Physiology and modelling of zinc allocation in aerobic rice

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Dit onderzoek is uitgevoerd binnen de C.T. de Wit onderzoekschool: Production Ecology and Resource Conservation.

Physiology and modelling of zinc allocation in aerobic rice

Wen Jiang

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. M.J. Kropff
in het openbaar te verdedigen
op vrijdag 29 februari 2008
des morgens te elf uur in de Aula

Wen Jiang (2008)
Physiology and modelling of zinc allocation in aerobic rice

Wen Jiang – [S.l.: s.n.]. Ill.
PhD thesis Wageningen University. – With ref. –
With summaries in English, Dutch and Chinese.
ISBN: 978-90-8504-902-9

Abstract

Wen Jiang, 2008. Physiology and modelling of zinc allocation in aerobic rice. PhD thesis, Wageningen University, Wageningen, The Netherlands. With summaries in English, Dutch and Chinese, 118 pp.

Zinc (Zn) deficiency in humans is widespread in many regions of the world, especially in the developing world. Rice, the staple food of more than half of the world's population, is potentially an important source of Zn for people whose diet consists mainly of cereal grain. Therefore, this thesis aimed at exploring the allocation of Zn in rice plants, as a basis for establishing the potential for enhancing their grain Zn mass concentration (ZnMC).

Two solution culture experiments, covering wide ranges in Zn supply levels, showed that increased Zn supply resulted in increased plant Zn uptake throughout crop development and in higher ZnMC in all plant organs, but to varying degrees. With higher plant Zn uptake, ZnMC increased most in stems, and least in grains. Two apparent barriers for Zn transport were identified, one between stem and rachis and one between bran and endosperm, since their ZnMCs strongly differed at high plant ZnMC.

Using radioactive ^{65}Zn applied to root or leaf after flowering, we found that when rice plants were grown under sufficient or surplus Zn supply, most of the Zn accumulated in the grains originated from uptake by roots after flowering, rather than from Zn remobilised from leaves.

On the basis of the results of the above studies on Zn (re-)allocation in rice plants, and derived relations between Zn mass fractions in different organs, we developed a descriptive simulation model to increase quantitative understanding of the relevant processes involved in grain zinc accumulation. Results from an independent field experiment were used for model validation. Results showed that the model allowed reproduction of recognizable patterns of ZnMC for a wide range of absolute values, and simulated grain ZnMC was in satisfactory agreement with observed values, with a mean normalized gross error of 8–11%. Further testing under different conditions is necessary to build confidence in its general applicability.

To assess genotypic variation in grain ZnMC, we proposed two new indices: low-Zn tolerance index for grain yield (TIY) and grain Zn mass concentration (TIZnMC). We found TIY and TIZnMC effective in identifying genotypes that perform well in terms of yield and grain ZnMC, respectively, under both Zn-limited and Zn-sufficient conditions.

It is concluded that there is limited scope for enhancing ZnMC in rice endosperm by simply increasing the Zn supply to rice plants, not enough to attain values necessary from a human nutritional point of view, because zinc allocation to the endosperm is limited, while observed genotypic differences indicate scope for improvement through breeding.

Keywords: Zinc, rice, *Oryza sativa*, grain, Zn mass concentration, biofortification.

Preface

In 2002, I was offered the opportunity to start a PhD with Wageningen University, The Netherlands. During the study, I have benefitted greatly from many people that have contributed to the successful completion of this work. Without their help, the thesis would not have been finished.

I am deeply indebted to my promotors and my supervisor in Wageningen, Professors Paul Struik and Herman van Keulen, and Dr. Tjeerd Jan Stomph. I very much appreciated that Professor Paul gave me intensive guidance and intellectual stimulation, and what impressed me was that he could reply to my frequent emails and give comments on my draft papers in time, even during weekends, although he is so busy. I am deeply grateful to Professor Herman, who gave me such warm and patient guidance, especially on my modelling work, as I had no experience on modelling. I also want to thank his wife Clara for preparing such delicious dinners for PhD students.

Tjeerd Jan Stomph, my daily supervisor, really gave me intensive and patient daily supervision on my research, I learned a lot from him. He often put forward some exciting research questions, which guided me to further research. In addition, it might have been related to our traditional Chinese education system, I used to follow the teacher's idea. Tjeerd Jan often reminded me "don't only follow us, try to understand and formulate your own ideas", which will be in my mind in my future research life, and I will also show this to my students. And I really enjoyed the pleasant time with his family.

I am also thankful to my supervisor in China, Professor Ming Zhao; he offered me this opportunity to study in Wageningen, and gave me support during my research. Also special thanks to my teachers in China Agricultural University, Professors Huaqi Wang, Guanghui Xie, and Chunqin Zou, Xiaoguang Yang, Jianhe Yan, and the staff in the Soil and Fertilizer Institute of Mengcheng, Anhui, Wenkai Zhang and Quanhua Wang, who all gave much help during my experiments.

I am grateful for the helpful discussions with people within the INREF "From natural resources to healthy people" programme. Drs Ellis Hoffland and Maja Slingerland, thanks for your useful comments and suggestions on my research and many thanks to your families for the pleasant dinner times we shared. Moreover, many thanks to my colleagues and fellow PhD students in the China part of the programme, Jianfen Liang, Xiaopeng Gao and Yu Qin.

I am indebted to Gon van Laar; she gave me a lot of help when building my simulation model, and I really appreciated her great help in editing my thesis. And thanks for your delicious traditional Dutch dinner.

I would like to thank Drs Xinyou Yin, Peter Leffelaar and Bas Bouman, who gave me much guidance and support on my modelling chapter, and Dr. Yin, thanks for the pleasant stays with your family.

I would like to extend my thanks to all the people in the "Haarweg building" who made my stay in Wageningen more pleasant; it would be impossible to name them all, but a special thanks to Jenny and Wampie for their kind administrative support. I am also grateful to the staff from the former TUPEA for the operational help with computers and software. I would like to thank Dr. Nico and his wife Rita for their hospitality and for inviting PhD students to have dinner together at their place.

I also would like to thank the MSc students in Wageningen and in China, Pieter-Jelte Lindenbergh, Dirk Hardeman, Lingna Jin and Tangqun Fan, who gave me much help during my experiments.

I would like to thank all my Chinese friends in Wageningen: Chunming Liu, Beizhong Han, Shipeng Li, Hanping Mao, Wenshan Guo, Fuyu Ma, Lizhen Zhang, Jinzhong yang, Qingdao Zou, Zhongqui Sun, Qi Jing, Huaidong Du, Li Jia, Lei Zhang, Qin Ji, Lingzhi Li, Xuefeng Li, Yuna Her, Suying Chang, Yuntao Ma, Jiayou Zhong and Ying Lei, Nan Luo. Thanks for the pleasant time we spent together in Wageningen. And many thanks to my colleagues from China Agricultural University and the Chinese Academy of Agricultural Sciences.

I would like to express my gratitude to my father and my mother, and my brother and sister-in-law. They always encouraged me to go further.

Finally, my special thanks to my husband, Hongsheng Zhang. During the past five years, without his understanding and support, I could not have continued and finished my study.

Wen Jiang
Wageningen, The Netherlands,
January, 2008

Contents

Acronyms

HI	Harvest index
MNGE	Mean normalized gross error
RDA	Recommended Dietary Allowance
RMSE	Root mean square error
TIY	Low-Zn tolerance index for grain yield
TIZnMC	Low-Zn tolerance index for grain Zn mass concentration
TIZnMCY	Low-Zn tolerance index for grain Zn mass concentration and grain yield
YEI	Grain yield efficiency index
ZnMC	Zinc mass concentration
ZnMCEI	Grain Zn mass concentration efficiency index
ZnMCYEI	Grain Zn mass concentration and yield efficiency index

CHAPTER 1

General introduction

Project background

This thesis work forms part of a larger interdisciplinary programme "From natural Resources to Healthy People". The programme aims to develop agriculture- or food-based interventions to alleviate nutritional deficiencies of the urban and rural poor, by investigating the efficiency of transfer of zinc (Zn) from the soil to the human body (for the programme description see pages 113 and 114). The programme included research on soil-plant Zn uptake aspects, Zn transport and accumulation in the grain, and grain technology and processing aspects of zinc in raw, polished rice, and research on the efficiency of zinc uptake from zinc enriched rice by humans. The contribution of the present thesis to this programme is to analyse the Zn storage, translocation, re-allocation and grain accumulation of Zn in rice plants.

Zn deficiency in human populations

Zinc deficiency in humans is widespread. Over 30% of the world's population may suffer from zinc deficiency (Welch *et al*., 2005). Zn deficiency is especially prevalent among resource-poor women and children. Zinc has multiple roles in basic cellular functions in all living organisms and is required for the normal development and functioning of non-specific and acquired immunity in humans (Shankar & Prasad, 1998). People who suffer from severe zinc deficiency show stunted growth, have slowly healing wounds, and become mentally retarded (Whitaker, 1998; Prasad & Bose, 2001). Yet, the most common deficiencies are of a less dramatic nature and lead to slight stunting, poorer mental development and poor immune system functioning. In China, average intake of zinc is 85.6% of its Recommended Dietary Allowance (RDA), and in Gansu province, the average intake of zinc is only 64.8% of the RDA (Ger *et al*., 1996). A survey from 19 provinces and districts in China revealed that 60% of the children suffered from Zn deficiency (Ma & Kou, 2003). In China, for the poorer part of the rural population, most people are used to eating a diet with a relatively high proportion of cereal grains, however, Zn density (or Zn mass concentration, mg Zn kg^{-1} dry matter) in cereal grains for human consumption is generally low (Table 1), therefore, its increase is being considered as a sustainable, long-term solution to human Zn deficiency (Rengel *et al*., 1999; Welch *et al*., 2005).

Table 1. Zn mass concentration in staple food.

Source	Zn (mg kg^{-1} dry weight)	Reference
Brown rice	33	Pedersen & Eggum (1983)
	22.8	Liang *et al*. (2007)
White rice	18	Pedersen & Eggum (1983)
Whole maize	22	Wolnik *et al*. (1985)
Whole wheat	31	Wolnik *et al*. (1983)

Rice as a main staple food crop

Rice is the world's most important staple crop, providing food for over half of the world's population, and rice grains are also important dietary sources of zinc for many resource-poor families globally. For the developing countries as a whole, rice accounted for 47% of the cereal production in 2000. In China, rice contributed 24% of the country's crop production (2001–2002) (FAO, 2002).

However, rice production consumes huge quantities of water: water consumption of an irrigated paddy rice crop is as high as 12,000–15,000 m^3 per hectare. So new methodologies and production technologies for rice are necessary in some of the production areas because water resources are under threat. In China, rice production is now in a transition from traditional lowland rice cultivation to a promising new cultivation system of 'aerobic rice', with much lower water requirements (Bouman *et al*., 2002). Rice varieties are being developed for aerobic cultivation conditions by crossing lowland varieties with upland varieties. These varieties can be directly sown and grown in irrigated but non-puddled fertile soils (Wang & Tang, 2000; Bouman *et al*., 2002). Aerobic rice cultivation has also been developed and adopted in other parts of the world, e.g., Brazil. In 2030, one-third of the rice is expected to be produced under aerobic cultivation (Wang & Tang, 2000; Bouman *et al*., 2002). Research has been done on soil Zn availability and plant Zn uptake in these systems (Gao *et al*., 2006), and it was found that Zn bio-availability was reduced under aerobic soil conditions compared to flooded conditions. The consequence of this was that the cultivation shift from aerobic to lowland conditions decreased rice Zn mass concentrations in the shoot and in the grains. Fertilization of the aerobic rice crops could remediate the lower Zn uptake but it was observed that the Zn harvest index was lower at higher Zn application levels (Gao *et al*., 2006). The physiology of the Zn partitioning over different plant organs is still poorly documented in both aerobic rice and lowland rice. In this research, we conducted experiments to understand and quantify the process of Zn allocation in rice cultivars developed for aerobic cultivation.

Biology of Zn allocation into rice grain

Accumulation of Zn in grains is controlled by a number of physiological processes as indicated in Figure 1, and several barriers have to be overcome to accumulate more zinc in the edible parts of plants (Welch & Graham, 2002).

Zn uptake by root

The root-soil interface is the first and most important barrier to affect Zn uptake (Welch & Graham, 2002). To increase Zn uptake by roots, the Zn availability in the rhizosphere must be increased (Welch, 1995), which could be done by enhanced release rates of root-cell H^+, metal chelating compounds and/or reductants, by increasing root absorptive surface area (fine roots and root hairs) and by association with mycorrhizal fungi (Liu *et al.*, 2000; Ryan & Angus, 2003; Gao *et al*., 2007). It is found that when the rice cultivation system was changed from flooded to aerobic conditions, plant Zn uptake decreased, which might be caused by changes in pH, dissolvable organic carbon (DOC) or redox in the rhizosphere, and by the differences in transpiration and diffusion between the two systems (Gao, 2007).

Zinc transport to shoot

Zinc transport in plants takes place both through the xylem and the phloem. Following absorption by the roots, Zn is rapidly transported via the xylem to the shoots (Riceman & Jones, 1958). Adequate Zn supply leads to a high proportion of Zn located in the shoots, while with toxic levels of Zn supply, a higher proportion of total Zn may

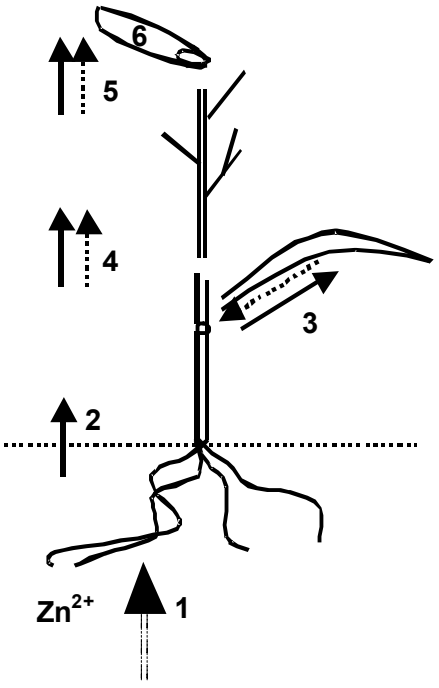

Figure 1. Schematic representation of Zn transport in the rice plant. The double arrow at the bottom indicates transport within the soil to the rhizosphere and into the roots, the solid arrows indicate xylem transport, the dashed arrows indicate retranslocation through phloem transport from leaves. The processes include: (1) Zn uptake by root, and internal Zn transfer from (2) root to stem, (3) stem to leaves and *vice versa*, (4) stem to rachis, (5) rachis to bran, and (6) bran to the endosperm.

accumulate in the roots, like in beans (White *et al*., 1979). The form in which Zn is actually transported in xylem sap remains unclear. The types and amounts of Zn would be affected by the composition, pH and redox potential of the xylem sap (Welch, 1995; Liao *et al*., 2000). Computer simulations run on xylem sap composition suggest that Zn is mainly transported in xylem of soybean as Zn-citrate complex and in tomato as Zn-citrate or malate complexes (White *et al*., 1981).

Zinc remobilization

Zinc appears to be the most mobile of all micronutrients and its remobilization is closely related to leaf senescence (Marschner, 1995; Uauy *et al*., 2006). Zn can be re-translocated from old leaves of wheat to young leaves and roots via the phloem (Erenoglu *et al.*, 2001). In soybean, it was found that 37.5% of the dose of foliar ^{65}Zn finally accumulated in grain, although plants were grown under sufficient Zn supply (Khan & Weaver, 1989), but the contribution of remobilization to grain Zn is unknown in rice. Transport of metals within phloem is thought to be via the positive hydrostatic pressure gradient developed from the loading of sucrose into the phloem from mature actively photosynthesizing leaves and unloading of sucrose into the sink tissues such as rapidly growing tissues, apical root zones and reproductive organs (MacRobbie, 1971; Hocking, 1980; Welch, 1995).

Loading of zinc into grains

Grain Zn accumulation apparently comes from different pools of Zn within the plant (Pearson *et al*., 1996b). In wheat, Zn reaches the developing wheat grain via the phloem (Herren & Feller, 1994; Pearson & Rengel, 1995b). Before Zn is loaded into the developing grain, the xylem is discontinuous (Zee & O'Brien, 1970) and the xylem-phloem exchange occurs in the rachis and to a lesser extent in the peduncle, lemma and palea (Pearson & Rengel, 1995b). Loading Zn to the phloem may be a rate-limiting step, and the saturation of membrane transporters may reduce the grain loading of Zn (Pearson *et al.*, 1996a). In wheat, once Zn enters the grain phloem, Zn is initially mostly transported to the crease and pericarp tissues, but as the grain matures increasingly more to the endosperm and the embryo. In mature wheat grains, the relatively largest amounts of Zn remain stored within the pericarp tissues (Pearson *et al*., 1998). However, in rice, the pathway of Zn loading to the grain is completely different from that in wheat. There is no discontinuity between rachis and grain vascular bundle (Zee, 1971; Krishnan & Dayanandan, 2003). Furthermore, a symplastic continuity exists between the cells of the vascular trace, chalaza, nucellar projection and nucellar epidermis (Figure 2) (Thorne, 1985; Krishnan & Dayanandan, 2003). The further transport of mineral nutrients from the outer grain tissues to the endosperm is

inwards through the apoplast from the nucellar epidermis that completely encircles the endosperm (except near the vascular trace) (Krishnan & Dayanandan, 2003). However, Gao *et al.* (2005) found that enhancing the rate of plant Zn uptake through fertility management did not translate into an increased grain Zn loading, Zn harvest index was reduced, which indicates some limitation must occur during Zn transport to and loading into the grain. Therefore, this thesis will focus on studying the internal Zn allocation in rice plants and exploring the most limiting transfers.

Dynamics of Zn partitioning in rice plant

As mentioned above, the final Zn mass concentration (mg kg^{-1}) in the rice grain is a function of its availability in the soil, the uptake capacity of the roots, the demand of the growing crop and the redistribution within the plant. In fact, the integrated effect

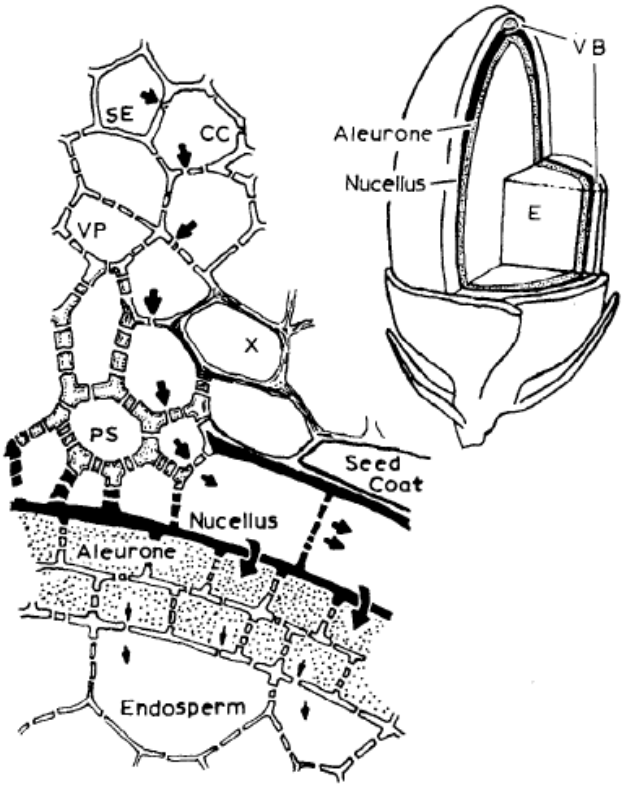

Figure 2. Schematic representation of the pathway of unloading assimilates and nutrients in a developing caryopsis of rice. Curved arrows (entering the endosperm transfer cells) represent what is thought to be facilitated transport. SE: sieve element; CC: companion cell; VP: vascular parenchyma; PS: pigment strand; X: xylem. The darkened cell walls of the nucellus represent cuticles. *Inset:* section through the grain, illustrating the circumferential position of the nucellus and aleurone transfer cells away from the single vascular bundle. E: endosperm; VB: vascular bundle. Cell sizes are approximate (Source: Thorne, 1985).

of these factors can be studied through Zn-crop models that describe the dynamics of the element in the soil, including its chemical transformations, the development of the uptake capacity of the root system, the dynamics of the Zn demand in the (various organs of the crop) and the mobility of the Zn in the crop. Simulation models have extensively been used to study various aspects of crop physiology (Charles-Edwards, 1981; Michalov, 1986; Hahn, 1987; Goudriaan & Monteith, 1990; Denison, 1992; Ingestad & Agren, 1992; Van Ittersum *et al*., 2003), including nutrient accumulation in soil-grown plants (Nye & Tinker, 1977; Van Veen & Frissel, 1981; Van Keulen & Seligman, 1987). Nitrogen-limited plant growth has been modelled for crops such as spring wheat (Van Keulen & Seligman, 1987; Weiss & Moreno-Sotomayer, 2006), rice (Drenth *et al*., 1994 (ORYZA-N); Bouman *et al*., 2001 (ORYZA2000)), and maize (Tittonell *et al*., 2006) and phosphorus-limited growth for maize (Radersma *et al*., 2005), wheat and beans (Daroub *et al*., 2003). However, the dynamics of zinc in the soil-plant system strongly differs from those of nitrogen and phosphorus in a number of aspects, including Zn uptake and Zn partitioning between organs. Therefore, simply using existing modelling approaches may not be possible and experimentation and modelling will both be needed to increase our quantitative understanding of the relevant processes involved in Zn uptake and partitioning in rice plants.

Aim and outline of the thesis

As mentioned above, there is still too little known on the physiological processes governing Zn allocation in rice plants to effectively design methods to improve grain Zn mass concentrations to the level required for an adequate human nutrition. Therefore, the main objectives of the present studied were:

- To quantify the allocation of Zn in rice plants developed for aerobic cultivation and to explore the potential of grain Zn accumulation (Chapter 2);
- To identify the sources of Zn allocated to the grain (Chapter 3);
- To develop a model for the Zn partitioning in rice (Chapter 4); and
- To develop some new indices for high grain Zn mass concentration and high grain yield screening in aerobic rice (Chapter 5).

Following this introduction chapter, in Chapter 2, we describe a study on the zinc allocation within rice plants. Two controlled-condition experiments were carried out with four rice cultivars developed for aerobic cultivation, grown at a wide range of Zn supply rate. The distribution of Zn between plant organs at different development stages was quantified over this range of Zn supply levels, and the potential of zinc accumulation in rice grain was explored. Furthermore, we investigated the change of the Zn distribution over endosperm and outer grain layers within rice grain at increasing plant Zn levels.

In Chapter 3, we investigated the Zn uptake and distribution after flowering and identified the sources for the Zn allocated to the grain. Radioactive ^{65}Zn was applied to the root at flowering or 15 days after flowering; the aim of the study is to verify if rice plants continue to take up Zn after flowering, and to investigate how much of this Zn is allocated to the grain. We applied ^{65}Zn to the flag leaf and the lowest senescent leaf at flowering, to assess and quantify the potential for Zn (re-)translocation from leaves and its role in grain Zn accumulation.

In Chapter 4, based on the experimentally identifying and quantifying of Zn allocation in rice plants reported in Chapters 2 and 3, we developed a model for Zn partitioning in rice plants. Data from Chapter 2 were used for model calibration, and an additional field experiment with five Zn application levels was conducted to validate the model.

In Chapter 5, we present low-Zn tolerance indices for grain Zn mass concentration and grain yield. A screening experiment with 16 accessions of aerobic rice in pots with and without Zn application under greenhouse conditions and a second screening of 14 accessions under field conditions in a low-Zn soil with or without Zn fertilization were conducted to test the merits of these indices in comparison to the major Zn efficiency indices from the literature.

In Chapter 6, a comprehensive overview of the obtained quantification of the allocation of Zn in rice and the possible sources of Zn for grains are discussed. The conclusions are used to further discuss the potential for increasing the grain Zn mass concentration in rice and the most promising approaches to increase grain Zn mass concentration in farmer fields.

CHAPTER 2

Does increased Zn uptake enhance grain Zn mass concentration in rice?

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Abstract

Rice is the worlds' most important cereal and potentially an important source of zinc (Zn) for people who eat mainly rice. To improve zinc delivery by rice, plant Zn uptake and internal allocation need to be better understood. This study reports on within-plant allocation and potential zinc accumulation in the rice grain in four so-called aerobic rice cultivars (Handao297, K150, Handao502 and Baxiludao). Two controlled-condition experiments were carried out, one with a wide range of constant zinc concentrations in the medium and one with a range of plant growth rate related supply rates. In both experiments, increased Zn supply induced increased plant Zn uptake rate throughout crop development, both when expressed as daily Zn uptake (μg d^{-1}) or as daily Zn uptake per gram of plant dry matter (μg g^{-1}). Zn mass concentration in all plant organs increased with an increase in Zn supply, but to various degrees. At higher uptake levels, the zinc mass concentration (ZnMC) in stems increased most, while the ZnMC in grains increased least. The increase in leaf ZnMC was generally small, but at toxic levels in the medium, also leaf ZnMC increased significantly. A milling test showed that when Zn mass concentration in brown rice increased from 13 to 45 mg kg^{-1}, Zn mass concentration in polished rice grains (endosperm) increased from 9 to 37 mg kg^{-1}, but remained 3–5 times lower than that in the bran. Irrespective of the zinc mass concentration in the brown rice, around 75% of total grain Zn was present in the endosperm. It appears that regulation of grain Zn loading differs from regulation of Zn loading to other organs. In both cultivars there was a major difference in ZnMC between bran and endosperm (120 and 30 mg kg^{-1}, respectively), suggesting a barrier for Zn transport between the two tissues. There seems to be a second barrier between stem and rachis, as their ZnMCs also differed greatly (300 and 100 mg kg^{-1}, respectively) in both cultivars at higher plant ZnMC. It is concluded that there is too little scope from a human nutrition perspective to enhance ZnMC in rice endosperm by simply increasing the Zn supply to rice plants, because zinc allocation to the endosperm is limited, while observed genotypic differences indicate scope for improvement through breeding.

Keywords: Aerobic rice, grain quality, nutrient uptake, *Oryza sativa* L., plant Zn distribution, zinc, zinc allocation.

INTRODUCTION

Zinc (Zn) deficiencies constitute a major public health problem in many countries, especially in regions where people rely on monotonous diets of cereal-based food (Prasad, 1984; Welch, 1993), as the Zn mass concentration (mg Zn kg^{-1} dry matter) in the grains of staple crops, such as cereals, is generally low. Increasing the Zn mass concentration in the grains of these crops is considered a sustainable way to alleviate human Zn deficiency (Graham, 1984; Prasad, 1984; Graham & Welch, 1996; Rengel *et al*., 1999; Frossard *et al*., 2000; Von Braun *et al*., 2005). Considerable efforts are undertaken to reach this goal through breeding (Graham *et al*., 1999; Cakmak *et al*., 2000; Toenniessen, 2002; Vasconcelos *et al*., 2003), but understanding of the underlying processes is still partly lacking and would facilitate such breeding programmes (Clemens *et al*., 2002).

Earlier studies have shown that an easy and direct way to increase the Zn mass concentration in cereal grains is to apply Zn fertilizer, either to the soil or to the leaves. Zn mass concentration in grains could be increased 1.5–3.5 fold in wheat (Yilmaz *et al*., 1997; Kalayci *et al*., 1999). In rice under field conditions, Gao *et al*. (2006) observed no effect of a 23 kg ha^{-1} application of Zn, while Jiang *et al*. (Chapter 5), using a broader range of genotypes, observed only a 1.75-fold increase through fertilization. Maximum Zn mass concentrations reported in these two studies on rice (< 30 mg kg^{-1}) are too low to meet the nutritional demand in humans (pers. comm. Prof. Michael Zimmermann, ETH Zurich), so further increases are needed. On the other hand, based on the difference in the zinc harvest index between fertilized and unfertilized plants reported by Gao *et al*. (2006), we hypothesize that enhancing plant zinc uptake mainly results in increased storage of Zn in vegetative tissues (hypothesis 1). Enhancing crop Zn nutrition through improved soil management could improve crop Zn mass concentration, however, the potential to increase Zn mass concentration in the rice grain may be limited. In soybean, the Zn mass concentration in seeds increased only from 56 to 167 mg kg^{-1} when Zn supply to the medium was increased thousand-fold (Raboy *et al*., 1984). There is no conclusive evidence for the existence of such a limitation to Zn mass concentration in grains of rice, nor on the level that can be reached. We, therefore, hypothesize that there is a physiological upper limit to grain zinc mass concentration in rice that restricts endosperm levels below the 30–40 mg Zn kg^{-1} (polished rice), required from a human nutritional point of view (hypothesis 2).

Pearson *et al*. (1996b) reported that the Zn-deficient wheat grain is not a strong sink for Zn, while at high Zn concentrations in nutrient solutions, a protective barrier prevents excessive Zn accumulation in the wheat grain. The question arises whether this also holds for rice. In wheat, there is a xylem discontinuity at the base of the grain (Zee & O'Brien, 1970) and xylem-phloem exchange occurs in the rachis and to a

lesser extent in the peduncle, lemma and palea (Pearson & Rengel, 1995b). Loading Zn to the phloem may be a rate-limiting step due to saturation of membrane transporters (Pearson *et al*., 1996b), and the interactions between the xylem and phloem transport systems may play an important role in the regulation of zinc transport to the maturing grains (Herren & Feller, 1994). However, in rice, there is no xylem discontinuity (Zee, 1971; Krishnan & Dayanandan, 2003), so the transport of Zn from the stem into the rachis and the grain vascular bundle through the xylem remains continuous during grain filling. Furthermore, a symplastic continuity exists between the cells of the vascular trace, chalaza, nucellar projection and nucellar epidermis (Thorne, 1985; Krishnan & Dayanandan, 2003). The further transport of minerals to the endosperm is inwards through the apoplast from the nucellar epidermis through the aleuron cells into the endosperm. This step may play an important role in the regulation of zinc transport to the endosperm. It can, therefore, be expected that the Zn mass concentration in the rachis and the bran are comparable, while there is a drop between the outer grain tissues and the endosperm, as observed in both, wheat (Pearson *et al*., 1996a; Pearson *et al*., 1998; Ozturk et al., 2006) and rice (Ren *et al*., 2006). During the milling or polishing process, the outer tissues (bran) are removed, resulting in relatively low levels of Zn in the parts of grain used for consumption (endosperm). The effect of Zn fertilization on the partitioning of Zn to the bran and endosperm tissues is not known. Assuming that the transport from outer tissues to the endosperm will increase when Zn mass concentrations in the outer layers increase, we hypothesize a proportional increase in the Zn mass concentration in bran and endosperm when Zn uptake in brown rice is increased (hypothesis 3).

With increasing shortage of fresh water available for agriculture, rice production in China, but also in e.g. Brazil, is now in a transition from traditional lowland rice cultivation, with a very high consumption of water, to the promising new cultivation system of 'aerobic rice', with much lower water requirements. Seeds are sown directly and crops are grown without standing water in irrigated, but non-puddled fertile soils with aerobic conditions in the root zone (Wang & Tang, 2000; Belder *et al*., 2005; Bouman *et al*., 2007). Varieties suitable for this new system are developed by crossing lowland rice with upland rice. Recently, availability and uptake of Zn in these systems have been investigated (Gao *et al*., 2006). This chapter reports on accumulation and partitioning of zinc in rice cultivars from the aerobic rice breeding programme of China Agricultural University as a further step towards understanding Zn allocation to the grains, through verification of the three hypotheses formulated above.

MATERIAL AND METHODS

Two experiments were carried out under controlled conditions. In Experiment 1, rice was grown in pots filled with acid-washed coarse sand at a wide range of constant Zn supply levels. The range covered levels from sufficient to toxic. As a follow-up, Experiment 2 was carried out on aerated hydroponics, as this allowed further control over Zn application throughout the experiment. Zn was applied every three days, in gradually increasing amounts, in proportion to the increase in dry matter accumulation of the plants. This method of application aimed at maintaining constant plant Zn levels, ranging from 10 to 200 mg kg^{-1}, thus avoiding severe deficiency and toxicity.

Experiment 1 (sand culture)

Individual pots were filled with 1 kg of quartz sand. The sand was previously washed thoroughly with 5% HCl, subsequently rinsed with tap water and finally with double-de-ionized water, and air-dried. Each pot was supplied with 2 litre of a Yoshida solution culture medium (Yoshida, 1976) with an initial pH of 6.0 ± 0.1, and buffered by KOH and HCl. The medium was refreshed every week. Zn, as ZnSO4.7H2O, was added at seven levels. As a contamination of 0.15 μmol Zn $l^{-1}$was detected in the medium without Zn addition, actual Zn levels in the media were 0.15, 0.165, 0.30, 15.15, 150, 750, 1500 and 2250 μmol l^{-1}.

Seeds of two aerobic rice cultivars popular in China, Handao297 and K150, were surface-sterilized by washing in 70% ethanol for 1 min and soaking in 1% sodium hypochlorite for 5 min, and pre-germinated in double-deionized water for 24 h. Five seeds were sown per pot, and plants were thinned to three seedlings per pot one week after emergence. Plants were grown under ambient temperature and light in the greenhouse at China Agricultural University, Beijing, during the summer season of 2003.

