Bioavailability of Zinc to Aerobic Rice

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Bioavailability of Zinc to Aerobic Rice

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Abstract

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Zinc deficiency is a wide-spread constraint for crop production and human health. This thesis should contribute to alleviation of Zn deficiency problems and aimed at identifying soil and plant factors affecting Zn bioavailability in rice (*Oryza sativa* L.). Two main research questions were: 1) what is the consequence of a cultivation shift from flooded to aerobic rice on Zn bioavailability and 2) which mechanisms control Zn mobilization by aerobic rice? Field experiments demonstrated that the cultivation shift from flooded to aerobic may increase Zn deficiency problems. Lower Zn uptake and mass fraction in shoot and brown rice were observed in aerobic fields. Results of a soil incubation experiment and modeling showed that the difference found in plant Zn uptake between the two cultivation systems was orders of magnitude lower compared to what can be expected based on chemical equilibria in the bulk soil. This discrepancy suggests that soil chemical properties such as pH, DOC level and redox conditions in rhizosphere need consideration. We observed considerable variation among aerobic rice genotypes in tolerance to Zn deficiency under both field and pot conditions. In a pot experiment we found that variation in tolerance was mainly associated with Zn uptake. Plant Zn uptake from low Zn soils can be increased by both Zn-mobilizing rhizosphere processes and inoculation with arbuscular mycorrhizal fungi. We demonstrated that mycorrhizal inoculation significantly increased Zn uptake, but only in genotypes with a low inherent Zn uptake. High Zn mobilization by a combination of chemical rhizosphere and mycorrhizal effects seems impossible and may not be feasible as a target for breeders. In a rhizotron experiment and a nutrient solution experiment, aerobic rice genotypes responded to Zn deficiency with increased root exudation of malate. Genotypes with a higher Zn uptake showed a stronger increase in malate exudation in response to Zn deficiency. These results confirm our hypothesis that genotypic variation in Zn uptake of aerobic rice can partly be explained by root exudation of malate.

Key words: Arbuscular mycorrhiza, Exudation, *Oryza sativa*, Rhizosphere, Rice, Soil, Zinc

Preface

During the past four years I conducted laboratory and field works in China and in the Netherlands, resulting in the present doctor's thesis. Many persons and institutions have contributed to the successful completion of this work. This thesis would not have been as it is without the help and support of a great number of people.

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I am indebted to the assistance of Thomas for helping me to finish the modeling part in Chapter III. Thom is thanked for his excellent supervision on Chapter V. In my view, Thom is one of the most intelligent persons I have ever met. Thanks for your inspiring comments!

I am also thankful for the helpful discussions with people within the INREF "From natural resources to healthy people" project. I am grateful for the encouragement and useful tips from TjeerdJan Stomph and Maja Slingerland. I would like to express my gratitude to my colleagues in China, namely Xinping Chen, Jianbo Shen, Lujiu Li, Xiaolin Li and Gu Feng, who shared their knowledge and experience with me and gave me useful tips for conducting the field, pot and lab experiments. Specially thanks to Prof. Huaqi Wang in China Agricultural University for providing seeds for this research.

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Xiaopeng Gao

Wageningen, The Netherlands,
February 2007

Contents

Chapter I

General Introduction

I. 1 Zinc as an essential nutrient for plants and humans

Zinc is a trace element found in all soils. It is an essential element for plants, animals and humans. As a component of proteins, Zn acts as a functional, structural or regulatory co-factor of a large number of enzymes. It is involved in many important plant physiological processes, for instance carbohydrate metabolism, protein metabolism, membrane integrity, starch formation and seed maturation (Brown et al., 1993; Fageria et al., 2002). In addition, Zn is of particular importance to human health. Zinc deficiency in the human body can result in undesirable consequences including growth retardation, dermatitis and impaired immune functioning (Welch, 1993).

I. 2 Zinc deficiency problems

Zinc deficiency has been reported in various parts of the world in annual crops (Cakmak, 2002). About 30% of the world's soils are Zn deficient (Alloway, 2004). Major regions are found in Australia, South-East Asia, South-West Asia and Central America (Figure 1).

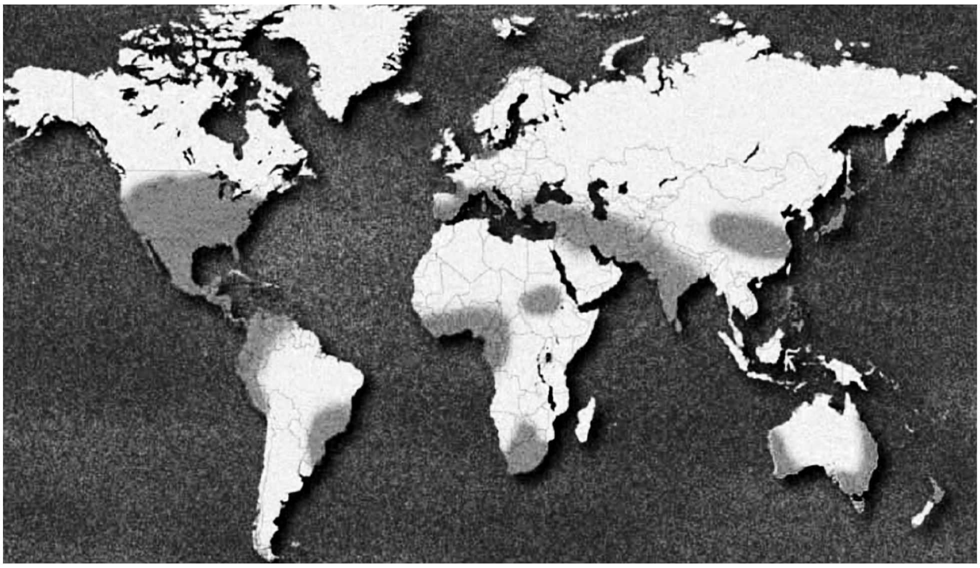

Figure 1. Geographic distribution of Zn deficient soils (gray areas) in the world (Alloway, 2004).

In China, soils of more than one third of the agricultural area are Zn deficient. These are predominantly found in the north and middle of China (Liu, 1996; Figure. 2).

Chemical forms (species) of Zn in soils differ largely in their availability to plants. The total Zn content is not a reliable index to reflect the capacity of soil to supply Zn for plants. To characterize the fraction of Zn in soils available for plant uptake, DTPA and HCl are frequently used as extractants for soils with high and low pH, respectively (Lindsay and Cox, 1985; Brennan and Gartrell, 1990). The critical levels for DTPA-Zn and HCl-Zn are 1.0 mg kg^{-1} and 1.5 mg kg^{-1}, respectively. Based on chemical analysis and the response of crop plants to Zn fertilization, China soils were divided into five classes differing in available Zn content (Figure. 2). The available Zn content in acid soils of South China is higher than that of calcareous soils in North China. Zinc may be strongly adsorbed on $CaCO_3$ or $MgCO_3$ (Katyal and Ponnamperuma, 1975) since carbonate has a high affinity for Zn. This is in accordance with the findings that Zn deficiency problems are mainly reported on plants grown on calcareous soils in North China, where the main crops affected are rice and maize (Takkar and Walker, 1993).

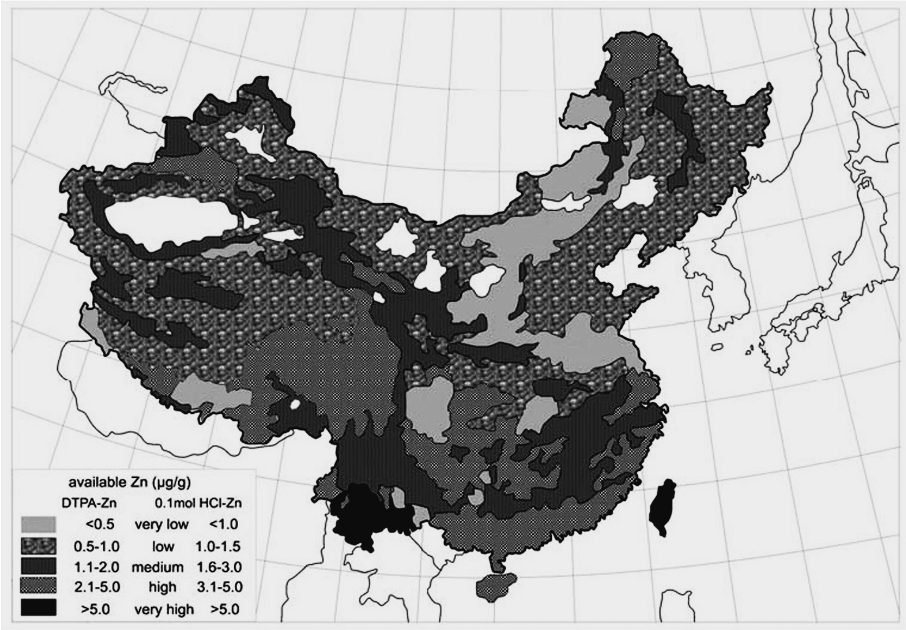

Figure 2. Zinc distribution in China soils (Liu, 1996).

Increasing the amount of bioavailable micronutrients in plant foods for human consumption is a challenge, which is particularly important for developing countries. Zinc deficiency affects over two billion people worldwide. Awareness is growing that low concentrations of Zn in staple food such as rice can adversely affect human health. This especially concerns low income countries such as China and many African countries where diets are cereal-based and low in vegetables and meat. It is estimated that about 60% of Chinese schoolchildren has blood or hair Zn concentrations below the critical level and 14.1% of total population in China is at risk of inadequate Zn intake (Chen, 2004). Increasing bioavailability of micronutrients in the edible part of staple crops through breeding and agricultural management is seen as a cost effective and sustainable way to alleviate micronutrient malnutrition in vulnerable groups. Some global joint projects are being conducted with the aim of improving micronutrient nutrition of humans. HarvestPlus is an international, interdisciplinary research program (http://www.harvestplus.org/) that seeks to reduce micronutrient malnutrition by harnessing the powers of agriculture and nutrition research to breed nutrient dense staple foods. Research in the present thesis was conducted in the context of the program "From Natural Resources to Healthy People" (http://www.inref.wur.nl/; pages 119-121 of this thesis), which aims to develop agriculture- or food-based interventions to alleviate nutritional deficiencies of human consumers, especially those from the urban and rural region in China and West Africa. Primary focus of the research described in this thesis is the investigation of the processes involved in the transfer of Zn from soil to plants.

I. 3 Aerobic rice

It has been estimated that half the world's population subsists completely or partially on rice (Datta, 2004). Rice feeds more than two billion people worldwide and is the number one staple food in Asia. Ninety percent of the world rice crop is grown and consumed in Asia. Because of water constraints, rice production worldwide, especially in Asia, is now in transition from the traditional high water-consuming lowland rice cultivation with flooded fields to a promising new cultivation system of "aerobic rice". Aerobic rice varieties are currently developed by crossing lowland with upland varieties and grown as a dry field crop in irrigated but non-flooded and non-puddled fertile soils (Bouman et al., 2005). In China, breeders have produced aerobic rice varieties with an estimated yield potential of 6-7 Mg ha^{-1} which are now being pioneered by farmers on some 190,000 ha in North China (Wang et al., 2002). A recent study showed that the water productivity (Crop yield/Water consumptively used in evapotranspiration, Kassam and Smith, 2001) of rice under aerobic conditions was 32-88% higher than under flooded conditions (Bouman et al., 2005).

The shift to aerobic cultivation predominantly occurs in North China, where the sustainability of lowland rice production is threatened by the increasing water scarcity (Wang et al., 2002). Soils in North China are mainly calcareous soils with a pH higher than 7, which are frequently Zn deficient. So far, however, breeding has mainly focused on the yield of aerobic genotypes under sufficient Zn supply.

I. 4 Soil and plant factors affecting Zn availability

Zinc in soil may be found (i) in the soil solution, as the free ion Zn^{2+}, and associated with organic and inorganic ligands, (ii) on exchange sites of reactive soil minerals, (iii) bound by organic matter, (iv) occluded in oxides and hydroxides of Al, Fe and Mn, and (v) entrapped in primary and secondary minerals. The relative amount of each of these forms of Zn (comprehensively referred to as speciation) is affected by various soil physical and chemical properties. Only the mentioned soluble Zn (present either as the free Zn^{2+} or Zn-ligand complexes) is commonly supposed to be directly available to plants. As indicated in Figure 3, Zn can react with oxides, with clay minerals and with organic matter. Some chemical speciation models (e.g. MINTEQA2, Allison et al., 1991; GEOCHEM-PC, Parker et al., 1994) were used to show that $ZnSO_4^0$ and $Zn(OH)^+$ may comprise up to 10% of dissolved Zn in most soils under normal condition. Other inorganic ligands, such as Cl^-, NO_3^- and PO_4^{3-} are of less importance. Zinc also forms complexes with organic ligands, for instance organic acids and dissolved organic carbon (DOC).

Soil properties including pH, redox potential, organic matter and pedogenic oxides exert the most significant influence on the adsorption-desorption reactions of Zn in soils and thus regulate the concentration of Zn dissolved in soil solution

(Guadalix and Pardo, 1995). These factors are expected to change upon the shift from lowland to aerobic cultivation, which induces a change in several of the main soil chemical processes and therefore the Zn bioavailability to plant roots. The net consequence of all these changes for Zn bioavailability is hard to predict because some changes favour an increase of Zn bioavailability, whereas others cause a decrease. It is also difficult to extrapolate results from the literature, as these concern different soils, and different soil composition implies that the properties as well as the processes controlling Zn availability may differ.

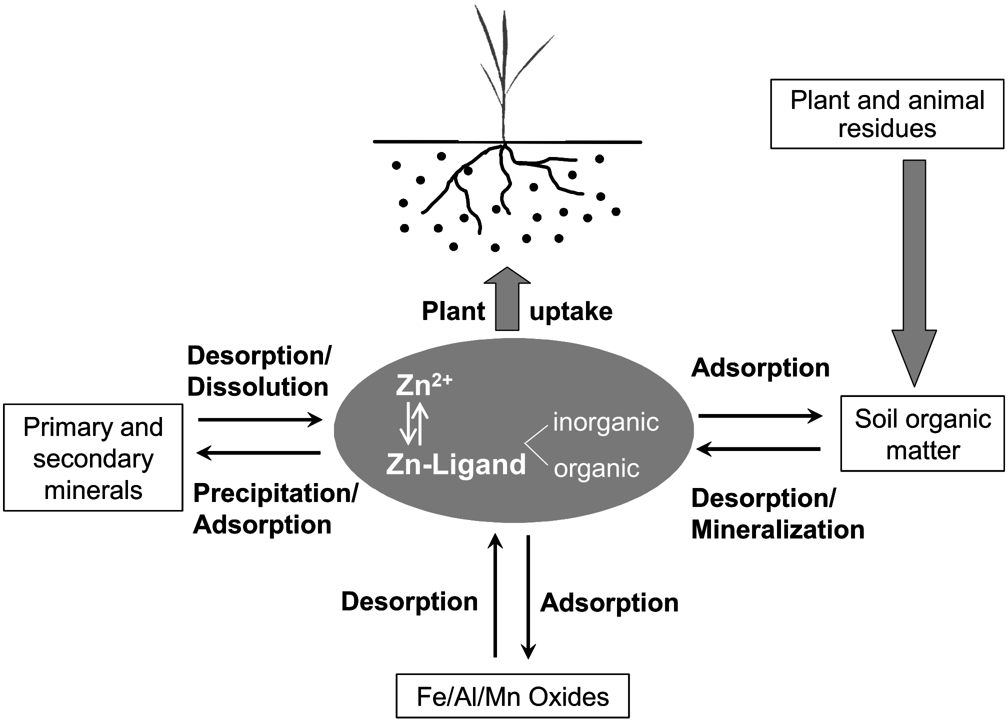

Figure 3. Soil processes affecting Zn availability (▭ solid phase; ⬬ soil solution).

Two major plant mechanisms improving Zn uptake by plants are: (1) the increase of the surface area for Zn acquisition including root and mycorrhizal hyphal surface and (2) root-induced changes in rhizosphere (Figure 4, Marschner, 1998).

As a rule in nutrient acquisition, a large root surface area (fine roots and long root hairs) is of key importance, specifically for immobile nutrients such as Zn (Marschner, 1998). The nutrient absorbing surface area can also be enhanced by mycorrhizal colonization of roots. A beneficial effect of mycorrhizal colonization on Zn uptake has been shown for many crops, which is mainly contributed to an enhanced exploration of a larger soil volume (Smith and Read, 1997).

Conditions in the rhizosphere differ in many respects from those in the bulk soil, e.g. pH, redox potential and concentrations of low and high molecular weight exudates. Many studies showed that root-induced changes in rhizosphere are of particular relevance to the dynamics and mobilization of Zn. One example is that the rhizosphere of lowland rice may be significantly acidified as a result of H^+ released by Fe oxidation, which may lead to solubilisation of Zn (Boekhold, 1992; Temminghoff et al., 1995; Kirk and Bajita, 1995). Another example is the exudation of Zn chelators such as phytosiderophores (Tolay et al., 2001; Suzuki et al., 2006) or citrate (Hoffland et al., 2006), which may increase plant tolerance to Zn deficiency.

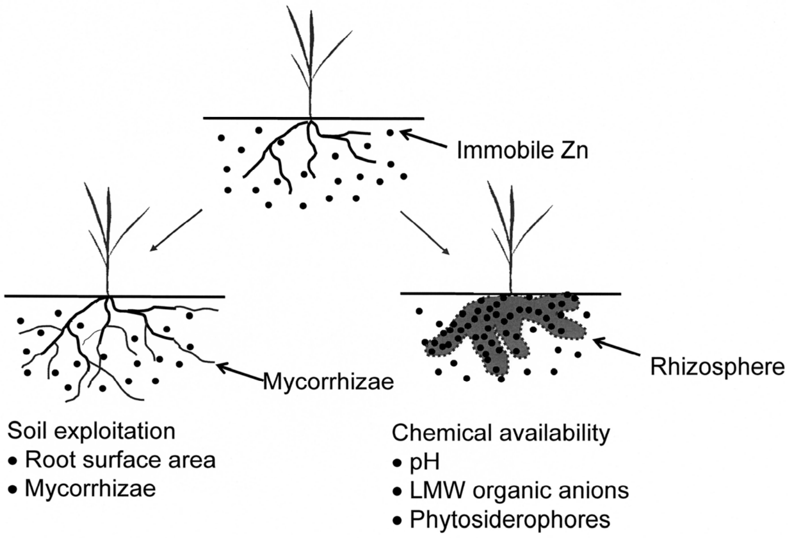

Figure 4. Plant factors affecting Zn uptake by plants.

I. 5 Zinc efficiency

Zinc efficiency is defined as the capacity of a cultivar to grow and yield well on a low Zn soil, and it is usually expressed as the ration of shoot dry weight under Zn deficiency over that under adequate Zn supply (Graham et al., 1992). Genotypes of plants vary widely in Zn efficiency, as reported for maize (Ramani and Kannan, 1985), wheat (Cakmak et al., 2001), common bean (Hacisalihoglu et al., 2004) and lowland rice (Sakal et al., 1989). A mechanistic explanation of differential Zn efficiency among genotypes of crop plants is still lacking. It may, however, be assumed that more than one mechanism is often responsible (Graham and Rengel, 1993). Hacisalihoglu and Kochian (2003) thoroughly reviewed the possible factors involved, including: 1) Seed Zn content, 2) Uptake of Zn from Zn-deficient soil, 3) Translocation of Zn from root to shoot, 4) Shoot Zn concentration, 5) Biochemical Zn utilization.

Despite its complexity, understanding the mechanisms of variation in Zn efficiency can greatly contribute to the selection and breeding of genotypes with higher tolerance to Zn deficient soils. Even though some progress has been made in this field (Cakmak et al., 1998; Erenoglu et al., 2002; Hacisalihoglu et al., 2003, Kochian, 1993; Rengel, 2001; Wissuwa et al., 2006), most of these studies have either used too few genotypes to allow a general conclusion, or have used a large set of genotypes, but have examined only one or two traits.

Since the concept of aerobic rice is new, there is no reported study dealing with variation in Zn efficiency among aerobic rice. In this thesis, we expect to find variation in Zn efficiency among aerobic rice varieties, which can be used as a tool to investigate the mechanisms on acquisition of Zn by aerobic rice.

I. 6 Project background

This project is in the context of an interdisciplinary program entitled “From Natural Resources to Healthy People”. The program started in 2002 and aims to develop sustainable agriculture- or food-based interventions to alleviate nutritional deficiencies of human consumers, especially those from the urban and rural region.

In Figure 5, the boxes in the middle form a chain representing the flow of nutrients from natural resources (soil) to the human body (health). The interventions (technologies) aiming at increasing bioavailability of nutrients in this food chain approach are indicated. Agricultural practices can contribute to higher uptake and allocation of nutrients to edible plant parts. Food processing/ preparation can concentrate desired nutrients in food products and inactivate anti-nutritional factors. Dietary study can lead to a good recommendation on food constitution to human consumers. The contribution of the present thesis to this program is to look at the soil-plant interaction, focusing on the efficiency of Zn transfer from soil to the plants (at beginning of food chain, Figure 5; see also pages 119-121 of this thesis).

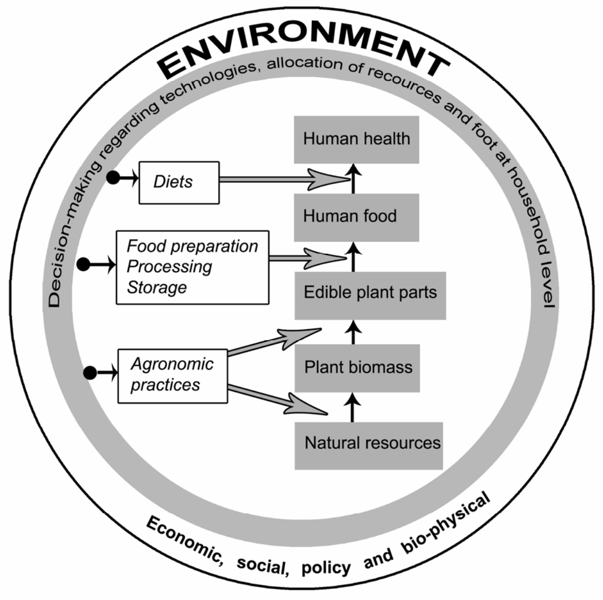

Figure 5. Food-chain framework in this program.

I. 7 Aim and outline of the thesis

This thesis aimed at identifying soil and plant factors that affect Zn bioavailability in aerobic rice. It does so by answering the following two research questions: 1) what is the consequence of a cultivation shift from flooded to aerobic rice on Zn bioavailability and 2) which mechanisms control Zn mobilization by aerobic rice? Experiments were conducted under both field and greenhouse conditions in China. To interpret the soil chemical causes that may affect Zn bioavailability to plants, experimental soil chemistry data were modeled.

Following this introduction chapter, chapters II and III deal with field experiments on the evaluation of the consequence of the shift from flooded to aerobic on Zn bioavailability. Chapter IIII addresses the first research question. It describes a field experiment conducted on a calcareous soil in Beijing region, North

China and focuses on the effect of aerobic compared to flooded conditions on crop biomass production, grain yield and Zn uptake. Chapter III gives the results of a similar experiment conducted in Anhui region, Mid China. The soils in Anhui region have a lower Zn level and a lower pH than the soils in the Beijing region. By using this soil as an example, we characterized and modeled both flooded and aerobic systems with geochemical analysis to identify the dominant processes that control the solid-solution partitioning of Zn in soils and understand the mechanisms of different bioavailability of Zn between two cultivation systems.

Chapter IV investigates and explains the variation in the capacity of genotypes to thrive well in soils with low Zn availability. A pot experiment was conducted with 23 rice genotypes grown on a low Zn soil. The aim of this study is to assess the variation in Zn efficiency among aerobic rice genotypes. We also investigated whether this variation in Zn efficiency can be associated with seed Zn content, Zn uptake, Zn translocation from root to shoot and/or shoot Zn concentration.

In addition to this screening experiment (Chapter IV), we focused on mycorrhizal effects (Chapter V) and rhizosphere effects (Chapter VI) that may possibly be involved in Zn uptake by aerobic rice. With six aerobic rice genotypes varying in Zn uptake efficiency, we examined the potential of arbuscular mycorrhizal fungi (AMF) inoculation as a means to increase Zn uptake by aerobic rice. Furthermore, we tested if there is variation in mycorrhizal responsiveness based on growth and Zn uptake among aerobic rice genotypes, and how this variation is related to Zn uptake in the nonmycorrhizal condition (Chapter V). With these same six genotypes, we conducted a rhizotron experiment and a nutrient solution experiment to find out if genotypic variation in Zn uptake is related to the capacity to exude low molecular weight organic anions into the rhizosphere in response to Zn deficiency. The effect of malate exudation on Zn availability in soil solution was evaluated by a malate-soil extraction experiment (Chapter VI).

This thesis ends with a general discussion (Chapter VII). This chapter discusses the relationship among the individual experiments and feeds back on the two research questions given above. Future research aims regarding the Zn availability to plants are also discussed.

Chapter II

From Flooded to Aerobic Conditions in Rice Cultivation: Consequences for Zinc Uptake

Xiaopeng Gao, Chunqin Zou, Xiaoyun Fan, Fusuo Zhang and Ellis Hoffland

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Abstract

Scarcity of water causes a shift from flooded to aerobic conditions for rice production in Zn deficient areas in North China. This shift alters soil conditions that affect Zn availability to the crop. This paper concerns the effect of aerobic compared to flooded conditions on crop biomass production, grain yield and Zn content. A field experiment was done with six rice genotypes (*Oryza sativa* L.) grown on a calcareous soil, both with (23 kg Zn ha^{-1}) and without Zn fertilization. Sampling was conducted at tillering and physiological mature stage. Zinc concentration in the shoots was significantly lower at both stages in plants grown in the aerobic field. At maturity, Zn uptake, biomass production, grain yield and Zn-harvest index [grain Zn/(shoot + grain Zn)] were lower under aerobic cultivation. Rice genotypes including aerobic rice and lowland rice differ in degree of response to low Zn supply. A twofold difference was found among aerobic genotypes in grain yield and Zn uptake. Also Zn-harvest index varied significantly. Zinc application affected neither grain yield nor grain Zn content, although it significantly improved biomass production in both systems in most genotypes. These results demonstrate that introduction of aerobic rice systems on calcareous soils may increase Zn deficiency problems.

Key words: Aerobic, Flooded, Lowland, Rice, Zinc, Zn-harvest index

II. 1 Introduction

Zinc deficiency in cereal plants is a well-known problem that causes reduced agricultural productivity all over the world (Cakmak et al., 1999; Fageria et al., 2002). In addition, it causes widespread Zn deficiency in humans, especially in developing countries where diets are cereal-based and poor in animal and fish products (Cakmak et al., 1999; Frossard et al., 2000).

Because of water constrains, rice production in China is now undergoing important changes from traditional high water-consuming lowland (paddy) rice cultivation to a promising new cultivation method of "aerobic rice". Aerobic rice is grown as a dry field crop in irrigated but non-flooded and non-puddled fertile soils (Bouman et al., 2002). In China, breeders have produced aerobic rice varieties with an estimated yield potential of 6-7 Mg ha^{-1} which are now being pioneered by farmers on some 190,000 ha in North China where water is increasingly getting scarce and where water scarcity makes lowland rice uneconomic (Wang et al., 2002).

Many of the soils under flooded rice cultivation in North China are Zn-deficient (DTPA-extractable Zn < 1.0 mg kg^{-1}; Sims and Johnson, 1991). The major causes are high pH, high carbonate content and low redox potential (Marschner, 1993; Mandal et al., 2000). Iron oxidation by root-released oxygen causes a reduction of the rhizosphere pH and limited release of Zn from highly insoluble fractions (Kirk and Bajita, 1995). Many factors that determine Zn bioavailability are expected to change after a shift to aerobic cultivation. Bulk soil pH may either increase or decrease depending on the original soil pH (Liu, 1996). Redox potential will increase (Gao et al., 2002), causing Fe oxidation, with concomitant acidification, precipitation of $Fe(OH)_3$ and adsorption of Zn on these oxides. Increased nitrification may cause plants to take up NO_3^- instead of NH_4^+ (Sanchez, 1976; Voesenek and Veen, 1994), which also causes the rhizosphere pH to increase. Organic matter, onto which Zn can be adsorbed, will be oxidized. Furthermore, reduction of the water content of the soil may restrict Zn transport towards the plant root (Yoshida, 1981). The consequence of all these changes for Zn bioavailability is hard to predict.

The shift from flooded to aerobic conditions sets the problem of Zn deficiency in rice in a new perspective. Considerable effort has been attributed to identifying

Zn-efficient genotypes to improve productivity on Zn-deficient soils. Many studies have shown that there is considerable variation in tolerance to Zn deficiency in the lowland rice germplasm (Sakal, 1977; Sakal et al., 1989; Singh et al., 1981). The mechanism, by which a genotype thrives well in Zn-deficient soil whereas others fail, is not well understood. The change of water management from flooded to aerobic conditions will raise new scientific questions on this subject. Few papers report on Zn efficiency and the mechanisms that are involved in Zn efficiency for lowland rice (Sakal et al., 1989; Doberman and Fairhurst, 2000). Yet, studies regarding the variation in Zn efficiency within and among aerobic rice varieties are not available.

In order to evaluate the consequences of this shift in cultivation system we compared Zn efficiency of rice genotypes under aerobic and flooded cultivations. We used one lowland and five aerobic genotypes to assess genotype variation in the response to this shift.

II. 2 Materials and methods

A field experiment was conducted in 2003 at an experimental station of China Agricultural University in Dongbeiwang, a northern suburb of Beijing. The soil is a calcareous alluvial soil (calcareous Cambisoil, ISSS, ISRIC, and FAO, 1998). Previous crops on this site showed Zn deficiency, but due to Zn application in the previous year Zn availability is above critical levels (Sims and Johnson, 1991; Table 1). This field was used in the previous 3 years for research on water use efficiency and nitrogen use efficiency of rice on both aerobic and flooded fields. The flooded field in this experiment had been under flooded conditions and aerobic field under aerobic conditions in the earlier years (2000-2002). Some soil properties are given in Table 1.

The experiment was designed as a randomized complete block with a split-plot arrangement and four blocks under both aerobic and flooded cultivation, with four replicates. Main plots were Zn fertilizer rates (0 and 23 kg ha^{-1} Zn as $ZnSO_4$, 3 days prior to sowing) and subplots were different rice genotypes (*Oryza sativa* L.). The area of each subplot was 6 m^2 (3 x 2 m). Flooded and aerobic areas were separated by 6 m-wide protection rows, on which aerobic rice was sown.

