anthesis), which

was 2 times higher than that of IR68144 (**Figure 4A**). In both IR64 and IR68144, more than half of the Zn accumulated in the grains was taken up before heading (during periods I and II) (**Figure 4A**). Around 80% or more of the Zn deposited in stems and roots was taken up in periods I and IV (**Figure 4D**,**E**). More than 65% of the Zn in hulls and leaves was accumulated in period I by both genotypes (**Figure 4B**,**C**).

**Remobilization of $^{68}$Zn from "Old Tissues" to "New Tissues" at Vegetative and Reproductive Stages.** After the supply of $^{68}$Zn was stopped, the total amount of $^{68}$Zn did not change during the following 3 weeks in either genotype. Higher total contents of $^{68}$Zn were found in the high Zn density genotype IR68144 (**Table 1**). In both genotypes very little (< 0.01 $\mu$g plant$^{-1}$) $^{68}$Zn was detected in old leaves (leaves 1−4), as $^{68}$Zn was continuously remobilized from old tissues (roots, stems, and older leaves) into new tissues (new leaves and tillers). This redistribution was more pronounced in the high Zn density genotype IR68144 (**Table 1**). The $^{68}$Zn contents of roots, stems, and leaves significantly decreased in both genotypes after stopping Zn supply ($p < 0.05$) (**Table 1**), showing that $^{68}$Zn stored in these tissues was remobilized. The reduction of $^{68}$Zn accumulated in the stem and leaf 5 was more marked in IR68144 than in IR64 (**Table 1**). As **Table 2** shows, the net export rate of $^{68}$Zn from stems was up to 2 times higher in IR68144 than in IR64, whereas the opposite situation was found for $^{68}$Zn export from the roots. In both genotypes large amounts of $^{68}$Zn were transported into the new leaves and tillers after $^{68}$Zn supply was stopped. The effect was more pronounced in the high Zn density genotype IR68144 (**Table 1**). The new leaves (leaves 6−8) and tillers of IR68144 had gained 3.3 and 7.3 $\mu$g of $^{68}$Zn plant$^{-1}$, respectively, 21 days after $^{68}$Zn supply was stopped, which were 1.3 and 2.0 times the amount found in IR64 (**Table 1**).

Similar results were obtained in both genotypes for the reproductive growth stage. The accumulation of $^{68}$Zn in the developing grains increased continuously with time ($p < 0.05$), although no external $^{68}$Zn was supplied (**Table 3**). The new accumulation of $^{68}$Zn in the grains (with hulls) increased by 38.6 and 29.3 $\mu$g plant$^{-1}$ in IR68144 and IR64, respectively, during 21 days without Zn supply. The $^{68}$Zn amount accumulated in IR68144 grains was 1.24 times that of IR64 at day 21, accounting for 41.4 and 33.4 $\mu$g plant$^{-1}$, respectively. The accumulated grain $^{68}$Zn was mostly retranslocated from stems and roots. After the supply of $^{68}$Zn was stopped at heading stage, accumulation of $^{68}$Zn in the old tissues (stems, roots) decreased significantly in both genotypes ($p < 0.05$) (**Table 3**). Two times more $^{68}$Zn was stored in the roots of IR64 than in those of