Experiment 2 (solution culture)

In the second experiment, different aerobic rice cultivars were used, as field observations indicated potentially larger genotypic differences (unpublished data). Seeds of the aerobic rice cultivars Handao502 and Baxiludao were treated before germination as described for Experiment 1. After pre-germination, seeds were planted in quartz sand washed with 5% HCl, and only received double de-ionized water. After 15 days, the seedlings were transplanted into foam disks fitted in the lids of 70-litre containers. Fifty-six seedlings were planted in each of the containers filled with half strength Hoagland solution (pH 5.6 ± 0.1) without zinc for each cultivar. Following the method used by Hoffland *et al*. (2000) for P, Zn (as $ZnSO_4$) was added every three days to the solution on the basis of expected dry matter increase, and seven target plant

Zn mass concentrations for total plant dry matter (10, 15, 25, 50, 100, 150 and 200 mg kg^{-1}). For each treatment, the amount of Zn (Zn_t, in µg) that would be needed for the expected new biomass to attain the target mass concentration, was calculated according to the formula:

$$Zn_t = (W_t - W_{t-1}) \times [Zn]_{target} \quad \text{with} \quad W_t = W_{t-1} \times e^{r\Delta t}$$

in which, r is the relative plant growth rate (in d^{-1}), estimated from a previous growth rate experiment; W_{t-1} is the dry weight per plant at time t–1 (in g); W_t is the dry weight per plant at time t (in g); Δt is the time interval between two applications of Zn, i.e. time t minus t–1 (in d, three days in our experiment); $[Zn]_{target}$ is the desired plant Zn mass concentration (in µg g^{-1}). Total Zn applied between start and end of the experiment for the seven target levels was: 142, 166, 251, 350, 558, 768, 979 µg $plant^{-1}$. Plants were grown in a glasshouse set to maintain a day/night temperature of 28 °C/ 21 °C at the Chinese Academy of Agricultural Sciences, Beijing, during the summer season of 2005. Light intensity was about 85% of natural light intensity and 1000 µmol m^{-2} s^{-1} light was supplemented when it was cloudy.

Plant harvest

In Experiment 1, plants were harvested 45 days after emergence, at flowering, 15 days after flowering, and at physiological maturity (30 days after flowering). Harvested plants were partitioned into roots, stems, sheaths, leaf blades, grains, other panicle tissues (rachis, rachillae and glumes combined) and senescent parts (leaf sheaths and blades combined).

In Experiment 2, plants were harvested at 45 days after emergence (30 days after transplanting), at panicle initiation, at flowering, 15 days after flowering and at physiological maturity (30 days after flowering). Harvested plants were partitioned into roots, stems, leaf blades, sheaths, senescent leaf blades, senescent sheaths, rachis (rachillae included), glumes, and grains.

All harvested plant materials in both experiments were washed in 0.1% HCl and subsequently rinsed a first time with tap water and a second time with de-ionized water. Total dry matter and grain weight were determined after oven-drying of the plant samples at 75 °C for 48 hours.

Chemical analyses

Zn mass concentrations of the plant samples were determined after grinding with a stainless-steel blade blender to a particle size of 0.25 mm. Dried ground plant samples (0.50 g) were digested in a bi-acid mixture (HNO_3:$HClO_4$ = 4:1) and Zn was determined by atomic absorption spectroscopy (AAS SPECTRAA-55; Varian Australia,

Mulgrave, Australia) at wavelength 213.9 nm. Zinc analyses were checked with certified Zn values in standard samples obtained from the Wageningen Evaluating Programmme for Analytical Laboratories (WEPAL, Wageningen University, The Netherlands). Zn mass concentrations (mg kg^{-1}) in individual plant organs were expressed on a dry weight basis. Zn content (µg $organ^{-1}$ or $plant^{-1}$) was calculated as dry weight multiplied by Zn mass concentration.

Polishing test

Brown rice samples from treatments of Experiment 2 for which enough grains were left, were polished with the use of a Pearlest rice mill (Kett Electric Laboratory, Tokyo, Japan). To minimize Zn contamination, all rubber parts of the mill had been replaced with special zero Zn emitting rubber by the Plant Nutrition Laboratory of the University of Adelaide, Australia (pers. comm. James Stangoulis). The mill separates pericarp, tegmen and aleurone layers (bran) from endosperm (white rice) in a way comparable to commercial milling. Bran was thus separated in two steps, outer layers were removed in a first polishing during 20 seconds, a second polishing of 40 seconds removed the final bran, leaving commercially acceptable white rice. The three fractions were collected separately and digested without further grinding with nitric acid and hydrogen peroxide in a closed vessel using the HotBlock system (A.I. Scientific, Clontarf, Qld, Australia). Digests were analysed using the ICP-AES of the Waite Analytical Services, Adelaide, Australia.

We assume that the contamination of the endosperm surface during polishing can be neglected. Mass balances based on dry matter of the different fractions and ZnMCs obtained for these fractions and based on ZnMC fractions assessed on samples of unpolished grains showed that this assumption was valid.

Statistical methods

Both experiments were conducted in a completely randomized design with three replications for each treatment and each cultivar. Analysis of variance was performed on the data on grain yield, harvest index and Zn harvest index (the ratio of Zn content in the dehulled grain to Zn content in the shoot). Means were compared with the post hoc Tukey's Honestly Significant Differences test ($P < 5\%$). All statistical analyses, including presented regression analyses were performed with SAS (Anonymous, 2001).

RESULTS

Treatment effects on grain yield and harvest index

Application of extreme levels of zinc in Experiment 1 resulted in lower plant grain and total dry matter production, both at the lowest and at the highest supply levels (Table 1). Higher supply levels resulted in shoot dry weight and grain yield decrease, for both Handao297 and K150, indicating serious toxicity effects. Grain production was more reduced than vegetative biomass production, resulting in harvest index reduction, especially at the lowest Zn supply level (Table 1).

In Experiment 2, there were no significant treatment effects of Zn supply level on grain yield and harvest index neither for Baxiludao nor Handao502 nor for shoot dry weight of Handao502 (Table 2). Only for Baxiludao a significantly lower shoot dry weight was observed at the lowest Zn supply level.

Zn uptake by rice plants

With an increase in Zn supply in Experiment 1, the rate of Zn uptake increased, for both cultivars either when calculated as daily Zn uptake (μg d^{-1}) (Table 1, data on total uptake) or as daily Zn uptake per gram of plant dry matter (μg g^{-1}) (Figures 1A–D). Essentially, the trends were similar during the period between panicle initiation and flowering and during the grain filling period, but Zn uptake rates in K150 were higher than in Handao297 before flowering, while the uptake rates of the two cultivars were comparable during grain filling (Figures 1A–D). Rates were lower during grain filling in both cultivars.

Also in Experiment 2 (Figures 1E, F) the rate of Zn uptake per gram dry matter increased as more Zn was supplied. During grain filling, the uptake rate increased in Handao502 compared to that before flowering, but in Baxiludao Zn uptake rate decreased slightly after flowering, both when calculated as daily Zn uptake (μg d^{-1}) (data not presented) and as daily Zn uptake per gram plant dry matter (μg g^{-1}). The realized total plant ZnMCs at the different Zn treatment levels (Table 2) corresponded relatively well to the targets at the higher levels, but clearly exceeded the targets at the lower levels. Comparable trends were found at the earlier harvests (data not presented).

Zn distribution among organs

The distribution of zinc among organs varied with level of Zn supply and development stage (Figures 2 and 3). At lower Zn supply levels and during grain filling, Zn content (μg $plant^{-1}$) decreased in the leaves (Experiment 2) or in leaves and sheaths combined (Experiment 1) for all tested cultivars (Figures 2A, B and 3A, B). At maturity in both experiments, at the lower Zn supply levels, 20–30% of total plant Zn was stored in the

Table 1. Effect of zinc concentration in the applied nutrient solution on plant Zn uptake, average Zn mass concentration in shoot and brown rice grains, shoot and grain dry matter production per plant and harvest index for the two tested varieties, Handao297 and K150 (Experiment 1).

Zn concentration in medium (μmol l^{-1})	Shoot Zn uptake (μg $plant^{-1}$)		Zn mass concentration (mg kg^{-1})				Dry matter (g $plant^{-1}$)				Harvest index[1]	
			Shoot		Grain (dehulled)		Grain (dehulled)		Shoot			
	Handao297	K150	Handao297	K150	Handao297	K150	Handao297	K150	Handao297	K150	Handao297	K150
0.15	599	594	52.8	55.9	51.8	30.4	2.34	2.94	9.98	9.36	0.24	0.32
0.165	859	487	58.9	40.1	56.8	28.3	4.05	4.03	13.1	10.5	0.31	0.38
0.30	925	613	53.1	47.9	63.6	28.9	5.03	4.18	15.6	11.4	0.33	0.37
1.65	1,070	760	70.5	59.8	55.4	26.9	3.00	4.50	13.7	11.6	0.22	0.39
15.15	1,350	1,520	106	105	56.8	38.8	2.82	4.59	11.3	13.0	0.25	0.35
150	7,890	5,450	603	389	81.2	53.4	3.30	4.42	11.6	12.5	0.28	0.35
750	13,100	8,740	875	768	102	57.9	3.94	2.64	13.2	10.2	0.29	0.26
2250	15,900	11,000	1,540	1,200	106	70.6	2.09	2.52	8.7	8.22	0.24	0.30
Treatment effects												
Zn							$P < 0.001$		$P < 0.001$		$P < 0.001$	
Var.							$P = 0.005$		$P < 0.001$		$P < 0.001$	
Zn × Var.							$P = 0.003$		$P < 0.001$		$P = 0.679$	
Tukey's HSD (0.05)												
Zn							0.82		2.30		0.06	
Var.							0.26		0.73		0.02	
Zn × Var.							1.25		3.49		0.09	

[1] Harvest index = Brown rice dry weight/above-ground biomass dry weight.

Table 2. Effect of zinc treatment on total Zn uptake, total above-ground dry matter, brown rice dry matter and harvest index for the two tested varieties Handoa502 and Baxiludao (Experiment 2).

Total plant target ZnMC [1] (mg kg^{-1})	Total Zn uptake (μg plant^{-1})		Zn mass concentration (mg kg^{-1})				Dry matter (g plant^{-1})				Harvest index [2]	
			Shoot		Grain (dehulled)		Grain (dehulled)		Shoot			
	Handao 502	Baxi-ludao	Handao 502	Baxi-ludao	Handao 502	Baxi-ludao	Handao 502	Baxi-ludao	Handao 502	Baxi-ludao	Handao 502	Baxi-ludao
10	127	134	22.9	31.4	20.7	21.6	1.23	1.22	4.28	3.72	0.29	0.32
15	147	162	27.3	32.7	21.4	23.2	1.36	1.38	4.35	4.46	0.31	0.30
25	222	214	42.9	39.7	31.2	26.1	1.35	1.40	4.40	4.61	0.31	0.30
50	315	303	60.8	59.7	35.0	34.2	1.36	1.44	4.55	4.39	0.30	0.33
100	459	547	87.1	97.7	41.6	39.7	1.44	1.55	4.61	4.91	0.31	0.32
150	572	575	117	118	44.8	40.7	1.38	1.45	4.32	4.34	0.32	0.33
200	892	826	189	180	49.4	49.2	1.32	1.30	4.05	3.86	0.33	0.34
Treatment effects												
Zn							$P = 0.051$		$P = 0.003$		$P = 0.110$	
Var.							$P < 0.001$		$P = 0.565$		$P = 0.015$	
Zn × Var.							$P = 0.285$		$P = 0.829$		$P = 0.285$	
Tukey's HSD (0.05)												
Zn							0.12		0.47		0.04	
Var.							0.04		0.16		0.12	
Zn × Var.							0.27		1.03		0.08	

[1] ZnMC:Zn mass concentration

[2] Harvest index = Brown rice dry weight/above-ground biomass dry weight.

dehulled grain. However, at higher Zn supply levels, Zn content in all non-grain organs remained more or less constant (roots, leaves and sheaths) or continued to increase after flowering (stem and panicle structure), where the increase in vegetative plant parts was much larger than in the grain, so at maturity in Experiment 2, dehulled grain contained only 10% of total plant Zn (Figures 3C, D) and in Experiment 1 an even smaller proportion (3–4%) (Figures 2C, D). The importance of uptake after flowering seemed cultivar-dependent in both experiments.

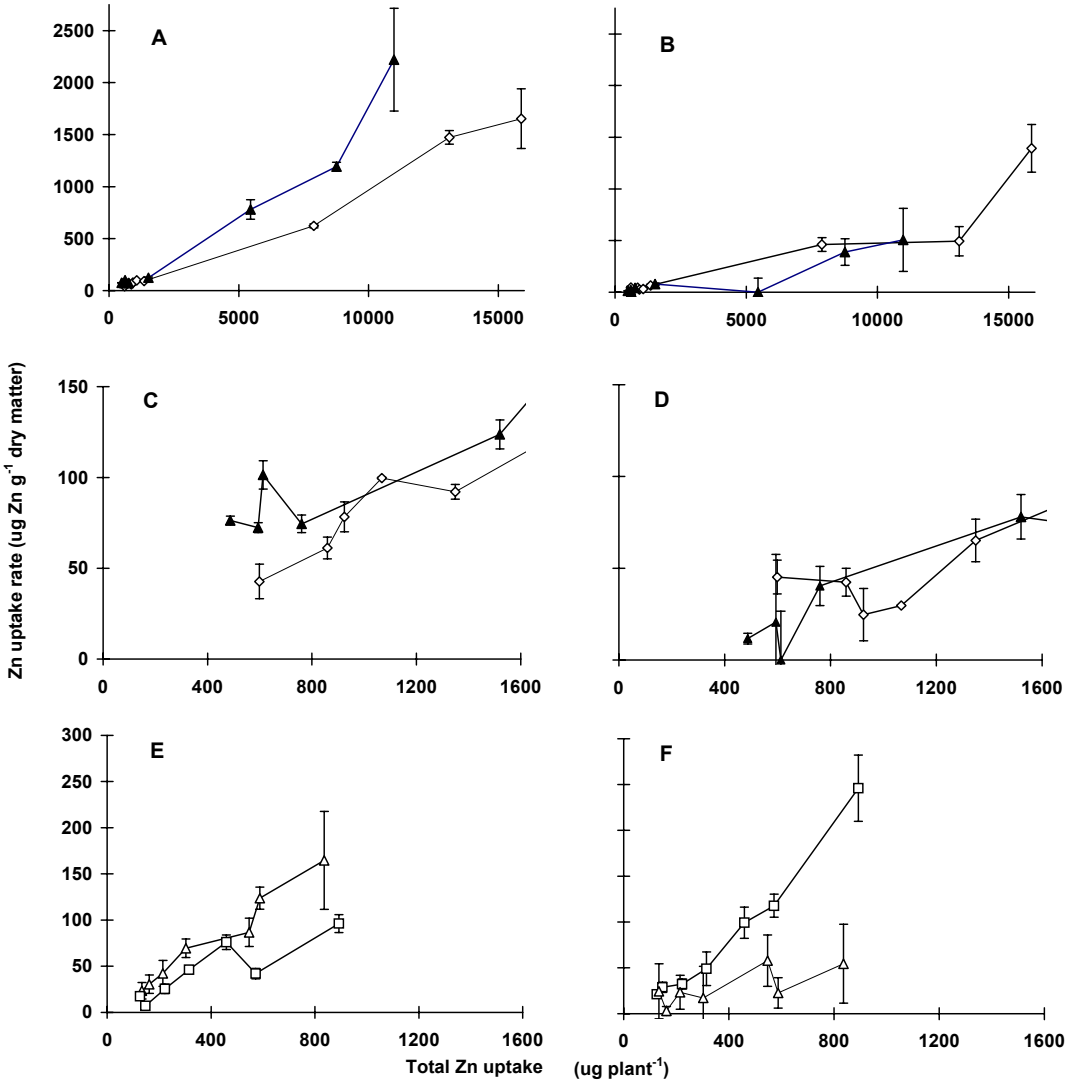

Figure 1. The change of Zn uptake rate per gram accumulated dry matter (mean ± SE, n = 3) with increasing total zinc uptake per plant between panicle initiation and flowering (A, C and E) and between flowering and maturity (B, D and F). Graphs A and B show the full range of Zn treatments for Experiment 1, graphs C and D show the lower range of total Zn uptake values for Experiment 1 and graphs E and F show the full range of treatments for Experiment 2. Cultivars are indicated as Handao297: open diamonds, K150: closed triangles, Baxiludao: open triangles and Handao502: open squares.

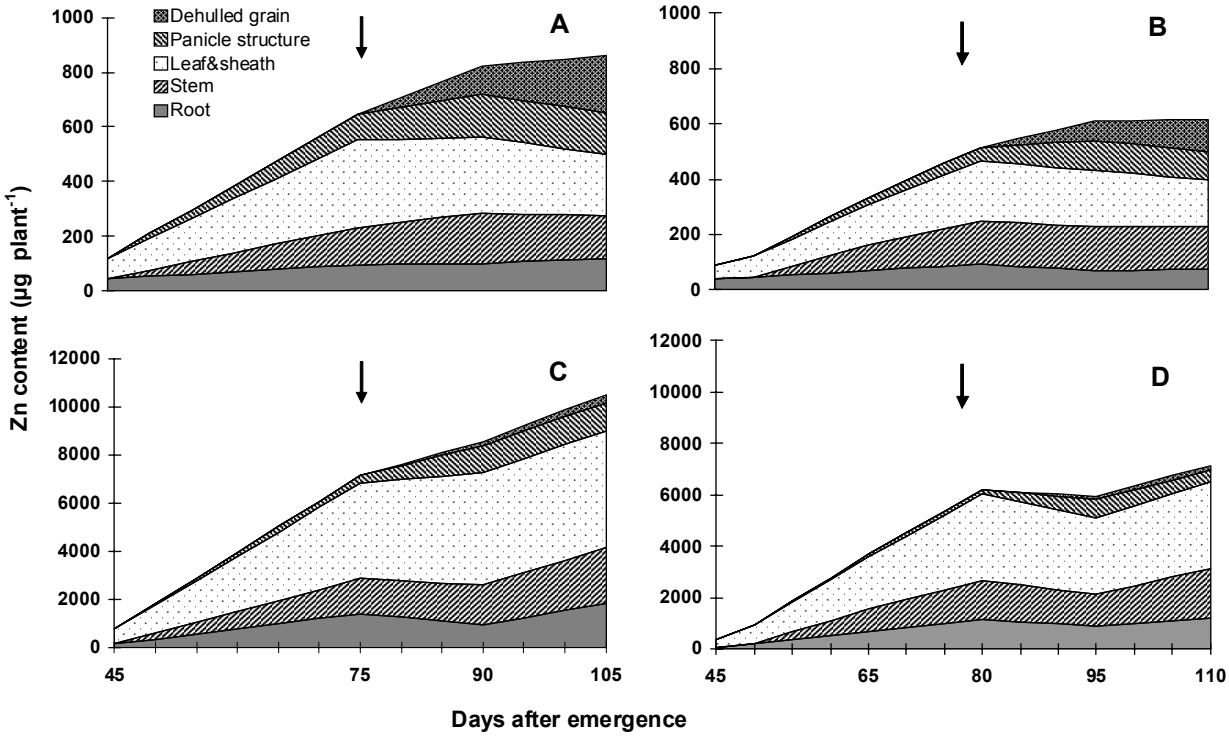

Figure 2. Cumulative Zn content (µg plant^{-1}) and its distribution among individual plant organs of aerobic rice cultivars Handao297 (A and C) and K150 (B and D) between 45 days after emergence and full maturity in Experiment 1. Data are the average of Zn content from plants grown at the lower Zn supply levels (0.15–1.65 µmol l^{-1}, A, B) or from plants grown at the higher Zn supply levels (150–750 µmol l^{-1}, C, D).

Zn mass concentration in individual plant organs

With increasing Zn supply levels, the Zn mass concentration in all individual organs increased (Figures 4 and 5). However, the increase in Zn mass concentration in stems and rachis was much larger than that in grains. In both experiments, Zn mass concentration in green leaves increased at a rate comparable to that in the grains up to the point where the Zn mass concentration in the stem reached about 300 mg kg^{-1}, beyond which point (data from Experiment 1 only) the Zn mass concentration in the leaves increased similarly to that in other vegetative plant parts (Figure 4). The exact point where the Zn mass concentration in leaves started to increase stronger seemed to differ between the tested cultivars (cf. Figure 4A).

Zn levels in the bran and endosperm

The milling test with rice from Experiment 2 indicated that a large proportion of total Zn in the grain had accumulated in the endosperm part, with an average value of 78%

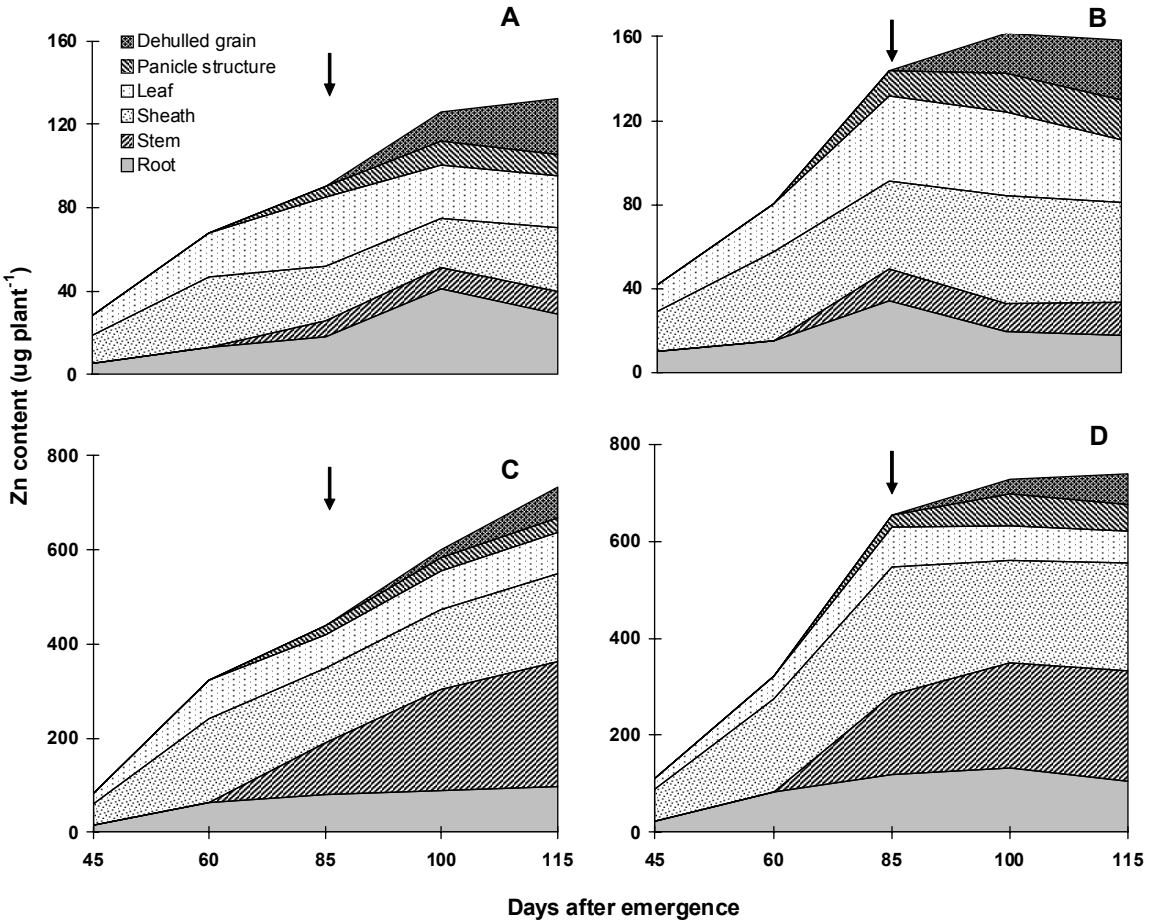

Figure 3. Cumulative Zn content (µg plant^{-1}) and its distribution among individual plant organs of aerobic rice cultivars Handao502 (A and C) and Baxiludao (B and D) at different harvesting dates between 45 days after emergence and full maturity in Experiment 2. Data are the average of Zn content from plants supplied with lower Zn levels (127–162 µg Zn plant^{-1}, graphs A, B) and higher Zn levels (572–892 µg Zn plant^{-1}, graphs C, D).

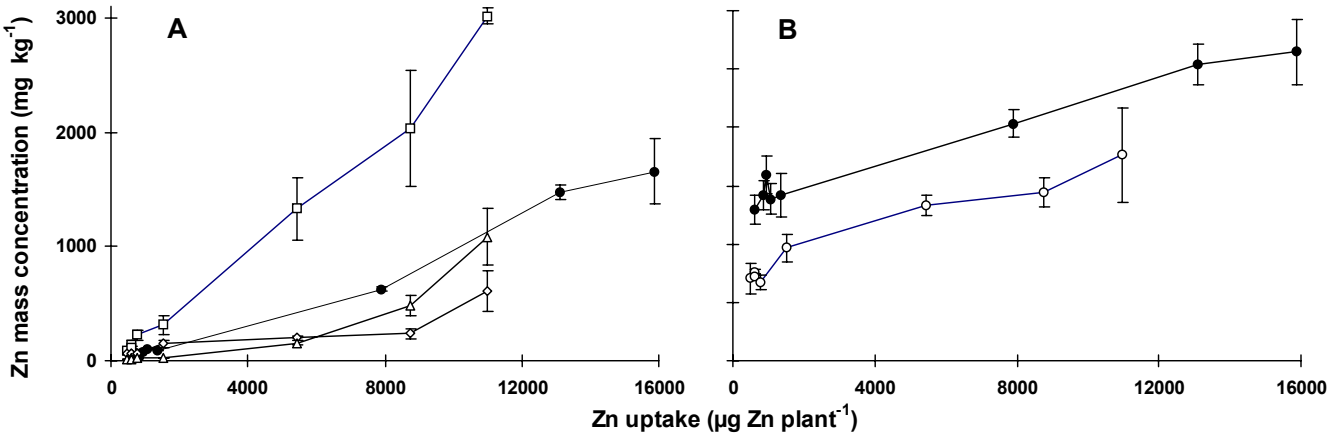

Figure 4. Zn mass concentration at maturity in Experiment 1 for Handao297 (closed symbols) and K150 (open symbols), (A) for stems (squares), rachis (diamonds) and leaves (triangles), and (B) for grains (circles). Error bars indicate standard deviations (n = 3) for individual data points, when not visible, standard deviation limits fall within symbols.

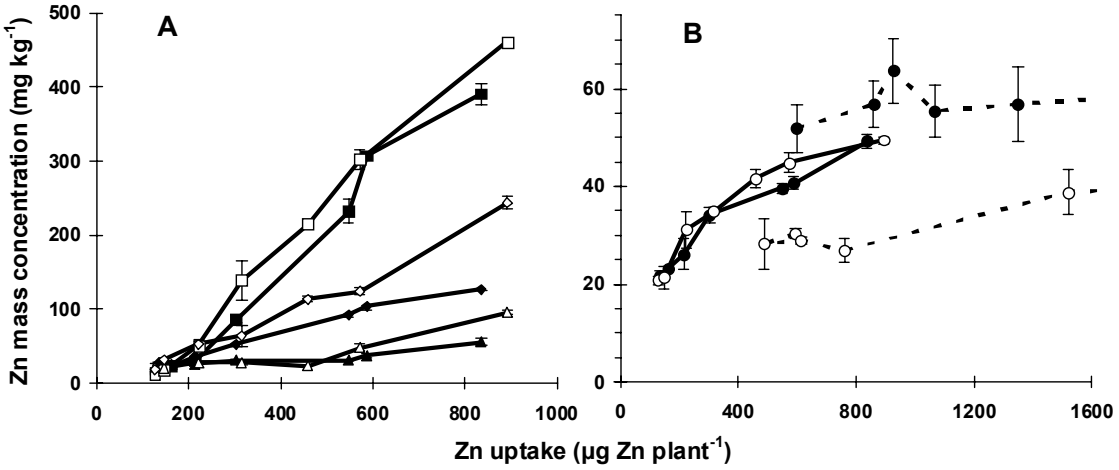

Figure 5. Zn mass concentration at maturity in Experiment 2 for Baxiludao (closed symbols) and Handao502 (open symbols); (A) for stems (squares), rachis (diamonds) and leaves (triangles), and (B) for grains (circles and solid lines). In (B) also data from Experiment 1 are given for grains of Handao297 (closed symbols, dotted lines) and K150 (open symbols, dotted lines). Error bars indicate standard deviations (n = 3) for individual data points, when not visible, standard deviation limits fall within symbols.

in Handao502 and of 73% in Baxiludao (Figure 6). The proportion was independent of the Zn mass fraction in the dehulled grain that varied due to differences in Zn supply levels. Zn mass concentration in bran and endosperm varied considerably, but both increased linearly with increasing Zn mass concentration in the brown rice (Figure 7). However, in absolute terms, the increase in Zn mass concentration in the bran was higher than that in the endosperm in both cultivars (Figure 7). In Baxiludao, the Zn mass concentration increased from 60 to 145 mg kg^{-1} in the bran obtained after 20 s milling, and from 46 to 108 mg kg^{-1} in the bran obtained between 20 and 60 s milling, and only from 9 to 37 mg kg^{-1} in the endosperm. The larger variation in the data on bran Zn mass concentration for Handao502 is due to the fact that almost all the grains of this cultivar broke during milling, whereas all grains of Baxiludao remained intact.

DISCUSSION

In the introduction, three hypotheses were presented which we tested in two experiments. In accordance with hypothesis 1, an increased Zn uptake by the rice plants resulted in a stronger increase in the ZnMC and Zn content in the vegetative parts than in the reproductive parts (Table 1 and Figures 1–3). Although the increased Zn uptake under higher Zn-supply conditions leads to higher Zn contents and Zn mass concentrations in all organs, the additional Zn taken up mainly accumulates in stems

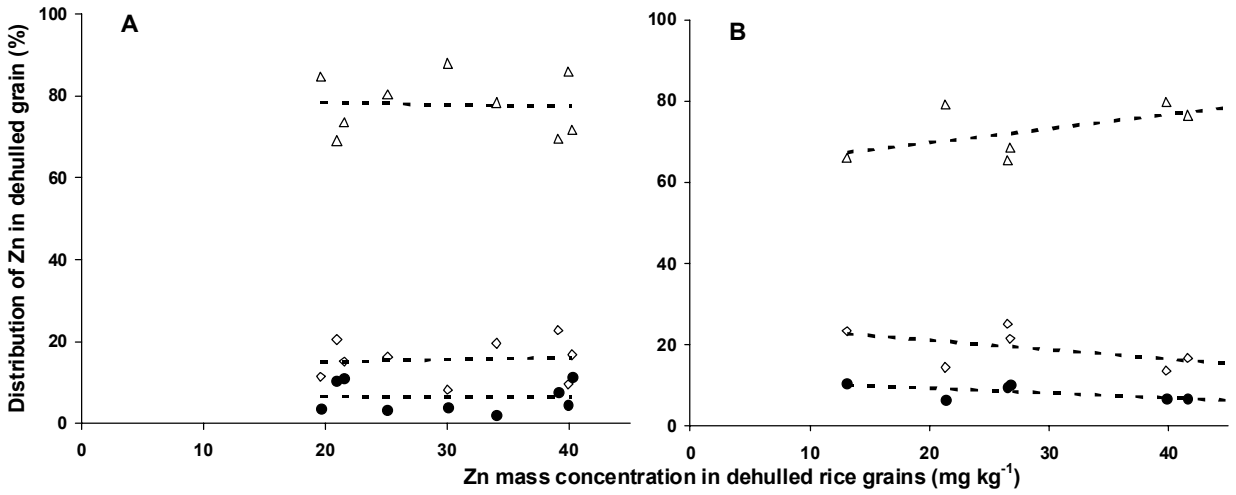

Figure 6. Distribution of Zn (% of total grain Zn content) between bran taken off after 20 s milling (closed circles), bran taken off between 20 and 60 s milling (open diamonds) and endosperm (open triangles) as a function of Zn mass concentration in the dehulled (brown) rice grain for Handao502 (A) and Baxiludao (B) from Experiment 2. Broken lines indicate linear regression lines; slopes were in no cases significantly different from zero ($P > 0.05$).

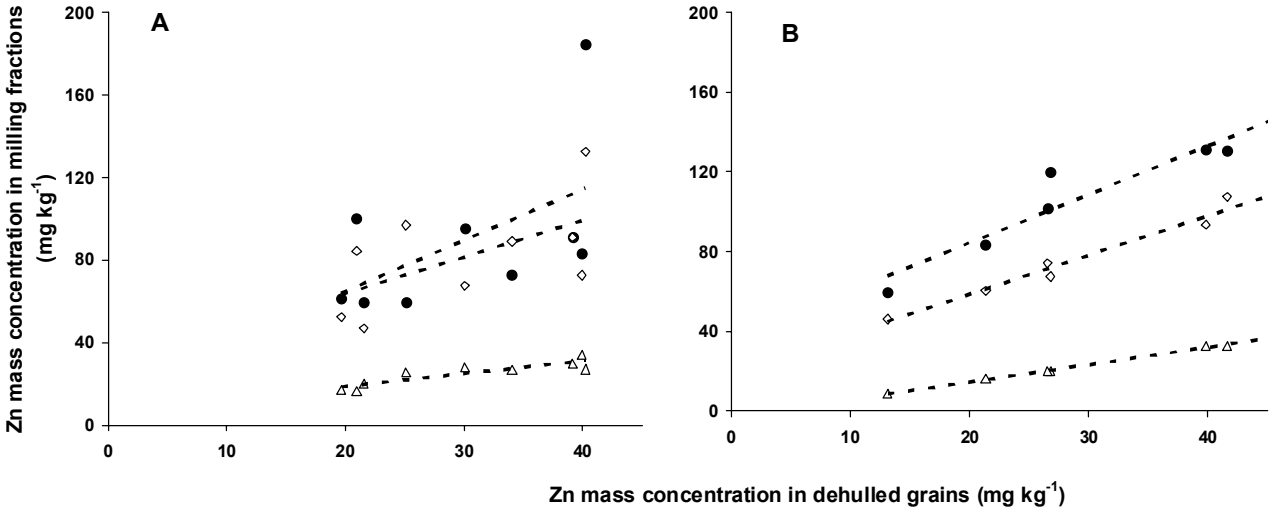

Figure 7. Zn mass concentration in bran taken off after 20 s milling (closed circles), bran taken off between 20 and 60 s milling (open diamonds) and endosperm (open triangles) as a function of Zn mass concentration in the dehulled (brown) rice grain for Handao502 (A) and Baxiludao (B) from Experiment 2. Broken lines indicate linear regression lines; slopes were significantly different between bran and endosperm ($P < 0.05$).

and sheaths. While over the investigated range of Zn-supply levels Zn mass concentration in stems increased from 20 to 400 mg kg^{-1}, the mass concentrations in the dehulled grains (brown rice) increased only from 20 to 50 mg kg^{-1}, so a factor 8 less (Figure 5). The Zn harvest index decreased considerably with increasing total plant Zn content in both experiments. This is consistent with previous reports on wheat by Herren & Feller (1994), and on rice by Fageria (2001) and Gao *et al*. (2006).