All rice genotypes (*Oryza sativa* L.) were known to have similar growth duration. Zinc concentration of the seeds in mg kg^{-1} was: 15.3, 18.6, 26.9, 26.5, 17.0, 20.2, for Qiuguang, Han297, Han277, Han72, 89B271-17hun (89B) and K150, respectively. Genotype Qiuguang is a japonica lowland genotype, widely used in North China. The other genotypes are aerobic varieties. Han297 is a widely used genotype. Han277, 89B and Han297 had shown relatively high Zn efficiency in preliminary experiments, whereas K150 and Han72 had shown a low Zn efficiency.

Seeds were sown in rows 20 cm apart on May 12 at a rate of 150 kg ha^{-1}. Nitrogen was applied as urea (225 kg N ha^{-1}), of which 40% was applied at sowing, 30% at tillering and 30% at booting stage. Thirty-nine kg P ha^{-1} as $NH_4H_2PO_4$ and 75 kg K ha^{-1} as KCl were applied as base fertilizers at sowing. The plots were kept free of weeds by an application of pre-emergence herbicide and hand weeding after crop establishment.

Two water treatments as aerobic and flooded cultivation were imposed. For aerobic cultivation, the irrigation was commenced at sowing and at visual symptoms of drought stress. Four sprinkler irrigations were applied: on May 12, May 21, July 15 and August 13. The fields that were used for rice cultivation under flooded conditions were submerged from June 16 (35 days after sowing (DAS)) until September 25 (130 DAS) with water delivered using flexible hoses connected to a deep groundwater well. Prior to submergence, these fields received the same irrigation as the aerobic fields.

The crop was sampled twice because plant Zn deficiency symptoms usually show early in crop development. The first sampling was at the tillering stage (July 5, 53 DAS); the other at the physiological maturing stage when the grains become hard and have about 20 % moisture in them (Oct 15, 156 DAS, Yoshida, 1981). At the tillering stage, two 50 cm segments of a row were sampled. Shoot samples from these two segments were bulked. At maturing stage, 1.5 m^2 (2.5 x 0.6) in the center of each plot was harvested to determine grain and straw yield. Samples were washed briefly in 0.1% HCl followed by tap water and deionized water. Total dry matter and grain weight were then determined by oven drying the sampled plants at 80 °C for 72 h.

After grinding, the oven dried sub-samples of straw and whole grains were digested in acid mixture (HNO_3 + $HClO_4$) (Jackson, 1973). Zinc in plant digests was analyzed using an atomic absorption spectrophotometer (Pye Unicam SP 9 800, Cambridge, UK).

Statistical analysis of the data was performed using the SAS analytical software (SAS, 1990). Analysis of variance (ANOVA) was employed and LSD ($P < 0.05$) was used to test the difference among treatments.

Table 1. Physical and chemical properties of the soil. All analyses were done before fertilizer application.

Depth (cm)	Texture	Bulk density (g cm^{-3})	pH (H_2O)	CEC (cmol kg^{-1})
0-30	Loam	1.33	8.0	11.1

O.M.[1] (g kg^{-1})	Total N (g kg^{-1})	Olsen-P (mg kg^{-1})	NH_4OAc-K (mg kg^{-1})	DTPA-Zn (mg kg^{-1})
21.4	1.17	34.6	145	2.0

[1] O.M. = Organic matter

II. 3 Results

II. 3. 1 Tillering stage

Cultivation effects: Cultivation significantly affected shoot dry weight and shoot Zn concentration, but did not affect shoot Zn content (Table 2, Figure 1). Shoot Zn concentrations were lower under aerobic than under flooded conditions (Figure 1). This effect of cultivation was similar for both Zn rates (Table 2) and for all genotypes except Han297. In aerobic fields, shoot Zn concentration without Zn application in three genotypes (Qiuguang, K150 and Han297) was below the sufficiency level for adequate growth (20 mg kg^{-1}), but well above the level below which Zn is deficient (10 mg kg^{-1}; Dobermann and Fairhurst, 2000). Zinc application resulted in an increase in shoot Zn concentration to levels above sufficiency level for all genotypes (data not shown).

Genotype and Zn application significantly affected shoot dry weight, shoot Zn concentration and shoot Zn content (Table 2). Shoot dry weight of the genotypes

Qiuguang, K150 and Han297 in aerobic fields was significantly increased when Zn was applied. This effect of Zn application was absent in flooded fields (Figure 2).

Table 2. Results of three factor ANOVA (*P*-values) for shoot dry weight, shoot Zn concentration and shoot Zn content at tillering stage.

Sources	DF	Shoot dry weight	Shoot Zn concentration	Shoot Zn content
Cultivation (C)	1	0.0001	0.0001	0.4067
Zn rate (Zn)	1	0.0001	0.0001	0.0001
Genotype (G)	5	0.0001	0.0030	0.0001
C * Zn	1	0.0918	0.9892	0.6122
C * G	5	0.1939	0.8247	0.8709
Zn * G	5	0.9655	0.4057	0.3575
C * Zn * G	5	0.0689	0.4854	0.8382

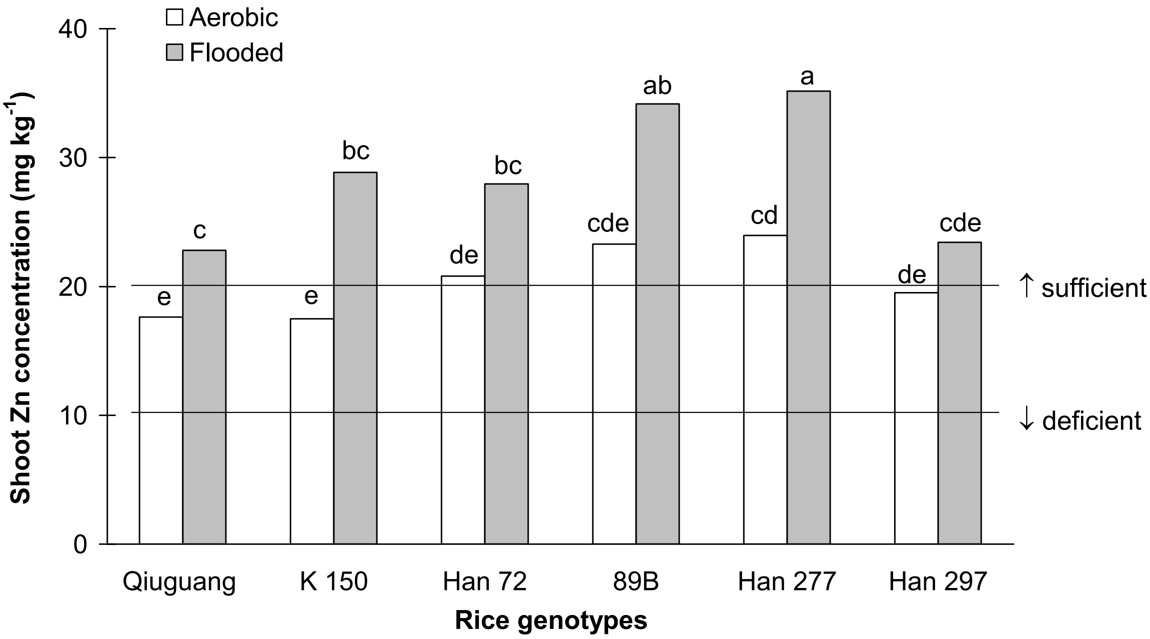

Figure 1. Cultivation effect on shoot Zn concentration of plants without Zn application at tillering stage. Means with the same letter are not significantly different ($P < 0.05$).

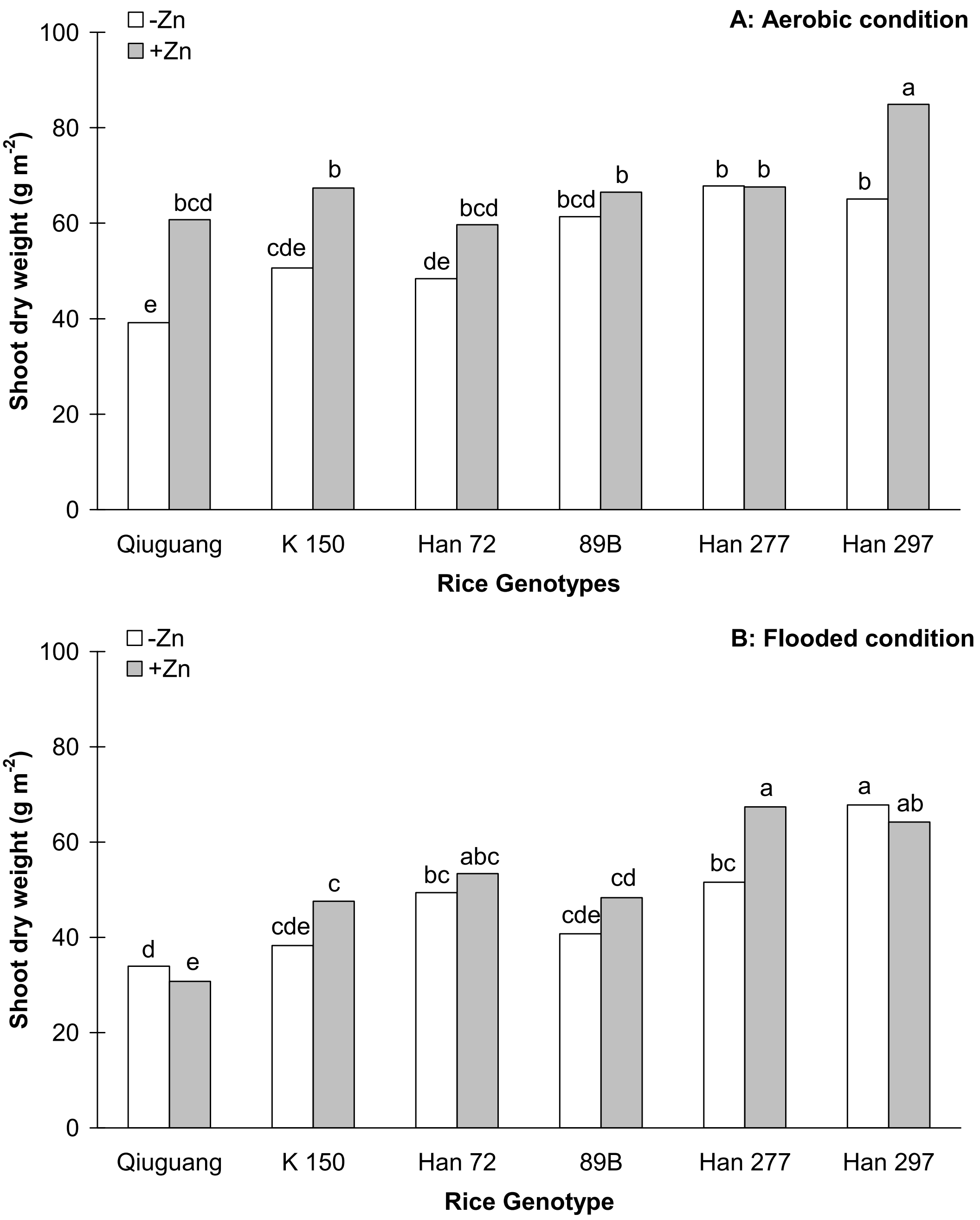

Figure 2. Effect of Zn application on shoot dry weight under aerobic (A) and flooded (B) condition at tillering stage. Means with the same letter are not significantly different ($P < 0.05$).

Genotype effects: Shoot dry weight, shoot Zn concentration and shoot Zn content varied significantly among genotypes (Table 2). Zinc efficiency, defined as the capacity of a genotype to maintain growth under low Zn conditions (Graham et al., 1992), was higher for aerobic genotypes (Table 3). There was considerable variation among aerobic genotypes.

No interaction effects were found among cultivation type, Zn application rate and genotype (Table 2). Zinc application increased the level of all three parameters given in Table 2, independent of cultivation type and genotype.

Table 3. Zinc efficiency of rice genotypes grown under aerobic condition.

	Genotypes					
	Lowland	Aerobic				
	Qiuguang	K150	Han72	89B	Han277	Han297
Zn efficiency*	64	75	81	92	100	77

*Zn efficiency = shoot dry weight (-Zn) / shoot dry weight (+Zn) x 100%

II. 3. 2 Mature stage

Cultivation effects: Similar to the results of tillering stage, the cultivation system had a significant effect on shoot Zn concentration (Table 4). Shoot Zn concentration was lower in aerobic than in flooded fields for genotypes K150, 89B and Han297 (Figure 3). Grain Zn concentration was unaffected (Table 4).

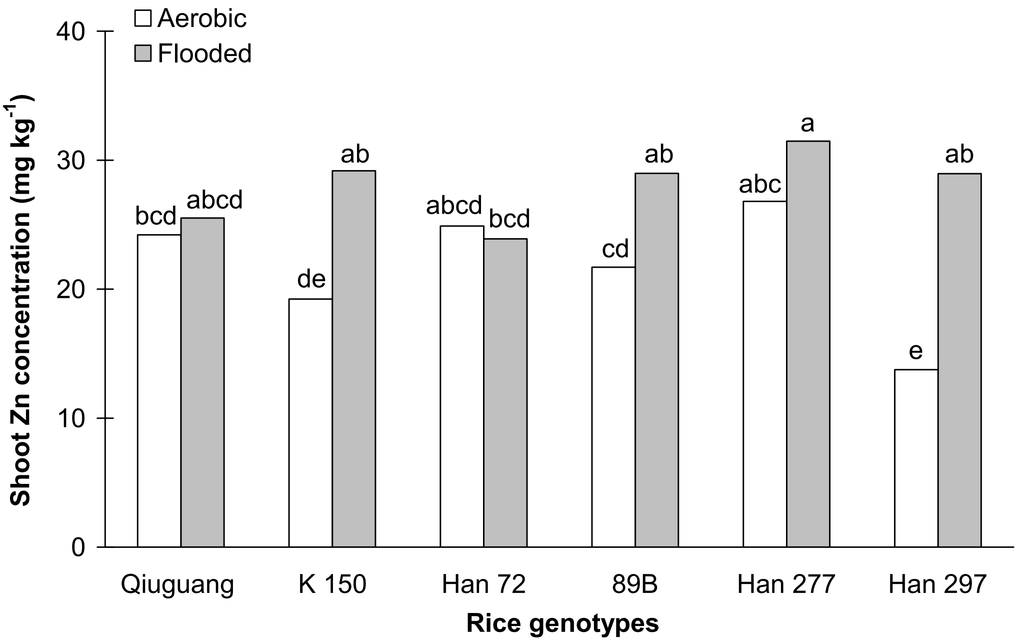

Figure 3. Cultivation effect on shoot Zn concentration of plants without Zn application at mature stage. Means with the same letter are not significantly different (*P* < 0.05).

Grain yield was significantly affected by cultivation for all genotypes. This was, however, not the case for shoot dry weight. Plants grown under aerobic conditions had significant lower grain yields than those grown under flooded conditions (Table 4; Figure 4). This effect of cultivation system was similar for both Zn rates (Table 4).

Harvest index under aerobic conditions was significantly lower than under flooded conditions (Table 4, 5). The same was true for Zn-harvest index (Table 5), defined as the grain Zn content (in g or mol) divided by the shoot Zn content, which was 27% lower under aerobic cultivation.

Genotype effects: Genotypes varied significantly in shoot Zn concentration but not in grain Zn concentration (Table 4). Genotypes 89B, Han277 and Han297 yields were significantly higher than yields of the other three genotypes under aerobic conditions (Figure 4). Genotype 89B also had the highest harvest index, both for dry matter and for Zn. Significant variation in Zn-harvest index was found among the genotypes (Table 5).

Zinc application effects: Zinc application significantly increased shoot Zn concentration and shoot dry weight. Grain yield, grain Zn concentration (Table 4), and harvest index (Table 5), however, were unaffected. This effect of Zn application was independent of cultivation system (Table 4). Zn-harvest index was lower under the high Zn application rate (Table 5).

Table 4. Results of three factor ANOVA (*p*-values) for shoot dry weight, grain yield, shoot Zn concentration, grain Zn concentration, harvest index and Zn-harvest index at mature stage.

Sources	DF	Shoot dry weight	Grain yield	Shoot Zn concentration	Grain Zn concentration	Harvest index	Zn-harvest index
Cultivation (C)	1	0.0028	0.0001	0.0001	0.7908	0.0001	0.0001
Zn rate (Zn)	1	0.0022	0.4873	0.0003	0.4828	0.0149	0.0043
Genotype (G)	5	0.0001	0.0001	0.0003	0.1032	0.0001	0.0003
C * Zn	1	0.1555	0.8768	0.5566	0.5022	1.0000	0.6085
C * G	5	0.0120	0.0001	0.0044	0.0656	0.0001	0.0001
Zn * G	5	0.5920	0.7184	0.8593	0.9824	0.9411	0.8397
C * Zn * G	5	0.1452	0.9908	0.0031	0.8680	0.4300	0.1449

Harvest index = grain yield / (grain yield + shoot dry weight)

Zn-harvest index = grain Zn content / (shoot + grain Zn content)

Table 5. Effect of cultivation, Zn rate and genotype on harvest index and Zn-harvest index.

Treatment	Harvest index (%)	Zn-harvest index (%)
Cultivation		
Aerobic	31 b	33 b
Flooded	50 a	45 a
Zn rate (kg ha^{-1})		
0	39 a	42 a
23	42 a	37 b
Genotype		
Qiuguang	38 b	35 bc
K150	40 b	41 ab
Han72	37 b	37 bc
89B	47 a	47 a
Han277	40 b	33 c
Han297	40 b	41 ab
Average	40	39

Within treatments, means followed by the same letter are not significantly different at $P < 0.05$ according to LSD.

II. 4 Discussion

Our results demonstrate that there is reason for concern for increased Zn deficiency problems as a result of cultivation change from flooded to aerobic conditions in calcareous soils with high pH. Under aerobic conditions, Zn shoot concentration (Figure 1, 3), grain yield and Zn-harvest index (Table 5) were significantly lower than under flooded conditions. The fact that biomass production, grain yield and shoot Zn concentration responded positively to Zn application (Table 2, 4) indicates that Zn was a growth-limiting factor in our experiment. Our results are in line with Giordano and Mortvedt (1974), who reported that Zn deficiency symptoms were more pronounced under moist conditions compared to flooded conditions on a soil with pH 7.5.

From a human nutritional point of view, high Zn concentration in the grain is of paramount importance. However, no significant differences were found in grain Zn concentration between two cultivation systems in this study. From flooded to aerobic cultivations, no significant reduction in grain Zn concentration was observed (Table 4). It was not clear whether this was related to Zn status in tested soil in our study. To carefully evaluate the effect of cultivation system on Zn concentration of the edible part of the whole grain and Zn distribution within grain need to be investigated in more detail.

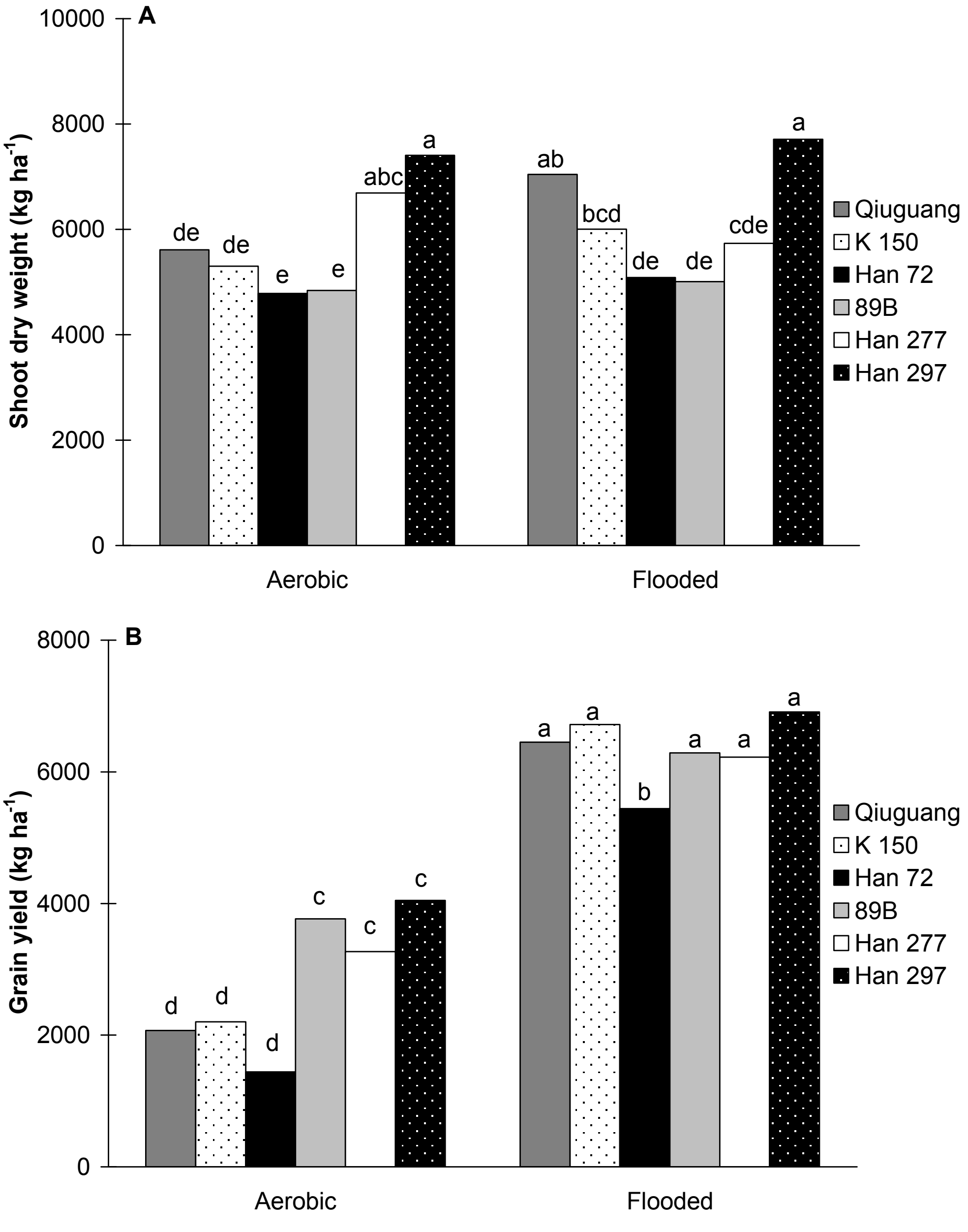

Figure 4. Shoot dry weight (A) and grain yield (B) of six genotypes under aerobic and flooded conditions when no Zn was applied. Means with the same letter are not significantly different ($P < 0.05$).

Under aerobic conditions, the newly developed aerobic rice genotypes showed a higher Zn efficiency than the lowland genotype Qiuguang (Table 3). This indicates that breeding has been successful in this respect. Also, Zn-harvest index was higher for most aerobic genotypes compared to the lowland genotype, although not significantly in most cases (Table 5). The variation among aerobic genotypes shows that there are ample opportunities for breeders to improve Zn efficiency.

Zinc application significantly decreased Zn-harvest index (Table 5) and did not affect grain Zn content and grain yield (Table 4). This is in agreement with many other studies on wheat (Rengel et al., 1999) and indicates that Zn application to the soil cannot fully meet the objective of increasing the Zn level in edible portions of rice. Development of rice cultivars with higher Zn efficiency and higher Zn-harvest index seems therefore a more promising strategy than fertilization for the purpose of increasing Zn content of the grain. Foliar application of Zn should be considered as an alternative strategy to increase Zn-harvest index and at the same time raise grain Zn concentration.

In line with Gao et al. 2005 (Chapter IV), we found considerable genotype variation in Zn efficiency and yield under aerobic conditions. Our results confirm preliminary results demonstrating that K150 is a Zn inefficient genotype (Table 3). Already at tillering, K150 showed a low shoot Zn content (results not shown) and concentration (Figure 1), and a relatively strong response to Zn fertilization (Figure 2). Genotypes 89B and Han277 were more efficient and showed sufficient shoot Zn concentrations (Figure 1), no response to Zn application at tillering stage (Figure 2), and relatively high yields (Figure 4). The results for these genotypes confirm that Zn deficiency has its impact already in early growth stages (Forno et al., 1975). No effect of Zn application was found on grain yield (Table 4). This is largely in agreement with previous results showing that Zn deficiency occurs in the first few weeks after sowing and plants can spontaneously recover 6-8 weeks later (Forno et al., 1975).

It is likely that drought contributed to the yield reduction under aerobic cultivation (Figure 4). Drought has been identified as a major constraint causing yield loss in rice in Asia (Widawaky and O'Toole, 1996), even for aerobic varieties (Bouman et al, 2005). No results of any rice cultivars that consistently grow better in aerobic soil than in flooded soil can be found. It is generally concluded that aerobic conditions in itself imply a low level of stress for rice, particularly if the relative humidity is low (Dingkuhn et al., 1989). An interaction between drought stress and Zn deficiency is highly likely, because low water availability will hamper soil Zn transport towards the roots. It may also hamper Zn transport within the plant.

Acknowledgements

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Chapter III

From Lowland to Aerobic Rice Cultivation: Analysis of Reduced Zinc Uptake

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Abstract

With decreasing water availability for agriculture and increasing demand for rice, rice production in Asia is now in a transition from flooded to aerobic cultivation systems. This paper concerns the effect of aerobic compared to flooded conditions on Zn bioavailability. A field experiment was conducted with a rice crop grown on a low Zn soil, under both flooded and aerobic conditions. Sampling was conducted at tillering and mature stage. The mass fraction of Zn in shoots and Zn uptake was significantly lower at both stages in plants grown in the aerobic field, indicating the reduced Zn bioavailability. To understand the underlying mechanisms, a controlled lab soil incubation experiment in combination with the geochemical analysis by a fully parameterized model was done. Results showed that the difference found in plant Zn uptake between two cultivation systems was rather small compared to what can be expected based on modelling. The individual effects of soil factors e.g. redox potential and pH have been demonstrated to cause orders of magnitude of variation in Zn concentration, compared to 15% difference in plant Zn uptake. This difference between the experimental observations and the model calculations leads to the suggestion that it may lead to erroneous interpretations if soil chemical factors are considered individually instead of in an integrated way that allows for synergistic and antagonistic effects. We speculated this discrepancy could be partly caused by the root-induced chemical changes in the rhizosphere, where the local acidity, DOC and redox conditions can be completely different from those of the bulk soils on which the geochemical analysis are based. Besides this rhizosphere effects, the observed lower Zn uptake in aerobic fields compared to flooded fields might also be partly caused by the different rates in transpiration and diffusion between two systems.

Key words: Aerobic, Flooded, Geochemical analysis, Incubation, Rice, Zinc

III. 1 Introduction

As the most important staple food in Asia, rice production is now in transition from the traditional high water-consuming lowland rice to a promising new production system of "aerobic rice" because of water shortage. Aerobic rice varieties are currently being developed by crossing lowland with upland varieties and grown as a dry field crop in irrigated but non-flooded and non-puddled fertile soils (Bouman et al., 2005). Studies on such new production systems for aerobic rice have just begun in some Asian countries, mainly focusing on yield potential and drought resistance (Yang et al., 2005; Kato et al., 2006). However, it has also been shown that after conversion towards aerobic cultivation, Zn deficiency may develop (Wang et al., 2002).

Zinc deficiency is one of the most common micronutrient disorders in rice, for both lowland varieties grown under flooded conditions (Doberman and Fairhurst, 2000) and upland or aerobic varieties under aerobic conditions (Fageria, 2001). Zinc deficiency has been associated with a wide range of soil conditions including high pH, high bicarbonate content, high organic matter and low redox potential (Alloway, 2004). Sharma et al. (2002) and Singh et al. (2002) reported Zn deficiency in raised beds and in direct seeded rice systems under an alternate wetting and drying irrigation regime. Zinc deficiency symptoms were observed for aerobic rice in North China, 2 or 3 years after fields were shifted from flooded to aerobic conditions (Wang et al., 2002). Thus, it is highly relevant to investigate the possible consequences of the shift from lowland to aerobic cultivation and the underlying mechanisms of changes in the bioavailability of Zn.

Previous research that compared Zn availability for plants in either flooded or aerobic conditions revealed that the results are conflicting. Yoo and James (2003) reported reduced uptake of Zn by rice, wheat and barley upon flooding. Kashem and Singh (2001) also found that flooded conditions significantly reduced the mass fraction and uptake of Zn in rice grown in contaminated soils. The most severe Zn deficiency was observed in the most poorly drained areas along a toposequence of soils with increasingly poorer drainage downslope (Van Breemen and Castro, 1980). Such deficiency had been attributed to high pH-values (Trierweiler and Lindsay, 1969), high organic matter contents (Van Breemen and Castro, 1980) and low redox potential, leading to precipitation of $Zn(OH)_2$ or ZnS (Lindsay, 1972;

Ponnamperuma, 1972) in poorly drained soils. Contrary to these results, we observed a smaller uptake of Zn by rice plants grown in aerobic conditions compared with flooded conditions on a calcareous soil (Gao et al., 2006; Chapter II). This result is in agreement with Giordano and Mortvedt (1974), who reported that Zn deficiency symptoms were more pronounced in moist fields than in flooded fields, and with the already mentioned observations of Wang et al. (2002). In view of the many factors that are involved in Zn uptake (Wissuwa et al., 2006), it is clear that understanding of the various processes should complement correlative approaches, such as provided by Van Breemen and Castro (1980). Chemical processes have been suggested as highly relevant in the previously cited literature.

Zinc availability for plants is influenced by the distribution of Zn over different chemical forms in soils, i.e., Zn speciation. Soil Zn can be present: (1) dissolved in soil solution, (2) bound as exchangeable Zn to negatively charged compounds, such as adsorbed onto metal oxides or organic matter, (3) included as insoluble Zn in clay minerals or metal oxides, or (4) held in primary minerals such as ZnS and $Zn(OH)_2$ (Kiekens, 1995). Only the first, dissolved fraction is commonly supposed to be directly available for plant uptake. It appears that Zn bioavailability in soil is mostly regulated by adsorption-desorption reactions and solubility relations between the solution and solid phases (Lindsay, 1991; Catlett et al., 2002). Behavior of Zn in aerobic soils differs significantly from flooded soils (Dutta et al., 1989), because of drastic differences in physical, chemical and electro-chemical properties of soils (Ponnamperuma, 1972). Soil properties including pH, redox potential, organic matter and pedogenic oxides exert the most significant influence on the adsorption-desorption reactions of Zn in soils and thus regulate the amount of Zn dissolved in soil solution (Guadalix and Pardo, 1995). These properties are expected to change after a shift from lowland to aerobic cultivation. Bulk soil pH will change from pH 7 towards the original soil pH (Liu, 1996). Redox potential will increase (Gao et al., 2002), causing the formation of Fe and Mn oxides, onto which Zn might be absorbed. Meanwhile, Zn solubility may increase by the oxidation of sulfide and consequent dissolution of ZnS (Castro, 1977). The content of organic matter may decrease because of oxidation. Predicting the overall consequence of these changes on soil Zn dynamics is difficult and which processes dominate may differ for different soils. For the assessment of the availability of Zn for plants, often soil extractions are used (Chuan et al., 1996; Dutta et al., 1989, Sajwan and Lindsay, 1988), which cannot reveal the availability of Zn in soil *in situ*, especially when the soil was kept under flooded condition. The recent progress of *in situ* pore water sampling in combination with geochemical modelling of Zn speciation and adsorption (Schröder et al., 2006) is very promising for understanding which processes dominate, how they differ for aerobic and anaerobic conditions, and how this affects Zn availability. A major advantage is that the available model has been parameterized, validated, and successfully applied in a purely predictive way for a broad range of soils and chemicals, under both aerobic and anaerobic conditions (Schröder et al., 2006).