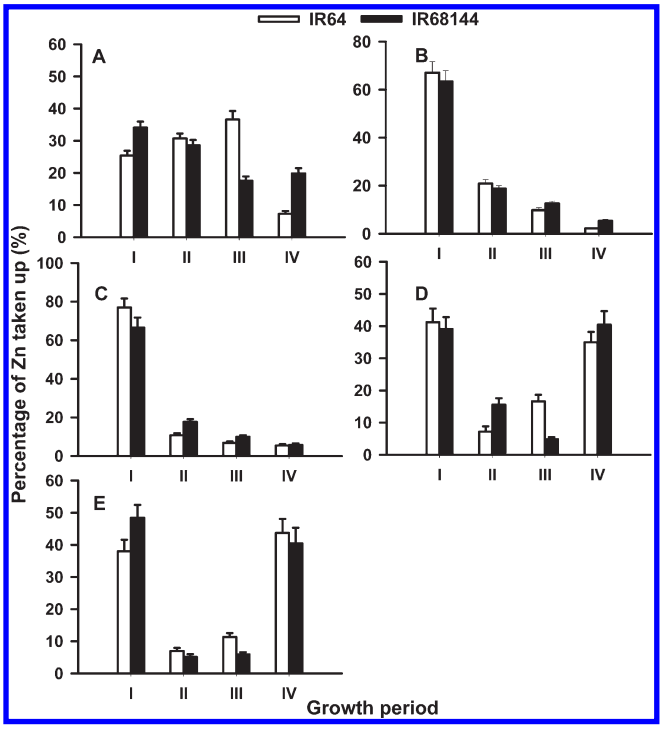


**Figure 4.** Contribution of Zn (in %) taken up during subsequent growth stages to the amounts of Zn allocated at harvest in different plant parts of the two genotypes, IR68144 (high Zn density) and IR64 (low Zn density): (**A**) grains, (**B**) hulls, (**C**) leaves, (**D**) stems, and (**E**) roots. The entire growth period was divided into four stages: (I) seedling, (II) tillering, (III) heading + anthesis, and (IV) grain filling. The bars with the same color sum to 100% for each genotype. Values shown represent means (bars) and standard errors (error bars) calculated from three replicate plants ($n = 3$) in each case.

**Table 2.** Net Export Rate of $^{68}$Zn from Roots and Stems of the Two Rice Genotypes IR68144 and IR64 after Zn Supply Was Stopped[a]

| | net export rate (ng day$^{-1}$ plant$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | root | | | stem | | |
| genotype | 7 days | 14 days | 21 days | 7 days | 14 days | 21 days |
| IR68144 | 296 ± 13 | 147 ± 17 | 81 ± 11 | 657 ± 46 | 438 ± 29 | 386 ± 38 |
| IR64 | 405 ± 31 | 248 ± 20 | 204 ± 20 | 361 ± 26 | 249 ± 18 | 260 ± 24 |

[a] An unpaired two-tailed $t$ test was used to analyze significant differences between controls and treatments. The differences between the two genotypes were significant at $p > 0.05$ for roots and stems at all three times. All data represent means ± standard errors for three replicates ($n = 3$).

**Table 1.** Zinc Remobilization in Different Tissues of the High Zn Density Genotype IR68144 and the Low Zn Density Genotype IR64 Harvested during Vegetative Growth at Various Times after Treatment[a]

| | | | $^{68}$Zn accumulation in different rice tissues ($\mu$g plant$^{-1}$) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| genotype | time (days) | total $^{68}$Zn ($\mu$g plant$^{-1}$) | roots | stems | leaf 1 | leaf 2 | leaf 3 | leaf 4 | leaf 5 | leaf 6 | leaf 7 | leaf 8 | tiller |
| IR68144 | 0 | 26.1 ± 1.8a | 5.2 ± 0.5a | 16.3 ± 1.2a | | | | | 1.3 ± 0.1a | | | | 3.3 ± 0.3c |
| | 7 | 25.4 ± 1.5a | 4.1 ± 0.3b | 11.7 ± 1.0b | | | | | 0.7 ± 0.1b | 1.8 ± 0.2a | | | 7.1 ± 0.7b |
| | 14 | 25.4 ± 1.2a | 3.7 ± 0.3bc | 10.1 ± 1.0bc | | | | | 0.5 ± 0.0c | 1.3 ± 0.1b | 1.6 ± 0.2a | | 8.2 ± 0.8b |
| | 21 | 25.6 ± 2.1a | 3.3 ± 0.3c | 8.2 ± 0.8c | | | | | 0.2 ± 0.0d | 1.1 ± 0.0b | 1.0 ± 0.1b | 1.2 ± 0.2 | 10.6 ± 1.0a |
| IR64 | 0 | 17.3 ± 0.9a | 6.0 ± 0.6a | 7.2 ± 0.8a | | | | | 1.2 ± 0.2a | | | | 2.9 ± 0.4c |
| | 7 | 17.5 ± 1.1a | 4.5 ± 0.4b | 5.9 ± 0.6b | | | | | 1.1 ± 0.1a | 1.2 ± 0.2a | | | 4.7 ± 0.5b |
| | 14 | 16.8 ± 0.9a | 3.8 ± 0.4bc | 5.0 ± 0.5b | | | | | 0.4 ± 0.0b | 1.1 ± 0.2a | 0.9 ± 0.1a | | 5.5 ± 0.7ab |
| | 21 | 16.3 ± 1.2a | 3.2 ± 0.3c | 3.5 ± 0.4c | | | | | 0.3 ± 0.0b | 1.0 ± 0.1a | 0.8 ± 0.1a | 0.8 ± 0.1 | 6.6 ± 0.7a |