In addition, in both experiments rice plants took up more zinc when Zn supply was increased. Also Zn uptake per gram dry matter increased with increasing Zn supply (Figure 1). The increase in uptake rate continued even when total Zn uptake was indeed in excess of the amount required for optimum plant growth and started to reduce grain yield (Table 1, Figure 1). The results over the large range of Zn levels applied in Experiment 1 indicate that internal allocation to different organs seems to be regulated in different ways. With increasing Zn uptake, plants first allocate the additional Zn to vegetative organs with (relatively) low metabolic activity (stems and sheaths), while leaves and grains only show moderate increases in ZnMC. With continuing increase in uptake, the rate of increase in ZnMC of the leaves changes (the highest levels Figure 4A), which coincides with the observed negative effect on dry matter accumulation (Table 1). The increase in ZnMC in grains, though, remains moderate.

After flowering, rice plants continued to take up Zn, well beyond halfway through the grain filling period (Figure 1). This is consistent with previous reports on paddy rice (Verma & Tripathi, 1983), on cotton (Constable *et al*., 1988), and on red spring wheat (Miller *et al*., 1994). After flowering, the Zn uptake rate decreased in K150 and Baxiludao, but not very much in Handao297 or Handao502 (Figure 1), which could be due to a gradual reduction in uptake capacity of the roots in the former two cultivars. At lower Zn supply levels, the Zn taken up after flowering seems to accumulate mostly in the grain, which is accompanied by net Zn remobilization from the leaves and transport to the grain, as Zn content in leaves decreased during grain filling (Figures 2 and 3). Under field conditions this may also occur, as plant-available Zn in the soil is often not sufficient (Miller *et al*., 1994), especially not under aerobic field conditions, where plant-available Zn is lower than in flooded conditions (Gao *et al*., 2006). However, at higher Zn supply levels, grain Zn accumulation could be fully accounted for by Zn uptake during grain filling, while the observed increase in leaf Zn content during grain filling seems to imply no or a very limited role for remobilization (Figures 2 and 3). This is in line with results from a study with labelled Zn, in which we found that translocation of leaf-applied Zn during grain filling hardly contributed to grain Zn accumulation, while Zn taken up via roots during grain filling was readily allocated to the grain (Jiang *et al*., 2007; Chapter 3).

The second hypothesis was that there are physiological upper limits to grain Zn accumulation, restricting grain ZnMC after polishing to values below 50 to 60 mg kg^{-1}. In Experiment 2, such high levels were not reached in polished rice. However, in Experiment 1 grain ZnMC in brown rice reached 100 mg kg^{-1}. The grains from Experiment 1 were not polished, but assuming a similar linear trend in both endosperm and bran ZnMC as for the grains from Experiment 2, endosperm values of 50 mg kg^{-1} could have been reached. Thousand-grain weights were not recorded, but we can assume that grain sizes remained unaffected by treatments. In other words, there does not seem to be an absolute physiological upper limit, but the ZnMCs in vegetative tissues at which in the grains the level, desirable for human nutrition, can be reached, are much higher than can ever be realized under field conditions. In rice, therefore, fertility management alone will not sufficiently improve zinc supply from staple-based diets, and further breeding efforts are needed. This is comparable to what was found in wheat, barley, rye and oats (Ekiz *et al*., 1998), where fertilization increased grain Zn mass concentrations from around 8 to around 20 mg kg^{-1} but contrasts with the much larger options for improvements through fertility management that seem to exist in sorghum where fertilization raised grain Zn mass concentration to around 60 mg kg^{-1} (Traoré, 2006).

In line with hypothesis 3 the Zn mass concentration in the endosperm increased proportionally with Zn mass concentration in the outer grain tissues or bran. At low zinc uptake or zinc supply levels, Zn mass concentrations in stem, rachis and endosperm are similar (all 20 to 40 mg kg^{-1}). Only the Zn mass concentration in the bran seems slightly higher (60 mg kg^{-1}) (Figures 5 and 7). At the higher uptake or supply levels, however, there is a clear difference in Zn mass concentration between the stem and the rachis in Handao502 and Handao297 (Figures 4A and 5A), and this difference is even larger in Baxiludao (Figure 5A) and K150 (Figure 4A). There are no structural barriers known to us that could explain this difference, so there must be a physiological regulation mechanism that might operate differentially in different cultivars and hence could be addressed through breeding. In Baxiludao, the Zn mass concentrations in bran and rachis seemed comparable at the higher Zn mass concentrations in vegetative tissues, but again there was a clear difference in Zn mass concentrations between bran (120 mg kg^{-1}) and endosperm (30 mg kg^{-1}) (Figure 7B). The variability in the data for Handao502 makes it difficult to assess the magnitude of the differences in Zn mass concentrations. If we assume that the higher values in the bran are more realistic, while the lower ones are due to contamination with endosperm, as a result of the breaking of the grain during milling, the ZnMCs in rachis and bran would also be very similar. Anyway, there was also an appreciable difference in Zn mass concentration between bran and endosperm in Handao502 (Figure 7A). The

outcome of such a genotype-specific physiological regulation mechanism is a saturation-type relation between total plant Zn mass concentration and grain Zn mass concentration. Under lower Zn-supply conditions, the bran could maintain a higher Zn mass concentration than both, the stem and the rachis, indicating active accumulation in the outer grain tissues (Figures 5 and 7). However, at higher Zn supply levels, Zn mass concentrations in the rachis and the bran were lower than those in the stem (Figures 5 and 7). Our data suggest, there may well be more than one rate-regulating step in the internal plant transport towards the grain endosperm.

The consequence of this physiological regulation is that it is difficult to enhance the Zn mass concentration in the dehulled rice grain by simply increasing Zn supply. Under field conditions, where plant Zn mass concentrations are very low, there is some scope for increasing Zn uptake and thereby slightly increasing grain Zn levels (Yilmaz *et al*., 1997; Kalayci *et al*., 1999; Gao *et al*., 2006), but the attainable level will be limited to the level at which further grain Zn accumulation seems to be down-regulated. Breeding target could be to enhance this level of maximum accumulation, while further research into the exact tissues in which the regulation is strongest and into the genes involved in this regulation could support these breeding efforts.

CHAPTER 3

Uptake and distribution of root-applied or foliar-applied ^{65}Zn after flowering in aerobic rice*

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Abstract

We investigated the uptake and distribution of Zn either applied to the roots or to the leaves in rice during grain development. Plants of two aerobic rice cultivars were grown in a nutrient solution with either sufficient Zn or surplus Zn. Root treatment with 1 week's supply of both ^{65}Zn and unlabelled Zn to roots started at flowering or 15 days after flowering. Foliar treatment with ^{65}Zn applied to the flag leaf or to senescent leaves was carried out at flowering. When ^{65}Zn was applied to roots, plants continued to take up Zn after flowering, even beyond 15 days after flowering, irrespective of cultivar and Zn nutritional status of the plants. During the one week of supply of both ^{65}Zn and unlabelled Zn, which either started at flowering or 15 days after flowering, the absorbed ^{65}Zn was mainly distributed to roots, stem and grains. Little ^{65}Zn was allocated to the leaves. Following a week of ^{65}Zn supply directly after flowering, under sufficient Zn or surplus Zn, the proportions of total ^{65}Zn uptake allocated to the grains continued to change during grain filling (9–33%). This Zn mainly came from the roots but under sufficient Zn supply also from the stem. With ^{65}Zn applied to leaves (either the flag leaf or the lowest senescent leaf), both cultivars showed similar Zn distribution within the plants. About 45–50% of the ^{65}Zn absorbed was transported out of the ^{65}Zn treated leaf. From that zinc, over 90% was translocated to other vegetative organs; little was partitioned to the panicle parts, and even less to the grains. These results suggest that in rice plants grown under sufficient or surplus Zn supply, most of the Zn accumulated in the grains originates from uptake by roots after flowering, not from Zn remobilization from leaves.

Keywords: Grain quality, nutrient distribution, nutrient uptake, *Oryza sativa* L., zinc.

* Published in Annals of Applied Biology 150 (2007), 383-391.

INTRODUCTION

Zinc (Zn) is an essential micronutrient for plants (Barak & Helmke, 1993) and humans (Buyckx, 1993). Important basic plant processes affected by zinc include photosystem II activity, carboxylation, and energy dissipation. Zn deficiency may even cause photodamage (Monnet *et al*., 2005). Moreover, zinc is an essential component of thousands of plant proteins (Broadley *et al*., 2007). Zn deficiency in soils is common in many parts of the world, including the rice growing areas of India (Karak *et al*., 2006) and China (Gao *et al*., 2006). Zn deficiency in the soil can be remedied by Zn fertilizer supply either applied through the roots or through the leaves. Crop responses to Zn fertilizer can be substantial (Broadley *et al*., 2007).

In contrast, in many other terrestrial environments Zn has accumulated through anthropogenic dispersion (Collins, 1981). Some of these environments include agro-ecosystems and home gardens and allotments. Contamination of arable land by excessive Zn can be a major stress factor for crop plants, as Zn is highly phytotoxic (Broadley *et al*., 2007). On heavily contaminated soils, yield reductions are common, although the risk to human health is limited (Alexander *et al*., 2006). Some plants are known to be hyperaccumulators of zinc and therefore may have potential to clean soils polluted with Zn (Li *et al*., 2006), although their low productivity could be a major limiting factor for success (Ebbs & Kochian, 1997). Also polluted sediments can be 'phytoremediated' by growing hyperaccumulators of zinc (e.g., Arreghini *et al*., 2006).

Zinc deficiency in humans is widespread in many regions of the world (Graham & Welch, 1996; Hambidge, 2000), especially in some developing countries where a large proportion of dietary Zn intake is derived from consumption of cereals. Zn deficiency in humans causes stunted growth, affects the immune system, causes fatigue, and impedes the mental and psychomotor development (Frossard *et al*., 2000; West & Verhoef, 2002; Traoré, 2006). As Zn density (Zn mass fraction) in grains for human consumption is generally low, its increase is being considered as a sustainable, long-term solution to human Zn deficiency (Rengel *et al*., 1999).

Zn accumulated in grains mainly originates from Zn uptake by roots after flowering, as shown in paddy rice (Verma & Tripathi, 1983), cotton (Constable *et al*., 1988), and sorghum (Traoré, 2006). Therefore, enhanced Zn supply to the root environment during grain filling (e.g., through late fertilization) may be needed to realize the required high grain Zn mass fraction.

Grotz & Guerinot (2006) recently reviewed the molecular aspects of Zn homeostasis in plants. Considerable efforts have also been made to elucidate the crop physiology of uptake, distribution and remobilization of zinc during grain filling. During grain filling, roots and stems are the largest Zn sources for allocation of Zn to the grains. However, grains can also accumulate Zn remobilized from leaves, as has

been shown for soybean (Khan & Weaver, 1989) and wheat (Pearson *et al*., 1995; Pearson & Rengel, 1995b), but not yet for rice.

Most research on Zn allocation in cereals has been carried out under limited or even marginal zinc supply during grain filling. The relative roles of uptake, allocation, and remobilization of Zn during grain filling under sufficient or surplus Zn supply are not known.

The objectives of the research described in this chapter are the following:

- To assess whether and to what extent rice plants can continue to take up Zn after flowering;
- To quantify the distribution of ^{65}Zn absorbed by the roots after flowering among the different plant organs;
- To identify the sources of Zn allocated to the grains; and
- To assess and quantify the potential of Zn applied to leaves after flowering to enhance the grain Zn mass fraction under conditions of sufficient and surplus plant Zn supply.

MATERIAL AND METHODS

Plant culture

Pre-culture phase

Seeds of rice (cv. Handao502 and cv. Baxiludao) were surface-sterilized by washing in 70% ethanol for 1 min and soaking in 1% sodium hypochlorite for 5 min. Seeds were then pre-germinated in double de-ionized water for 24 h. After pre-germination, seeds were planted in quartz sand washed with 5% HCl. For two weeks, only double de-ionized water was supplied.

Culture phase

After 14 days, the seedlings were transplanted into foam disks fitted in the lids of 20-L containers. Fifty-six seedlings (including spare ones) of each cultivar were planted in each of two containers filled with half strength Hoagland solution (pH 5.5) without zinc. Two plant Zn mass fraction levels (sufficient: 20 mg kg^{-1}; surplus: 200 mg kg^{-1}) were attained by applying Zn to the culture solution, following a method similar to the one described by Hoffland *et al*. (2000). Zn, as $ZnSO_4.7H_2O$, was added to the nutrient solution every three days. The amount to be added per plant (Zn_t, in µg) was calculated according to the formula:

$$Zn_t = (W_t - W_{t-1}) \times [Zn]_{desired}, \quad \text{with} \quad W_t = W_{t-1} \times e^{r\Delta t}$$

in which, r is the relative plant growth rate (in d^{-1}), derived from a previous growth rate experiment; W_{t-1} is the dry weight per plant at time t–1 (in g); W_t is the dry weight per plant at time t (in g); Δt is the time interval between two applications of Zn, i.e. time t minus t–1 (in d). Note that in our experiments, Δt was three days; $[Zn]_{desired}$ is the desired plant Zn mass fraction (in mg kg^{-1}), i.e. 20 and 200 mg kg^{-1}, respectively in these experiments. Plants were grown in a glasshouse, set to maintain a day/night temperature of 28 °C/21 °C, light intensity was about 85% of natural light intensity and 1000 µmol m^{-2} s^{-1} light was supplemented when it was cloudy.

Treatments with labelled Zn

We carried out two different experiments with labelled Zn (^{65}Zn). In the first experiment, ^{65}Zn was supplied to the roots, whereas in the second experiment ^{65}Zn was applied to leaves.

Experiment with ^{65}Zn supplied to roots

Before application of ^{65}Zn, plants were transferred to 1-L pots with a nutrient solution. Unlabelled Zn supply continued according to the calculation method explained above (culture phase) in order to maintain sufficient or surplus Zn mass fraction levels in the plants. All lower senescent leaves were removed. Either at flowering or 15 days after flowering, 50 µl $^{65}ZnSO_4$ solution containing 148 kBq ^{65}Zn was added to the nutrient solution in each pot. Plants were exposed to these nutrient solutions, containing both labelled and unlabelled Zn, for one week. At the end of that week, three plants per treatment combination and per cultivar were harvested for ^{65}Zn uptake analysis, and the three remaining plants were transferred to 1-L pots with a nutrient solution without $^{65}ZnSO_4$ (but with their respective original levels of sufficient or surplus unlabelled Zn) for harvest at maturity. At the end of the ^{65}Zn treatment, roots of all plants were washed for 10 min using 250 ml of 1mM $CaSO_4$, followed by 250 ml of 1mM Na EDTA (ethylene di-amine tetra-acetic acid sodium salt) to remove extracellular Zn (Von Wiren *et al*., 1996).

Experiment with ^{65}Zn applied to leaves

The tip section (5 cm length) of the test leaf (flag leaf or the lowest senescent leaf) of the intact plants was immersed in 5 ml $^{65}ZnSO_4$ solution, containing 450 kBq ^{65}Zn and 0.01% L-77, a leaf surface wetting agent, in an Eppendorf tube for 10 seconds. Foliar application took place 6 h before the beginning of the night period, and the application procedure was repeated the next day. Plants were harvested at maturity.

Plant harvest and Zn analysis

Plants not treated with ^{65}Zn were harvested either at flowering or 15 days after flowering (DAF), and partitioned into different components. When harvested at flowering, plants were partitioned into roots, stems, leaves (leaf blades), sheaths, panicles and new tillers*. At 15 DAF, the harvested plants were partitioned into roots, stems, leaves (leaf blades), sheaths, rachis, glumes, grain, and new tillers.

Plants that received ^{65}Zn through the roots, either during the week after flowering or during the third week after flowering, were harvested at the end of the one-week treatment or at maturity. The harvested plants were partitioned into roots, stems, green leaves, senescent leaves (i.e. senescent leaf blades), sheaths, senescent sheaths, rachis (rachilla included), glumes, (dehulled) grains, and new tillers.

Plants that had been provided with ^{65}Zn through the leaf tips were harvested at maturity and were partitioned into 12 components: the leaf blade with ^{65}Zn applied, the sheath of the leaf with ^{65}Zn applied, other green leaves, other green sheaths, senescent leaves, senescent sheaths, rachis (rachilla included), grains, glume, stem, roots, and new tillers. The ^{65}Zn-treated leaves were cut and washed for about 10 min in 10 mM $ZnSO_4$ solution to remove ^{65}Zn adsorbed on the leaf surface within the leaf apoplasmic spaces (Erenoglu *et al*., 2002).

All plant material was dried at 75 °C for 48 hours and ground. The plant material harvested before labelled Zn was applied, was powdered and 0.5 g of the powder was digested in a bi-acid mixture (HNO_3:$HClO_4$ = 4:1). Zn was determined by atomic absorption spectroscopy (AAS SPECTRAA-55; Varian Australia, Mulgrave, Australia) at wavelength 213.9 nm.

Of the plant material harvested after exposure to ^{65}Zn, 20 mg powdered material was completely digested in HNO_3. The activity of ^{65}Zn was determined by a Liquid scintillation counter (Beckman LS6000IC, Beckman Coulter, Fullerton, CA, USA).

Calculation of total Zn uptake after ^{65}Zn application to the roots

Atomic mass of radioactive Zn (^{65}Zn) is equal to the average of the five naturally occurring Zn isotopes. It has been shown that in transport of Zn in rice, the lighter isotopes (^{64}Zn) have a slight preference, but given the small difference, this will not have played a significant role in our experiment (Weiss *et al*., 2005). In the experiment with supply of radioactive Zn to the roots, total Zn uptake by plants included not only ^{65}Zn, but also unlabelled Zn. ^{65}Zn content could be detected and calculated. Uptake of unlabelled Zn was calculated on the basis of the ratio of ^{65}Zn and unlabelled Zn applied to the root at the onset of the treatment with radioactive Zn.

* The term ‘new tillers’ is used for sprouts without panicles at the end of the growing period.

Table 1. Effect of Zn supply levels on Zn mass fraction before ^{65}Zn treatment in plant organs of rice harvested at flowering or 15 days after flowering.

			Zn mass fraction in individual plant organ (mg kg^{-1})							
Stage	Zn status	Cultivar	Root	Stem	Leaf	Sheath	Panicle			New tillers
Flowering	Sufficient	Handao502	46	17	28	14	17			26
		Baxiludao	75	16	27	18	31			25
	Surplus	Handao502	232	373	67	168	59			169
		Baxiludao	377	355	83	246	72			201
	Significance (*P*)									
	Zn		≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001			≤ 0.001
	Cultivar		≤ 0.001	n.s.	≤ 0.05	≤ 0.01	≤ 0.01			≤ 0.05
	Zn × Cultivar		≤ 0.001	n.s.	≤ 0.01	≤ 0.05	n.s.			≤ 0.05
			Root	Stem	Leaf	Sheath	Rachis	Glumes	Grain	New tillers
15 days after flowering	Sufficient	Handao502	82	13	24	13	23	33	22	23
		Baxiludao	75	18	29	19	19	17	26	30
	Surplus	Handao502	324	684	112	262	188	67	35	227
		Baxiludao	653	455	70	215	125	78	41	204
	Significance (*P*)									
	Zn		≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001
	Cultivar		≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	n.s.	≤ 0.01	≤ 0.01
	Zn × Cultivar		≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	n.s.	≤ 0.001

n.s., not significant; Zn, zinc.

Statistical methods

Experiments were conducted in a completely randomized design. Data on Zn mass fraction before application of ^{65}Zn were subjected to two-way (Zn levels and cultivars) analysis of variance (ANOVA). All statistical analyses were performed with SAS (SAS Institute Inc., 1989). Results for ANOVA were considered significant at $P \leq 0.05$, $P \leq 0.01$ or $P \leq 0.001$.

RESULTS

Effect of Zn supply on Zn mass fraction in different parts of aerobic rice

Surplus Zn supply resulted in higher Zn mass fractions than sufficient Zn supply in all plant organs of both cultivars, when plants were harvested at flowering or 15 DAF (Table 1). Zn mass fractions were 6–10 times higher in the new tillers and 30–50 times in the stem (Table 1). The Zn mass fraction in the parts of the panicle, other than the grains, was also considerably higher: approximately 10-fold in the rachis and about 2–5-fold in the glumes. Surplus Zn supply, however, resulted in only a 1.6 times higher Zn mass fraction in the grains. Differences in Zn mass fraction between the two cultivars were significant in most plant organs, except for the stem at flowering and the glumes at 15 DAF (Table 1).

^{65}Zn uptake after one week of supply of both ^{65}Zn and unlabelled Zn to the roots

Plants continued to take up Zn (including ^{65}Zn and non-^{65}Zn) after flowering and even beyond 15 DAF (Figure 1). Continued Zn uptake was even observed under surplus Zn supply, albeit usually at a lower rate than under sufficient Zn supply. Plants took up more Zn under sufficient Zn supply than under surplus Zn supply ($P < 0.05$), except plants of cv. Baxiludao in the period 15–22 DAF that took up very little Zn at sufficient Zn supply compared to plants of cv. Handao502 (Figure 1B). A significant Zn level × cultivar interaction ($P \leq 0.01$) was observed for both periods of ^{65}Zn application, i.e. that started at flowering or at 15 DAF. Data suggest that Baxiludao accumulated the majority of the Zn needed for grain growth earlier during grain filling than Handao502, at least when there was no surplus supply.

Relative distribution of ^{65}Zn after ^{65}Zn supply to the roots at flowering

Following 1 week of supply of both ^{65}Zn and unlabelled Zn, initiated at flowering, the ^{65}Zn taken up was mainly allocated to roots (34–48%), followed by stem (24–31%), and grains (7%–23%); little ^{65}Zn absorbed was allocated to leaves (less than 1% to green or senescent leaves) (Table 2). This pattern was observed in both cultivars. When plants were grown until maturity, following a week of ^{65}Zn supply directly after

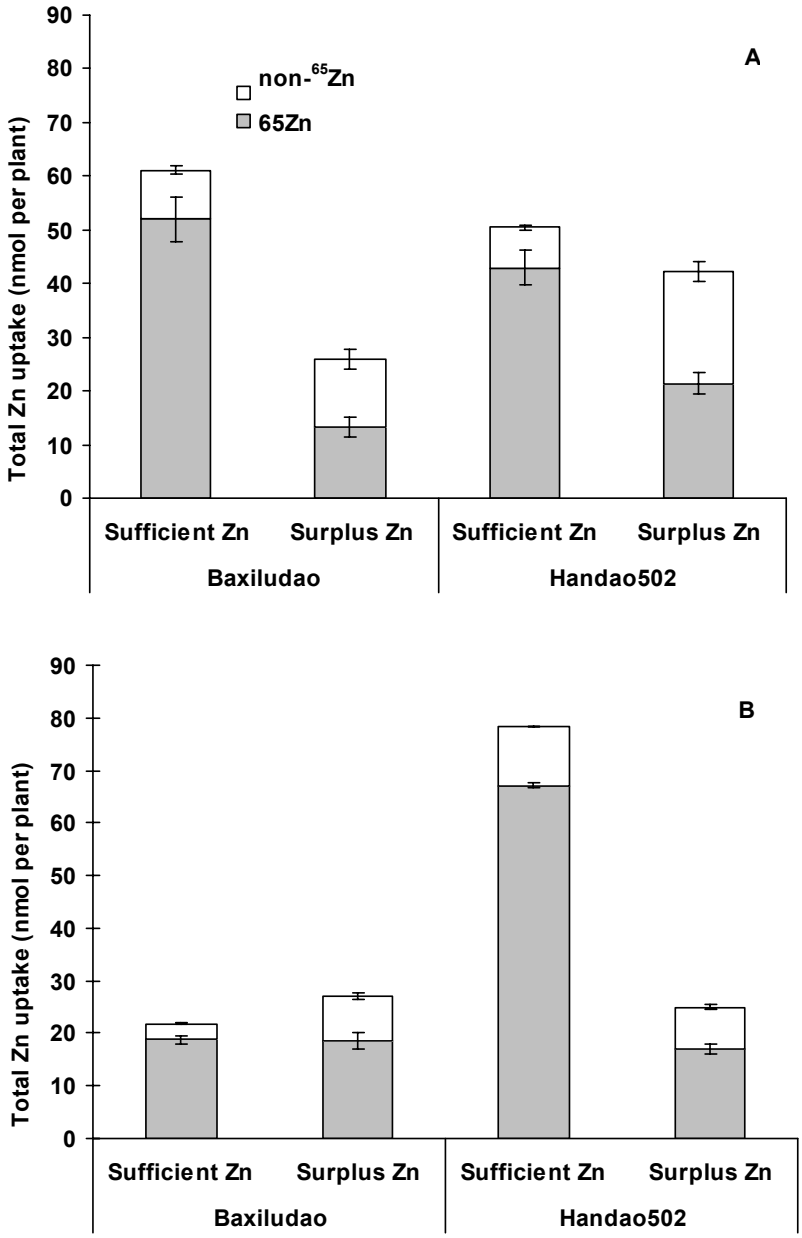

Figure 1. Total Zn (^{65}Zn and non-^{65}Zn) uptake (mean ± SE, n = 3) by the entire plant following 1 week of supply of both ^{65}Zn and non-^{65}Zn to the nutrient solution at flowering (A) or 15 days after flowering (B). Plants of the two cultivars were grown under sufficient or surplus Zn supply.

flowering, large proportions of ^{65}Zn (change in proportion of the total ^{65}Zn uptake present in the grains: 9–33%) continued to be allocated to the grain, mainly from the roots, but in plants grown under sufficient Zn supply also from the stem (Table 2). In contrast, plants grown under surplus Zn supply, did not show a decline in relative distribution of ^{65}Zn in the stem during grain filling. Zn distribution to the grains was affected by plant Zn nutritional status: more ^{65}Zn was allocated to the grains in the

Table 2. Relative distribution of ^{65}Zn among individual plant organs (n = 3) at the end of 1 week of supply of ^{65}Zn initiated at flowering (i.e. at 7 DAF), under both sufficient Zn and surplus Zn conditions, and the relative distribution at maturity stage (Maturity).

			Relative distribution of labelled Zn (%)[1] or change in proportion (%-points)									
Stage	Zn status	Cultivar	Root	Stem	Leaf	Senescent leaf	Sheath	Senescent sheath	Rachis	Glume	Grain	New tillers
7 DAF	Sufficient	Handao502	41	31	0	0	7	0	2	3	13	3
		Baxiludao	35	24	0	0	4	8	1	3	23	1
	Surplus	Handao502	48	30	1	0	5	1	2	4	7	3
		Baxiludao	34	27	1	0	12	7	2	3	15	0
Maturity	Sufficient	Handao502	20	7	1	0	2	6	1	8	46	9
		Baxiludao	18	18	1	1	4	8	2	4	42	3
	Surplus	Handao502	18	41	0	0	6	5	2	2	16	10
		Baxiludao	11	36	2	0	8	5	2	3	34	0
Change in proportion of ^{65}Zn[2]	Sufficient	Handao502	–21	–24							+33	
		Baxiludao	–17	–6							+19	
	Surplus	Handao502	–30	+11							+9	
		Baxiludao	–23	+6							+19	

DAF, days after flowering; Zn, zinc.

[1] Values are expressed in percent of total ^{65}Zn uptake.

[2] Relative distribution of ^{65}Zn to organs at maturity minus the relative distribution at 7 DAF.

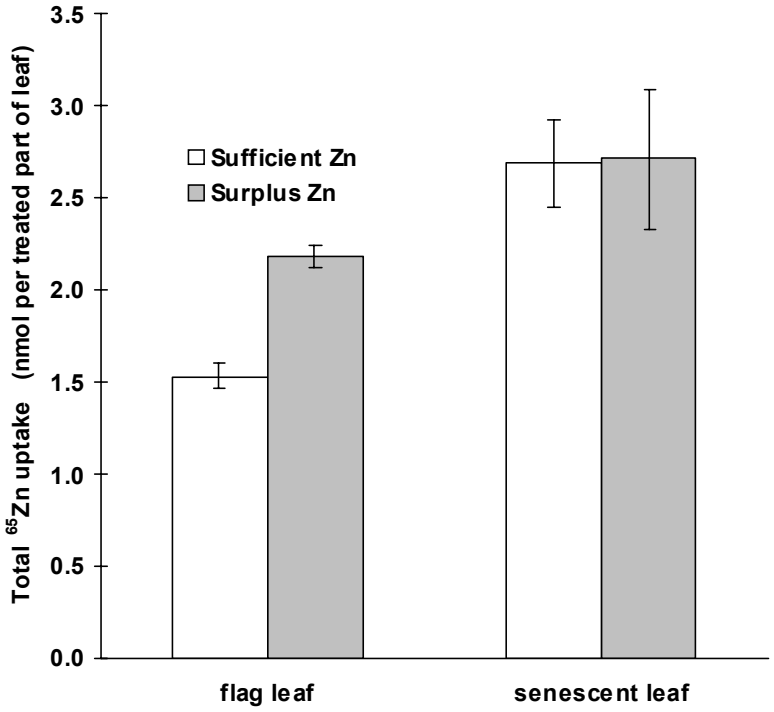

Figure 2. Total uptake (mean ± SE, n = 3) of ^{65}Zn by the leaf when ^{65}Zn was applied to the flag leaf or to the lowest senescent leaf at flowering in aerobic rice (cv. Handao502). The tip section of the treated leaf (flag or the lowest senescent leaf) was immersed in 5 ml $^{65}ZnSO_4$ solution (containing 450 kBq ^{65}Zn and 0.01% L-77) in an Eppendorf tube for 10 seconds. Before and after treatment, plants were grown under sufficient or surplus Zn supply. Plants were harvested at maturity.

plants grown under sufficient Zn supply than in the plants grown under surplus Zn supply, with a difference of about 6–8% a the end of one week of applying both ^{65}Zn and unlabelled Zn, and a difference of 8–30% at maturity. During the period between cessation of ^{65}Zn supply and maturity, total ^{65}Zn in the plant did not change (data not presented), so loss of ^{65}Zn from the plants to the solution can be neglected.

Relative distribution of ^{65}Zn after ^{65}Zn supply to the roots at 15 DAF

The distribution of ^{65}Zn uptake following 1 week of supply of both ^{65}Zn and unlabelled Zn to the roots, initiated at 15 DAF, was comparable to that following initiation of the ^{65}Zn treatment at flowering. ^{65}Zn uptake was initially mainly allocated to the roots (21–36%), stems (24–39%), and grains (11–19%), and hardly to the leaves (Table 3). However, during the period from cessation of the ^{65}Zn supply until maturity, little ^{65}Zn was translocated to the grains from other plant parts, and Zn was mainly re-allocated between vegetative organs.

Table 3. Relative distribution of ^{65}Zn among individual plant organs (n = 3) at the end of 1 week of supply of ^{65}Zn applied 15 DAF (i.e. at 22 DAF), under both sufficient Zn and surplus Zn conditions, and the relative distribution at maturity (Maturity).

			Relative distribution of labelled Zn (%)[1] or change in proportion (%-points)									
Stage	Zn status	Cultivar	Root	Stem	Leaf	Senescent leaf	Sheath	Senescent sheath	Rachis	Glume	Grain	New tillers
22 DAF	Sufficient	Handao502	31	32	0	0	14	0	2	2	19	0
		Baxiludao	30	39	0	0	7	3	1	1	19	1
	Surplus	Handao502	36	24	2	0	16	0	5	6	11	0
		Baxiludao	21	27	4	0	21	3	3	5	15	2
Maturity	Sufficient	Handao502	14	34	1	1	6	3	2	6	22	11
		Baxiludao	15	31	1	0	8	5	2	3	23	11
	Surplus	Handao502	12	45	1	0	13	4	4	6	15	0
		Baxiludao	7	40	5	2	16	3	3	6	11	5
Change in proportion of ^{65}Zn[2]	Sufficient	Handao502	–17	+2							+3	
		Baxiludao	–15	–8							+4	
	Surplus	Handao502	–24	+21							+4	
		Baxiludao	–14	+13							–4	

DAF, days after flowering; Zn, zinc.

[a] Values are expressed in percent of total ^{65}Zn uptake.

[b] Relative distribution of ^{65}Zn to organs at maturity minus the relative distribution at 22 DAF.