The objective of this work is to determine differences in plant Zn uptake between lowland and aerobic rice cultivation systems. Both systems are characterized with geochemical analysis and modelling to identify the dominant processes that control the solid-solution partitioning of Zn in soils. The considered soil is compared with those considered in cited literature, to explain apparent disagreements.

III. 2 Materials and methods

III. 2. 1 Crop growth

A field crop experiment was conducted in 2004 at Shou city (116° 78' E, 32° 57' N), Anhui province, China. Major soil properties are presented in Table 1. The experimental field had been used for a lowland rice - wheat rotation system for more than ten years. Rice plants showed Zn deficiency symptoms in the last two years in flooded fields. This is in accordance with the soil DTPA-Zn level, which is below the critical level (0.8 mg kg^{-1}, Doberman and Fairhurst, 2000).

The experiment was conducted under two water regimes with four replicates. The area of each plot was 36 m^2 (6 × 6 m). To prevent seepage between plots, the flooded and aerobic plots were separated by installing plastic sheets in the bunds down to a depth of 60 cm. At sowing, all plots received 225 kg N ha^{-1} as urea, 39 kg P ha^{-1} as KH_2PO_4 and 75 kg K ha^{-1} as KCl to ensure these elements were not yield-limiting factors. Rice (*Oryza sativa* L.) seeds were sown in rows 20 cm apart in mid May at a density of 150 kg ha^{-1}. In the aerobic plots, the irrigation was commenced at sowing and at visual symptoms of drought stress. Three irrigations were applied: on 1 day after sowing (DAS), 35 DAS and 92 DAS. The flooded plots were submerged at 3 weeks after sowing. A 3 cm water level was maintained until 2 weeks before harvest in October.

Table 1. Physical and chemical properties of the soil. All analyses were done before fertilizer application.

Depth (cm)	Texture (<2 µm %)	pH (H_2O)	CEC (cmol kg^{-1})	C (g kg^{-1})	Total N (g kg^{-1})	P-Olsen (mg kg^{-1})
0-30	40	6.0	20.9	15	1.0	18.5

NH4OAc-K	DTPA-Zn	HNO_3-Zn	Element measured in aqua regia (mg kg^{-1})					
(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	Ca	Fe	Mn	P	S	Zn
201	0.3	4.2	4424	31368	283	415	19	41

The crop was sampled at tillering stage (53 days DAS) and mature stage (146 DAS, about 20% moisture in grains, based on Yoshida, 1981). At tillering stage, shoot samples were collected from two 50 cm segments. At mature stage, 9 m^2 (3 × 3 m) in the center of each plot was harvested. Samples were washed shortly in 0.1% HCl followed by deionized water. Shoot dry weight and grain yield were determined after drying the samples at 80 °C for 72 h. Zinc mass fraction in rice shoot was determined after digestion in an acid mixture (HNO_3 + $HClO_4$). Digests were analyzed with an atomic absorption spectrophotometer (Pye Unicam SP 9 800, Cambridge, UK). Zinc analyses were validated using the certified Zn values in standard samples obtained from Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL, Wageningen University, the Netherlands). Shoot Zn uptake was calculated by multiplying shoot dry weight with shoot Zn mass fraction.

All data were tested and met the requirements on normality (Kolmogorov-Smirnov) and homogeneity of variance (Levene's test). Analysis of variance was done on the data of shoot dry weight, grain yield, shoot Zn uptake and Zn mass fraction in shoot. Means were compared with Tukey's Honestly Significant Differences test at the 5% level of probability. All analyses were performed with SAS Release 8.02 (SAS Inc.).

III. 2. 2 Soil incubation

To obtain representative solution samples under field conditions that are of use for comparison with modelling is quite difficult because of soil variability, also on small distances, and the high sensitivity of Zn concentration in solution for such variability (Schröder et al., 2006). Hence, to chemically characterize the soil solution, homogenized samples were incubated under well-controlled conditions. Soils used for the incubation experiment were collected from the above experimental field. Soils were air-dried (25-30 °C), sieved (2 mm), and thoroughly mixed to increase homogeneity. Dry soils were then pre-incubated at field water capacity level (70% water holding capacity) for one week before the experiment started.

Treatments included flooded and aerobic conditions with four replicates. For flooded conditions, the soils were continuously kept flooded with a 2-cm water layer. For aerobic conditions, the soils were continually kept at field water capacity. Rhizon soil moisture samplers (SMS MOM, Rhizosphere Research Products, Wageningen, The Netherlands), with a polyethersulfone membrane of 10 cm length with a pore diameter < 0.2 μm, were used to sample pore water. Before use, the Rhizon samplers were cleaned with 0.28 M HNO_3 and checked for leakage. Rhizon soil moisture samplers were used to sample pore water at 15 cm soil depth. A low pressure was build up inside the Rhizon samplers by a 10 mL syringe (300912, Becton Dickinson, EDC, Temse, Belgium).

The weighted 258 g wet soils (200 g dry soil at field water capacity) were placed separated into eight 250 mL plastic beakers. During filling, Rhizon samplers were placed at 15 cm soil depth. Redox potential electrodes were inserted into the flooded beakers. At the bottom of the aerobic beakers, a filter holder with filter was placed prior to filling such that there is air below the soil. Three hollow tubes were inserted through the bottom to the air for each beaker to provide fresh air from the bottom. The amount of pore water sampled by Rhizons was around 6 mL for each aerobic beaker and around 9 mL for each flooded beaker. Pore water was sampled three times, i.e., when the redox potential in flooded beakers was 200, 0 and -170 mV, respectively. The pH was determined immediately after sampling. DOC (Dissolved organic carbon) was determined on a TOC analyzer (SK12, Skalar, Breda, The Netherlands). Element concentrations including Ca, Cu, Fe, Mg, P, Zn of pore water were determined by ICP-MS (Spetroflame Modula, Spectro, Kleve, Germany).

Means of pH, DOC and metal concentrations between aerobic and flooded treatments were compared with Tukey's Honestly Significant Differences test at the 5% level of probability.

III. 2. 3 Geochemical modelling

Speciation calculations were done with the object-oriented modelling framework ORCHESTRA (Meeussen, 2003). The model set-up and parameterisation is based on Schröder et al. (2005). Equilibrium constants for minerals and soluble species used for calculation are taken from the MINTEQA2 database (Allison et al., 1991). Constants from other sources are summarised in Table 2.

Table 2. Soluble and mineral species and *log* Ksp values used for calculation from other sources than Allison et al., 1991.

species or mineral	*log* K_{sp}	ref
$Ca^{2+} + Cl^{-} = CaCl^{+}$	-1.00	Lindsay, 1979
$Ca^{2+} + 2Cl^{-} = CaCl_{2(aq)}$	0.00	Lindsay, 1979
$Fe^{2+} + CO_3^{2-} = Fe^{(II)}CO_{3(aq)}$	4.38	Stumm and Morgan, 1995
$Fe^{3+} + H_2O + CO_3^{2-} = Fe(OH)CO_{3(aq)} + H^{+}$	-3.83	Bruno et al., 1992a
$Fe^{2+} + 2CO_3^{2-} = Fe^{(II)}(CO_3)_2^{2-}$	7.10	Bruno et al., 1992b
$Mn^{2+} + CO_3^{2-} = MnCO_{3(aq)}$	4.90	Stumm and Morgan, 1995
$Zn^{2+} + 2\ CO_3^{2-} = Zn(CO_3)_2^{2-}$	9.63	Parkhurst and Appelo, 1999
$Zn_3(PO_4)_2 \cdot 4H_2O + 4H^{+} = 3Zn^{2+} + 2H_2PO_4^{-} + 4H_2O$	3.80	Lindsay, 1979
$ZnFe_2O_4 + 8H^{+} = Zn^{2+} + 2Fe^{3+} + 4H_2O$	9.85	Lindsay, 1979
$Zn_5(OH)_6(CO_3)_2 + 10H^{+} = 5Zn^{2+} + 2CO_{2(g)} + 8H_2O$	45.00	Preis and Gamsjäger, 2001
$Zn_2SiO_4 + 4H^{+} = 2Zn^{2+} + H_4SiO_4$	15.33	Parkhurst and Appelo, 1999
$Zn_2Al(OH)_6Cl + 6H^{+} = 2Zn^{2+} + Al^{3+} + Cl^{-} + 6H_2O$	23.50	Ford and Sparks, 2000

As shown in Table 1, the soil used has a low concentration of Zn, and the 0.43 HNO_3 extractable fraction is only 10% of the concentration measured in aqua regia. Thus, the major part of the Zn seems not to be exchangeable and is therefore not available to plant uptake but might be bound in e.g. the lattice of clay minerals. Therefore, we used the HNO_3-extractable amounts as input for the generic model calculations.

To calculate the adsorption of metals to solid phases and DOC, we used the consistent NICA model (Kinniburgh et al., 1999) parameterized by the generic NICA parameter set (Milne et al., 2003). We assumed 50% of soil organic carbon (SOC) to be present as humic acid (HA) and 30% of DOC to be present as fulvic acid (FA), based on a carbon content of organic matter of 50% (Schachtschabel et al., 1992). The remaining fractions of SOC and DOC were assumed to be non-reactive. The pe was derived from the redox potential (Eh) as: pe = Eh/0.059, where Eh is expressed in volt.

Adsorption of metals and anions by iron oxide was calculated by the CD-MUSIC model (Hiemstra and Van Riemsdijk, 1996), assuming a specific surface area of 50 m^2 g^{-1} (Cornell and Schwertmann, 1996). The sorption parameters used are given in (Schröder et al., 2005). Sorption on magnetite (Fe_3O_4) was modelled similar to goethite (Dixit and Hering, 2003; Cornell and Schwertmann, 1996). Sorption to manganese (hydr)oxides was described by the surface complexation model of Tonkin et al. (2004), using a specific surface area of 50 m^2 g^{-1}. Clay adsorption of Ca, Zn, and Mn was calculated by a simple ion exchange model with a constant CEC and we used exchange coefficients for Zn/Ca, Fe/Ca and Mn/Ca of 1 (Bruggenwert and Kamphorst, 1982). The CEC of clay (0.46 cmol kg^{-1} clay) is derived by subtracting the calculated CEC of the organic matter and goethite at the given pH (0.025 cmol kg^{-1}) from the CEC measured (Table 1). The activity of Fe^{3+} is controlled by the presence of goethite, and the activity of PO_4^{3-} by hydroxyapatite. SiO_4^{4-} activity is calculated in equilibrium to quartz. We used the total amounts of Ca measured in soil as input parameter. The pCO_2 is calculated from the amount of IOC measured in pore water.

A similar approach was used by Schröder et al. (2006) to predict several heavy metals including Zn in river floodplain soils with excellent results. This work also indicated the importance of Zn/Fe interactions on Zn concentrations in solution.

III. 3 Results

III. 3. 1 Plant Zn uptake

Water management (aerobic or flooded) significantly affected shoot Zn uptake at both tillering and mature stages (Table 3). Shoot Zn uptake was 20% and 15% lower under aerobic conditions than flooded conditions at tillering and mature stage, respectively. A lower shoot Zn mass fraction was found in aerobic fields. Shoot dry weight and grain yield were slightly but significantly lower under aerobic conditions than flooded conditions.

III. 3. 2 Soil incubation

Figure 1 shows the change of redox potential as a function of time after flooding started in the soil incubation experiment. The initial redox potential was 425 mV. During the first 10 days after flooding, redox potential decreased rapidly to 100 mV. After that, the decrease of redox potential was slower. It took 5, 42 and 148 days of flooding to reach a redox potential 200mV, 0 mV and –170 mV, respectively, which covers the range of redox potentials measured in the aerobic and flooded fields.

The concentration of Zn in pore water showed little change during the incubation period, indicating a minor effect of redox potential on Zn availability (Table 4). There was no significant difference in the measured Zn concentration in pore water between aerobic and flooded conditions at 5 and 42 days after flooding. A slightly but significantly higher Zn concentration in pore water was found in aerobic conditions at the 148th of day sampling.

A significant difference was found for pore water pH, which under aerobic conditions was higher than under flooded conditions (Table 4). With the increase of incubation time, pH showed little changes under aerobic conditions whereas for the flooded soil, pH gradually approached that of the aerobic conditions.

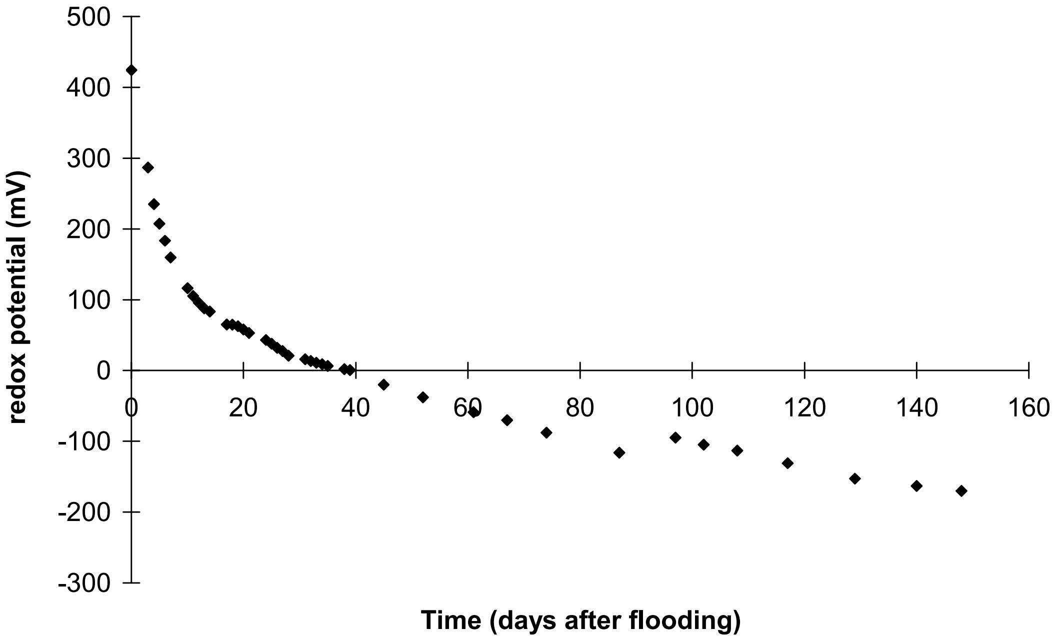


Figure 1. Dynamics of the redox potential under flooded conditions.

For both flooded and aerobic samples, DOC concentrations in pore water decreased with the extent of incubation time (Table 4). However, a higher DOC concentration was maintained in flooded samples throughout the experiment. Higher Ca, Fe and Mn concentrations were found in pore water samples from the flooded soil. With the extent of incubation time, the concentrations of Fe and Mn showed a tendency to increase in samples from flooded soil and a contrasting tendency was found in samples from the aerobic soil.

Table 3. Effect of cultivation on shoot dry weight, straw yield, grain yield, shoot Zn mass fraction and Zn uptake.

Cultivation	Tillering stage			Mature stage			
	Shoot dry weight ($g\ m^{-2}$)	Shoot Zn mass fraction ($mg\ kg^{-1}$)	Zn uptake ($g\ ha^{-1}$)	Straw yield ($Mg\ ha^{-1}$)	Grain yield ($Mg\ ha^{-1}$)	Shoot Zn mass fraction ($mg\ kg^{-1}$)	Zn uptake ($g\ ha^{-1}$)
Flooded	47.1 a[1]	14 a	6.4 a	11.4 a	5.2 a	32 a	349 a
Aerobic	42.9 b	11 b	5.1 b	10.5 b	4.7 b	29 b	297 b

[1] Within treatments, Per column means followed by the same letter are not significantly different (Tukey, *P* = 0.05).

Table 4. Effect of water regimes on pH, DOC and element concentrations in pore water collected from the incubation experiment. Samplings were conducted at 5, 42, 148 days after incubation started.

Treatment	Redox potential	pH	DOC	Metal concentration				
				Ca	Cu	Fe	Mn	Zn
	(mv)		(mg L^{-1})	(mg L^{-1})	(µg L^{-1})	(mg L^{-1})	(mg L^{-1})	(µg L^{-1})
5th day								
Flooded	200	6.1 b[1]	86 a	205 a	4.2 ab	5.7 a	6.7a	15.0 a
Aerobic	425	6.7 a	92 a	85 b	6.1 a	0.1 b	2.4b	11.1 a
42nd day								
Flooded	0	6.7 b	82 a	479 a	1.1 b	59.2 a	19.0a	10.1 a
Aerobic	-	6.9 a	33 b	74 b	2.6 a	0.1 c	1.1c	9.0 a
148th day								
Flooded	-170	6.5 b	39 a	370 a	0.9 b	43.0	16.0a	8.8 b
Aerobic	433	6.8 a	13 b	79 b	2.3 a	n.d.[2]	0.2b	10.6 a

[1] Per column means followed by the same letter are not significantly different (Tukey, *P* = 0.05).

[2] n.d. means not detective.

III. 3. 3 Geochemical modelling

Figure 2 shows the concentrations of Zn as a function of the redox potential for different pH values as calculated by the model, without attempting to calibrate it further to the data.

The calculated Zn concentrations are rather constant over a wide pe range, but are strongly dependent on pH. ZnS is formed below -180 mV. For the present conditions, formation of ZnS and FeS_2 are both thermodynamically feasible and in the later case, the large amounts of Fe and the small amounts of S present in soil may preclude the formation of ZnS at all. Assuming equilibrium with ferrihydrite (Lindsay, 1979) does not change the modelling results much, except that at high pH and pe the concentration of Zn decreases because of the formation of a mineral phase. The presence of bicarbonates only has minor influence on the Zn partitioning, where concentrations up to 200 mg L^{-1} IOC (inorganic carbon content) were tested.

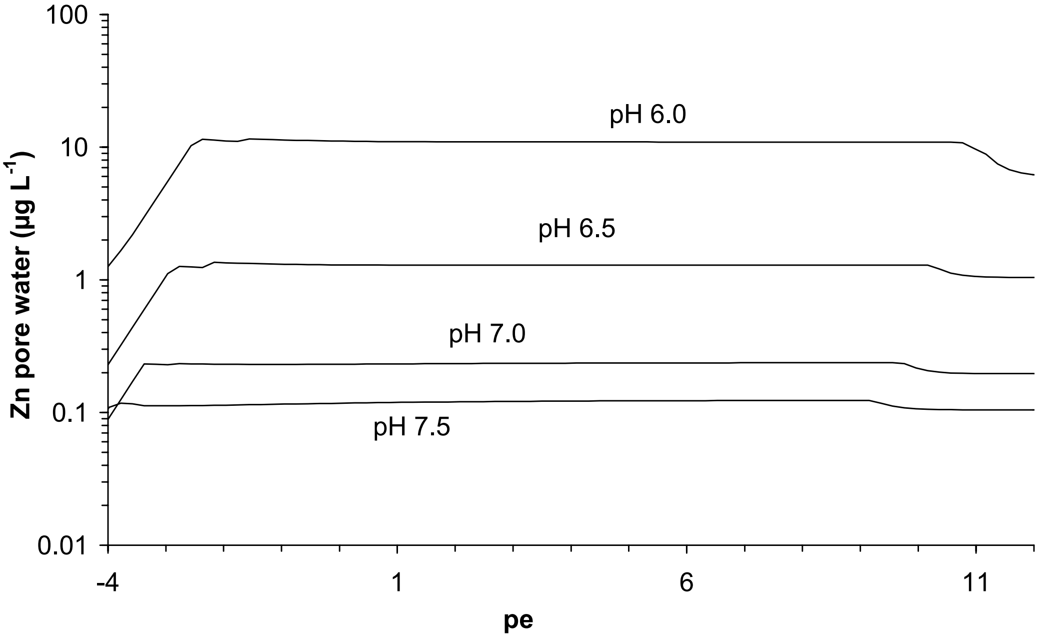
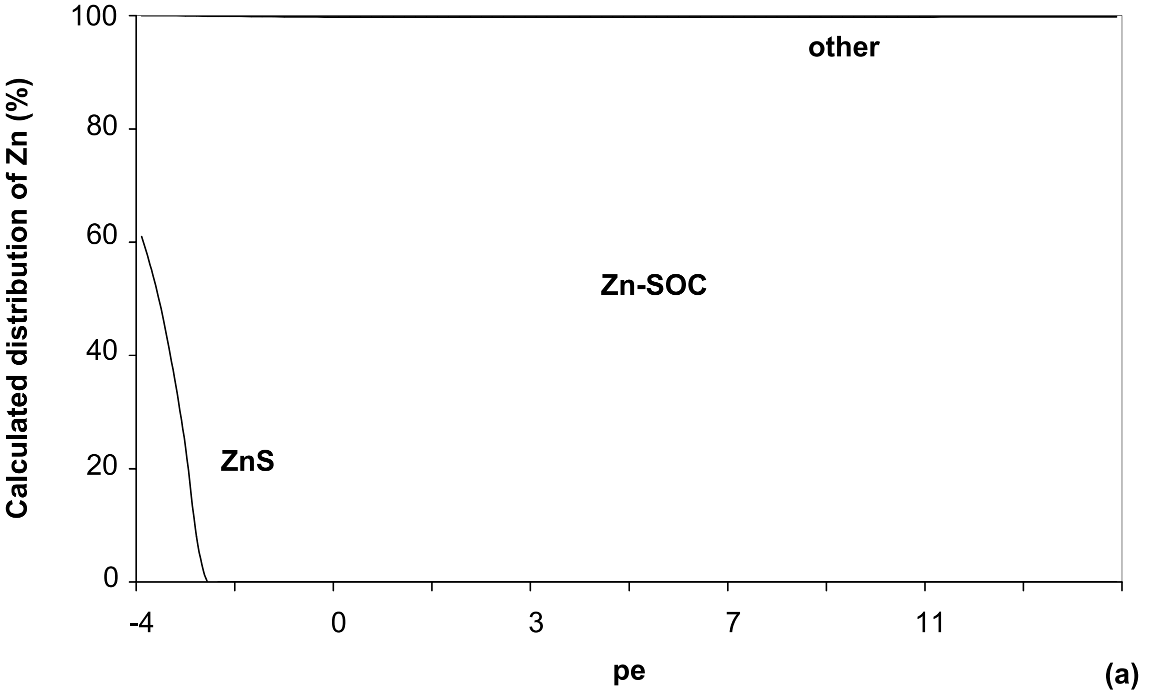


Figure 2. Zinc concentration in pore water as a function of the pe at pH 6.0, 6.5, 7.0, and 7.5 as calculated by the geochemical model.

Figure 3 shows the distribution of Zn in the solid phase and the water phase at pH 6.5. In the solid phase, binding to organic matter dominates, but at very low redox potential, it can also partly be present as ZnS. In the water phase, the distribution is dominated by the free Zn^{2+} cation, and sorption to DOC is limited. However, for pH values larger than 7.0, Zn in pore water can be equally distributed as Zn^{2+} and DOC bound Zn (data not shown). A large part of Ca in soil is not present as a mineral phase but adsorbed by either clay or organic matter. With lower pe and larger Fe concentrations, the Ca concentrations measured in the pore water becomes larger too (Table 4). Both Ca and Fe concentrations are therefore strongly correlated with the redox potential (data not shown) and can outcompete Zn, which is mainly bound to organic matter.

Figure 4 and 5 shows the calculated dependency of the Zn concentrations on the pH and the Ca concentrations. At lower pH values, the Zn concentration decreases up to 2 log-units for every pH-unit and about one log-unit above pH 7.0, whereas Zn concentrations increase with approximately a 1:1 ratio with the Ca concentrations at positive pe values. Both variables thus influence the Zn concentration in pore water quite strongly.



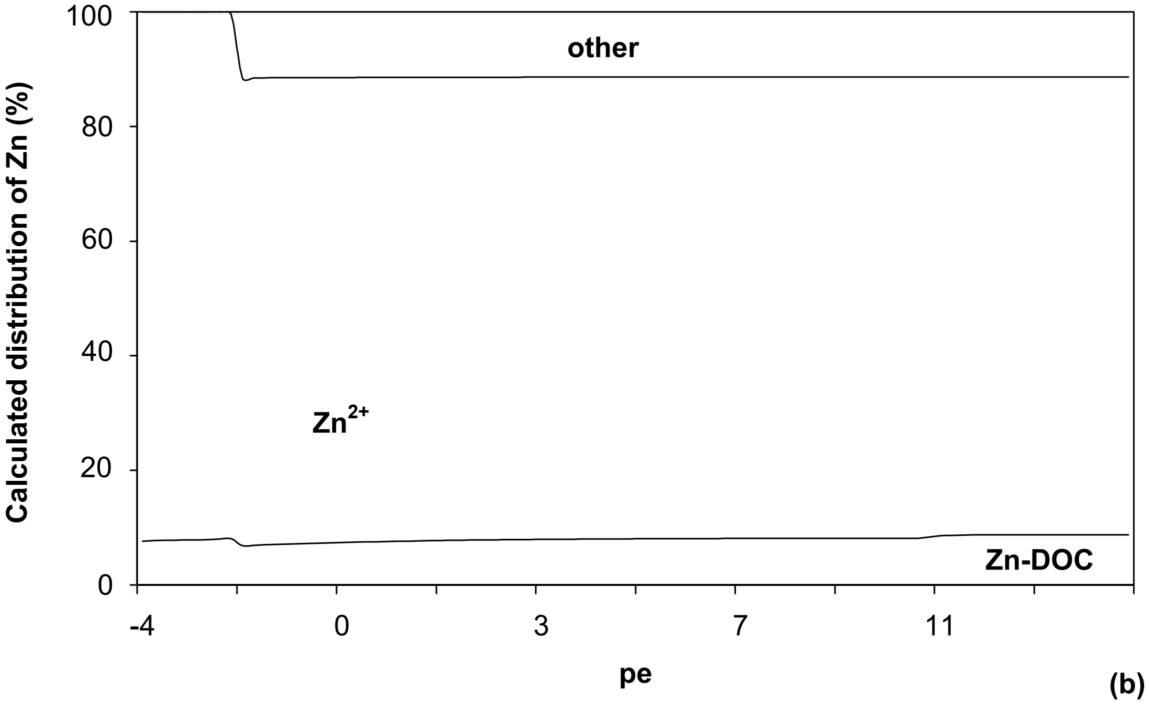


Figure 3. Calculated distribution of Zn in the solid phase (a) and soluble phase (b) for different redoxpotentials at pH 6.5 as calculated by the geochemical model.

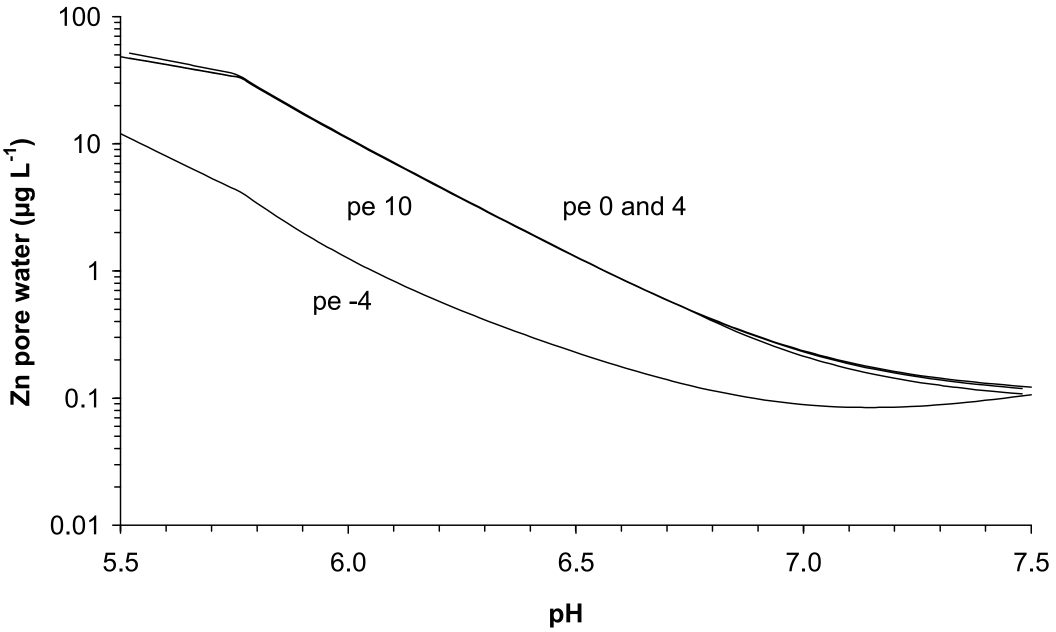


Figure 4. Zinc concentration in pore water as a function of the pH at pe -4, 0, 4, and 10 as calculated by the geochemical model.

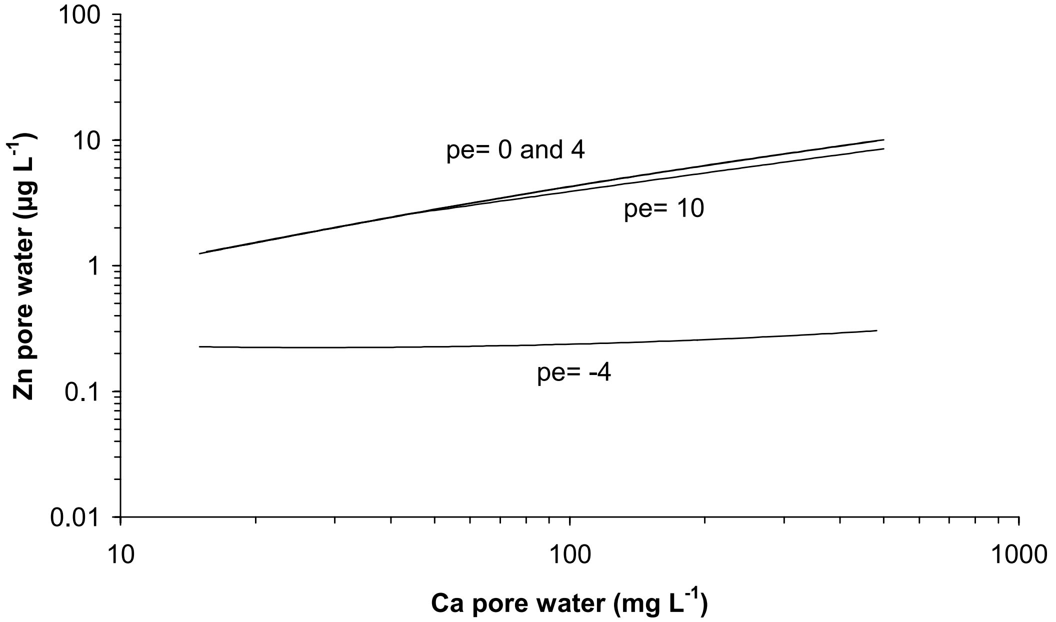


Figure 5. Zinc concentration in pore water as a function of the Ca concentration at pH 6.5 and pe -4, 0, 4, and 10 as calculated by the geochemical model.