[a] Plants were treated with 1.0 $\mu$M $^{68}$Zn for 10 days until tillering stage (45 days after transferring to nutrient solution) and harvested 0, 7, 14, or 21 days after treatment. Leaf positions 1−8 were counted from bottom to top. Less than 0.01 $\mu$g plant$^{-1}$ of $^{68}$Zn was detected in leaves 1−4 of both genotypes, regardless of sampling time. Values shown represent means ± SE ($n = 3$) for three different replications. ANOVA was performed in one way with LSD test; different letters in the same column indicate significance at $p < 0.05$.

IR68144 on day 0. This amount reduced by 5.6 $\mu$g plant$^{-1}$ after 21 days in IR64 and by 3.1 $\mu$g plant$^{-1}$ in IR68144 (**Table 3**). On the other hand, 1.5 times more $^{68}$Zn was stored in the stems of IR68144 than in IR64 at day 0 and decreased by 32.9 $\mu$g plant$^{-1}$ at day 21 after Zn supply was stopped, as compared to 20.5 $\mu$g plant$^{-1}$ for IR64 (**Table 3**). In comparison with roots and stems, $^{68}$Zn in nonflag and flag leaves was less decreased in both genotypes. A significant reduction was observed only after a long time in the absence of $^{68}$Zn supply (**Table 3**).

**Remobilization of Foliar-Applied $^{68}$Zn into Grains.** Foliar-applied $^{68}$Zn significantly increased grain Zn concentrations in both genotypes (**Figure 5**). Total Zn was increased by approximately 10%, independent of application stage ($p < 0.05$). The grain $^{68}$Zn concentrations increased with duration of treatment in both genotypes. The accumulation of grain $^{68}$Zn was almost twice as high in IR68144 as in IR64 after foliar application (**Figure 5**). Thus, the difference between the two genotypes was much higher than after root application, where the grain Zn concentration of IR68144 was 1.34 times that of IR64 at harvest. Interestingly, foliar application of $^{68}$Zn significantly ($p < 0.05$) decreased the transport of Zn from roots to grains in IR68144 after the booting stage. This was not found in IR64. The percentages of $^{68}$Zn were higher for the grains and hulls of the high Zn density rice genotype (IR68144) than for IR64 for both treatment times (**Table 4**). The opposite was the case in the flag leaves, where the percentage of $^{68}$Zn was greater in IR64 than in IR68144. The percentages of $^{68}$Zn increased with treatment time in grains, hulls, and leaves, but decreased in stems and flag leaves. These data show that $^{68}$Zn allocated in stems and flag leaves was remobilized and translocated to grains and other leaves.

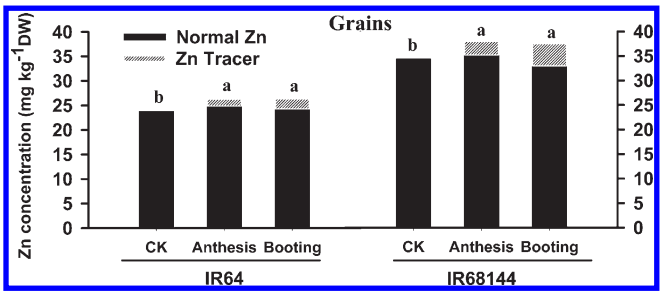


**Figure 5.** Effect of foliar $^{68}ZnSO_4$ on the concentrations of $^{68}$Zn and normal Zn in grains of two rice genotypes. Five milliliters of 1.0 mM $^{68}ZnSO_4$ was daubed on the flag leaf of the main stem at booting stage or anthesis stage, respectively, until 10 days before harvest. CK refers to no foliar $^{68}ZnSO_4$ application. Means denoted by the same letter did not differ significantly ($p < 0.05$).