Uptake and translocation of foliar-applied ^{65}Zn

Exogenously applied ^{65}Zn could be absorbed by the leaf, when applied to the flag leaf or to the lower senescent leaf after flowering (Figure 2). For plants grown under sufficient or surplus Zn supply, the distribution of ^{65}Zn absorbed by the leaf was similar. At maturity, about 45% of the total ^{65}Zn absorbed had moved out of the ^{65}Zn-treated flag leaf, and 50% out of the ^{65}Zn-treated senescent leaf. Most of the ^{65}Zn transported out of the treated leaf, however, was translocated to other vegetative organs, such as roots, other green leaves and sheaths. Little was allocated to the panicle parts, and even less to the grains (Figure 3).

DISCUSSION

Zinc deficiency in rice can be corrected by the application of inorganic salt ($ZnSO_4.7H_2O$), but application in chelated forms, such as Zn-EDTA, is more efficient (Karak *et al*., 2006). In hydroponically grown plants, Zn deficiencies are often difficult to induce when non-buffered solutions are used (Degrijse *et al*., 2006). When present, aqueous complexes increase Zn uptake.

In our research using $ZnSO_4.7H_2O$ as the Zn source in hydroponics, surplus Zn supply resulted in higher Zn mass fractions in the plant, especially in roots and stem. In the panicle parts, the Zn mass fraction in the rachis was much higher than in the glumes and grains (Table 1), which supports findings in wheat (Miller *et al*., 1994; Pearson *et al.*, 1995). This seems to indicate that the major barrier to more transport into the grains is between the rachis and the grain, as has also been observed in e.g. wheat (Zee & O'Brien, 1970, 1971). In wheat, the xylem discontinuity (O'Brien *et al*., 1985; Miller *et al*., 1994; Pearson *et al*., 1995, 1998) could play a role, but there is no evidence of such a discontinuity in rice (Zee, 1971); the exact place and reason for the sharp decline in mass fractions between rachis and grain remains to be elucidated, but the molecular approaches as described by Grotz & Guerinot (2006) may be instrumental in obtaining further insight.

After flowering, aerobic rice cultivars indeed continued to take up Zn, even beyond 15 DAF, and even under surplus Zn supply (Figure 1). This is consistent with reports for paddy rice (Verma & Tripathi, 1983), cotton (Constable *et al*., 1988), and red spring wheat (Miller *et al*., 1994) grown under field conditions. Of total plant Zn, 36% (rice), 50% (cotton), or 10% (wheat) was taken up between flowering and maturity. Plants grown under sufficient Zn supply took up more ^{65}Zn than plants grown under surplus Zn, when ^{65}Zn was supplied at flowering (Figure 1). When ^{65}Zn was applied at 15 DAF, Baxiludao took up much less ^{65}Zn than Handao502 under sufficient Zn supply. This may be associated with the shorter grain filling period in Baxiludao (26 d) than in Handao502 (31 d), under both, sufficient Zn and surplus Zn condition.

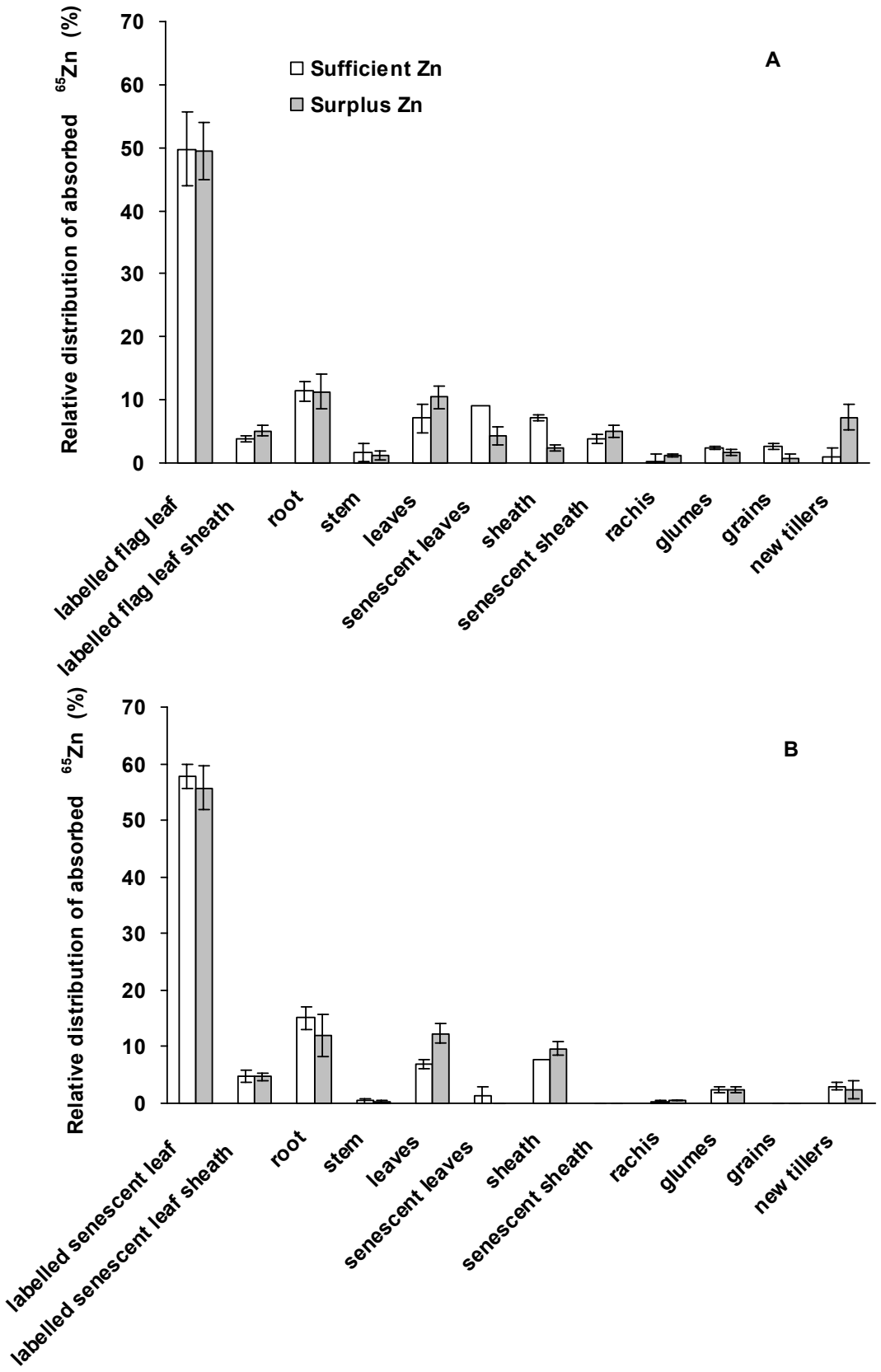

Figure 3. Relative distribution within the plant (mean ± SE, n = 3) of total foliar applied ^{65}Zn. ^{65}Zn was applied to the (A) flag or (B) to the lowest senescent leaf at flowering of rice plants (cv. Handao502) grown under sufficient or surplus Zn supply.

In aerobic rice, the ^{65}Zn taken up by roots after flowering was mainly allocated to the roots, the stem and the grains, whereas little ^{65}Zn was distributed to leaves (Tables 2 and 3). So, apparently in rice, transport of Zn taken up after flowering is directed towards the grains and the intermediate Zn storage organs (roots and stem). In wheat, however, a large proportion of root-supplied ^{65}Zn at early or late grain filling was transported to the leaves, both under low and sufficient Zn conditions (Pearson & Rengel, 1995b). Zn content in leaves continued to increase until 14 DAF before it started to decrease in both low-Zn and sufficient-Zn plants (Pearson & Rengel, 1994). So in contrast to rice, wheat leaves may still require Zn during grain filling.

In wheat, adequate transport of Zn from stem and leaves to developing grain is possible (Pearson & Rengel, 1994, 1995b). This suggests the involvement of phloem transport. Furthermore, during the period from cessation of ^{65}Zn supply at flowering until maturity, large amounts of ^{65}Zn were transported out of the root and even the stem (only under sufficient plant Zn conditions) and mainly allocated to the grains. In wheat, after cessation of Zn supply during grain filling, large amounts of Zn in the roots and stems were rapidly remobilized during the first 14 DAF (Pearson & Rengel, 1994). Under field conditions this may also occur, as plant-available Zn in the soil is often insufficient, and most of the Zn in the grain originates from other plant parts, especially from roots and stems (Miller *et al*., 1994). In wheat, foliar-applied ^{65}Zn can be translocated to leaves above the treated leaf, to leaves below the treated leaf and to root tips (Haslett *et al*., 2001). Haslett *et al.* (2001) confirmed by stem girdling that the ^{65}Zn transport was indeed via the phloem. Their final conclusion was that Zn is highly mobile in the phloem of wheat.

In our study with rice, after ^{65}Zn was applied to either the flag leaf or a senescent leaf during grain filling, about 45−50% of the ^{65}Zn absorbed by the treated leaf was re-allocated in plants, grown under both, sufficient or surplus Zn conditions (Figure 3). Most of the Zn remobilized from the leaf was translocated to roots, leaves and sheaths, whereas panicle parts received not much and especially grains received very little. This does not concur with the findings in soybean reported by Khan & Weaver (1989). They found that in plants that were grown under sufficient Zn supply, 37.5% of the dose of foliar ^{65}Zn finally accumulated in the grain. In wheat, the Zn content in leaves decreased during grain filling (Miller *et al*., 1994; Pearson & Rengel, 1994), either because of leaf senescence or of limited Zn supply during grain filling. It is unclear from our research to what extent Zn remobilized from the leaf, would contribute to accumulation of Zn in the grains when the plants would be grown under limited Zn conditions, but this was not the aim of our research.

Our findings suggest that in rice plants, grown under sufficient or surplus Zn supply, most of the Zn accumulated in the grains originates from concurrent uptake by

roots after flowering, not from remobilization from leaves. So, enhanced root uptake capacity after flowering potentially is an effective way to enhance the grain Zn mass fraction in rice. Characterization of the phloem transport of Zn in rice panicles and grains during grain development does require further work.

CHAPTER 4

Can Zn transport and partitioning in rice plants be modelled?

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Abstract
We developed a descriptive simulation model for the dynamics of Zn transport and partitioning in aerobic rice varieties. Uptake of zinc and crop dry matter accumulation are defined as forcing functions. Internal (re-)distribution is based on defined relations between total plant zinc mass concentration and individual organ zinc mass concentrations.
1. In the calibration run, we simulated Zn mass concentrations in the various organs mostly within ±15% of the observed values, with leaf and grain Zn mass concentrations simulated most accurately, with a mean normalized gross error (MNGE) of 6–8%.
2. In the validation run with independent data, the simulated Zn mass concentrations for roots were far from the observed values, with MNGEs of 28% and 22% for variety Handao502 and Baxiludao, respectively. Simulated grain Zn mass concentration was in satisfactory agreement with observed values, 11% and 8% MNGE, and a root mean square error of 2.5 and 1.2 mg kg^{-1} for Handao502 and Baxiludao, respectively. The descriptive simulation model adequately reproduced the main patterns in experimental data, but further testing under different conditions is necessary to build confidence in its general applicability.

Keywords: Crop modelling, Zn allocation, rice, grain, Zn mass concentration.

INTRODUCTION

Mechanisms of Zn uptake and partitioning in plants have been studied intensively as a basis for increasing Zn mass concentration in cereal grains to enhance human nutritional value (Chapters 2 and 3; Pearson & Rengel, 1995a, b; Pearson *et al*., 1998; Grusak *et al*., 1999; Welch & Graham, 2002; Traoré, 2006; Gao *et al*., 2006). However, some major crop-physiological issues remain unclear and critical relations are poorly understood. Final Zn content in the grain is a function of its availability in the soil, the uptake capacity of the roots, Zn demand of the growing crop and Zn partitioning within the plant. The integrated effect of these factors can be studied through soil-crop models that describe the dynamics of Zn in the soil, including its chemical transformations, the development of the uptake capacity of the root system, the dynamics of Zn demand in the (various organs of the) crop and its mobility within the crop.

Simulation models have extensively been used to study various aspects of crop physiology (Michalov, 1986; Hahn, 1987; Charles-Edwards, 1981; Goudriaan & Monteith, 1990; Denison, 1992; Ingestad & Agren 1992; Van Ittersum *et al*., 2003), including nutrient accumulation in soil-grown plants (Nye & Tinker, 1977; Van Veen & Frissel, 1981; Van Keulen & Seligman, 1987). Nitrogen-limited plant growth has been modelled for crops such as wheat (Van Keulen & Seligman, 1987; Weiss & Moreno-Sotomayer, 2006), rice (ORYZA-N: Drenth *et al*., 1994; ORYZA2000: Bouman *et al*., 2001), and maize (Tittonell *et al*., 2006) and phosphorus-limited growth for maize (Radersma *et al*., 2005), wheat and beans (Daroub *et al*., 2003). However, the dynamics of zinc in the soil-plant system strongly differ from those of nitrogen and phosphorus. For instance, crops continue to take up Zn beyond Zn toxicity levels in plant tissues and substantial uptake of Zn after flowering can take place (Chapter 2).

In Chapters 2 and 3, we describe studies on Zn (re-) allocation in rice plants under a range of experimental conditions, showing that zinc accumulation in the grain is limited, and that barriers for Zn transport might exist between stem and rachis and between bran and endosperm. Moreover, most of the Zn accumulated in the grains of rice plants grown under sufficient or surplus Zn supply originated from Zn uptake by roots after flowering, not from Zn remobilized from leaves. On the basis of these studies, quantitative relations between Zn mass concentrations in various plant organs and those in the total plant have been derived that can be used in modelling of internal plant allocation.

In order to integrate our understanding and quantify internal plant Zn-dynamics in rice, the objective of this study was to develop a descriptive simulation model for the partitioning of Zn in rice plants following uptake by the roots, calibrate the model on

the basis of a limited number of data sets and validate it on independent data sets, including different cultivars.

MATERIAL AND METHODS

Model description

The basic relations incorporated in the model (Figure 1), operating with a daily time-step, are:

(1) Daily rates of Zn uptake by the root system (RZUp) and of dry matter accumulation of individual organs (roots, stems, leaves (green and senescent), sheaths (green and senescent), rachis, glumes and grains) are given as forcing functions, derived from experimental data.

(2) A 'target' Zn mass concentration of individual plant organs (TZMCorgan) is defined as a function of the Zn mass concentration of the total plant (PZMC) (Figure 2), calculated as the ratio of integrated Zn uptake (ZUp) and integrated dry matter accumulation (WP).

(3) Zn demand of each organ (ZDorgan) is defined as the difference between the target Zn content (TZMCorgan times organ weight (Worgan)) and its actual Zn content (AZorgan), with negative values indicating 'surplus' Zn, potentially available for translocation.

(4) Allocation of Zn among live organs (ZUporgan) (senescent organs do not import Zn) is governed by the ratios of Zn demand of each of the organs to total Zn demand of the crop.

(5) When daily Zn uptake exceeds total Zn demand, excess Zn is assumed to be stored in the root system.

(6) If daily Zn uptake does not meet total Zn demand, 'translocatable' Zn (TrZorgan) from the live organs (if available), supplements Zn available for partitioning, assuming a time constant for translocation (TCTr) of five days. If demand is still not met, Zn may also be mobilized from senescent organs. Translocatable Zn from senescent organs is defined as the Zn in excess of a fixed non-remobilizable residual concentration (RESZMC), set to 15 mg kg^{-1} dry matter (based on own unpublished data).

(7) Daily net flow of Zn to each organ (NZorgan) is the difference between acquisition from uptake (ZUporgan) and loss through translocation (ZTrorgan).

(8) Zn storage in senescent organs is equal to the difference between cumulative Zn uptake (ZUp) and total Zn content in live organs (TAZ).

(9) Zn mass concentrations of individual organs are derived from cumulative net Zn flow for each organ (AZorgan) and the weight of the organ (Worgan).

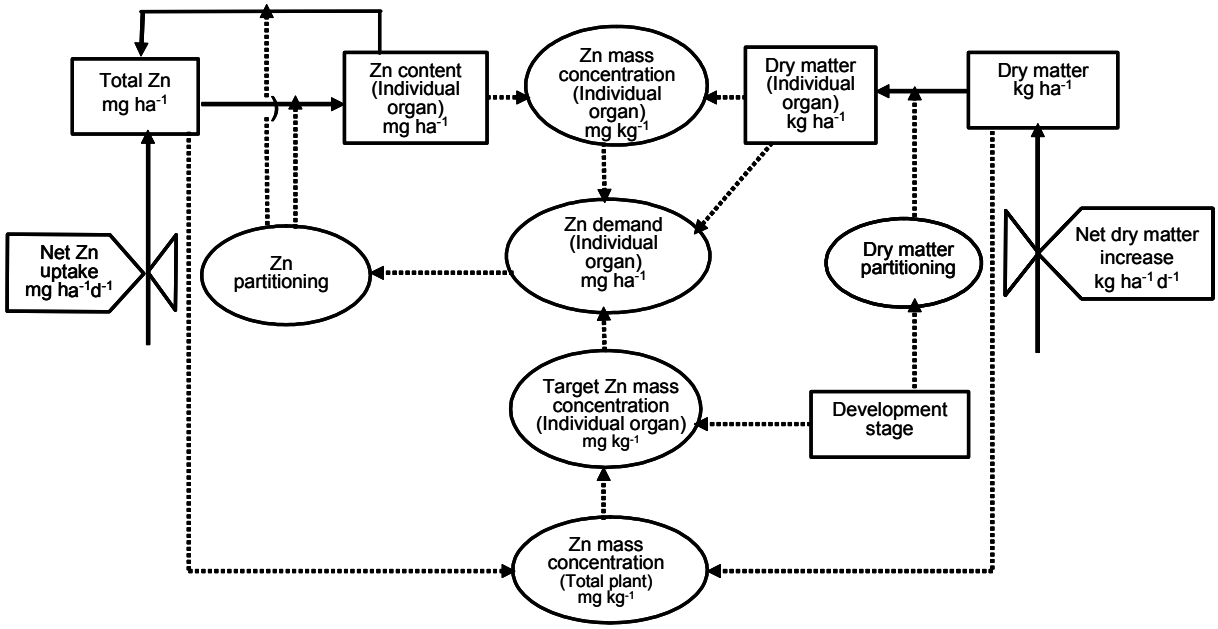

Figure 1. Relational diagram of the Zn allocation model. Rectangles, valves and circles are state, rate and intermediate variables, respectively, solid arrows indicate material flows, broken arrows indicate information flows.

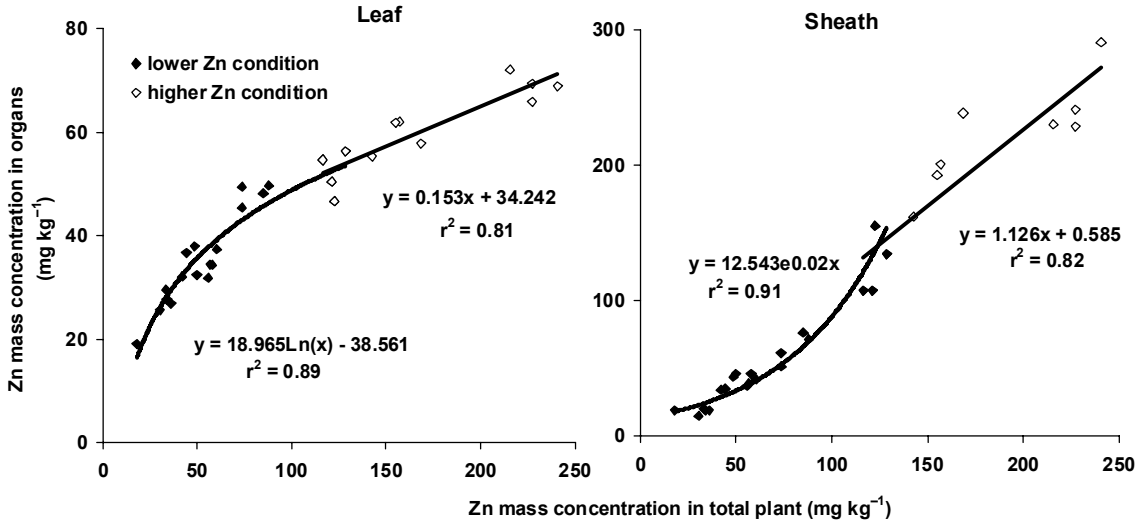

Figure 2. Correlations between Zn mass concentrations in individual plant organs and Zn mass concentration in total plant at flowering for Baxiludao, as an example of the regressions summarized in Table 1.

Experimental data

Solution culture experiments were conducted at a wide range of Zn supply levels (Chapter 2), the results of which were used to parameterize and calibrate the model, while data from a field experiment with five Zn levels were used for model validation.

Solution culture

For this experiment, described in detail in Chapter 2, rice cvs Handao502 (a newly developed cultivar) and Baxiludao, both specifically recommended for aerobic growing conditions, were selected. The application of Zn was determined by seven target plant Zn mass concentrations (10, 15, 25, 50, 100, 150, 200 mg kg^{-1} dry matter) and assumed plant growth over three day periods as described by Hoffland *et al*. (2000) for phosphorus (Chapter 2). At each harvest, five plants per container were sampled.

Field experiment

The field experiment was carried out in Mengcheng, Anhui province, China (33°55' N, 116°15' E) in 2004. The soil at the experimental site is a Shajiang black soil (vertisol, Anonymous, 1998) with pH 6.8. DTPA-extractable Zn was 0.30–0.40 mg kg^{-1} soil, i.e. well below the critical Zn concentration of 0.5 mg kg^{-1}. The experimental design was a split-plot, with three replications, main plots were five levels of Zn supply, i.e. 0, 2.5, 5.0, 10 and 20 kg Zn ha^{-1} as $ZnSO_4.7H_2O$, and subplots the same two rice accessions (Handao502 and Baxiludao) as used in the solution culture experiment. Each subplot was 15 m^2, sown to 10 rows at an inter-row distance of 0.25 m and 85 plants per meter within the row.

Composite fertilizer ($N\text{-}P_2O_5\text{-}K_2O$: 12-18-10) at the rate of 50 kg P ha^{-1} and 75 kg N ha^{-1} and Zn fertilizer (only in the +Zn plots) were incorporated before planting and 50 kg N ha^{-1} was top-dressed as ammonium nitrate at maximum tillering. Weeds were controlled by a pre-emergence herbicide and hand weeding. Plants were grown under rainfed conditions, with supplemental irrigation from a deep well, directly following sowing and at flowering, applied through flexible hoses connected to a subsurface pipe system.

In both, the field and the solution culture experiment, plants were harvested five times: at the seedling stage, at panicle initiation (with the young panicle 1 mm in length), at flowering (50% of the plants in a plot showing anthesis), 15 days after flowering and at physiological maturity. In the field experiment, two 1-m segments of row were sampled each time. The harvested plants were partitioned into individual organs.

All plant material was dried at 75 °C for 48 hours. Dried plant samples were ground in a stainless steel mill and passed through a 0.25-mm sieve before analysis. Sub-samples were digested in a bi-acid mixture (HNO_3:$HClO_4$ = 4:1). Zn was determined by atomic absorption spectroscopy (SPECTRAA−55; Varian Australia, Mulgrave, Australia).

Parameterization of the model

Zn mass concentrations of individual plant organs were closely related to the total plant Zn mass concentration (Figure 2). Regression analyses of the data from the solution culture experiment and some additional data at low ZnMC (18 mg kg^{-1} in the total plant) from D. Hardeman (unpublished MSc thesis, Wageningen University) were used to estimate model parameters describing the relations for each cultivar and organ. The results showed that both, shape and coefficients of the regression lines varied with crop development stage, and among organs. Shapes of the regression lines are illustrated in Figure 3 and a complete list of parameters is given in Table 1.

Model evaluation

Simulated and measured values of Zn mass concentrations in organs were graphically compared, and root mean square error (RMSE) and mean normalized gross error (MNGE) were calculated to evaluate model performance during calibration and validation:

$$\text{RMSE} = \left(\frac{\sum_{i=1}^{n} (Yi - Oi)^2}{n} \right)^{0.5} \qquad \text{MNGE (\%)} = \frac{100}{n} \left(\sum_{i=1}^{n} \frac{Abs(Yi - Oi)}{Oi} \right)$$

where, *Yi* and *Oi* are simulated and observed values, respectively.

RESULTS

Calibration data set

Typical examples of comparisons between simulated and measured Zn mass concentration are given for sufficient and surplus Zn supply conditions for Handao502 (Figure 4) and Baxiludao (Figure 5). For both cultivars, simulated time courses of Zn mass concentration in general showed good agreement with observed values for both Zn supply levels and for all organs, although occasionally the simulated time course deviated from the observed values. Comparison of simulated and observed values for all data sets of the calibration (Figure 6) showed that simulated Zn mass concentrations were mostly within ±15% of the observed values, with a mean normalized gross error (MNGE) of 6–18% for the various organs (Table 2). Simulated leaf and grain Zn mass concentrations were in relatively good agreement with observed data, with a mean normalized gross error (MNGE) of 6–8% (Table 2), and a root mean square error (RMSE) of 5.2–6.1 mg kg^{-1} for the leaves, and 2.4–3.2 mg kg^{-1} for the grains for both cultivars (Table 2).

Table 1. Summary of the parameter values for the model for Baxiludao and Handao502.

Organs	Stages	PZMC[1] < 100 mg kg^{-1}				PZMC ≥ 100 mg kg^{-1}			
Handao502		a	b	r^2	Curves[3]	a	b	r^2	Curves
Root	Seedling	1.230	–6.0	0.94	Linear	1.037	11.064	0.88	Linear
	S-PI[2]	1.469	0	0.91	Linear	0.949	71.186	0.95	Linear
	PI-F	1.883	–17.937	0.93	Linear	0.832	77.770	0.93	Linear
	F-F15	1.808	0	0.40	Linear	1.222	16.230	0.99	Linear
	F15-M	1.689	0	0.87	Linear	1.605	–8.009	0.95	Linear
Stem	PI-F[4]	6.0	0.041	0.88	Expon.	2.436	–69.656	0.98	Linear
	F-F15	8.0	0.033	0.84	Expon.	2.880	–23.229	0.99	Linear
	F15-M[5]	5.491	0.043	0.91	Expon.	2.305	10.0	0.97	Linear
Leaf	Seedling	20.348	–43.359	0.85	Log.	0.448	15.338	0.92	Linear
	S-PI	12.285	–13.609	0.74	Log.	0.833	–58.269	0.94	Linear
	PI-F	12.911	–15.202	0.95	Log.	0.272	18.633	0.94	Linear
	F-F15	8.880	–8.067	0.63	Log.	0.553	–26.202	0.99	Linear
	F15-M	7.276	–1.626	0.57	Log.	0.643	–32.285	0.99	Linear
Sheath	Seedling	18.987	0.023	0.89	Expon.	1.856	–38.844	0.99	Linear
	S-PI	22.567	0.019	0.88	Expon.	1.196	39.116	0.96	Linear
	PI-F[4]	13.0	0.013	0.68	Expon.	1.223	–50.109	0.95	Linear
	F-F15[6]	8.0	0.020	0.76	Expon.	1.521	–107.47	0.99	Linear
	F15-M[6]	13.324	0.017	0.86	Expon.	1.370	–72.827	0.92	Linear
Rachis	PI-F	0.694	4.607	0.83	Linear	0.220	43.006	0.79	Linear
	F-F15	0.872	0	0.91	Linear	0.690	20.352	0.98	Linear
	F15-M	1.213	–5.0	0.95	Linear	1.271	–18.801	0.92	Linear
Glume	PI-F15	17.143	–28.064	0.86		One model			Logarithmic
	F15-M	19.528	–37.66	0.96		One model			Logarithmic
Grain	F-F15	6.325	0.126	0.81		One model			Logarithmic
	F15-M	13.538	–20.886	0.97		One model			Logarithmic
Baxiludao									
Root	Seedling	1.196	5.0	0.91		One model			Linear
	S-PI	1.196	5.0	0.91		One model			Linear
	PI-F	1.920	–20.0	0.41	Linear	2.064	–107.53	0.94	Linear
	F-F15	2.339	–25.0	0.93	Linear	3.658	–221.61	0.87	Linear
	F15-M	2.339	–25.0	0.93	Linear	3.477	–165.29	0.91	Linear
Stem	PI-F	10	0.024	0.85	Expon.	1.570	–5.731	0.98	Linear
	F-F15	7.85	0.033	0.96	Expon.	2.055	–7.913	0.95	Linear
	F15-M	7.85	0.033	0.96	Expon.	1.433	103.55	0.88	Linear
Leaf	Seedling	12.407	–13.320	0.74	Log.	0.336	5.869	0.95	Linear
	S-PI	13.828	–22.403	0.53	Log.	0.301	0.515	0.94	Linear
	PI-F	18.965	–38.561	0.87	Log.	0.153	34.242	0.82	Linear
	F-F15	9.738	–6.164	0.77	Log.	0.063	32.344	0.82	Linear
	F15-M	7.876	–2.883	0.78	Log.	0.235	8.334	0.95	Linear
Sheath	Seedling	17.052	0.023	0.96	Expon.	1.807	2.944	0.99	Linear
	S-PI	21.206	0.022	0.89	Expon.	1.648	18.061	0.95	Linear
	PI-F	12.543	0.020	0.92	Expon.	1.126	0.585	0.82	Linear
	F-F15	8.377	0.019	0.67	Expon.	0.785	4.493	0.93	Linear
	F15-M	13.391	0.017	0.97	Expon.	1.481	–72.650	0.94	Linear
Rachis	PI-F	0.358	25.866	0.90	Linear	0.144	49.059	0.85	Linear
	F-F15	0.613	0.000	0.95	Linear	0.600	0.000	0.40	Linear
	F15-M	0.871	–2.791	0.91	Linear	0.274	70.436	0.85	Linear
Glume	PI-F15	27.568	–69.102	0.76		One model			Logarithmic
	F15-M	21.307	–47.842	0.95		One model			Logarithmic
Grain	F-F15	11.073	–16.012	0.79		One model			Logarithmic
	F15-M	15.029	–29.555	0.94		One model			Logarithmic

[1] PZMC: Zn mass concentration in total plant; [2] S, F, F15, M indicate seedling, flowering, 15 DAF, maturity stage, respectively. [3]Logarithmic: y=a×log(TZMC)+b; Exponential: y=a×exp(b×TZMC); Linear: y=a×b×TZMC; [4, 5, 6] the TZMC level for the switch is 50 mg kg^{-1}, 90 mg kg^{-1}, or 105 mg kg^{-1}, respectively.

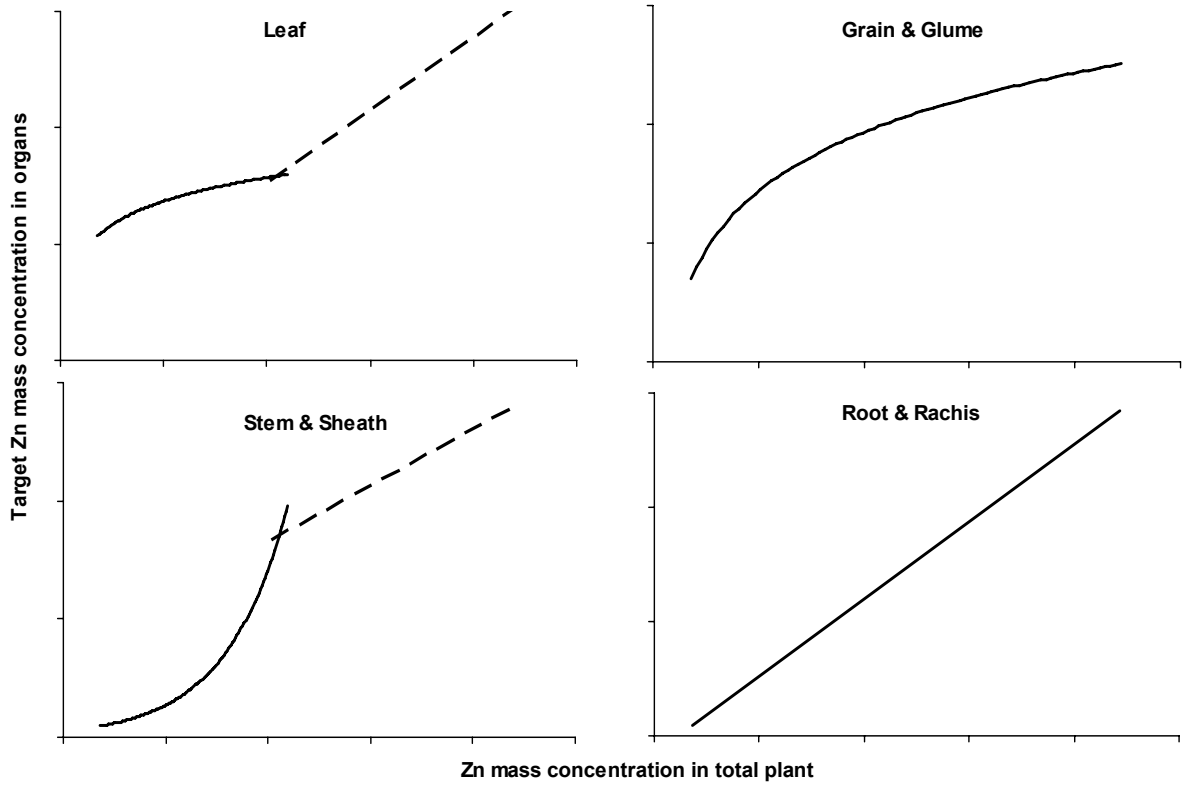

Figure 3. Zn mass concentrations in individual plant organs as a function of the Zn mass concentration in the total plant. The solid and dashed lines were used in the model to determine target Zn mass concentrations in individual organs.

