AGRICULTURAL BIOFORTIFICATION OF RICE THROUGH MICRONUTRIENTS AVAILABILITY CONTROL AND VARIETAL SELECTION.

（土壌の微量要素可給性の制御と品種選択による
米の農業生物学的栄養強化）

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Dedication

This dissertation work is dedicated to:

In memory of my grandmother Hortencia Muecke Perez (Tita) who gave me inspiration and power to finish my studies.

To my daughter Heidy, my mother, brothers and friends for their encourage and support throughout this lasting process.

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CHAPTER I

GENERAL INTRODUCTION

1.1 The challenges of micronutrients malnutrition

Micronutrient malnutrition is a well-documented health problem which increasingly affects nearly half of the world population. Although developing countries have the most severe situations in terms of starvation and undernourishment, the phenomena is not endemic of these regions. The developing world is also facing malnutrition; coexisting with obesity, overweight and other related non-communicable diseases due to consumption of low micronutrient-rich foodstuff (FAO, 2012). Thus, there is need to alleviate micronutrient deficiencies by increasing the density micronutrients mainly in cereals destined for human consumption.

Developing countries have an important challenge of increasing crop production to meet the food demand for their growing populations as well as supplying essential nutrients to support human health. The change began in the 1960´s, when the so-called ¨green revolution¨ saved the world from hunger, by exponentially increasing staple crops production through the use of high yield varieties (HYV) and high doses of agro-chemicals. However, intensive cropping systems also increased the demand of nutrients and inputs causing erosion, degradation of soil capabilities, depletion in micronutrients availability, contamination of water bodies (Pingali 2012) and an interminable list of constraints. Nowadays, the low availability of iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B) and other micronutrients not only cause reduction in plant growth and yield of HYV (Das, 2014) but also overlapping clinical diseases to populations in China and other Asian countries which are associated with lower density of micronutrient in foodstuffs (Yang *et al*., 2007). The reduction of soil micronutrient availability and increase in consumption of less micronutrient-rich foodstuffs and milled cereals (e.g. rice, wheat and corn) have contributed to the growth of sub-clinical manifestation of micronutrient malnutrition or "Hiden hunger"(Welch, 2001) becoming a social and public health issue. The World Bank (2011), clearly summarized this situation stating: "The effects of malnutrition can be devastating and enduring. It can impede behavioral and cognitive development, educability, and reproductive health, thereby undermining future work productivity".

1.2 Clinical manifestations of micronutrient deficiency

The most prevalent micronutrient deficiencies in developing countries are iron, iodine and zinc affecting mainly poor women, infants and children under 5 years (Welch, 2001). For example, Fe deficiency which is the widespread nutritional disorder in the world, it causes anemia contributing to 20% of all maternal deaths (WHO, 2015 On the other hand, clinical manifestation of Zn deficiency affects two billion people worldwide (Prasad, 2003). Its prevalence might entail diarrhea, pneumonia, alterations in pregnant women (defects at birth and mortality in suckling), mental and physical impairment, immune dysfunction, skeletal anomalies, skin lesions and many others (Prasad, 2008). The deficiencies of Cu increase the risk to suffer anemia and other pathologies (Griffith et al., 2009) while Mn deficiencies result in heavy bleeding during menstrual periods causing loss of Fe, Zn and Cu in the menstrual blood (Eades, 2000)

1.3 Addressing micronutrient deficiencies

In an effort to prevent and control micronutrient deficiencies, some nutritional interventions have been adopted for instance food fortification, dietary diversification, nutrient supplementation and biofortification (Fig. 1). However, there is a high pressure to reduce deficiency effects and thus short term nutrient interventions (1) are preferred by policy-makers because in their perspective, malnutrition is a disease that must be "treated". Unfortunately, there is no documented evidence about the effectiveness of nutritional interventions in a reasonable short term as none have proven to be sustainable in the long term (Welch 2001).

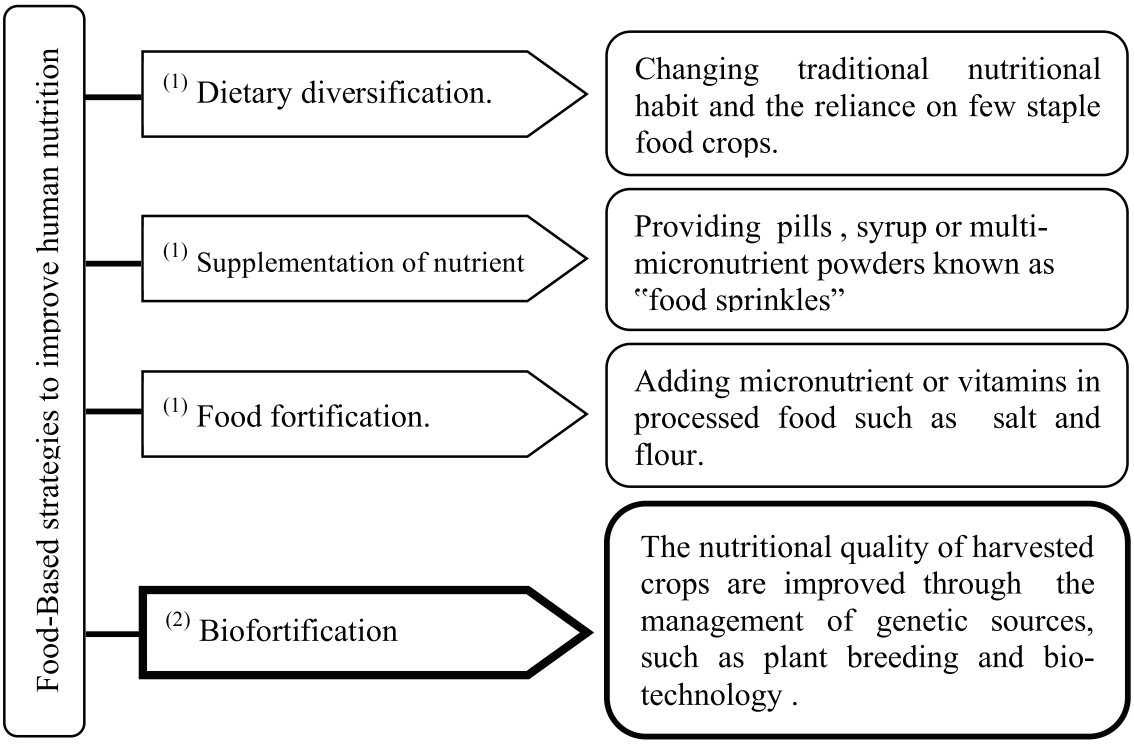

Figure 1.1 Adoption of public health interventions as a way to minimize the burden of malnutrition in developing nations.

In developing countries, most of these programs are costly because they require a considerable number of facilities and infrastructure and the penetration rates are low in remote areas (Bishai and Nalubola, 2002).

In contrast to short term interventions, Biofortification was designed to improve the nutritional quality of staple crops through conventional plant breeding or biotechnology (Hotz et al., 2007), providing a sustainable linkage between agricultural interventions and nutrition outcomes. Thus Biofortification appears as the most cost-effective strategy, with higher penetration rates in vulnerable populations than other nutritional interventions (Meenakshi *et al.*, 2007). Nevertheless biofortification through plant breeding as well as previous strategies failed in one aspect; They did not consider the availability of micronutrients in soil as the main concern to increase their bioavailability in plant. Even though high micronutrient varieties are developed, their biofortified potential under soil micronutrient deficiencies can only be partially expressed. Against this back-drop, it was necessary to develop a new concept of biofortification oriented towards the proper use of soil and agricultural practices; an action termed agronomic biofortification (White and Brown, 2010). Therefore, the adoption of plant-breeding strategies to enrich micronutrients bioavailability in grains alongside proper agronomic practices through application of fertilizer either organic or inorganic, irrigation and soil management strategies can become in the most suitable ways to increase the density of micronutrients in staple crops (Figure 1.2).

The dimensions of biofortification can reach almost all staple crops. However rice (*Oryza sativa L.*) in our opinion is one of the best candidates to reduce malnutrition in vulnerable populations for many reasons.

1. It is the main staple crop for more than half of the world's population.
2. It covers the second largest production area after wheat.
3. It provides more than 25 % of dietary energy supply and 20 % of dietary protein intake in the developing countries.
4. Is able to grow in a wide number of soil types, including alkaline and acidic soils from tropical to sub-tropical and temperate latitudes.

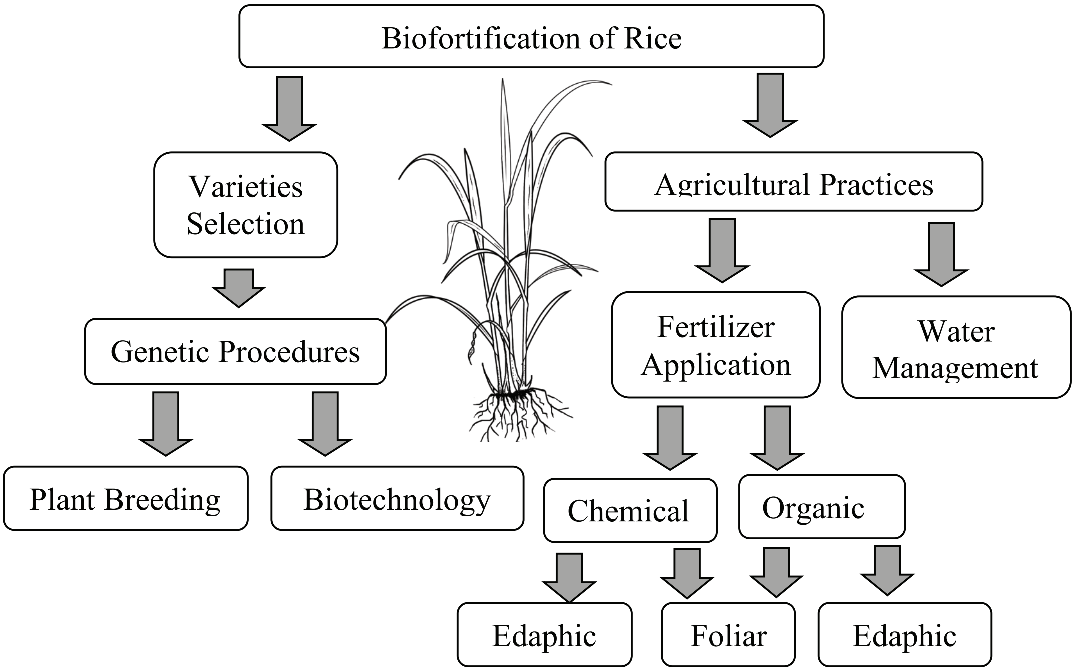

Figure 1.2. The process of biofortification of rice comprises the combination of advanced breeding technologies and proper agricultural practices

1.4 Correcting micronutrients availability in paddy fields

As aforementioned, micronutrient availability has become a key limitation to productivity, stability and the sustainability of soils (Brady and Weil) The concentration of micronutrient in soil, is controlled by the amount of minerals contained in the original parent material, redox potential, weathering processes, organic matter contents and the action of microorganisms. Despite the concentration in soil, only a small fraction (less than 0.2%) of the nutrients supply is dissolved in soil with remaining amount bounded (98%) in organic matter and relatively inorganic compounds (Larcher 1995). This section provides a brief overview of how micronutrient availability and uptake can be improved through agricultural biofortification practices.

1.4.1 Chemical fertilizer.

Many researchers assume that if the total concentration of micronutrients in soil is phytoavailable it would be enough to support the plant nutrient demand (White and Brown, 2010) However availability of micronutrients is conditioned by a number of factors:

1- Soil pH and electrochemical changes associated with agricultural practices are the most important factors governing micronutrient availability.

2- Contents of organic matter complexes that bind nutrients limiting their availability to plants.

3- Soil parent materials (e.g. Acidic soil, Calcareous soil)

4- Synergic and antagonist interaction among elements which either depress or increase availability in soil.

5- Climatic conditions causing changes in pH which consequently affects micronutrients availability.

6- Physiological mechanism developed by plants for micronutrients acquisition e.g. Aerenchyma, exudates, phytosiderophores, mycorrhization.

Inorganic micronutrient fertilizers are frequently used by rice growers to improve soil chemical and physical properties. These applications have had significant effects on the accumulation of micronutrients in grain (Khoshgoftarmanesh *et al.*, 2010) Although chemical fertilizers are readily available for plants, their application can become ineffective due adsorption, precipitation and oxidation reactions triggered by the interaction of above mentioned factors (White and Broadly 2008). For rice production, deficiencies of iron (Fe) and manganese (Mn) are common only in upland or calcareous soils (Dobermann and Fairhurst, 2000). Although these elements are recommended by agronomists, its use as inorganic fertilizers in rice has very little if any effect because of the rapid precipitation as oxides and phosphates in soil (Baker and

Pilbeam 2015) Rice plants have developed strategies to cope with deficiencies by taking up Fe and other micronutrients even when their availability is low in soil (see section 1.5). Unlike Fe and Mn, Zn fertilizer has higher solubility in soil, application of $ZnSO_4$ to highly Zn deficient soils, not only improved grain yield but also enhanced Zn concentration in wheat twofold (Cakmack 2008) deficiencies are observed mainly as pH increase by reduction of redox potential in normal condition of paddy field. Cu is another effective element that can be applied as $CuSO_4$ in order to correct Cu deficiencies but care must be taken because the range between Cu deficiency and toxicity levels is narrow. (Dobermann and Fairhurst, 2000)

1.4.2 Organic matter amendments to improve micronutrient contents

Soil organic matter amendment (e.g. Farm-yard manures, crop residues, slurry) is reported as an excellent source of Fe, Zn, Cu and Mn and other elements not provided by inorganic fertilizers. In contrast to chemical fertilization the use of organic amendment offers advantages that improve soil properties i.e.: (Brady and Weil, 2008)

1. Improves soil physicochemical and biological properties.
2. Improves cation exchange capacity.
3. Increases water holding capacity.
4. Promotes soil microbial activity.

With regard to the relationship between availability in soil and concentration in grain, reported that any improvement in soil micronutrient concentration is closely correlated with the concentration observed in grain (White and Brown, 2010). Although OM fertilization is a sustainable and environmentally friendly approach, OM applications alone may not meet crop demand for nutrients therefore cannot be considered as substitute of chemical fertilization in short term applications (Dawe *et al* 2003). In addition, metal complexation with organic ligands in soil solution is generally found to increase the mobility of soil micronutrients while chelation processes reduce cellular metal uptake (Khoshgoftarmanesh, 2010). Thus OM application in combination with inorganic fertilizer is advisable to meet crop demands and reduce risks (IRRI 2012). Studies conducted by Shahid et al (2013) demonstrated that, the combination of NPK+farmyard manures in rice-rice system is the best alternative to improve soil properties and availability of micronutrients in soil.

1.4.3 Water management controlling electrochemical conditions

Since rice is mainly produced under two different ecosystems irrigated lowland and upland (Dobermann and Fairhurst, 2000), differences in soil micronutrient availability can be expected. The duration of soil submergence in flooded systems cause changes in soil physico-chemical properties by depletion or diffusion of O_2 which affect the redox potential and consequently the availability of micronutrients and other essential nutrients. (Figure 1.3).

At the beginning, aerobic microorganisms use the available O_2 trapped in soil pores for respiration. Thus, O_2 depletes in few hours, remaining little amount in upper layer supplied by diffusion from atmosphere or by oxygenated water . Facultative and obligate anaerobic microorganisms take over further biochemical reactions using substances other than O_2 as the terminal electron acceptor for metabolism. After O_2 is depleted, NO_3-N is reduced followed progressively by Mn^{4+}, Fe^{3+}, SO_4^{2-} and CO_2 Due to the H^+ consumption in the reduction reactions, pH tends to move toward neutrality as a result of the buffering capacity of the soil (Brady and Weil, 2008).

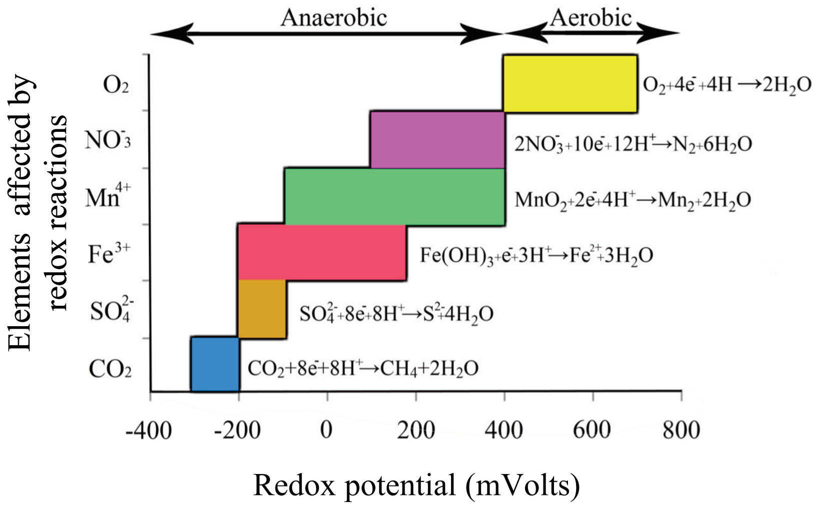

Figure 1.3 The effect of changes in redox potential E_h on elements that are used by microorganisms as electron acceptor. Diagram shows the theoretical Oxidation-reduction reaction that take place when soil is water saturated. [Modified from DeLaune and Reddy, 2005]

Rice growers have traditionally assumed that permanent flooding conditions are a synonym of high yield keeping them reticent to grow rice on upland ecosystems. Certainly upland varieties always have produced lowest yields than their lowland counterparts. However this traditional way to produce rice withdraws two to three times more water than other crops (Tuong et al. 2005), is responsible for soil degradation and leaching of essential nutrients as well as representing one of the most important methane and greenhouse gases emission source (Bouman and Tuong, 2007). These characteristics of irrigated systems along with the current increasing rice demand and water scarcity have forced farmers and policy-makers to change paradigms in considering other alternatives to reduce cost and save water inputs.

Experiences from Madagascar and India by the adoption of System of Rice Intensification SRI, other experiences in Brazil using sprinkler in bred upland varieties and most recently the adoption of Alternative Dry and Wetting (AWD) proposed by IRRI have demonstrated that these alternatives are able to save water and inputs without compromising on production (Bouman and Tuong, 2007). Although, the adoption of these alternatives have also allowed researchers to understand the dynamics of some micronutrients in soil little is known about its effects on micronutrient concentration in rice grain.

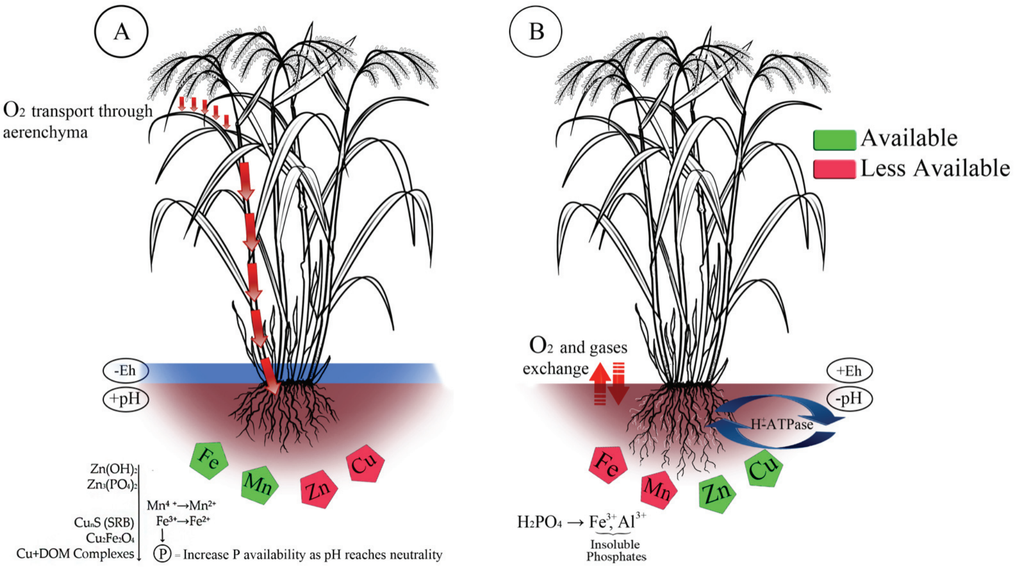

Figure 1.3. Effects of water management and plant adaptive mechanisms to uptake micronutrients and gases exchange. A- Irrigated anaerobic conditions; B- Aerobic upland conditions.

Establishment of flooding conditions reduces Fe and Mn to more soluble forms. (Fig. 1.3), therefore, Fe and Mn deficiency generally do not occur under such condition. In certain environments Mn and Fe toxicity can appear causing damage to plants and affecting availability of other nutrients (Dobermann and Fairhurst, 2000). Taking advantage of some cultivation practices to minimize the toxicity such as mid-summer drainage: *Nakaboshi* (in Japanese)” is commonly adopted in Japan, to mitigate the negative effects of anaerobic condition in paddy field (De Datta 1981).

A change in pH reduces Zn availability which is the most common micronutrient disorder in lowland rice, (Das 2014). Zn availability tend to decrease by precipitation as $Zn(OH)_2$ or reaction with S in acid soils. Cu is also affected by flooding. Cu ions are bound very tightly on organic matter surface, clay particles and complexes with Fe, S Al and Mn. (Dobermann and Fairhurst, 2000)

When soil is saturated with water rice plants use structures called aerenchyma to provide the movement of O_2 from the shoots to the roots (Marschner, 2012). Their role is to supply O_2 for roots respiration and growth under anaerobic condition by allowing a small zone of oxygenated soil around the roots and providing an aerobic environment for microorganisms that can prevent the damage from phytotoxic soil components such as Fe Mn and some heavy metals (Visser et al., 1997). Aerenchyma is also responsible for CO_2 and CH_4 exchange (Miyata, 2000) .

1.4.4 Foliar fertilization

Currently foliar fertilization approach produces a faster response for correction of physiological micronutrient deficiencies which are temporally ameliorated. Despite this there is insufficient evidence about the advantages in rice production since its effectiveness is often variable and unpredictable, thus it may not meet crop demand for nutrients. As a strategy to increase micronutrient contents in rice, experiments conducted by Fang *et al*., (2008) showed that Zn and Se foliar application have a positive impact on grain contents due its medium-high mobility. Other cations such as Fe and Mn have some limitation due to the abundance of negative charges in the apoplastic space and low mobility, which may limit their translocation to seeds (Millaleo *et al*., 2010). Another limitation is the low penetration rate due waxy cuticle present on leaves (hydrophobic leaves). (Fernandez and Brown, 2013) One of the major factors to ensure foliar fertilizer efficiency is in the timing application.

1.5 Micronutrients uptake

Interaction between root surface and soil elements (free ions and ions complexed onto colloids or organic particles) is driven by three components: root interception, mass flow and diffusion. They are the mechanisms responsible for the fluxes of water and dissolved micronutrients from the bulk soil to the root surface (Marschner, 2012). Once micronutrients and water enter the plant through roots; the movement upward in the xylem occurs in three different pathways: the apoplast, symplast and transmembrane movement (White and Brown, 2010). Upon micronutrient deficiencies stress, the rhizosphere uptake Fe via the Strategy II system which allows interaction with soil by releasing organic acid compounds e.g phytosiderophores. There is evidence that under certain conditions Zn Cu, Mn and other micronutrient use these chelating molecules as metal transporters (Bashir *et al*., 2013).

1.5.1 Timing and remobilization

In terms of micronutrients accumulation the demand for micronutrients is a function of growth stage based on the expression of mineral transporter genes. Therefore timing of fertilizer application and water management could play a crucial role in micronutrient availability and uptake. Approximately, 36% of total Zn in rice grain is taken up directly from soil between anthesis and maturity stages Jiang et al., (2007) while 60% of Cu in grain is absorbed at filling stage (Garnett et al., 2005) Likewise, Graham et al., (1988) reported large accumulation of Mn up to saturation point in roots and stem under high Mn availability at early plant stages. A large number of transporters are involved in movement of nutrient ions. The overexpression of some genes such as OsYSL´s are reported as responsible for the increase of Fe and Mn in rice grain during anthesis and seed formation stages which are probably the active Fe uptake period of rice. (Ishimaru et al 2010).