Figure 6 shows the effect of enlarged Fe^{2+} concentrations on the Zn concentration in pore water. At both pH 6.0 and pH 7.0 values, the effect that involves Fe^{2+} on the concentration of Zn in pore water is important only at the small redox potentials.

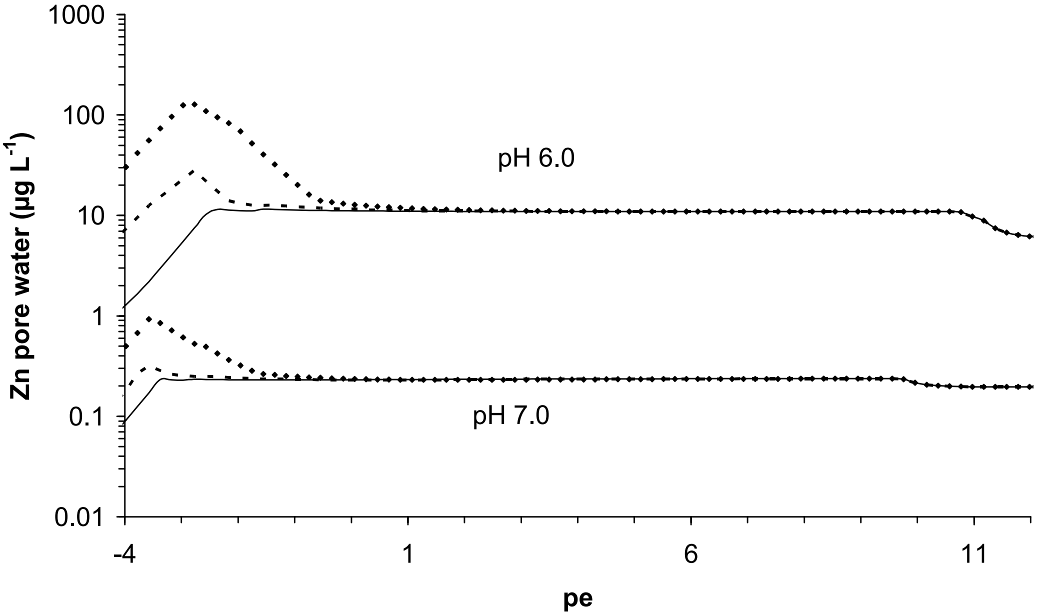


Figure 6. Zinc concentration in pore water as a function of the pe at pH 6.0 and 7.0 and enlarged Fe^{2+} concentrations. Solid line: uncalibrated model; dotted line: twofold Fe^{2+} concentrations; diamonds: fourfold Fe^{2+} concentrations.

III. 4 Discussion

Similar as in previous work (Gao et al., 2006; Chapter II), plants took up less Zn in aerobic fields than in flooded fields. The difference in Zn uptake is small but statistically significant. The cause for difference can be diverse, such as water stress for the aerobic cultivation, and Zn speciation in soil and soil solution. In this paper, the role of speciation is the main focus and the main tools are the geochemical model and experimental data. By not calibrating the model, it is ascertained that only the current understanding of speciation with currently available parameters is used. The advantage of this approach is that the used model is consistent with a broad selection of publications, whereas the disadvantage that only trends can be considered is not a very limiting disadvantage for the homogenized incubation samples. Also, the kinetic effects are not accounted for in the current study. So, no attempt has been made to obtain (or fit) a better agreement between model and the geochemical measurements. This implies that differences in predicted and measured Zn concentration levels occur, however, this does not invalidate the trends that are discussed.

Differences in Zn speciation between aerobic and flooded conditions are often attributed to differences in redox potential which may lead to precipitation as a sulfide, organic matter content, pH, and competition of different cations (Van Breemen and Castro, 1980; Schröder et al., 2006; Wissuwa et al., 2006). As Figure 2 reveals, the total Zn concentration in pore water is rather constant for a large range of the redox potentials according to the geochemical modelling. Only for redox potentials smaller than observed in this research, Zn in solution may drop significantly due to formation of ZnS in Figure 2. Hence, the similar concentrations of Zn found for different redox potentials in Table 4 are in agreement with the modelled independency of Zn as a function of redox potential. Stieger and Feller (1994) also reported that Zn availability in soils is not sensitive to changes of redox potential.

However, the dependency of dissolved Zn on soil pH is completely different for the experimental observations and the model calculations (Figure 2). For pH ranging from 6.1 to 6.9, differences in dissolved Zn would be expected of almost two orders of magnitude according to the calculations. Such differences are absent in Table 4 and are also absent if Zn uptake is compared, and also disagree with the larger effect of pH than redox potential on Zn solubility in contaminated soils (Chuan et al., 1996). Also Yoo and James (2003) attributed the decreased Zn solubility in acid soils to the increase of pH due to flooding rather than to the decrease of redox potential. Similar results were reported by Mandal et al. (1992). There has been considerable research on the effect of soil pH on the mobility or the availability of Zn. Charlatchka and Cambier (2000) have shown that pH variations influence Zn solubility, where it does not matter whether the pH changes are induced by reductive dissolution, organic acid formation or other processes. It has been shown that upon flooding the pH of acid soils increases towards neutral values (Ponnamperuma, 1972) as is observed in the incubation experiment. It is plausible that the discrepancy between observed and modelled pH effect is caused by additional processes.

Upon a decrease of the redox potential during prolonged flooding, oxides of Fe(III) and Mn(III, IV) become unstable and dissolve, due to Fe and Mn reduction (Stumm and Sulzberger, 1992). That this process occurs is suggested by increasing Fe and Mn concentrations as shown in Table 4, and has several consequences which can either increase or decrease dissolved Zn concentrations. The first effect is that formed Fe^{2+} and Mn^{2+} are more soluble than the oxidized forms, and will compete with Zn for sorption sites at soil organic matter, clay and other oxides. This effect causes dissolved Zn to increase. Also the dissolution of oxides, onto which Zn may have been adsorbed, will lead to an increase of dissolved Zn. The Fe(III) and Mn(III, IV) oxide-bound Zn-fraction was suggested to be an important control on the solubility and bioavailability of Zn in soils by Luo and Christie (1998). As Figure 3 indicates the oxide bound Zn fraction is relatively small for the present soil and not expected to be very important. The release of Fe and Mn, which outcompete Zn at organic matter sorption sites, may be responsible that the change of pH for the flooded case from 6.1 to 6.7 (day 42) and 6.5 (day 148) is not associated with decreasing Zn in solution as suggested by Figure 2. Whereas these processes are very plausible, an objective quantification with the model is not possible, because it is virtually impossible to experimentally quantify the ratio of Fe(II)/Fe(III), which is kinetically determined, with sufficient accuracy for a solution. Moreover, the ratio of ionic Fe and suspended Fe-colloids is difficult to assess. Both ratios play a role if Fe(II) is reoxidized (e.g. after sampling) since the formed Fe(III) colloids may very effectively scour the solution for Zn and surpress measured Zn concentrations (Mandal and Hazra, 1997). Another effect that involves Fe^{2+} is important only at the low redox potentials and illustrated in Figure 6. If a significant part of the Fe measured in pore water is present as Fe^{2+}, it will inhibit the formation of ZnS. Furthermore, since insufficient S is present in this soil to immobilize all Fe in the form of FeS, the increased Fe concentration may surpress Zn-binding by SOC.

Other studies have showed an enhanced Zn availability at lower redox potential. Charlatchka and Cambier (2000) showed that at a fixed pH, reducing conditions enhance the mobility of Zn in contaminated soils. Chuan et al. (1996) analyzed dissolved Zn in a polluted paddy soil at different fixed pH values and observed higher Zn solubility at lower redox potential. Similar as in the present paper, they attributed this effect to reductive dissolution of Fe and Mn. For the present soil, it may be noted that also the increase of Ca in solution, for the flooded case, leads to enhanced competition for sorption sites with Zn, and therefore cause a significant buffering of Zn in solution.

Of major interest is the understanding of why different studies seem to be in disagreement. The results of this work suggest at least a few causes for apparent discrepancies, even if these suggestions cannot be made definitive with the available information. For instance, extensive flooding periods may lead to a considerable decrease in the concentration of Zn if the formation of insoluble ZnS occurs (Carbonell-Barrachina et al., 2000) and is well known for anaerobic sediments (Schröder et al., 2006). This process, which decreases Zn concentrations in solution by orders of magnitude and therefore would be highly relevant for Zn uptake, requires sufficient sulfur, sufficiently low redox potentials (long term inundation), and absence of other metals that might outcompete Zn for sulfur. In this study, the geochemical modelling showed that ZnS is formed below –180 mV, among others because of the low sulfur content of this soil. In the lab incubation experiment, the lowest redox potential was –170 mV, hence ZnS formation would not be expected. For other soils with higher sulfide content, precipitation of ZnS may dominate the Zn solubility in soils and then a lower Zn availability can be expected under flooded conditions. This could be also a possible factor to explain the conflicting results regarding the comparison of Zn availability to plants between flooded and aerobic conditions.

This work suggests several other factors than sulfur content and achievable redox conditions, that need to be taken into account in future Zn-rice availability studies. The concentrations of redox sensitive oxides, that may supply cations of e.g. Fe and Mn to solution and that may sorb part of the present Zn under aerobic conditions is important. This pool of Zn and of cations that may compete with Zn should be compared with other Zn sorbing phases, such as clay colloids and soil organic matter.

For the present soil, DOC-complexed Zn is relatively low. For other soils, as has been suggested in literature, this may not be the case. For plant uptake, concentrations of Zn in solution may not be limiting Zn uptake if other species migrate faster towards plant roots (such as DOC-Zn) or if desorption from e.g. soil organic matter occurs rapidly.

Whereas the present work indicates some probable processes that affect Zn availability for rice, the absence of a pH effect in Zn uptake might be due to other factors. A prominent one would be that Zn uptake, similarly to e.g. water uptake (De Jong van Lier et al., 2006), depends on chemical conditions close to the soil-root interface (the 'rhizosphere'). At this interface, conditions can be different, and therefore be represented erroneously by those of the bulk of soil as characterized in the present paper and most cited papers. In particular, if rhizosphere pH-values are relatively constant and independent of bulk soil pH-changes as affected by respectively flooded and aerobic conditions, the latter might lead to wrong expectations such as those mentioned earlier in this section.

Rhizosphere controls the bioavailability of Zn through changes in pH, redox potential and microbial population. Changes in pH are brought by the excretion of protons (H^+), hydroxyl (OH^-) or bicarbonate (HCO_3^-) ions due to cation/anion imbalance in the plant, the evolution of CO_2 by respiration, and the excretion of low molecular weight organic acids. The form of N supply has a major role in the cation/anion uptake ratio and its subsequent effect on rhizosphere pH (Marschner, 1993). As a consequence of cultivation shift from flooded paddy soil to aerobic soil, the dominant form of N taken up by rice roots is shifted from NH_4^+ to NO_3^-. Generally, to maintain electro neutrality within the plant cell, rhizosphere pH decreases when NH_4^+ forms are used and increases when NO_3^- forms are used because of the release of H^+ and OH^- or HCO_3^-, respectively (Troelstra et al., 1985; Gahoonia et al., 1992). A difference of 1-2 pH units between rhizosphere and bulk soil has been reported frequently (e.g. Marschner and Romheld, 1983; Hinsinger, 1998). This rhizosphere pH increase after a shift from flooded to aerobic conditions may to some extent explain the reduced plant Zn uptake in aerobic fields. Moreover, plant roots alter the redox potential in the rhizosphere soil directly by excreting CO_2, and indirectly through the supply of readily available carbon for enhanced microbial respiration. In particular, root absorption and assimilation of NH_4^+ and NO_3^- consume 0.31 mol O_2 mol^{-1} NH_4^+ and 1.5 mol O_2 mol^{-1} NO_3^-, respectively (Bloom et al., 1992). Hence, when roots use NO_3^- as N source in aerobic fields, the rhizosphere redox potential declines more rapidly than when they use NH_4^+ in flooded fields. Also, the enhanced activity of microorganisms in rhizosphere is important in relation to the bioavailability of Zn. More research is needed to clarify the difference in rhizosphere environment between flooded and aerobic fields and the effect on plant Zn uptake.

The difference found in plant Zn uptake between aerobic and flooded fields might also be partly caused by the different rates in transpiration and diffusion between two systems. In aerobic fields, the irrigation was conducted at visual symptoms of drought stress. It is assumed the averaged soil moisture tension in the root zone during the growing season under this cultivation is around 90 kPa and above (Wang et al., 2002). Wopereis et al. (1996) showed that rice transpiration rate decreases with the decreasing soil moisture content when the soil moisture tension is higher than 70 kPa. A lower transpiration rate in aerobic fields than flooded fields was expected in the current study, which may decrease the mass flow of Zn from soil to plants and consequently decrease Zn uptake by plants. Grifferty and Barrington (2000) reported that transpiration rate of young wheat plant significantly affected plant Zn uptake and the lower transpiration rate reduced plant Zn uptake. Another possibly involved mechanism to explain lower Zn uptake in aerobic fields is the lower rates of dissolution and diffusion, which may restrict Zn transport towards the plant root (Yoshida, 1981).

III. 5 Conclusion

In this paper, we showed that Zn uptake by rice was significantly, yet, in a geochemical sense only to a modest degree, affected by cultivation under either aerobic or flooded conditions. Though this limited effect has been reported before in the scientific literature, it might be unexpected from a soil chemical point of departure, where the individual effects of e.g. redox potential and pH have been demonstrated to involve orders of magnitude of heavy metal concentrations variation.

A significant redox effect as such might be expected provided that sufficient sulfur is present in soil to immobilize Zn at low redox potentials. A large pH effect may be expected, but might also be completely compensated as in the present study if redox-controlled metal oxide dissolution leads to compensating effects. This observation leads to the suggestions, that (i) it may lead to erroneous interpretations if soil chemical factors are considered individually instead of in an integrated process-related way that allows for synergistic and antagonistic effects as mentioned, and (ii) the present paper suggests that at least the trio pH-redox-oxide should be considered simultaneously.

Some processes can not be easily quantified with sufficient accuracy. An example is so-called 'rhizosphere' processes in the field. However, it is plausible that local acidity, DOC levels caused by excretion of complexing organic acids, and perhaps different redox conditions may be completely different from bulk properties on which most availability studies are based. With geochemical models as used in this paper, understanding can be obtained of what happens in the rhizosphere, despite the limitations of such models.

Acknowledgements

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Chapter IV

Tolerance to Zinc Deficiency in Rice Correlates with Zinc Uptake and Translocation

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Abstract

To study variation in Zn efficiency (ZE) among current Chinese rice genotypes, a pot experiment was conducted with 15 aerobic and 8 lowland rice genotypes. Aerobic rice is currently bred by crossing lowland with upland rice genotypes, for growth in an aerobic cultivation system, which is saving water and producing high yields. A Zn deficient clay soil was used in our screening. Zinc deficiency resulted in a marked decrease in shoot dry matter production of most genotypes after 28 days of growth. Genotypes were ranked according to their tolerance to Zn deficiency based on ZE, expressed as the ratio of shoot dry weight at Zn deficiency over that at adequate Zn supply. Substantial genotypic variation in ZE (50-98%) was found among both lowland and aerobic genotypes. Zinc efficiency correlated significantly ($P < 0.05$) with Zn uptake ($R^2 = 0.34$), Zn translocation from root to shoot ($R^2 = 0.19$) and shoot Zn concentration ($R^2 = 0.27$). The correlation with seed Zn content was insignificant. In stepwise multiple regression analyses, variation in Zn uptake and Zn translocation explained 53% of variation in ZE. Variation in Zn uptake could be explained only for 32% by root surface area. These results indicate that Zn uptake may be an important determinant of ZE and that mechanisms other than root surface area are of major importance in determining Zn uptake by rice.

Key words: Aerobic, Lowland, Rice, Translocation, Tolerance, Uptake, Zinc efficiency

IV. 1 Introduction

Because of water constraints, rice production in China is now in transition from the traditional high water-consuming lowland rice cultivation to a promising new cultivation system of "aerobic rice". Aerobic rice varieties are currently developed by crossing lowland with upland varieties and grown as a dry field crop in irrigated but non-flooded and non-puddled fertile soils (Bouman et al., 2002). Their yield potential is higher than for traditional upland varieties that are adapted to infertile soils. The soils that are used for aerobic cultivation are frequently Zn deficient. So far, however, breeding has mainly been focused on the yield of aerobic genotypes under sufficient Zn supply.

Zinc deficiency in cereal plants, including rice, is a well-known problem that causes reduced agricultural productivity all over the world (Fageria et al., 2002). Fertilization is not always an option to resolve Zn deficiency because of agronomic and economic factors, such as the relatively high cost of fertilizer (Graham and Rengel, 1993). Alternatively, exploiting genetic variability to breed staple crops with high Zn efficiency (ZE) could offer a sustainable and cost-effective way to overcome Zn deficiency problems. The term ZE is defined as the capacity of a genotype to grow well under Zn-deficient conditions, and ZE is usually expressed as the ratio of shoot dry weight under Zn deficiency over that under adequate Zn supply (Graham et al., 1992). Hence, it is relevant to investigate whether there is variation in ZE among aerobic genotypes.

Genotypes of crop plants can vary widely in ZE, as reported for maize (Ramani and Kannan, 1985), wheat (Cakmak et al., 2001), common bean (Hacisalihoglu et al., 2004) and lowland rice (Sakal et al., 1989). Mechanisms responsible for genotypic variation in ZE were thoroughly reviewed by Rengel (2001) and Hacisalihoglu and Kochian (2003). There seem to be many uncertainties on mechanisms that control tolerance to Zn deficiency. Most likely, there is no single mechanism in any crop species. The expression of high ZE in cereals including wheat, rye, barley, triticale and oat was related to enhanced uptake and translocation capacity of Zn into shoots and higher amounts of physiologically active Zn in leaf tissues (Cakmak et al., 1998).

For aerobic rice, we previously showed ZE variation among five aerobic rice genotypes under the field conditions (Gao et al., 2006; Chapter II). The aim of this study is to assess the variation in ZE for a larger set of Chinese rice genotypes, including some newly developed aerobic genotypes. We also investigate whether this variation in ZE can be attributed to seed Zn content, Zn uptake, Zn translocation from root to shoot and/or shoot Zn concentration.

IV. 2 Materials and methods

A greenhouse pot experiment was conducted in Hefei city (117° 16' E, 31° 51' N), Anhui province, China, to determine ZE for different rice (*Oryza sativa* L.) genotypes. Treatments include 2 Zn levels and 23 rice genotypes. Zinc levels were -Zn (no Zn applied) and +Zn (5 mg Zn kg^{-1} of soil). Zinc was applied together with the other nutrients as a solution of $ZnSO_4 \cdot 7H_2O$, and was equivalent to 10 kg Zn ha^{-1}. Of the 23 rice genotypes, 15 were aerobic and 8 were lowland genotypes. Of the aerobic genotypes, K150, Han297, Han72, 89B271-17hun and Han277 had shown ZE in a previous field experiment ranging from 75% (K150) to 100% (Han277) (Gao et al., 2006; Chapter II). Of the lowland genotypes, IR26 and IR8192-31 are known to be Zn-inefficient and Zn-efficient, respectively (Doberman and Fairhurst, 2000). They were used in this experiment as references. A Zn-deficient soil was collected from Shou city, Anhui province, China. Some characteristics of the soils: soil texture clayey, pH (H_2O) 6.5, organic matter 1.7%, and DTPA-extractable Zn 0.32 mg kg^{-1} soil. The treatments were combined in a completely randomized design with 3 replicates.

Twenty seeds of one genotype were sown per plastic pot containing 2 kg soil. At sowing time, each pot received a basal application of 150 mg N as $Ca(NO_3)_2$, 44 mg P as KH_2PO_4 and 83 mg K as KCl. Deionized water was added in amounts sufficient to bring the soil water content to 80% of field capacity. After emergence, the plants were thinned to 12 seedlings per pot. The pots were watered daily with deionized water to 80% of field capacity. The temperature in the greenhouse was 30±3 °C during the day and 23±3 °C during the night. Plants were grown under natural day length and light intensity in July 2004.

Plants were harvested 28 days after germination, because Zn deficiency problems are usually most severe in the first 2-4 weeks of growth (Doberman and Fairhurst, 2000). Shoots were cut off at ground level and soil was washed from the roots with tap water. Digital root images were made with a scanner (Epson Expression/STD 1600 scanner). The resulting grayscale images were analyzed with WinRHIZO root analysis software (Regent Instruments, Quebec, Canada). All root images were analyzed for root length and surface area. Shoots and roots were rinsed in deionized water, oven dried at 70 °C for 48 h, and weighed. ZE was calculated as the ratio of shoot (root) dry weight under Zn deficiency over that under adequate Zn supply. Dried plant and seed samples were digested in acid mixture (HNO_3 + $HClO_4$) for Zn analysis (Jackson, 1973). Zinc in plant digests was analyzed with an atomic absorption spectrophotometer (Pye Unicam SP 9 800, Cambridge, UK). Zinc analyses were checked using the certified Zn values in standard samples obtained from Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL, Wageningen University, Netherlands).

Statistical analysis of the data was performed using the SAS analytical software (SAS, 1990). Multiple regression analysis (stepwise) was employed and LSD ($P < 0.05$) was used to test the difference among treatments.

IV. 3 Results

IV. 3. 1 Zinc deficiency symptoms

Visual symptoms of Zn deficiency, such as reduced shoot elongation and whitish-brown necrotic patches on leaves, appeared 3-4 weeks after sowing. There were large differences in the severity of symptoms among 23 rice genotypes. Genotypes K150, Han297, Yuefu, Xieyou10 and IR26 showed severe deficiency symptoms and other genotypes such as Han 44, Hongkelaoshuya, Jindao305 and IR8192-31 showed very light or no symptoms in -Zn treatments. At +Zn treatments all genotypes grew well and no symptoms appeared.

IV. 3. 2 Dry matter production and Zn efficiency

Zinc deficiency resulted in a marked decrease in the dry weight of both shoot and root for most rice genotypes (Table 1). Shoot-based ZE varied from 50 to 98%. The ranking on shoot ZE was largely but not fully in accordance with that based on root ZE. The aerobic genotypes Hongkelaoshuya, Han44 and lowland genotypes IR8192-31, Jindao305 were genotypes most tolerant to Zn deficiency, with ZE values around 95%. The aerobic genotypes K150 and lowland genotypes Yuefu, Xieyou10, IR26 were the most intolerant genotypes with ZE values from 50% to 61%. As a group, lowland genotypes had generally lower ZE than aerobic genotypes.

IV. 3. 3 Factors determining ZE

Shoot Zn concentration at the -Zn treatment was within or below the marginal range of 10-20 mg kg^{-1} (Dobermann and Fairhurst, 2000) for all genotypes (Table 2). Shoot Zn concentration and ZE were positively and significantly (P = 0.007) related, with shoot Zn concentration explaining 27% (adj. R^2) of variation in ZE. Zinc application resulted in an increase to on average 93 mg kg^{-1} dry weight (Table 2), which is considered above sufficiency level. Under the +Zn treatment, no Zn toxicity symptoms were found and all genotypes could grow well. This indicates that plant growth in the -Zn treatment was limited by Zn availability indeed.

Seed Zn concentration of genotypes ranges from 9.7 to 15.4 mg kg^{-1} for aerobic rice and 9.2 to 30.3 mg kg^{-1} for lowland rice genotypes (Table 3). Zinc content in the seed ranged from 0.22 to 0.40 μg seed^{-1} for aerobic rice and 0.20 to 0.63 μg seed^{-1} for lowland rice genotypes (Table 3). Two genotypes from IRRI had higher Zn concentration and Zn content in seeds than others. Probably, this is because they originate from fields with high Zn availability. There was no significant correlation between ZE and seed Zn concentration (adj. R^2 = -0.02; P = 0.42) or seed Zn content (adj. R^2 = 0.04; P = 0.18).

Zinc uptake by plants was calculated as the difference between total plant Zn content and seed Zn content. It ranged from 5.2 to 35.3 μg pot^{-1} for aerobic genotypes and 7.3 to 31.6 μg pot^{-1} for lowland genotypes under -Zn treatment (Table 3). Zinc uptake and ZE were positively related (Figure 1). Zinc uptake explained 34% (adj. R^2) of the variation in ZE. Zinc application on average increased Zn uptake by plants with a factor 10-15 (data not shown).

Table 1. Plant dry weight and Zn efficiency (ZE) of 23 rice genotypes grown in a Zn-deficient soil with (+Zn = 5 mg kg^{-1}) and without (-Zn) Zn application. Per pot, 12 plants were grown for 28 days.

Genotypes	Shoot (g pot^{-1})			Root (g pot^{-1})		
	-Zn	+Zn	ZE (%)	-Zn	+Zn	ZE (%)
Aerobic rice						
K150	0.53	1.05	50	0.13	0.21	62
Han297	1.55	2.23	70	0.18	0.27	67
89B271-muzhuxi	1.85	2.65	70	0.49	0.49	100
89D108-11-1	1.87	2.65	71	0.46	0.62	74
91B-8-30-3	1.71	2.36	72	0.27	0.44	61
Han9	1.87	2.51	75	0.30	0.42	71
Han72	1.30	1.73	75	0.12	0.14	86
91B-te-3	1.97	2.55	77	0.34	0.43	80
89B271-17hun	1.26	1.62	78	0.08	0.13	62
Han502	1.74	2.15	81	0.26	0.33	77
Baxiludao	1.61	1.98	81	0.20	0.21	95
90B-10-1	1.95	2.33	84	0.40	0.42	95
Han277	2.17	2.51	87	0.39	0.41	95
Han44	1.66	1.87	89	0.22	0.26	84
Hongkelaoshuya	1.86	1.99	94	0.37	0.37	100
Mean	1.66	2.15	77	0.28	0.34	81
Lowland rice						
Yuefu	0.85	1.14	60	0.11	0.18	61
Xieyou10[#]	1.89	3.09	61	0.20	0.56	36
IR26	1.60	2.50	64	0.15	0.24	63
Qiuguang	1.17	1.79	65	0.19	0.26	73
Guofeng1[#]	2.29	3.46	66	0.27	0.66	41
Shennong99-8	0.65	0.98	67	0.09	0.11	82
Jindao305	1.15	1.28	90	0.13	0.17	78
IR8192-31	2.03	2.06	98	0.17	0.17	100
Mean	1.45	2.04	71	0.16	0.29	67
LSD (0.05)	0.30	0.41	17	0.07	0.08	29

Table 2. Zinc concentration in the seeds, shoots and roots of 23 rice genotypes grown in a Zn-deficient soil with (+Zn = 5 mg kg^{-1}) and without (-Zn) Zn supply.

Genotypes	Shoots (mg kg^{-1})		Roots (mg kg^{-1})	
	-Zn	+Zn	-Zn	+Zn
Aerobic rice				
K150	7.8	98	29.0	155
Han297	8.7	87	26.3	202
89B271-muzhuxi	12.9	96	23.4	299
89D108-11-1	8.3	97	22.0	268
91B-8-30-3	11.1	85	28.3	173
Han9	8.3	91	24.0	145
Han72	8.6	84	22.9	133
91B-te-3	11.6	105	26.0	236
89B271-17hun	13.7	67	30.7	183
Han502	15.7	95	24.0	130
Baxiludao	10.8	116	22.3	222
90B-10-1	12.5	85	21.4	192
Han277	9.3	111	25.3	171
Han44	10.7	94	28.0	145
Hongkelaoshuya	13.5	107	23.7	199
Mean	10.9	95	25.2	190
Lowland rice				
Yuefu	13.0	89	28.1	123
Xieyou10#	9.6	105	22.0	193
IR26	9.6	83	30.7	215
Qiuguang	8.3	83	29.3	252
Guofeng1#	8.6	110	27.5	194
Shennong99-8	12.2	85	20.4	174
Jindao305	11.6	87	23.7	233
IR8192-31	17.3	86	24.6	160
Mean	11.3	91	25.8	193
LSD (0.05)	2.4	13	6.1	25

Root surface area of genotypes under -Zn treatment ranged from 88 to 560 cm^2 pot^{-1} for aerobic rice and from 132 to 360 cm^2 pot^{-1} for lowland rice genotypes (Table 4). Root surface area correlated significantly with Zn uptake, but only explained 32% of variation in Zn uptake (Figure 2).

To assess whether the translocation of Zn from root to shoot is a factor that is involved in ZE, the percentage of Zn present in the shoot was calculated. It varied from 52 to 90% in the -Zn treatment (Table 3). The Zn efficient genotypes Hongkelaoshuya, Han44 and Han297 all translocated around 74% to the shoot. The inefficient genotype K150 translocated only 52% of its Zn to the shoot. The regression between Zn translocation as independent and ZE as dependent variable was positive and significant (Figure 3).

Multiple regression analysis (stepwise) was conducted to determine the combination of factors that best explained the variation in ZE statistically. The overall regression was significant ($P = 0.01$) with the coefficient of determination (adj. R^2) of 0.53. Contributing variables were Zn uptake (partial $R^2 = 0.34$) and Zn translocation to the shoot (partial $R^2 = 0.19$). Addition of seed Zn content and shoot Zn concentration as predictors did not further improve the model. In some studies, ZE is also expressed by taking into account the biomass potential of genotypes [ZE = (Dry weight at low Zn level / Experimental mean dry weight at low Zn) / (Dry weight at high Zn level / Experimental mean dry weight at high Zn)] (Graham, 1984; Fageria, 2001). In current study, we also tested the multiple stepwise regressions for ZE defined in this way. Similar to the results described above, adj. R^2 is 0.50 and contributing variables were Zn uptake (partial $R^2 = 0.35$) and Zn translocation to the shoot (partial $R^2 = 0.15$).