## DISCUSSION

In this study, we found that more than half of the grain Zn found at harvest had been taken up during vegetative growth (periods I and II). This means that large amounts of the Zn deposited in the grains at maturity had been retranslocated from other plant parts and not been transported directly after uptake to the grains in the grain-filling stage. Generally, the amount of Zn transported into grains is one of the standards to characterize Zn use efficiency. In both low and high Zn density genotypes, only small percentages of the Zn accumulated during periods II and III were still found in roots and stems at maturity, whereas large portions of the Zn taken up during these growth periods were translocated to the grains and hulls. Thus, irrespective of yield, periods II and III appear to the growth stages of the highest Zn use efficiency.

**Table 3.** Zinc Remobilization in Different Tissues of the High Zn Density Genotype (IR68144) and the Low Zn Density Genotype (IR64) at Reproductive Stage[a]

| | | | $^{68}$Zn accumulation in different rice tissues ($\mu$g plant$^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|
| genotype | time (days) | total $^{68}$Zn ($\mu$g plant$^{-1}$) | roots | stems | nonflag leaves | flag leaves | grains (with hulls) |
| IR68144 | 0 | 83.6 ± 2.1a | 4.3 ± 0.4a | 54.8 ± 2.3a | 14.8 ± 0.5a | 5.8 ± 0.8a | 3.8 ± 0.4d |
| | 7 | 81.6 ± 0.8a | 2.6 ± 0.2b | 46.8 ± 3.1b | 13.8 ± 1.0a | 5.2 ± 0.5a | 13.2 ± 2.3c |
| | 14 | 82.1 ± 1.3a | 1.9 ± 0.3c | 30.9 ± 1.7c | 14.8 ± 0.8a | 3.8 ± 0.4b | 30.7 ± 2.9b |
| | 21 | 80.2 ± 4.4a | 1.2 ± 0.1d | 21.9 ± 1.3d | 11.6 ± 0.6b | 4.0 ± 0.4b | 41.4 ± 3.3a |
| IR64 | 0 | 70.6 ± 1.3a | 8.2 ± 1.0a | 37.2 ± 1.3a | 17.0 ± 1.2a | 4.0 ± 0.4a | 4.1 ± 0.3d |
| | 7 | 68.5 ± 3.9a | 4.9 ± 0.3b | 28.8 ± 2.7b | 15.4 ± 1.3ab | 3.3 ± 0.7ab | 16.1 ± 0.9c |
| | 14 | 69.8 ± 0.7a | 3.4 ± 0.2c | 20.2 ± 1.9c | 16.5 ± 1.1ab | 3.6 ± 0.3ab | 26.1 ± 2.8b |
| | 21 | 70.0 ± 3.3a | 2.6 ± 0.2c | 16.7 ± 1.1d | 14.5 ± 0.6b | 2.8 ± 0.2b | 33.4 ± 2.3a |

[a] Plants were treated with 1.0 $\mu$M $^{68}$Zn for 30 days until the heading stage (75 days after transferring to nutrient solution) and harvested after 0, 7, 14, or 21 days, respectively. Values represent means ± SE ($n = 3$) for three different replications. Different letters in the same column indicate significance at $p < 0.05$.

**Table 4.** Relative Distribution of $^{68}$Zn among Individual Plant Tissues of Two Rice Genotypes at Harvest after Daubing $^{68}ZnSO_4$ on Flag Leaf at Different Growth Stages[a]

| | | relative distribution of $^{68}$Zn (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | main stem | | | | | | tiller | |
| growth stage | genotype | grains | hulls | flag leaves | nonflag leaves | stems | roots | grains | stems + leaves |
| anthesis | IR68144 | 5.9 | 2.2 | 77.2 | 2.8 | 10.9 | nd[b] | 0.0 | 1.0 |
| | IR64 | 4.1 | 1.6 | 82.4 | 2.3 | 8.7 | nd | nd | 0.9 |
| booting | IR68144 | 8.9 | 3.3 | 71.4 | 5.2 | 9.7 | nd | 0.1 | 1.6 |
| | IR64 | 4.8 | 2.0 | 79.2 | 4.5 | 7.9 | 0.1 | 0.1 | 1.6 |
| change in fraction of applied $^{68}$Zn[c] | IR68144 | +3.0 | +1.1 | −5.8 | +2.4 | −1.2 | | | |
| | IR64 | +0.7 | +0.4 | −3.2 | +2.2 | −0.8 | | | |

[a] Values are expressed in percentage of total $^{68}$Zn uptake. All data are means of three replications. [b] nd, no $^{68}$Zn detected. [c] Relative distribution of $^{68}$Zn to tissues at booting stage minus the relative distribution at anthesis stage.