1.6 The study Objective

From the foregoing discussion we can conclude that, the use of agricultural biofortification could be a complementary approach to the on-going breeding programs. most of these strategies represent an feasible way to improve micronutrient availability in soil and consequently increase concentration in grain. The objectives of this study are:

1. Assess the influence of soil properties and irrigation management on morphology and yield parameters.
2. Determine the effects of irrigation management and timing on the availability of micronutrients and contents in rice grain.
3. Determine correlation between soil micronutrients availability and grain concentration.
4. Compare the effectiveness of different agricultural practices regarding to micronutrient availability in soil grain micronutrient concentration
5. Establish differences in term of essential nutrient contents in grain, between upland and lowland genotypes

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CHAPTER II

Assessment of the Influence of Water Management on Yield Component and Morphological Behavior of Rice at Post-heading Stage

2.1. INTRODUCTION

The world's irrigated rice production consumes about 34–43 % of the global irrigation water (Bouman et al. 2007). Increase in freshwater demands for industrial and domestic purposes has resulted in a shortage of irrigation water and is threatening rice production. With the current situation, it is projected that by 2025 more than 15 million ha will face water scarcity (Tuong and Bouman 2003). This situation does not bode well for the world to satisfy the increased rice demand due to population growth, especially when the predictions say, production must increase more than 40 % by 2030 to cope with the challenge of food self-sufficiency, with fewer water and land resources and higher cost of production (Khush 2005).

It is well known that, irrigation assures proper plant growth, effectiveness of fertilizer, and integrated management of weeds and pests. However, continuous flooding generally adopted in rice production, withdraws two to three times more water than other crops (Tuong et al. 2005). The adoption of water saving (WS) technologies such as alternative wetting and drying, SRI, and saturated soil culture has been fostered due to the reduction in water requirement by up to 30 % and increase in water use efficiency with either little or no negative effects on rough grain yield (Bouman et al. 2007). Indeed, some authors have reported slight increase in rice yield using adequate timing of water management and soil moisture (Liu et al. 2013; Wang et al. 2014). Likewise, the shifting from flooding to upland condition in WS culture promotes root distribution, soil organic matter decomposition, and stimulates nitrification–denitrification processes (De Datta 1981). Changes in Eh by the establishment of soil aerobic condition are able to either increase the availability of micronutrient such as Zn and Cu or inhibit toxic effects caused by Fe and Mn reduction in soils (Dobermann and Fairhurst 2000).

However, such technologies have some limitations since they require a certain level of specialization concerning soil water control, i.e., water layer depth, percolation, evapotranspiration, seepage, and timing for establishment of WS period. On the other hand, care must be taken in paddy fields because water stress promotes some disorders which retard phenological development, reduce tillering and panicle emission, increase leaf senescence (De Datta 1981), and grain yield can be drastically reduced by more than 70 % (Bouman et al. 2007). Processes such as photosynthesis and water use efficiency may be increased under upland conditions (Fukai et al. 1985). Wopereis et al. (1996) found that 50 % flowering was delayed under drought conditions compared with irrigated treatments as well as reduction of grain weight when the stress was promoted at the reproductive stage. Grigg et al. (2000) evaluated flooding condition and flush irrigation throughout all growth phases and observed that 58 % rough grain yield was reduced and the leaf area index was below 3.8 m^2m^{-2} in flush irrigation system, following panicle initiation.

As observed above, rice growth phases are reference points for rice culture and agronomic practices. Emphasis has been placed on the reproductive stage which is referred to as the critical stage for nutrient demand (De Data 1981; Dobermann and Fairhurst 2000) as well as for translocation and remobilization of nutrients (Sperotto et al. 2012). Therefore, most researchers recommend avoiding the use of WS techniques during the reproductive stage and keep irrigation until 10–15 days prior to harvest, due to increased risk of spikelet sterility (De Datta 1981). Since during post-heading stage reliance on water and farmer's labor demand reduces, rice-weed competition considerably declines by canopy closure (Ampong-Nyarko 1991). It is likely that those farmers who cannot afford WS techniques because of all the risks aforementioned could try to use WS culture at post-heading, in correspondence with their will, needs, and local conditions without any negative effects on yield and biomass production. However, there is almost no reference about the influence of WS period during grain filling stage upon morphological behavior and yield components. The study aimed at ascertaining whether WS technique during the post-heading stage could affect morphological behavior as well as rice yield parameters in order to increase water use efficiency in paddy rice production.

2.2 MATERIAL AND METHODS

2.2.1 Experiment site and soil properties

Pot experiments were conducted in a greenhouse at Shimane University ($35^{0}29'$ N $133^{0}04'$E) during the rice growing season in Japan (May - October). For this experiment, plastic buckets (Ø=24cm x 25cm height) were filled with 7.5 kg of two soil types, *Typic Fluvaquent* “Grey Soil” (GS) from a lowland paddy field and *Typic Paleudult* “Red Soil” (RS) collected from a recently developed upland field. Table 2.1 shows some physical and chemical characteristics of the soils used for the experiment. Twenty-days old seedlings of rice (*Oryza sativa L.*) semi-dwarf variety *Koshihikari* were transplanted in the study.

2.2.2 Experimental design

The experiment was set up in a randomized block design separated for five water irrigation treatments with four replications for each soil type (Fig 2.1), where T1 was continuously flooded throughout the rice growing period, T2, T3, and T4 were flooded from transplanting until 4, 3, and 2 weeks after heading (WAH), respectively, then were drained and kept in a soil moisture level approximately 35 ± 3 % (v/v), which was equal to field capacity. The treatment (T5) was kept in the same soil moisture throughout the growing stages in order to mimic upland systems.

Table 2.1 Soil physical and chemical properties.

Soil characteristics.	Grey Soil	Red Soil
Sand %	22.8	29.6
Silt %	37.8	48.7
Clay %	39.4	21.7
Bulk Density g cm^{-3}	1.39	1.2
Soil textural class	Clay Loam	Clay Loam
Soil Color	2.5Y6/3	10YR5/4
pH	4.9	5.3
EC mSm^{-1}	3.9	4.1
Total Carbon (mg g^{-1})	19.7	10.4
Total Nitrog. (mg g^{-1})	1.8	1.03
Avail P (mg kg^{-1})	42.9	261.2
Avail Sulfur (mg kg^{-1})	29.8	24.8

During the flooded period, water depth was kept at ± 5 cm. Chemical fertilizers were incorporated into soil (N:P:K—45:65:60 kg ha^{-1}) following the Shimane prefecture recommended fertilizer doses for Koshihikari production. N was split into four applications, 1st-20 % basal, 2nd-30 % at tillering stage, 3rd- 40 % at panicle initiation, and 4th- 10 %, 10 days after panicle initiation. P was applied 100 % during basal dose. Meanwhile, K was split into two applications, 50 % at basal and 50 % at panicle initiation. Apart from the name of the treatments, i.e., T1–T5, we classified soil conditions into two, aerobic and anaerobic conditions since soil aerobic/anaerobic status was closely related to the results on plant morphology and yield. Aerobic condition was defined as the soil condition with the period in which the soil moisture level was kept at the field capacity, while anaerobic condition was done as flooded soil condition. Namely, T1 was always in anaerobic, T5 was in aerobic, and T2–T4 had two periods of the conditions.

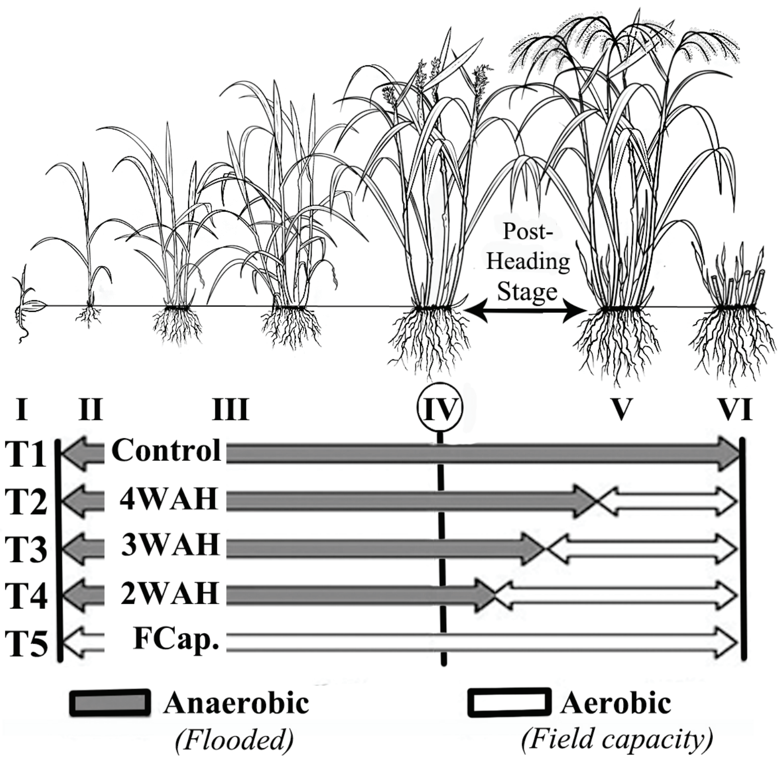

Figure 2.1. Schematic representation of experimental design at different irrigation treatments applied during the growth season. (I) Sowing, (II) Transplanting, (III) Tillering, (IV) Anthesis, (V) Maturity (VI) Harvest.

2.2.3 Plant morphology and yield component.

Plant morphological behaviors such as height, phyllochrons ‘‘periods in day between the sequential emergence of leaves in the culm’’, and total number of tillers, which include unproductive tillers, were recorded at 25, 50, 75 DAT. Yield parameters (panicle length, 1000 grains weight, spikelets per panicle, and percentages of filled and sterile grain) were recorded after harvest. Effective tillers ‘‘fully exerted panicles’’, aboveground biomass (leaf, sheath, and shoot), and root samples were collected, counted and weighed after harvest. Roots were well washed in order to remove attached soil and debris. All samples were oven dried at 70 °C for 72 hours. Grains were threshed by hand and the yield in 14 % moisture content was calculated using the following equation (1):

$$Gy_{14} = G \times \left[\frac{(100-m)}{86}\right] \quad (1)$$

Where "Gy_{14}" is the grain yield (g) per plant adjusted to 14 % moisture content, "G" is the weight filled grain (g) per plant at harvest and "m" is grain moisture content (g) (Dobermann and Fairhurtst, 2000).

2.2.4 Soil analysis

Soil samples were collected from 5, 10, and 15 cm depth in the bucket after harvest. Soil samples were air dried, grounded, and sieved (Ø = 2 mm) for all the subsequent analyses, which were conducted following the Japan Soil Environment Analysis Methods Committee (2003). Soil pH was measured using the electrode method (D-24 HORIBA, Japan) at a soil–water ratio of 1:2.5. Eh was measured using the electrode method (PNR-41, DKK-TOA Co., Japan). Available micronutrients (Avail. Fe, Zn and Mn) were extracted using the diethylene-triaminepentaacetic acid (DTPA-TEA) extracting solution (Lindsay and Norvell 1978) and determined by Inductively Coupled Plasma Spectroscopy (ICPE-9000; Shimadzu, Japan).

2.2.5 Statistical analysis

Data was statistically analyzed by one–way analysis of variance (ANOVA) ($p<0.05$) to determine the influence of water management on rice yield components and soil properties. Significant differences among the treatments were determined by Tukey´s honestly significant difference (HSD) test ($p<0.05$) for multiple means comparison. All statistical analyses were performed using IBM SPSS Statistic v20.0 (IBM SPSS 2011, Chicago IL, USA).

2.3 RESULTS

2.3.1 Phenology and plant growth.

Plant growth parameters under different water treatments during growing season (25, 50, 75 DAT) are summarized in Table 2.2. Some significant differences between anaerobic and aerobic conditions were observed for both soils. In spite there was no significant difference ($P<0.05$) in plant height and the number of phyllochroms in soil conditions at 75 DAT, the mean value of plant height in GS was 6% taller than that of RS. Likewise flooded treatments recorded higher amount of tillers over field capacity treatment in each soil. At harvest, (Table 2.3) GS-T2 and T3 which were drained at post heading stage had the higher total number of tillers; 23 and 21 tillers respectively among all treatments. Unlike other treatments, GS-T5 showed more stunted plants and lower total number of tillers compared to the control.

Table 2.2 Morphological behavior of respective water conditions in two soil types at 25, 50, 75 DAT and harvest stage.

Treatments	Grey Soil			Red Soil		
	Height (cm)	Phyllochrom	TN Tiller	Height (cm)	Phyllochrom	TN Tiller
			25 DAT			
Anaerobi	46.6 ±2.9	5.9 ±0.2	3.0 ±1.0	43.9 ±2.0	5.8 ±0.3	2.3 ±1.3
Aerobic	42.3 ±1.7	5.5 ±0.1	2.8 ±0.5	39.9 ±2.0	6.0 ±0.2	2.5 ±0.6
Mean	44.4 ±3.2	5.7 ±0.3	2.9 ±0.9	41.9 ±2.6	6.0 ±0.3	2.4 ±1.2
			50 DAT.			
Anaerobi	86.2 ±2.4	10.1 ±0.1	12.9 ±2.0	78.9 ±2.6	10.1 ±1.9	6.4 ±1.9
Aerobic	98.0 ±4.7	9.0 ±0.4	9.8 ±2.1	83.5 ±3.0	9.0 ±2.1	5.3 ±2.1
Mean	92.1 ±5.6	9.5 ±0.5	11.3 ±2.3	81.2 ±3.2	9.5 ±2.0	5.8 ±2.0
			75 DAT.			
Anaero	104.2 ±2.8	13.0 ±0.0	13.4 ±1.9	101. ±5.2	12.9 ±0.6	7.5 ±1.9
Aerobic	109.3 ±1.5	11.3 ±0.2	11.0 ±0.8	99.8 ±4.7	11.2 ±0.2	6.3 ±2.1
Mean	106.7 ±3.3	12.2 ±0.7	12.2 ±2.0	100. ±5.0	12.0 ±0.9	7.0 ±1.9

Values represent means ± Standard Deviation.

Meanwhile in RS the measured parameters did not show any significant difference ($P<0.05$) among its flooded treatments T1-T4 and T5. On RS, stunting plants were observed during the early tillering and tiller emission seemed to stagnate as the tillering phase progressed forward.

Table 2.3 Morphological parameters at harvest.

		Grey Soil				Red Soil			
		Height (cm)	Phyllochroms	TN Tiller	Vegetative Cycle (Days)	Height (cm)	Phyllochroms	TN Tiller	Vegetative Cycle (Days)
	T1	110 ±4.9	13.0 ±0.0	16.0 ±1.8	139	103.3 ±0.8	13.0 ±0.	10.0 ±0.9	139
Treatments	T2	109 ±1.9	13.0 ±0.0	18.8 ±1.7	139	107.3 ±4.0	13.0 ±0.	9.7 ±1.5	142
	T3	108 ±1.3	13.0 ±0.0	21.3 ±2.1	139	103.0 ±6.2	13.2 ±0.	9.75 ±1.5	139
	T4	115 ±3.4	13.0 ±0.0	23.3 ±3.1	139	99.7 ±5.5	13.0 ±0.	9.3 ±2.3	142
	T5	109 ±1.8	13.2 ±0.5	15.3 ±2.1	152	99.8 ±4.7	13.0 ±0.	10.3 ±1.0	152
	Mean	110 ±3.6	13.0 ±0.2	18.9 ±3.7	142	102.6 ±4.6	13.0 ±0.	9.9 ±1.3	143

Values represent means ± Standard deviation N=4.

The enlargement of the vegetative cycle and a delay of phenological phases by 13 days were observed at T5 in both soils. However, the number of phyllochroms was similar regardless of water treatment and soil type. Based on plant observation, the 12th phyllochroms in the culm of *Koshihikari* variety coincided with the panicle initiation stage, which was used as reference point in our experiment. RS treatments appeared yellowish in meristematic tissues and had some brown spots in lower leaves indicating a nutritional disorder (Fig 2.2).

2.3.2 Rice plant traits.

The number of effective tillers was associated with the number of tillers holding panicles. In our results, both water management (aerobic, anaerobic) and soil type, exerted influence on the number of effective tillers (Table 2.4). Although results showed that GS-T4 had the largest number of effective tillers (14) that were 10% and 29% larger than GS-T1 and GS-T5, respectively, no significant difference was found among T1 and treatments drained at post-heading stage. Likewise, RS-T1 number of effective tillers was 9% larger than T5, but without significant differences. RS showed lower effective tillers rate, which was significantly reduced between 30-48% compared with the mean observed in GS (Fig 2.2).

Figure 2.2 Significance difference were observed in plants that grew in Grey soil (left) and plant grew in Red soil (right). Reduction in dry-mass is attributable to soil nutritional disorders.

The percentage of filled grains per panicle did not show significance differences between the control and drained treatments. However the effect of water stress was evident on the percentage of filled grain at T5 in both soils.GS and RS were 18% and 11% respectively, lower than the T1. Adoption of WS period reduced grain sterility as in 15% and 14% for GS and RS respectively and significantly increased in agreement with the percentage of filled grain. T5 in both soils had higher sterility than the other treatments. The combination of high temperature >35°C registered in the greenhouse and evaporation rates, caused leaf rolling and early senescence in lower leaves. During reproductive stage, the water stress in T5 might have promoted the grain sterility. Apart from water treatment, other yield parameters such as 1000 grain weight, panicle length and total grain per panicle, did not show any significant differences ($P<0.05$) on each soil. Furthermore we noticed a slight increase in panicle length by 5% and the number of spikelets per panicle by 10% in GS, over RS. but it was not significantly different.

A shift from continuous flooding (T1) to aerobic conditions in T2, T3and T4 resulted into no significant difference ($P<0.05$) in grain yield on each soil (Table 2.4). However, GS-T2 and GS-T3 showed slightly higher yield increase by 8% and 5%, respectively above GS-T1 and doubled in T5. With regard to RS, T1 had a significantly higher yield than drained treatments (T4-2). When compared with RS-T1, the yield average of RS-drained treatments and RS-T5 it was found that they were lower by14% and 34% respectively. It should be noted that the reduction of yield of over 45% in flooded RS compared with GS, may be attributed to nutritional disorders triggered by water management and soil properties. For treatments under permanent aerobic conditions, other factors such as the high sterility, the low number of effective tillers and low tolerance of Koshihikari to continuous water stress were the most determining factors to the low yield.

A similar trend was observed in above ground biomass (leaf and stems) production except in the RS-T5 which showed a 30% significant increase in biomass above the RS-T1. The differences observed between total number of tillers (Table 2.3) and the number of effective tillers (Table 2.4) was attributed to a considerable number of unproductive tillers that appeared after panicle initiation when 50% of total dose of N was applied and WS period was established. The emergence of new shoots was more evident in T2, T3 and T4 of GS, while RS barely produced new shoots. On root biomass production, no significant differences were observed across all the treatments regardless of water treatment in both soils. However the adoption of WS period at post heading stage in GS, the root biomass increased by more than 10% and 40% in T1 and T5 respectively. In terms of root growth, all treatments, except T5, in both soils developed a red Fe layer (plaque) coating root surface as a consequence of flooding condition (Fig 2.3). In T2, T3 and T4, growth of white root tips arose mainly in GS that developed after soil was shifted to aerobic condition.

Figure 2.3 Visual effects of irrigation system on roots. A) roots in upland system B) roots coated by a layer of ferrous iron as consequence of permanent anaerobic conditions.

Table 2.4 Effects of water management in each soil type on rice plant components.

	Treatments	N	Effective Tillers	Panicle Length (cm)	Number of Spikelets Panicle^{-1}	1000 Grain Weight (g)	Filled Grain (%)	Sterility (%)	Grain Yield 14% Moist. (g)	Shoots Dry mass (g)	Root Dry mass (g)
Grey Soil	T1	4	12.3 ±1.3^{A}	20.3 ±2.2^{A}	136.0 ±15.2^{A}	23.4 ±1.0^{A}	87.1 ±1.6^{A}	12.90 ±1.6^{A}	39.2 ±2.1^{A}	55.4 ±4.8^{AB}	19.9 ±2.0^{A}
	T2	4	13.0 ±3.3^{A}	21.3 ±2.2^{A}	135.9 ±16.7^{A}	23.2 ±0.6^{A}	86.2 ±1.1^{A}	13.80 ±1.1^{A}	40.1 ±5.3^{A}	59.7 ±6.5^{AB}	22.0 ±8.1^{A}
	T3	4	12.8 ±1.0^{A}	19.5 ±1.3^{A}	137.8 ±8.8^{A}	23.0 ±1.1^{A}	87.6 ±2.0^{A}	12.40 ±2.0^{A}	41.0 ±2.6^{A}	63.9 ±9.3^{A}	20.4 ±1.7^{A}
	T4	4	13.5 ±1.3^{A}	22.3 ±1.0^{A}	137.7 ±9.3^{A}	22.8 ±0.4^{A}	88.7 ±3.2^{A}	11.26 ±3.2^{B}	42.2 ±0.9^{A}	62.3 ±13.3^{AB}	23.1 ±1.9^{A}
	T5	4	10.5 ±3.7^{B}	18.0 ±2.6^{A}	105.7 ±24.5^{B}	22.5 ±0.7^{A}	71.3 ±13.9^{B}	28.69 ±13.9^{A}	19.6 ±5.5^{B}	41.0 ±12.6^{B}	15.6 ±5.9^{A}
Mean		20	12.4 ±2.4	20.3 ±2.3	130.6 ±19.1	23.0 ±0.8	84.2 ±8.8	15.81 ±8.8	36.4 ±9.3	56.4 ±12.3	20.1 ±5.4
Red Soil	T1	4	8.5 ±1.3^{a}	19.0 ±2.9^{a}	123.6 ±9.2^{ab}	22.5 ±0.4^{a}	85.4 ±1.8^{ab}	14.61 ±1.8^{ab}	23.4 ±3.9^{a}	23.4 ±3.4^{a}	12.4 ±1.9^{a}
	T2	4	7.3 ±1.5^{a}	18.0 ±2.5^{a}	130.6 ±17.6^{a}	22.9 ±0.9^{a}	86.9 ±4.0^{ab}	13.14 ±3.9^{ab}	21.4 ±1.4^{ab}	21.4 ±1.9^{a}	14.0 ±2.7^{a}
	T3	4	6.5 ±0.6^{a}	20.5 ±1.7^{a}	129.5 ±15.0^{a}	22.4 ±0.8^{a}	91.8 ±2.7^{a}	8.25 ±2.7^{b}	20.1 ±3.2^{ab}	21.7 ±1.8^{a}	12.8 ±5.1^{a}
	T4	4	7.0 ±1.6^{a}	19.5 ±1.5^{a}	119.8 ±14.5^{ab}	23.1 ±0.3^{a}	87.2 ±4.7^{ab}	12.78 ±4.7^{ab}	19.0 ±1.9^{ab}	23.5 ±6.8^{a}	11.6 ±4.1^{a}
	T5	4	7.8 ±2.4^{a}	19.8 ±2.2^{a}	94.6 ±19.9^{b}	22.5 ±0.5^{a}	76.1 ±13.2^{b}	23.89 ±13.2^{a}	15.5 ±3.7^{b}	30.5 ±8.8^{a}	12.7 ±2.1^{a}
Mean		20	7.4 ±1.6	19.4 ±2.4	119.7 ±19.4	22.6 ±0.6	85.5 ±7.9	14.53 ±7.9	19.9 ±3.8	24.1 ±5.9	12.7 ±3.1

Values represent means ± standard deviation. Different capital letters in same column denote significance differences among treatments in Grey soil, while, different lower case letters in same column denote significance differences among treatments in Red soil at $P<0.05$.

2.3.3 *Influence of water management on soil properties*

Water management affected soil electrochemical condition in both soils. Soil Eh decreased up to-280 mV and -245mV for GS and RS, respectively, few days after soil flooding. The pH in all flooded treatments, tended to attain neutrality, increasing from its original pH 4.85 to 5.48-5.56 units for GS and from 5.31 to 6.16 - 6.39 units in RS (Fig. 2.3a). Regardless of WS period established at different time during post heading stage, no significant differences were noticed in pH for GS. However, RS pH decreased significantly as the WS period was established, drained treatments reduced their pH in 0.15 pH unit compared with control , while T5 was 0.23 units lower.