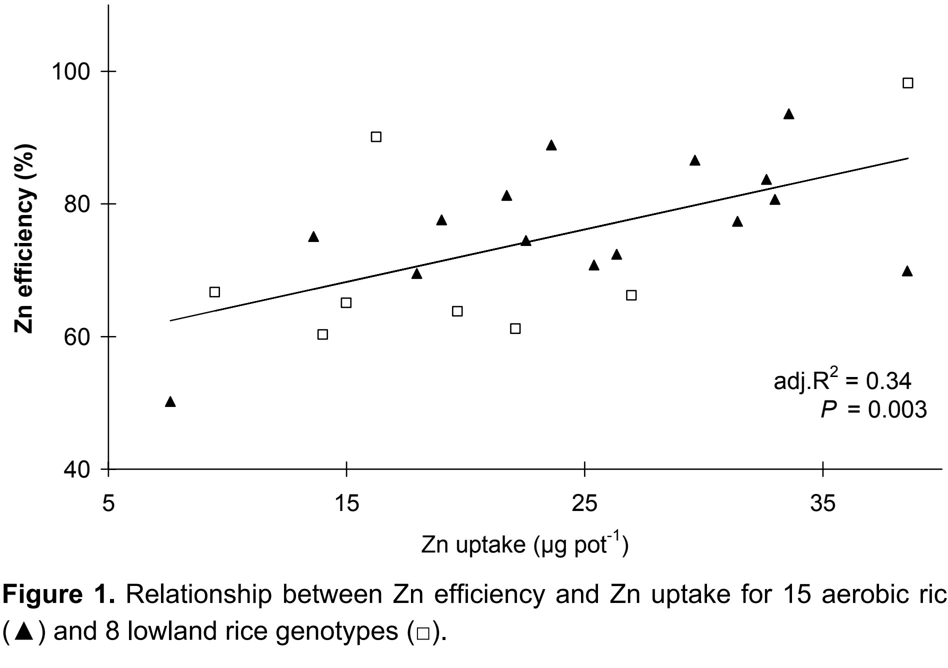

Figure 1. Relationship between Zn efficiency and Zn uptake for 15 aerobic rice (▲) and 8 lowland rice genotypes (□).

Table 3. Plant parameters related to Zn efficiency (All data are from the -Zn treatment).

Genotypes	Seed Zn concentration (mg kg^{-1})	Seed Zn content (µg $seed^{-1}$)	Zn uptake (µg pot^{-1})	Zn in shoot (%)
Aerobic rice				
K150	11.1	0.22	5.2	52.4
Han297	12.5	0.37	13.9	73.7
89B271-muzhuxi	11.7	0.29	35.3	61.5
89D108-11-1	9.9	0.26	22.5	60.4
91B-8-30-3	10.9	0.26	23.4	71.4
Han9	9.7	0.26	19.7	68.0
Han72	15.4	0.33	9.9	80.3
91B-te-3	12.7	0.31	28.0	72.2
89B271-17hun	10.8	0.26	16.1	89.9
Han502	15.2	0.40	28.6	81.8
Baxiludao	9.7	0.23	19.2	79.2
90B-10-1	10.0	0.25	29.8	74.2
Han277	14.5	0.37	25.6	67.3
Han44	12.0	0.27	20.6	74.6
Hongkelaoshuya	11.3	0.28	30.4	74.1
Mean	11.8	0.29	21.9	72.1
Lowland rice				
Yuefu	9.5	0.21	11.7	78.1
Xieyou10[#]	17.0	0.41	17.6	80.4
IR26	27.8	0.49	14.3	76.4
Qiuguang	10.4	0.23	12.4	63.7
Guofeng1[#]	12.8	0.24	24.3	72.4
Shennong99-8	9.2	0.20	7.3	81.8
Jindao305	9.6	0.21	14.0	80.8
IR8192-31	30.3	0.63	31.6	89.5
Mean	15.8	0.33	16.7	77.9
LSD (0.05)	1.2	0.12	5.4	6.6

Table 4. Root surface area of 23 rice genotypes grown under -Zn conditions.

Genotypes	Root surface area (cm^2 pot^{-1})
Aerobic rice	
K150	213
Han297	252
89B271-muzhuxi	549
89D108-11-1	560
91B-8-30-3	267
Han9	460
Han72	220
91B-te-3	383
89B271-17hun	88
Han502	347
Baxiludao	325
90B-10-1	468
Han277	475
Han44	266
Hongkelaoshuya	273
Mean	343
Lowland rice	
Yuefu	173
Xieyou10[#]	290
IR26	185
Qiuguang	292
Guofeng1[#]	360
Shennong99-8	134
Jindao305	132
IR8192-31	222
Mean	224
LSD (0.05)	102

IV. 4 Discusstion

The present study showed a substantial genotypic variation in tolerance to Zn deficiency among rice genotypes, not only for lowland varieties but also for newly bred aerobic varieties (Table 1). This variation among both lowland and aerobic genotypes offers opportunities for breeding as a tool to resolve Zn deficiency problems in rice. Our previous study showed variation in ZE among five aerobic rice genotypes under field conditions (Gao et al., 2006, Chapter II). The current study confirms these results. The five genotypes used in the field experiment showed the same order of ZE in the present pot experiment, suggesting that the results presented here are a good reflection of the field performance of the genotypes.

Multiple regression analysis showed that Zn uptake is the most important factor statistically explaining variation in ZE among the considered rice genotypes. Our results are in agreement with the results obtained with wheat (Cakmak et al., 1997a; Graham et al., 1992), chickpea (Khan et al., 1998) and common bean (Hacisalihoglu et al., 2004).

The uptake of the relative immobile Zn^{2+} by plant roots can be determined by root uptake surface area, root-induced chemical rhizosphere changes and the physiological uptake capacity per g root or per cm^2 root. An increase in uptake surface area can be achieved by either larger root system or higher mycorrhizal colonization. In the current study, variation in Zn uptake could only for 32% be explained by root surface area (Figure 2), which is consistent with the results on wheat (Dong et al., 1995). This emphasizes that there must be important additional mechanisms that determine Zn uptake. Our study does not distinguish between rhizosphere effects and uptake kinetics. The latter did not explain variation in ZE among wheat cultivars (Hacisalihoglu et al., 2001). If this is also true for rice, then rhizosphere effects could play a major role in Zn uptake. These rhizosphere effects may involve acidification of the rhizosphere (Kirk and Bajita, 1995) and exudation of Zn chelators (Tolay et al., 2001). Alternatively, or in addition, mycorrhizas could play a major role (Purakayastha and Chhonkar, 2001).

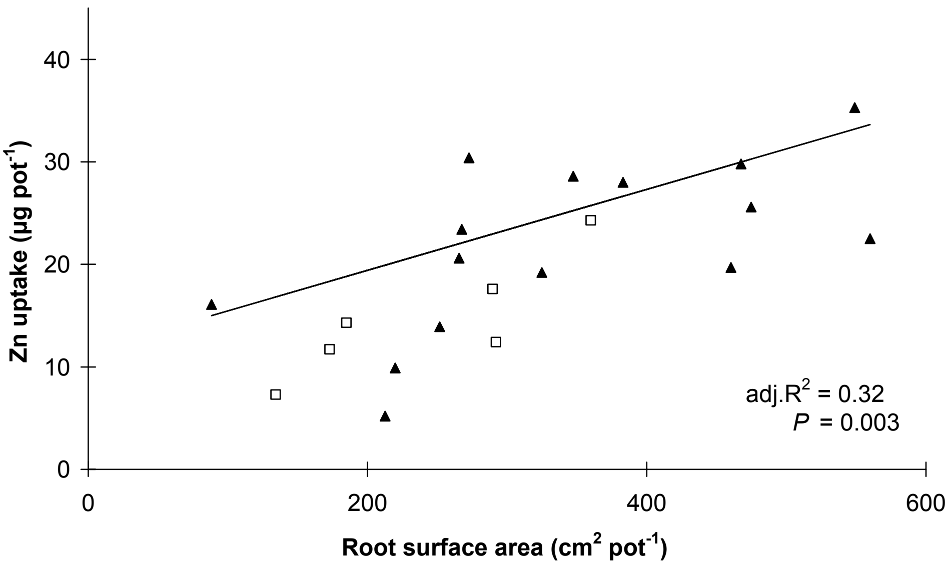

Figure 2. Relationship between Zn uptake and root surface area for 15 aerobic (▲) and 8 lowland rice genotypes (□).

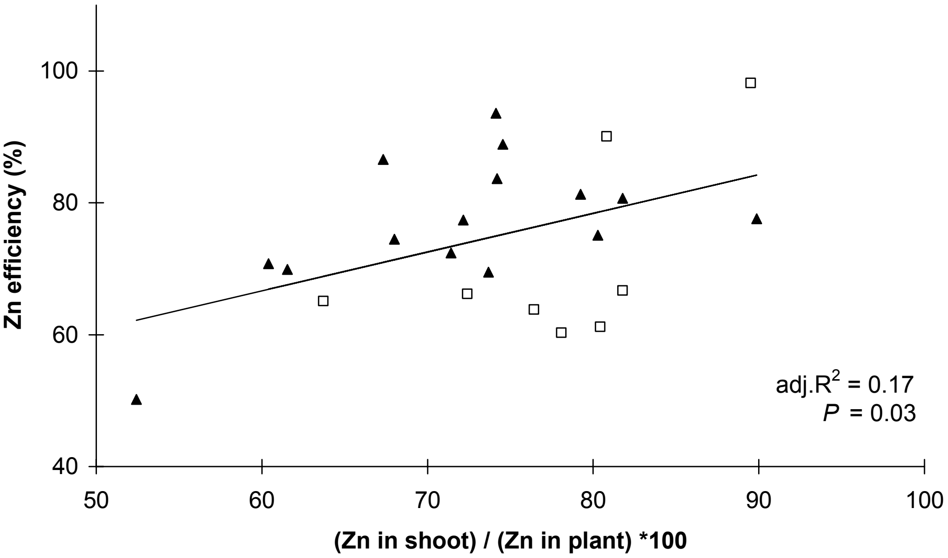

Figure 3. Relationship between Zn efficiency and Zn translocation for 15 aerobic rice (▲) and 8 lowland rice genotypes (□).

The second statistically significant variable determining ZE was Zn translocation from root to shoot. Zinc absorbed by roots can be rapidly transported to the shoots (Longnecker and Robson, 1993). Higher Zn translocation was thought to be a mechanism to explain the genotypic differences in ZE among chickpea (Khan et al., 1998) and wheat genotypes (Grewal et al., 1996). Other studies on wheat and common bean, however, showed that no correlation between Zn translocation to the shoot and ZE (Kalayci et al., 1999; Erenoglu et al., 2002; Hacisalihoglu et al., 2004).

Shoot Zn concentration and ZE were positively related, contrary to what was found for wheat (Cakmak et al., 1997a), chickpea (Khan et al., 1998) and common bean (Hacisalihoglu et al., 2004). This indicates the low internal requirement is not a mechanism involved in ZE of rice. Shoot Zn concentration was not included in the multiple regression analysis as a contributing factor to variation in ZE because it correlates with both Zn uptake and Zn translocations.

We could only explain 53% of variation in ZE with Zn uptake and Zn translocation to the shoots. Similarly, a large unexplained variation in ZE was reported for wheat (Cakmak et al., 2001). The unexplained variation in ZE might be related to differences in biochemical Zn utilization and Zn retranslocation from older into younger tissues in shoots (Hacisalihoglu and Kochian, 2003). ZE was found to be positively correlated with the activity of the Zn-requiring enzyme Cu/ZnSOD for wheat (Cakmak et al., 1997b; Hacisalihoglu et al., 2003) and black gram (Pandey et al., 2002). This enzyme protects plants from oxidative damage. This suggests that Zn efficient genotypes may be able to maintain well functioning of this enzyme under low Zn conditions. Zinc retranslocation from old parts to the young parts of shoot was suggested as one possible mechanism affecting ZE in common bean (Hacisalihoglu et al, 2004), wheat (Torun et al., 2000) and rice (Hajiboland et al., 2001).

In the current study, we only examined the possible mechanisms of ZE at seedling stage. It is possible that additional mechanisms of ZE are operative at later stages in the life cycle of the plant. A Zn efficient barley genotype was able to remobilise greater amounts of Zn from vegetative to reproductive tissues compared with a Zn-inefficient genotype (Genc et al., 2004).

Our study confirms that tolerance to Zn deficiency is a complex trait in which many plant characteristics are involved. Even though we took into account the four characteristics generally considered to be the most obvious, we could only explain 53% of variation in ZE. We also confirmed that a large set of genotypes needs to be considered in order to get a complete view on crop tolerance to Zn deficiency.

Acknowledgements

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Chapter V

Mycorrhizal Responsiveness of Aerobic Rice Genotypes is Negatively Correlated with their Zinc Uptake when Nonmycorrhizal

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Abstract

Plant Zn uptake from low Zn soils can be increased by Zn-mobilizing chemical rhizosphere processes. We studied whether inoculation with arbuscular mycorrhizal fungi (AMF) can be an additional or an alternative strategy. We determined the effect of AMF inoculation on growth performance and Zn uptake by rice genotypes varying in Zn uptake when nonmycorrhizal. A pot experiment was conducted with six aerobic rice genotypes inoculated with *Glomus mosseae* or *G. etunicatum* or without AMF on a low Zn soil. Plant growth, Zn uptake and mycorrhizal responsiveness were determined. AMF-inoculated plants produced more biomass and took up more Zn than nonmycorrhizal controls. Mycorrhizal inoculation, however, significantly increased Zn uptake only in genotypes that had a low Zn uptake in the nonmycorrhizal condition. We conclude that genotypes that are less efficient in Zn uptake when nonmycorrhizal are more responsive to AMF inoculation. We provide examples from literature allowing generalization of this conclusion on a trade off between mycorrhizal responsiveness and nutrient uptake efficiency.

Key words: Arbuscular mycorrhiza, Genotypic variation, *Glomus etunicatum*, *Glomus mosseae*, Mycorrhizal responsiveness, Rice, Root colonization, Zinc uptake

V. 1 Introduction

Low zinc (Zn) availability is a constraint to plant production on about one-third of agricultural soils worldwide. In addition, Zn deficiency affects over two billion people (Welch and Graham, 2002), mostly in developing countries where diets are cereal-based and low in meat, fish and vegetables. Increase of Zn levels in cereal grains through increase of Zn uptake is therefore needed.

Total Zn in soils with low bioavailable Zn is generally high enough to support high yields for many years. Consequently, genotypes with a high Zn-mobilizing capacity can perform relatively well (Rengel and Marschner, 2005). Thus, plant Zn uptake from low Zn soils can, apart from Zn fertilization (Rengel et al., 1999), be enhanced by engineering Zn efficiency in plants (Ramesh et al., 2004), selecting genotypes that inherently can take up Zn efficiently (Hacisalihoglu and Kochian, 2003) or by inoculation with arbuscular mycorrhizal fungi (AMF). In a previous paper (Gao et al., 2005; Chapter IV) we showed large variation in Zn uptake among nonmycorrhizal aerobic rice genotypes. In the current study we investigate whether inoculation of these genotypes with AMF could further increase Zn uptake. We thereby address the question whether efficient chemical nutrient-mobilizing rhizosphere processes can be combined with high mycorrhizal responsiveness in one genotype.

A beneficial effect of mycorrhizal colonization on Zn uptake has been shown for maize (Faber et al., 1990; Liu et al., 2000; Sharma et al., 1992), pigeneo pea (Wellings et al., 1991), wheat (Khare et al., 1998; Ryan and Angus, 2003) and wetland rice (Purakayastha and Chhonkar, 2001). Upon colonization, the mycelium of the AMF increases the nutrient absorbing surface area of the symbiosis, enhances exploration of a larger soil volume and thereby increases uptake, specifically of immobile nutrients such as P and Zn (Smith and Read, 1997).

Variation in responsiveness to inoculation with AMF among plant genotypes has been often documented but the genetic basis for this variation remains poorly understood. Studies in wheat (Hetrick et al., 1995) and maize (Kaeppler et al., 2000) indicated that there is a genetic basis for dependency on or responsiveness to AMF. Hetrick et al. (1992) suggested that modern breeding practices have produced cultivars that are highly dependent on fertilizers and show a reduced dependency on and responsiveness to the mycorrhizal symbiosis. It was then implied that there is considerable potential for redesigning crops that show an enhanced dependency on and responsiveness to AMF (Ryan and Graham, 2002). Zhu et al. (2001) also highlighted the importance of including mycorrhizal responsiveness in breeding programs for maximizing nutrient uptake efficiency. While this suggestion that modern breeding practices have limited the mycorrhizal contribution to nutrient uptake is intuitively plausible, it has been challenged, however, by Kaeppler et al. (2000) who alternatively proposed that selection for environmental stability may automatically be manifested in a decreased mycorrhizal response.

Aerobic rice varieties are currently developed for the new water-saving aerobic cultivation system (Bouman et al., 2005) by crossing high-yielding lowland with traditional upland varieties. So far, breeding has mainly been focused on yield and drought resistance of aerobic genotypes. Previously we have shown that there is considerable variation in Zn uptake from low Zn soils among newly bred genotypes (Gao et al., 2005; Chapter IV). We are unaware of previous reports on genotypic variation in mycorrhizal responsiveness based on Zn uptake in any plant species. Here we test if there is variation in mycorrhizal responsiveness based on growth and Zn uptake among aerobic rice genotypes, and how this variation is related to Zn uptake in the nonmycorrhizal condition.

We test the following hypotheses: (i) AMF-colonized rice plants are more efficient in mobilizing Zn from a low Zn soil than nonmycorrhizal plants; (ii) significant genotypic variation in mycorrhizal responsiveness exists among rice genotypes; (iii) high mycorrhizal responsiveness based on Zn uptake does not combine with inherent plant factors involved in efficient Zn mobilization in the rhizosphere. We discuss previous papers on other plant species and nutrients to evaluate if our conclusion on this third hypothesis can be generalized.

V. 2 Materials and methods

V. 2. 1 Experimental design and conditions

A pot experiment was conducted in a greenhouse of China Agricultural University, Beijing. Treatments included six rice genotypes and three mycorrhizal treatments in a factorial design with three replicates.

Six aerobic rice genotypes were selected because of their previously shown variation in Zn uptake and Zn efficiency (Gao et al., 2005; Chapter IV). Han 72, Han 44, Han 297 and 91B-8-30-3 are newly bred varieties in China Agricultural University. K 150 is a variety bred by Liaoning Academy of Agricultural Science, North China. Hongkelaoshuya is a traditional upland variety from Yunnan Province, South China. The genotypes had similar root surface area and similar root length at low soil Zn levels. Zinc uptake, however, calculated as the difference between plant Zn content after 28 days of growth and seed Zn content, ranged from 5.2 μg pot^{-1} to 30.4 μg pot^{-1} (Gao et al., 2005; Chapter IV).

Mycorrhizal treatments included a nonmycorrhizal control (-AMF) and inoculation with the mycorrhizal fungus *Glomus mosseae* (BEG167) or *Glomus etunicatum* (BEG168). Both were originally isolated from a high pH soil (pH_{H2O} 8.2) in North China. The inoculum consisted of colonized root segments and attached rhizosphere soil from maize grown under P and Zn deficient conditions in a glasshouse for 2 months.

A clay soil was collected from Shou city, Anhui province, China. Major characteristics: pH_{H2O} 6.5, organic matter 1.7%, DTPA-extractable Zn 0.3 mg kg^{-1}, and P-Olsen 18.5 mg kg^{-1}. The Zn status is much below the critical level (0.8 mg kg^{-1}) and the P status is high (> 10 mg kg^{-1}; Dobermann and Fairhurst, 2000). In a previous experiment, this soil induced Zn deficiency symptoms in most of the genotypes used (Gao et al., 2005; Chapter IV). Growth of all genotypes responded to Zn application. The soil was sterilized by autoclaving at 120 °C for 2 h, and air-dried. DTPA-extractable Zn was only slightly increased due to autoclaving but was on average still 0.3 mg kg^{-1}.

Eight seeds of one genotype were sown per pot containing 2 kg soil. At sowing time, each pot received a basal application of 150 mg kg^{-1} N as $Ca(NO_3)_2$ and 83 mg kg^{-1} K as KCl. In the +AMF treatments, inoculum (200 g) was mixed uniformly with the soil. In the -AMF treatment, an equivalent amount of sterilized inoculum together with the filtrate (<0.25 μm) of unsterilized soil was added to provide a similar microflora apart from the mycorrhizal fungus. The plants were thinned to four seedlings per pot one week after emergence. The pots were watered daily with deionized water, maintaining water content at 15% (w/w). The temperature in the greenhouse was 30±3 °C during the day and 23±3 °C during the night. Plants were grown under natural day length and light intensity in July 2005.

V. 2. 2 Harvest and analyses

Plants were harvested two months after germination, at tillering stage. Shoots were cut off at ground level and soil was washed from the roots with tap water. Shoots and roots were rinsed in deionized water. Roots were cut into 1 cm segments and mixed thoroughly. A subsample of 0.2 g fresh weight per pot was taken to determine mycorrhizal root colonization as described by Phillips and Haymann (1970). Briefly, 1 cm root samples were cleared in 10% KOH, acidified in 2% HCl and stained with 0.05% trypan blue in lactophenol. Thirty 1 cm root segments were randomly selected and mounted parallel to each other on a slide. Each root was observed under a microscope (200-400×) and rated according to the range of classes indicated by Trouvelot et al. (1986). The computer program MYCOCALC (www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html) was used to calculate the percentage of root length colonized by mycorrhizal fungi.

The shoots and remaining roots were oven-dried at 70 °C for 48 h, and weighed. Dried and ground plant samples were digested in acid mixture (HNO_3 + $HClO_4$) for Zn analysis (Jackson, 1973). Zinc in plant digests was analyzed with an atomic absorption spectrophotometer (Pye Unicam SP 9 800, Cambridge, UK). Zinc analyses were checked using the certified Zn values in standard samples obtained from Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL, Wageningen University, the Netherlands). Zinc uptake was calculated as the difference between plant Zn content and seed Zn content.

Mycorrhizal responsiveness (MR) was calculated as:

$$MR = [(\text{Plant dw}_{+AMF} - \text{Plant dw}_{-AMF}) / \text{Plant dw}_{-AMF}] \times 100$$

(Hetrick et al., 1992).

Mycorrhizal Zn responsiveness (MZnR) was calculated similarly:

$$MZnR = [(\text{Zn uptake}_{+AMF} - \text{Zn uptake}_{-AMF}) / \text{Zn uptake}_{-AMF}] \times 100.$$

V. 2. 3 Statistical analysis

All data were tested and met the requirements on normality (Kolmogorov-Smirnov) and homogeneity of variance (Levene's test). Analysis of variance was done on data on shoot and root dry weight, shoot Zn mass fractions and Zn uptake. Means were compared with Tukey's Honestly Significant Differences test at the 5% level of probability. All analyses were performed with SAS Release 8.02 (SAS Inc.).

V. 3 Results

V. 3. 1 Plant growth and mycorrhizal responsiveness

Inoculation with either *G. mosseae* or *G. etunicatum* significantly increased shoot dry weight of aerobic rice (Table 1, 2). The average mycorrhizal responsiveness based on plant dry weight of six rice genotypes was 48% for *G. mosseae* and 27% for *G. etunicatum* (Table 2). *G. mosseae*-colonized plants had significantly higher root dry weights than *G. etunicatum*-colonized and nonmycorrhizal plants.

There was an interaction (*P* = 0.002) between genotype and AMF treatment (Table 1). Genotypes Han 72 and 91B-8-30-3 did not increase shoot dry weight upon inoculation with either of the two AMF species (Table 2). Genotypes Han 44 and Hongkelaoshuya responded differently depending on the AMF species. Shoot dry weight of genotypes K 150 and Han 297 increased upon inoculation with either of the two AMF species.

Obvious symptoms of Zn deficiency (chlorotic leaves with brown necrotic spots on leaves) showed at the end of the experiment. There was no difference in the severity of symptoms among AMF treatments. The genotypes Han 44, 91B-8-30-3 and Hongkelaoshuya showed less symptoms than the other three genotypes.

Table 1. *P*-values of two-way analysis of variance for shoot and root dry weight, shoot Zn mass fractions and Zn uptake.

Source	DF	Dry weight		Shoot Zn mass fraction	Zn uptake
		Shoot	Root		
Mycorrhizas (M)	2	<0.0001	0.003	0.48	<0.0001
Genotypes (G)	5	<0.0001	<0.0001	0.03	<0.0001
M × G	10	0.002	0.32	0.02	0.002

V. 3. 2 Zinc uptake and mycorrhizal Zn responsiveness

Shoot Zn mass fraction of all plants ranged between 13.2 and 22.7 mg kg^{-1} (Figure 1). Neither *G. mosseae* nor *G. etunicatum* affected shoot Zn mass fraction (Table 1; Figure 1). Zinc uptake was significantly increased by inoculation with AMF (Table 1, 3). For *G. mosseae* and *G. etunicatum* mycorrhizal Zn responsiveness was on average 54% and 23%, respectively.

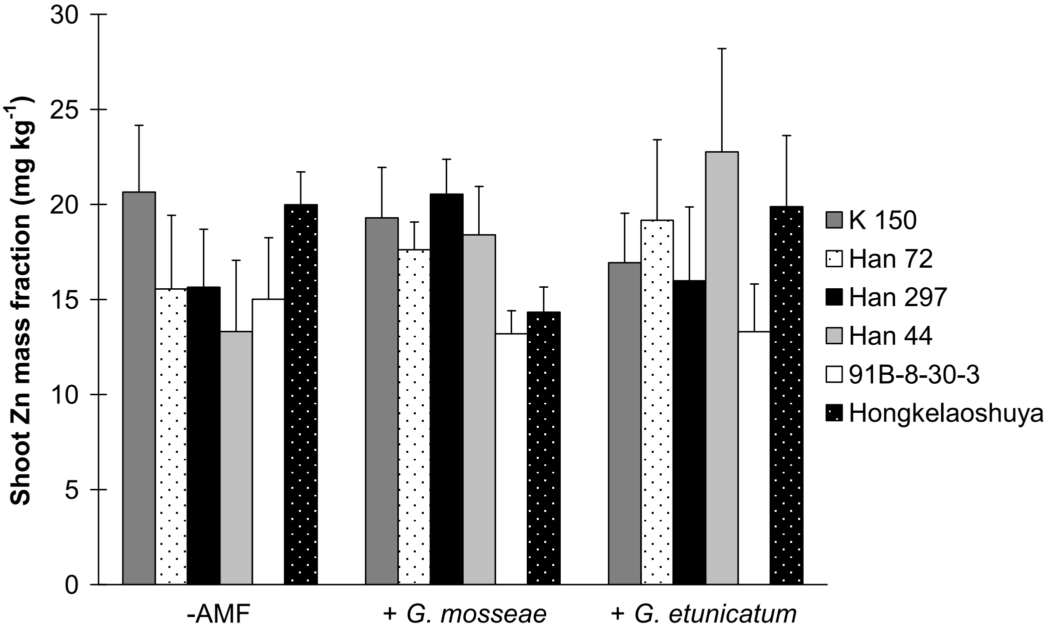

Figure 1. Shoot Zn mass fraction of six rice genotypes for three AMF treatments.

There was genotypic variation ($P < 0.0001$) in Zn uptake among the six rice genotypes (Table 1, 3). Also, the interaction between genotype and AMF treatment was significant for Zn uptake. Inoculation with *G. mosseae* significantly increased Zn uptake of genotypes K 150, Han 72 and Han 297 with 117%, 88% and 112%, respectively. For the other three genotypes, no significant effect of inoculation on Zn uptake was found (Table 3). Inoculation with *G. etunicatum* significantly increased Zn uptake of genotype K 150. Mycorrhizal Zn Responsiveness varied between 19% and 117% for *G. mosseae* and between -7% and 170% for *G. etunicatum* (Table 3). On this Zn deficient soil, the genotypes with low Zn uptake when nonmycorrhizal (K 150, Han 72 and Han 297) were more responsive with respect to Zn uptake than the three genotypes with higher Zn uptake when nonmycorrhizal (Han 44, 91B-8-30-3 and Hongkelaoshuya). Plant Zn uptake by nonmycorrhizal plants was negatively correlated with MZnR ($P = 0.006$) and MR ($P = 0.04$) for both mycorrhizal fungi (Figure 2).

Table 2. Plant dry weight in response to AMF inoculation and mycorrhizal responsiveness (MR) of six rice genotypes.

Rice genotype	Shoot dry weight (mg pot^{-1})			Root dry weight (mg pot^{-1})			MR (%)	
	-AMF	+ *G. mosseae*	+ *G. etunicatum*	-AMF	+ *G. mosseae*	+ *G. etunicatum*	+ *G. mosseae*	+ *G. etunicatum*
Han 72	201 a[1]	303 a	272 a	36 a	53 a	47 a	50	35
K 150	193 b	456 a	532 b	36 b	59 ab	73 a	125	164
Han 297	373 b	695 a	588 a	108 a	155 a	123 a	77	48
Han 44	571 ab	673 a	408 b	93 b	141 a	53 a	23	-31
91B-8-30-3	489 a	664 a	637 a	109 a	137 a	109 a	34	25
Hongkelaoshuya	507 b	711 a	647 a	123 a	156 a	102 a	38	19
Mean	389 c	584 a	514 b	84 b	117 a	85 b	48	27

V. 3. 3 Mycorrhizal root colonization

No root colonization by AMF was observed in the uninoculated plants. Variation in root colonization was found ($P < 0.0001$) among the six rice genotypes. There was no interaction between genotype and AMF treatment ($P = 0.1$; Table 4). Root colonization of AMF-inoculated plants ranged from 28% to 58% (Table 5). On average, root colonization by G. *mosseae* was slightly but significantly higher than by G. *etunicatum*. Neither MR (*P* = 0.9) nor MZnR (*P* = 0.6) correlated with root colonization.