Table 2 Root mean square error (RMSE, mg Zn kg^{-1} organ dry matter) and mean normalized gross error (MNGE, %) between simulated and observed values for Zn mass concentration in different plant organs for the complete datasets used for calibration and validation.

	Calibration				Validation			
	RMSE		MNGE		RMSE		MNGE	
	Handao 502	Baxilu-dao	Handao 502	Baxilu-dao	Handao 502	Baxilu-dao	Handao 502	Baxilu-dao
Root	13.8	34.0	10	12	8.6	7.3	28	22
Stem	23.9	13.9	18	11	3.1	3.7	17	18
Sheath	14.8	18.9	15	11	4.9	3.7	19	16
Leaf	6.1	5.2	8	8	3.7	4.7	11	17
Rachis	9.8	6.7	15	12	6.6	1.8	22	9
Glume	5.1	6.6	12	16	2.0	2.4	5	13
Grain	2.4	3.2	6	7	2.5	1.2	11	8

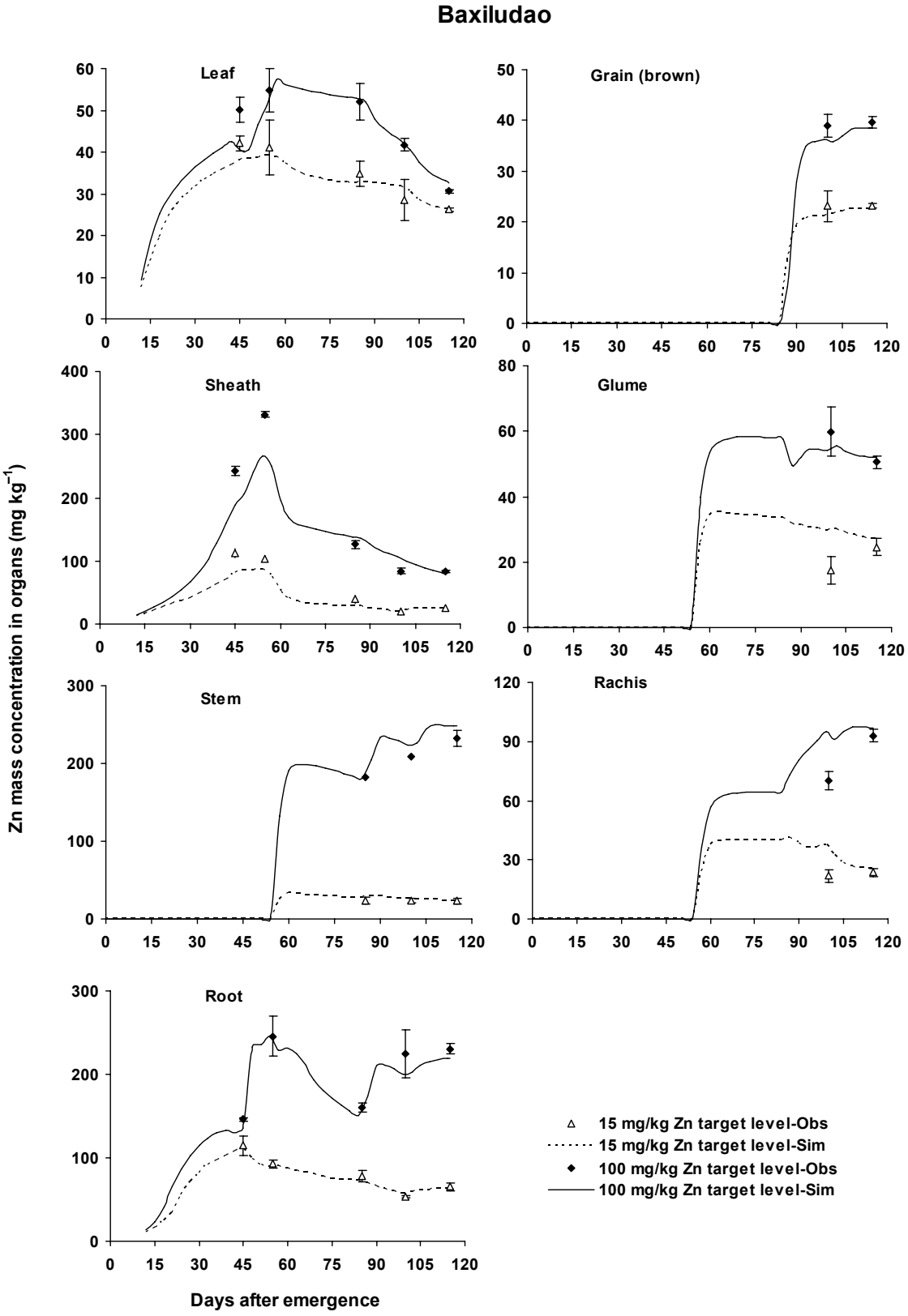

Figure 4. Simulated (lines) and observed (symbols) Zn mass concentration in individual organs for Handao502 in the nutrient solution experiment at two target Zn mass concentrations.

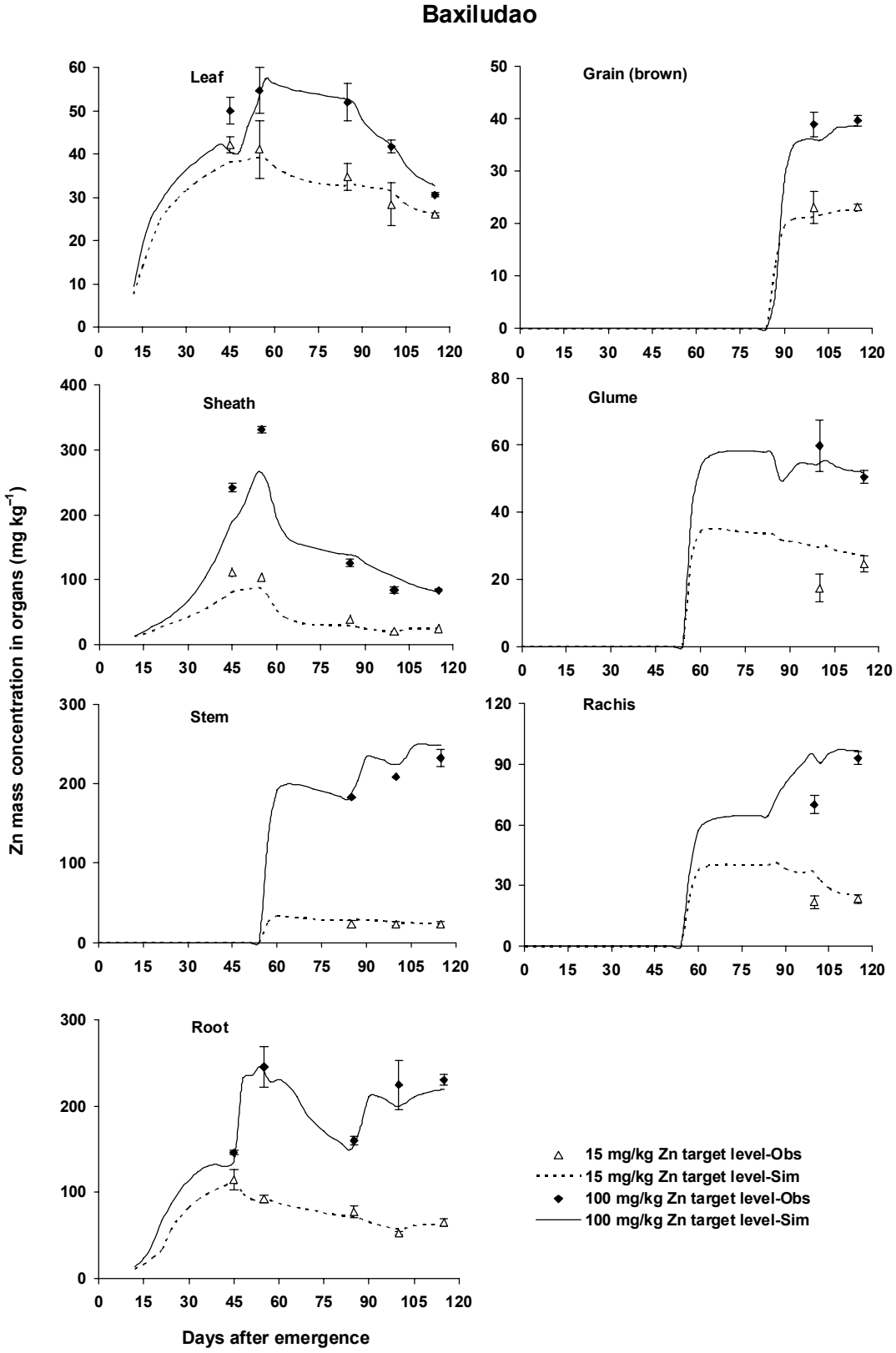

Figure 5. Simulated (lines) and observed (symbols) Zn mass concentration in individual organs for Baxiludao in the nutrient solution experiment at two target Zn mass concentrations.

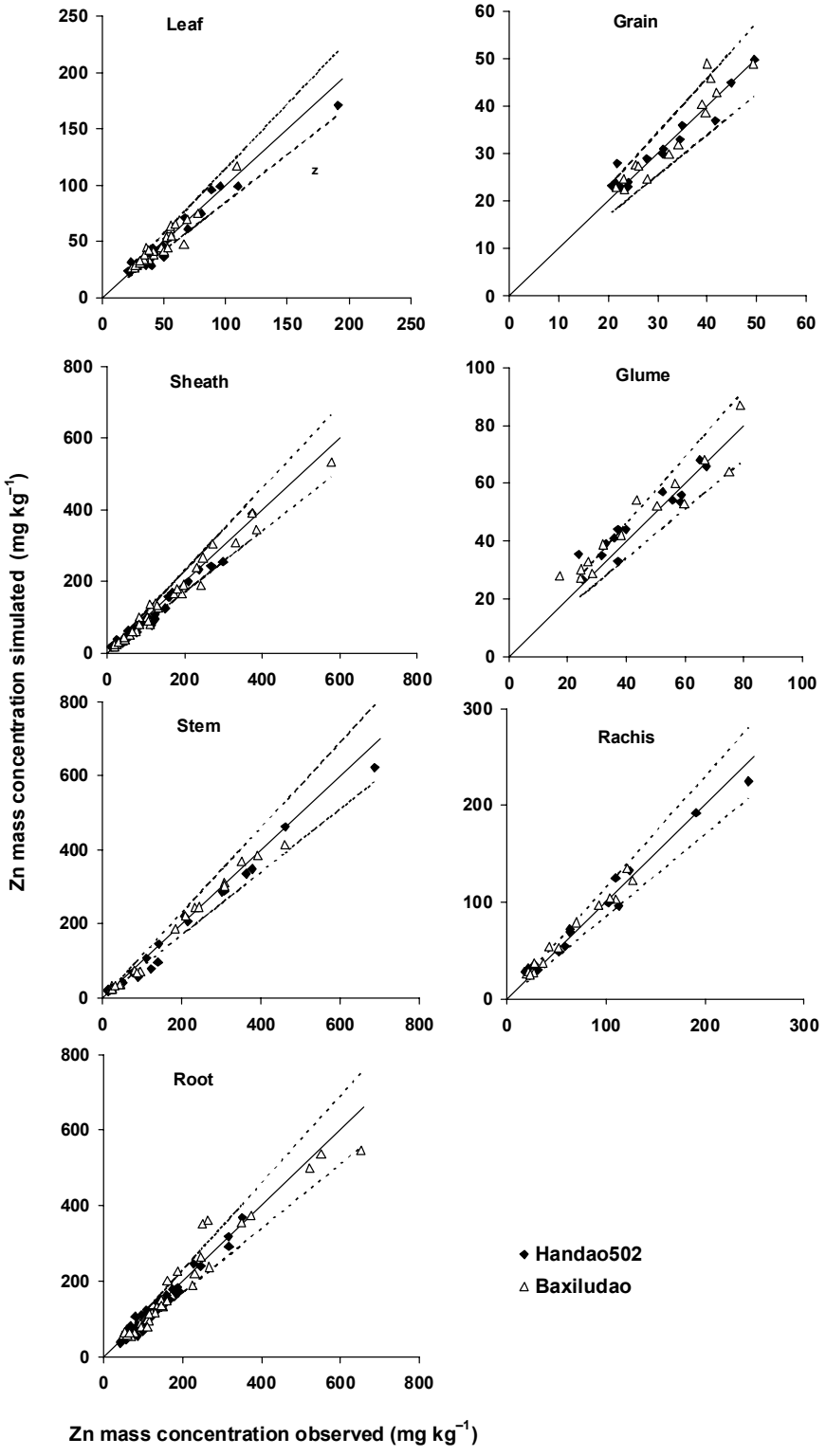

Figure 6. Comparison of simulated and observed values for Zn mass concentration in individual organs for the whole data set from the nutrient solution experiment (at various development stages and for all Zn treatments) for cultivars Handao502 and Baxulidao. Solid lines are 1:1 lines and dotted lines indicate 15% deviation from the observed values for both cultivars.

Validation data set

Simulated dynamics of Zn mass concentrations for the various organs for the 0 and 20 kg Zn ha^{-1} treatments for varieties Handao502 and Baxiludao were similar to the observed patterns (Figures 7 and 8). Simulated root Zn mass concentrations for Handao502 exceeded the measured values towards the end of the growth period, indicating inaccurate root-shoot partitioning. Simulated Zn mass concentrations in leaf blades matched measured values well, especially in Handao502, although in the early stages the model underestimated the mass concentration; simulated values for the sheaths agreed well with observations. For Baxiludao, mass concentrations in the stem were underestimated at the high Zn level, as for Handao502 in both treatments at the first sampling; in the later samplings the differences were small. Glume Zn mass concentrations tended to be underestimated for Baxiludao and those in the rachis for Handao502. Grain Zn mass concentrations were underestimated at the high Zn level in Handao502. Overall, for the root, a large part of the simulated values deviated more than ±15% from the observed values (Figure 9), with mean normalized gross error (MNGE) of 28% for Handao502 and 22% for Baxiludao (Table 2). For the leaf blades, simulated values were mostly within ±15% of the observed values (Figure 9), with a root mean square error (RMSE) of 3.7–4.7 mg $kg^{-1}$and a mean normalized gross error (MNGE) of 11–17%, for both, Handao502 and Baxiludao. For the glume, MNGE was 5% for Handao502 and 13% for Baxiludao. For the grain, MNGE was 11% and the root mean square error (RMSE) 2.5 mg kg^{-1} for Handao502 and for Baxiludao, 8% and 1.2 mg kg^{-1}, respectively.

DISCUSSION

To create a consistent framework for integration of a large set of widely varying data on Zn mass concentrations in different (aerobic) rice varieties, a descriptive dynamic model was developed for allocation and translocation of Zn among plant organs, using total plant Zn uptake and dry matter accumulation in plant organs as forcing functions. The simple model allowed reproduction of recognizable patterns of Zn mass concentrations for a wide range of absolute values, created in both culture solution and soil media. In the validation data set, simulated root Zn mass concentrations deviated substantially from the observed values (Figures 7 and 8), which could not be improved without negatively affecting the agreement for other organs. This might be due to the different culture conditions. Under solution culture conditions, some of what is observed as Zn in roots could have been Zn on roots still, which leads to overestimation of the Zn allocated to roots under equilibrium conditions and thus would explain overestimation of the modelled Zn in roots under field conditions. In addition, under field conditions there is the problem of correctly assessing total root

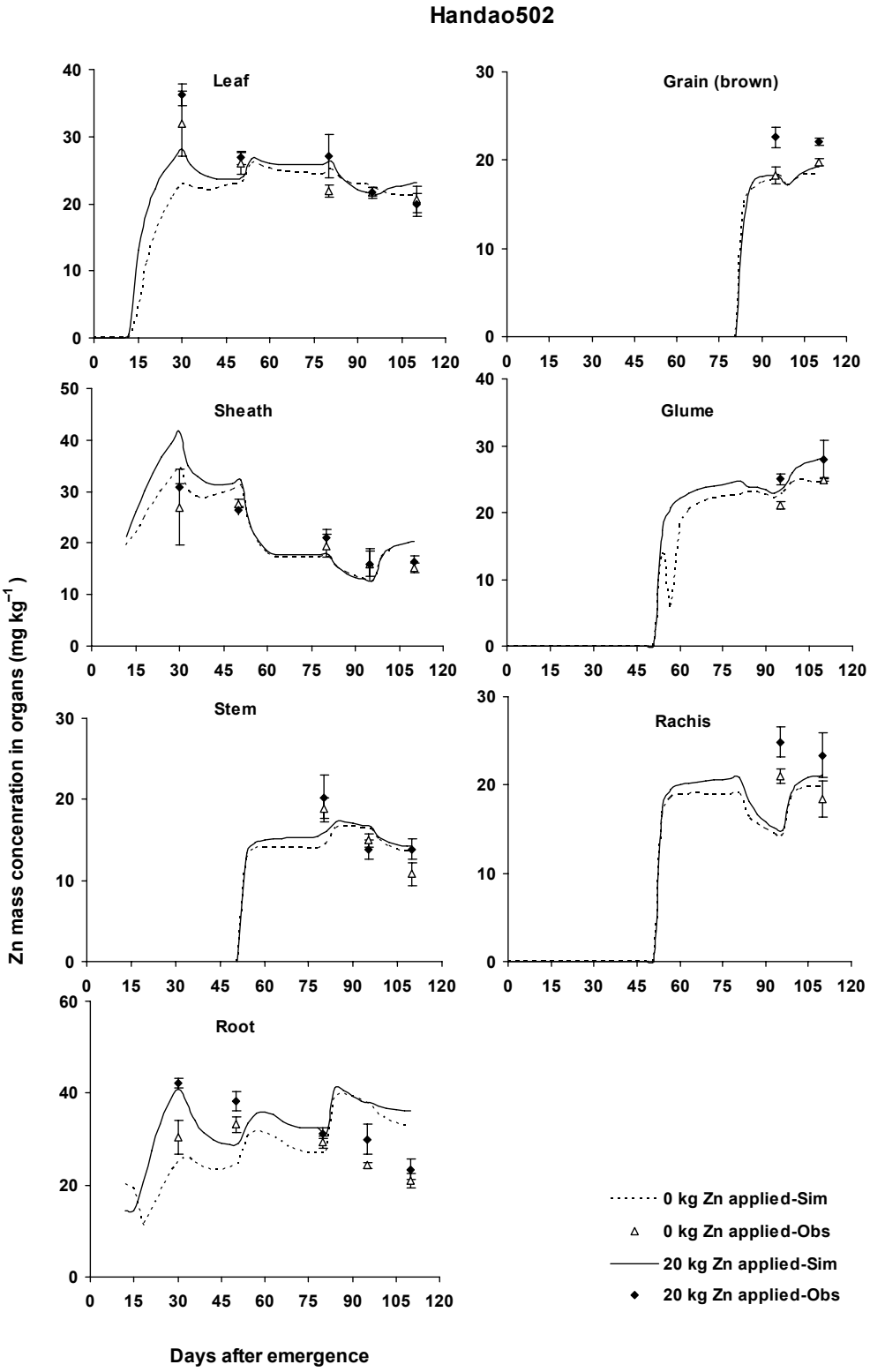

Figure 7. Simulated (lines) and observed (symbols) Zn mass concentrations in individual organs for Handao502 in the field experiment at two Zn supply levels.

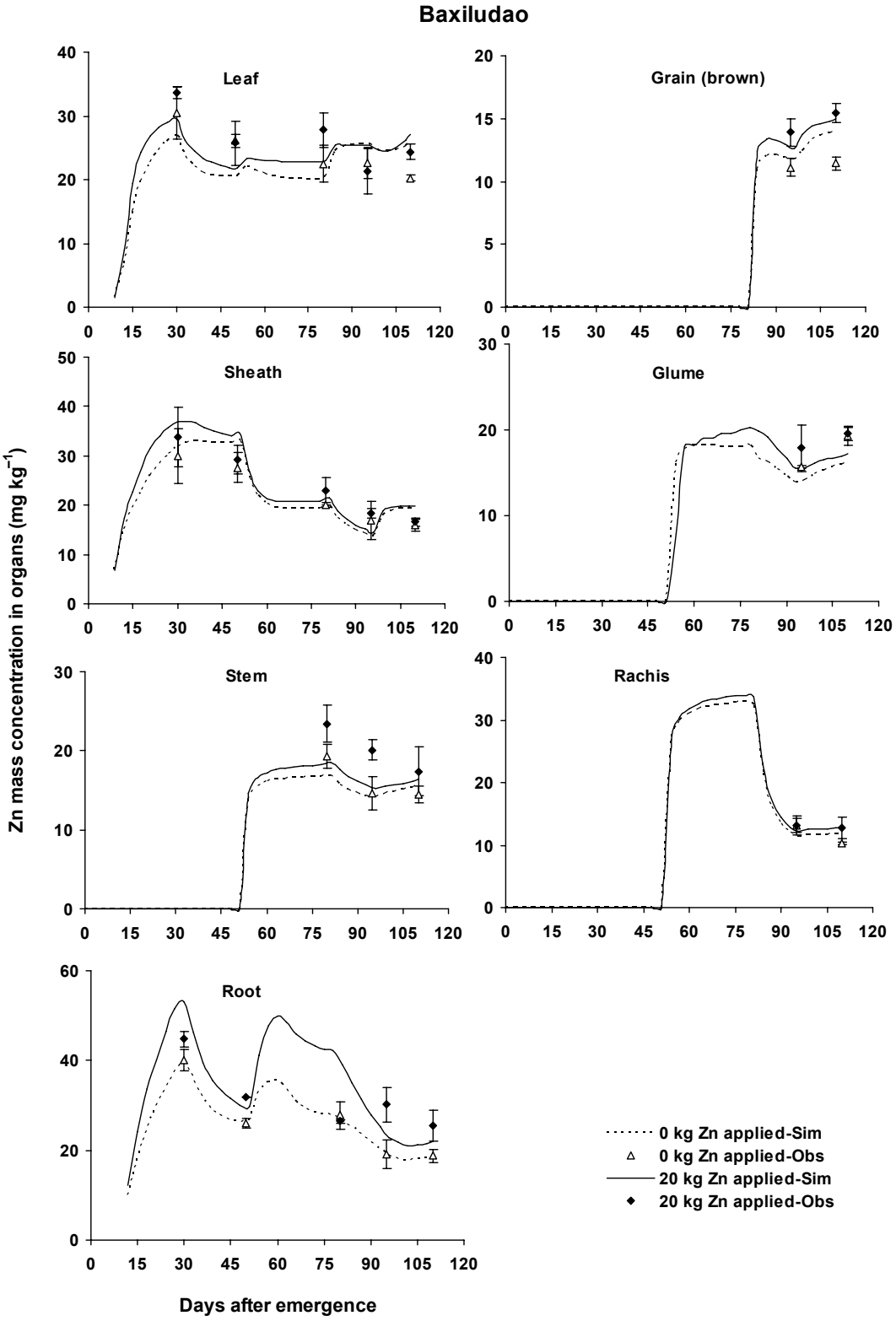

Figure 8. Simulated (lines) and observed (symbols) Zn mass concentrations in individual organs for Baxiludao in the field experiment at two Zn supply levels.

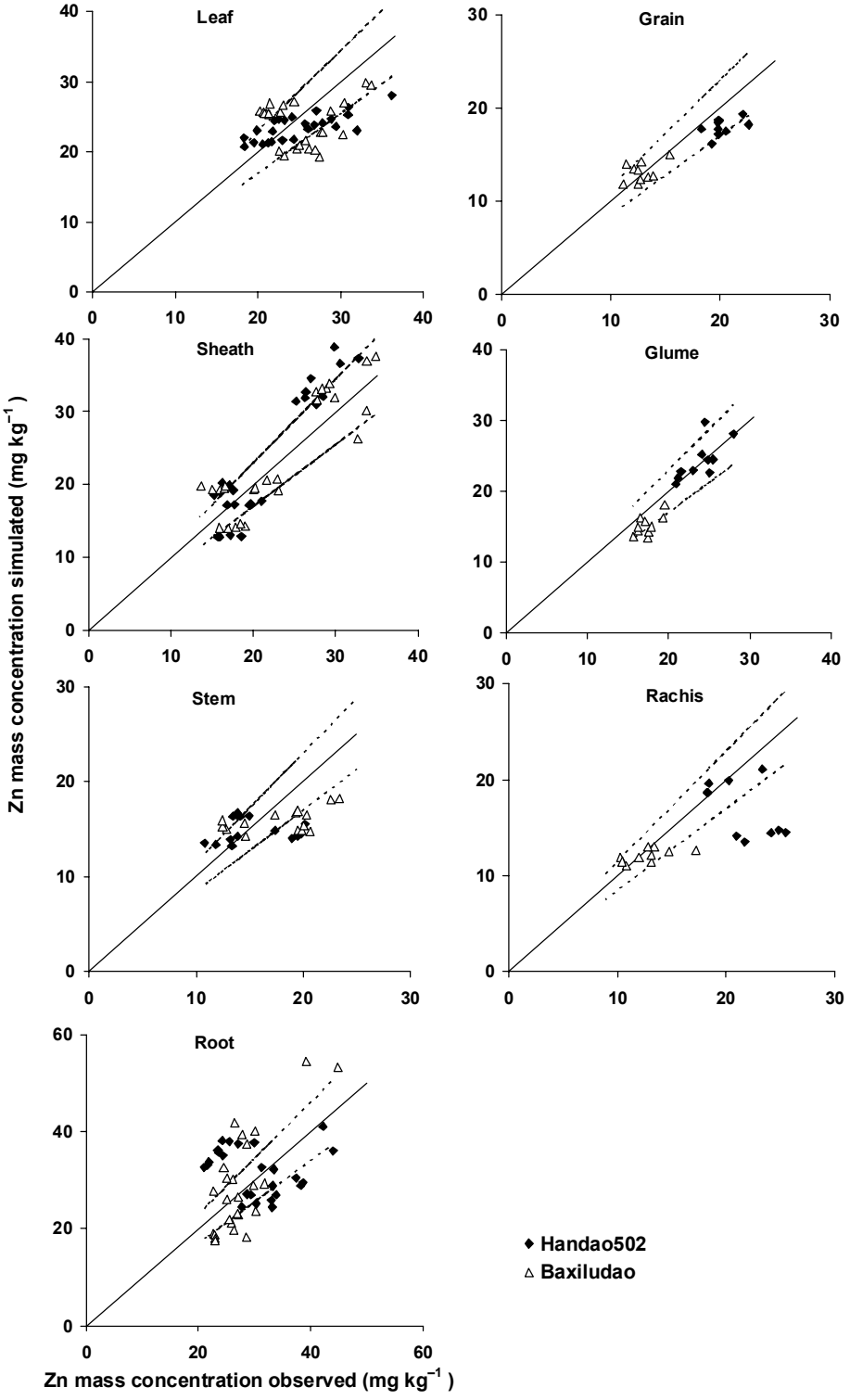

Figure 9. Comparison of simulated and observed values for Zn mass concentration in individual organs for the whole data set from the field experiment (at various development stages and for all Zn treatments) for both cultivars. Solid lines are 1:1 lines and dotted lines indicate 15% deviation from the observed values for both cultivars.

biomass and root Zn mass concentration, as we did not recover all roots. Therefore, sampling error may lead to a wrong estimation. In this model, simulated grain Zn mass concentration was in satisfactory agreement with observed values for both varieties.

In this validation data set, the dynamics of Zn partitioning under high plant Zn status could not be validated, because under field conditions high concentrations are difficult to attain (Gao, 2007). The results of the model suggest that to attain grain Zn mass concentrations of the order of 40 mg kg^{-1} under field conditions, Zn mass concentration in the total plant should at least be doubled.

In conclusion, the model satisfactorily reproduced the general picture of Zn distribution among plant organs, including the grain, for given patterns of total Zn uptake. It may therefore serve as a basis for quantification of the consequences of changes in the system, such as increased zinc uptake during different growth phases, or higher Zn mass concentrations in non-grain organs within the plant, on grain zinc accumulation and grain Zn mass concentration. However, since performance of the model strongly 'hinges' on the relations given in Figure 3 and Table 1, more experimental data for rice are required to test whether these relations apply under a wider range of conditions and how much the relations vary among more contrasting rice cultivars. Based on the current descriptive simulation model, a more explanatory model might be developed by replacing empirical relations with more causal and/or physiologically-based relations, such as the minimum and maximum Zn transport and/or accumulation rates for different organs at different development stages, and the fraction of translocatable Zn from different vegetative organs.

CHAPTER 5

Screening indices for grain yield and grain zinc mass concentration in aerobic rice

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Abstract

Zinc is an important micronutrient, both for crop growth and human nutrition. In rice production, yields are often reduced and Zn mass concentrations in the grains are often low when Zn is in short supply to the crop. This may result in malnutrition of people dependent on a rice-based diet. Plant breeding to enhance low-Zn tolerance might result in increased yield and nutritional quality, but requires effective selection criteria, embedded in physiological insight in the Zn husbandry of the crop. Using existing and newly developed low-Zn tolerance indices, this study presents results of screening experiments carried out in high- and low-Zn soils, using 16 accessions of aerobic rice under greenhouse conditions (to conceptualize the indices) and 14 accessions under field conditions (to validate the indices). In these experiments, the different Zn levels did not result in differences in grain yield. Therefore, additional data from the literature, in which Zn level did have an effect on grain yield, were used to further check the validity of the indices. The grain yield efficiency index (YEI) and the grain Zn mass concentration efficiency index (ZnMCEI), the grain Zn mass concentration and yield efficiency index (ZnMYEI), and the low-Zn tolerance index for grain yield (TIY) and for grain Zn mass concentration (TIZnMC) were applied to evaluate the genotypic low-Zn tolerance performance in attaining (relatively) high grain yield, high grain Zn mass concentration, or both. The results indicate that ZnMCEI is different from YEI and that the low-Zn tolerance indices were the better tools to identify superior genotypes. Amongst the indices tested, TIY and TIZnMC were closely correlated with grain yield and grain Zn mass concentration, respectively. Therefore, TIY was effective in screening for high stability and high potential of grain yield, and TIZnMC was effective for grain Zn mass concentration under low and high soil Zn conditions. Genotypic differences in yield and grain Zn mass concentration were shown to be unrelated and therefore deserve separate attention in breeding programmes. Combining TIY and TIZnMC in a single low-Zn tolerance index could be considered, but did not seem to be superior to using the two separate indices.

Keywords: Breeding, low-zinc tolerance, *Oryza sativa* L., harvest index, zinc efficiency.

INTRODUCTION

Zinc is an important micronutrient for crop growth and human nutrition. In rice production, and especially in aerobic rice production, when Zn is in short supply to the crop, yields are often reduced (Gao *et al*., 2006) and Zn mass concentrations in the grains are often low (Chapters 2 and 3). This may result in Zn malnutrition of people dependent on a rice-based diet.

Micronutrient malnutrition (often called 'hidden hunger') has been estimated to afflict over two billion people, especially resource-poor women and children in the developing world, and their numbers are increasing (Buyckx, 1993; McGuire, 1993; Yip & Scanlon, 1994; Hambidge, 2000; Von Braun *et al*., 2005). Crop products constitute the primary source of all micronutrients for humans, especially in developing countries. For instance, in China, 70–85% of the Zn intake is derived from plant sources (Yang *et al.*, 2000). Therefore, enhancing the Zn mass concentration in grains destined for human consumption is being considered a sustainable long-term solution for combating Zn malnutrition (Graham, 1984; Graham & Welch, 1996; Rengel *et al*., 1999; Frossard *et al*., 2000; Von Braun *et al*., 2005).

Zn mass concentration in grain might be increased by applying Zn fertilizer to the soil or directly to the plants (Broadley *et al*., 2007). Continued fertilization in excess of crop uptake could lead to problems; hence judicious use should be advocated. For the short term, it is relevant that Zn application might lead to higher grain yield in low-Zn soil. However, it was shown in wheat (Kalayci *et al*., 1999) and in rice (Gao *et al*., 2006) that in currently available varieties, under field conditions, grain Zn mass concentration is not easily increased by fertilization.

Therefore, development of varieties that would combine high yields with high grain Zn mass concentrations in situations without high levels of available Zn is a desirable breeding goal. Hence, to evaluate Zn efficiency of varieties in breeding programmes, indices based on both, grain yield and grain Zn mass concentration, and embedded in physiological insight in the Zn husbandry of the crop, are needed. In Chapters 2, 3 and 4 (cf. Jiang *et al*., 2007), we showed that the final mass of Zn in the grain is a function of Zn availability in the soil, the capacity of the roots to take up Zn, the Zn demand of the growing crop, and the partitioning of Zn within the canopy. However, a large proportion of Zn is sequestered in the vegetative parts of the canopy and in the panicle structure, so that relatively little of the Zn accumulates in the grains, although stimulating Zn uptake after flowering increases Zn mass concentration in the grains. We also showed that the physiological mechanisms of Zn husbandry in relation to grain Zn mass concentration are cultivar-specific, demonstrating the potential of selection for increased Zn efficiency and increased Zn mass concentration in the grains, independent of grain yield.

Currently, two indices relating to 'Zn efficiency' are widely used. One is the grain yield efficiency index, first defined by Graham (1984) as the ratio of [yield of a genotype at low soil Zn level/experimental mean yield at low soil Zn] to [yield of a genotype at high soil Zn level/experimental mean yield at high soil Zn], to classify genotypes into efficient (grain yield efficiency index exceeding 1) and inefficient (grain yield efficiency index in the range of 0.0–0.5) groups. A genotype with a high grain yield efficiency index has the ability to produce a relatively high yield under Zn-limited soil conditions compared to its own yield under Zn-sufficient conditions and to yields of other genotypes tested. This agronomic definition is meaningful to a plant breeder selecting genetic material in the field.