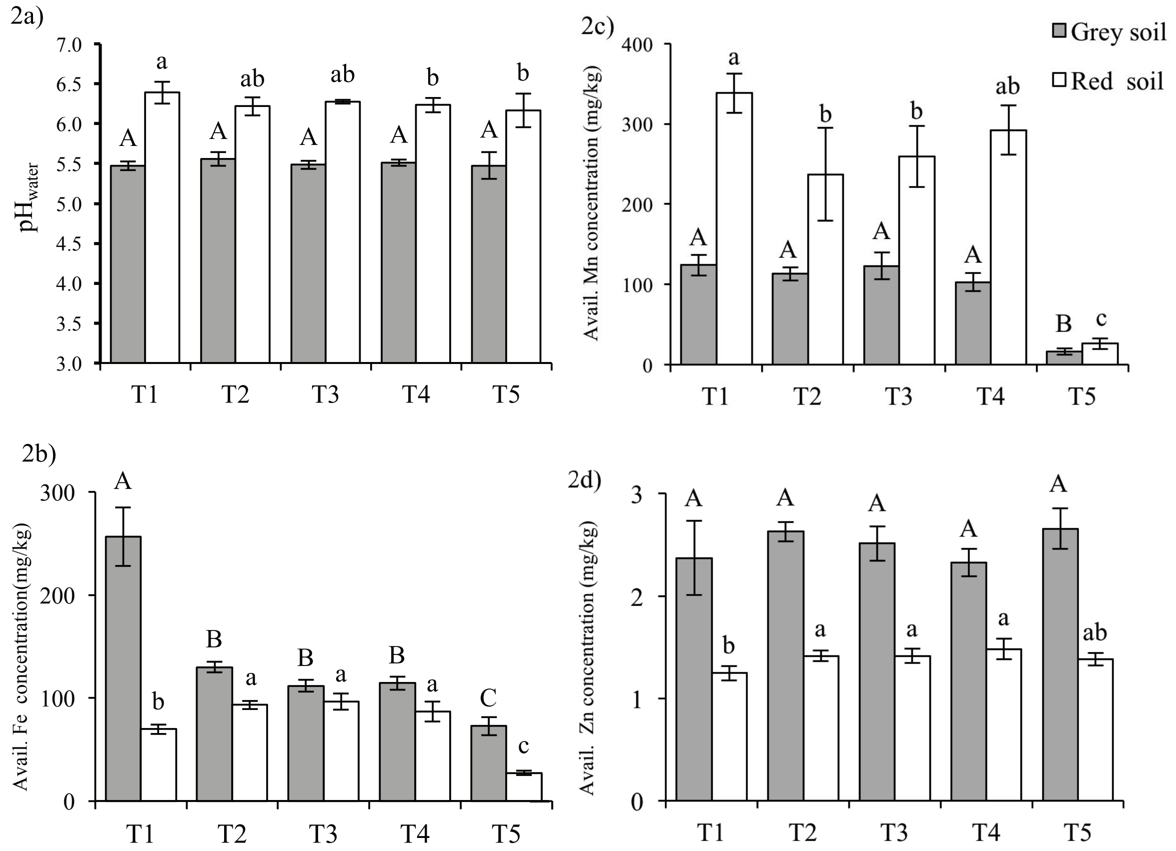

Figure 2.3 Influence of water management and soil type on soil pH and micronutrient contents. Bars in the graphs are the means and error bars indicating standard deviation (N=12). Capital letters indicate significant differences among treatments in GS, while lower-case letters indicate significant differences among treatments in RS at P<0.05.

Micronutrient availability was also affected by water management. GS exhibited higher available Fe which decreased as its flooding period became shorter from T1 to T2–T4 and to T5. Although in GS-T1 the available Fe was highest among all treatments, no toxicity symptom was observed. The effect of flooding on high Fe availability (Fig. 2.3b) was reverted when treatments T2, T3 and T4 underwent re-oxidation, decreasing its Fe availability by around 54% in GS. Meanwhile, in RS, available Fe was inconsistent between T1 and post-heading drained treatments (T2-4) compared with the results in GS. RS-T5 showed available Fe less than half of RS-T1. Redox reactions and increase of pH, significantly increased the availability of Mn in T1, T2, T3 and T4 over T5 in each soil. Available Mn in T1 was eight fold and thirteen fold higher than that in T5 (16.23 and 26.23 mg kg^{-1}) in GS and RS, respectively.

The high available Mn in RS (Fig 2.3c) could be responsible for some nutritional disorders observed under flooding conditions, i.e. spots in lower leaves and yellowish meristematic tissues. Available Zn increased by WS period and permanent aerobic condition being higher in GS than RS (Fig. 2.3d). Unlike GS in which large differences were not found among treatments, the RS treatments subjected to WS period and permanent aerobic condition increased its Zn availability compared with control.

2.4. DISCUSSION.

2.4.1 Morphological behavior and grain yield

Establishment of WS period at post heading stage does not affect negatively any of the morphological parameters or yield component. The plant growth and biomass production considerably increased at the first growing stages in both soil condition because of the high efficiency of N fertilizer and a notable increase in physiological activity (Hasanuzzaman 2010). With the enhancement in leaf area index and shoot biomass generally increase CO_2 fixation by photosynthesis and consequently biomass production contributing to the accumulation of carbohydrates (Seneweera, 2011) and other nutrients, that are remobilized during the grain filling stage (Fageira, 2013). In general, under permanent aerobic conditions (T5), almost all morphological components were reduced in each soil. It might be because rice in response to water scarcity, plant could suppress leaf emission, tillering and regulate photosynthesis (Alfonzo 2008; Xu et al., 2010). Thus these facts could also explain the delay in phenological phases of more than 10 days at T5.

In addition, the combination of RS characteristics and flooded condition during early and active tillering increased the available Mn until toxic levels, depressing tiller production (Table 2.2) more than the effect of establishment of WS period, as was noticed in RS-T1, T2, T3 and T4. Similar constraint also was reported by Noda (2007) in Shimane prefecture. Therefore, the change in redox reactions after flooding, was the main factor promoting the increase of Mn availability (Saharawat, 2012; Brady and Weil, 2014). The excessive contents of Mn, caused toxicities in rice plants, leading to disorders in photosynthetic processes (Millaleo et al., 2010) stunted plant and the reduction in tiller emergence rates (Dobermann and Fairhurtst, 2000).

The Koshihikari variety was developed as an improved lowland variety. In Japan and other countries it is highly appreciated for its good yield potential and taste. Previous study conducted by Matsuo and Mochizuki (2009) suggested that Koshihikari does not have the ability to grow well in upland systems. In spite of this limitation, our treatments, T2, T3, T4 which were turned into aerobic condition from flooded one, did not show significant differences in morphology and yield parameters either among them or compared with continuous flooding of each soil. These treatments in GS even slightly increased shoot and root dry mass and grain yield compared with Control.

The increase of the total number of tillers experienced on drained treatments in GS was promoted by the establishment WS period and the subsequent increment of soil temperature. Under such conditions, organic matter decomposes easily, increasing the availability of nutrients (Brady and Weil 2014). Although the emission of new tiller at late growth stages sometimes has not been considered by farmers, our result suggested that early culture of *Koshihikari* accompanied with a proper WS period at the post-heading stage could produce enough tillers for ratoon cropping. Therefore WS will ensure a reserve of water for the double cropping that not only will generate a surplus in grain yield but could also reduce time labor, soil degradation and inputs. Besides that, establishment of WS period favors rhyzosphere enhancement (Table 4), which agreed with the previous reports (Alfonzo 2008; Mitchell et al. 2012). Root elongation, contribute to increasing root-soil surface contact and likely physiological rhyzosphere activity. Hence it will improve the uptake of N, P and K and other essential nutrients, compared with the continuous flooding condition (Yang et al 2004).

The adoption of WS period at post-heading stage keeping the soil close to its field capacity threshold did not reduce grain yield in either soil compared with their respective continuous flooded treatments. The water requirement changes through the distinct rice growing stages, being the later stages (milk, dough and full ripening) less water demanding (De Datta 1981). Additionally, leaf senescence at later stages is invariably bounded with the reduction of the chlorophyll content and the photosynthetic processes, (Lu et al.2002) this decline presumably will reduce the water demand toward full ripening stage

The dramatic decrease of yield observed in drained RS treatments (Table 2.4) compared with the GS were not due to the effect of aerobic conditions during the post heading stage, but rather from the soil chemical properties aforementioned. Likewise the permanent aerobic condition (T5) lead to a significant reduction in grain yield in 50% and 44% lower than their continuous flooding treatments at GS and RS respectively. Experiments with high yielding lowland varieties such as IR20 settled in aerobic rice conditions under furrow irrigation (De Datta 1973) and sprinklers (McCauley 1990) achieved a considerable water input reduction lesser than continuous flooding but grain yield was shrink in 67% and 20-30% respectively. Previously, and regardless of soil type Wopereis et al.(1996) reported that the response of rice plant to water stress arise when soil water potential drops below -100 kPa. Under such condition of water stress, leaf expansion and tillering decline substantially affecting the grain yield performance.

In our experiment, the reduction in grain yield was closely associated with the reduction of total biomass, caused either by the low tolerance of Koshihikari to permanent aerobic condition in both soils or the Mn toxicities in RS. The correlation matrix at Table 2.5 showed a strong positive correlation between grain yield and the most important morphological components such as the above ground (Leaf & stems) biomass ($r=0.85$), root biomass ($r=0.65$) and number of panicles ($r=0.82$).at $P<0.01$. These morphological components also showed strong correlation among each other. As was aforementioned, with the establishment of WS period at post-heading stage all these components were improved or kept comparable with permanent flooding condition. Similar correlation between yield and dry biomass have been reported in three different cultivars (*Mineasahi, Hinohikari* and *Akebono*) by Shiratsuchi et al (2007)

The percentage of sterile grain was the only parameter that showed a negative correlation $r=-0.35$ at $P<0.05$. However the effect of sterility on grain yield was mainly ascribed to the high Mn availability (toxic levels) in flooded treatments (Dobermann and Fairhurtst, 2000).or drought stress under permanent aerobic conditions. Regardless of soil type the adoption of WS period at post-heading stage reduced the sterility (Table 2.4) in almost all the treatments.

Table 2.5 Correlation coefficient for the relationship among grain yield and the most important morphological components.

Morphological components and grain yield	Root Biomass	Leaf stems Biomass	Total Biomass	Total Panicles	Fill grain (%)	Sterility (%)
Leaf & Stems Biomass	0.72^{**}					
Total Biomass	0.80^{**}	0.98^{**}				
Total Panicles plant^{-1}	0.62^{**}	0.86^{**}	0.87^{**}			
Fill Grains (%)	0.08	-0.04	0.10	-0.06		
Sterility (%)	-0.08	0.04	-0.10	0.06	-1.00^{**}	
Grain yield	0.66^{**}	0.85^{**}	0.93^{**}	0.82^{**}	0.35^{*}	-0.35^{*}

** and * represent significant correlation at $P<0.01$ and $P<0.05$ respectively.

2.4.2 Changes in soil properties conditioned by water management

After a few days of flooding, soil became reduced condition, -280 mV and -245mV for GS and RS, respectively. This altered Fe and Mn forms from their oxidation states to reduced states, i.e. ferrous and to manganous ions, which are more soluble and preferentially available form for rice. The chemical reduction of Fe and Mn affect directly pH because both consume H^+ ions, increasing the pH in the soil solution (Brady and Weil 2014). Although Fe takes an active part in photosynthetic and N fixation processes (Marschner 2012), in irrigated systems Fe toxicity often arises, lead by the effects of Eh reduction. Then, when soil was turned to WS regime at post-heading stage all the processes became reversibly aerobic. This increases the Eh reduced the Fe and Mn availability in the soil solution. In our results, available Fe sharply decreased in T2, T3 and T4 compared with T1 (Fig.2.4b) while the Mn availability in GS-T2, T3 and T4 was kept comparable to T1 until the harvest stage (Fig.2.4c). Under paddy field conditions, Fe oxidation occurs at lower Eh (+200~ -200 mV) than Mn (+400~-100 mV) (Kyuma 1997).Therefore, Fe availability decreased faster in time than Mn as the Eh increased after the establishment of WS period. This implies that Mn reducing state could prevail for longer period even after harvesting. Result showed that WS at post-heading stage not only reduced the water consumption, but also could control the Fe availability if toxicity symptoms appear.

Unlike GS that showed mean Mn concentration of 730 mgkg^{-1}in flag leaf, the RS-T1, T2, T3 and T4 treatments increased the flag leaf Mn concentrations higher than 2300 mg kg^{-1} (data not shown) which exceeded toxicity levels >800-2500 mg kg^{-1}, (Dobermann and Fairhurtst 2000). This high Mn level in the leaf possibly suppressed rice growth, which might counteract positive effect of WS period applied in RS-T2, T3 and T4. It is recommended to combine with incorporation of straw to replenish Si and K removed from the field in order to alleviate Mn toxicity or other costly measures such as fertilizer management (IRRI, 2014). Alternatively to reduce Mn toxicity, "mid-summer drainage: *Nakaboshi* (in Japanese)" is also a possible option, which is soil water condition drier than the field capacity and commonly practiced in Japan by rice growers in order regulate the nutrient supply at later stages and mitigate the negative effects of anaerobic condition of paddy field (De Datta 1981).The adoption of WS at post-heading stage as we observed in the present study can be a feasible option without compromising yield decline by Mn toxicity even in such soil types.

Regarding to the available Zn in both soils, it did not significantly differ among the treatments. Although a slight increase was observed in RS T2, T3, T4 and T5 (fig. 2.4d) it was not as we expected. Zn availability in soil increases as pH decrease by reduction of Eh under aerobic condition (Brady and Weil 2014). The water contents in soil close to the soil field capacity might not inhibit Zn availability. Presumably other factor such as soil organic matter contents, soil parent material and rhyzosphere activity had more strong influence on it and its uptake by plant. Zn availability in GS ranged from 2.30 to 2.60 mg kg^{-1}. The soil availability was adequate for rice production in agreement with the recommendation of 1.5 $mgkg^{-1}$by Dobermann and Fairhurst (2000). Whereas RS showed Zn availability below the level above mentioned, which implies a clear Zn deficiency. Although Zn availability increased significantly when WS period was established as well as in permanent aerobic conditions, it seems that under surplus Zn availability water management did not exert significant influence on its availability as we observed in GS The high concentration of available P that was observed in RS before transplanting (Table 2.1) seem to be the main cause for the reduction of Zn availability. Decline of Zn in almost all the paddy field, arise under flooding conditions when the pH reach the neutrality Zn precipitate as ZnOH or bound with P resulting in insoluble Zinc-Phosphates (IRRI 2014). The deficiency of Zn besides the Mn toxicities clearly contrasted with the poor morphological behavior and stunted plant in RS in the present study.

2.5. CONCLUSIONS

In many regions, lowland rice production has been practiced based on the assumption that continuous flooding condition is related to high yields, however, nowadays this practice is becoming unsustainable because water shortage and increasing water demand. In this study we evaluated the effect of the adoption of WS period at post heading stage for two different soils on plant morphology and grain yield. Results demonstrated that, regardless of soil type, reduction of water input by the establishment of WS period at post-heading stage did not have a negative effect on morphological behavior and grain yield. The use of WS rather allows to increase root and aboveground biomass, which had a significant impact on rough grain yield and reduction of the percentage grain sterility. Concerning soil properties the use flooding regime throughout growing season or WS period after heading stage affected Eh and consequently the soil pH and micronutrients availability. The potential toxicity of Mn under flooding condition was the main factor reducing suitability of red soil for rice production. However by establishment of aerobic condition this constraint can be controlled or even reduced to its optimal levels. Logically, implementation of WS period at post heading stage could save a considerable amount of irrigation water, which in turn would improve water use efficiency.

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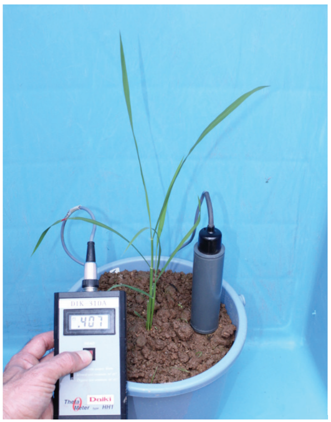
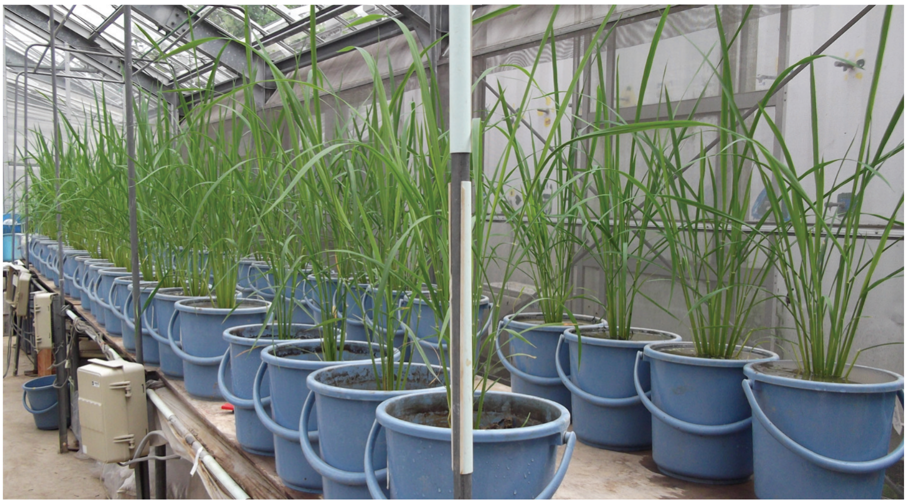


Moisture Field Cap. Control

Rice Panicle Initiation



Second Pot Experiment

Soil Analysis

Plant at Harvest Season

CHAPTER III

Control of Micronutrients Availability in Soil and Concentration in Rice Grain through Field Water Management.

3.1. INTRODUCTION

Micronutrient deficiencies in soil have been identified as one of main factors affecting crop yield, food quality and human health (Yang et al., 2007; Alloway, 2008). In recent years, emphasis on agriculture, environment, demographic growth and micronutrient malnutrition has increasingly taken center stage in policy discussions on food security in developing countries. The inhabitants of these countries are prone to suffer from undernourishment, caused by inadequate food intake or intake of foods of poor nutritional quality (Bain et al., 2013). New approaches have been applied in achieving sustainable improvements in the micronutrient status of vulnerable populations. One of them is the "genetic biofortification" which has been accepted as a cost-effective and sustainable strategy in order to ameliorate the malnutrition (Cakmak, 2008; Meenakshi et al., 2007). The development of new improved crops varieties, through advanced breeding techniques as well as the screening of germplasm, has allowed for identification of varieties with high potential of accumulating micronutrient in edible parts. Some of the major successes have been observed in crops such as wheat, maize, beans, sweet potato, cassava and rice.

Rice (*Oryza sativa* L.) is the main staple crop for more than half of the world population (FAO, 2013). In spite of its high consumption, the low content of micronutrient in rice required to meet whole dietary elements necessary to keep a balanced diet in humans, calls for concern. Rice as a major cereal has become a priority for current research works. New findings suggest that micronutrients content in rice grain depends not only on genotypic variables, but also on environmental factors, such as the endogenous soil mineral contents, soil properties (Cakmak, 2008; Sperotto et al., 2013) water management (Dobermann & Fairhurst, 2000; Liao et al., 2013) and climate conditions (Najafi-Ghiri et al., 2013).

“Agronomic biofortification” through the application of fertilizer (inorganic or organic), modification of cultivation systems, soil management and new irrigation strategies have proved effective in enriching micronutrients content in rice grain by controlling the availability of soil micronutrients for plant (White & Broadley, 2009). Unlike genetic biofortification, the agronomic biofortification has shown to be a short-term approach, more accessible for developing countries which are facing the burden of malnutrition and where the advanced technologies (e.g. plant breeding and genetic engineering) still remain unreachable (Cakmak, 2008).

Since soil micronutrient availability is highly associated with the contents in plant and grain quality; its availability has become the key limitation factor to productivity, stability and sustainability of rice production in many countries such as Australia, China, India and United States (Bell & Dell, 2008). Studies conducted by Yang et al., (2007) in China, reported that such deficiencies in soil overlapped areas where population exhibits health micronutrients imbalance, affecting more than 40% of the population. Usually those deficiencies are afforded by the applications of chemical fertilizer (edaphic and foliar) or organic amendment. The application of Zn fertilizer has had a positive response in wheat (*Triticum aestivum* L.), (Cakmak, 2008) , sorghum (*Sorghum bicolor* L.) and rice (Gao et al., 2012). Iron is another important micronutrient for plant and human health. Major Fe deficiencies have been reported mainly in sandy and calcareous soils (Brady & Weil, 2014). However, under such condition application of inorganic Fe fertilizer often results in ineffective practice due the conversion to ferric form (Rengel et al., 1999). Besides that, the micronutrients content in grain could be controlled by genetic factor meaning that the application of fertilizer even in excess could not increase the content in grain and would rather trigger toxicity symptoms. Likewise micronutrient demand for physiological processes and plant uptake rates could also vary in function of different growing stages and environmental condition. (Maschner, 2012)

Rice as well as other crops needs essential micronutrients in very small amount compared to macronutrient. Almost all the soil contains enough micronutrients to support the plants growth demands, however, micronutrients availability often is governed by some soil properties (White & Broadley, 2009). For example, changes in soil redox potential and pH are the most important properties that could affect micronutrient availability in paddy (Tao et al., 2007; Brady & Weil, 2014). Likewise, soil organic matter and its decomposition processes have a significant and direct impact on the availability of micronutrients (Marschner & Rengel, 2007). Other factors such as synergistic and antagonist interactions among micronutrients and essential elements could also often affect micronutrient uptake by crops (Fageira, 2002).

In order to increase the contents of micronutrients in grain, understanding the dynamics of micronutrient at different growing stages is also very important. Some studies have pointed out that the period between panicle initiation to maturity is considered as high demand for grain micronutrient accumulation. For instance, concerning Zn uptake, Jiang et al., (2007) reported that 36% of total Zn in rice grain was taken up directly from soil between anthesis and maturity stages, similar patterns has been described in wheat during grain filling stage (Pearson & Rengel, 1994). For Fe, as Inoue et al*.,* (2009) observed, Fe-trasnporter gene OsYSL15 was highly expressed during the anthesis and seed formation stage, these stages probably are the active Fe uptake period of rice. Copper (Cu) on the other hand is essential in flower fecundation (Dobermann & Fairhurst, 2000). Therefore, its demand should significantly increase at anthesis.

With regards to above factors influencing the micronutrient availability in soil, plant uptake and the content in rice grain, little information is available about the effect of water management over micronutrient concentration in grain during grain filling stage. Since changes in soil water condition are strongly associated with micronutrient availability and plant uptake; shifting soil water condition could be a suitable approach to control soil micronutrient availability and to improve the nutritional value of rice in developing countries where farmers do not have easy accesses to fertilizers or other costly approaches. Therefore, the aim of this present study was to assess the effect of water management on the micronutrient contents in rice grain and availability of soil at grain filling stage.

3.2 MATERIALS AND METHODS

3.2.1 Experiment site and soil properties

The effects of water management on soil properties and micronutrients content in rice grain, were evaluated through a pot experiment conducted in a greenhouse at Shimane University (35°29′N 133°04′E), from 2011-2012 during rice growing season in Japan (May15th/Oct15th). Plastic bucket (Ø=24cm x 25cm height) were filled with 7.5 kg of air dry "Gray Lowland" soil Typic Fluvaquent (USDA, 2010). The general soil characteristics are presented in Table 3.1. The semi-dwarf (cv. Koshihikari) rice variety was used in this study.

3.2.2 Experimental design

The pot experiment was laid out in a randomized block design of five water irrigation treatments (Fig. 3.1) with four replications. Control was flooded from transplanting to harvest. The heading stage (50% of the panicle exertion) was the benchmark for the next customized irrigation.

Table 3.1 Physical-chemical properties of Typic fluvaquent soil “Gray lowland” collected at paddy field in Shimane prefecture, Japan

Soil characteristics.	Value
Sand (%)	22.8
Silt (%)	37.8
Clay (%)	39.4
pH	4.85
EC (mSm^{-1})	3.87
Bulk Density (g cm^{-3})	1.39
Total C (g kg^{-1})	17.6
Total N (g kg^{-1})	1.6
NH_4-N (mg kg^{-1})	4.91
NO_3 –N (mg kg^{-1})	8.22
Available P (mg kg^{-1})	34.9

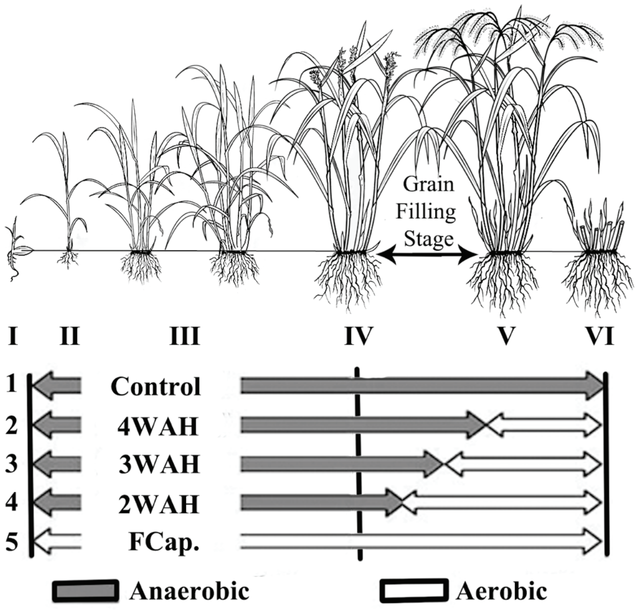


Figure 3.1 Schematic representation of the experimental design at with different irrigation treatments were applied during the growth season. (I) Sowing, (II) Transplanting, (III) Tillering, (**IV**) Heading, (**V**) Maturity Stage, (VI) Harvest. Period between (↔) IV and V was considered as grain filling stage.