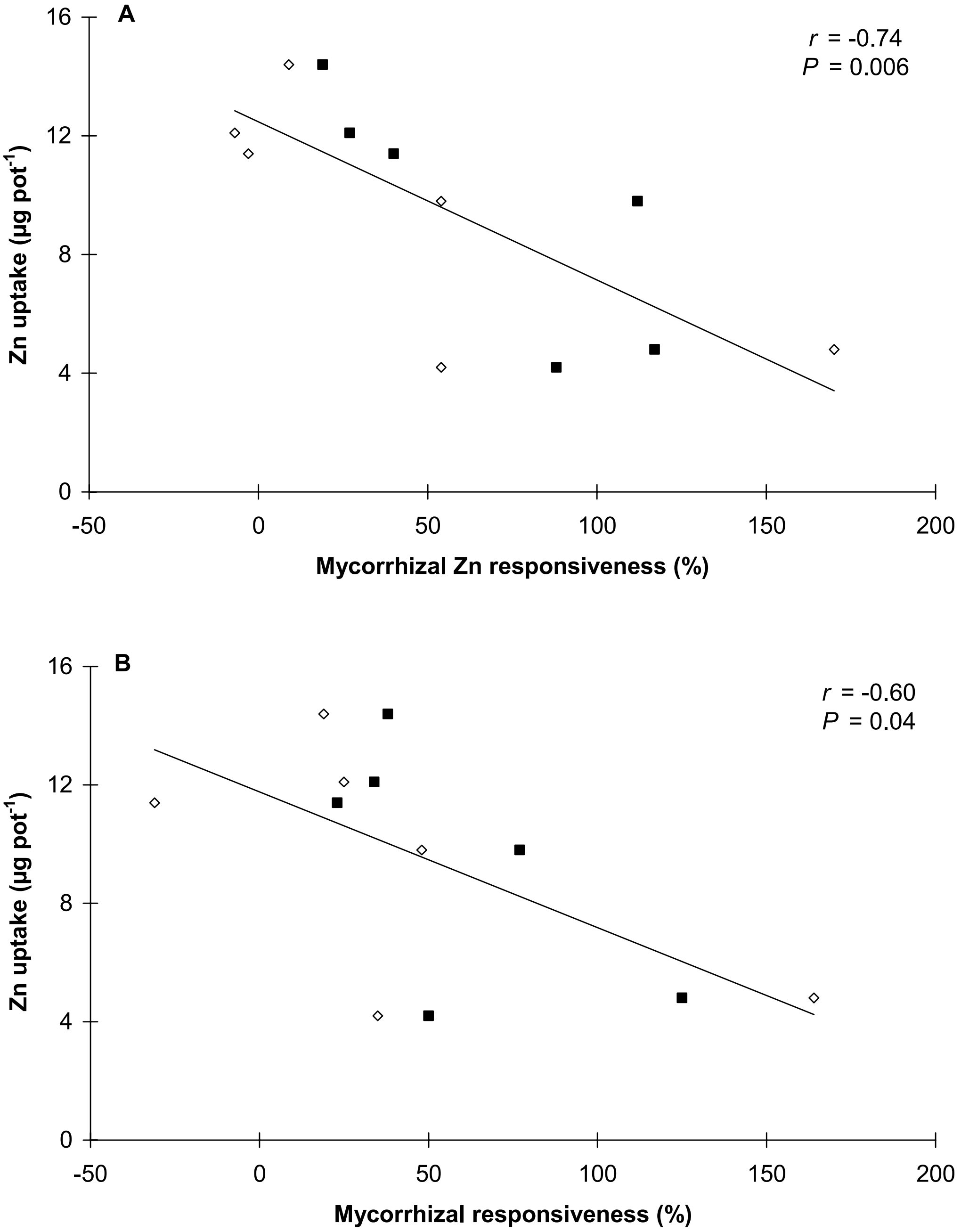

Figure 2. Correlation between Zn uptake when nonmycorrhizal and mycorrhizal Zn response (**A**) or mycorrhizal response (**B**) of six rice genotypes for two AMF species (■ *G. mosseae*; ◊ *G. etunicatum*).

Table 3. Zinc uptake in response to AMF inoculation and mycorrhizal response based on Zn uptake (MZnR) of aerobic rice genotypes.

Genotype	Zn uptake (µg pot^{-1})			MZnR (%)	
	-AMF	+ *G. mosseae*	+ *G. etunicatum*	*G. mosseae*	*G. etunicatum*
Han 72	4.2 b[1]	8.0 a	6.5 ab	88	54
K 150	4.8 b	10.5 a	13.1 a	117	170
Han 297	9.8 b	20.8 a	13.7 b	112	54
Han 44	11.4 a	15.9 a	11.1 a	40	-3
91B-8-30-3	12.1 a	15.4 a	11.3 a	27	-7
Hongkelaoshuya	14.4 a	17.1 a	15.7 a	19	9
Mean	9.5 c	14.6 a	11.9 b	54	23

[1] Per row means followed by the same letter are not significantly different (Tukey, $P = 0.05$)

V. 4 Discussion

We demonstrated a beneficial effect of AMF inoculation on Zn uptake by aerobic rice (Table 3). The effect in our study was equal to (*G. mosseae*) or smaller (*G. etunicatum*) than reported for lowland rice (Purakayastha and Chhonkar, 2001), confirming the potential of AMF to increase Zn uptake from low Zn soils. Also in a heavy metal-contaminated soil, two upland rice varieties inoculated with *G. mosseae* took up more Zn than uninoculated plants (Zhang et al., 2005). This suggests that the mycorrhizal effect on Zn uptake is independent on the Zn status of the soil. All studies done so far were pot studies, in which rooting density is high compared to a field condition. Verification under field conditions is necessary.

A beneficial effect of AMF inoculation on biomass is frequently attributed to increased P uptake. In our experiment, however, it is highly unlikely that increased P uptake explains increased biomass production and thus increased Zn uptake. Firstly, the P status of the soil was high and the Zn status was low (Dobermann and Fairhurst, 2000). So Zn, and not P, was the growth-limiting nutrient, which is confirmed by the appearance of Zn deficiency symptoms. P deficiency symptoms were not recorded. Secondly, Zn uptake (Table 3) and biomass production (Table 2) were significantly correlated ($P < 0.05$ for both *G. mossae* and *G. etunicatum*). Thirdly, Zn mass fractions for all samples but three (Figure 1) were below the level required for sufficient growth (20 mg kg^{-1}; Dobermann and Fairhurst, 2000) and similar to those found in a previous field experiment where a significant response to Zn application was shown (Gao et al., 2006; Chapter II). Earlier studies (Baon et al., 1993; Zhu et al., 2001) showed a negative correlation between mycorrhizal response and P utilization efficiency (PUE – the inverse of P mass fraction). An increase in P mass fraction (and hence a decrease in PUE) upon mycorrhizal inoculation is likely to be explained by luxury uptake of P and differential nutrient limitation by mycorrhizal and nonmycorrhizal plants. In the present study, Zn mass fractions were low but hardly differed between nonmycorrhizal plants and mycorrhizal plants (Table 1), indicating that plants under both treatments were Zn-limited. Therefore, no other nutrients but Zn limited the growth of both nonmycorrhizal and mycorrhizal plants, indicating that the growth response of rice plants was completely explained by the mycorrhizal contribution to Zn uptake.

There was large genotypic variation in MZnR (Table 3), which was not related to variation in root colonization. Zinc uptake under nonmycorrhizal conditions was significantly negatively related to MR and MZnR for both mycorrhizal fungi (Figure 2). This correlation indicates that genotypes with high responsiveness to AMF colonization are inherently less efficient to take up Zn from low Zn soils. This confirms our hypothesis that mycorrhizal Zn responsiveness correlates negatively with Zn uptake in the nonmycorrhizal condition.

Table 4. P-values from two-way analysis of variance for root colonization. Noninoculated controls were not included in this analysis.

Source	DF	Root colonization
Mycorrhizas (M)	1	0.02
Genotypes (G)	5	<0.0001
M × G	10	0.10

A negative correlation between mycorrhizal responsiveness and nutrient uptake in the nonmycorrhizal condition has been reported repeatedly. Koide et al. (1988) showed that cultivated oats had a lower P uptake and were more responsive to mycorrhizal inoculation than wild oats. A tomato genotype with a lower P uptake when nonmycorrhizal was also more responsive to mycorrhizal colonization than a more efficient genotype (Bryla and Koide, 1998). The authors concluded that plants that possess mechanisms for acquiring phosphorus efficiently, may be less dependent on (and responsive to) mycorrhizal colonization. For barley, Baon et al. (1993) observed that the cultivar with the lowest P uptake when nonmycorrhizal (Shannon) showed the largest mycorrhizal responsiveness. Wright et al. (2005), who compared an African landrace with a European high-yielding variety of maize, showed that the African landrace combined a higher nutrient uptake when nonmycorrhizal with a lower mycorrhizal response. And Kaeppler et al. (2000) showed that P uptake of maize inbred lines was negatively correlated with mycorrhizal responsiveness. These latter authors pointed out that variation in mycorrhizal responsiveness could be due to variation of plants to efficiently acquire nutrients and grow well under conditions of low nutrient availability. If there is substantial variation in that latter character (due to other mechanisms through which plants could perform well on nutrient-deficient soils), plants without these other mechanisms perform poorly in the nonmycorrhizal condition and hence will derive more benefit from mycorrhizal inoculation. Under that interpretation a low responsiveness to mycorrhizal inoculation is an almost unavoidable consequence of the possession of other, sometimes more important nutrient acquiring mechanisms of a plant. It would then be important to determine the relative contribution of plant and mycorrhizal fungus to nutrient uptake efficiency, to target those processes that make the larger contribution to nutrition (Smith et al., 1992). A dominant focus on mycorrhizal symbiosis and mycorrhizal responsiveness could then lead to selection of plants that are less able to cope with low soil nutrient levels. In the case of aerobic rice, the variation in rhizosphere mobilization potential outweighs a mycorrhizal contribution (Table 3). Even though AMF do contribute to Zn uptake, selecting and breeding plants on the basis of maximum mycorrhizal response will likely not lead to the most efficient cultivars.

Inoculation with AMF did not significantly increase Zn uptake beyond levels that were found in genotypes with an inherently high uptake (Table 3). This indicates that mechanisms other than mycorrhizal formation were more efficient in Zn mobilization. Root-induced chemical changes in rhizosphere could play a major role in Zn uptake. These rhizosphere effects may involve Fe oxidation and acidification of the rhizosphere (Kirk and Bajita, 1995) and exudation of Zn chelators such as phytosiderophores (Tolay et al., 2001) or citrate (Hoffland et al., 2006). In this case, the mycorrhizal symbiosis is apparently just one of several nutrient-acquiring specializations (Pate, 1994).

Table 5. Main effects of inoculation of rice genotypes with AMF species on root colonization.

Treatments/ Levels		Root colonization (%)	
Genotypes	Han 72	57	a[1]
	K 150	50	a
	Han 297	48	a
	Han 44	58	a
	91B-8-30-3	55	a
	Hongkelaoshuya	28	b
AMF	*G. mosseae*	53	A
	G. etunicatum	46	B

[1] Within genotypes or AMF treatment, per column means followed by the same letter are not significantly different (Tukey, P = 0.05).

Acknowledgements

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Chapter VI

Malate Exudation by Aerobic Rice Genotypes Varying in Zinc Uptake Efficiency

Xiaopeng Gao

Abstract

Plant Zn uptake from low Zn soils can be increased by Zn-mobilizing chemical rhizosphere processes. With six aerobic rice (*Oryza sativa* L.) genotypes varying in Zn uptake efficiency, a rhizotron experiment and a nutrient solution experiment were conducted to find out if genotypic variation in Zn uptake is related to the capacity to exude low molecular weight anions into the rhizosphere in response to Zn deficiency. In both experiments under very different conditions, plants responded to Zn deficiency with increased root exudation of malate. On average, the rhizosphere malate concentration at Zn deficiency increased 64% compared to that at adequate Zn supply in the rhizotron experiment. Similarly, under solution culture conditions, malate exudation rate at Zn deficiency was 41% higher than that at adequate Zn supply. A main effect of genotypic variation was identified under both conditions. Aerobic rice genotypes with a higher Zn uptake showed a stronger increase in malate exudation than the genotypes with a lower Zn uptake. With a soil extraction experiment, we furthermore quantified the effect of malate exudation on Zn availability in soil solution. Results showed that increased malate concentrations in soil solution can increase Zn bioavailability. The estimated rhizosphere Zn concentration for the efficient genotype is 15% higher than that for the inefficient genotype. These results highlight the importance of rhizosphere effects for Zn uptake by plants and confirm our hypothesis that the genotypic difference in Zn uptake of aerobic rice is partly explained by the capacity to exude malate into the rhizosphere.

Key words: Aerobic rice, Genotypic variation, Malate, Rhizosphere, Root exudation, Tolerance, Zinc uptake

VI. 1 Introduction

Low zinc (Zn) availability is a constraint to rice production in many areas (Dobermann and Fairhurst, 2000). Zinc deficiency in crops is associated with soil properties including high pH, high organic matter content, and high carbonate content (Alloway, 2004). Zinc deficiency can be corrected by Zn application to the soil or plant. This approach, however, is not always feasible because of agronomic and economic factors, such as the relatively high cost of fertilizer (Graham and Rengel, 1993). Alternatively, development of rice cultivars with high Zn efficiency, which was defined as the capacity of a genotype to grow or yield well on a low Zn soil (Graham et al., 1992), could offer a sustainable and cost-effective way to overcome Zn deficiency problems. It is therefore highly relevant to screen Zn efficiency variation among plant genotypes and explore the underlying mechanisms. We previously showed large variation in Zn efficiency among aerobic rice genotypes under both field (Gao et al., 2006; Chapter II) and pot conditions (Gao et al., 2005; Chapter IV). We emphasized that Zn uptake is the most important mechanism explaining variation in Zn efficiency among rice genotypes at early vegetative stage. Recently, similar findings were reported for bread wheat (Genc et al., 2006) and maize (Furlani et al., 2005).

As the major mechanism contributing to Zn efficiency, Zn uptake by soil grown plants can be enhanced either by 1) the adjustment of surface area where Zn uptake occurs including root and mycorrhizal hyphal surface and 2) root-induced changes in rhizosphere (Marschner, 1998). We recently reported that variation in Zn uptake was explained only for 32% by root surface area (Gao et al., 2005; Chapter IV) and was not explained by the genotypic variation in responsiveness to inoculation with arbuscular mycorrhizal fungi (Gao et al., 2007; Chapter V). Based on these results, we suggested a major involvement of rhizosphere processes in tolerance of aerobic rice to low Zn availability. Root-induced changes in the rhizosphere could significantly enhance Zn bioavailability to plants (Marschner, 1998). These rhizosphere effects may involve iron oxidation and consequent acidification of the rhizosphere (Kirk and Bajita, 1995) and exudation of Zn chelators such as phytosiderophores or low molecular weight organic anions (LMWOAs). A beneficial effect of phytosiderophores on tolerance to Zn deficiency has been shown in several studies for graminaceous plant species, including wheat (Zhang et al., 1991; Walter et al., 1994), barley (Erenoglu et al., 2000), sorghum and maize (Hopkins et al., 1998). For rice, however, we are not aware of any report on phytosiderophore exudation.

Rice plants have been found to exude LMWOAs. When grown hydroponically or in soil, malate and citrate exudation by rice roots was reported in several studies (Aulakh et al., 2001; Bhattacharyya et al., 2003; Hajiboland et al., 2005; Hoffland et al., 2006). Exudation rates frequently respond to environmental conditions. Malate exudation increased as a response to higher bicarbonate levels in the root growth medium (Yang et al., 2003; Hajiboland et al., 2005). Recently, rice was found to respond similarly to P and Zn deficiency by increased citrate exudation (Hoffland et al., 2006). It was also suggested that the citrate exudation capacity of rice genotypes was related to their tolerance to Zn deficiency. Due to the absence of a reliable method for sampling *in situ* in the rhizosphere, however, these studies were conducted under hydroponic conditions, which eliminate the soil-root aspect entirely. Recent advances in rhizotron techniques (Göttlein et al., 1999) and *in situ* extraction of soil solution (Wang et al., 2004; Shen and Hoffland, 2007) provided the means to overcome this problem.

It is proposed that LMWOAs can increase soil Zn availability in two ways: 1) they are probably exuded with protons as counter ions (Jones, 1998). Thereby they reduce the rhizosphere pH and increase Zn availability. In addition, 2) the anions can chelate Zn. It was shown that organic anions such as malate and citrate can release Mn from synthetic MnO_2 (Jauregui and Reisenarer, 1982) and Fe from goethite and ferrihydrite (Jones et al., 1996). The effects of LMWOAs on soil Zn availability, however, to our awareness have never been quantified.

Aerobic rice varieties are currently developed for the new water-saving aerobic cultivation system (Bouman et al., 2005) by crossing high-yielding lowland with traditional upland varieties. In this study, we tried to explain the variation in Zn uptake among aerobic rice genotypes in terms of variation in LMWOAs exudation. We tested the following hypotheses: i) roots of aerobic rice exude more LMWOAs as a response to Zn deficiency; ii) genotypic variation in Zn uptake is related to LMWOA exudation rate; iii) increase of LMWOA concentration in rhizosphere soil solution can increase Zn bioavailability.

VI. 2 Materials and methods

Six aerobic rice genotypes were selected because of their previously shown variation in Zn uptake and Zn efficiency (Gao et al., 2005; Chapter IV). Han 72, Han 44, Han 297 and 91B-8-30-3 (91B) are newly bred varieties in China Agricultural University. K 150 is a variety bred by Liaoning Academy of Agricultural Science, North China. Hongkelaoshuya (Hongke) is a traditional upland variety from Yunnan Province, South China. The genotypes had similar root surface area but showed large variation in Zn uptake (Figure 1, Gao et al., 2005; Chapter IV). Three genotypes (Hongke, 91 B and K 150) were selected for a rhizotron experiment. Five genotypes (Hongke, Han 44, Han 297, Han 72 and K 150) were selected for a nutrient solution experiment.

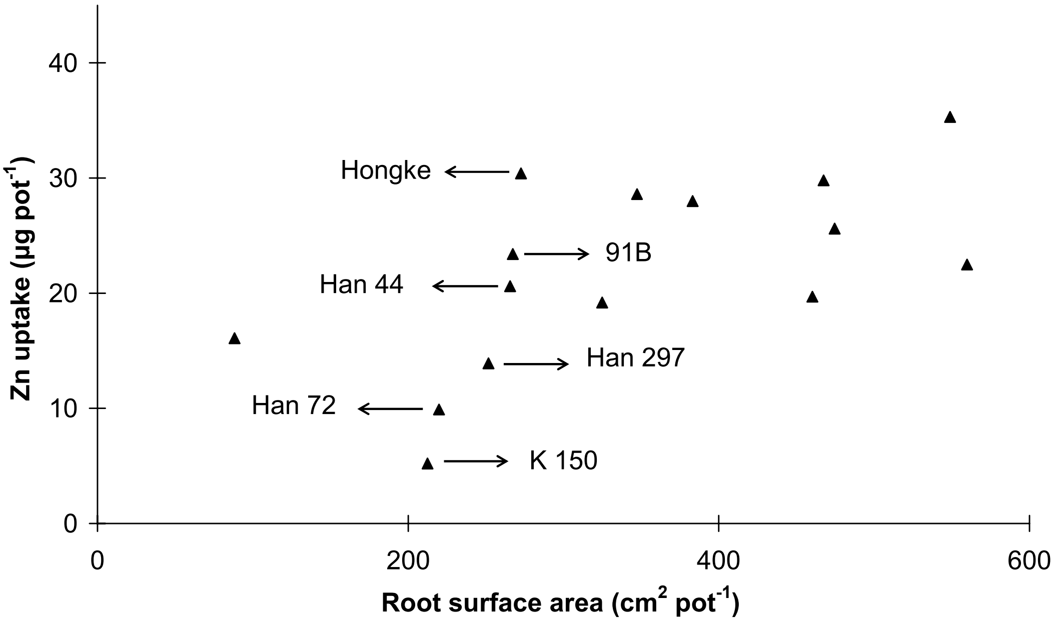

Figure 1. The selected six aerobic rice genotypes (from Gao et al., 2005; Chapter IV).

VI. 2. 1 Rhizotron experiment

A rhizotron lysimeter experiment was conducted in a growth chamber (14 hours photoperiod at 70 Watts m^{-2}, day/night temperature of 25/18 °C). Treatments included two Zn levels: -Zn (no Zn applied) and +Zn (5 mg Zn kg^{-1} soil), and three aerobic rice genotypes.

Rhizotrons (33 cm long, 20 cm wide, 1.5 cm deep) with an inner volume of 900 cm^3 were filled with a clay soil that was collected from Shou city, Anhui province, China. Major soil characteristics: pH_{H2O} 6.5, organic matter 1.7%, DTPA-extractable Zn 0.3 mg kg^{-1}, and P-Olsen 18.5 mg kg^{-1}. The Zn status is below the critical level (0.8 mg kg^{-1}; Dobermann and Fairhurst, 2000). In a previous experiment, plant growth responded significantly to application of 5 mg Zn kg^{-1} soil. Soils were air-dried, sieved at 2 mm, then homogenized and moistened at 20% (w/w) before filling. One side of the rhizotron consisted of a PVC sheet with 5×5 mm grid of holes for installation of lysimeters similar to Göttlein et al (1999). The opposite side was made of a transparent Plexiglass plate and was covered by a removable dark PVC sheet. The purpose was to observe root growth and locate the sampling points. During the experiment, the rhizotrones were positioned at an angel of 30° to force the roots to grow along the Plexiglas plate side. The microlysimeters had polyethersulfone suction cups with maximum pore size 0.2 µm (Rhizosphere Research Products, Wageningen, The Netherlands; Shen and Hoffland, 2007). The suction cup was glued inside a 5 cm polyetheretherketon (PEEK) to facilitate handling. Before installation, every lysimeter was sterilized with 10% H_2O_2 and checked for leaks.

Four seeds of one genotype were sown per rhizotron containing 1.3 kg dry soil. At sowing time, each rhizotron received a basal application of 150 mg kg^{-1} N as $Ca(NO_3)_2$, 44 mg P as KH_2PO_4 and 83 mg kg^{-1} K as KCl. The plants were thinned to two seedlings per pot one week after emergence. The soil was watered daily with deionized water, maintaining water content at 20% (w/w).

The soil solution sampling was conducted at the 28th day after sowing, when symptoms of Zn deficiency for -Zn plants and a growth response to Zn application were visual. The lysimeters were carefully installed in the soils around the selected root tips by inserting them into the holes on the rhizotron. For each treatment, six root tips were selected to collect the rhizosphere soil solution. Another two points, which are 3 cm far away from the root system, were selected as bulk soil. A low pressure was build up inside each lysimeter using a 5 mL syringe. The pressure was maintained for 2 hours. The volume of sampled soil solution was around 500 μL for each lysimeter. LWMOAs, including tartrate, malate, lactate, acetate, maleate, citrate, succinate and fumarate, were analyzed by reversed phase HPLC (SHIMADZU 10A, Imtakt, Kyoto) in the ion suppression mode. Separation was conducted on a 250×4.6 mm reversed phase column (Alltima C-18). The mobile phase was 25 mmol KH_2PO_4 L^{-1} (pH=2.5), with a flow rate of 1 mL min^{-1} at 28 °C and UV detection at 214 nm. The sample injection volume was 20 μL. Identification of organic acids was performed by comparing retention time and absorption spectra with those of standards.

After collection of the soil solution, shoots were harvested and dried at 70 °C for 72 h and weight was recorded. Zinc efficiency was calculated as the ratio of shoot dry weight at Zn deficiency over that at adequate Zn supply.

VI. 2. 2 Nutrient solution experiment

A nutrient solution experiment was conducted in a greenhouse (16 h photoperiod at 70 Watts m^{-2}, day/night temperatures of 20/16 °C and 80% humidity) in March 2005 at Wageningen University, the Netherlands. Treatments included five aerobic rice genotypes and two Zn levels (0 and 0.5 mg L^{-1}).

Rice seeds were surface sterilized with 10% H_2O_2 for 15 min, rinsed thoroughly with deionized water and germinated on moist filter paper for 2 d at 25 °C. After germination, the seeds were transferred to moist quartz sand in the green house at room temperature. When the second leaf emerged, the seedlings were transplanted to 50 L plastic containers containing continuously aerated nutrient solution. The complete nutrient solution contained (in mmol L^{-1}) 0.25 KH_2PO_4, 1.25 KNO_3, 1.25 $Ca(NO_3)_2$, 0.5 $MgSO_4$ and (in mg L^{-1}) 0.5 B as H_3BO_3, 0.5 Mn as $MnSO_4$, 0.05 Zn as $ZnSO_4$, 0.02 Cu as $CuSO_4$, 0.01 Mo as $(NH_4)_6Mo_7O_{24}$ and 4.6 Fe as Fe-EDTA. The pH was adjusted to and maintained at 6.5 by daily titration. In the -Zn treatment $ZnSO_4$ was omitted. Thirty plants were grown per 50 L container.

Plants were harvested 25 days after transplanting. Roots were rinsed with deionized water and put individually (n = 5) in a test tube containing 20 mL deionized water for 30 min. The plants were then removed and the solution was filter-sterilized (0.2 µm). The first 10 mL that passed the filter were discarded to avoid effects of filtering on the malate concentration. The sterile samples were stored immediately in previously sterilized containers at -18 °C until analysis. Plant roots and shoots were oven dried at 70 °C for 72 h and weighed.

Malate concentration was determined spectrophotometrically using an enzymatic method. In the presence of malate dehydrogenase and a glycylglycine buffer (pH 10.0), nicotinamide-adenine dinucleotide (NAD) was added to the sample to convert the malate in samples into oxaloacetate. Meanwhile, NAD was converted to NADH. By trapping the oxaloacetate in a subsequent reaction catalyzed by the enzyme glutamat-oxaloacetate transaminase, the equilibrium of this reaction lies completely on the side of NADH. The amount of NADH formed is stoichiometric with the amount of malate present in the sample. The NADH concentration was determined by means of its absorbance at 340 nm. The detection limit for this assay is 0.5 µM. All chemicals were from Roche Diagnostics GmbH, Mannheim, Germany.

VI. 2. 3 Soil extraction experiment

The same soil as used in rhizotron lysimeter experiment was used for the soil extraction experiment. Three series of extraction solutions were prepared.

1) Malic acid solutions with different concentrations (0, 0.5, 1, 50, 100 mM) were prepared with deionized water. $CHCl_3$ was also added to a final concentration to 0.15% (v/v) to prevent microbial degradation of malate. The $CHCl_3$ concentration needed was determined in a preliminary experiment.
2) Malic acid solutions with concentrations 0.5, 1, 50, 100 mM were prepared as 1). The pH of these solutions was then adjusted with NaOH to 7.14, which was the original pH for 0 mM malic acid.
3) Deionized water and 0.1 mM HCl were used to prepare solutions with pH values 1.57, 2.01, 2.21, 2.38, 3.13, 3.29, 3.35 and 3.52, respectively. These values include the variation among the measured pH in solutions of series 1.

So, there were altogether 17 solutions prepared for the extraction (Table 2). With series 2) and series 3), we can distinguish the malate ligand effect from pH reduction effect on Zn bioavailability.

Three gram of dry soil was placed in a centrifuge tube. To each tube, 30 mL extraction solution was added. The tubes were then shaken mechanically for 48 h at room temperature (20 °C). After shaking, the pH in the suspensions was measured. The suspensions were then centrifuged at 3000 g for 10 minutes. Supernatants were filtered (0.45 µm) and analyzed for malate and Zn. Malic acid concentration was determined spectrophotometrically using an enzymatic method (see above). Zinc concentration was determined by ICP-MS(Spetroflame Modula, Spectro, Kleve, Germany).

VI. 2. 4 Statistical analysis

All data met the requirements of normality (Kolmogorov-Smirnov) and homogeneity of variance (Levene's test). Analysis of variance was done on data on malate concentration in soil solution in rhizotron experiment and shoot dry weight and malate exudation rate in nutrient solution experiment. Means were compared with Tukey's Honestly Significant Differences test at the 5% level of probability. Multivariate regression analysis was conducted with data from the soil extraction experiment. All analyses were performed with SAS Release 8.02 (SAS Inc.).

VI. 3 Results

VI. 3. 1 Plant growth

Aerobic rice plants in both rhizotron and nutrient solution experiments showed a positive growth response to Zn application (Table 1). Zinc efficiency under rhizotrons condition was 55, 69 and 91% for genotypes K 150, 91B and Hongke, respectively. Zinc efficiency of five genotypes ranged from 56-80% in nutrient solution experiment. The highest Zn efficiency was found for genotype Hongke in both experiments. Genotype K 150 showed a low Zn efficiency in both experiments. The genotypes showed similar order of Zn efficiency in two experiments. Compared to the rhizotron, the difference in Zn efficiency between the efficient genotype Hongke and the inefficient genotype K 150 became smaller when grown in the nutrient solution condition.

Table 1. Shoot dry weight and Zn efficiency of aerobic rice genotypes in rhizotron and nutrient solution experiments.

	Genotypes	Shoot dry weight (mg plant^{-1})		Zn efficiency (%)
		-Zn	+Zn	
Rhizotron				
	K 150	151	274	55
	91B	217	316	69
	Hongke	275	302	91
	Mean	214	297	
Nutrient solution*				
	Han 297	277	491	56
	K 150	293	420	70
	Han 72	369	481	76
	Han 44	405	531	77
	Hongke	522	656	80
	Mean	373	516	

* Zn: $P < 0.0001$; Genotype: $P < 0.0001$; Zn × Genotype: $P = 0.28$

VI. 3. 2 Root exudates

Malate, lactate and maleate were LMWOAs identified in the rhizosphere soil solution of all three genotypes for rhizothon experiment. Maleate levels, however, were very low (< 0.002 mM, data not shown). Other LMWOAs such as tartrate, acetate, citrate, succinate, and fumarate were below detection levels. Malate concentration was significantly affected by Zn level, genotype and their interaction (Figure 2A). Lactate was significantly affected by genotype but not affected by Zn level and the interaction (Figure 2B). In general, the LMWOA concentration in rhizosphere soil solution was higher than in bulk soil solution.

Also in the nutrient solution experiment, a main effect of Zn level on malate exudation was found (Figure 3). The increase in malate exudation rate as a response to Zn deficiency was found for all genotypes except Han 297. There was a less significant main effect of genotype ($P = 0.08$). Similar to the rhizotrons experiment, there was a significant interaction between Zn level and genotype.