Our results indicate that Zn mobility in rice plants is important for Zn density in rice grains. Increased root uptake does not necessarily result in enhanced Zn accumulation in rice grains (*28*). Excess metal may be deposited in root vacuoles and other tissues. Zinc is transported from roots to shoots, mainly with the transpiration stream. Thus, major bottlenecks in rice plant biofortification may be root−shoot barriers (*20*). In this study, the high Zn density genotype IR68144 accumulated more Zn in grains (plus hulls), stems ,and leaves, but less Zn in roots, than the low Zn density genotype IR64 (**Figure 1**), suggesting that the high Zn density genotype transported more Zn from roots to shoots and that the capacity of xylem loading is key for the high Zn density in grains. New findings demonstrate that root−shoot translocation of Zn is controlled by heavy metal transporting ATPases (HMAs) (*29*). These HMAs are thought to transport Zn across the plasma membrane of root vascular cells into the xylem for transport to the shoot. Thus, to enhance the rates of Zn translocation from roots to shoots for biofortification purposes, increasing the expression of HMA4 or similar genes may be critical (*30*).

Our results also indicate that grain Zn density is closely associated with the ability of Zn translocation from old tissues to new tissues at both early and late growth stages, especially the remobilization of Zn from stems to grains. The ability of Zn remobilization in plants has been suggested to be an important factor in grain Zn accumulation (*19*, *31*,). However, there is no report on Zn transport from old tissues to new tissues in rice plants during early growth stages (vegetative) or late growth stages (reproductive). In this study, we found that more remobilization of Zn occurred in the high Zn density genotype IR68144. Without supply of external Zn more $^{68}$Zn was retranslocated from "old tissues" to "new tissues" in IR68144 than in IR64 during vegetative and reproductive growth. Retranslocation of $^{68}$Zn from flag leaves to grains was twice as high in the former genotype when $^{68}$Zn was applied to the flag leaves during booting or anthesis. This is consistent with previous work (*28*) that also suggested a higher capacity of Zn remobilization in the high Zn density genotype. Therefore, we conclude that retranslocation of Zn from vegetative tissues such as stems and old leaves to grains, even during the reproductive growth stage, is an important factor for Zn density in rice grains, as reported for other plants (*20*).

It remains unclear whether stem Zn is transported into grains directly through the rachis or through phloem unloading from leaves. It has been suggested that Zn must be transferred from xylem to phloem before entering the grain in wheat and barley, as the xylem is discontinuous at the base of the seeds in these plants (*32*). However, there is no evidence of such a discontinuity in rice (*20*, *33*). Thus, Zn might be loaded directly from the xylem into the nucellar epidermis and aleuron cells. In the present study, we found that the concentration of $^{68}$Zn in flag leaves and other leaves slightly decreased after the supply of $^{68}$Zn was stopped, but the concentration of $^{68}$Zn decreased much more in stems and roots, indicating that Zn was relocated from these parts into the developing grains (**Tables 1** and **3**). Most $^{68}$Zn applied to the flag leaves remained in that tissue. Only a small portion was relocated to the grains and other tissues (Figure**5**; **Table 4**). In previous studies, when radioactive Zn ($^{65}$Zn) was applied to leaves and roots at flowering stage, mainly root-applied Zn was found in the grains (*16*). On the basis of these facts, we suggest that in rice Zn remobilized in roots and stems is loaded into the grains primarily through the xylem of the rachis at grain filling. However, the transfer of a small portion of foliar-applied $^{68}$Zn to the grains indicates that also leaf Zn can be remobilized and transferred into the grains through the phloem (**Figure 5**). Most of the $^{68}$Zn exported from the treated leaves was translocated into the grains (**Table 4**). This contrasts with previous findings where most of the $^{65}$Zn that left treated leaves was translocated to other vegetative organs, whereas only very small amounts were allocated to the panicle parts and even less to the grains (*16*). This discrepancy may be attributed to differences in the times and rates of Zn application. In the present study, a high concentration (1.0 mM $^{68}ZnSO_4$) and long time (several weeks) of stable isotope was used to trace Zn allocation in rice plants. In contrast, radioactive isotope tracing was usually applied in low concentration over a short time only. Further investigation is needed to assess to what extent rice plants can remobilize Zn into grains via phloem transfer from leaves.