Treatments where flooded-irrigation was stopped during the post-heading at four week after heading "4WAH," three week after heading "3WAH," and two weeks after heading "2WAH," and kept in a soil 1evel approximately 35±3 % (v/v) moisture, equivalent to ±80% of its field capacity level. The last treatment was kept at the same moisture condition, 80% of field capacity conditions "FCap" throughout growth period in order to mimic the conditions of upland systems. Treatments under flooding condition were ±5cm water depth. Distilled water was used for irrigation to avoid micronutrient supply through it. Chemical fertilizer (N:P:K) at doses of 45:65:60 kg ha^{-1} respectively was applied based on crop demand as follows: N was split in four application, 1st-20% basal, 2nd-30% at tillering stage, 3rd-40% at panicle initiation and 4th-10% 15 days after panicle initiation (PI). P was applied 100% of dose basal. K was split in two application 50% basal and 50% at panicle initiation.

3.2.3 Plant and rice grain chemical analysis

Plant samples were collected at harvest. Flag leaves were first washed with tap water and twice with distilled water. Thereafter they were oven dried at 40°C for 48 hours and dehusked in rotating rubber roll, (Satake THU35B, Japan). For micronutrient analysis, flag leaves and grain samples were ground into fine powder in agate grinding jars, using a mixer mill (MM200, Retsch GmbH, Haan, Germany). Grain and plant samples were oven-dried 12 hours at 80 °C. Subsequently 0.5 g of sample was digested in 2.5 ml HNO_3 within Teflon vessel. All samples were oven heated at 160°C for 4 hours, kept resting overnight and diluted with distilled water up to 25 ml (Koyama & Sutoh, 1987). The concentrations of Fe, Zn, Cu and Mn were determined by Inductive Coupled Plasma Spectroscopy (ICPE-9000, Shimadzu, Japan).

3.2.4 Soil sampling and analysis

Soil samples were collected from 5, 10 and 15 cm depth in the bucket after harvest. They were immediately stored in ZIPLOC hermetic plastic bag and refrigerated in order to maintain original chemical soil properties. Portions of each sample was oven dried at 105 °C to measure the moisture content to calculate the necessary amount for all the subsequent analyses following standard analyses methods (Japan Soil Environment Analysis Methods Committee, 2003). The concentration of micronutrients (Fe, Zn, Cu and Mn was analyzed by mixing 10 g of soil with 20 ml of diethylene triamine pentaacetic acid (DTPA-TEA) extracting solution (Linsay & Norvel, 1978). The solution obtained was filtered and analyzed in ICPE-9000. Soil pH was determined by the soil-water ratio of 1:2.5 and electric conductivity (EC) soil-water ratio of 1:5 using electrode method (D-24 HORIBA, Japan). Redox potential was measured in situ from transplanting period to 25 DAT at 10cm depth using electrode method (PNR-41, DKK-TOA Co., Japan).

3.2.5 Statistical analysis

To determine the influence of water management on soil properties, micronutrient concentration in soil, plant and rice grain accumulation, data were statistically analyzed by one–way analysis of variance (ANOVA). Significant differences among the treatments were determined by Turkey's honestly significant difference (HSD) test ($P<0.05$) for multiple means comparison. Correlation analysis between grain micronutrient concentration and soil properties was done using Pearson Product Moment Correlation (PPMC). All statistical analysis was performed using IBM SPSS Statistic v20.0 (IBM SPSS, 2011. Chicago IL, USA).

3.3 RESULTS

3.3.1 Soil properties

Effects of flooding on soil redox potential (*Eh*) (Fig.3.2a.) were observed from transplanting until early tillering stage (25 DAT). There was a sharp decrease over time in the *Eh* at the 10 cm depth, from +467 to -379 mVolt (mV). This suggested that depletion of O_2 and an increase in electron activity mediated by microorganisms respiration was highly marked in the first five days. However, after 17 days Eh values achieved an apparent stability, without significant changes. As a result of changes in the soil electrical conductivity in the flooded treatments, the pH increased from initial pH 4.85 (transversal dashed line) at transplanting (Fig 3.2b). In the control which was flooded throughout the growth stage, pH was 6.18 at the harvest stage, significantly higher than that of the other treatments that were drained after heading at 4, 3, 2 WAH. In the drained treatments, pH ranges declined between 5.54-5.58 without significant differences among them. Results show that pH acquires specific values at specific soil moisture. FCap treatment showed the lower pH of 5.24.

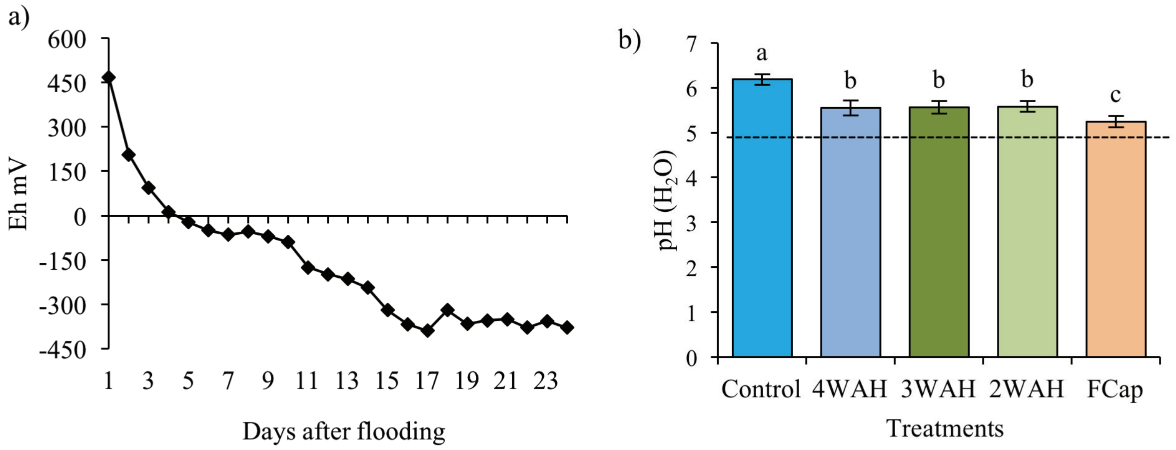


Figure 3.2 Influence of soil flooding on soil electrochemical properties a).Soil Redox Potential b) Soil pH(H_2O). [dashed line represent the pre-flood pH value].

3.3.2 Influence of Water Management on grain micronutrient contents and yield

One–way analysis of variance (ANOVA) showed that, there were statistically significant differences between means of micronutrient concentration in grain (P-value < 0.05) Table 3.2 Therefore resulted in rejection of the null hypothesis which stated that all the data came from groups with the same mean. In this case, subsequent analysis will help us to find a significant difference between pairs of means in at least one of the treatments.

Table 3.2 One–Way analysis of variance (ANOVA) for the concentration of micronutrients in grain.

		Sum of	df	Mean	F	Sig.
Grain-Fe Concentration	Between Groups	139.438	4	34.860	9.888	0.000
	Within Groups	123.385	35	3.525		
	Total	262.824	39			
Grain-Zn Concentration	Between Groups	204.186	4	51.046	11.431	0.000
	Within Groups	156.292	35	4.465		
	Total	360.477	39			
Grain-Cu Concentration	Between Groups	36.593	4	9.148	17.769	0.000
	Within Groups	18.020	35	0.515		
	Total	54.613	39			
Grain-Mn Concentration	Between Groups	3043.854	4	760.963	16.706	0.000
	Within Groups	1594.257	35	45.550		
	Total	4638.111	39			

The concentration of micronutrients in rice grain (Fig. 3.3) differed significantly among irrigation treatments. The Fe concentration in grain ranged from 19.01 mg kg^{-1} to 13.95 mg kg^{-1}. 4WAH, Control and 2WAH showed the higher Fe concentration with no large significant differences, while 3WAH and FCap were 10% and 23% respecctively lower than Control. In contrast to Fe, Zn concentration in rice grain was more sensitive to changes in water management (Fig. 3.3b). Treatments FCap and 2WAH showed values 35.40 mg kg^{-1} and 33.70 mg kg^{-1} respectively, representing 18% and 11% significantly higher than Control.

The concentration of Cu in grain clearly rose due the establishment of aerobic condition (Fig 3.3c). Treatments FCap and 2WAH showed higher concentrations of 4.82 and 4.11 mg kg^{-1} respectively without significant difference between them. Both treatments almost doubled their concentration in comparison with Control, 4WAH and 3WAH which were not significantly different. The trend observed for Mn grain concentration was not as we expected for the different water treatments. As soil anaerobic conditions were more prolonged, grain Mn concentration decreased. Values varied from 31.52 mg kg^{-1} to 55.68 mg kg^{-1}, treatments 2WAH and FCap rose their Mn concentration by 65-76 % respectively higher than Control (Fig.3.3d).

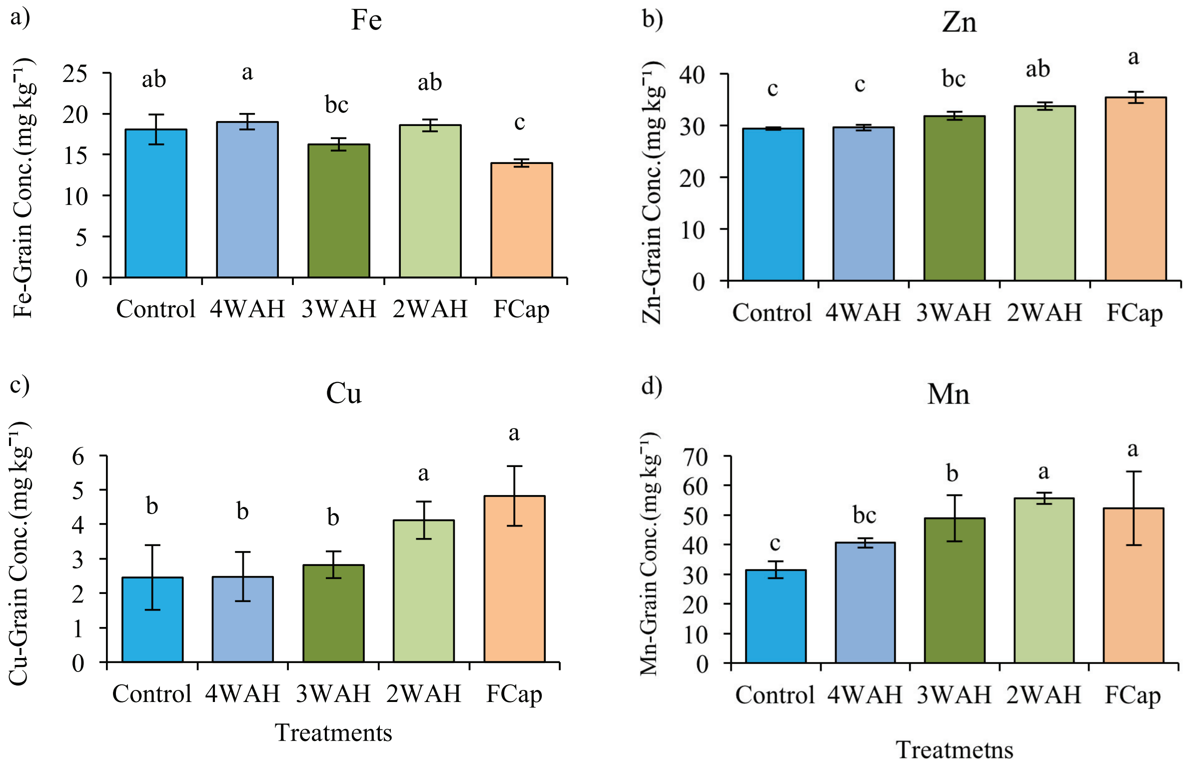


Figure 3.3 Concentration of micronutrients (Fe, Zn, Cu and Mn) in rice grain, influenced by water management.

The effects of soil drainage on grain micronutrient concentration during early grain filling stage, around 75-80 DAT, was beneficial particularly from treatment 2WAH where the period of flooding was the shortest. That period was identified as high demand for micronutrient acquisition and accumulation. Despite of changes in soil properties, the transition from flooded to aerobic conditions increased grain yield and root density by 7% and 7.3% respectively compared to the control (Table 3.3). Although aboveground plant biomass (leaf and stem weight) showed higher values for drained treatments , we observed that the increase was due the emergence of some new shoots after of the aerobic conditions were established. These tillers did not produce panicle at the time of harvest.

Table 3.3 Comparison of dry matter weight among different water managements. Values represent means of dry matter weight, on each part of the rice plant.

	Control	4WAH	3WAH	2WAH	FCap.
Grain yield (g plant^{-1}).	30.1^{b}	30.5^{ab}	31.3^{ab}	32.2^{a}	15.7^{c}
Leaf & Stems weight(g plant^{-1}).	101.0^{b}	108.6^{ab}	115.9^{a}	119.4^{a}	72.0^{c}
Roots weight (g plant^{-1}).	13.7^{a}	12.9^{ab}	13.9^{a}	14.7^{a}	12.5^{b}

* Means followed by different lowercase letter represent the significant difference among treatments. at $P<0.05$

3.3.3 Soil micronutrient availability and water management.

Soil micronutrient availabilities were clearly influenced by water managements. The Fe availability (Fig.3.4a) significantly decreased from Control to FCap. The decreasing change was more evident after the re-oxidation of treatments 4, 3, 2 WAH where the concentration was reduced by more than 20 %. Treatments 3WAH, 2WAH and FCap showed a slight increase in Zn availability by 6, 11and 12% over Control, respectively, although differences were not statistically significant (Fig. 3.4b).

Cu availability increased sharply as aerobic condition were established. Figure 4c shows how Cu availability increase from flooded condition to 4WAH in a short time. Cu availability reached its peak values as result of decreasing pH which is maintained across subsequent treatments.

Availability of Cu did not show significant difference between the drained (4, 3, 2WAH) and FCap treatments. FCap and 2WAH were >20% significantly higher than Control. Likewise, Mn availability (Fig. 3.4d) remained high across all the treatments regardless of flooding schedule. These treatments did not showed significant differences among them. Availability of Mn range from 187 mg kg^{-1} to 228 mg kg^{-1}. Control was six fold higher in available Mn than FCap.

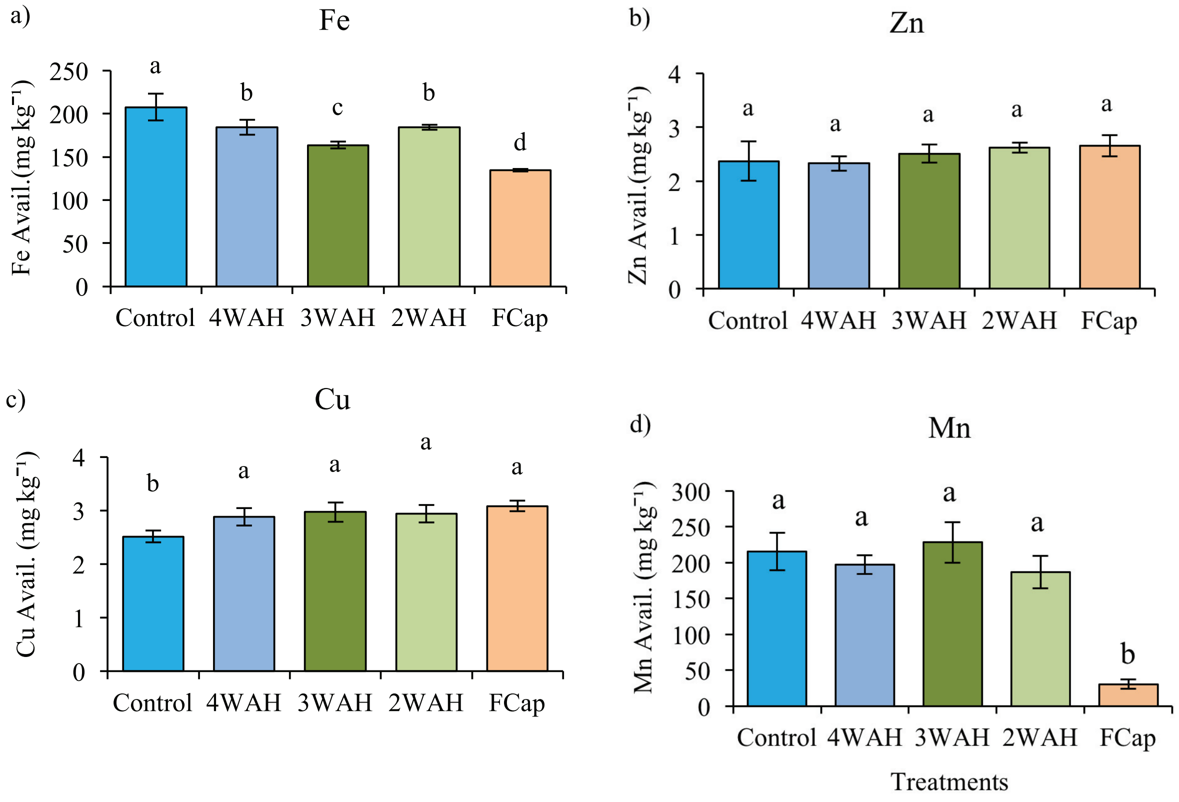


Figure 3.4 Micronutrients availability (Fe, Zn, Cu and Mn) in soil.

3.4. DISCUSSION

3.4.1 Effect of water management on electro-chemicals soil conditions

At first, for better understanding of the results of present study, we simply summarize the effect of Soil Eh and pH change with water management on micronutrient availability in soil. Soil flooding conditions caused modifications in soil pH and Eh. After its establishment, soil pores were water saturated and Eh started to decrease progressively (Fig.3.2a). The depletion of O_2, triggered new processes driven mainly by strict facultative anaerobic microbes that thrive under these environments. They take over further biochemical reactions, using substances other than O_2 as the terminal electron acceptor to metabolize organic compounds (Berkowitz, 2014). In the present study, the pH increase under flooding condition (Fig.3.2b), could be led by Eh reduction and the hydrogen consumption, during the reduction process of MnO_2 and $Fe(OH)_3$ and other transition elements demanded in microbes metabolism (Brady & Weil, 2014). The transition from flooding to aerobic condition during grain filling stage, reduced pH suggesting that establishment of aerobic condition could have affected the Eh and therefore pH could be progressively restored toward its initial value, as soil is re-oxidized (Zhou et al., 2014). Redox reactions (i.e. gain and losses of electrons) can define both the soil pH and the availability of micronutrients, (White & Broadley, 2009).

3.4.2 Fe dynamics

In our result, Fe availability (Fig 3.4a) was over the critical level in soil (5 mg kg^{-1}) for deficiency and below 300 mg kg^{-1} in leaf for toxicity (Dobermann & Fairhurst, 2000). The availability of Fe in soil was significantly higher in flooded than drained treatments, which implies that Fe availability in soil depends on soil Eh. Therefore water management becomes the major controlling factor to consider in promoting Fe plant uptake. Results also showed that even when soil became aerobic, Fe availability decreased at 2, 3 and 4WAH, but the grain Fe concentration was kept comparable in all the treatments except FCap. Moreover, transition from flooding to aerobic and re-oxidation of Fe^{2+} to less soluble form at 2WAH and the other drained treatments, did not affect substantially the concentration of Fe in grain. Remobilization of Fe from tissues to the grain may explain this phenomena. Studies conducted on rice by Sperotto et al., (2013) suggested that leaves were the main source to supply Fe to the seeds by remobilization from old tissues. Besides, release of organic acids (phytosiderophores) in rhizosphere might also contribute to Fe acquisition under aerobic condition and deficiency stress (Nozoye et al., 2013). Rice plant, during the tillering and reproductive stages is able to accumulate Fe as ferritin a “Fe-reserve protein” (Da Silveira et al., 2009). This Fe is remobilized via phloem by metal-transporters such as OsIRT1, OsIRT2, OsYSL15, and OsNRAMP5 which enhance their activity during grain filling stage (Bashir et al., 2013). In addition, the enhancement of Fe availability in soil, inhibited the availability of Zn and Cu in soil (Dobermann & Fairhurst, 2000) and the concentration of Zn, Cu and Mn in grain as showed correlation matrix (Table 3.4) therefore the re-oxidation of Fe to less soluble form from 2WAH, might facilitate the uptake of such micronutrients and improve the concentration in grain

3.4.3 Zn dynamics

Regarding available Zn values, in soil were above the critical levels (0.8 mgkg^{-1}) for deficiency (Dobermann & Fairhurst, 2000). The concentration of Zn in grain as well as the concentration in soil was the highest in FCap, and also tended to increase by transition from Control, to 2, 3 ,4WAH (Fig 3.3b and 3.4b). These enhancements in grain positively correlated ($P<0.01$) with the Zn availability in soil (see table 3.4). Although Zn availability in soil might be affected by several factor (Sadeghzadeh, 2013) apparently, the reestablishment of aerobic condition, pH declining and reduction of Fe to less available form were the major factor controlling Zn availability. The timing in which irrigation was stopped, also played an important role for Zn acquisition. Unlike FCap, which achieved high grain Zn but low yield, 2WAH showed the best performance in terms of Zn grain concentration and grain yield (Table 3.3). The period in which soil was re-oxidized coincided with grain filling stage, which has been identified as high demand period not only for the uptake and remobilization of Zn but also other essential elements (Pearson & Rengel, 1994; Bashir et al.,

2013) Many researchers found that Zn is translocated from old tissues to flag leaf and afterwards remobilized to the grain (Rengel et al., 1999; Wu et al., 2010). However, the higher percentage of Zn moved into the grain is taken up directly by root, during post-heading stage (Jiang et al., 2007; Sperotto et al., 2013). Although the availability in soil did not show significant differences with control, 2WAH responded more positively than other treatments. In comparison with 2WAH, treatments 3 and 4WAH did not increase their Zn grain concentration even when aerobic condition could have facilitated availability of Zn. Under flooding condition, Zn can be precipitated as ZnS and $Zn(OH)_2$ or reduced its transport and root uptake by HCO_3 which is the predominant anion at such condition (Dobermann & Fairhurst, 2000). The small enhancement in root density (Table 3.3) observed in 2WAH, also could be another positive factor that facilitated a larger soil contact area (Yang et al., 2004) and therefore, increase Zn uptake by plant.

3.4.4 Cu dynamics

Available Cu in soil is held mainly as a free cupric ion (Cu^{2+}) which is the preferred form for plant (Hoang et al., 2008). In our results the Cu behavior in soil and grain was broadly similar with the trend observed in Zn. Results showed that the water condition at this period in which soil was re-oxidized at early grain filling stage, i.e. two weeks after heading, was determining factor controlling the Cu contents in grain. Figure 3.3c and 3.4c, showed a close relationship between soil Cu availability and grain concentration. Furthermore, the shifting from anaerobic to aerobic condition promote enhancement of Cu solubility and bioavailability as well as better root uptake (Liao et al 2013). On the other hand the lower Cu availability observed in treatments where anaerobic condition was kept longer, could be ascribed to the formation of Cu-ferrite ($Cu_2Fe_2O_4$), Cu-Organic matter bounds and formation of insoluble CuS (Dobermann and Fairhurst, 2000). Soil Zn availability did not significantly increase when the aerobic conditions were established, but Cu did (Fig 3.4b and 3.4c). This suggested that Cu is able to increase in availability easier than did Zn. Garnett et al., (2005) reported that more than 60% of Cu in grain was absorbed at filling stage, which explains the importance of timing in water management to increase grain Cu concentration. Enhancement of Cu availability is advisable in soils where Cu do not exceed toxic level for plant and humans (Xu et al., 2006).

3.4.5 Mn dynamics

Despite of establishment of aerobic condition, scheduled at 2, 3 ,4 WAH, soil Mn availability remained high (Fig. 3.4d) without significance changes compared with control. Since Mn is reduced at higher potential (+400~ -100mV) than Fe (+200~ -200 mV) (Kyuma, 1997), the Mn availability in soil can be kept stable longer than Fe in drained treatments of which the moisture condition changed. Although Fe and Mn take active parts in redox reactions in soil and both are able to increase their availability after soil flooding, presumably, high Fe availability observed in soil, adversely inhibited Mn uptake by roots, because of the antagonist interaction in soil (Dobermann & Fairhurst, 2000; Millaleo et al., 2010; Das, 2014). Alam, (1985) reported that a concentration of Mn in leaves and stems of 7 weeks plants were increased when Fe application ranged 0-30 mg kg^{-1} in soil, but above 30 mg kg^{-1} application reduced Mn concentration in plant. Other studies conducted by Pearson & Rengel, (1994) concluded that Mn concentration in flag leaf and other active leaves increased during grain filling stage, but remobilization to the grain was not carried out, regardless of the leaf concentration. Suggesting that, continuous uptake from soil seems to be the main source of grain Mn. However its behavior still remains unclear, therefore further investigations are needed to elucidate this issue.