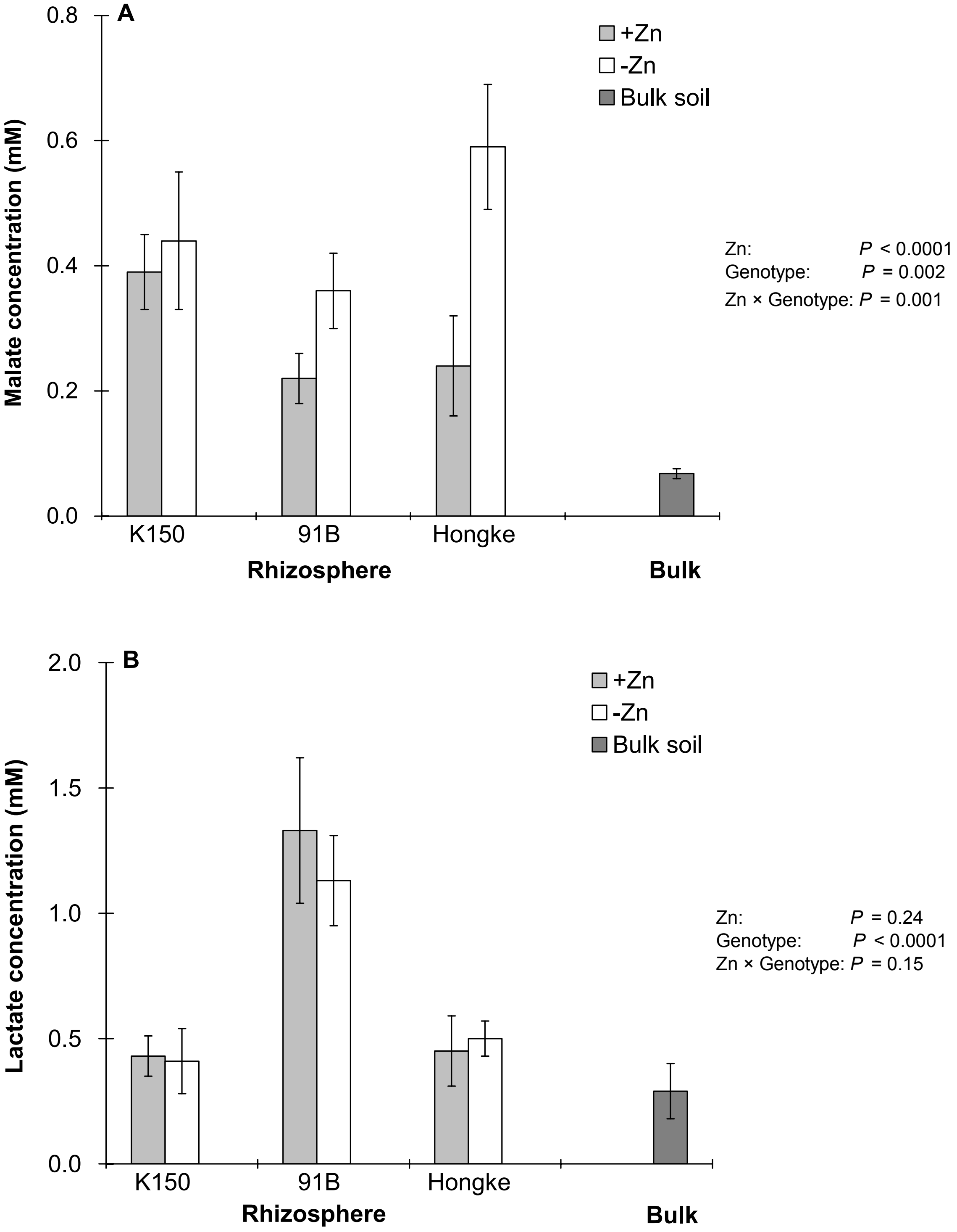

Figure 2. Malate and lactate concentration in the rhizosphere and bulk soil solution of three aerobic rice genotypes in the rhizotron experiment.

The increase in malate exudation in response to Zn deficiency varied among genotypes, as indicated by the positive interaction between Zn level and genotype. At Zn deficiency in rhizotrons, malate concentration in the rhizosphere soil solution increased 150, 65 and 12% for Hongke (efficient), 91B (intermediate) and K 150 (inefficient). Similarly, at Zn deficiency in nutrient solution experiment, the efficient genotypes as Hongke and Han 44 showed stronger increase in malate exudation rate than the inefficient genotypes. At Zn deficiency, the malate exudation rate increased 70% and 122% for Hongke and Han 22, respectively. The inefficient genotypes Han 297 and K 150 showed no or a less strong response. There was a significant and positive correlation between the malate exudation rate at Zn deficiency of the genotypes and their Zn uptake from a low Zn soil in a previous screening experiment (Figure 4). Plant Zn uptake in this regression was taken from a previous screening experiment (Gao et al., 2005; Chapter IV) because no Zn was presented in -Zn treatment in the current nutrient solution experiment.

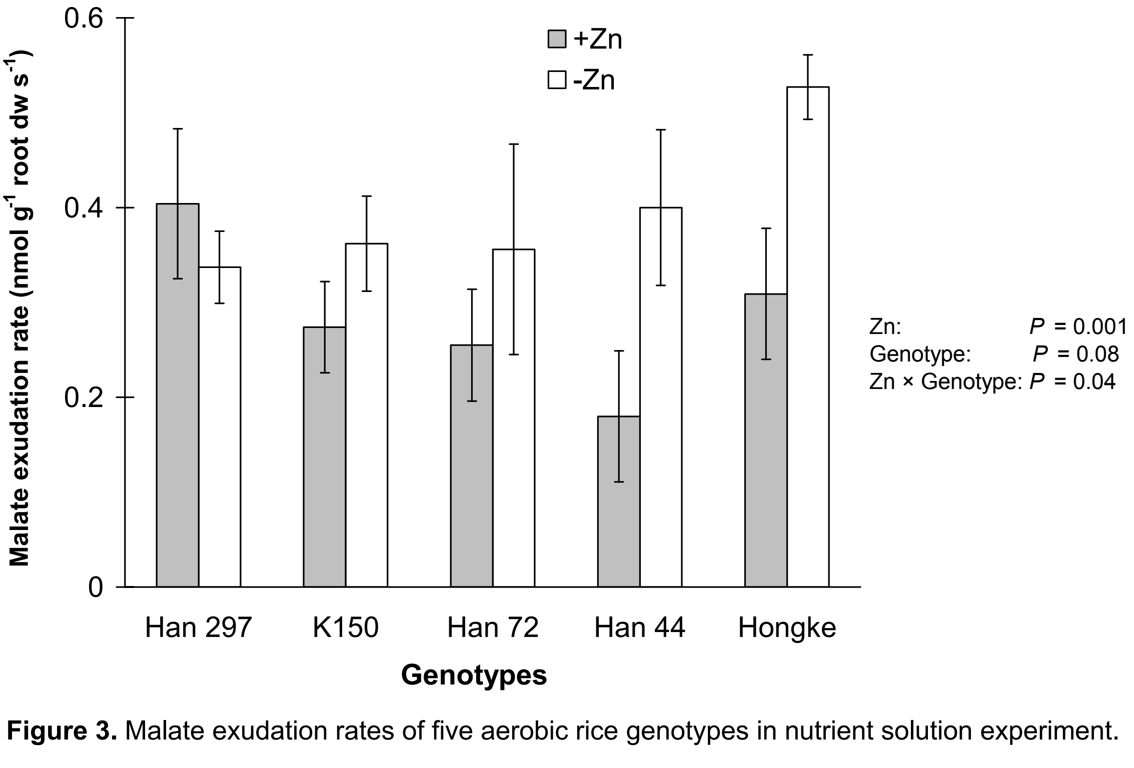

Figure 3. Malate exudation rates of five aerobic rice genotypes in nutrient solution experiment.

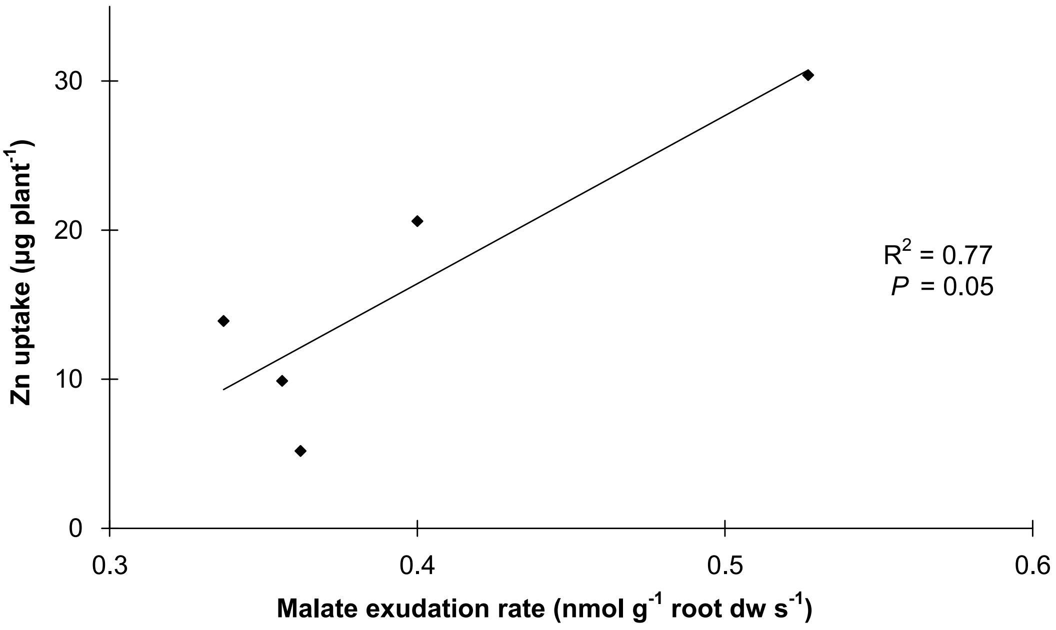

Figure 4. Relationship between the malate exudation rates of five aerobic rice genotypes at Zn deficiency on nutrient solution and their Zn uptake from a low Zn soil (cited from Gao et al., 2005; Chapter IV).

VI. 3. 3 Soil extraction

In general, the soil extraction procedure changed the pH of the extractant towards the original soil pH level (6.5; Table 2). The malate concentration decreased after soil extraction. More Zn was detected in solutions with higher malate concentration and lower pH.

The relationship between pH and pZn in the solution after extraction was derived from the extractions in which no malate was added (Table 2). There is a statistically significant positive relationship between pH and pZn (Figure 5). The regression can be written as: pZn = 0.35 pH + 4.93, $R^2 = 0.8$, $P = 0.004$.

To assess the overall effects of pH in combination with malate chelation on Zn availability in soil solution, multivariate regression analysis was conducted. The data from Table 2, where malate was added, were used for this analysis. The overall regression was expressed as: pZn = 5.09 + 0.1 pH + 0.2 pMalate ($R^2 = 0.92$, $P < 0.0001$). Both pH and pMalate contributed pZn significantly with *P* values of 0.03 and 0.005, respectively.

Table 2. pH, malate concentration and Zn concentration before and after soil extraction.

Before extraction		After extraction		
pH	Malate concentration (mM)	pH	Malate concentration (mM)	Zn concentration (μM)
7.14	0	6.38	0	0.07
3.50	0.5	5.95	0.2	0.32
3.30	1	5.73	0.5	0.49
2.34	50	2.94	43.0	1.86
2.21	100	2.77	62.0	2.98
7.14	0.5	6.71	0.1	0.32
7.14	1	6.44	0.5	0.43
7.14	50	6.65	44.5	0.62
7.14	100	6.75	71.4	1.50
1.57	0	2.24	0	3.50
2.01	0	3.78	0	0.51
2.21	0	4.53	0	0.17
2.38	0	4.65	0	0.15
3.13	0	5.90	0	0.04
3.29	0	6.09	0	0.12
3.35	0	6.20	0	0.10
3.52	0	6.36	0	0.16

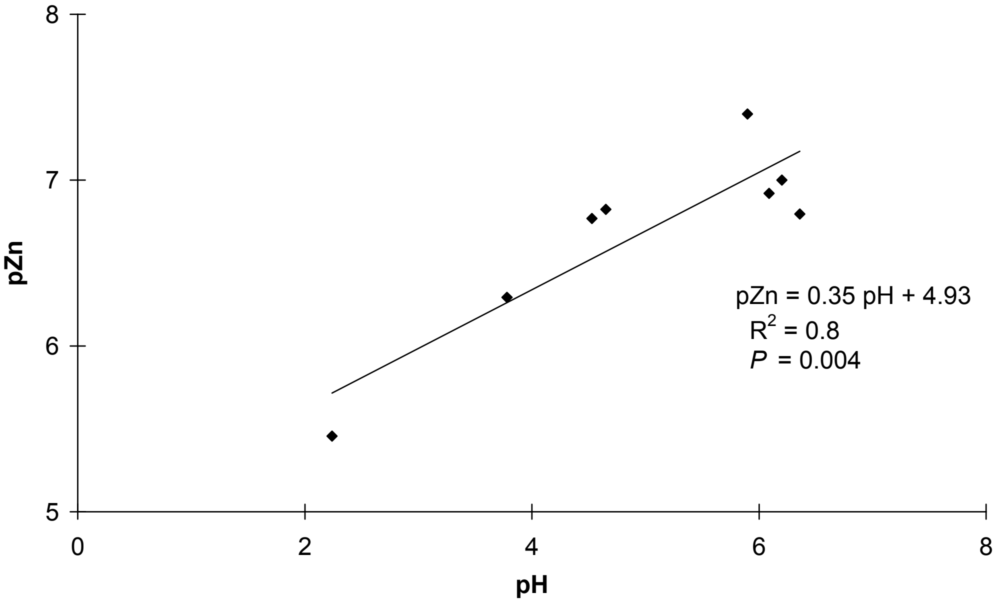

Figure 5. Relationship between pH and pZn in soil extraction experiment.

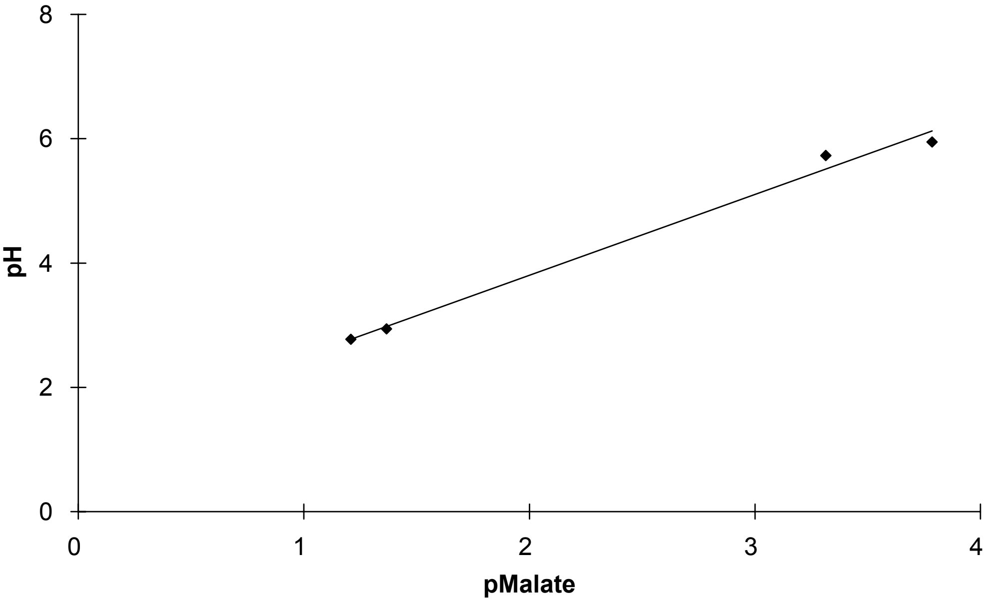

Figure 6. Relationship between pH and pMalate in soil extraction experiment.

The relationship between pH and pMalate was derived from the extractions in which malate was added and pH was not adjusted (Table 2). The plot appears linear and the resulting model is: pH = 1.2 + 1.3 pMalate (R^2 = 0.99, P = 0.005; Figure 6). With this model, pH of the rhizosphere soil solution collected from plants at -Zn treatment in rhizotrons experiment was estimated (Table 3). Rhizosphere pH was evaluated to be 5.4 and 5.6 for the efficient Hongke and inefficient K 150 respectively. With the derived multivariate regression, Zn concentration in rhizosphere soil solution of plants at -Zn treatment was evaluated. The modeled Zn concentration was 0.47 µM for the inefficient K 150 and 0.54 µM for the efficient Hongke.

Table 3. Estimated pH and Zn concentration in the rhizosphere soil solution of plants grown in rhizotrons (-Zn treatment).

Genotypes	Measured malate concentration (mM)	Estimated pH	Estimated Zn concentration (µM)
K 150	0.44	5.6	0.47
91 B	0.36	5.7	0.45
Hongke	0.59	5.4	0.54

VI. 4 Discussion

In both plant experiments, we showed a significant plant growth response to Zn application (Table 1), indicating plants in -Zn treatments suffered from Zn deficiency. The order of Zn efficiency among the genotypes was similar to results from previous pot (Gao et al., 2005; Chapter IV) and field experiments (Gao et al., 2006; Chapter II). The finding that variation in Zn efficiency among genotypes became smaller when grown on nutrient solution (Table 1) suggested the importance of rhizosphere effects in determining Zn efficiency. In the nutrient solution experiment, rhizosphere effects on plant Zn availability were absent and the genotypic variation in Zn efficiency was smaller.

We showed in two types of experiments that aerobic rice genotypes respond to Zn deficiency with increased root exudation of malate (Figure 2A and Figure 3). LMWOAs exudation by rice roots was also reported as a response to bicarbonate (Hajiboland et al., 2005), Al toxicity (Ma et al., 2000; Chen et al., 2006) and P deficiency (Hoffland et al., 2006). Citrate and malate are the common responsive LMWOAs identified in rice root exudates at nutrient stress. Citrate exudation was found as a response to nutrient deficiency including Zn (Hoffland et al., 2006), Cu and Mn deficiency (Neumann and Römheld, 2001). Malate exudation was reported as a response to bicarbonate stress, which is frequently associated with Zn deficiency in calcareous soils (Yang et al., 2003; Hajiboland et al., 2005). Further research is needed to understand why malate is responsive in some types of stress and citrate in others. Lactate was the other organic anion identified in rhizosphere soil solution. Lactate, however, has only one carboxyl group and has a low affinity for metals. The stability constants of organic anion-Zn complexes (at 25 °C and zero ionic strength) is 2.12 for lactate and 3.32 for malate (Martell and Smith, 1977), indicating that malate has a higher affinity for Zn than lactate.

The positive correlation between root malate exudation at Zn deficiency on nutrient solution and plant Zn uptake suggested that rhizosphere effect is one of the important processes to determine plant tolerance to Zn deficiency (Figure 4). This was also supported by the fact that a higher rhizosphere malate concentration and a stronger increase at Zn deficiency were found for the efficient genotype (Figure 2A). These results confirm a previous study, in which a positive relationship between citrate exudation capacity of rice genotypes and their tolerance to Zn deficiency was suggested (Hoffland et al., 2006).

With a soil extraction experiment, we quantified the effect of malate exudation on Zn availability in soil solution. Both pH reduction and malate chelation increased Zn availability in soil solution. This confirms our hypothesis that increased malate concentrations in soil solution can increase Zn bioavailability. We assumed protons as the counter ions exuded with malate by plant roots. In the rhizotron experiment, rhizosphere solution pH was not measured because of sample volume limitations. With the linear regression between pH and pMalate (Figure 6), however, we modeled the rhizosphere soil solution pH of three rice genotypes at Zn deficiency. Zinc concentration in rhizosphere soil solution was further modeled and the estimated Zn concentration for the efficient Hongke is 15% higher than that for the inefficient K 150 (Table 3). This confirms our hypothesis that the genotypic difference in Zn uptake of aerobic rice is partly explained by their capacity to exude malate into the rhizosphere. The difference in estimated rhizosphere concentration among different genotypes, however, was much smaller than we expected. Zinc uptake by the efficient Hongke was five times higher than the inefficient K 150 (Figure 1; Gao et al., 2005; Chapter IV). Comparatively, only a 15% difference (between Hongke and K 150) or no difference (between 91 B and K 150) was found in the current estimated Zn concentration in rhizosphere. This small difference may be explained by the limitation of the rhizotron lysimeter rhizosphere soil solution sampling technique. Even though this technique can extract soil solution samples at millimeter scale, it is hypothesized that it vastly underestimates soil solution organic anion concentrations (Jones et al., 2003). On the one hand, the organic acid release from root tips is only in a few μm scales. On the other hand, the diffusion coefficients of organic acids in soil are very low (Darrah, 1991). More investigations are required to carefully evaluate the role of organic acids in increasing Zn bioavailability to plants.

Chapter VII

General Discussion

In this final Chapter the main results of the previous Chapters are shortly summarized and discussed in a more general context. We feed back on the two research questions presented in the General Introduction. Future research aims regarding the Zn availability to plants are also presented.

1. What is the consequence of a cultivation shift from flooded to aerobic rice on Zn bioavailability?

With the field experiments on two different soils in two different years (Chapter II, III), we demonstrated that the cultivation shift from flooded to aerobic condition may increase Zn deficiency problems. Both on a calcareous soil in Beijing region and on a clay soil in Anhui region, we found lower plant Zn uptake and lower shoot Zn mass fraction in aerobic fields than flooded fields. It is reported that Zn deficiency symptoms were observed for aerobic rice in North China, 2 or 3 years after fields were shifted from flooded to aerobic conditions (Wang et al., 2002). The current studies have demonstrated that increased Zn deficiency problems show already in the first year after cultivation shift.

To understand the underlying mechanisms of reduced Zn bioavailability in aerobic fields compared to flooded fields, we characterized and modeled both systems in Anhui with geochemical analysis to identify the dominant processes that control the solid-solution partitioning of Zn in soils. Geochemical analysis showed that the difference found in plant Zn uptake between two cultivation systems was rather small compared to what can be expected based on modelling. The individual effects of soil factors e.g. redox potential and pH have been demonstrated to cause orders of magnitude of variation in Zn concentration, compared to 15% difference in plant Zn uptake. This observation leads to the suggestion that it may lead to erroneous interpretations if soil chemical factors are considered individually instead of in an integrated way that allows for synergistic and antagonistic effects. We speculated this discrepancy could be partly caused by the root-induced chemical changes in the rhizosphere, where the local acidity, DOC and redox conditions can be completely different from those of the bulk soils on which the geochemical analysis are based. Besides this rhizosphere effects, the observed lower Zn uptake in aerobic fields compared to flooded fields might also be partly caused by the different rates in transpiration and diffusion between two systems.

In practise, the shift to aerobic cultivation predominantly occurs in North China, where the sustainability of lowland rice production is threatened by increasing water scarcity (Wang et al., 2002). Soils in North China are mainly calcareous soils with pH higher than 7. Based on the geochemical analysis, the increase of soil pH upon the ongoing shift to aerobic cultivation and the relative high bicarbonate content would further increase Zn deficiency problems. Zinc fertilization should be recommended to local farmers in these areas, by either soil or foliar applications. The genotypic variation in aerobic rice varieties in tolerance to Zn deficiency under field conditions (Chapter II) can be used as an alternative tool to overcome the Zn deficiency problems.

From a human nutritional point of view, it is highly relevant to investigate the effect of the cultivation shift on the mass fraction of Zn in rice grains. The field experiment conducted on a calcareous soil in Beijing region showed little difference in mass fraction of Zn in whole grain between flooded and aerobic conditions (Chapter II). Another field experiment with similar design, which was conducted on a clay soil in Anhui region, showed a slightly but significantly decreased Zn mass fraction in brown rice under aerobic fields compared to flooded fields (Table 1). There are several possible reasons to explain the observed different effects. Firstly, we analyzed the whole grain in Beijing experiment but the duhulled brown rice in Anhui experiment. As shown in Table 1, the mass fraction of Zn is 5 times higher in hull than brown rice. If we consider the calculated Zn mass fraction in whole grain, again there was no difference between two cultivation systems. Secondly, the Beijing field has a higher soil Zn status than Anhui field. DTPA-extractable Zn is 2.0 and 0.3 mg kg^{-1} for Beijing and Anhui, respectively. In both experiments, we observed a lower Zn harvest index in aerobic fields than in flooded fields, which was caused by a reduced harvest index (dry matter based). In general, the lower Zn mass fraction in brown rice and lower Zn harvest index in aerobic fields compared to flooded fields indicates a decreased Zn bioavailability to the human consumer of grain. Possible soil and crop management options to increase Zn mass fraction in grain will be discussed later in this Chapter.

Table 1. Cultivation effects on Zn mass fraction in grain and Zn harvest index on a low Zn soil in Anhui.

Cultivation	Zn mass fraction (mg kg^{-1})			Zn harvest index (%)
	Brown rice	Hull	Whole	
Flooded	18 a[1]	68 a	24 a	34 b
Aerobic	16 b	69 a	23 a	29 a

[1] Per column means followed by the same letter are not significantly different (Turkey, $P = 0.05$).

2. Which mechanisms control Zn mobilization by aerobic rice?

Knowledge of Zn acquisition by rice is necessary to develop appropriate strategies to prevent Zn deficiency in crop and human consumers. The genotypic difference in Zn efficiency, which is defined as the capacity of a cultivar to grow and yield well on a low Zn soil (Graham et al., 1992), offers a tool to answer this question. In this thesis, we reported the variation in Zn efficiency among aerobic rice in both field (Chapter II) and pot (Chapter IV) experiments. The genotypes used in two conditions showed similar ranking based on Zn efficiency, suggesting that soil-based pot screening experiments can be useful for routine screening for Zn efficiency at field level.

To identify the mechanistic explanation of differential Zn efficiency among aerobic rice genotypes, a greenhouse pot experiment was conducted with 15 aerobic genotypes and 8 lowland genotypes grown on a low Zn soil (Chapter IV).

Considerable genotypic variation in Zn efficiency was demonstrated. As a whole, 53% of the observed variation in Zn efficiency was associated with Zn uptake and translocation from roots to shoots. We assumed that the unexplained proportion of the variation was ascribed to biochemical utilization and translocation of Zn from older to younger parts of the plant. These results confirmed that Zn efficiency is indeed a complex trait. This was concluded for Exacum (Riseman and Craig, 2000) and wheat (Genc et al., 2006), too. In the current study, we only examined the possible mechanisms of Zn efficiency at seedling stage. It is possible that additional mechanisms of Zn efficiency are operative at later stages in the life cycle of the plant, which deserves consideration to get a complete view on crop tolerance to Zn deficiency.

As the major mechanism contributing to Zn efficiency, Zn uptake by soil grown plants can be enhanced either by 1) the adjustment of surface area where Zn uptake occurs including root and mycorrhizal hyphal surface and 2) root-induced changes in rhizosphere (Marschner, 1998). In the current study, variation in Zn uptake was explained for 32% by root surface area (Chapter IV), which is consistent with results on wheat (Dong et al., 1995). On the one hand, this result confirms the positive contribution from the increase in root surface area on Zn uptake by plants. On the other hand, it emphasizes that there must be important additional mechanisms that determine Zn uptake. As reported in lowland rice, root-induced rhizosphere changes such as the acidification of the rhizosphere (Kirk and Bajita, 1995) and exudation of Zn chelators (Hoffland et al., 2006) may play important roles.

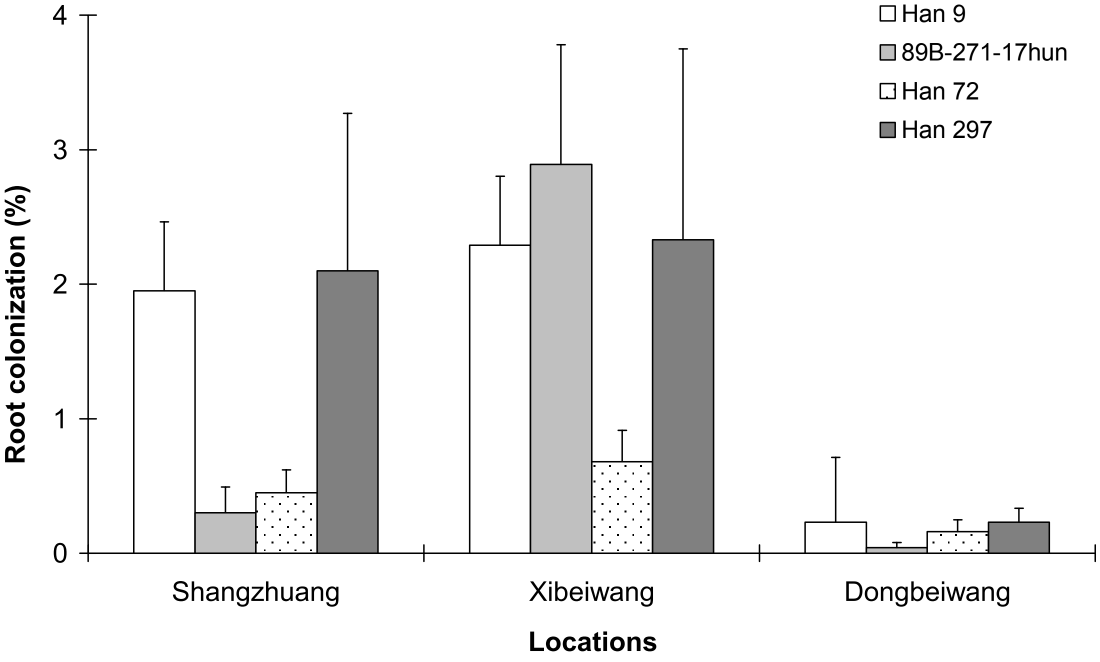

Figure 1. Root colonization of four aerobic rice genotypes in three different locations of Beijing, China (Means ± SD). For each sample site, root samples of five individual plants were collected. Roots were cut into 1 cm segments and mixed thoroughly. A subsample of 1 g fresh weight per pot was taken to determine mycorrhizal root colonization as described by Phillips and Haymann (1970).

A field survey was conducted with four aerobic rice genotypes in three different locations to get insight in colonization of aerobic rice roots by arbuscular mycorrhizal fungi (AMF). The results showed very low mycorrhizal root colonization under natural conditions, ranging between 0.1% and 2.9% (Figure 1). This means AMF colonization must have been very low in the screening experiment (Chapter V). So, genotypic variation in Zn uptake observed in this screening experiment cannot be explained by a differential mycorrhizal effect. Again, this conclusion emphasized that the root-induced changes in rhizosphere are potential mechanisms of importance in aerobic rice, as reported in lowland rice in a recent research (Wissuwa et al., 2006).

In a pot experiment with six genotypes growing on a low Zn soil (Chapter V), we studied whether inoculation with AMF can be an additional or an alternative strategy to increase rice Zn uptake. We reported a negative correlation between plant Zn uptake by nonmycorrhizal plants and Mycorrhizal Zn responsiveness. This indicates that genotypes are either responsive to AMF, or have alternative mechanisms to mobilize Zn. A combination of the two seems impossible and may not be feasible as a target for breeders. In the case of aerobic rice and Zn, the variation in rhizosphere mobilization potential outweighs a mycorrhizal contribution (Table 3, Chapter V). Given the low AMF colonization under natural field conditions (Figure 1) and excess use of phosphorus fertilizers in Chinese soils (Zhang et al., 2004), it is reasonable to expect little benefit from mycorrhizal colonization or inoculation on Zn uptake by aerobic rice under field conditions. On the other hand, it deserves emphasis that only two kinds of fungi and six genotypes were used in this study. More investigations with a larger set of genotypes and fungi are necessary to confirm this unpromising result. Also, the responsiveness of Zn uptake by different plant species on different mycorrhizal fungi needs to be characterized, which would clarify the role of crop rotation in this particular case.