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## LITERATURE CITED

(1) Broadley, M. R.; White, P. J.; Hammond, J. P.; Zelko, I.; Lux, A. Zinc in plants. *New Phytol*. **2007**, *173*, 677–702.
(2) Coleman, J. E. Zinc enzymes. *Curr. Opin. Chem. Biol*. **1998**, *2*, 222–234.
(3) Cakmak, I. Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytol*. **2000**, *146*, 185–205.
(4) Krämer, U.; Clemens, S. Functions and homeostasis of zinc, copper, and nickel in plants. *Molecular Biology of Metal Homeostasis and Detoxification*; Springer: Berlin, Germany, 2006; pp 216−271, Springer
(5) Cakmak, I. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? *Plant Soil* **2008**, *302*, 1–17.
(6) Sara, E. W.; Janet, M. P.; Kenneth, H. B. Use of national food balance data to estimate the adequacy of zinc in national food supplies: methodology and regional estimates. *Public Health Nutr*. **2005**, *8*, 812–819.
(7) Zimmernann, M. B.; Hurrell, R. F. Improving iron, zinc and vitamin A nutrition through plant biotechnology. *Curr. Opin. Biotechnol*. **2002**, *13*, 142–145.
(8) Senadhira, D.; Graham, R. D. Genetic variation in iron and zinc concentrations in brown rice. *Micron* **1999**, *3*, 4–5.
(9) Graham, R. D.; Welch, R. M.; Bouis, H. E. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. *Adv. Agron*. **2001**, *70*, 77–142.
(10) Yang, X. E.; Ye, Z. Q.; Shi, C. H.; Zhu, M. L.; Graham, R. D. Genotypic differences in concentrations of iron, manganese, copper, and zinc in polished rice grains. *J. Plant Nutr*. **1998**, *21*, 1453–1462.
(11) Graham, R. D.; Senadhira, D.; Beebe, S.; Iglesias, C.; Monasterio, I. Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. *Field Crop Res*. **1999**, *60*, 57–80.
(12) Hacisalihoglu, G.; Kochian, L. V. How do some plants tolerate low levels of soil zinc? Mechanisms of zinc efficiency in crop plants. *New Phytol*. **2003**, *159*, 341–350.
(13) Genc, Y.; Verbyla, A. P.; Torun, A. A.; Cakmak, I.; Willsmore, K.; Wallwork, H.; McDonald, G. K. Quantitative trait loci analysis of zinc efficiency and grain zinc concentration in wheat using whole genome average interval mapping. *Plant Soil* **2009**, 49–66.
(14) Widodo; Broadley, M. R.; Rose, T.; Frei, M.; Pariasca-Tanaka, J.; Yoshihashi, T.; Thomson, M.; Hammond, J. P.; Aprile, A.; Close, T. J.; Ismail, A. M.; Wissuwa, M. Response to zinc deficiency of two rice lines with contrasting tolerance is determined by root growth maintenance and organic acid exudation rates, and not by zinc-transporter activity. *New Phytol*. **2010**, *186*, 400–414.
(15) Gao, X. P.; Zhang, F. S.; Hoffland, E. Malate exudation by six aerobic rice genotypes varying in zinc uptake efficiency. *J. Environ. Qual*. **2009**, *38*, 2315–2321.
(16) Jiang, W.; Struik, P. C.; Lingna, J.; van, K. H.; Zhao, M.; Stomph, T. J. Uptake and distribution of root-applied or foliar applied $^{65}$Zn after flowering in aerobic rice. *Ann. Appl. Biol*. **2007**, *150*, 383–391.
(17) Marschner, H. *Mineral Nutrition of Higher Plants*; Academic Press: London, U.K., 1995; 889 pp.