3.4.6 Relationship among soil, plant and grain micronutrients composition.

The effect of water management on soil micronutrients availability and its relationship with the concentration in grain and flag leaf are shown in Table 3.4. The availability of Fe, Zn and Cu in soil significantly correlated with their respective concentrations grain. Soil pH, redox reactions, plant physilogy (Growth stage) and rhyzosphere activity are the main factors controlling the availability in soil and concentration of micronutrients in grain (Dobermann & Fairhurst, 2000; Gao et al., 2012; Marchner, 2012) mediated by the transition from flooding to aerobic condition. Mn detected in soil did not correlate with Mn concentration in flag leaf and showed a negative correlation with grain Mn ($p<0.05$). Fe availability in soil correlated negatively with the concentration of Zn and Cu in plant and grain ($p<0.01$). This observation suggested that availability of Fe in soil could be attributed to antagonistic interaction, namely inhibition of Zn, Cu and Mn uptake (Dobermann & Fairhurst, 2000) Negative effects of Fe excess on Cu and Mn shoots concentration have been observed in *Indica* varieties (Yoshihara et al 2010).

Table 3.4 Correlation matrix between soil micronutrients availability and micronutrient concentration in flag leaf and grain.

		Soil Micronutrient				Flag leaf Micronutrient				Grain		
		Fe	Zn	Cu	Mn	Fe	Zn	Cu	Mn	Fe	Zn	Cu
Soil	Zn	-0.33*										
	Cu	-	0.43**									
	M	0.71**	-	-								
Flag leaf	Fe	0.20	0.32	0.13	0.06							
	Zn	-	0.41	0.38	-	-0.14						
	Cu	-	0.47*	0.61**	-	0.20	0.77**					
	M	-0.43	0.55*	0.59**	-0.41	0.68*	0.31	0.72**				
Grain	Fe	0.63**	-0.38*	-	0.55**	0.31	-	-	-0.27			
	Zn	-	0.39*	0.46**	-	0.31	0.73**	0.86**	0.76*	0.2		
	Cu	-	0.45**	0.51**	-	0.34	0.72**	0.93**	0.80*	0.2	0.87*	
	M	-	0.48**	0.59**	-0.34*	0.46*	0.28	0.64**	0.85*	0.2	0.72*	0.79*

**and* represent significant correlation at $P<0.01$ and $P<0.05$ respectively.

Concentration of Fe, Zn, Cu and Mn in flag leaf correlated positively with their respective concentration in grain. Moreover, concentration of Zn, and Cu in grain and flag leaf correlated significantly ($p< 0.01$) among each other. Studies conducted by Ishimaru et al., (2005) revealed that the expression of ZIP´s gene family are actively related with the Zn and Cu uptake and transport system. The same mechanism may control the behavior of Zn and Cu in plant, which brought the similar results of Zn and Cu in the present study.

3.5 CONCLUSIONS

Soil water management changes the soil Eh and pH, which control the availability of micronutrient in soil and consequently the concentration in rice grain. When those changes occur during grain filling stage, micronutrients such as Zn, Cu and Mn increase their concentration in the grain, likely due the reduction of pH and their interaction with other soil properties. Although water management did not exerted significant changes on soil Fe availability, the re-oxidation of Fe to less available form, promoted the increase of Zn, Cu and Mn in grain.

Although researchers have been focused to increase the concentration of micronutrient in grain through breeding strategies, if soil management in relation to water condition is not applied, those strategies inevitably will fail. Therefore establishment of aerobic condition starting from 2 weeks post-anthesis till maturity by proper water management such as intermittent irrigation, seems to be the most feasible strategy to increase the concentration of micronutrients in rice grain. Furthermore, this technique did not result in grain yield losses.

Our results exhibited the importance of soil environmental managements in realization of the biofortification program. Plant breeding strategies must work alongside agronomic biofortification techniques. In this particular case of rice, the combination between water management and plant breeding could be the most viable option in order to ensure the supply of micronutrients from the soil and overcome malnutrition in vulnerable populations.

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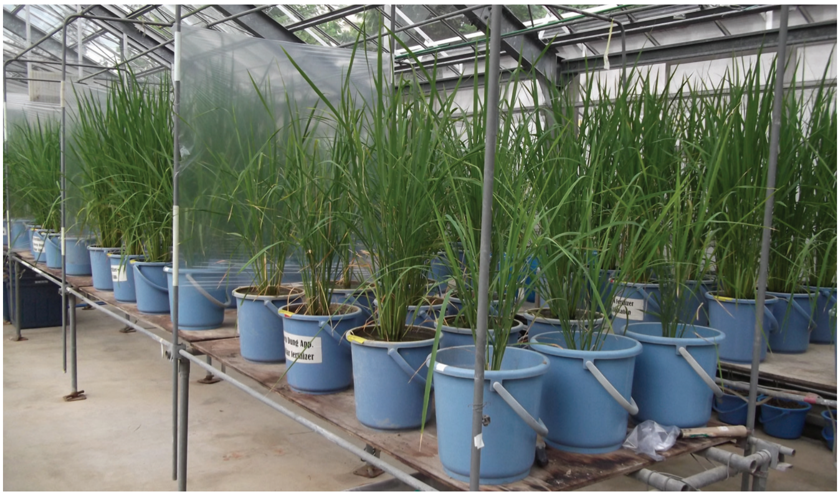

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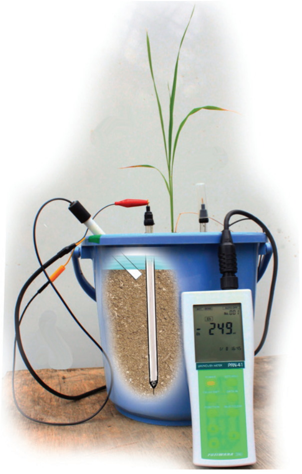

Redox Potential Measurement

Foliar Fertilizer Spraying

Harvest Season

Soil Sampling

CHAPTER IV

Agricultural Approaches and Water Management as a Way to Increase the Nutritive Value of Rice Grain

4.1 INTRODUCTION

Application of fertilizer either chemical or organic is a common approach in maintaining and improving the biological and physicochemical properties of soil as well as supplying essential elements for rice plant growth (Baker and Pilbeam, 2015). However, intensive monoculture, improper soil management and in appropriate fertilizer use have resulted in progressive depletion of micronutrient availability as a consequence of soil degradation, leaching of nutrients, salinity and other injuries. As a domino effect, the degradation of soil capabilities and deficiency of micronutrients has not only reduced the grain yield but also reduced the density of micronutrient contents in staple crops. Nowadays, the low availability of micronutrients in soil is overlapping populations in China and other Asian countries, which are facing the burden of malnutrition (Yang *et al*., 2007). Recent reports of FAO (2015) point out that 2 billion people suffer from one or more micronutrient deficiencies and keep growing.

In order to cope with the scourge of malnutrition in vulnerable populations, several approaches have been implemented (See Fig. 1.1). However, implementation of these and other alternatives still faces a number of hurdles (Berry *et al.,* 2012) and do not address the deficiency of micronutrients in soil, which is the most important constraint.

Generally, the improvement of soil capability implies the increase in micronutrients availability (De Datta, 1981; Yang *et al*., 2007) and consequently the concentration of micronutrients in tissues and grain can be improved. Application of inorganic fertilizer represents a faster method to increase yield and satisfy crop micronutrients demand (Baker and Pilbeam, 2015). However, the application of inorganic fertilizer does not necessarily imply an increase in soil micronutrients availability and plant uptake, even when yield did. Application of micronutrients directly to the soil has the risk to react with soil minerals and organic matter therefore the availability could decrease (Khoshgoftarmanesh *et al*., 2010). Unlike chemical fertilizer, the adoption of organic amendment techniques have been fostered as the most feasible and sustainable approaches to restore soil capabilities. One of the more widespread organic fertilization is the application of farmyard manures (FYM) as source of CN and micronutrients (Fe, Zn, Mn and Cu) to offset the low content of soil organic matter in soils (Gao *et al*., 2000). In spite of its benefits to soil, organic fertilizer cannot be considered as a substitute to chemical fertilization in short term applications) because nutrients are released into the soil progressively as organic matter decomposes and the process relies on many environmental factors (FAO, 2007). Therefore in this regard, a combination with inorganic fertilizers is advisable (IRRI, 2012). Studies conducted by Shahid *et al*.,(2013) demonstrated that, the combination of NPK+FYM in rice-rice system was the best alternative to improve soil properties and availability of micronutrients in soil. Combining organic/inorganic fertilizers increases N efficiency by enhancing microbial activity and yield (Pan *et al*., 2009).

Another agronomical approach widely used to correct micronutrients deficiencies is, foliar fertilization (FF). This technique is considered an efficient short-term approach to increase mineral contents in rice grain (He *et al*., 2013). However FF has several limitations such as low penetration and translocation rates of some micronutrients and absorption efficiency which can be affected by the nature of plants (e.g. waxy cuticle, hairiness) and environmental factors (Maschner, 2012). According to several studies, FF is considered as a nutrient corrective approach and as the organic amendments, it cannot be a substitute for chemical fertilization (Fageria *et al*., 2009).

From the foregoing discussion, and based on our previous results about the establishment of water saving techniques (Timing of establishment), its effects controlling micronutrient availability and accumulation in grain (See chapter 2 and 3), we can assume that fertilization approaches (e.g. organic, inorganic and foliar applications) to improve soil micronutrient contents, in combination with water management might increase nutritive value of rice grain. however little Thus, the aim of this study was to investigate the combined contribution of three common agricultural approaches (water management, soil amendment and foliar fertilization) in increasing Fe, Zn Cu and Mn accumulation in grain and assess its effects on plant morphological behavior.

4.2 MATERIALS AND METHODS

The effect of different fertilization approaches on the concentration of micronutrients in grains under different water management was evaluated through a pot experiment conducted in a greenhouse at Shimane University. The fertilizer approaches comprised the application of Sheep Farm Yard Manure (FYM) collected from the Animal Research Facility at Shimane University, chemical fertilizer (NPK) and foliar fertilizer ($FeSO_4$; $ZnSO_4$; $CuSO_4$ and Mn reagent). The semi-dwarf (cv. Koshihikari) rice variety (20 days old) seedlings were transplanted into plastic buckets (Ø=24cm x 25cm height) during rice growing season in Japan (May/Oct). Each bucket was filled with 6.5 kg of air dried “Gray Lowland” soil Typic Fluvaquent (USDA, 2005).

4.2.1 Experimental design

The three fertilizer approaches were used in combination or singly in the pot experiment. The fertilizer treatments were as follows:

T1. Control, No fertilizer (NF)

T2. 200g of Sheep dung (FMY).

T3. 200g of FMY + Foliar fertilization (FMY +FF).

T4. 100g of FMY + Chemical Fertilizer (FMY +CF).

T5. 100g of FMY + Chemical Fertilizer + Foliar Fertilization (FMY +CF+FF).

T6. Chemical Fertilizer (CF).

T7. Chemical Fertilizer + Foliar Fertilization (CF+FF).

Each of the above fertilizer treatments had four different water regimes (Wc) where Wc1 was flooded at ±5cm water depth from transplanting to harvest. Wc2: alternative wetting and drying (AWD), customized from early tillering to panicle initiation, where flooding condition was established until 50% of anthesis and then AWD re-established again up to harvest on a 2-3 days flooding interval Wc3: flooding conditions were established from transplanting and stopped at 2WAH keeping soil 1evel approximately 35± 3 % (v/v) of moisture, similar to ±80% of its field capacity; Wc4 which was kept at field capacity (FCap.) conditions throughout the growth period in order to mimic the conditions of upland systems. All these were replicated three times. Treatments in which organic material was required were flooded one week before transplanting.The experiment was laid out in a complete randomized block design.

Chemical fertilizer (N:P:K) was applied in doses of 45:65:60 kg ha-1 respectively basing on recommendations of Shimane prefecture. Each element was applied as follows: [$(NH_4)_2SO_4$] N was split in four applications, 1st-20% as basal application, 2nd-30% at tillering stage, 3rd-40% at panicle initiation and 4th-10% 15 days after panicle initiation. [Na_2HPO_4] P was applied 100% as basal application. [KCl] K was split in two applications; 50% as basal application and 50% at panicle initiation while foliar application Foliar fertilizer (FF) was applied in doses of 1% $FeSO_4$, 0.5% ZnSO4, 0.5% CuSO4 and 0.5% Mn reagent were applied at panicle initiation, booting and heading stage.

4.2.2 *Data collection and analysis*

Plant growth parameters such as plant height, number of panicles, grain yield and SPAD "chlorophyll contents" in flag leave were measured after harvest. Plants were harvested and oven dried at 70 °C for 72 hours. Grains were oven dried at 40°C for 48 hours and grain yield (14 % moisture content) was recorded, thereafter dehusked in rotating rubber roll, (Satake THU35B, Japan).

For grain micronutrient analysis, all samples were ground into fine powder in agate grinding jars using a mixer mill (MM200, Retsch GmbH, Haan, Germany). Rice powdered samples were oven-dried 12 hours at 80 °C. Subsequently 0.5 g of sample was digested in 2.5 ml HNO_3 with teflon vessel. All samples were oven heated at 160°C for 4 hours, kept resting overnight and diluted with distilled water up to 25 ml (Koyama and Sutoh, 1987). The concentrations of Fe, Zn, Cu and Mn were determined by Inductive Coupled Plasma Spectroscopy (ICPE-9000; Shimadzu, Japan).

3.2.3 *Soil sampling and analysis*

Soil samples were collected from 5 and 15 cm depth in each bucket immediately after harvest. They were stored in ZIPLOC hermetic plastic bagsand refrigerated at (temperature) in order to maintain their original soil chemical properties. Portions of each sample were oven dried at 105 °C to calculate the moisture content necessary for all the subsequent analyses following standard analyses methods (Japan Soil Environment Analysis Methods Committee,2003). The concentration of micronutrients (Fe, Zn, Mn and Cu) was analyzed by mixing 2 g soil with 20 ml of 0.1N Hydrochloric acid. The solution obtained was filtered and the concentration of micronutrients determined by Inductive Coupled Plasma Spectroscopy (ICPE-9000; Shimadzu, Japan). Soil pH was determined by the soil-water ratio of 1:2.5 and electrical conductivity (EC) by 1:5 soil-water ratiousing the glass electrode method (D-24 HORIBA, Japan). Soil Redox potential (*Eh*) of flooded treatments was measured in situ from first day after flooding (DAF) to 45 DAF at 10cm depth using electrode method (PNR-41, DKK-TOA *Co., Japan*).

4.2.4 *Statistical analysis*

To determine the influence of the different fertilizer applications in combination with water management on soil properties, micronutrient availability as well as the concentration of miconutrient rice grain, data were statistically analyzed by (ANOVA). Significant differences among the treatments were determined by Turkey´s honestly significant difference (HSD) test ($P<0.05$) for multiple means comparison. All statistical analyses were performed using IBM SPSS Statistic v20.0 (IBM SPSS, 2011. Chicago IL, USA).

4.3 RESULTS

4.3.1 Effects of different fertilizer approaches and water management on micronutrient availability

Results in Table 4.1 show that, despite fertilization practices and water management, plant height was not affected. Treatments which grew under "permanent flooding condition" Wc1 were slightly higher than Wc4. The combination of fertilizer application (NPK+FYM) in T4 and T5 showed largest number of panicles, in almost all water regimes. Significant differences between NPK+FYM treatments and control were found in Wc1 and Wc3. Regardless of fertilization practices, the effect of water management was clearly observed at Wc2 where the establishment of AWD produced higher number of panicles per pot than the rest of water regimes. Meanwhile

Wc4 had the lowest values of panicles. Furthermore total dry matter did not show significant difference between fertilization treatments in Wc1 and Wc2. In these water regimes, total dry matter mean values in fertilizer treatment T1 were significantly lower than other treatments in which fertilizer amendments were applied. Treatments in which soil was improved by addition of FYM (for 200g or 100g +NPK) were more responsive in terms of dry matter accumulation. Application of FF did not have any significant effects with regard to plant height, number of panicles and dry matter accumulation.

During our greenhouse experimentation, the temperatures were high $>35^{O}$C during the flowering period which negatively affected grain yield. This condition caused a high percentage of grain sterility and consequently high variation in grain yield. Nevertheless we noticed that treatments T4, T5, T6 and T7 alongside with proper water management, promoted maximum grain yield and the response to warming were much higher than T1, T2 and T3.

Permanent aerobic condition Wc4 showed significantly lower grain yield compared to other treatments, likely due to the effects of water stress. With FF application, we could not find any significant difference among treatments suggesting that FF had very little influence on plant morphological behavior.

Although, contents of chlorophyll "SPAD" cannot be grouped with plant morphological parameters, its contents are associated with plant health, vigor and estimation of plant N content. Regardless of fertilization approaches applied, it was observed that treatments that received FYM i.e. T2, T3, T4 and T5 produced vigorous plants with chlorophyll contents >30 SPAD units at harvest time. These treatments were statistically different from the control and this could be an indicator of remnant physiological activity.

Table 4.1 Morphological components and grain yield response to fertilization and water management.

Treatments			Wc1		Wc2		Wc3		Wc4	
Height (mm)	T1	NF	110.00	±2.00^{a}	102.67	±2.52^{b}	107.00	±2.00^{a}	107.00	±5.29^{a}
	T2	FYM	109.00	±4.36^{a}	107.67	±4.04^{ab}	109.33	±2.52^{a}	104.00	±3.61^{a}
	T3	FYM+FF	108.33	±0.58^{a}	104.33	±3.06^{b}	106.67	±3.06^{a}	105.67	±0.58^{a}
	T4	FYM +CF	114.00	±2.65^{a}	113.33	±2.31^{a}	109.33	±1.15^{a}	111.00	±1.00^{a}
	T5	FYM +CF+FF	110.33	±1.53^{a}	114.67	±0.58^{a}	110.67	±1.53^{a}	103.67	±8.14^{a}
	T6	CF	112.33	±3.06^{a}	108.33	±4.16^{ab}	111.67	±3.79^{a}	110.33	±3.06^{a}
	T7	CF+FF	109.67	±1.15^{a}	109.00	±2.00^{ab}	109.00	±1.73^{a}	106.33	±10.02^{a}
Number of Panicles	T1	NF	27.33	±0.58^{ab}	30.33	±2.31^{a}	29.67	±1.53^{bc}	15.33	±3.79^{a}*
	T2	FYM	28.33	±4.62^{ab}	44.33	±19.86^{a}	26.00	±6.08^{bc}	11.33	±0.58^{a}*
	T3	FYM+FF	24.00	±6.56^{b}	42.00	±6.00^{a}*	23.33	±5.51^{c}	21.00	±8.66^{a}
	T4	FYM +CF	34.33	±4.73^{a}	40.00	±6.08^{a}	35.33	±2.52^{ab}	25.33	±4.04^{a}*
	T5	FYM +CF+FF	37.00	±6.56^{a}	41.67	±7.64^{a}	39.33	±2.31^{a}	23.67	±2.52^{a}*
	T6	CF	27.00	±2.00^{ab}	29.67	±4.73^{a}	31.67	±1.15^{abc}	21.33	±2.52^{a}
	T7	CF+FF	29.00	±0.00^{ab}	28.00	±2.65^{a}	28.67	±1.15^{bc}	19.67	±10.50^{a}
Total Dry Matter (g plant^{-1})	T1	NF	62.89	±1.58^{a}	70.41	±11.49^{a}	57.78	±0.67^{b}	47.88	±5.10^{b}*
	T2	FYM	73.79	±32.31^{a}	82.98	±32.56^{a}	81.79	±6.55^{a}	56.58	±20.39^{ab}
	T3	FYM+FF	78.63	±24.11^{a}	87.13	±15.21^{a}	77.93	±14.80^{ab}	74.40	±7.98^{a}
	T4	FYM +CF	102.91	±17.35^{a}	82.03	±20.00^{a}	85.25	±5.68^{a}	70.99	±7.71^{ab}
	T5	FYM +CF+FF	79.30	±5.17^{a}	88.93	±9.01^{a}	88.65	±10.02^{a}	77.38	±3.32^{a}
	T6	CF	57.38	±2.37^{a}	60.66	±4.54^{a}	68.41	±2.54^{ab}*	70.61	±2.40^{ab}*
	T7	CF+FF	57.43	±1.97^{a}	65.33	±7.95^{a}	67.73	±5.39^{ab}	65.92	±6.71^{ab}
Grain Yield (g plant^{-1})	T1	NF	32.84	±2.42^{bc}	29.61	±3.28^{bc}	32.97	±11.31^{bc}	14.23	±0.83^{a}*
	T2	FYM	26.36	±6.44^{b}	27.72	±1.59^{c}	28.98	±1.86^{bc}	18.79	±2.49^{a}*
	T3	FYM+FF	21.45	±4.58^{b}	28.11	±10.40^{c}	25.84	±8.20^{c}	20.25	±3.37^{a}
	T4	FYM +CF	54.01	±10.33^{ab}	50.76	±4.92^{a}	44.83	±6.01^{ab}	11.52	±6.91^{a}*
	T5	FYM +CF+FF	47.72	±3.77^{ab}	40.92	±3.91^{bc}	42.07	±7.72^{abc}	18.59	±15.48^{a}*
	T6	CF	54.72	±2.14^{a}	44.24	±2.94^{ab}*	55.26	±1.59^{a}	14.50	±2.69^{a}*
	T7	CF+FF	42.33	±14.68^{abc}	43.43	±5.19^{ab}	40.41	±4.23^{abc}	13.12	±7.51^{a}*
SPAD	T1	NF	21.51	±1.58^{b}	31.13	±1.16^{ab}	24.47	±2.01^{d}	25.70	±8.07^{b}
	T2	FYM	37.20	±7.45^{a}	38.89	±6.11^{a}	32.57	±2.23^{bc}	38.86	±1.43^{a}
	T3	FYM+FF	36.57	±6.63^{a}	34.73	±1.72^{ab}	42.70	±1.22^{a}*	32.50	±2.42^{ab}
	T4	FYM +CF	30.93	±3.33^{ab}	33.77	±1.59^{ab}	34.12	±5.22^{bc}	30.60	±3.40^{ab}
	T5	FYM +CF+FF	31.50	±1.50^{ab}	34.00	±2.89^{ab}	35.90	±3.54^{ab}	34.53	±1.37^{ab}
	T6	CF	22.47	±1.87^{b}	28.33	±3.15^{b}*	30.30	±1.06^{bcd}*	26.67	±1.99^{b}
	T7	CF+FF	28.00	±2.25^{ab}	33.70	±2.82^{ab}*	26.60	±1.47^{cd}	24.10	±5.20^{b}

Values represent means ± SD "standard deviation". Lower case letters in same column denote significant differences among fertilizer treatments, while (*) over same row denote significant differences among water sub plots at $P<0.05$.

4.3.2 Variation in soil micronutrients and soil electric conditions as mediated by fertilizer amendments and water management

Effects of flooding on *Eh* were observed in flooded treatments from 1DAF to 45 DAF. There was found a sharp decrease on *Eh* in FYM amended treatments from +414 mV to -321 mV (Fig. 4.1). In the control treatments the reduction was much more discrete and smaller than the FYM treatments. This could be attributed to the depletion of O_2 and an increase in electron activity as mediated by respiration of microorganisms by the addition of FYM. After 45 days the *Eh* leveled in spite of fertilizer application.

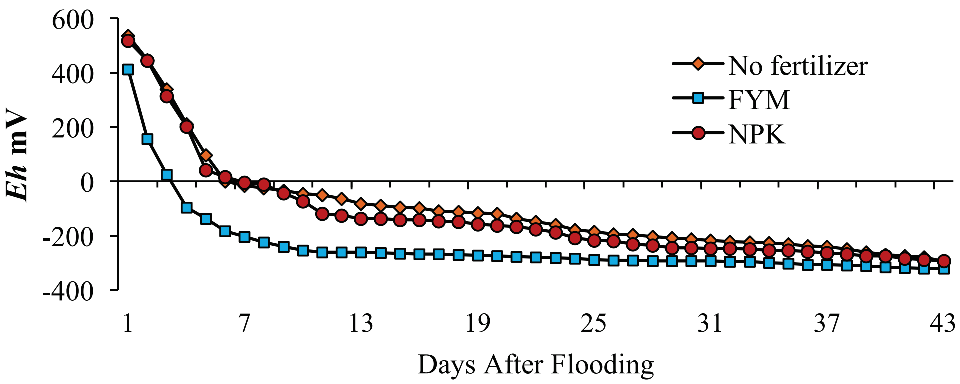

Figure 4.1 Effect of soil physicochemical condition and water management on the *Eh.*

Results of soil micronutrient availability are shown in Figure 4.2. Results in Fig. 4.2a show that soil Fe availability was not significantly different among fertilizer treatments. Fe availability ranged between 25.25 and 547.0 mgkg^{-1} with a coefficient of variation of 60 %. The most significant differences were found in water management regimes where Wc1 and Wc2 showed high Fe availability (>200mgkg^{-1}), while Wc3 and Wc4 were two and eight times fold of continuous flooding respectively. Concerning Zn availability in soil, values ranged between 9.10 and 49.23 mgkg^{-1} with a coefficient of variation of 41 %. Addition of FYM in T2 and T3 increased Zn availability (>20 mgkg^{-1}) which was significantly higher compared to control and other treatments (Fig 4.2b). Apparently, the availability of Zn in soil corresponds to the high contents of decomposable OM in FYM. Although, changes in water regimes did not show any significant differences from T1 to T5, Zn availability was significantly lower inWc1 compared to Wc2, Wc3 and Wc4 in fertilizer treatments including NPK alone. Cu was another micronutrient in which its availability was controlled by complexation with organic matter and changes in electrochemical conditions induced by water management. Cu availability ranged between 2.34 and 10.50 mgkg^{-1} with a coefficient of variation of 30%. Under water management regimes, Wc3 showed higher Cu availability. Almost all treatment at Wc1 and Wc2 had low Cu availability that could be attributed to the duration of flooding condition and shifting in *Eh*. Significant differences were found in T2 and T3 which were lower than control. However, Cu availability was similar for all fertilizer treatments in Wc3 and Wc4 (Fig 4.2c). The availability of Mn in irrigated treatments had similar trends as was observed in Fe. All fertilizer treatments under Wc1, Wc2 and Wc3 exceeded 200 mgkg^{-1} without any statistical differences between them. However, under Wc4 significant differences in fertilization were observed. Wc4 in combination with FYM (except T4 "aerobic") achieved higher Mn availability than control and NPK treatments (T6 and T7) which were significantly lower (Fig. 4.2d).