With six aerobic rice genotypes having similar root surface area but different Zn uptake, we found that the genotypes with a higher Zn uptake showed a stronger response in malate exudation than the genotype with a lower Zn uptake (Chapter VI). This observation was confirmed both in a rhizotron experiment and in a nutrient solution experiment under very different conditions. We furthermore quantified the effect of malate exudation on Zn availability in soil solution by a malate-soil extraction experiment. The increased malate concentrations in soil solution can increase Zn bioavailability. However, the estimated concentration of Zn in the rhizosphere for the efficient genotype was only 15% higher than that for the inefficient genotype, compared to the 5 fold difference in plant Zn uptake. The small difference in estimated concentration of Zn in rhizosphere may be partly accounted for by the limitation from rhizothon-lysimiter technique, which may vastly underestimate soil solution organic acid concentrations. On the other hand, this small difference also indicated the importance of investigations on other possible mechanisms such as exudation of phytosiderophores. It was reported that Zn deficiency increases the secretion of phytodiderophores from wheat (Cakmak et al., 1994) and barley (Suzuki et al., 2006) roots into the rhizosphere. The detailed mechanism of the release of phytosiderophores by rice roots and its consequent effect on plant Zn uptake, however, has never been illuminated, even though rice was proven to produce small amounts of phytosiderophores (Zhang et al., 1998). Further investigations are necessary to quantify the contribution of rhizosphere effects on plant Zn acquisition. Introduction of models into the complex plant-soil system may be an interesting and plausible approach to link soil chemistry and plant physiology, as done by Geelhoed et al. (1999), Kirk (2002) and Wissuwa (2003). Also, we only investigated the acquisition of Zn by aerobic rice at seedling stage. In view of various factors affecting or responsible for Zn uptake efficiency, more investigations regarding time course of uptake are necessary.

3. Soil and crop management options to increase Zn mass fraction in crop grains

From a human nutritional point of view, high Zn mass fraction in the grain is of paramount importance. Increase of Zn mass fraction in grains of staple food crops through both plant breeding and nutrition management in the soil-plant system is urgent (Frossard et al., 2000). In this part, we shortly discuss the possibilities and limitations of applying fertiliser, plant breeding or biotechnology to increase Zn mass fraction in grains of crop plants.

Many studies have shown the beneficial effects of Zn application on correcting Zn deficiency and increasing grain yield of various cereal crops (Hergert et al., 1984; Cakmak et al., 1999). In most cases, however, Zn mass fraction in crop grains does not increase to the desired levels to meet human requirements even by using high rates of fertilizer application (Rengel et al., 1999). The observed increase in Zn level is far below the target level (50-80% more Zn than in existing popular cultivars) of HarvestPlus, which is an international and interdisciplinary research program aiming at reducing micronutrient malnutrition by agricultural approaches (http://www.harvestplus.org/).

Long-term fertilization with $ZnSO_4$ on an alkaline soil did not increase Zn mass fraction in grain of maize (Payne et al., 1988). Srivastava et al. (1999) showed that soil Zn application at transplanting only increased grain Zn mass fraction of lowland rice from 18 mg kg^{-1} to 25 mg kg^{-1}. Similarly, we recently found little increase in Zn mass fraction in rice grains with soil application of Zn as 23 kg ha^{-1} on a calcareous soil (Chaper II). This result was repeated on a clay soil in a different year. Again, soil application at sowing showed a minor influence on Zn mass fraction in brown rice (Figure 2). Similar results were reported within the INREF program by Jiang et al. (personal communication), who concluded from a nutrient solution experiment that increasing Zn uptake of aerobic rice mainly increased allocation to other organs rather than to the grain. These results emphasized the limited potential to increase Zn mass fraction in rice grains by fertiliser application.

In contrast, Zn application as soil or foliar or combination both could increase seed Zn concentration of wheat by a factor of 3 or more (Yilmaz et al., 1997). The extent of increase in Zn concentration in crop grains by Zn fertilization seemed to be influenced by crop species, crop genotypes and soil types. Our results, in combination with similar results from Srivastava et al. (1999) and Jiang et al. (personal communication) clearly showed that rice is more difficult than other crops in this particular case. More investigations are required to understand these differences. Zinc fertilization does increase yield of rice in both experimental sites, even though its effect on increasing the mass fraction of Zn in grains is not promising. In this case, it is still important to recommend local farmers to use Zn fertilizers on their fields, especially where soils are low in available Zn. Also, we only evaluated the fertilization effect of Zn at one stage (sowing), as one form ($ZnSO_4$) and by one method (soil application). It is suggested in sorghum production that Zn fertilizer combined with organic amendment could significantly increase grain yield and grain Zn mass fraction (Traore, 2006). Some research questions need to be addressed regarding the interactive effects between Zn sources, application methods and application stages.

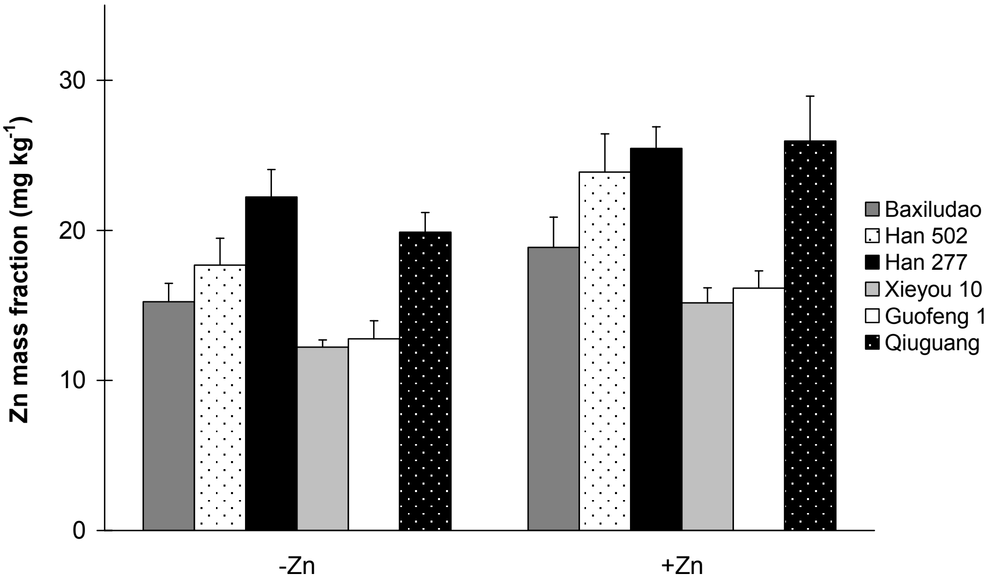

Figure 2. Effects of Zn application on Zn mass fraction in brown rice of six rice genotypes (Means ± SD). Data are from a field experiment with rice plants grown aerobically on a low Zn soil. The experiment was designed as a randomized complete block with Zn fertilizer rates (0 and 10 kg ha^{-1} Zn as $ZnSO_4$) as main plots and six different rice genotypes as subplots. At harvest, rice grains were dehulled and Zn mass fractions in brown rice were then determined after digestion in an acid mixture (HNO_3 + $HClO_4$).

An alternative approach to increase Zn mass fraction in crop grains is to exploit the genetic variation within plant genome. In the current study, however, Zn mass fraction in rice grains among genotypes either did not vary (Chapter II) or only showed a small range of variation (12-22 mg kg^{-1}, Figure 2). Again, the absence of genotypic variation in grain Zn mass fraction may be related to the limited number of genotypes used in our experiments. In contrast to our results, Yang et al. (1998) did report a large variation (8-95 mg kg^{-1}) in Zn mass fraction of polished rice among 285 rice genotypes. Studies at IRRI (Graham et al., 1999) have also shown a 4-fold difference in grain Zn concentration among 939 rice genotypes, ranging from 14 mg kg^{-1} to 58 mg kg^{-1}. Also within the INREF program, Liang et al. (2007) reported a 3 fold variation in Zn mass fraction in brown rice among 56 varieties of

Chinese rice, ranging from 13-39 mg kg^{-1}. These results clearly indicate that, to increase potential for an optimal mass fraction of Zn in rice grains, the breeding strategy should include as many genotypes as possible.

Many studies showed the importance of rhizosphere effects on Zn mobilization and uptake by plants, such as on rice (Hoffland et al., 2006, Gao et al., 2005; Wissuwa et al., 2006) and wheat (Cakmak et al., 1996). Knowledge about the physiological and molecular mechanisms of Zn mobilization in the rhizosphere is necessary. On the one hand, this will pave the way for the engineering of plants with enhanced capacity to take up more Zn from the soils. On the other hand, the progress in our understanding offers the possibility to improve Zn bioavailability by manipulating plant rhizosphere. For example, the form of N supply has a major role in the cation/anion uptake ratio and its subsequent effect on rhizosphere pH (Marschner, 1998). It is possible to manipulate the rhizosphere pH through appropriate use of N compounds, thereby controlling the transformation, mobility and bioavailability of Zn. In the rice rhizosphere, oxidation reactions on root surface can result in the formation of Fe plaque, which accumulates large amounts of Zn. Consequently, Zn uptake by rice plants can increase as Fe plaque formed, but decreased at high amounts of Fe plaque (Zhang et al., 1998). Another example is that a newly developed "non-flooded film mulching" in rice production can significantly increase the bioavailability of Zn compared to the conventional flooded cultivation (Liu et al., 2004). The underlying mechanisms were contributed to the increase of soil temperature and well developed rice root system. These several cases indicate that adjusting rhizosphere processes through optimizing soil and water management in crop production can effectively improve the bioavailability of Zn. The practical application of the accumulated knowledge for rhizosphere management to improve plant tolerance to Zn deficiency and Zn mass fraction of edible parts in sustainable agricultural production systems is a major challenge for the future. Special attention should be paid at an agro-ecosystem level (Zhang et al., 2004).

Genetic engineering approaches have been applied to increase the mass fraction of Zn in crop grains. At present, knowledge of the genes controlling specific steps of Zn cycling in soil-plant system is still rudimentary, but increasing rapidly. Transformation and overexpression of Zn transporters from Arabidopsis in barley (*Hordeum vulgare*) can increase plant Zn uptake and seed Zn content (Ramesh et al., 2004). Recently, a NAC (NAM-B1, DQ869673) gene was identified in wheat, which can accelerate senescence and increase Zn remobilisation from leaves to developing grains (Uauy et al., 2006). These results show the contribution of molecular genetic tools to increase Zn efficiency in crops and Zn translocation into food.

Cropping systems such as rotation and intercropping may have numerous advantages in terms of increasing availability of micronutrients including Zn. In a Chinese peanut (*Arachis hypogaea* L.)/maize (*Zea mays* L.) intercropping example, the excretion of phytosiderophores by maize into the rhizosphere plays an important role in improving Fe nutrition of peanut crops intercropped by maize crops (Zuo et al., 2000). Enhanced release of phytosiderophores by plants may mobilize Zn in the soil and enhance Zn uptake (Zhang et al., 1991). Investigations are needed to quantify the role of rhizosphere processes in intercropping systems. Karlen et al. (1994) concluded that crop rotation may increase the availability of Zn. With several India Alfisols and Inceptisols, Mandal et al. (2000) found a higher Zn desorption under flooded-dried condition than the continuously flooded control, indicating rice (*O. sativa* L.) - maize (*Zea mays* L.) rotation can use Zn fertilizer more efficiently than continuously flooded rice. In the rice (*O. sativa* L.) – wheat (*T. aestivum* L.) rotation, use of manure maintained higher soil available Zn compared to the same rotation fertilized with inorganic fertilizers (Kumar and Yadav, 1995). However, these advantages from managing cropping system have been studied more from the standpoint of increasing crop yield. The potential of managing cropping system to increase micronutrient density, especially Zn, in crop grains should be the subject of a vigorous research effort in the future.

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.

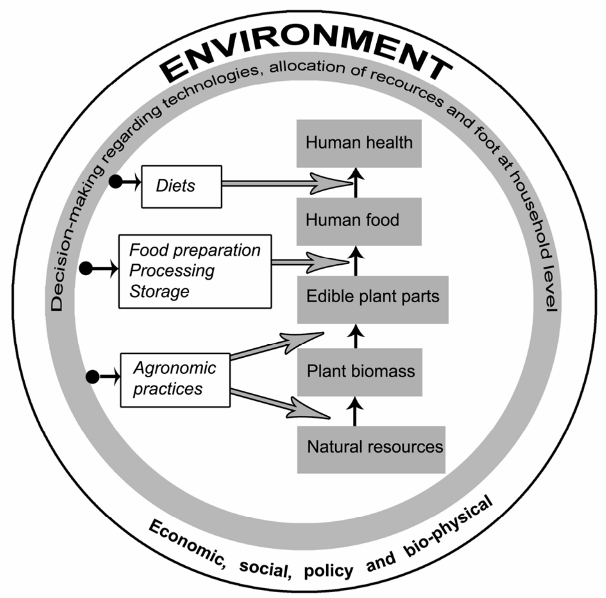

Diagram of the food chain

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 & management and their interaction on micronutrient/phytic acid molar ratio in seed. This has led to recommendations on choice of genotype, fertiliser 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.

Summary

This thesis forms part of an interdisciplinary program titled "From natural resources to healthy people", which aims to develop sustainable agriculture- or food-based interventions to alleviate nutritional deficiencies in human consumers. This research focused on the first step in the food chain, Zn transfer from the soil to the plant.

Zn deficiency is a problem that adversely affects the world food production and human health. This latter specifically holds for developing countries, in which diets mainly consist of cereals such as rice. Knowledge of Zn acquisition by rice is necessary to develop appropriate strategies to prevent its deficiency in crop and human body. In China, an extra practical dimension is formed by the current change in rice cultivation. This is changing from lowland (flooded) to aerobic. This thesis aims at identifying the soil and plant factors affecting Zn bioavailability in aerobic rice. The two main research questions addressed were: 1) what is the consequence of a cultivation shift from flooded to aerobic rice on Zn bioavailability and 2) which mechanisms control Zn mobilization by aerobic rice? The first question was answered by field experiments on two different soils in two different years. To understand the underlying mechanisms on difference in Zn bioavailability between two cultivation systems, a lab soil incubation experiment in combination with geochemical analysis was conducted. The later question was answered by investigating genotypic variation in Zn uptake efficiency among aerobic rice varieties.

With the field experiments on two different soils in two different years, we have demonstrated that the cultivation shift from flooded to aerobic conditions may increase Zn deficiency problems. Plants took up less Zn in aerobic fields. A lower mass fraction of Zn in shoot and brown rice was observed under aerobic conditions compared to flooded conditions. We also observed a decreased Zn harvest index in aerobic fields, which was caused by a decreased dry matter based harvest index. These results indicated that the cultivation shift decreased bioavailability not only for crops but also for human consumers.

A controlled lab soil incubation experiment was conducted to understand the underlying mechanisms of reduced Zn bioavailability in aerobic fields compared to flooded fields. To this end, use was made of a fully parameterized geochemical model with which a comparison was made between observations and major trends according to current geochemical understanding. Zinc uptake by rice was significantly, yet, in a geochemical sense only to a modest degree, affected by the cultivation under either aerobic or flooded conditions. Though this limited effect has been reported before in the scientific literature, it might be unexpected from a soil chemical point of departure, where the individual effects of e.g. redox potential and pH have been demonstrated to involve orders of magnitude of heavy metal concentrations variation. This difference between the experimental observations and the model calculations leads to the suggestion that it may lead to erroneous interpretations if soil chemical factors are considered individually instead of in an integrated way that allows for synergistic and antagonistic effects. The different chemical properties such as local acidity, DOC levels caused by excretion of complexing organic acids, and perhaps different redox conditions in rhizosphere compared to the bulk of the soil need careful considerations when comparing two cultivation systems.

With 23 rice genotypes grown on a low Zn soil, we conducted a pot screening experiment to assess the genotypic variation in Zn efficiency in aerobic rice. Zn efficiency is defined as the capacity of a genotype to grow or yield well on a low Zn soil. The observed considerable variation in Zn efficiency confirmed our findings under field conditions. The genotypes used in both conditions showed similar ranking based on Zn efficiency, suggesting that soil-based pot screening experiments can be useful for routine screening for Zn efficiency at field level. This variation offers opportunities for breeding as a tool to resolve Zn deficiency in aerobic rice.

Regression analysis was conducted to determine the combination of factors that best explained the variation in Zn efficiency and results showed that 53% of the observed variation was associated with Zn uptake (34%) and translocation from roots to shoots (19%). We assumed the unexplained proportion of the variation was ascribed to biochemical utilization and translocation of Zn from older to younger parts of the plant. These results confirmed that Zn efficiency is indeed a complex trait as reports on Zn efficiency in other crops have also shown.

Plant Zn uptake from Zn deficient soils can be increased by both Zn-mobilizing rhizosphere processes and inoculation with arbuscular mycorrhizal fungi (AMF). In a pot experiment with six genotypes growing on a low Zn soil, we studied whether these two strategies can be combined in one genotype. A pot experiment was conducted with six rice genotypes inoculated with and without AMF on a Zn deficient soil. On average, AMF-inoculated plants produced 41% higher biomass and took up 39% more Zn compared to nonmycorrhizal controls. Mycorrhizal inoculation, however, significantly increased Zn uptake only in genotypes that had a low Zn uptake in the nonmycorrhizal condition. This indicates that a combination of rhizosphere effect with mycorrhizal effect seems impossible and may not be feasible as a target for breeders. Inoculation with AMF did not significantly increase Zn uptake beyond levels that were found in genotypes with an inherently high uptake, indicating that mechanisms other than mycorrhizal formation are more efficient in Zn mobilization. Root-induced chemical changes in rhizosphere could play a major role in Zn uptake.

To facilitate measurements of changes in Zn bioavailability in the rhizosphere of the aerobic rice root, a modefied rhizotron technique in combination with *in situ* extraction of soil solution was used. Soil solution from close to the root tips was sampled by inserting the lysimeters into the holes on the rhizotron. With the same six genotypes as used in mycorrhizal study, we conducted a rhizotron experiment and a nutrient solution experiment to find out if genotypic variation in Zn uptake is related to the capacity to exude low molecular weight organic anions into the rhizosphere in response to Zn deficiency. In both experiments under very different conditions, plants responded to Zn deficiency with increased root exudation of malate. On average, the rhizosphere malate concentration at Zn deficiency increased 64% compared to that at adequate Zn supply in the rhizotron experiment. Similarly, under solution culture conditions, malate exudation rate at Zn deficiency was 41% higher than that at adequate Zn supply. A main effect of genotypic variation was identified under both conditions. Aerobic rice genotypes with a higher Zn uptake showed a stronger increase in malate exudation than the genotypes with a lower Zn uptake. The effect of malate exudation on Zn availability in soil solution was evaluated by a malate-soil extraction experiment. Results showed that increased malate concentrations in soil solution can increase Zn bioavailability. For the rhizotron experiment, the estimated rhizosphere Zn concentration for the efficient genotype is 15% higher than that for the inefficient genotype, though this difference was smaller than the previously found 5 fold difference in Zn uptake. This discrepancy was partly attributed to the underestimation of malate concentrations in rhizosphere, which would be caused by the limitation of the *in situ* lysimeter rhizosphere soil solution sampling technique. On the other hand, this discrepancy also indicated the importance of investigations on other possible mechanisms such as exudation of phytosiderophores. These results highlight the importance of rhizosphere effects for Zn uptake by plants and confirm our hypothesis that the genotypic difference in Zn uptake of aerobic rice is partly explained by the capacity to exude malate into the rhizosphere.

Although we made some progress in the subject on bioavailability of Zn in aerobic rice, some questions remain. In the first place, the current outcomes on the two research questions indicate that sufficient understanding of chemical changes in rhizosphere is still lacking. The difference in chemical changes in rhizosphere between aerobic and flooded conditions needs to be carefully compared to evaluate the consequence of the cultivation shift on bioavailability of Zn. Regarding the mechanisms of Zn mobilization from low Zn soils by crop plants, introduction of computer models into the complex plant-soil systems research may be an interesting and plausible approach. In the General Discussion, we also shortly discussed the potential to increase Zn level in rice grains by soil and crop management options.

Samenvatting

Dit proefschrift beschrijft de resultaten van een project dat deel uitmaakt van een interdisciplinair onderzoeksprogramma getiteld "*From Natural Resources to Healthy People*". Doel van dit programma is om duurzame interventies te ontwikkelen die voedingsdeficiënties bij mensen helpen verminderen. Daarbij wordt een ketenbenadering toegepast. Dit project is gericht op de eerste stap in de voedselproductieketen: opname van zink (Zn) uit de bodem door de plant.

Zink gebrek is een beperkende factor voor de wereldvoedselproductie en voor de gezondheid van een groot deel van de wereldbevolking. Het komt vooral voor bij mensen in Zuidelijke landen, waar het dieet eenzijdig is en voornamelijk of geheel bestaat uit granen zoals rijst. Kennis van Zn mobilisatie uit de bodem door rijst planten kan bijdragen aan voorkoming van Zn gebrek in het gewas en bij de consument van het graan. In China geeft de verandering van teeltsysteem een extra dimensie aan de problematiek van Zn gebrek. Gedwongen door watergebrek verandert men van natte rijstteelt, waarbij het veld vrijwel het volledige groeiseizoen onder water staat, naar geïrrigeerde teelt. Dit laatste systeem wordt "aerobe rijstteelt" genoemd.

Doel van dit proefschrift was om vast te stellen welke bodem- en plantfactoren de biologische beschikbaarheid van Zn voor aerobe rijst bepalen. De twee belangrijkste onderzoeksvragen waren: 1) wat is het gevolg van de verandering van teeltsysteem voor de biologische beschikbaarheid van Zn en 2) welke processen spelen een rol bij Zn mobilisatie uit Zn deficiënte bodems door de aerobe rijst plant. Voor het beantwoorden van de eerste vraag zijn veldproeven gedaan op twee verschillende bodems, in twee verschillende jaren. Om de achterliggende bodemchemische processen beter te begrijpen, is een incubatie experiment gedaan en is een geochemisch model gebruikt. Voor het beantwoorden van de tweede vraag zijn genotypes van aerobe rijst (*Oryza sativa* L.) onderzocht die variëren in de mate waarin ze in staat zijn goed te groeien op Zn arme bodems.

De veldproeven lieten zien dat de verandering van natte naar aerobe rijstteelt de problemen van Zn gebrek verergeren. De planten namen minder Zn op wanneer ze groeiden op aerobe velden. De massafractie Zn in de spruit en bruine rijst korrel was ook lager bij aerobe teelt. Ook de hoeveelheid Zn in het graan ten opzichte van de hoeveelheid in de spruit was lager. Dit laatste werd veroorzaakt door een lagere *harvest index*. Deze resultaten duiden erop dat het Zn probleem niet alleen toeneemt voor het gewas, maar ook voor de consument van het graan.

Om beter te begrijpen welke mechanismen verantwoordelijk zijn voor de lagere Zn beschikbaarheid onder aerobe omstandigheden is een bodemincubatie experiment uitgevoerd onder gecontroleerde omstandigheden. Ook werd gebruik gemaakt van een volledig geparameteriseerd geochemisch model. Zink opname door het gewas was weliswaar lager onder aerobe omstandigheden, maar vanuit een bodemchemisch oogpunt was de verlaging gering. Vaak veroorzaakt geringe variatie in redox potentiaal of pH al ordegrootte verschillen in concentraties zware metalen. Dat dit in het veld niet gebeurde, duidt erop dat het nodig is om veranderingen in bodemchemische parameters in samenhang tot elkaar te beschouwen. Bovendien is het bij de vergelijking van de twee teeltsystemen van belang niet alleen veranderingen in de bodem als geheel te beschouwen, maar vooral ook veranderingen in de rhizosfeer in beschouwing te nemen. Daar kunnen pH en DOC afwijken door worteluitscheiding van organische zuren. Ook de redox potentiaal kan afwijken van die in de bodem als geheel.

In een potproef is bepaald of er variatie is in Zn efficiëntie is onder 23 genotypes van aerobe rijst. Zink efficiëntie is het vermogen van een genotype of gewas om goed te groeien of produceren op een Zn arme bodem. De variatie in Zn efficiëntie zoals vastgesteld in deze potproef, bevestigde de variatie die ook in het veld was gevonden. De rangorde in efficiëntie van genotypes was bovendien hetzelfde, onafhankelijk van de opkweekmethode (veld *vs.* pot). Potproeven kunnen daarom goed gebruikt worden voor het bepalen van variatie in Zn efficiëntie. Om vast te stellen welke plant factoren Zn efficiëntie bepalen, is regressie analyse gedaan. Deze wees uit dat 53% van de waargenomen variatie kon worden verklaard uit een combinatie van Zn opname (34%) en Zn translocatie van wortel naar spruit (19%). We speculeren dat de onverklaarde variatie kan worden toegeschreven aan biochemische gebruiksefficiëntie en translocatie van Zn van oudere naar jongere delen van de plant. Deze resultaten bevestigen dat Zn efficiëntie een complexe eigenschap is.

De opname van Zn door de plant uit Zn deficiënte bodems kan worden verhoogd door rhizosfeerprocessen en door inoculatie met arbusculaire mycorrhizaschimmels (AMF). In een potproef met zes aerobe rijst genotypes hebben we onderzocht of de combinatie van deze twee leidt tot hogere Zn opname. Planten van deze genotypes werden gezaaid op een Zn deficiënte grond en werden wel of niet geïnoculeerd met AMF. Gemiddeld genomen produceerden de geïnoculeerde planten 41% meer droge stof en namen ze 39% meer Zn op dan de niet-geïnoculeerden. Inoculatie leidde echter alleen tot toename van Zn opname bij genotypes die zonder mycorrhizas relatief weinig Zn opnamen. Dit betekent dat een combinatie van inherent hoge Zn opname en een relatief grote verhoging van de opname door AMF kolonisatie niet voorkomt. Het is dus niet voor de hand liggend dat door veredeling dit wel bereikt kan worden. Inoculatie met AMF leidde bij genotypes met een inherent lage Zn opname niet tot een Zn opname die hoger was dan de Zn opname door niet-geïnoculeerde planten van genotypes met een inherent hoge opname. Waarschijnlijk zijn daarom rhizosfeer processen effectiever voor het mobiliseren van Zn dan kolonisatie door AMF.

Om de mogelijkheid van veranderingen van Zn beschikbaarheid in de rhizosfeer te kunnen inschatten, is een *rhizotron* techniek gebruikt in combinatie met *in situ* extractie van de bodemoplossing. De bodemoplossing rond de wortelpunten werd verzameld door microlysimeters aan te brengen via gaatjes in de *rhizotron*. Dezelfde genotypes die ook waren gebruikt in het mycorrhiza experiment werden zowel in deze *rhizotrons* als op voedingsoplossing opgekweekt. Doel was om vast te stellen of de gevonden genotypische variatie in Zn efficiëntie kon worden verklaard met variatie in de uitscheiding van laag moleculaire organische anionen in de rhizosfeer als reactie op Zn gebrek. In beide experimenten, onder zeer verschillende omstandigheden, reageerden de planten op Zn gebrek door uitscheiding van malaat. Gemiddeld nam de malaat concentratie in de rhizosfeer bij Zn gebrek toe met 64%. Op voedingsoplossing nam de concentratie toe met 41%. Genotypes met een hogere Zn opname lieten een sterkere toename zien in malaat exudatie. Het effect van verhoogde malaat concentratie in de bodemoplossing op de beschikbaarheid van Zn werd onderzocht in een bodem extractie experiment. Dit toonde aan dat hogere malaat concentraties inderdaad leiden tot een hogere Zn concentratie in de bodemoplossing. De verhoging in malaat concentratie die bij een efficiënt genotype werd gevonden in het *rhizotron* experiment zou echter slechts leiden tot een toename van 15% voor de Zn concentratie in de rhizo-sfeer bodemoplossing, terwijl de Zn opname met een factor 5 hoger was dan bij een inefficiënt genotype. Deze discrepantie kan waarschijnlijk verklaard worden uit de tekortkomingen van het *rhizotron* experiment. Een andere mogelijke verklaring zou kunnen zijn dat andere rhizosfeer processen in belangrijke mate bijdragen aan Zn mobilisatie door efficiënte genotypes bijvoorbeeld uitscheiding van fytosideroforen. Deze resultaten bevestigen het belang van rhizosfeer effecten voor opname van Zn door planten. Ze bevestigen ook onze hypothese dat variatie in Zn opname onder genotypes mede verklaard kan worden met variatie in de capaciteit om bij Zn gebrek malaat uit te scheiden.

Hoewel ons inzicht in het onderwerp biologische beschikbaarheid van Zn voor aerobe rijst is vergroot, blijven er nog vragen onbeantwoord. Ten eerste schiet ons begrip Zn speciatie in de rhizosfeer tekort. De verschillen in rhizosfeer chemie onder natte en aerobe condities moeten zorgvuldig onderzocht worden om het gevolg van de verandering van teeltsysteem voor biologische beschikbaarheid van Zn te kunnen begrijpen. Bij het beter gaan begrijpen hoe gewassen Zn kunnen mobiliseren uit Zn deficiënte bodems kunnen modellen een nuttig instrument zijn. In de *General Discussion* worden ook kort mogelijke gewas- en bodembeheersmaatregelen besproken die kunnen bijdragen aan verhoging van het Zn gehalte in rijst korrels.

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Curriculum Vitae

Xiaopeng Gao was born on December 10th, 1978 in Shanxi, China. He attended primary and secondary school in Shanxi and graduated from the secondary school in 1995. In September of the same year, he started to study agronomy in Shanxi Agricultural University. He received his bachelor's degree in 1999. From 2000 to 2002, he followed an MSc program in Department of Plant Nutrition in China Agricultural University, obtaining his MSc degree in October 2002. The MSc project was on investigating the effects of silicon on improving plant water use efficiency. Following graduation, he was admitted to the Wageningen University PhD program. The focus of his PhD research was on the bioavailability of zinc to aerobic rice. The main outcomes are described in this thesis.

PE&RC PhD Education Statement Form

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 22 credits (= 32 ECTS = 22 weeks of activities)

Review of Literature (5.6 credits)

- Bioavailability of zinc for aerobic rice

Writing of Project Proposal (7 credits)

- Bioavailability of zinc for aerobic rice

Post-Graduate Courses (2.8 credits)

- Advanced statistics (PE&RC 2003)
- WU-CAAS Autumn school “from plant production to healthy food” (Beijing, 2003)
- Scientific writing (WGS 2004)
- Project and time management (WGS, 2006)

Deficiency, Refresh, Brush-up and General Courses (7.3 credits)

- Basic statistics (PE&RC 2003)
- SOQ-20306 Chemical interactions in soil, water, sediment (Department Soil Quality, 2003)
- ORYZA 2000 Modelling (Wageningen and China Agricultural University, 2005)

PE&RC Annual Meetings, Seminars and Introduction Days (1.1 credit)

- PE&RC day on “ethics in science” (2002)
- PE&RC weekend (2003)

International Symposia, Workshops and Conferences (7 credits)

- XV International plant nutrition colloquium; oral presentation. (Beijing, 2005)
- 18 th World conference of soil science; oral presentation. (Philadelphia, 2006)