The second index commonly used is the ratio of yield at low Zn level to yield at high Zn level (Graham *et al*., 1992; Cakmak *et al*., 1994; Rengel & Graham, 1995). This index could reflect the genotype's ability to cope with Zn deficiency relative to its own yield under non-limiting conditions. This index is of interest to crop physiologists and soil scientists, as it may form the basis for further study of the mechanisms underlying Zn efficiency, including root system geometry, chemical modification of the root-soil interface, and internal Zn redistribution.

Within a given experiment, the ratio of [experimental mean yield at high Zn] to [experimental mean yield at low Zn] will be identical for all entries. Within one experiment, the two indices therefore differentiate between the entries in an identical way and only differ by a constant factor. However, for breeders, their performance under different environmental conditions (i.e. weather and/or soil) is of interest. In this chapter, we, therefore, only use the grain yield efficiency index.

Another candidate for evaluation is the stress tolerance index (STI) (Fernandez, 1993), used to compare genotypic performance across years or environments where stress is common. STI is the product of [*YP*/*XP*], [*YS*/*XS*] and [*XS*/*XP*], where *YP* and *YS* are the yields of a given genotype in non-stressed and stressed environments, respectively, and *XP* and *XS* the mean yields of all tested genotypes in non-stressed and stressed environments, respectively. Higher values of STI for a genotype indicate greater stress tolerance and higher yield potential. STI has been found effective in identifying genotypes that perform well under both stress and non-stress conditions (Porch, 2006). This index has the potential to support identification of genotypes, lines or varieties that perform relatively well under stress, but also take advantage of favourable conditions by yielding high in terms of production and/or quality.

The indices described above are all related to the yield of the varieties and not to quality criteria, such as Zn mass concentration. This study was, therefore, carried out to test the merits of these indices in screening genotypes for grain Zn mass concentration and grain yield, separately and in combination. In the study, rice

accessions were used specifically bred for favourable performance under aerobic soil conditions (Bouman *et al*., 2002; Yang *et al*., 2005). Such aerobic soil conditions are potentially reducing soil Zn availability, thus increasing the need to select for improved Zn efficiency and necessitating enhanced cultivar performance (Gao *et al*., 2006).

MATERIAL AND METHODS

The study comprises three data sets. A greenhouse experiment was set up to conceptualize the screening indices under relatively controlled conditions. A field experiment was carried out to validate these indices under real-life conditions. In the greenhouse experiment we did not observe significant effects of Zn on grain yield or harvest index (although Zn uptake, Zn efficiency and Zn mass concentrations were strongly affected). In the field experiment, grain yield was affected by Zn level, but the harvest index was not. Therefore, we identified a data set from literature to verify our results in conditions where Zn did affect grain yield and harvest index (Giordano & Mortvedt, 1974).

Both, the greenhouse experiment and the field experiment consisted of a diverse set of genotypes. However, due to the poor ecological adaptation of some of the genotypes used in the greenhouse study, we could not carry out the field experiment with the same material. Some accessions caused considerable leverage in the regression analyses. The literature data set for verification also consisted of a diverse data set, but with cultivars not included in our own experimentation.

Greenhouse experiment

A pot experiment was carried out in a greenhouse at China Agricultural University, Beijing, China, from 24 May until 15 October 2003. Plants were grown in pots containing 7.5 kg soil (pH 6.8, DTPA-extractable Zn 0.3–0.4 mg kg^{-1}, i.e. well below the critical Zn concentration of 0.5 mg kg^{-1}; same soil as in the field experiment reported below), either without amendment or amended with 10 mg Zn kg^{-1} soil, added as $ZnSO_4.7H_2O$. A basal fertilization of 200 mg N kg^{-1} soil as $Ca(NO_3)_2$ and 100 mg P kg^{-1} soil as KH_2PO_4 was applied to all pots. All nutrients were mixed thoroughly with the soil before sowing. Sixteen aerobic rice (*Oryza sativa* L.) accessions were used in the experiment. Seeds were obtained from the Aerobic Rice Research Center of China Agricultural University; Zn mass concentration in the de-hulled grain ranged between 9.7 and 15.4 mg kg^{-1}. The experiment was set up in a completely randomized factorial design (16 accessions × 2 Zn levels) with three replicates. Ten seeds were sown in each pot, and the plant stand was thinned to four seedlings per pot soon after emergence. Pots were watered daily with de-ionized water

to 80% of field capacity. Plants were cultivated under natural temperature and natural light during the summer season. Before flowering (1 September 2003), all pots were moved into a glasshouse, set to maintain a temperature of 30 ± 1 °C during the day and 21 ± 1 °C during the night. Light intensity was about 85% of natural light intensity and 1000 μmol m^{-2} s^{-1} light was supplemented when it was cloudy. At physiological maturity (30 days after flowering), plants were harvested to determine dry weights and Zn mass concentrations.

Field experiment

The field experiment, comprising 14 aerobic rice (*Oryza sativa* L.) accessions, was carried out in Mengcheng, Anhui province, China (33°55' N, 116°15' E) in 2004. Because photoperiod and temperature in the field were very different from those in the greenhouse, only four accessions tested in the greenhouse experiment were also tested in the field experiment. The soil at the experimental site was a Shajiang black soil (vertisol; Anonymous, 1998) with pH 6.8. DTPA-extractable Zn was 0.30–0.40 mg kg^{-1} soil, i.e. well below the critical Zn concentration of 0.5 mg kg^{-1}. Seeds were obtained from the Aerobic Rice Research Center of China Agricultural University; Zn mass concentration in the de-hulled grain was 12.7–19.4 mg kg^{-1}. The experimental design used was a split-plot, with three replications, main plots were two levels of Zn (+Zn, 22.5 kg ha^{-1} added as $ZnSO_4.7H_2O$, and –Zn, no Zn added) and subplots were 14 aerobic rice accessions. Plant spacing within the row was 0.15 m, and distance between rows 0.25 m. Composite fertilizer ($N–P_2O_5–K_2O$: 12–18–10) at the rate of 50 kg P ha^{-1} and 75 kg N ha^{-1} and Zn fertilizer (only in the +Zn plots) were incorporated before planting and 50 kg N ha^{-1} was top-dressed as ammonium nitrate at tillering. Plants were grown under rainfed conditions, with supplemental irrigation one day after sowing and at the flowering stage. Plants were sampled at physiological maturity to determine dry weights and Zn mass concentrations.

Measurement of Zn mass concentrations

Plant samples from both experiments were transported to the laboratory and partitioned into shoot (except panicle), panicle and grain. Samples were rinsed three times with double-de-ionized water, and then oven-dried at 75 °C for 48 h. Each component was weighed and the grain was de-hulled. Dried plant samples were ground in a stainless steel mill and passed through a 0.25-mm sieve before analysis. Sub-samples of 0.5 g of the dried and ground samples were digested in a bi-acid mixture (HNO_3:$HClO_4$ = 4:1). Zn was determined by atomic absorption spectroscopy (SPECTRAA-55; Karian Australia, Mulgrave, Australia).

Definition of Zn efficiency indices

The following indices for Zn efficiency were calculated:

Grain yield efficiency index (YEI) (Graham, 1984):

$$YEI = (Y_L/\tilde{Y}_L)/(Y_H/\tilde{Y}_H),$$

Zn mass concentration efficiency index (ZnMCEI):

$$ZnMCEI = (ZnMC_L/\tilde{Z}nMC_L)/(ZnMC_H/\tilde{Z}nMC_H),$$

Grain Zn mass concentration and yield efficiency index (ZnMCYEI):

$$ZnMCYEI = (YEI)\ (ZnMCEI),$$

Low-Zn tolerance index for grain yield (TIY) (based on Fernandez, 1993):

$$TIY = (Y_L/\tilde{Y}_L)\ (Y_H/\tilde{Y}_H)\ (\tilde{Y}_L/\tilde{Y}_H) = (Y_L)\ (Y_H)/(\tilde{Y}_H)^2\ ,$$

Low-Zn tolerance index for grain Zn mass concentration (TIZnMC):

$$TIZnMC = (ZnMC_L/\tilde{Z}nMC_L)\ (ZnMC_H/\tilde{Z}nMC_H)\ (\tilde{Z}nMC_L/\tilde{Z}nMC_H)$$
$$= (ZnMC_L)\ (ZnMC_H)/(\tilde{Z}nMC_H)^2,$$

Low-Zn tolerance index for grain Zn mas concentration and grain yield (TIZnMCY):

$$TIZnMCY = (TIY)\ (TIZnMC),$$

where, Y_H is the genotypic yield at high Zn, and Y_L the genotypic yield at low Zn; $\tilde{Y}_H$ is the mean yield over all genotypes at high Zn, and $\tilde{Y}_L$ the mean yield at low Zn. $ZnMC_H$ is the genotypic grain Zn mass concentration at high Zn, and $ZnMC_L$ the genotypic grain Zn mass concentration at low Zn; $\tilde{Z}nMC_H$ is the mean grain Zn mass concentration over all genotypes at high Zn, and $\tilde{Z}nMC_L$ the mean grain Zn mass concentration at low Zn.

Data analysis

Regression analysis and analysis of variance (ANOVA) were performed with SAS (Anonymous, 2001).

In addition to data from own greenhouse and field experiments, the data set from Giordano & Mortvedt (1974) was added to the correlation analyses between all defined indices.

RESULTS

Grain yield and grain Zn mass concentration

Zn treatment did not significantly affect grain yield or harvest index in the greenhouse, but did affect grain yield in the field experiment (Table 1). Accessions significantly differed in grain yield and harvest index (Table 1). Tables 2 and 3 show the effect of Zn and genotype on grain Zn yield (i.e. the mass of Zn per plant present in the grain at

Table 1. Significance of F values derived from ANOVA of the effects of accessions of aerobic rice, Zn level and their interaction for various variables. The coefficients of variation (CV) of these variables are also presented.

Experiment	Variables	Accessions	Zn level	Zn level × Accessions	CV (%)
Greenhouse	Grain yield	**[1]	NS	NS	8.3
	HI[2]	**	NS	NS	8.5
	Grain ZnMC[3]	**	**	**	11.7
	Grain Zn yield	**	**	**	15.3
	Shoot Zn content	**	**	**	13.6
	Zn use efficiency	**	**	**	13.0
	Zn harvest index	**	**	**	17.0
Field	Grain yield	**	*	NS	8.6
	HI	**	NS	**	6.2
	Grain ZnMC	**	**	**	13.7
	Grain Zn yield	**	**	*	20.0
	Shoot Zn content	**	**	NS	15.1
	Zn use efficiency	**	*	NS	13.5
	Zn harvest index	**	**	**	11.1

[1] *, ** Significant at 5 and 1% levels, respectively; NS means not significant.

[2] HI: harvest index = grain dry weight/total shoot dry weight.

[3] Grain ZnMC: grain Zn mass concentration.

the end of the growing period), shoot Zn content (i.e. the mass of Zn per plant in the above-ground plant dry matter), Zn use efficiency (i.e. the shoot dry matter production per unit of Zn uptake) and Zn harvest index (i.e. grain Zn yield divided by shoot Zn content). In the greenhouse experiment (Table 2), additional Zn supply increased grain Zn yield and shoot Zn content for all genotypes, but reduced Zn use efficiency and had a variable effect on Zn harvest index. Genotypes showed large variation in all characteristics listed in Table 2. In the field experiment (Table 3), additional Zn supply increased grain Zn yield, shoot Zn content and Zn harvest index in most genotypes, but not in all. Zn use efficiency was not affected by application of Zn, and was consistently higher than in the greenhouse experiment.

Under low Zn conditions, grain yield varied between 2.5 and 4.5 g plant^{-1} in the greenhouse and between 213 and 457 g m^{-2} in the field experiment (Tables 4 and 5). Zn application significantly increased Zn mass concentration in the grains (Grain

Table 2. Grain Zn yield, shoot Zn content, Zn use efficiency and Zn harvest index in the greenhouse experiment. The accessions are grouped as indicated in Table 4.

	Grain Zn yield (µg Zn/plant)		Shoot Zn content (µg Zn/plant)		Zn use efficiency (g shoot dry matter/µg Zn)		Zn harvest index	
	−Zn	+Zn	−Zn	+Zn	−Zn	+Zn	−Zn	+Zn
90B10-1	102	729	781	4810	0.022	0.003	0.13	0.15
91B8-30-3	105	598	1010	5480	0.018	0.003	0.10	0.11
Handao277	184	959	816	6090	0.020	0.003	0.23	0.16
91BTe3	96	552	972	6750	0.017	0.002	0.10	0.08
89B271Mozhuxi	117	627	932	8970	0.018	0.002	0.13	0.07
Handao9	100	537	762	5790	0.018	0.003	0.13	0.09
Handao72	114	580	780	5270	0.020	0.003	0.15	0.11
89D108-11-1	127	617	1100	7050	0.013	0.002	0.12	0.09
TB Mozhuxi	119	529	989	6070	0.017	0.003	0.12	0.09
K150	140	590	892	5720	0.015	0.002	0.16	0.10
89B271-17Hun	148	520	946	5300	0.019	0.003	0.16	0.10
Handao99-19	114	401	571	4950	0.018	0.002	0.20	0.08
Hongkelaoshuya	106	552	1320	5550	0.012	0.003	0.08	0.10
Handao502	133	567	1000	6430	0.017	0.003	0.13	0.09
Baxiludao	118	499	808	5210	0.020	0.003	0.15	0.10
Handao297	143	557	1170	3990	0.014	0.004	0.12	0.14
Mean	123	588	928	5840	0.017	0.003	0.14	0.10
SED	40		335		0.001		0.01	

ZnMC; Tables 1, 4 and 5), and there were strong interactions between Zn level and accession (Table 1). Grain ZnMC responded differently to Zn fertilization among accessions. Four accessions in the greenhouse experiment and five accessions in the field experiment were found to show a markedly strong response to Zn fertilization, with an increase in grain ZnMC exceeding 3 times the standard error of the difference between means (SED). Other accessions were less responsive to Zn fertilization (Tables 4 and 5). Accessions tested in both, the greenhouse and the field experiment, strongly varied in yield, grain Zn mass concentration and partitioning of dry matter and Zn. Under low soil-Zn conditions without Zn supply, grain Zn mass concentration varied from 27.3 (Handao9) to 50.5 (89D108-11-1) mg kg^{-1} in the greenhouse

Table 3. Grain Zn yield, shoot Zn content, Zn use efficiency and Zn harvest index in the field experiment. The accessions are grouped as indicated in Table 4.

	Grain Zn yield (µg Zn/m^2)		Shoot Zn content (µg Zn/m^2)		Zn use efficiency (g shoot dry matter/µg Zn)		Zn harvest index	
	−Zn	+Zn	−Zn	+Zn	−Zn	+Zn	−Zn	+Zn
Qinai-3hun	4140	7450	14500	18600	0.06	0.05	0.29	0.40
Henghan1	3620	5710	16400	15900	0.05	0.05	0.22	0.36
Handao7	4650	9750	16400	22900	0.07	0.05	0.28	0.42
Handao65	4820	7420	14900	16300	0.07	0.06	0.32	0.46
Liaohan109	5990	8900	13500	19300	0.07	0.06	0.44	0.46
Yunnanhandao	5410	8320	20400	22300	0.07	0.07	0.27	0.37
90B290	11100	12000	20800	24000	0.07	0.06	0.53	0.49
91B8-14	5070	5900	15200	17300	0.08	0.07	0.33	0.34
91BTe9-7	7040	6720	17100	18400	0.07	0.07	0.41	0.37
Haogelao-5	6560	5920	18500	19300	0.07	0.07	0.36	0.31
Handao297	4850	8660	15000	17600	0.08	0.07	0.32	0.49
Handao502	6860	10200	23000	23800	0.07	0.06	0.30	0.43
Baxiludao	5400	7390	19700	20700	0.08	0.08	0.27	0.36
Hongkelaoshuya	7910	9440	28800	27300	0.05	0.06	0.28	0.35
Mean	5960	8120	18200	20300	0.07	0.06	0.33	0.40
SED	1009		2075		0.006		0.029	

experiment and from 12.0 (Baxiludao) to 26.3 (Hongkelaoshuya) mg kg^{-1} in the field experiment. With additional Zn, grain Zn mass concentration varied from 28.9 (Handao99-19) to 57.5 mg kg^{-1} (89D108-11-1) in the greenhouse experiment and from 16.3 (Haogelao-5) to 29.6 (Qinai-3hun) mg kg^{-1} in the field experiment (Tables 4 and 5). Among the four accessions used in both, the greenhouse and the field experiment, grain Zn mass concentration was lowest in Baxiludao and highest in Hongkelaoshuya.

Low-Zn tolerance indices

Accessions differed in the indices YEI, ZnMCEI, ZnMCYEI, TIY, TIZnMC, and TIZnMCY (Tables 4 and 5). Handao277 (in the greenhouse experiment) and 90B290 (in the field experiment) were outliers, combining very high values for TIY and TIZnMC, which indicates good performance (low-Zn tolerance) under low-Zn conditions, with high grain yield and high grain Zn mass concentration potentials at

Table 4. Grain yield, grain Zn mass concentration, and Zn efficiency indices of 16 accessions tested in the 2003 greenhouse experiment. The accessions are grouped into: a first set of four accessions with an increase in grain ZnMC exceeding 3 times the standard error of the difference between means (SED), a set of eight accessions with a difference less than 3 times the SED and four accessions also reported in Table 5 for the field experiment, and with differences less than 3 times the SED.

Accessions	Grain yield (g plant^{-1})		Grain ZnMC[1] (mg kg^{-1})		YEI[2]	ZnMCEI	ZnMCYEI	TIY	Rank	TIZnMC	Rank	TIZnMCY
	−Zn	+Zn	−Zn	+Zn								
90B10-1	3.2	3.3	31.7	55.6	0.99	0.67	0.67	0.84	13	1.00	4	0.85
91B8-30-3	3.7	3.5	28.6	42.8	1.07	0.78	0.84	1.03	8	0.70	12	0.72
Handao277	4.5	4.5	41.2	53.7	1.02	0.89	0.91	1.60	1	1.26	2	2.01
91BTe3	3.4	3.5	27.9	39.5	1.00	0.82	0.83	0.96	10	0.63	14	0.60
89B271Mozhuxi	4.0	3.9	29.5	40.0	1.03	0.86	0.89	1.25	2	0.67	13	0.83
Handao9	3.7	3.9	27.3	35.0	0.97	0.91	0.88	1.14	3	0.54	16	0.62
Handao72	3.6	3.7	32.1	39.7	1.00	0.94	0.93	1.05	6	0.72	11	0.76
89D108-11-1	2.5	2.7	50.5	57.5	0.96	1.02	0.98	0.55	16	1.65	1	0.90
TB Mozhuxi	3.0	3.0	40.3	44.4	1.01	1.06	1.06	0.71	14	1.02	3	0.72
K150	3.5	3.7	40.5	40.2	0.97	1.17	1.13	1.02	9	0.93	6	0.94
89B271-17Hun	3.8	3.5	39.2	37.0	1.09	1.24	1.36	1.06	5	0.82	8	0.88
Handao99-19	3.4	3.5	34.0	28.9	0.99	1.37	1.36	0.94	11	0.56	15	0.52
Hongkelaoshuya	2.8	3.2	38.1	43.7	0.89	1.02	0.91	0.70	15	0.95	5	0.67
Handao502	3.7	3.6	36.5	40.0	1.05	1.07	1.11	1.05	7	0.83	7	0.87
Baxiludao	3.2	3.4	37.4	37.1	0.96	1.17	1.12	0.86	12	0.79	10	0.67
Handao297	3.6	3.9	39.5	36.0	0.95	1.28	1.22	1.12	4	0.81	9	0.91
SED	0.21		3.07									
Mean	3.45 $=\bar{Y}_L$	3.53 $=\bar{Y}_H$	35.9 $=\bar{Z}nMC_L$	42.0 $=\bar{Z}nMC_H$								

[1] Grain ZnMC: grain Zn mass concentration. [2] see text for definitions: YEI, Grain yield efficiency index; ZnMCEI, Grain Zn mass concentration efficiency index; ZnMCYEI, Grain Zn mass concentration and yield efficiency index; TIY, Low-Zn tolerance index for grain yield; TIZnMC, Low-Zn tolerance index for grain Zn mass concentration; TIZnMCY, Low-Zn tolerance index for grain Zn mass concentration and grain yield.

Table 5. Yield and Zn mass concentration and Zn efficiency indices of 14 accessions tested in the 2004 field experiment. The accessions are grouped into: a first set of five accessions with an increase in grain ZnMC exceeding 3 times the standard error (SED), a set of five accessions with an increase less than 3 times the SED and four accession also reported in Table 4 for the greenhouse experiment, of which the first two with differences exceeding 3 times the SED.

Accessions	Grain yield (g plant^{-1})		Grain ZnMC[1] (mg kg^{-1})		YEI[2]	ZnMCEI	ZnMCYEI	TIY	Rank	TIZnMC	Rank	TIZnMCY
	−Zn	+Zn	−Zn	+Zn								
Qinai-3hun	244	252	17.1	29.6	1.02	0.75	76	0.45	13	1.01	4	0.45
Henghan1	213	221	16.9	25.9	1.01	0.85	87	0.35	14	0.87	5	0.30
Handao7	353	439	13.1	21.9	0.85	0.78	66	1.14	6	0.57	11	0.65
Handao65	290	300	16.5	24.8	1.02	0.87	89	0.64	11	0.81	6	0.52
Liaohan109	424	428	14.1	20.9	1.04	0.88	92	1.33	4	0.59	9	0.78
YunnanHandao	457	503	12.0	16.5	0.96	0.95	90	1.69	1	0.39	14	0.65
90B290	437	440	25.3	27.0	1.04	1.22	128	1.41	2	1.36	2	1.93
91B8-14	281	301	18.0	19.5	0.98	1.21	119	0.62	12	0.70	7	0.43
91Bte9-7	372	385	18.9	17.4	1.02	1.42	145	1.05	7	0.65	8	0.69
Haogelao-5	361	364	18.3	16.3	1.04	1.46	152	0.96	9	0.59	9	0.57
Handao297	385	416	12.5	20.7	0.97	0.79	77	1.18	5	0.52	12	0.61
Handao502	351	376	19.5	27.0	0.98	0.94	93	0.97	8	1.05	3	1.02
Baxiludao	444	410	12.0	18.0	1.14	0.87	101	1.33	3	0.43	13	0.58
Hongkelaoshuya	300	318	26.3	28.5	0.99	1.21	120	0.76	10	1.49	1	1.05
SED		22.9		1.9								
Mean	351	368	17.2	22.4								
	$=\tilde{Y}_L$	$=\tilde{Y}_H$	$=\tilde{Z}nMC_L$	$=\tilde{Z}nMC_H$								

[1, 2] For explanation see notes in Table 4.

Table 6. Linear correlation coefficients between grain yield, grain Zn mass concentration and Zn efficiency indices, for the greenhouse and field experiment and for additional data from the literature (Giordano & Mortvedt, 1974). (Note: autocorrelation exists in all cases)

Indices Terms	Trials	YEI[3]	ZnMCEI	ZnMC -YEI	TIY	TIZn-MC	TIZn-MCY	TIZn-MCY[5]
LYIELD[1]	F[2]	0.18	0.12	0.16	0.98**	–0.36	0.46	
	G	0.57*[4]	–0.12	0.03	0.98**	–0.35	0.56*	0.09
	D	0.77*	0.22	0.64	0.95**	–0.28	0.88**	
HYIELD	F	–0.15	0.04	0.01	0.98**	–0.39	0.43	
	G	0.26	–0.08	–0.01	0.98**	–0.35	0.57*	0.02
	D	–0.07	–0.29	–0.17	0.79*	–0.50	0.62	
LGZnMC	F	0.12	0.59*	0.57*	–0.28	0.91**	0.64*	
	G	–0.24	0.47	0.41	–0.31	0.84**	0.39	0.50
	D	0.29	0.57	0.49	–0.28	0.91**	0.21	
HGZnMC	F	–0.08	–0.32	–0.32	–0.52	0.84**	0.33	
	G	–0.07	–0.57*	–0.59*	–0.16	0.84**	0.53*	0.45
	D	–0.26	–0.35	–0.29	–0.49	0.85**	–0.15	
YEI	F&G				NS			
ZnMCEI	F&G					NS		
ZnMCYEI	F&G						NS	

[1] LYIELD/HYIELD is grain yield at low/high Zn level. LGZnMC/HGZnMC is grain Zn mass concentration at low/high Zn level. [2] F, Field experiment; G, Greenhouse experiment; D: data derived from Giordano & Mortvedt (1974). [3] YEI, Grain yield efficiency index; ZnMCEI, Grain Zn mass concentration efficiency index; ZnMYEI, Grain Zn mass concentration and yield efficiency index; TIY, Low-Zn tolerance index for grain yield; TIZnMC, Low-Zn tolerance index for grain Zn mass concentration; TIZnMCY, Low-Zn tolerance index for grain Zn mass concentration and grain yield. [4] *, ** is significantly correlated at 5% and 1% level, respectively. [5] Linear correlation coefficient when the outlier accession Handao277 was excluded. NS, non significant.

high Zn supply compared to all other accessions tested. Handao99-19 and Henghan1 both showed low Zn efficiency, with low grain yield and low grain Zn mass concentration under low-Zn conditions. The ranking of the accessions was non-consistent for the different indices, although YEI and TIY were both based on grain yield, whereas ZnMCEI and TIZnMC were both based on grain Zn mass concentration (Tables 4 and 5). There was no significant correlation between YEI and TIY, nor between ZnMCEI and TIZnMC (Table 6).

Correlations between Zn-efficiency indices and grain yield or grain Zn mass concentration

In both screening experiments, TIZnMC was strongly correlated with grain Zn mass concentration, and TIY was correlated with grain yield under both low- and high-Zn conditions. TIZnMCY was correlated with grain yield, but only in the greenhouse experiment, and was not consistently correlated with grain Zn mass concentration (Table 6). The other indices, such as YEI and ZnMCEI, were not consistently correlated with either grain yield or grain Zn mass concentration. Thus, TIY was effective in identifying accessions with high and stable grain yield potential, whereas TIZnMC was effective in identifying accessions with high grain Zn mass concentration. The correlations of the combination of TIY and TIZnMC (TIZnMCY) to grain yield and grain Zn mass concentration were always weaker than those of the individual component indices (Table 6).

Test of indices with additional data

As in the greenhouse experiment no Zn effect on grain yield and harvest index was observed, and in the field experiment no effect on harvest index, we used an additional data set in which significant effects of Zn supply on these characteristics were observed (Giordano & Mortvedt, 1974). It was found that the results of the correlation analyses of all indices with grain yield and grain Zn mass concentration, described above, for this additional data set were similar to those found for our own data sets. This means that the two low-Zn tolerance indices also perform well under conditions in which Zn availability has more pronounced effects on crop performance.

DISCUSSION

Genotypic variation in grain Zn mass concentration

Genotypic variation in grain Zn mass concentration in rice has been reported by Giordano & Mortvedt (1974), Yang *et al.* (1998), Fageria (2001), Gregorio (2002) and Gao *et al*. (2005). We also observed strong variation in grain Zn mass concentration among tested accessions, in both, the greenhouse and the field experiment, and Zn supplementation resulted in significantly higher grain ZnMC (Tables 1, 4 and 5). This genotypic variation was associated with variation in Zn use efficiency and Zn harvest index. The observed significant interaction between Zn application and accession indicates a significant genotype by environment interaction. Grain ZnMC was correlated with panicle structure ZnMC. However, grain ZnMC was not correlated with Zn harvest index (the ratio of grain Zn content to shoot Zn content) or Zn mass concentration in the shoot, except in the situation without Zn supplementation in the

greenhouse experiment (data not shown). This suggests that the differences in grain Zn mass concentration among genotypes were due to a difference in loading ability of Zn from the panicle to the grains, and were not directly determined by Zn harvest index or shoot Zn content. This is consistent with the results of Grusak *et al*. (1999), indicating that the ability to maintain xylem influx into the panicle during seed formation and the ability to load the grain from that xylem are essential for realizing a high grain Zn mass concentration.

Zn efficiency or low-Zn tolerance indices in screening

Genotypes characterized by high grain yield efficiency indices (YEI) have the ability to produce relatively high yields under Zn-limited soil conditions compared to their own yield under Zn-sufficient soil conditions and compared to yields of other tested accessions (Graham, 1984). However, in this study, we found no consistent correlation between grain yield efficiency index and grain yield (Table 6).

Genotypic variation in Zn efficiency has been studied in various crops, including bean (Ambler & Brown, 1969; Hacisalihoglu *et al*., 2004), wheat (Graham & Rengel, 1993; Cakmak *et al*., 1997; Kalayci *et al*., 1999; Hacisalihoglu *et al*., 2003) and rice (Fageria, 2001; Gao *et al*., 2005). Insights are increasing in the mechanisms underlying high Zn efficiency, i.e. how the plant is able to maintain reasonable growth rates and yields under conditions of low Zn availability in the growth medium. Potential mechanisms include relatively efficient root Zn uptake and translocation, and effective and efficient biochemical utilization of Zn; however, a multitude of questions with respect to these mechanisms still remains (Rengel & Graham, 1995; Cakmak, 2000; Hacisalihoglu *et al*., 2001, 2003; Hacisalihoglu & Kochian, 2003). It is essential to note, though, that a mechanism, such as efficient biochemical utilization of low Zn levels in leaves for production, has no inherent contribution to high grain Zn loading capability and may in fact be fully unrelated.

Similarly to the grain yield efficiency index, the grain Zn mass concentration efficiency index (ZnMCEI) is not highly correlated with grain Zn mass concentration, neither under low nor under sufficient Zn conditions. Hence, ZnMCEI only reflects the accession's ability to produce a relatively high grain Zn mass concentration under Zn-limited soil conditions, compared to its own grain Zn mass concentration under Zn-sufficient soil conditions, and not its ability to use high Zn-availability conditions to attain a high grain Zn mass concentration.

The two new Zn indices, derived from drought research, i.e. the low-Zn tolerance index for grain yield (TIY) and the low-Zn tolerance index for grain Zn mass concentration (TIZnMC), attain higher values for genotypes characterized by greater low-Zn tolerance in terms of grain yield or grain Zn mass concentration, respectively,

and higher yield or grain Zn mass concentration potential, under low and sufficient soil Zn conditions, respectively. Moreover, in all experiments, TIY and TIZnMC were highly correlated with grain yield, and grain Zn mass concentration, respectively (Table 6).

However, TIZnMCY, the combination of TIY and TIZnMC, did not correlate with either grain yield or grain Zn mass concentration (when the outlier accession Handao277 was excluded) (Table 6). So, TIY and TIZnMC are effective in identifying genotypes that perform well in terms of yield or grain Zn mass concentration, respectively, under both Zn-limited and Zn-sufficient conditions, but an effective indicator for a combination of the two characteristics could not be identified.

Based on TIY, the ranking of the four common varieties in both experiments (Handao297, Handao502, Hongkelaoshuya, Baxiludao) was different in the greenhouse from that in the field experiment (Table 6). In other studies, different rankings in a set of genotypes have been observed in different experimental years at the same site (Kalayci *et al*., 1999), which could be the result of differences in plant-available Zn, and, therefore, in Zn-stress intensity between experiments. However, the overlap in terms of accessions between the two experiments was too limited for any further analysis.

CONCLUSIONS

For rice breeding programmes, the two indices, TIY and TIZnMC, appear promising for screening genotypes for a combination of high low-Zn tolerance based on both, grain yield and grain Zn mass concentration, and for exploration of higher Zn availability through higher yield and grain Zn mass concentration potentials under non-stressed conditions. As the two indices give different rankings and the correlation between the compound index TIZnMCY and either yield or grain ZnMC is much weaker than for the individual indices, it seems important to separate the analyses of both traits in breeding programmes.

CHAPTER 6

General discussion

Zinc (Zn) deficiency is a widespread problem in regions where the human diet is mainly based on cereals. Over 30% of the world's population shows symptoms of zinc deficiency, many of them live in Asia. In China, average intake of zinc is 85% of its Recommended Dietary Allowance. Symptoms of severe zinc deficiency include stunted growth, mental retardation and poor recovery after illness or inflammation.

For many Chinese, the staple food is rice and the diversity in dietary intake is low for the resource-poor. However, the Zn mass concentration in rice grains for human consumption is generally low. A logical step to overcome Zn deficiency is to try to increase the bio-availability of Zn in rice grain by influencing the Zn husbandry of the crop, either through breeding or through agronomy. Such an approach, however, requires fundamental insight into the Zn husbandry. Therefore, this thesis addressed the following research questions:

- How is Zn allocated in rice plants and what is the potential for accumulation of zinc in the rice grain?
- What are the sources of Zn allocated to the grain in rice?
- Can Zn allocation in the rice plant be modelled?
- What indices could be suggested as screening tools for high grain Zn mass concentration in rice?
- What are effective approaches to increase Zn mass concentration in rice grains?

How is Zn allocated in rice plants and what is the potential for accumulation of zinc in the rice grain?