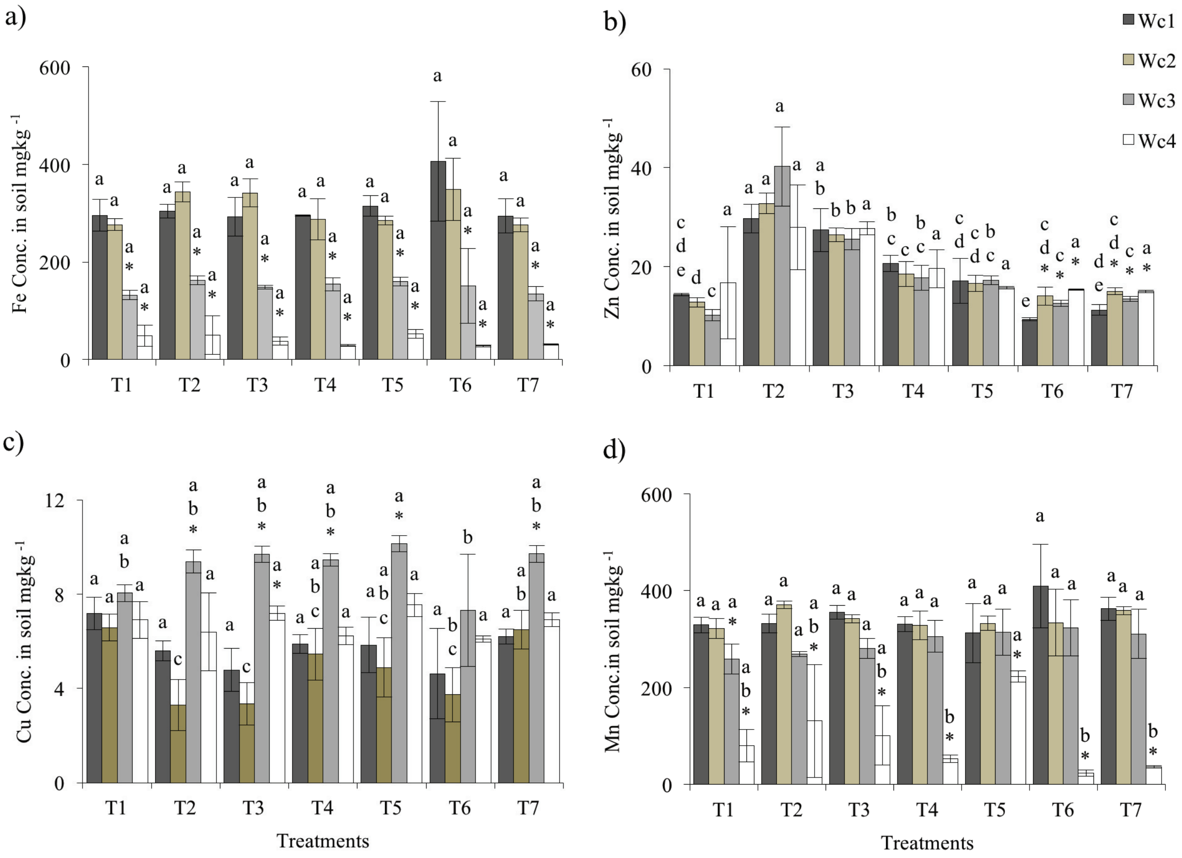

Figure 4.2.Availability of Fe, Zn, Cu and Mn in soil under different fertilizer combinations and water management.(N=3). (*) mean statistically differences among water regimes on each treatment. T1. Control (NF); T2. 200g FYM; T3. 200g FYM+FF; T4. 100g of FYM+CF; T5. 100g of FYM + CF+FF; T6. CF and T7. CF+FF.

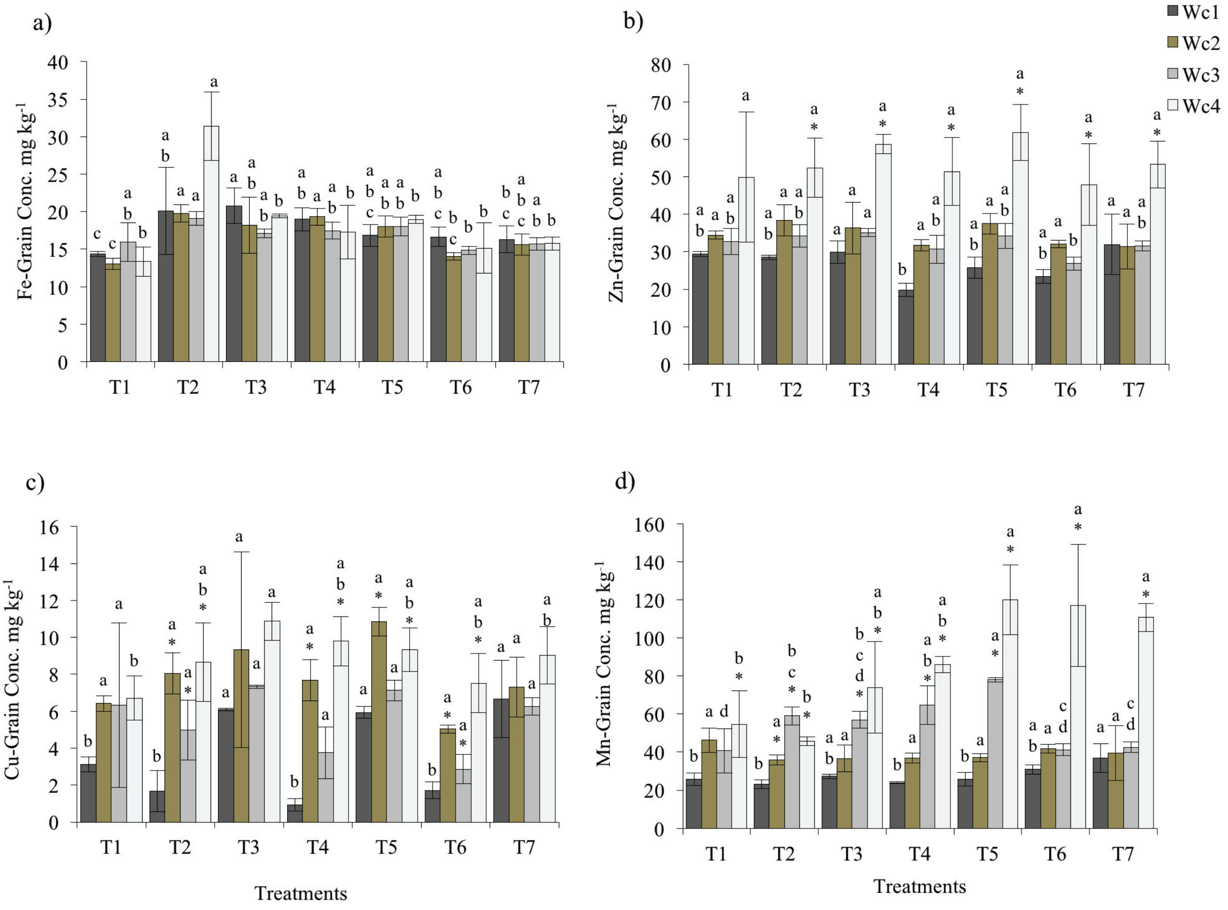

Figure 4.3 Concentration of Fe, Zn, Cu and Mn in grain at different fertilizer combinations and water management. (N=3). (*) mean statistically differences among water regimes on each treatment. T1. Control (NF); T2. 200g FYM; T3. 200g FYM+FF; T4. 100g of FYM+CF; T5. 100g of FYM + CF+FF; T6. CF and T7. CF+FF.

4.3.3 Concentration of Micronutrients in grain

Concentration of different micronutrients in rice grain under the influence of different agricultural approaches is shown in fig. 4.3. The mean concentration of Fe was 17.56 $mgkg^{-1}$ and ranged between 14.18 to 22.58 $mgkg^{-1}$. The grain Fe concentration was significantly higher in almost all treatments compared with the control. Treatments T2 and T3 (200mg FYM) and integrated application of fertilizer (T4 and T5(100g FYM+NPK)) slightly increased their concentration above T6 and T7 visualizing some advantages of FYM over chemical fertilizer. Although comparisons among water regimes did not show clear significant differences and had erratic behaviors; it was observed that permanent flooding condition "Wc1" had an upward trend. In our results, no significance differences were observed by the application of FF, evidencing the low mobility of Fe by its effect (Fig 4.3a).

Unlike Fe concentration, the enhancement in *Eh* had a distinctly positive impact on the concentration of Zn in grain. With exception of T7, it was found that treatments T1 to T6 under less reductive (Wc2-4) soil conditions increased grain Zn concentration (Fig. 4.3b). Although the incorporation of FYM for T2 and T3 increased the concentration of Zn over other treatments, no significant differences were observed. Application of $ZnSO_4$ as a FF affects grain Zn concentration to greater or lesser extent. In our results, a positive effect on Zn by the combination of water management and foliar fertilization was found. They were very much perceptible at Wc4-T3, T5 and T7 which increased its concentrations over their similar fertilization treatments by12%, 20% and 11% respectively.

The concentration of Cu was also largely affected by water management and FF. The establishment of permanent flooding condition Wc1 reduced availability in soil and consequently the Cu grain concentrations. This reduction was exacerbated due the inclusion of decomposable OM, promoting reduced conditions in T2 and T4, and showing the lowest concentrations 1.68 and 0.92 mgkg^{-1} respectively (Fig. 4.3c). The result suggests the high dependency of Cu on soil electrochemistry and contents of OM. Regardless of soil fertilization and water condition, Cu concentration in grain increased as consequence of foliar fertilizer. Treatments T2, T5 and T7 at Wc1 were significantly higher in Cu concentration compared to control. Cu as well as Zn, seems to have high mobility in leaves during anthesis, favoring their accumulation in grain.

Regarding the concentration of Mn in grain, the results were consistent with the availability observed in soil. In spite of agronomical approaches, significant differences were not found at Wc1 and Wc2 among treatments (Fig. 4.3d). However Mn concentration in treatments T2, T3, T4 and T5 for subplots Wc3 and Wc4 increased significantly above concentration observed in control, probably due to FYM application. In NPK fertilizer treatments "T6 and T7",Wc4 was significantly different from other treatments including control. The effect of FF on Mn concentration in grain was negligible. No significant differences were found between non-FF and FF treatments.

In general the influence of water management and soil fertilizer amendment were responsible for the concentration of micronutrients in grain to greater or lesser extent (Table 4.2). Fe Zn, Cu and Mn were clearly affected by the combination of both factors. However, in Zn and Cu concentration, FF acted as a third factor, excreting high influence on grain concentration. In terms of relationship among micronutrients concentration and the application of agricultural techniques, results showed that concentration of micronutrient in grain varied greatly in the same variety despite its genetic stability. Dynamics of each micronutrient under different soil conditions and fertilizer treatments are shown in Fig. 4.4.

Table 4.2 Interaction among grain micronutrients concentration and agricultural practices.

	Fe			Zn			Cu			Mn		
	Fertilizer	Water Manag	Fertilizer * Water Manag	Fertilizer	Water Manag	Fertilizer * Water Manag	Fertilizer	WaterMa nag	Fertilizer * WaterMa	Fertilizer	WaterMa nag	Fertilizer * WaterMa
F	26.61	4.72	5.07	3.13	86.23	0.74	10.06	38.16	1.61	8.74	128.05	7.68
Sig.	0.000	0.005	0.000	0.010	0.000	0.761	0.000	0.000	0.089	0.000	0.000	0.000
Partial Eta	0.74	0.20	0.62	0.25	0.82	0.19	0.52	0.67	0.34	0.48	0.87	0.71

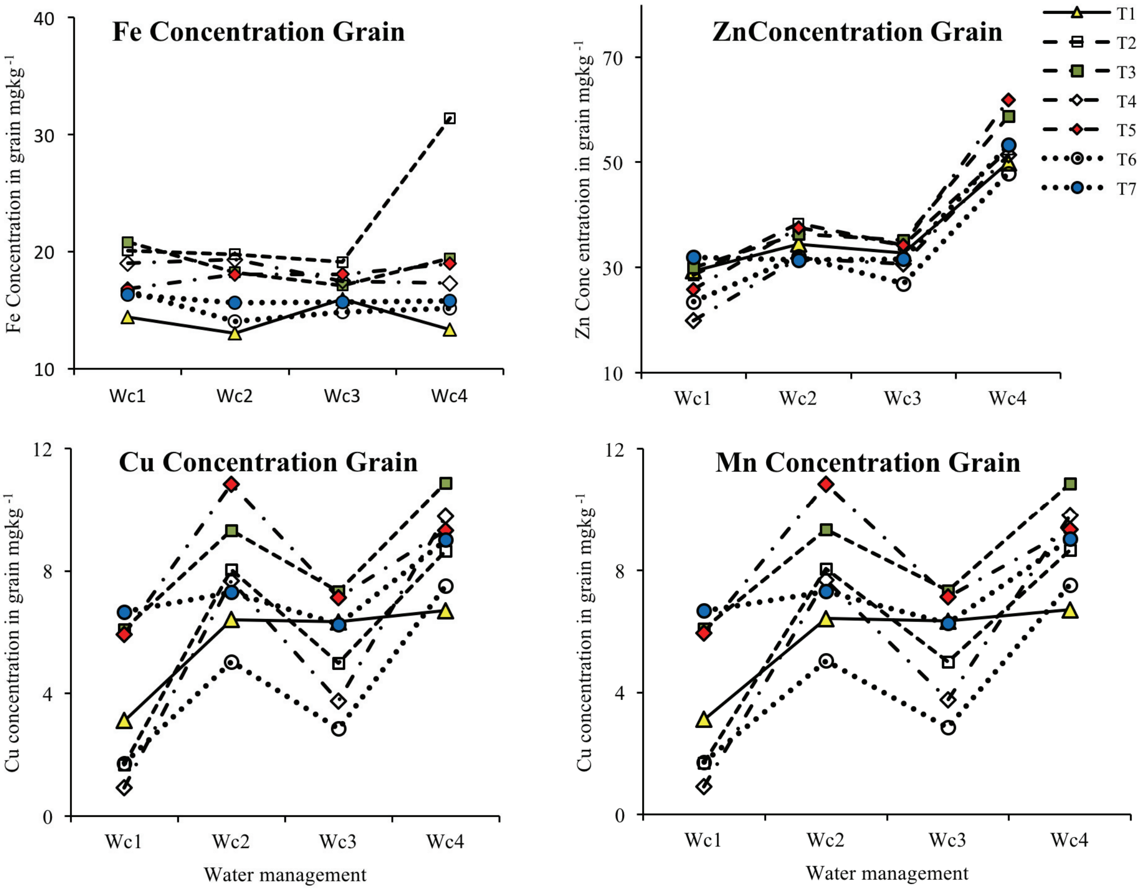

Figure 4.4 Representation of the estimated marginal mean between water management and fertilizer applications using grain micronutrient concentration as dependent variable at $p<0.05$. T1. Control (NF); T2. 200g FYM; T3. 200g FYM+FF; T4. 100g of FYM+CF; T5. 100g of FYM + CF+FF; T6. CF and T7. CF+FF

Results indicate that adding FYM was the key factor to increase Fe concentration; T1, T6 and T7 tended to be lower despite of different water control. Zn concentration was highly associated with *Eh*, increasing in Wc2 to Wc4 where aerobic conditions were established partially or permanently at growth stages. Concentration of Zn in grain was in correspondence with availability observed in soil. On the other hand, Cu concentration varied from one soil condition to another, but the trend tended to grow with the establishment of aerobic condition, in similar ways as was observed in Zn. Although Mn also showed a growing tendency from Wc2 to Wc4 there was not a clear pattern to follow mainly because there was no correspondence with the concentration measured in soil. More studies are therefore needed to elucidate the Mn behavior in rice grain.

4.4 DISCUSSION

4.4.1 Impact of fertilization and water management on morphology and grain yield

Chemical and organic fertilizers improve physicochemical soil properties providing micronutrients which are readily soluble and available for plants. In general, our results showed that either application of NPK fertilizer or FYM directly into the soil not only improved soil capabilities but also promoted an enhancement in some plant characters, such as number of panicles, total dry matter and grain yield (Table 4.1). Combination of FYM with NPK (T4-T5) was the most effective approach to enhance yield and morphological components. It is well known that OM is able to improve soil properties, increase the cation exchange capacity (CEC) and water holding capacity, while chemical fertilizer provide readily available macronutrients for plant (Brady and Weil 2014). Hence the combination of both could be responsible for better nutrition and consequently the improvement in yield and dry matter (Shahid *et al*., 2013; Ali *et al*., 2009).

Likewise, the good nutritional status achieved in soil due soil amendment, could have reduced the sterility in grain (Dobermann and Fairhurst, 2000).

Regarding the effect of water management on morphological behavior, we confirmed that, deliberate changes in soil electrochemistry using Wc2 and Wc3 treatments, exerted a positive effect on the growth parameters and yield. This observation supports our findings in our previous experiments (See Table 3.4). This is based on the assumption that soil transition from anaerobic to aerobic condition reduces electron activity causing significant transformation on N and micronutrients availability (Ashton, 2013), thus more sustainable rice water management practices such as SRI and AWD have reduced water and fertilizer inputs achieving high yield and dry matter accumulation (Bouman *et al.,* 2007). Our results reveal that there is a high potential in the combination of water management and the application of NPK alongside FYM, to increase or maintain comparable yield components at the same level of traditional rice production techniques. Unlike irrigated treatment, the permanent field capacity conditions inWc4-treatments, caused water stress which was the main constraint to increase dry matter and yield. However, in that condition yield response was higher by addition of high rates of organic manure in T2-T5. As aforementioned, increasing water holding capacity is one of the most important features of the application of organic manure for drought system. However other characteristics of less importance such as increased porosity, bulk density and high stability of aggregates, jointly would make possible better plant performance under such conditions (Vanlauwe *et al*., 2001). Although the application of farmyard manure have significant impacts on soil physical chemical and biological properties, (Brady and Weil, 2014) care must be taking when using high rates of manures. In our own experience we observed that high rates of OM in FYM treatments, resulted in retarding growth and yellowish leaves until 20-25 DAT, after which the plants recover very well but the vegetative cycle was bit enlarged. We suspect that, the stress was caused by the sharp decline of *Eh* during seedling stages and the anoxic condition. Under these conditions plant roots system and microorganism might compete for oxygen triggering plant stresses (Masunaga, personal Communication). With regard to FF of micronutrient, no significant effects were seen in morphological behavior or grain yield. Our results are consistent with a deep revision about this topic made by Fageria *et al*., (2009) which concluded that foliar fertilization may not necessarily increase yield but may favor nutritional value of grain when is applied during reproductive stages.

4.2 *Micronutrient availability in soil and its influence in Grain contents*

The advantages achieved by the application of organic and inorganic fertilizer or even combination of both was barely noticeable on the availability of Fe in soil. First because Fe availability measured prior to this experiment was sufficient to meet the plant demand (See Chapter 2 and 3). Secondly, the contents of Fe in sheep manure and NPK fertilizer was not high enough to increase soil Fe pool. The establishment of flooding conditions and the increase in electron activity, stimulated by microbes in treatments where FYM was added, could be the key factor to achieving the peak in Fe availability (Brady and Weil, 2014). In spite of the fact that Fe availability was significantly reduced in aerobic conditions (e.g. Wc3 and Wc4), the concentration did not fall below critical values $<2 \mathrm{mgkg}^{-1}$,(Dobermann and Fairhurst, 2000) keeping concentration high enough to meet plant demands. Likewise, it was found that, the contents in grain increased in T2 and T3 slightly compared to the control and chemically treated treatments. Addition of organic matter improves microbial activity which is highly associated with Fe availability under flooding conditions. Microbes use Fe as electron acceptor transforming it to its most available form for rice (Brady and Weil, 2014) and consequently concentration in grain could be increased. Moreover, the formation of Fe and Zn chelates with OM holds these micronutrients in more available form for plant (Khoshgoftarmanesh *et al*., 2010).

Regarding the effects of FF on the concentration of Fe in grain, no evidence was found in favor of its beneficial effects. The feasibility of Fe foliar spraying can be limited for several factors such as low penetration rates due hydrophobic nature of leaves and its low to intermediate mobility throughout the phloem (Maschner, 2012). A review about this topic made by Sperotto (2013) mentioned "restrictions to the mobility of Zn and Fe supplied as cations can be expected due to the abundance of negative charges in the apoplastic space, which may limit their translocation to other plant compartments" some authors recommend the use of chelates instead. Besides that, recent studies demonstrated that, in a given soil type, if Fe availability is enough to support plant growth, the application of foliar Fe and other micronutrients would become irrelevant (Aciksoz *et al*., 2011). Ideally, our FF treatments aimed to increase contents in grain rather than make deficiency corrections. Since Fe availability was higher enough to meet plant demands, we decided to use 1% $FeSO_4$ instead the recommended 2-3 % dose (Dobermann and Fairhurst, 2000) in order to minimize the risk of toxicity. The decision might have impeded a relationship between Fe concentration in grain and FF.

Concentration of Zn in soil was above the critical level 2.0 $mgkg^{-1}$ to consider deficiencies (Dobermann and Fairhurst, 2000). Thus, the concentration of Zn in soil was not considered as limiting factor. Although the application of FYM increased significantly Zn in soil concentration, the increase in grain was not in accordance with our expectation. This is in agreement with Gao *et al*., (2006) who stated that increasing Zn in soil does not affect its concentration in grain. Therefore, water management and foliar fertilization would be responsible to increase the contents of Zn in grain.

The transition from flooding to soil aerobic condition in Wc2 and Wc3 not only entailed physicochemical changes (e.g pH, Eh) but also promoted tillering and root enlargement (see also chapter 2 and 3). Increasing soil-root contact, consequently can improve the efficiency for N, P and K and other essential nutrients uptake (Yang *et al.,* 2004). Our results suggested that there is high root micronutrient uptake when redox potential is deliberately changed. Generally, transition to aerobic condition may increase Zn availability because Fe is less available due re-oxidation (Rengel *et al*., 1999). In order to meet Fe requirements, the rhizosphere is able to exude organic chelating molecules called phytosiderophores (Marschner, 2012) which under certain conditions cations such as Zn Cu, Mn and other micronutrient can use these chelating molecules as metal transporters (Vonwiren *et al*., 1996; Meda *et al*., 2007). This plant uptake strategy combined with root enlargement could justify in part the increase of micronutrients in grain observed when *Eh* was increased.

Grain Cu concentration was controlled by the addition of FYM and water management (Table 4.2). As a reference, the application of 200g of FYM in T2 increased Cu concentration >20% in Wc2 and Wc4 compared to control. However in T2-Wc1 it reduced by >80% compared with T1-Wc1. Addition of FYM has been identified as a good Cu source (Gao *et al*., 2000). But establishment of anaerobic condition in combination with decomposable OM promote the formation of insoluble Cu compounds such as Cu-ferrite and CuS (Dobermann and Fairhurst, 2000), therefore, aerobic condition in presence of decomposable OM could increase Cu availability to plant and thus its concentration in grain. Appropriate management of FYM application and irrigation might allow farmers to control either Cu deficiencie including concentration in grain or pollutant effects. As corrective approach, we observed a notorious effect of FF in promoting the Cu concentration in grain. This feature suggests that Cu has intermediate to high mobility in plant. Generally, the application of Cu has been highly fostered to control fungal diseases and to reduce grain sterility (Dobermann and Fairhurst, 2000) but the literature about its effects on rice grain concentration is very limited. Thus, the behavior of this micronutrient from leaves to the panicle remains unclear.