(18) Haslett, B. S.; Reid, R. J.; Rengel, Z. Zinc mobility in wheat: uptake and distribution of zinc applied to leaves or roots. *Ann. Bot.* **2001**, *87*, 379–386.
(19) Erenoglu, B.; Nikolic, M.; Römheld, V.; Cakmak, I. Uptake and transport of foliar applied zinc ($^{65}$Zn) in bread and durum wheat cultivars differing in zinc efficiency. *Plant Soil* **2002**, *241*, 251–257.
(20) Stomph, T. J.; Jiang, W.; Struik, P. C. Zinc biofortification of cereals: rice differs from wheat and barley. *Trends Plant Sci.* **2009**, *14*, 123–124.
(21) Stanley, H.; Zlotkin; Claudia, S.; Seth, O. A. Demonstrating zinc and iron bioavailability from intrinsically labeled microencapsulated ferrous fumarate and zinc gluconate sprinkles in young children. *J. Nutr.* **2006**, *136*, 920–925.
(22) Weiss, D. J.; Mason, T. F. D.; Zhao, F. J.; Kirk, G. J. D.; Coles, B. J.; Horstwood, M. S. A. Isotopic discrimination of zinc in higher plants. *New Phytol.* **2005**, *165*, 703–710.
(23) Moynier, F.; Pichat, S.; Pons, S. L.; Fike, D.; Balter, V.; Albarède, F. Isotopic fractionation and transport mechanisms of Zn in plants. *Chem. Geol.* **2009**, *267*, 125−130.
(24) Krishnan, S.; Karabi, D.; Vilas, P.; Swapan, K. D. Rice caryopsis structure in relation to distribution of micronutrients (iron, zinc, $\beta$-carotene) of rice cultivars including transgenic indica rice. *Plant Sci.* **2009**, *177*, 557–562.
(25) Chen, W. R.; Yang, X. E.; He, Z. L.; Feng, Y.; Hu, F. H. Differential changes in photosynthetic capacity, 77 K chlorophyll fluorescence and chloroplast ultrastructure between Zn-efficient and Zn-inefficient rice genotypes (*Oryza sativa*) under low zinc stress. *Physiol. Plant.* **2008**, *132*, 89–101.
(26) Yang, X. E.; Römheld, V.; Marschner, H.; Chaney, R. L. Application of chelator-buffered nutrient solution technique on zinc nutrition study in rice. *Plant Soil* **1994**, *163*, 85–94.
(27) Rengel, Z. Physiological responses of wheat genotypes grown in chelator-buffered nutrient solutions with increasing concentrations of excess HEDTA. *Plant Soil* **1999**, *215*, 193–202.
(28) Jiang, W.; Struik, P. C.; van, K. H.; Zhao, M.; Jin, L. N.; Stomph, T. J. Does increased Zn uptake enhance grain Zn mass concentration in rice? *Ann. Appl. Biol.* **2008**, *153*, 135–147.
(29) Hussain, D.; Haydon, M. J.; Wang, Y.; Wong, E.; Sherson, S. M.; Young, J.; Camakaris, J.; Harper, J. F.; Cobbett, C. S. P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in *Arabidopsis*. *Plant Cell* **2004**, *16*, 1327–1339.
(30) Hanikenne, M.; Talke, I. N.; Haydon, M. J.; Lanz, C.; Nolte, A.; Motte, P.; Kroymann, J.; Weigel, D.; Kramer, U. Evolution of metal hyperaccumulation required cis-regulatory changes and copy number expansion of HMA4. *Nature* **2008**, *453*, 391–395.
(31) Pearson, J. N.; Rengel, Z. Uptake and distribution of $^{65}$Zn and $^{54}$Mn in wheat grown at sufficient and deficient levels of Zn and Mn: II. During grain development. *J. Exp. Bot.* **1995**, *46*, 841–845.
(32) Miller, R. O.; Jacobsen, J. S.; Skogley, E. O. Aerial accumulation and partitioning of nutrients by hard red spring wheat. *Commun. Soil Sci. Plan.* **1994**, *25*, 1891–1911.
(33) Zee, S. Y. Vascular tissue and transfer cell distribution in the rice spikelet. *Aust. J. Biol. Sci.* **1971**, *25*, 411–414.

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