Under a wide range of zinc supply rates provided throughout the rice crop's development, under both nutrient solution and sand culture conditions (Chapter 2), internal plant Zn distribution and the potential to enhance grain Zn mass concentration were studied. We found that the distribution of zinc among organs varied with level of Zn supply and development stage (Chapter 2). Trend lines for the relations between organ Zn mass concentrations and total plant zinc mass concentration, however, had rather high correlation coefficients (Chapter 4). Before flowering, when Zn availability was low, about 20–30% of Zn uptake was present in the leaf blades. When Zn supply was more than sufficient, a larger proportion of total Zn accumulated in root, stem and sheath. After flowering, when Zn supply was low, Zn content in leaf and sheath decreased over time, and at maturity, 20% of total plant Zn was present in the dehulled

grain (= brown rice). However, when more Zn was supplied, Zn mass concentration in root, leaf blade and sheath remained more or less constant, but the Zn mass concentration continued to increase after flowering in stem and panicle. The increase in Zn content in the vegetative plant parts was much larger than that in the grain, so at maturity only 10% of the total plant Zn was present in the dehulled grain. As a consequence, when toxic Zn levels were applied, grain Zn mass concentration tripled to around 90 mg Zn kg^{-1} rice, while mass concentrations in the vegetative plant parts increased 30-fold to above 1500 mg Zn kg^{-1}. Pearson *et al*. (1996b) reported that the Zn-deficient wheat grain is not a strong sink for Zn, while at high Zn concentrations in nutrient solutions, a protective barrier seemingly prevents excessive Zn accumulation in the grain. In this study, comparison of the Zn mass concentration of different plant organs could indicate where within the plant there are limitations to Zn loading.

From root to stem and to rachis: the root to stem transfer does not seem limiting the Zn loading of above-ground plant parts. Between stem and rachis, there is also no drop in Zn mass concentration under sufficient Zn supply conditions. However, when surplus levels are supplied, Zn mass concentration in the rachis is lower than that in the stem (Table 1), indicating a transfer limitation between the two organs.

From rachis to bran: in the panicle parts, there is no limitation between rachis and outer grain tissue layers (bran, Table 1), and the bran could maintain an even higher Zn mass concentration than both, the stem and the rachis, indicating active Zn accumulation in the outer grain tissues (Table 1). This is consistent with the reports by

Table 1. Zn mass concentration in different organs in the solution culture experiment of Chapter 2.

	Zn mass concentration (mg kg^{-1})	
	Sufficient Zn supply	Excess Zn supply
Grain (brown rice)	20	45
Endosperm	20	30
Bran	60	100
Glumes	25	60
Rachis	25	120
Leaf blades	25	50
Stem	25	300
Root	25−60	200
Sheath	25	120

Krishnan & Dayanandan (2003) and Thorne (1985) that a symplastic continuity exists between the cells of the vascular trace, the chalaza, the nucellar projection and the nucellar epidermis.

From bran to endosperm: the further Zn transport from bran to the endosperm is inwards through the apoplast from the nucellar epidermis through the aleuron cells into the endosperm. This step may play an important role in the regulation of zinc transport to the endosperm. Indeed, a milling test showed that the Zn mass concentration in the endosperm was one-third of that in the bran (Table 1). However, the low Zn mass concentration in endosperm might be due to the dilution by the large quantity of starch in the endosperm; probably most Zn in the endosperm is located in the membrane, and the cell membrane surface area in the endosperm might possibly determine how much Zn could potentially accumulate in the endosperm. Testing of this hypothesis requires further research and the findings could support targeting of breeding efforts.

What are the sources of Zn allocated to the grain in rice?

In order to investigate the relative contribution to grain Zn accumulation of Zn uptake by the root and direct allocation during grain filling and that of Zn remobilization from the leaves after flowering, we applied radioactive ^{65}Zn to different organs. Radioactive zinc was applied to roots either at flowering or 15 days after flowering or at flowering only to a leaf (the flag leaf or the latest senescing leaf). The treated rice plants were grown under either sufficient Zn or surplus Zn conditions (Chapter 3). In aerobic rice, the ^{65}Zn taken up by roots after flowering was mainly allocated to the roots, the stem and the grains, and during the period from cessation of ^{65}Zn supply at flowering until maturity, large amounts of ^{65}Zn were transported out of the root and even the stem (only under sufficient plant Zn conditions) and mainly allocated to the grains. And we found after ^{65}Zn was applied to either the flag leaf or a senescent leaf during grain filling, about 45–50% of the ^{65}Zn absorbed by the treated leaf was re-allocated in plants, and most of the Zn remobilized from the leaf was translocated to roots, leaves and sheaths, whereas panicle parts received not much and especially grains received very little. Our findings suggest that in rice plants, grown under sufficient or surplus Zn supply, most of the Zn accumulated in the grains originates from concurrent uptake by roots after flowering, not from remobilization from leaves (Chapter 3).

Can Zn allocation in the rice plant be modelled?

With the allocation of Zn in rice plants experimentally identified and some relationships quantified (Chapters 2 and 3), and in order to increase quantitative understanding of the relevant processes involved in grain zinc accumulation in rice

plants, we developed a descriptive simulation model (Chapter 4). Data from the experiments reported in Chapter 2 were used for parameterization and calibration and an independent field experiment was conducted for model validation. The descriptive model reproduced recognizable patterns of Zn mass concentrations over a wide range of observed values, created experimentally in both solution culture and soil media. Simulated grain Zn mass concentration was in satisfactory agreement with observed values, with a mean normalized gross error (MNGE) of 8–11%, and a root mean square deviation (RMSD) of 1.2–2.5 mg Zn kg^{-1} dry matter at simulated grain mass concentrations between 12 and 23 mg Zn kg^{-1}. With this model, we can generate the general picture of Zn allocation within the rice plant, with total plant Zn uptake and dry matter accumulation in the various organs as model inputs, and it might thus be used as a basis for quantification of the consequences of changes in the system, such as supplemental zinc uptake during different growth phases, or the application of supplemental Zn in some non-grain organs within the plant, on grain zinc accumulation and grain Zn mass concentration. Based on the current descriptive simulation model, steps to develop it into a more explanatory model can be defined. For such a model, additional information is required, such as on the minimum and maximum Zn transport rates between organs during different development stages, and the fraction of re-translocatable Zn from the different vegetative organs to the grains.

What indices could be suggested as screening tools for high grain Zn mass concentration in rice?

Since genotypic variation in grain Zn mass concentration exists in rice (Giordano & Mortvedt, 1974; Yang *et al*., 1998; Fageria, 2001; Gregorio, 2002; Gao *et al*., 2005), there is a need for screening tools for high grain Zn mass concentrations. However, the present indices such as grain Zn efficiency and Zn efficiency are all related to the yield of the varieties, but not to quality criteria, such as grain Zn mass concentration. Therefore, we put forward two new indices (Chapter 5): a low-Zn tolerance index for grain yield (TIY) and one for grain Zn mass concentration (TIZnMC). The merits of these indices were tested on data from two screening experiments carried out in a low-Zn soil, with or without additional Zn application, using 16 accessions of aerobic rice under greenhouse conditions and 14 accessions under field conditions. Additional data from the literature were used to further check the validity of the indices under conditions where a yield effect of Zn application was observed, as opposed to the earlier mentioned two screens. We found that TIY and TIZnMF were effective in identifying genotypes that perform well in terms of yield or grain Zn mass concentration, respectively, under both Zn-limited and Zn-sufficient conditions. So, TIY and TIZnMF appear promising for screening genotypes for a combination of good

tolerance to low Zn conditions based on both grain yield and grain Zn mass concentration, and for exploration of higher Zn availability through higher yield and grain Zn mass concentration potentials under non-stressed conditions.

What are effective approaches to increase Zn mass concentration in rice grains?

Enhancing the Zn mass concentration in staple food grains destined for human consumption is considered a sustainable, long-term solution to combat Zn malnutrition (Graham, 1984; Graham & Welch, 1996; Rengel *et al*., 1999; Frossard *et al*., 2000; Von Braun *et al*., 2005). Based on our studies, we gave a comprehensive overview of the obtained quantification of the allocation of Zn in rice and we can now discuss the potential to increase the grain Zn mass concentration in rice and the most promising approaches to increase grain Zn mass concentration in crops grown in farmers' fields. In our field experiments (Chapters 4 and 5), we found that soil Zn availability remained too low, even after Zn application, so that grain Zn mass concentration (mg Zn kg^{-1} grain) increased only marginally, and was still far below the 60–90 mg Zn kg^{-1} grain considered satisfactory for human consumption. In nutrient solution experiments, we found that to attain 60 mg Zn kg^{-1} grain, shoot Zn mass concentration should exceed 150 mg Zn kg^{-1} (Figure 1), which is 5–7 times that observed under field conditions (20–30 mg Zn kg^{-1} in the shoot). To realize the desired grain Zn mass concentration, two approaches are proposed here.

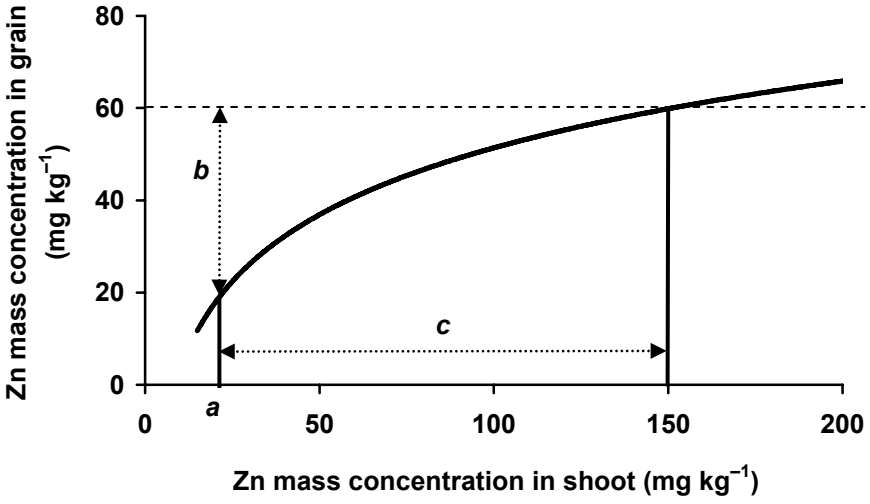

Figure 1. The relationship between shoot Zn mass concentration (mg kg^{-1}) and grain Zn mass concentration (mg kg^{-1}): *a* indicates the current Zn levels in shoot and in grain, *b* indicates the distance between current grain Zn mass concentration and the target one, *c* indicates the distance between current shoot Zn mass concentration and the target one.

One approach is to shorten *b* in Figure 1 by enhancing the Zn loading ability into grain under current plant Zn conditions (shoot Zn levels). It is found that the most rate-limiting steps to Zn transfer to the rice endosperm are those between stem and rachis, and between bran and endosperm. Therefore, breeding should focus on improving these pathways, to allocate more Zn to the grain, and thus increase zinc harvest index. In our screening experiment (Chapter 5), we found that genotypic differences exist in Zn harvest index in aerobic rice, so as a next step we could use more germplasm resources to screen for and select higher grain Zn loading ability genotypes for further breeding.

The other approach is to advance along arrow *c* in Figure 1 by improving plant zinc uptake capacity to increase Zn mass concentration in the shoot, especially during grain filling, which could be attained through breeding and/or improved soil management. In our field experiment, we only applied Zn once (before sowing); from the results of the ^{65}Zn experiment (Chapter 3) it follows that when Zn supply is sufficient, the major proportion of grain Zn comes from Zn uptake by roots after flowering. This suggests that supplying more Zn at or shortly before the flowering stage could enhance grain Zn accumulation. In wheat the grain Zn mass concentration could reach 70 mg kg^{-1} with Zn applied to both, soil and leaves just before flowering (personal communication Prof. I. Cakmak). However, in rice, the potential effects of Zn application at different stages and to both soil and leaves on grain Zn mass concentration under field conditions are still unclear and this needs to be addressed in the future, in conjunction with the economics of such applications from both a farmer's and a public health perspective. In addition, as the root Zn uptake ability must be regulated by some genes, it should be feasible to identify and tag such genes. Stacking improved uptake capacity with the earlier mentioned improved grain allocation could then lead to bigger breakthroughs than have been obtained so far in rice breeding for enhanced grain Zn levels.

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Summary

Zinc (Zn) deficiency in humans is widespread, especially in the developing world. In China, average intake of zinc is 85.6% of its Recommended Dietary Allowance. Zn deficiency is especially prevalent among the rural poor, whose diet consists mainly of cereal grains, as Zn mass concentration (mg Zn kg^{-1} dry matter) in cereal grains for human consumption is generally low. Therefore, increase in Zn mass concentration in cereal grains is being considered as a sustainable solution to human Zn deficiency. As rice is the staple food for more than half of the world's population, the research presented in this thesis aims at analysing storage, partitioning, translocation, re-allocation and grain accumulation of Zn in rice plants.

To study how Zn is allocated in rice plants and what the potential is for accumulation of zinc in rice grain, a nutrient solution and a sand culture experiment were conducted with four rice cultivars developed for aerobic cultivation (Handao297, K150, Handao502, and Baxiludao) grown at a wide range of Zn supply levels. We found that increased Zn supply resulted in increased plant Zn uptake rate throughout crop development and in higher Zn mass concentrations (ZnMC) in all plant organs, but relatively least in the grains (brown rice). It appears, therefore, that regulation of grain Zn loading differs from regulation of Zn loading to other organs. Within the rice grain, Zn mass concentration in the endosperm (polished rice) was 3–5 times lower than that in the outer layers (bran). Irrespective of the zinc mass concentration in the brown rice, around 75% of total grain Zn was present in the endosperm. The major difference in ZnMC between bran and endosperm (120 and 30 mg kg^{-1}, respectively at high Zn supply) suggests a barrier for Zn transport between the two tissues. A second barrier seems to exist between stem and rachis, as their ZnMCs also differed greatly (300 and 100 mg kg^{-1}, respectively) at high plant ZnMC. In other words, the poor zinc allocation to the endosperm limits the scope for enhancing ZnMC in rice endosperm by simply increasing the Zn supply to rice plants.

With radioactive ^{65}Zn, applied to either the roots or the flag leaf after flowering, we investigated the sources of Zn allocated to the rice grain, i.e., the relative contribution to grain Zn accumulation of Zn uptake by roots after flowering and of Zn remobilization from vegetative organs. We found that when rice plants were grown under sufficient or surplus Zn supply, most of the Zn accumulated in the grains originated from uptake by roots; under sufficient Zn supply conditions, also a substantial part of the Zn accumulated in the stem was remobilized, while remobilization from leaves contributed little Zn.

On the basis of the results of the above studies on Zn (re-)allocation in rice plants and derived quantitative relations between Zn mass concentrations in various plant organs, we developed a descriptive model for allocation and translocation of Zn among plant organs, using total plant Zn uptake and dry matter accumulation in its organs as forcing functions. The calibration and validation results showed that the descriptive model allowed reproduction of recognizable patterns of Zn mass concentrations over a wide range of observed values, created experimentally in both solution culture and soil media under controlled conditions and in the soil in a field study. Simulated grain Zn mass concentration was in satisfactory agreement with observed values, with a mean normalized gross error of 8–11%, and a root mean square error of 1.2–2.5 mg Zn kg^{-1} dry matter. Although the descriptive simulation model adequately reproduced the main patterns observed in the experiments, further testing under different conditions is necessary to build confidence in its general applicability. A more explanatory model might be developed by replacing empirical relations with more causal and/or physiologically-based relations, for processes and rates such as the minimum and maximum Zn transport and/or accumulation rates for different organs at different development stages, and the fraction translocatable Zn from different vegetative organs and the rates of translocation.

Since genotypic variation in grain Zn mass concentration exists, screening for such differences is an important breeding tool. We propose two new indices for screening: a low-Zn tolerance index for grain yield (TIY) and a low-Zn tolerance index for grain Zn mass concentration (TIZnMC), derived from drought stress research. These indices combined with existing indices (grain yield efficiency index (YEI) and grain Zn mass concentration efficiency index (ZnMCEI)), were tested with screening experiments in high- and low-Zn soils, using 16 rice accessions under greenhouse conditions and 14 rice accessions under field conditions. We found that TIY and TIZnMC were closely correlated with grain yield and grain Zn mass concentration, respectively. Therefore, TIY was effective in screening for high stability and high grain yield potential, and TIZnMC was effective in screening for grain Zn mass concentration under low and high soil Zn conditions.

In conclusion, the consequence of the observed physiological regulation is that it is difficult to enhance the Zn mass concentration in the rice grain to levels needed for improved human nutrition by simply increasing Zn supply. Under field conditions, where plant Zn mass concentrations are very low, there is some scope for increasing Zn uptake and thereby slightly increasing grain ZnMC, but the increase will be limited to the level at which further grain Zn accumulation seems to be down-regulated. To attain higher ZnMCs, one approach would be to enhance the Zn loading ability into grain under normal plant Zn conditions; the other approach is to improve Zn uptake

under aerobic conditions to increase Zn mass concentration in the shoot, especially during grain filling, which could be attained through breeding and/or improved soil management. Arguably, a combination of both approaches is the best way forward but this necessitates disentangling them during further breeding and physiological research.

Samenvatting

Gebrek aan zink (Zn) bij mensen is een veel voorkomend probleem, met name in ontwikkelingslanden. In China is de gemiddelde dagelijkse zink inname ca 85% van de aanbevolen hoeveelheid. Zn-gebrek komt vooral veel voor onder de plattelands-bevolking waarvoor granen het hoofdbestanddeel vormen van de dagelijkse voeding, aangezien de Zn-massaconcentratie (mg Zn kg^{-1} droge stof) in deze granen over het algemeen erg laag is. Het verhogen van de Zn-massaconcentratie in granen wordt daarom gezien als een duurzame oplossing voor Zn-gebrek bij mensen. Omdat rijst het hoofdvoedsel is voor meer dan de helft van de wereldbevolking, is het doel van het in dit proefschrift beschreven onderzoek om de opslag, de verdeling en herverdeling en de accumulatie van Zn in de korrel te analyseren.

Om te bestuderen hoe Zn in rijstplanten wordt verdeeld over de verschillende organen en wat de capaciteit is voor accumulatie in de korrel, werden twee experimenten uitgevoerd bij sterk uiteenlopende Zn bemestingsniveaus met in totaal vier rijstrassen die ontwikkeld zijn voor de teelt onder aerobe bodemomstandigheden (Handao297, K150, Handao502 en Baxiludao); in het ene experiment werd rijst op een voedingsoplossing geteeld, en in het andere in zand waaraan voedingsoplossing werd toegevoegd. We vonden dat een groter aanbod van Zn leidde tot een hogere opname door de planten gedurende de gehele groeiperiode en tot hogere Zn-massaconcentraties (ZnMC) in alle onderscheiden plantenorganen, zij het relatief het minst in de korrels ('bruine rijst'). Hieruit werd geconcludeerd dat de accumulatie in de korrel en die in de overige organen op een verschillende manier wordt gereguleerd. Binnen de rijstkorrel was de Zn-massaconcentratie in het endosperm (de gepolijste korrel) 3–5 keer lager dan in de buitenste lagen van de korrel (het slijpsel of de vliesjes). Onafhankelijk van de Zn-massaconcentratie in de bruine rijst, bevond zich ruwweg 75% van de totale hoeveelheid Zn in de korrel in het endosperm. Het grote verschil in ZnMC tussen slijpsel en endosperm (respectievelijk 120 en 30 mg kg^{-1} bij een hoog aanbod van Zn) maakt aannemelijk dat er een barrière is voor Zn-transport tussen deze beide weefsels. Tussen stengel en rachis lijkt zich een tweede barrière te bevinden, aangezien bij hoge massaconcentraties in de totale plant, ook tussen deze twee organen grote verschillen in ZnMC werden gevonden (respectievelijk 300 en 100 mg kg^{-1}). Met andere woorden, de belemmeringen voor transport naar het endosperm beperken de mogelijk-heden om de ZnMC in het endosperm van rijst te verhogen door enkel de zinkvoorziening van de plant te verbeteren.

We hebben de herkomst onderzocht van het Zn in de graankorrels, d.w.z. de relatieve bijdrage van Zn opgenomen door de wortels na de bloei en van Zn dat

herverdeeld werd vanuit de vegetatieve organen, door tijdens en na de bloei radioactief ^{65}Zn toe te dienen aan ofwel de wortels ofwel het vlagblad. We vonden dat wanneer rijstplanten met voldoende of met een overmaat aan beschikbaar zink werden geteeld, het grootste deel van het zink in de korrels direct vanuit de wortels werd aangevoerd; bij teelt onder omstandigheden met voldoende beschikbaar zink was ook een aanzienlijk deel afkomstig uit herverdeling vanuit de stengel, terwijl herverdeling vanuit de bladeren geen rol van betekenis speelde.

Op basis van de resultaten van bovengenoemde studies naar de (her-) verdeling van Zn in rijstplanten en van de hieruit afgeleide kwantitatieve relaties tussen de Zn-massaconcentratie in de gehele plant en die in de verschillende organen hebben we een beschrijvend model ontwikkeld voor de verdeling en herverdeling van Zn tussen de verschillende plantenorganen, waarin de opname van zink door de plant en de toename in drooggewicht van de organen als invoergegevens worden gebruikt. De resultaten van de calibratie en de validatie toonden aan dat het model de waargenomen veranderingen in Zn-massaconcentraties in de proeven met voedingsoplossing en met zandculturen in potten, uitgevoerd onder gecontroleerde omstandigheden en die uitgevoerd onder veldomstandigheden, op bevredigende wijze kon reproduceren. De gesimuleerde Zn-massaconcentraties waren in redelijke overeenstemming met de waargenomen waarden, met een gemiddelde genormaliseerde bruto-fout (Mean Normalized Gross Error) van 8–11%, en een standaardafwijking (Root Mean Square Error) van 1.2–2.5 mg Zn kg^{-1} drogestof. Hoewel het beschrijvende simulatiemodel de belangrijkste trends uit de experimenten redelijk kon reproduceren, moet het meer uitgebreid worden getest onder verschillende omstandigheden, om het vertrouwen in de meer algemene toepasbaarheid te doen groeien. Een meer verklarend model zou ontwikkeld kunnen worden door de empirische relaties te vervangen door meer verklarende en/of meer op fysiologische inzichten gestoelde verbanden voor verschillende processen en snelheden, zoals de minimale en maximale transport-snelheden naar of accumulatiesnelheden in de verschillende organen tijdens verschillende fenologische ontwikkelingsstadia, en de fractie van de Zn die voor herverdeling beschikbaar is vanuit de verschillende vegetatieve organen, alsmede de snelheid waarmee die beschikbaar komt.

Aangezien er genotypische variatie bestaat in de Zn-massaconcentratie van rijstkorrels, is een testprotocol voor deze verschillen een belangrijk middel bij de veredeling. We stellen hiertoe, naar analogie van methoden uit onderzoek naar droogtegevoeligheid van planten, twee nieuwe indices voor: een de index voor tolerantie voor lage Zn-niveaus t.a.v. de korrelopbrengst (TIY) en een andere t.a.v. de Zn-massaconcentratie in de korrel (TIZnMC). In combinatie met bestaande indices (de index voor efficiëntie van de korrelopbrengst (YEI) en de index voor efficiëntie van de

Zn-massaconcentratie in de korrel (ZnMCEI)) zijn de nieuwe indices getoetst aan de hand van twee experimenten waarin 16 rijstlijnen in potten werden geteeld in een kas en 14 rijstlijnen in het veld, beide in een grond die arm was aan zink en waaraan al dan niet extra zink was toegevoegd. We vonden dat TIY en TIZnMC nauw gecorreleerd waren met respectievelijk de korrelopbrengst en de Zn-massaconcentratie in de korrel. Hieruit bleek dat TIY een effectieve indicator was voor een stabiele en in potentie hoge korrelopbrengst en TIZnMC een effectieve indicator voor een relatief hoge Zn-massaconcentratie in de korrel, onder zowel Zn-beperkte omstandigheden als omstandigheden met adequate Zn-voorziening.

Concluderend lijkt de consequentie van de waargenomen fysiologische regulatie van zinktransport in de rijstplant te zijn dat het moeilijk is om de Zn-massa-concentratie in rijstkorrels te verhogen tot het voor menselijke voeding gewenste niveau alleen door verbetering van de zinkvoorziening tijdens de teelt. Onder veldomstandigheden, waar de Zn-massaconcentraties in het gewas erg laag zijn, is er wel enige ruimte om de zinkopname te verhogen en daarmee ook de ZnMC in de korrel, maar deze toename gaat niet verder dan het niveau waarop verdere toename in accumulatie van zink in de korrel wordt belemmerd door negatieve terugkoppelings-mechanismen. Om hogere ZnMCs in de korrel te bereiken zou ten eerste gezocht kunnen worden naar verhoging van de Zn opnamecapaciteit van de korrels bij normale zink massaconcentraties in de rijstplant, terwijl een alternatieve aanpak zou kunnen zijn om de Zn opname uit de bodem onder aerobe omstandigheden te verhogen met als doel de Zn-massaconcentratie in de bovengrondse delen te verhogen, met name tijdens de korrelvullingsperiode, hetgeen bereikt kan worden middels veredeling en/of door verbeterd beheer van de bodem. Hoewel open voor discussie, lijkt een combinatie van deze beide de beste strategie om verbeteringen te realiseren, waarbij de beide elementen wel gescheiden moeten worden geanalyseerd tijdens veredeling en fysiologisch onderzoek.

概要

人类锌的缺乏非常普遍，尤其是在一些发展中国家。在中国，人均锌日摄入量仅达到推荐摄入量的 85.6%。在一些乡村贫困地区，人们缺锌相对更普遍，因为在这些地区，人们的的饮食主要是一些谷类作物，而谷类作物籽粒可食用部分锌含量(mg kg^{-1})普遍比较低。因此，提高这部分锌的含量是解决人类锌缺乏的一项可持续措施。稻米是世界半数以上人口的主要粮食, 本论文主要研究锌在旱稻体内的储存、运转分配及籽粒部位锌的积累。

为了研究锌在旱稻体内运转分配以及籽粒锌积累的潜力，分别在水培和砂培条件下，以旱稻 297、K150、旱稻 502 和巴西陆稻为材料，进行不同供锌水平处理。 研究发现旱稻植株对锌的吸收随着供锌水平提高而增加，各器官锌的含量也随之不同程度地提高，但是籽粒中锌含量增加的幅度最小。因此，在对锌的运转调控机制上籽粒可能与其它器官不同。研究发现在籽粒内部，胚乳（精米）锌的含量比籽粒外皮层低 3–5 倍，但是对糙米来说，籽粒所有的锌约有 75%储存在胚乳中。籽粒外层和胚乳锌含量(高锌供给水平下分别为 120，30 mg kg^{-1})的显著差异表明在锌在从籽粒外层向内部胚乳运转过程中存在某种障碍。另外，锌从茎部向穗节部位运转过程中也存在障碍，因为两者锌的含量也存在显著差异（高锌供给水平下分别为 300，100 mg kg^{-1}）。换句话说，锌向籽粒内部胚乳运转的障碍最终限制了仅靠增加外源锌的供给来提高籽粒锌的含量的幅度。

通过开花期 ^{65}Zn 根部和叶片（旗叶和老叶）外施，研究了在旱稻植株体内锌含量不缺乏或者有多余情况下，籽粒锌积累主要来源。研究发现，籽粒锌积累主要来自于花后根部对锌的吸收，或者茎部锌的再运转 (不包括体内锌有多余情况下)，而极少来自叶片锌的再分配。

在以上对旱稻体内锌的运转分配（再分配）途径的研究，以及对植株各器官锌含量定量关系基础上，建立了有关锌在旱稻体内各器官运转分配的描述性模型，以根部不同时期锌的吸收以及植株干物质的积累为输入函数。分别以不同锌供给水平的水培试验和田间试验对该模型进行校正和验证，将模拟值和实测值进行比较，两者拟合程度较好。其中，籽粒锌含量模拟值跟实测值比较吻合，MNGE（mean normalized gross error）为 8–11%, RMSD (root mean square deviation)为 1.2–2.5 mg kg^{-1}。尽管该描述性模型模拟结果比较好，但是仍需要在其它试验条件下对该模型的适用性进一步验证。另外，对锌在各器官各时期的最大和最小运输或积累速率以及各营养器官可再分配锌的比例及再运转分配的速率等需要进一步研究确定，建立在生理基础定量化的解释性模型有待于进一步构建，来替代目前建立在经验性数量关系基础上的描述性模型。

已有研究表明旱稻基因型间籽粒锌含量存在显著差异，所以对旱稻进不同基因型间籽粒锌含量进行筛选是旱稻籽粒高锌育种的一项重要途径。本论文根据

前人有关抗旱胁迫筛选研究提出了两个分别以产量和籽粒锌含量为标准的旱稻耐低锌筛选指标，即 TIY （low-Zn tolerance index for grain yield）和 TIZnMC （low-Zn tolerance index for grain Zn mass concentration），本研究利用在温室条件下（16 个品种）和田间条件下（14 个品种）的旱稻籽粒锌筛选试验分别来对上述筛选指标以及目前已被广泛应用的其它相关指标如 YEI （grain yield efficiency index）和 ZnMCEI（grain yield efficiency index and grain Zn mass concentration efficiency index）进行比较验证。研究表明 TIY 和 TIZnMC 分别和籽粒产量、籽粒锌含量成显著相关，表明 TIY 指标可以用来有效地对旱稻品种间的耐低锌（以产量为标准）以及高锌条件下的产量潜力进行筛选鉴定，而 TIZnMC 可以用来有效地用来分别在低锌和高锌条件下籽粒高锌旱稻品种进行鉴定。

简而言之，对锌在旱稻体内的运输分配途径以及生理调控研究表明在仅靠增加外源锌的供给量来提高旱稻籽粒锌的含量，达到人体对锌的需求水平是很难实现的。在一般田间条件下，植株体内锌的含量非常低，虽然补施锌肥可以在一定程度上增加籽粒锌的含量，但是增加幅度很小。所以要进一步强化旱稻籽粒锌的含量，一个途径可以在目前植株体内锌含量水平基础上加强锌向籽粒部位的运转;另外一个途径可以通过田间土壤环境管理或者育种来增加旱稻植株体内锌的含量，尤其是开花后。当然，将这两途径进行结合应该是最好的方式，但是在结合之前有必要在育种和生理基础上对每个途径分别进行进一步的研究。