Generally, in paddy fields the Mn availability increases by reduction of *Eh* and the application of organic matter. (Mandal and Miltra, 1982; Marschner, 2012).Our results showed a Mn concentration of >40 mgkg^{-1}, high enough to meet plant requirements (Dobermann and Fairhurst, 2000).A good relationship with *Eh* was also noticed. However, no correspondence between Mn availability in soil and concentration in grain was observed. The use of FYM apparently could have accelerated Mn availability due the preferential order (Fig. 1.3) of microbes to use other element than O_2 as electron acceptor (Brady and Weil, 2014). Fertilization approaches were ineffective in increasing the Mn availability in soil but rather water management remains the key factor to increasing Mn. The conditions created by NPK+FYM and NPK at WC3 and Wc4 significantly increased Mn concentration but the causes are unknown due to lack of correspondence with soil availability. We suspect that reduction in Fe availability due to establishment of aerobic conditions which may have induced antagonistic effects with Mn (Table 3.4). Furthermore, application of FF did not exert any effect on Mn concentration in grain. Although Mn deficiencies are rare in paddy fields, foliar application has been recommended to correct deficiencies in upland regimes and calcareous soils. However, there is enough evidence that Mn has very low phloem mobility from leaves to grain (Millaleo *et al*., 2010). Likewise, Graham *et al*., (1988) reported large accumulation of Mn up to saturation point in roots and stem under high Mn availability at early plant stages. Studies in other crops (e.g. *Lupinus angustifolius*) showed storage and translocation of Mn from roots to seed development even when the availability of Mn was environmentally limited (Hannam *et al*., 1985).

Micronutrient concentration in FYM is variable and depends on the quality and composition of the animal feed and environmental factors. Therefore, the increase in soil only will be possible by using highly concentrated manures. However, care should be taken because manures contain other heavy metals including Cu which can become undesirable heavy metals in grains and in soil as pollutants.

4.5 CONCLUSION

The results obtained in this study showed that the use of agricultural practices can improve nutritional status of soil as well as morphological behavior to a greater or lesser extent. Although, the concentration of micronutrients in soil showed a considerable enhancement in almost all amended treatments, the availability in soil mainly relied on changes caused by water management on soil redox potential. The application of organic manure increased the concentration of Fe in grain for all treatments compared with control and chemical fertilizer application treatments. The concentration of Zn and Cu in grain produced the best results under drained and permanent upland condition while Mn concentration did not correspond to the concentration observed in soil. Presumably antagonism with some other micronutrients inhibited Mn uptake and accumulation in grain.

The application of foliar fertilizer ($ZnSO_4$ and $CuSO_4$) at reproductive stage in combination with the organic fertilization, allowed the increase in Zn and Cu concentration in grain compared with other treatments but foliar $FeSO_4$ did not improve Fe concentration in grain probably due to its low translocation rate.

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CHAPTER V

Characterization of Upland and Lowland Rice Genotypes Regarding to the Contents of Essential Nutrients and Phytic Acid in Grain

5.1 INTRODUCTION

Domesticated rice varieties are mainly produced in two different ecosystems: irrigated - rainfed lowland which accounts 80% of worldwide area, supporting approximately 92% total rice production, and rainfed upland, which makes up the remaining 20% production area, sustaining barely less than 8% of total rice production (Doberman & Fairhurst, 2000). Given its high consumption (no matter the growth ecosystem), rice provides 21% and 15% of the global dietary energy and protein supply, respectively (McClean et al., 2002). However, rice is a poor source of Vitamin A, thiamine, riboflavin, Zn, Fe and I (Juliano, 1993) while it is relatively rich in Se, Mg, Mn, K, P (Heinemann et al., 2005) as well as anti-nutritional (phytates) compounds (Bohn et al., 2008).

Deficiency of micronutrients or so called "hidden hunger" has become a major public health concern that affects one third of the world population, overlapping developing countries, and communities where rice is the staple crop. Reasons to justify hidden hunger are countless, however the displacement of landraces in favor of modern high yield varieties (HYV) during the green revolution had the major repercussion on nutritional biodiversity. In spite of the successfulness of HYV increasing the yield over 200%, its adoption exacerbated the prevalence of micronutrient malnutrition (Welch, 2001) due its high demand of fertilizer inputs and depletion of soil micronutrient pools. Hence, there is a consensus about the need to integrate improvement in grain quality (ex. plant breeding) and farming practices as the most practical way to increase the density of nutrients in grain.

Generally, the rice grain nutritional value is associated with plant genotypes, environmental conditions, and the availability of nutrients in soil (Yang et al., 2007). Unlike growers who often utilize high yield varieties in lowland ecosystems, farmers who produce rice under upland conditions are forced to use landraces which are distinguished by their drought tolerance, and low input demands. Under such conditions (upland) the availability of N, K, P, Mg, Ca, Si, Mn and Fe tend to be lower than lowland ecosystems either because these elements are involved in redox reactions or due their association with colloidal soil structure (Dobermann and Fairhurst, 2000). However, upland genotypes are genetically designed to optimize physiological processes and nutrient uptake where the modern HYV do not. In addition, the rice plant can alternatively use different strategies to uptake nutrients from soil by releasing organic compounds in rhyzosphere (e.g. phytosiderophores) even when their availability is limited by external factors (Marschner and Römheld, 1994), facilitating the flow of nutrients from roots and other tissues to the grain.

The differences between upland and lowland genotypes in regard to nutritional value has not been widely documented. Upland genotypes differ greatly in starch composition, digestibility, lipid contents and double contents of proteins (Frey and Becker, 2005). Studies carried at IRRI found that certain landraces had double the contents of Fe and Zn compared with HYV (Kennedy and Burlingame, 2003). Recent studies conducted by Zhang et al. (2013), reported that upland rice is more genetically diverse than the irrigated lowland rice. This suggests that rice polymorphism is associated with genotypes (Crusciol et al., 2008; Anuradha et al., 2012) and environmental interactions (Heinemann et al., 2005; Norton et al., 2014). Likewise, the notorious features regarding to nutritional value could be associated to the over expression of some specific genes to control nutrient remobilization and transport to the grain in response to deficiencies (Lian et al., 2006; Ishimaru et al., 2010).

Rice and anti-nutrients contents

As aforementioned, rice grain is a good source of P which is considered as a precursor of phytic acid [inositol hexakisphosphate] (Lott et al., 2000). Phytic acid is ubiquitous element considered as an antioxidant and anti-nutritional compound (Bohn et al., 2008). Its major function in the human body is the sequestration of free radicals involved in causing cancerous illness. However, its high contents in rice grain also inhibit the bioavailability of some important micronutrients. In cereals, phytates are rapidly accumulated in seeds during the ripening period. Regarding the rice grain content of phytates, " phytates exhibit a high affinity for all polyvalent cations"(e.g. Fe and Zn) (Graf and Eaton, 1990). Thus, in terms of human nutrition far beyond its antioxidant properties, phytic acid represents a limitation for vulnerable populations wherein rice constitutes the most important component of the diet. Consequently, the inhibition of nutrients bioavailability will cause serious imbalances on recommended dietary allowances (Yang et al., 2007). Therefore the screening for rice varieties for high nutritional value and low levels of phytic acid merits further evaluations.

Up to now, there are very few studies about the potential of rainfed upland ecosystems to produce rice with not only drought tolerance, but also higher nutritional value. The assessment of our germplasm resources will allow us to identify varieties with higher nutritional quality for vulnerable populations. Thus, the purpose of this study was to determine the differences in nutrients and phytic acid content in grain between upland and lowland rice genotypes.

5.2 MATERIAL AND METHODS

A total of 46 rice varieties, including six red pigmented cultivars from the collection of genetic resources of Shimane University were clustered into two groups; 18 upland genotypes (UpG) and 28 lowland genotypes (LwG) (Table 5.2 and 5.3). One month old seedlings (May 12th, 2010; May 20th 2013) were transplanted at a density of 22,2 plant/m2 (15cm x 30cm spacing) in a lowland irrigated experimental field of Shimane University. The main soil properties are shown in Table 5.1.

Table 5.1 Soil physicochemical properties.

Soil Type :	Sandy Loam
Clay (%)	8.44
Silt (%)	22.55
Sand (%)	69.01
pH $_{(H_2O)}$	6.77
pH $_{(KCl)}$	5.48
EC (mS/m)	8.58
Ex.Ca (cmol$_+$/kg)	11.92
Ex K (cmol$_+$/kg)	0.65
Ex. Mg (cmol$_+$/kg)	2.92
Ex. Na (cmol$_+$/kg)	0.33
Avail. P "Bray II" (mg/kg)	583.71
Fe (mg/kg)	1286.72
Zn (mg/kg)	13.48
Cu (mg/kg)	9.09
Mn (mg/kg)	315.33

Plots were fertilized with slow release chemical fertilizer Ube Kousan Nouzai Co/ltd,"Kumiai UCoat 364 for lowland rice" (N 13% :P_2O_5: 16% :K 14% :Mg 2%) at a dosage of 30.8 g/m^2 or 40 Kg/ha N in order to meet crop demand.

5.2.1 *Grain analysis*

The grains were oven dried at 40°C for 12 hours and de-hulled with a rice de-huller; (Type THU 35B, Sakate Co. Ltd. Tokyo, Japan). For the analysis of grain nutrient content, all samples were ground into fine powder in agate grinding jars, using a mixer mill (MM200, Retsch GmbH, Haan, Germany). Ground samples were further oven-dried for 12 hours at 80 °C. A portion of 0.5 g of each sample was digested in 2.5 ml HNO_3 in a Teflon vessel. Afterwards samples were oven heated at 160°C for 4 hours, cooled overnight, and diluted with distilled water up to 25 ml (Koyama and Sutoh, 1987). The concentrations of Al, Fe, Zn, Cu and Mn were determined by Inductive Coupled Plasma Mass Spectroscopy (8800 ICP-MS Triple Quad, Agilent Technologies, Japan). Other macronutrients such as Ca, K, Mg, Na and P were determined by Inductive Coupled Plasma Spectroscopy (ICPE-9000; Shimadzu, Japan).

5.2.2 *Phytic acid (PA) determination*

Contents of PA in rice grain were determined by the following method previously used by Dai et al., 2007; Wei et al., 2012. The ground grains (0.5 g) were placed in a centrifuge tube and extracted with 10 ml of 0.2N HCl. Samples were shaken for 2 hours at 30 r.p.m and centrifuged for 10 minutes at 10^4 rpm. The supernatant of 2.5 ml was transferred to new clean centrifuge tube, and 2 ml of 2% $FeCl_3$ solution was added. The mixture was shaken for 2 minutes by hand and was boiled for 30 minutes in a water bath. After cooling, the solution was centrifuged for 15 minutes at 10^4 r.p.m. The resulting supernatant was discarded, and the precipitate was washed 3 times with 5ml of extra pure water for each time. Thereafter 3 ml of 1.5 mol L^{-1} NaOH was added to the precipitate, shaken and centrifuged for 10 minutes at 10^4 r.p.m. The supernatant was discarded again, and 3 ml of 0.5 N HCl was added to dissolve the precipitate. Finally, samples were diluted with extra pure water up to 25 ml. The Fe concentration was analyzed by ICPE-9000. Phytic acid content in grains were calculated by multiplying Fe concentration by the Factor 4.2.

The molar ratio between *PA* and minerals was calculated based on molecular weight (M) of *PA* ($C_6H_{18}O_{24}P_6$: 660.04 gmol^{-1}) and cations (Fe:55.85, Zn:65.41, Ca:40.08) gmol^{-1}. Equation (1) (Tamanna et al., 2013).

$$Molar\ Ratio = \frac{PA_{\mathrm{conc.}}}{PA_{\mathrm{M}}} \bigg/ \frac{\mathrm{Cation}_{\mathrm{conc.}}}{\mathrm{M}} \tag{1}$$

5.2.3 *Statistical analysis*

To determine the significant differences between UpG and LwG nutrient concentrations and phytic acid contents, data were statistically analyzed by the independent sample T-test, assuming equality of variance (Levene's test) at $P<0.05$. All statistical analysis was performed using IBM SPSS Statistic v22.0 (IBM SPSS, 2014. Chicago IL, USA).

5.3 RESULTS

5.3.1 Effect of differents genotypes on the grain essential nutrient contents

3.1 Accumulation of nutrients in upland and lowland genotypes

Comparison, between UpG and LwG in terms of nutrient contents and PA are present in Table 5.2. T-test for independent sample analysis showed that the concentration of Fe, K, and P differed significantly ($p \leq 0.05$) The UpG exceeded LwG for Fe, K, and P concentration by 11%, 10%, and 7%, respectively. Likewise, it was noticed that the concentration of Fe, K and P in UpG exceeded the mean values obtained in LwG by 80%, 72% and 77%, respectively (Table 5.3 and 5.4). Regarding the other elements analyzed (Zn, Cu, Mn, Ca, Mg, Na and Al), no significance differences were observed. However, the concentration of Zn (4%), Ca (5%), Mg (5%) and Al (20%) in grain were greater in UpG compared to LwG. When comparing the nutrient contents of red, green, and white pigmented varieties, there were no significant differences (data not shown). However, it was observed that almost all red pigmented varieties had higher Zn concentrations as shown in Table 5.3 and 5.4.

Table 5.2 Student *t*-test to estimate differences in grain nutrient contents between UpG and LwG at $p<0.05$. between UpG and LwG.

	Fe	Zn	Cu	Mn	Ca	K	Mg	Na	P	Al	PA
UpG M (mgkg^{-1})	14.60	33.66	1.48	25.01	146.09	1276.3	1569.3	104.47	4113.9	2.10	3057.6
LwG M (mgkg^{-1})	13.20	32.48	1.56	24.37	138.70	1161.7	1496.9	101.41	3862.2	1.75	2933.4
GM (mgkg^{-1})	13.75	32.94	1.53	24.62	141.59	1206.54	1525.19	102.61	3960.67	1.89	2982.00
M Diff.(mgkg^{-1})	1.40	1.18	-0.08	0.64	7.39	114.56	72.40	3.07	251.75	0.35	124.20
% CV LwG	13.73	14.61	52.20	20.37	15.57	13.88	11.31	13.15	11.00	29.82	15.70
% CV UpG	10.82	14.96	50.66	22.31	16.11	12.78	7.47	14.65	8.52	32.37	8.05
Test t-test assuming equal variances											
Sig.	0.01*	0.43	0.73	0.69	0.29	0.02*	0.09	0.49	0.04*	0.08	0.24

M= mean observed on each group; GM general mean; MDiff.= mean differences between UpG/LwG´s observed mean; SD±= GM´s Standard Deviation and Sig* indicates a statistically significant differences between groups at $p\leq0.05$

5.3.2 Upland genotypes nutrients features

Detailed analysis of micronutrient concentrations (Fe, Zn, Cu and Mn) in grain of the UpG are listed in Table 5.3. The Fe concentration ranged from 11.39 mgkg^{-1} in *Rikotou Rikuu* to 17.43 mgkg^{-1} in *Senshou* with an average of 14.60 mgkg^{-1} dry matter basis which was significantly higher than LwG, and low CV=11% was observed among UpG. The variety *Houmanshiden Ine,* "Red pigmented" had greater Zn=45.88 mgkg^{-1} and Mn=41.04 mgkg^{-1} concentrations. The Fe concentration for *Senshou* and the Zn and Mn concentrations in *Houmanshiden Ine* varieties were not only the highest among UpG, but they were also the greatest among the 46 cultivars assessed. The concentration of Cu had a mean value of 1.48 mgkg^{-1}.Cu content varies greatly from one variety to another ranging from 0.68 mgkg^{-1} in *Oiran* to 3.25 mgkg^{-1} in *Hakamuri* with a considerable CV= 51%. Likewise, results confirmed that rice is a very good source of P, K, Mg and Ca. Among all of the nutrients required for grain production, P was the most abundant >3500 mgkg^{-1}. The cultivar *Okabo* had the highest concentrations of P = 4850.60 mgkg^{-1} and Mg =1817.73 mgkg^{-1} among both UpG and LwG while the lowest concentration of P was found in *Hakamuri* cultivar 3571.43 mgkg^{-1}. K was the third most abundant element, and its contents were between 1031.75 to 1640.16 mgkg^{-1}. The highest concentration of K was observed in *Kaneko*. Contents of Ca were between 111.78 mgkg^{-1} in *Dango* to 185.63 mgkg^{-1} in *Hirayama* with a CV= 16%. Na and Al were the least abundant elements with mean values of 104.47 mgkg^{-1} and 2.10 mgkg^{-1}, respectively.

5.3.3 *Lowland genotypes features*

Table 5.4 summarizes the chemical properties of the 28 LwG cultivars. The concentration of Fe decreased 10% compared with UpG. The variety *Shinriki Mochi*, had the highest concentrations of Fe (16.22 mgkg^{-1}) and Zn (43.71 mgkg^{-1}) among LwG while *Radin Goi Sesat* had the lowest concentrations of Fe and Zn, which were least among all of the cultivars tested. The CV=14% for Fe was much higher in LwG while Zn contents had a similar CV to UpG. The contents of Cu in LwG varies greatly from one cultivar to another just like in the UpG. Cu contents ranged from 0.59 mgkg^{-1} in *Kyoto asahi* to 3.91 mgkg^{-1} in *Nerica* which not only had the highest Cu concentration in LwG, but also among all of the varieties assessed. The highest contents of Mn in LwG was found in *Ishihiro* which was 35.70 mgkg^{-1}. Looking at the concentrations of macronutrients, the mean value of K was 1161 mgkg^{-1} which was significantly lower compared to UpG. Results show that the K concentration ranged between 873 mgkg^{-1} in *Radin Goi Sesat* to 1505 mgkg^{-1} in *Mansaku*. *Mansaku* also had the highest Mg concentration among the LwG. The highest concentrations of P, Ca and Na in LwG were observed in *Shinyamadaho II* variety which were 4594 mgkg^{-1}, 190 mgkg^{-1} and 131 mgkg^{-1}, respectively.

Table 5.3 Nutritional composition and accumulation of phytic acid (mg kg^{-1}) in grain of upland genotypes.

ID	Upland Cultivar	Fe	Zn	Cu	Mn	Ca	K	Mg	Na	P	Al	PA
JRC-03	Hinode	15.19	38.75	0.82	31.93	181.91	1411.53	1645.13	94.43	4239.56	2.12	3343.20
JRC-04	Senshou	17.43	35.54	1.53	23.66	128.75	1384.70	1541.80	104.05	4370.76	2.38	3007.20
JRC-06	Kaaneko	15.63	30.84	0.98	30.01	184.39	1640.16	1699.80	132.70	4637.18	2.75	3360.00
JRC-08	Okkam Modoshi	13.45	29.95	0.92	20.40	154.88	1314.74	1429.28	111.06	3720.12	2.53	2788.80
JRC-10	Hirayama	15.19	31.03	0.88	26.36	185.63	1526.95	1711.58	101.30	4501.00	2.39	3259.20
JRC-11	Kahei	16.84	29.56	0.81	22.02	156.62	1314.23	1625.49	91.90	3977.27	2.03	2956.80
JRC-12	Oiran	13.77	26.50	0.68	23.83	157.61	1417.98	1526.68	81.52	3908.10	2.72	2973.60
JRC-13	Bouzu Mochi	14.61	31.07	1.31	20.37	112.60	1051.59	1433.53	95.73	3655.75	2.84	2738.40
JRC-21	Akamai Kōchi(a)	14.52	28.07	0.88	17.82	152.20	1287.43	1571.86	97.80	4251.50	2.56	3225.60
JRC-25	Dango	16.81	36.28	2.48	29.69	111.78	1222.55	1646.71	94.31	4291.42	2.97	3024.00
JRC-29	Shichimenchō Mochi	14.74	40.01	2.31	24.67	135.07	1227.90	1576.62	135.56	4037.33	2.72	3192.00
JRC-40	Akamai Nagasaki(a)	14.00	29.77	1.27	19.79	159.27	1257.40	1538.46	87.77	4166.67	1.08	2721.60
JRC-43	Akamai	13.58	35.59	2.07	23.30	121.79	1134.12	1444.77	94.18	3895.46	1.32	3040.80
JRC-47	Okabo	15.00	39.45	1.33	28.62	143.92	1309.76	1817.73	107.57	4850.60	1.43	3242.40
JRC-48	Hakamuri	13.15	35.57	3.25	22.18	125.00	1031.75	1393.85	127.48	3571.43	2.00	2990.40
JRC-49	Rikutou rikuu 2	11.39	30.62	0.98	23.30	144.42	1240.04	1593.63	107.57	4108.57	0.91	2889.60
JRC-53	Raiden	12.11	31.41	1.51	21.16	119.07	1062.25	1408.10	97.83	3661.07	0.99	3578.40
JRC-54	Houmanshinden Ine(a)	15.33	45.88	2.57	41.04	154.69	1137.72	1641.72	117.76	4206.59	1.99	2704.80
Means		14.60	33.66	1.48	25.01	146.09	1276.27	1569.26	104.47	4113.91	2.10	3057.60
SD ±		1.58	5.03	0.75	5.58	23.53	163.05	117.23	15.30	350.54	0.68	246.24
(%) CV±		10.82	14.96	50.66	22.31	16.11	12.78	7.47	14.65	8.52	32.37	8.05
(%)Increasing over LwG		10.5*	3.6^{ns}	−	2.6^{ns}	5.3^{ns}	9.9*	4.8^{ns}	3.0^{ns}	6.5*	20.0^{ns}	4.0^{ns}

* Significant differences at $p<0.05$; ns = no significant differences; (−) = No increment was registered; PA= Phytic acid; (a) Red pigmented varieties.

Table.5.4 Nutritional composition and accumulation of phytic acid (mg kg^{-1}) in grain of lowland genotypes.

ID	Lowland Cultivar	Fe	Zn	Cu	Mn	Ca	K	Mg	Na	P	Al	PA
JRC-19	Wataribune	14.18	31.16	1.88	30.47	151.50	1205.00	1410.00	111.00	3510.00	1.95	2469.60
JRC-22	Mansaku	14.23	34.95	0.87	29.49	136.50	1505.00	1750.00	97.50	4525.00	2.39	3561.60
JRC-23	Ishijiro	14.39	35.43	1.53	35.70	155.07	1406.56	1650.10	123.26	4204.77	1.87	3124.80
JRC-24	Joushuu	14.09	29.86	0.85	24.97	143.00	1145.00	1605.00	98.50	3830.00	1.91	2940.00
JRC-26	Aikoku	12.77	30.24	0.60	23.29	124.75	1198.22	1538.46	108.97	4018.74	2.87	2856.00
JRC-27	Ginbouzu	15.64	35.80	1.11	22.27	122.80	1232.88	1629.16	103.72	4099.80	2.53	3964.80
JRC-28	Shinriki Mochi	16.22	43.71	2.86	32.03	133.00	1310.00	1700.00	95.50	4130.00	1.93	3124.80
JRC-31	Kameji	15.84	35.50	1.63	23.53	117.53	1100.60	1628.49	107.07	3730.08	2.02	2469.60
JRC-32	Omachi	13.14	35.35	3.00	26.74	116.37	1178.50	1449.70	86.29	3722.88	2.00	2973.60
JRC-33	Shinriki	12.21	38.19	2.40	22.52	124.01	1027.67	1353.75	115.12	3344.86	1.56	2452.80
JRC-34	Kyoutoasahi	15.64	35.35	0.59	27.82	158.55	1386.68	1471.17	117.79	4110.34	2.35	2587.20
JRC-35	Kabashiko	14.87	35.12	0.77	32.36	149.70	1357.29	1686.63	115.27	4316.37	1.81	3444.00
JRC-36	Sekiyama	15.26	38.18	1.21	30.00	152.86	1247.53	1696.25	97.63	4413.21	1.99	2805.60
JRC-37	Shinyamadaho 2	13.09	30.55	0.68	23.00	189.60	1391.09	1722.77	131.19	4594.06	1.60	4099.20
JRC-42	Touboshi [(a)]	11.86	29.04	1.01	22.09	174.56	1148.92	1489.15	101.58	4127.22	2.34	2889.60
JRC-44	Karahoushi[(a)]	13.20	37.67	2.22	21.86	113.59	1056.55	1378.97	116.07	3705.36	1.13	3108.00
JRC-45	Hiyadachitou	12.76	31.64	1.07	19.61	112.77	1152.69	1571.86	84.83	3842.32	1.91	2973.60
JRC-52	Aichiasahi	10.83	31.58	1.79	17.14	111.17	1002.96	1309.29	113.64	3320.16	1.04	2755.20
WRC-13	Asu	10.71	22.82	0.86	20.94	134.73	1117.76	1317.37	86.33	3493.01	0.79	2587.20
WRC-18	Qingyu(Seiyu)	10.88	27.76	1.37	15.96	104.58	1125.50	1479.08	87.65	3944.22	1.05	2906.40
WRC-33	Surjamukhi	14.43	29.88	1.23	18.92	133.70	954.27	1341.95	108.35	3499.01	1.56	2486.40
WRC-45	Ma sho	13.03	31.92	1.37	22.25	143.00	1095.00	1595.00	89.50	4300.00	1.45	2906.40
WRC-49	Padi perak	12.01	28.30	1.48	29.45	165.50	975.00	1315.00	97.00	3605.00	1.57	2553.60
WRC-61	Radingoi sesat	9.22	20.01	1.64	20.60	165.67	873.25	998.00	75.85	2814.37	2.21	2083.20
	Hatsubashi	12.28	33.00	1.17	18.90	116.27	1057.88	1487.03	88.82	3712.57	0.99	2956.80
	Sachikaze	11.03	31.68	2.67	22.12	133.47	1015.94	1369.52	98.61	3471.12	1.23	3528.00
	Sari Queen	11.40	30.98	1.83	21.71	163.86	925.74	1386.14	93.07	3415.84	1.08	2536.80
WAB	Nerica	14.40	33.73	3.91	26.49	135.42	1334.33	1582.34	89.29	4340.28	1.87	2990.40
Means		13.20	32.48	1.56	24.37	138.70	1161.71	1496.86	101.41	3862.16	1.75	2933.40
SD ±		1.81	4.74	0.81	4.96	21.60	161.24	169.26	13.34	424.65	0.52	460.48
CV (%)		13.73	14.61	52.20	20.37	15.57	13.88	11.31	13.15	11.00	29.82	15.70
(%)Increasing over UpG		−	−	5.4 ^{ns}	−	−	−	−	−	−	−	−

ns = no significant differences; (−) = No increment was registered; PA= Phytic acid; [(a)] Red pigmented varieties.