Appendix 1

Listing of the model

```
*---------------------------------------------------------------------*
*                    A model for Zn partitioning in rice              *
*                              February 2008                          *
*                              FSTWin-Version                         *
*                                                                     *
* Crop and Weed Ecology Group,                                        *
* Wageningen University, Wageningen, PO Box 430, 6700 AK Wageningen   *
* The Netherlands                                                     *
*                                                                     *
* Plant Research International,                                       *
* Wageningen University and Research Centre, P.O. Box 16,             *
* 6700 AK Wageningen, The Netherlands                                 *
*                                                                     *
* Qingdao Agricultural University, 266109 Qingdao, China              *
*                                                                     *
* Experimental data: Parameters and Functions from solution culture   *
*                  experiment 2005, Chapters 2 and 4                  *
* Aerobice rice  cv. BaxiLudao                                        *
* Target Zn supply level: 10mg Zn per kg dry matter                   *
*---------------------------------------------------------------------*

***1.  Initial conditions

INITIAL
INCON IWRT  = 0.001;  IWST  = 0.001; IWLV  = 0.001;   IWSH = 0.001
INCON IWSLV = 0.001;  IWSSH = 0.001; IZUP  = 0.001
INCON IWRAC = 0.001;  IWGLU = 0.01;  IWGR  = 0.001
INCON IZRT  = 0.;     IZST  = 0.;    IZLV  = 0.;      IZSH = 0.
INCON IZSLV = 0.;     IZSSH = 0.;    IZRAC = 0.
INCON IZGLU = 0.;     IZGR  = 0.
PARAMETER TCTR = 5.;  RESZ= 15.

***2.  Observed values

*** Section 1: Growth of the individual rice organs over time
***            for comparison with simulated data.

FUNCTION XWRTT   =  0.,0.00, 45.,0.54,  55.,1.09,  85.,2.14, ...
                                       100.,1.77, 115.,1.51
FUNCTION XWSTT   =  0.,0.00, 45.,0.00,  55.,0.00,  85.,3.49, ...
                                       100.,2.69, 115.,3.13
FUNCTION XWLVT   =  0.,0.00, 45.,1.59,  55.,3.14,  85.,5.18, ...
                                       100.,3.89, 115.,3.10
FUNCTION XWSHT   =  0.,0.00, 45.,1.08,  55.,2.36,  85.,3.85, ...
                                       100.,3.08, 115.,2.2
FUNCTION XWSLVT  =  0.,0.00, 45.,0.00,  55.,0.00,  85.,0.85, ...
                                       100.,1.45, 115.,1.42
FUNCTION XWSSHT  =  0.,0.00, 45.,0.00,  55.,0.00,  85.,0.67, ...
                                       100.,1.08, 115.,1.04
FUNCTION XWRACT  =  0.,0.00, 45.,0.00,  55.,0.00,  85.,0.75, ...
                                       100.,0.35, 115.,0.34
```

```
FUNCTION XWGLUT  =  0.,0.00, 45.,0.00,  55.,0.00,  85.,0.50, ...
                                       100.,1.90, 115.,1.30
FUNCTION XWGRT   =  0.,0.00, 45.,0.00,  55.,0.00,  85.,0.00, ...
                                       100.,3.38, 115.,6.09
FUNCTION XTWT    =  0.,0.00, 45.,3.22,  55., 6.59,  85.,17.42, ...
                                       100.,19.59, 115.,21.45

*** Section 2:  Total Zn uptake of plants over time
***             for comparison with simulated data.
FUNCTION XZUPT   =  0.,0.000, 45., 160.56, 55., 326.66, 85., 620.18, ...
                                          100., 719.86, 115., 670.08

*** Section 3:  Zn mass concentrations in individual plant organs over time
***             for comparison with simulated data.
FUNCTION XZRTT   = 0.,0.000, 45.,65.66,  55.,48.32,  85.,70.19, ...
                                       100.,54.75, 115.,59.04
FUNCTION XZSTT   = 0.,0.000, 45.,0.000,  55.,0.000,  85.,19.01, ...
                                       100.,18.92, 115.,22.32
FUNCTION XZLVT   = 0.,0.000, 45.,38.01,  55.,30.77,  85.,27.43, ...
                                       100.,28.66, 115.,23.86
FUNCTION XZSHT   = 0.,0.000, 45.,58.73,  55.,75.96,  85.,18.20, ...
                                       100.,18.10, 115.,22.46
FUNCTION XZRACT  = 0.,0.000, 45.,0.000,  55.,0.000,  85.,38.64, ...
                                       100.,20.14, 115.,27.05
FUNCTION XZGLUT  = 0.,0.000, 45.,0.000,  55.,0.000,  85.,38.64, ...
                                       100.,17.35, 115.,24.61
FUNCTION XZGRT   = 0.,0.000, 45.,0.000,  55.,0.000,  85.,0.000, ...
                                       100.,27.86, 115.,21.63
FUNCTION XPZMCT  = 0.,0.000, 45.,49.75,  55.,49.78,  85.,35.6, ...
                                       100.,36.67, 115.,33.47

*** 3. Run control

TIMER STTIME =  0.; FINTIM = 115.; DELT = 1.; PRDEL = 3.
PRINT AZRT, AZST, AZSH, AZLV, AZRAC, AZGLU, AZGR

TRANSLATION_GENERAL DRIVER='EUDRIV'


****4. Dynamic condition

DYNAMIC

*** Section 1

*   Dry weights
WRT   = INTGRL (IWRT,  GRT)
WST   = INTGRL (IWST,  GST)
WLV   = INTGRL (IWLV,  GLV)
WSH   = INTGRL (IWSH,  GSH)
WSLV  = INTGRL (IWSLV, GSLV)
WSSH  = INTGRL (IWSSH, GSSH)
WRAC  = INTGRL (IWRAC, GRAC)
WGLU  = INTGRL (IWGLU, GGLU)
WGR   = INTGRL (IWGR,  GGR)
TW    = WRT + WST + WLV+ WSH+ WSLV+ WSSH+ WRAC+ WGLU+ WGR
```

```
*   Growth rates
GRT   = INSW(TIME-45. ,  0.0144, GGRT)
GGRT  = INSW(TIME-85. , -0.001*TIME + 0.1033, TGRT)
TGRT  = INSW(TIME-100., -0.0247, -0.0174)

GST   = INSW(TIME-55. ,  0., GGST)
GGST  = INSW(TIME-85. ,  0.1162, SGST )
SGST  = INSW(TIME-100., -0.0534, 0.0294)

GLV   = INSW(TIME-45.,  0.0442, GGLV)
GGLV  = INSW(TIME-85., -0.0044*TIME+ 0.3727, 0.0022*TIME - 0.2911)

GSH   = INSW(TIME-10., 0.004, OGSH)
OGSH  = INSW(TIME-45., 0.0298, GGSH )
GGSH  = INSW(TIME-55., 0.1274, TGSH)
TGSH  = INSW(TIME-85., 0.0497,-0.0547)

GSLV  = INSW(TIME-55. , 0., GGSLV)
GGSLV = INSW(TIME-100., 0.0317, -0.0022)

GSSH  = INSW(TIME-55. , 0., GGSSH )
GGSSH = INSW(TIME-100., 0.0238,-0.0031)

GRAC  = INSW(TIME-55. ,  0., GGRAC)
GGRAC = INSW(TIME-85. ,  0.0252, SGRAC)
SGRAC = INSW(TIME-100., -0.0273, -0.0005)

GGLU  = INSW(TIME-55. , 0., GGGLU)
GGGLU = INSW(TIME-85. , 0.0168, OGGLU)
OGGLU = INSW(TIME-100., 0.0931, -0.0398)

GGR   = INSW(TIME-85. , 0., -0.003*TIME + 0.5033)


*** Section 2

*   Daily Zn uptake from the soil
ZUP   = INTGRL(IZUP, RZUP)
RZUP  = INSW(TIME-10. ,   0.3, RRZUP)
RRZUP = INSW(TIME-45. ,   0.287*TIME - 3.3881, TRZUP)
TRZUP = INSW(TIME-85. , -0.3412*TIME + 33.673, SRZUP)
SRZUP = INSW(TIME-100.,  6.6453,  -2.5104)

*   Zn mass concentration of the total plant (root plus shoot)
PZMC  = FCNSW(TIME, 0., 0.,PZMCF)
PZMCF = ZUP/TW

*   Target Zn mass concentrations of the different individual organs
*   as a function of PZMC
TZRT  = INSW(PZMC-100., INSW(TIME-56. , 1.196*PZMC+ 5., STZRT), ...
                        INSW(TIME-56. , 1.196*PZMC+ 5., STZRT))
STZRT = INSW(PZMC-100., INSW(TIME-86. , 1.920*PZMC-20., OTZRT), ...
                        INSW(TIME-86. , 2.064*PZMC-107.53, OTZRT))
OTZRT = INSW(PZMC-100., INSW(TIME-101., 2.339*PZMC- 25., ...
                2.339*PZMC- 25.), INSW(TIME-101.,3.658*PZMC-221.61, ...
                3.477*PZMC-165.29))
```

```
TZST  = INSW(TIME-56.,0., STZST)
STZST = INSW(PZMC-100., INSW(TIME-86., 10.*EXP(0.024*PZMC), OTZST), ...
                        INSW(TIME-86., 1.570*PZMC-5.731,OTZST))
OTZST = INSW(PZMC-100., INSW(TIME-101., 7.850*EXP(0.033*PZMC), ...
            7.850*EXP(0.033*PZMC)), INSW(TIME-101., 2.055*PZMC-7.913, ...
            1.433*PZMC + 103.55))

TZLV  = INSW(PZMC-100., INSW(TIME-46., 12.407*LOG(NOTNUL(PZMC))- ...
                13.32, OTZLV), INSW(TIME-46., 0.336*PZMC+5.869, STZLV))
STZLV = INSW(PZMC-100., INSW(TIME-56., 13.828*LOG(NOTNUL(PZMC))- ...
                22.403,OTZLV), INSW(TIME-56., 0.301*PZMC+ 5.515, OTZLV))
OTZLV = INSW(PZMC-100., INSW(TIME-86., 18.965*LOG(NOTNUL(PZMC))- ...
             38.561,TTZLV), INSW(TIME-86., 0.153*PZMC+ 34.242,TTZLV))
TTZLV = INSW(PZMC-100.,INSW(TIME-101., ...
           9.738*LOG(NOTNUL(PZMC))-6.164,7.876*LOG(NOTNUL(PZMC)) - ...
           2.883), INSW(TIME-101., 0.063*PZMC+32.344, 0.235*PZMC+8.334))

TZSH  = INSW(PZMC-100.,INSW(TIME-46., 17.052*EXP(0.023*PZMC), STZSH), ...
                       INSW(TIME-46., 1.807*PZMC+2.944, STZSH))
STZSH = INSW(PZMC-100., INSW(TIME-56. , 21.206*EXP(0.022*PZMC), ...
                OTZSH), INSW(TIME-56. , 1.648*PZMC+ 18.061, OTZSH))
OTZSH = INSW(PZMC-100., INSW(TIME-86. , 12.543*EXP(0.0195*PZMC), ...
                TTZSH), INSW(TIME-86. , 1.126*PZMC+ 0.585, TTZSH))
TTZSH = INSW(PZMC-100., INSW(TIME-101., 8.377*EXP(0.019*PZMC), ...
            13.391*EXP(0.017*PZMC)), INSW(TIME-101.,0.785*PZMC+4.493, ...
             1.481*PZMC- 72.65))

TZRAC  = INSW(TIME-55., 0., STZRAC)
STZRAC = INSW(PZMC-100., INSW(TIME-86., 0.358*PZMC+ 25.866,OTZRAC),  ...
                         INSW(TIME-86., 0.144*PZMC+ 49.059,OTZRAC))
OTZRAC = INSW(PZMC-100., INSW(TIME-101., 0.613*PZMC,  0.600*PZMC-   ...
           5.6778),INSW(TIME-101., 0.871*PZMC-2.791, 0.274*PZMC+70.436))

TZGLU  = INSW(TIME-55., 0., STZGLU)
STZGLU = INSW(TIME-101.,  27.568*LOG(NOTNUL(PZMC))- 69.102,...
                          21.307*LOG(NOTNUL(PZMC))- 47.842)

TZGR   = INSW(TIME-86., 0.,STZGR)
STZGR  = INSW(TIME-101., 11.073*LOG(NOTNUL(PZMC))- 16.012,...
                         15.029*LOG(NOTNUL(PZMC))- 29.555)

*** Section 3

*   Zn demand (ZDorgan)
ZDRT  =  MAX(0., TZRT  * WRT  - AZURT)
ZDST  =  MAX(0., TZST  * WST  - AZUST)
ZDLV  =  MAX(0., TZLV  * WLV  - AZULV)
ZDSH  =  MAX(0., TZSH  * WSH  - AZUSH)
ZDRAC =  MAX(0., TZRAC * WRAC - AZURAC)
ZDGLU =  MAX(0., TZGLU * WGLU - AZUGLU)
ZDGR  =  MAX(0., TZGR  * WGR  - AZUGR)

*   Total Zn demand (TZD)
TZD   =  ZDRT + ZDST + ZDLV + ZDSH +ZDRAC + ZDGLU + ZDGR

*   Fraction based on the Zn demand  (FZorgan)
FZRT  = FCNSW(TZD, 0., 0., ZDRT/  NOTNUL(TZD))
```

```
FZLV  = FCNSW(TZD, 0., 0., ZDLV/  NOTNUL(TZD))
FZST  = FCNSW(TZD, 0., 0., ZDST/  NOTNUL(TZD))
FZSH  = FCNSW(TZD, 0., 0., ZDSH/  NOTNUL(TZD))
FZRAC = FCNSW(TZD, 0., 0., ZDRAC/ NOTNUL(TZD))
FZGLU = FCNSW(TZD, 0., 0., ZDGLU/ NOTNUL(TZD))
FZGR  = FCNSW(TZD, 0., 0., ZDGR/  NOTNUL(TZD))
FT    = FZRT+ FZLV+ FZST+ FZSH+ FZRAC + FZGLU + FZGR

*   Translocatable zinc from individual organ (TRZorgan)
TRZRT  = MAX(0., AZURT - TZRT * WRT)
TRZST  = MAX(0., AZUST  - TZST  * WST)
TRZLV  = MAX(0., AZULV  - TZLV  * WLV)
TRZSH  = MAX(0., AZUSH  - TZSH  * WSH)
TRZRAC = MAX(0., AZURAC - TZRAC * WRAC)
TRZGLU = MAX(0., AZUGLU - TZGLU * WGLU)

*   Total translocatable Zn from individual life organs (TRZTL)
TRZTL  = (TRZRT + TRZST +TRZRAC+ TRZGLU +TRZLV+ TRZSH)/TCTR

*   Total translocatable Zn from senescing leafs and sheaths (TRZTS)
TRZTS  = MAX(0.,MIN(AZUSSL-(WSLV+WSSH)*RESZ,TZD-RZUP*DELT- ...
               TRZTL))/TCTR

*   Total Zn for partitioning (TZTR)
TZTR   = INSW(TZD-RZUP*DELT,TZD, TZTRP)
TZTRP  = MIN(TZD, RZUP*DELT + TRZTL + TRZTS)

*   Allocation of Zn to each organ (ZUPorgan)
ZUPRT  = TZTR * FZRT + MAX(0.,RZUP*DELT-TZD)
ZUPST  = TZTR * FZST
ZUPLV  = TZTR * FZLV
ZUPSH  = TZTR * FZSH
ZUPRAC = TZTR * FZRAC
ZUPGLU = TZTR * FZGLU
ZUPGR  = TZTR * FZGR

*   Net Zn flow to each organ (NZorgan)
NZRT   = ZUPRT  - TRZRT/ TCTR
NZST   = ZUPST  - TRZST/ TCTR
NZLV   = ZUPLV  - TRZLV/ TCTR
NZSH   = ZUPSH  - TRZSH/ TCTR
NZRAC  = ZUPRAC - TRZRAC/ TCTR
NZGLU  = ZUPGLU - TRZGLU/ TCTR
NZGR   = ZUPGR

*   Cumulative net Zn flow to each organ (AZUorgan)
AZURT  = INTGRL(IZRT, NZRT)
AZUST  = INTGRL(IZST, NZST)
AZULV  = INTGRL(IZLV, NZLV)
AZUSH  = INTGRL(IZSH, NZSH)
AZURAC = INTGRL(IZRAC, NZRAC)
AZUGLU = INTGRL(IZGLU, NZGLU)
AZUGR  = INTGRL(IZGR, NZGR)
ATZUP  = AZURT + AZUST + AZULV + AZUSH + AZURAC + AZUGLU + AZUGR
AZUSSL = ZUP - ATZUP
```

```
*   Zn mass concentration in each organ (AZorgan)
AZRT   =   MAX(0., AZURT / WRT)
AZST   =   MAX(0., AZUST / WST)
AZLV   =   MAX(0., AZULV / WLV)
AZSH   =   MAX(0., AZUSH / WSH)
AZRAC  =   MAX(0., AZURAC/ WRAC)
AZGLU  =   MAX(0., AZUGLU/ WGLU)
AZGR   =   MAX(0., AZUGR / WGR)

*** Section 4

*   Observed values (Zn mass concentration in organs for comparison with
*   simulated values)
XZRT   = AFGEN(XZRTT , TIME)
XZST   = AFGEN(XZSTT , TIME)
XZLV   = AFGEN(XZLVT , TIME)
XZSH   = AFGEN(XZSHT , TIME)
XZRAC  = AFGEN(XZRACT, TIME)
XZGLU  = AFGEN(XZGLUT, TIME)
XZGR   = AFGEN(XZGRT , TIME)

*   Observed values (Dry weight of each organ) for comparison with
*   simulated values)
XWRT   = AFGEN(XWRTT,  TIME)
XWST   = AFGEN(XWSTT,  TIME)
XWLV   = AFGEN(XWLVT,  TIME)
XWSH   = AFGEN(XWSHT,  TIME)
XWSLV  = AFGEN(XWSLVT, TIME)
XWSSH  = AFGEN(XWSSHT, TIME)
XWRAC  = AFGEN(XWRACT, TIME)
XWGLU  = AFGEN(XWGLUT, TIME)
XWGR   = AFGEN(XWGRT,  TIME)
XTW    = AFGEN(XTWT,   TIME)

*   Observed values (Total Zn uptake) for comparison with
*   simulated values)
XZUP   = AFGEN(XZUPT,  TIME)

END
STOP
ENDJOB
```

Appendix 2

List of variables used in the model

ATZUP	Total Zn content in live organs	µg plant^{-1}
AZGLU	Zn mass concentrations of glume	µg g^{-1}
AZGR	Zn mass concentrations of grain	µg g^{-1}
AZLV	Zn mass concentrations of leaves	µg g^{-1}
AZRAC	Zn mass concentrations of rachis	µg g^{-1}
AZRT	Zn mass concentrations of root	µg g^{-1}
AZSH	Zn mass concentrations of sheath	µg g^{-1}
AZST	Zn mass concentrations of stem	µg g^{-1}
AZUGLU	Cumulative net Zn flow for glume	µg plant^{-1}
AZUGR	Cumulative net Zn flow for grain	µg plant^{-1}
AZULV	Cumulative net Zn flow for leaves	µg plant^{-1}
AZURAC	Cumulative net Zn flow for rachis	µg plant^{-1}
AZURT	Cumulative net Zn flow for root	µg plant^{-1}
AZUSH	Cumulative net Zn flow for sheath	µg plant^{-1}
AZUSSL	Cumulative net Zn flow for senescing leaf and sheath	µg plant^{-1}
AZUST	Cumulative net Zn flow for stem	µg plant^{-1}
FZGLU	Fraction of Zn for partitioning to glume	-
FZGR	Fraction of Zn for partitioning to dehulled grain	-
FZLV	Fraction of Zn for partitioning to leaf blade	-
FZRAC	Fraction of Zn for partitioning to rachis	-
FZRT	Fraction of Zn for partitioning to root	-
FZSH	Fraction of Zn for partitioning to leaf sheath	-
FZST	Fraction of Zn for partitioning to stem	-
GGGLU	Intermediate variable	-
GGLU	Glume growth rate/lost	g plant^{-1} d^{-1}
GGLV	Intermediate variable	-
GGR	Dehulled grain growth rate	g plant^{-1} d^{-1}
GGRAC	Intermediate variable	-
GGRT	Intermediate variable	-
GGSH	Intermediate variable	-
GGSLV	Intermediate variable	-
GGSSH	Intermediate variable	-
GGST	Intermediate variable	-

GLV	Green leaf growth/lost rate	g plant^{-1} d^{-1}
GRAC	Rachis growth rate/lost	g plant^{-1} d^{-1}
GRT	Root growth rate	g plant^{-1} d^{-1}
GSH	Green sheath growth rate/lost	g plant^{-1} d^{-1}
GSLV	Senescent leaf blade increasing rate	g plant^{-1} d^{-1}
GSSH	Senescent leaf sheath increasing rate	g plant^{-1} d^{-1}
GST	Stem growth rate/lost rate	g plant^{-1} d^{-1}
IWGLU	Initial dry weight of glume	g plant^{-1}
IWGR	Initial dry weight of dehulled grain	g plant^{-1}
IWLV	Initial dry weight of green leaf blade	g plant^{-1}
IWRAC	Initial dry weight of rachis	g plant^{-1}
IWRT	Initial dry weight of root	g plant^{-1}
IWSH	Initial dry weight of green leaf sheath	g plant^{-1}
IWSLV	Initial dry weight of senescent leaf blade	g plant^{-1}
IWSSH	Initial dry weight of senescent leaf sheath	g plant^{-1}
IWST	Initial dry weight of stem	g plant^{-1}
IZGLU	Initial zinc mass concentration of glume	µg plant^{-1}
IZGR	Initial zinc mass concentration of dehulled grain	µg plant^{-1}
IZLV	Initial zinc mass concentration of green leaf blade	µg plant^{-1}
IZRAC	Initial zinc mass concentration of rachis	µg plant^{-1}
IZRT	Initial zinc mass concentration of root	µg plant^{-1}
IZSH	Initial zinc mass concentration of green leaf sheath	µg plant^{-1}
IZSLV	Initial zinc mass concentration of senescent leaf blade	µg plant^{-1}
IZSSH	Initial zinc mass concentration of senescent leaf sheath	µg plant^{-1}
IZST	Initial zinc mass concentration of senescent leaf sheath	µg plant^{-1}
IZUP	Initial zinc uptake	µg plant^{-1}
NZGLU	Net flow of Zn to glume	µg plant^{-1}
NZGR	Net flow of Zn to grain	µg plant^{-1}
NZLV	Net flow of Zn to leaves	µg plant^{-1}
NZRAC	Net flow of Zn to rachis	µg plant^{-1}
NZRT	Net flow of Zn to root	µg plant^{-1}
NZSH	Net flow of Zn to sheath	µg plant^{-1}
NZST	Net flow of Zn to stem	µg plant^{-1}
OTZLV	Intermediate variable	-
OTZRAC	Intermediate variable	-
OTZRT	Intermediate variable	-
OTZSH	Intermediate variable	-
OTZST	Intermediate variable	-

PZMC	Zn mass concentration of the total plant	$\mu g\ g^{-1}$
RESZ	Residual zinc in organs not available for retranslocation	$\mu g\ plant^{-1}$
RRZUP	Intermediate variable	-
RZUP	Daily rates of Zn uptake by the root	$\mu g\ plant^{-1}\ d^{-1}$
SGRAC	Intermediate variable	-
SGST	Intermediate variable	-
SRZUP	Intermediate variable	-
STZGLU	Intermediate variable	-
STZGR	Intermediate variable	-
STZLV	Intermediate variable	-
STZRAC	Intermediate variable	-
STZRT	Intermediate variable	-
STZSH	Intermediate variable	-
STZST	Intermediate variable	-
TCTR	Time constant for re-translocation	d
TGRT	Intermediate variable	-
TGSH	Intermediate variable	-
TRZGLU	Translocatable Zn from glume	$\mu g\ plant^{-1}$
TRZLV	Translocatable Zn from leave	$\mu g\ plant^{-1}$
TRZRAC	Translocatable Zn from rachis	$\mu g\ plant^{-1}$
TRZRT	Translocatable Zn from root	$\mu g\ plant^{-1}$
TRZSH	Translocatable Zn from sheath	$\mu g\ plant^{-1}$
TRZST	Translocatable Zn from stem	$\mu g\ plant^{-1}$
TRZTL	Total translocatable Zn from live individual organ	$\mu g\ plant^{-1}$
TRZTS	Total translocatable Zn from senescing leaf and sheath	$\mu g\ plant^{-1}$
TRZUP	Intermediate variable	-
TTZLV	Intermediate variable	-
TTZSH	Intermediate variable	-
TW	Total dry weight	$g\ plant^{-1}$
TZD	Total Zn demand	$\mu g\ plant^{-1}$
TZGLU	Target Zn mass concentration of glume	$\mu g\ g^{-1}$
TZGR	Target Zn mass concentration of dehulled grain	$\mu g\ g^{-1}$
TZLV	Target Zn mass concentration of leaf blade	$\mu g\ g^{-1}$
TZRAC	Target Zn mass concentration of rachis	$\mu g\ g^{-1}$
TZRT	Target Zn mass concentration of root	$\mu g\ g^{-1}$
TZSH	Target Zn mass concentration of leaf sheath	$\mu g\ g^{-1}$
TZST	Target Zn mass concentration of stem	$\mu g\ g^{-1}$
TZTR	Total Zn available for translocation	$\mu g\ plant^{-1}$

TZTRP	Intermediate variable	-
WGLU	Dry weight of glume	g plant^{-1}
WGR	Dry weight of dehulled grain	g plant^{-1}
WLV	Dry weight of green leaf blade	g plant^{-1}
WRAC	Dry weight of rachis	g plant^{-1}
WRT	Dry weight of root	g plant^{-1}
WSH	Dry weight of green leaf sheath	g plant^{-1}
WSLV	Dry weight of senescent leaf blade	g plant^{-1}
WSSH	Dry weight of senescent leaf sheath	g plant^{-1}
WST	Dry weight of stem	g plant^{-1}
XTW	Observed total plant dry weight	g plant^{-1}
XWGLU	Observed dry weight of glume	g plant^{-1}
XWGLUT	Table with time dependent XWGLU data	-
XWGR	Observed dry weight of dehulled grain	g plant^{-1}
XWGRT	Table with time dependent XWGR data	-
XWLV	Observed dry weight of green leaf blade	g plant^{-1}
XWLVT	Table with time dependent XWLV data	-
XWRAC	Observed dry weight of rachis	g plant^{-1}
XWRACT	Table with time dependent XWRAC data	-
XWRT	Observed dry weight of root	g plant^{-1}
XWRTT	Table with time dependent XWRT data	-
XWSH	Observed dry weight of green leaf sheath	g plant^{-1}
XWSHT	Table with time dependent XWSH data	-
XWSLV	Observed dry weight of senescent leaves	g plant^{-1}
XWSLVT	Table with time dependent XWSLV data	-
XWSSH	Observed dry weight of senescent sheaths	g plant^{-1}
XWSSHT	Table with time dependent XWSSH data	-
XWST	Observed dry weight of stem	g plant^{-1}
XWSTT	Table with time dependent XWST data	-
XZGLU	Observed Zn mass concentrations of glume	µg g^{-1}
XZGR	Observed Zn mass concentrations of grain	µg g^{-1}
XZLV	Observed Zn mass concentrations of leaves	µg g^{-1}
XZRAC	Observed Zn mass concentrations of rachis	µg g^{-1}
XZRT	Observed Zn mass concentrations of root	µg g^{-1}
XZSH	Observed Zn mass concentrations of sheath	µg g^{-1}
XZST	Observed Zn mass concentrations of stem	µg g^{-1}
XZUP	Observed total Zn uptake	µg plant^{-1}
ZDGLU	Zn demand of glume	µg plant^{-1}

ZDGR	Zn demand of dehulled grain	µg plant^{-1}
ZDLV	Zn demand of leaf blade	µg plant^{-1}
ZDRAC	Zn demand of rachis	µg plant^{-1}
ZDRT	Zn demand of root	µg plant^{-1}
ZDSH	Zn demand of leaf sheath	µg plant^{-1}
ZDST	Zn demand of stem	µg plant^{-1}
ZUP	Total Zn uptake by plant	µg plant^{-1}
ZUPGLU	Zn allocation to glume	µg plant^{-1}
ZUPGR	Zn allocation to dehulled grain	µg plant^{-1}
ZUPLV	Zn allocation to leaves	µg plant^{-1}
ZUPRAC	Zn allocation to rachis	µg plant^{-1}
ZUPRT	Zn allocation to root	µg plant^{-1}
ZUPSH	Zn allocation to sheath	µg plant^{-1}
ZUPST	Zn allocation to stem	µg plant^{-1}

Research programme

"From Natural Resources to Healthy People"

The research for this thesis has been part of the programme ***From Natural Resources to Healthy People: Food-based Interventions to Alleviate Micronutrient Deficiencies.*** This is one of the programmes sponsored by the Interdisciplinary Research and Education Fund (INREF) of Wageningen University. INREF aims to stimulate development-oriented research and education through programmes designed and implemented in partnership with research institutes in developing countries. The programmes aim to build relevant capacity in local research institutions to solve actual problems. The main partners in our programme were China Agricultural University, Beijing and the Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, both from China, the National Institute for Environment and Agricultural Research, INERA from Burkina Faso and the University of Abomey-Calavi from Benin. In total eight staff members from these institutes, including the author of this thesis, received a PhD training.

The micronutrient malnutrition problem

Chronic micronutrient deficiencies, particularly of vitamin A, iron and zinc, lead to impaired mental and physical development and decreased work output, and contribute to morbidity from infections. Pregnant women and children are vulnerable groups. Animal products are good sources of desired micronutrients, but most people in West Africa and China depend largely on sorghum and rice, respectively, for their daily food. These plant-based foods contain limited amounts of micronutrients while they also contain anti-nutritional factors such as phytic acid and polyphenols that inhibit absorption of micronutrients by humans.

Next to the nutritional quality, the production of enough food is an important problem as population growth leads to higher demands for food and more permanent cropping, both increasing pressure on natural resources. In West Africa, soil and water conservation measures are being developed to prevent soil erosion, nutrient and water losses and to maintain or even increase yields. In China, the introduction of aerobic rice systems aim to reduce water use per kg of rice, maintaining yields similar to the current flooded rice systems.

Programme strategies to improve the supply of micronutrients

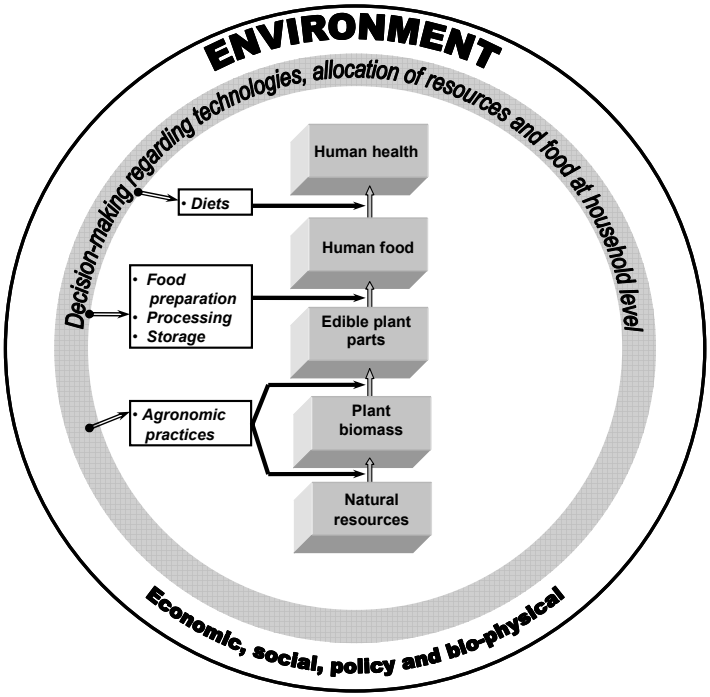
The increasing demand for food stipulates that improvements in food quality cannot be

accepted when they are at the expense of food quantity. Any solution should be in line with sustainable natural resource management.

The programme applied a food chain approach (figure) in sorghum and (aerobic) rice to explore synergies and trade-offs between different interventions along the chain.

Diagram of the food chain

The food chain approach is indicated showing how external conditions like the economic and bio-physical environment set the stage for decision making at the household level. These decisions in their turn determine practices which have a direct impact on the processes at different points in the food chain. Research in the programme has been done related to each of the three types of interventions.



Agronomic practices should aim to increase uptake and allocation of micronutrients from soil to edible plant parts, while keeping accumulation of anti-nutritional factors low. Research has focussed on effects of genotype, environment and management and their interaction on micronutrient/phytic acid molar ratio in seed. This has led to recommendations on choice of genotype, fertilizer and water use.

Food processing aims to concentrate desired micronutrients in end products and inactivate anti-nutritional factors. Research focussed on effects of milling and processing on micronutrient/phytic acid molar ratio in food, leading to recommendations on optimal combinations of unit operations.

Nutrition studies aim to validate the results in humans. Research focussed on dietary composition, determination of methods to measure impact and evaluation of effects of some of the proposed changes upstream in the food chain on micronutrient uptake in vulnerable groups. This has led to insight in sources of micronutrient and anti-nutritional factors and in the potential contribution of an intervention in the staple food.

At the end of the programme an analysis will be made to determine the relative impact of the different proposed measures along the chain for the final aim: improved micronutrient nutrition of targeted vulnerable groups.

Publications of the author

Jiang W, Zhao M, Van Keulen H, Stomph T J, Struik P C (2005) Effect of zinc applications on plant growth and zinc partitioning in aerobic rice. In: Eds. C.J. Li et al., Plant nutrition for food security, human health and environmental protection. Fifteenth International Plant Nutrition Colloquium, Tsinghua University Press, Beijing, pp. 418-419.

Jiang W, Struik P C, Jin L N, Van Keulen H, Zhao M, Stomph T J (2007) Uptake and distribution of root-applied or foliar-applied ^{65}Zn after flowering in aerobic rice. Annals of Applied Biology 150, 383-391.

Jiang W, Struik P C, Van Keulen H, Zhao M, Jin L N, Zhao M, Stomph T J (2008) Does increased Zn uptake enhance grain Zn mass concentration in rice? Submitted to Annals of Applied Biology.

Jiang W, Struik P C, Zhao M, Van Keulen H, Fan T Q, Stomph T J (2008) Screening indices for grain yield and grain zinc mass concentrations in aerobic rice. Submitted to NJAS-Wageningen Journal of Life Sciences.

Jiang W, Van Keulen H, Struik P C, Stomph T J (2008) Can Zn transport and partitioning in rice plants be modelled?

PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of Literature (5.6 ECTS)
- Allocation of zinc in plants

Writing of Project Proposal (7 ECTS)
- Physiology and modelling of zinc allocation in rice plant

Post-Graduate Courses (4.5 ECTS)
- Advanced English; CAU (2003)
- Advanced biological sciences; CAU (2003)
- WUR-CAAS autumn school "From plant production to healthy food"; WUR-CAAS, Beijing (2005)

Deficiency, Refresh, Brush-up Courses (5.9 ECTS)
- Simulation models on soil-crop system; CAU (2003)
- On systems analysis and simulation of ecological processes; PPS-WUR (2004)
- ORYZA2000 modelling training course; WUR-IRRI-CAU (2005)

Competence Strengthening / Skills Courses (1.2 ECTS)
- Techniques for writing and presenting a scientific paper; WGS (2006)

Discussion Groups / Local Seminars and Other Meetings (7.7 ECTS)
- Discussion group of crop cultivation and physiology; CAU&CAAS (2002-2006)
- Discussion group of crop and weed ecology (2002-2007)

PE&RC Annual Meetings, Seminars and the PE&RC Weekend (1.1 ECTS)
- PE&RC day: ethics in science (2002)
- PE&RC introduction weekend (2003)

International Symposia, Workshops and Conferences (7 ECTS)
- Symposium on plant modelling, simulation, visualization and their application; China (2003)
- Crop science conference; China (2003)
- XV international plant nutrition colloquium; China (2005)
- International aerobic rice workshop (oral presentation); China (2007)
- Zn crop conference; Turkey (2007)

Curriculum vitae

Wen Jiang was born on October 17th, 1972 in Shandong, China. She finished high school in 1993, and finished her Bachelor of Science degree in Agriculture, majoring in Agronomy, at Laiyang Agricultural College (presently Qingdao Agricultural University), Shandong province. After obtaining her first degree in 1997, she worked as a research assistant in Laizhou Seed Company, Shandong. From 2000 to 2002, she studied in Laiyang Agricultural College for an MSc degree. During these MSc studies, she obtained a scholarship from the International Rice Research Institute (IRRI) in the Philippines for her thesis research. This work focused on the physiology of water stress in rice. After obtaining her MSc degree in July 2002, in November the same year, she was admitted to the Wageningen University PhD programme of the Production Ecology and Resource Conservation graduate school, financed through the Interdisciplinary Research and Education Fund (INREF). The research topic was the physiology and modelling of Zn allocation in rice.

Wen Jiang currently holds a position as a teacher in the College of Plant Science and Technology at Qingdao Agricultural University.