5.3.3 *Phytic acid*

Although there was no significant difference in the concentration of PA in grain between UpG and LwG (Table 5.2), UpG had a greater concentration of PA compared to LwG. The concentrations differed widely among varieties ranging from 2705 mgkg^{-1} in *Houmanshiden Ine* to 3578 mgkg^{-1} *Raiden* in UpG while in LwG, the ranges were 2083 in *Radingoi* Sesat to 4099 mgkg^{-1} in *Shinyamadaho II.* Also, the coefficient of variation of LwG (16%) was larger than UpG (8%).

In addition, the inhibitory effect of PA over some multivalent metal ions was calculated for both genotypes (Table 5.5). These values are associated with micronutrient bioavailability and are used as an indicator of nutritional quality of the grain. In both LwG and UpG, the PA/Fe ratio was higher than the established critical value suggesting low bioavailability of Fe. Moreover, the molar ratio of PA/Fe was greater than the molar ratios of PA/Zn, PA/Mn and PA/Ca. The PA/Zn and PA/Ca ratios were below their critical values.

Table 5.5. Phytic acid/ multivalent metals Molar ratios.

PA Molar Ratio	Fe	Zn	Cu	Mn	Ca
UpG	17.73	9.00	199.38	10.16	1.29
LwG	18.80	8.95	181.39	10.00	1.30
Mean	18.36	8.97	188.20	10.07	1.30
Critical Value*	≥14	≥10	ND	ND	≥1.56

* Critical values were suggested by Tamanna et al., 2013, citing several authors.

5.4 DISCUSSION

Nutrient contents in the rice grains were in the order of P > Mg > K > Ca > Na > Zn > Mn > Fe > Cu for UpG as well as LwG. Significant variation was observed among cultivars suggesting that the accumulation of nutrients and favorable nutritional traits are strongly associated with genetic diversity and the environment (Anuradha et al., 2012; Chen et al., 2012). In terms of ecotypes and nutrient concentration, it was found that UpG had significantly greater Fe, K and P contents. This feature could give a new added value to upland rice varieties based on their nutritional value. Although other measured minerals did not differ significantly, concentrations were slightly higher than LwG with the exception of Cu. Our results are partially consistent with previous research by Crusiol et al.(2008) who reported that upland varieties were able to increase proteins, N, P, Ca, Mg, Fe, and Zn levels while S and Cu had reduced levels in polished grains. The results indicated that UpG seem to have a high efficiency in nutrient uptake and in nutrient accumulation in grain. The rice plant has developed physiological mechanisms to protect itself against water shortages and nutrient deficiencies. The plant is also able to control photosynthetic activity (Fukai et al., 1985), use alternative nutrient uptake strategies (Marschner and Römheld, 1994), and activate the expression of specific genes for drought tolerance (Liu et al., 2014) and nutrient transport (Ishimaru et al., 2010). We must assume that nutrient efficiency mechanisms vary between genotypes, and they could be differentially expressed for more than one mechanism as well as not expressed in the less-efficient genotype (Rengel, 1999). Despite that UpG grow under non-stressed conditions, these genotypes might retain inactive mechanisms to avoid or minimize the impact of abiotic stresses. Such mechanisms could represent an advantage over LwG with regard to nutrient efficiency even when those stresses have not been environmentally provoked.

Table 5.6 shows the relationship among the nutrients in the grain. It was found that Fe, Zn, and Mn contents positively correlated each other suggesting they could partially share similar mechanisms in rice grains. Fe is the most important element for electron transfer in both photosynthetic and respiratory reactions in chloroplasts and mitochondria (Inoue et al., 2009). Because Fe and Mn are actively enrolled in both processes, it is believed that they share the same translocation route controlled by OsYSL2 genes (Ishimaru et al., 2010). The relationship between Fe and Zn in rice has also been reported by the over-expression of several genes such as HvNAS1, OsNAS1-3 and TOM1 increasing their concentrations (Bashir, 2013).

Table 5.6 Correlation matrix Phytates and nutrients concentration in rice grain.

	Fe	Zn	Cu	Mn	Ca	K	Mg	Na	P
Zn	.560**								
Cu	-.024	.435**							
Mn	.493**	.594**	.165						
Ca	.101	-.189	-.391**	.354*					
K	.609**	.200	-.327*	.448**	.454**				
Mg	.680**	.508**	-.206	.490**	.211	.745**			
Na	.248	.409**	.038	.293*	.170	.247	.238		
P	.621**	.394**	-.234	.451**	.331*	.804**	.908**	.237	
PA	.258	.252	-.121	.123	.088	.511**	.584**	.259	.582**

**. Correlation is significant at the 0.01 level.

*. Correlation is significant at the 0.05 level.

Likewise, concentrations of Cu only correlated with the concentration of Zn r=0.435. Studies conducted by Ishimaru et al. (2005)revealed that the expression of ZIP´s gene family are actively involved with Zn and Cu uptake and transport systems. Thus, the use of the same transporter family would directly affect the concentration of Cu and Zn in grain.

With regard to colored genotypes, the concentration of micronutrients was not affected due the contents of *Anthocyanin* (responsible for red color). The concentration of Zn was slightly greater compared to the white and green genotypes. This result is partially in agreement with observations made by Yang (1998) and Frey and Becker (2005) who stated that colored genotypes contained high micronutrient contents such as iron and zinc.

Another factor that could contribute to the accumulation of nutrients is the content of phytates. Phytates in grain are used as storage sites for cations such as Ca, Mg, K, Zn and Fe, and for the development of young plants (Graf and Eaton, 1990; Lu et al., 2013). PA accounted for approximately 75% of total P in both phenotypes. Our results were comparable to those found by Lott et al., (2000) who found that PA in legumes and cereals accounted for 60-90% of the total phosphorus concentration. PA concentrations had a high positive correlation with total P, r=0.582 at $p<0.01$. Another function of PA is to store P in phosphate (PO_4^{3-})form as an energy source (Bohn et al., 2008). The positive correlation between PA and P suggest that the contents of PA in grain could be predicted through determination of total P in grain. Other elements such as K and Mg had a high correlation at $p <0.01$ with PA of r=0.511 and r=0.584, respectively. The affinity of PA with K and Mg primarily appear as K-Mg salt in the rice bran and embryo, and it rapidly accumulates in seed as the ripening period progresses (Reddy et al., 1989; Iwai et al., 2012). Although PA did not correlate with any other element, the chelator effect of its PO_4^{3-}groups lead to the formation of complexes with mineral cations like Zn, Cu, Fe, Ca and Mn (Bohn et al., 2008).This affirmation could be corroborated by the correlation between P and almost all of the elements assessed.

Also, UpG showed high nutrient contents. We found that 72% of UpG had higher concentrations of PA than the mean observed in LwG. Although it might seem like the high contents of PA in UpG could negatively affect mineral availability in the human diet, there is a simple way to predict its antagonistic behavior towards nutrient bioavailability in rice. One of the most accurate ways to determine the mineral bioavailability of nutrients and their absorption in humans is by the determination of PA/mineral ratio (Su et al., 2014)

Eventhough humans require all the nutrients assessed in our studies for good health, we focused on the bioavailability of Fe and Zn because they are the most prevalent deficiencies in the world (Bashir et al., 2013). Our results showed that PA/Fe ratio in both lowland (18.80) and upland (17.73) genotypes were above the recommended critical value ≥14 (Table 5.5). The higher values in LwG indicate lower bio-availability than UpG (Tamanna et al., 2013). In contrast to Fe, the PA/Zn was lower than critical value ≥10 and similar in both genotypes. This suggests that the contents of PA does not limit Zn bio-availability. However due the chelator effect of calcium phytate on Zn bio-availability, dietary ratios of Ca × PA/ Zn would be a better predictor of Zn absorption than is the PA/Zn ratio alone (Lopez et al., 1998).

5.5 CONCLUSIONS

It may be concluded from the results that, UpG are a better nutritional source of Fe, K and P than LwG. Results revealed that UpG might have higher nutrient use efficiencies than LwG because nutrient acquiring mechanisms under environmental stresses such as drought stress can activate specific genes or alternative nutrient uptake strategies are present even when the stresses were not environmentally provoked. Furthermore, the PA/metal ratios and the correlation between concentrations of phytates and P in grains could help to predict the inhibitor effect of phytic acid on nutrient bioavailability. Although our study focused on comparing the grain nutritional values between ecotypes (UpG and LwG), variation was observed in the concentration of nutrients among cultivars of the same group. This implies that genetic variability exists. Regardless of the low yield reported in UpG by different authors, the screening of our genetic resources allowed for the identification of genotypes with higher nutritional value which can further plant breeding programs.

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CHAPTER VI

SUMMARY

This study examined the feasibility of agricultural practices (irrigation and fertilization and selection of varieties) to increase the concentration of micronutrients in rice grain, emphasizing on effect of water management and timing to control the availability of micronutrients in soil. The study further compares genotypic differences between upland and lowland rice varieties in term of nutrients and phytic acid concentration in rice grain.

In an exploratory study, the effects of water saving strategy on rice micronutrient concentration during post-heading stage in two soil types (Typic Fluvaquent and Typic Paleudult) was assessed, as well as its impact on morphology, yield parameters and availability of micronutrients in soil. The results revealed that regardless of soil type, the establishment of water saving did not have negative effects on morphological traits, meanwhile the grain yield was kept comparable to the conventional cultivation patterns. Establishment of aerobic soil condition during post anthesis stage also reduced the grain sterility, as well as increased root and shoot dry-mass in both soil. These features not only assisted rice water use efficiency, but also have potential of increasing micronutrient uptake efficiency due to its enhanced root growth. Changes in soil pH and redox potential also played an important role controlling micronutrient availability and toxicity. It was observed that availability of Zn slightly increased with the establishment of aerobic conditions while the toxic level of Mn in Typic Paleudult was reduced to less harmful levels. Mn toxicity was responsible for poor morphological behavior with consequent reduction in yield parameters and feasibility of this soil for rice production. In summary, the water saving practice in the post-anthesis stage has the potential to improve water use efficiency and to control micronutrients availability in soil, without sacrificing yield parameters.

In subsequent study of water saving strategy, the degree of effect of the availability of micronutrient in soil on the biofortification of rice grain was significantly varied at different reproductive stages (anthesis to dough-milky grain). Changes in soil redox potential and pH, from two weeks after heading, altered concentration of Zn, Cu, and Mn in grain. Although rice Fe concentration did not considerablly changed as consequence of water management, probably soil re-oxidation brought Fe to less available form, reducing the antagonist reactions with aforementioned elements. The grain-filling stage seems to be the most active stage for micronutrient uptake and translocation to the grain. Timing at this stage can be taken as reference for any biofortification purpose. Thus, changes in soil electrochemical properties through implementation of water management strategies seems to be the most feasible and environmentally friendly way to increase availability of micronutrients in soil and the concentration in grain.

The application of farmyard manures (FYM) and other forms of organic matter contributed to soil micronutrients availability by changing physical, chemical, and biological soil properties. The combination of NPK-FYM was the best approach for improving biomass, number of panicles, yield and reduction of grain sterility. Regardless of fertilizer treatments, the implementation of Alternative Wetting and Drying (AWD) was the best approach to increase number of panicle per plant. The application of foliar micronutrients fertilizer did not exerted any influence on morphological parameters. Most importantly, effects of FYM were observed about changes in Redox potential (*Eh*) and Zn availability in soils. The availability of Fe and Mn were higher associated with the duration of anaerobic conditions and low *Eh.* While Cu showed its better availability in oxidative soil condition (Wc3 : Water saving from 2 weeks after heading), Wc4: Field capacity) alongside FYM and FYM+NPK treatments. FYM also increased Mn availability at Wc4 condition. Fe concentration in grain increased indistinctly of fertilized treatments over Control. Although the application of FYM slightly improved the concentration of Fe, Zn, and Cu in grain, water management was the main factor controlling Zn, Cu and Mn in grain. Foliar micronutrient fertilization showed a slight increase only for Zn and Cu grain concentration.

Selection of high micronutrient crop varieties is an important tool to provide more nutrients into human diets. The result of our survey in rice grain of 46 genotypes revealed that concentration of any essential nutrient contents in rice grain widely varies among genotypes; which confirms the existence of genetic variability. Upland varieties were significantly higher in Fe, K and P in comparison to lowland varieties. Even though other elements such as Zn, Ca, Mg and Al achieved a non-depreciable increment in upland genotypes, no significance differences were observed between both genotypes. Evaluation of some red-pigmented varieties showed high Zn concentration compared with brown-white pericarp varieties. However, the result is not conclusive and further studies are recommended on red-pigmented varieties. The result reveals that upland genotypes might have higher nutrient uptake ability than lowland genotypes. The nutrients acquiring mechanisms in upland genotypes could be retained in activated even when the environmental stress has not been provoked. An anti-nutrient (phytic acid) contents was evaluated based on "phytic acid-metal molar ratios" and its inhibitory effect on bioavailability of some multivalent ions (Fe, Zn, Cu, Mn and Ca). The result showed that lowland genotypes pose higher phytic acid inhibitory potential on Fe bioavailability than upland genotypes.

Use of proper agronomic practices in combination with the plant breeding strategies is the most viable option in order to increase in micronutrients value of crops edible parts and to overcome malnutrition in vulnerable populations. The results of present studies confirm that even high-micronutrient varieties developed under soil with micronutrient deficiencies; and their biofortified potential will not be expressed to the full extent. Therefore, if the increase of micronutrient availability and improvement of soil properties are not considered in biofortification strategies, those strategies will inevitably fail.

土壌の微量要素可給性の制御と品種選択による米の農業生物学的栄養強化

本研究は米の微量要素濃度を増加させるための農法（灌漑、施肥方法および品種選択）について実験調査を行った。特に、水管理の影響と土壌中の微量要素の可給度制御のタイミングについて試験を行った。また、米の栄養分およびフィチン酸濃度について、陸稲と水稲品種間の遺伝的差異も比較した。

2種の土壌（Typic Fluvaquent と Typic paleudult）を用いて、出穂期以降の期間の節水戦略が米の微量要素濃度に及ぼす影響を調べた。また、稲の形態、収量構成要素および土壌中の微量要素の可給度への影響についても調べた。栽培試験の結果、土壌種に関係なく、節水処理は稲の形態には悪影響を及ぼさず、米の収量は従来の水管理方法と同等であった。開花期以降の節水処理による好気的な土壌条件の成立は、両方の土壌で根と地上部乾物重を増加させ、また不稔籾を減少させた。これらの結果より、節水処理は稲の水利用効率を促すだけでなく、根の生長促進により微量要素の吸収効率を向上させた可能性を示している。土壌のpHと酸化還元電位の変化もまた、微量要素の可給性や毒性の制御に重要な役割を果たした。好気的条件はZnを可給度を増加させる一方、Typic Paleudult の過剰なMn濃度を毒性の無いレベルに低減させた。Mn毒性は稲の生育不良とそれに伴う収量減少をもたらし、この土壌の稲作への適用を制限している。以上のように、開花期以降の節水処理の適用により、収量を犠牲にする事無く水利用効率を向上させ、土壌中の微量要素の可給度を制御できる事が示された。

土壌の微量要素可給度の米の農業生物学的栄養強化に対する影響は、生殖成長期（開花期から登熟期）の段階で大きく異なっていた。出穂から2週間後以降、土壌の酸化還元電位とpHの変化は米のZn, Cu, Mn含量を変化させた。米のFe含量は水管理により大きく変化しなかった。節水処理による土壌の再酸化はFeを不可給化し、Zn, Cu, Mnの植物吸収における拮抗作用を低減させたと考えられた。登熟期は微量要素の吸収と籾への移流が最も盛んな時期であり、農業生物学的栄養強化の目的において考慮すべきタイミングである。このように、水管理による土壌の電気化学的性質の制御は、土壌の微量要素可給度と米の微量要素含量を増加させるための最も実現性があり、かつ環境親和性の高い方法であると考えられた。

堆厩肥（FYM, Farmyard Manure）や他の有機物の施用は、土壌の生物、物理、化学的特性を変化させ微量要素の可給度を増す。NPK-FYMの肥料の組み合わせが稲の乾物重、穂数、収量、不稔の減少を最も改善した。施肥処理区に関係なく、AWD（Alternative Wetting and Drying、間断灌漑）処理が穂数を最も大きくした。微量要素の葉面散布は、稲の形態に全く影響しなかった。FYM施用の影響で最も重要だったのは土壌の酸化還元電位(Eh)とZn可給度の変化であった。FeとMn可給度は嫌気的条件が長くなりEhが下がるとともに高くなった。一方、Cuの可給度は酸化的な土壌条件(Wc3:出穂2週間以降の節水灌漑, Wc4:圃場容水量処理)とFYMとFYM+NPK処理で向上した。FYMはまたWc４処理のMn可給度も高めた。米のFe濃度は肥料処理区と対象区で明確な差はなかった。FYMの施用は米のFe, Zn, Cu含量を少し増加させたが、水管理条件が米のZn, Cu Mn含量の主な制御要因であった。微量要素の葉面散布はZnとCuのみ少し増加させた。

微量要素含量の高い作物品種を選抜することは、人の食物中の栄養価を高める重要な方法である。46品種の米の分析の結果、米の必須栄養素含量は品種で大きく異なっており遺伝的な変動がある事を示した。陸稲品種は水稲品種に比べてFe,K,P含量が有意に高かった。Zn, Ca, Mg, Alのような他の元素は陸稲品種でやや高かい程度であったが、いくつかの赤米品種は有意に高いZn含量を示した。しかし、今回の結果ですべてを結論づける事はできず、赤米品種については更なる研究が求められる。本研究の結果、陸稲品種は水稲品種よりも高い養分吸収能を有する可能性が示された。陸稲品種の養分獲得機構が環境ストレスのかかっていない時でも活性状態に維持されている可能性が考えられる。養分価を減ずるフィチン酸含量をフィチン酸/金属元素のモル比と、多価イオン(Fe, Zn, Cu, Mn, Ca)の可給化阻害効果をもとに推定した。その結果、水稲品種はフィチン酸によるFeの栄養価低減の影響が大きい可能性が示唆された。

品種改良戦略とともに適当な農法を組み合わせて利用することは、作物可食部の微量要素栄養価を高め貧困層の栄養失調問題を克服するための最も有望な方法である。本研究の結果は、微量要素含量の高い品種であっても土壌中が欠乏レベルでは、その特性を十分に発揮できない事を示している。従って、土壌の微量要素可給度を上げる事と土壌特性の改善を考慮する事無しに農業生物学的栄養戦略は成し得ない。

APPENDIX 1

Effect of Agricultural practices and water management on plant morphology

Morphology	Treatments	N	Wc1	SD	Wc2	SD	Wc3	SD	Wc4	SD
Height	NF	3	110.00	2.00	102.67	2.52	107.00	2.00	107.00	5.29
	OM	3	109.00	4.36	107.67	4.04	109.33	2.52	104.00	3.61
	OM+FF	3	108.33	0.58	104.33	3.06	106.67	3.06	105.67	0.58
	OM+CF	3	114.00	2.65	113.33	2.31	109.33	1.15	111.00	1.00
	OM+CF+FF	3	110.33	1.53	114.67	0.58	110.67	1.53	103.67	8.14
	CF	3	112.33	3.06	108.33	4.16	111.67	3.79	110.33	3.06
	CF+FF	3	109.67	1.15	109.00	2.00	109.00	1.73	106.33	10.02
	Total	21	110.52	2.80	108.57	4.79	109.10	2.64	106.86	5.41
Plant Weight	NF	3	62.89	1.58	70.41	11.49	57.78	0.67	47.88	5.10
	OM	3	73.79	32.31	82.98	32.56	81.79	6.55	56.58	20.39
	OM+FF	3	78.63	24.11	87.13	15.21	77.93	14.80	74.40	7.98
	OM+CF	3	102.91	17.35	82.03	20.00	85.25	5.68	70.99	7.71
	OM+CF+FF	3	79.30	5.17	88.93	9.01	88.65	10.02	77.38	3.32
	CF	3	57.38	2.37	60.66	4.54	68.41	2.54	70.61	2.40
	CF+FF	3	57.43	1.97	65.33	7.95	67.73	5.39	65.92	6.71
	Total	21	73.19	20.73	76.78	17.66	75.36	12.40	66.25	12.73
Grain Yield	NF	3	32.84	2.42	29.61	3.28	32.97	11.31	14.23	0.83
	OM	3	26.36	6.44	27.72	1.59	28.98	1.86	18.79	2.49
	OM+FF	3	21.45	4.58	28.11	10.40	25.84	8.20	20.25	3.37
	OM+CF	3	54.01	10.33	50.76	4.92	44.83	6.01	11.52	6.91
	OM+CF+FF	3	47.72	3.77	40.92	3.91	42.07	7.72	18.59	15.48
	CF	3	54.72	2.14	44.24	2.94	55.26	1.59	14.50	2.69
	CF+FF	3	42.33	14.68	43.43	5.19	40.41	4.23	13.12	7.51
	Total	21	39.92	14.14	37.83	9.83	38.62	11.15	15.86	6.84
N Panicle	NF	3	27.33	0.58	30.33	2.31	29.67	1.53	15.33	3.79
	OM	3	28.33	4.62	44.33	19.86	26.00	6.08	11.33	0.58
	OM+FF	3	24.00	6.56	42.00	6.00	23.33	5.51	21.00	8.66
	OM+CF	3	34.33	4.73	40.00	6.08	35.33	2.52	25.33	4.04
	OM+CF+FF	3	37.00	6.56	41.67	7.64	39.33	2.31	23.67	2.52
	CF	3	27.00	2.00	29.67	4.73	31.67	1.15	21.33	2.52
	CF+FF	3	29.00	0.00	28.00	2.65	28.67	1.15	19.67	10.50
	Total	21	29.57	5.64	36.57	9.96	30.57	5.93	19.67	6.64
SPAD	NF	3	21.51	1.58	31.13	1.16	24.47	2.01	25.70	8.07
	OM	3	37.20	7.45	38.89	6.11	32.57	2.23	38.86	1.43
	OM+FF	3	36.57	6.63	34.73	1.72	42.70	1.22	32.50	2.42
	OM+CF	3	30.93	3.33	33.77	1.59	34.12	5.22	30.60	3.40
	OM+CF+FF	3	31.50	1.50	34.00	2.89	35.90	3.54	34.53	1.37
	CF	3	22.47	1.87	28.33	3.15	30.30	1.06	26.67	1.99
	CF+FF	3	28.00	2.25	33.70	2.82	26.60	1.47	24.10	5.20
	Total	21	29.74	6.85	33.51	4.06	32.38	6.22	30.42	6.09

Effect of Agricultural practices and water management on grain micronutrient contents

Grain	Treatments	N	Wc1	SD	Wc2	SD	Wc3	SD	Wc4	SD
Cugrain	NF	3	3.12	0.41	6.41	0.42	6.34	4.45	6.70	1.20
	OM	3	1.68	1.12	8.05	1.11	4.99	1.62	8.65	2.13
	OM+FF	3	6.09	0.08	9.33	5.31	7.32	0.09	10.87	1.03
	OM+CF	3	0.92	0.34	7.67	1.12	3.75	1.41	9.79	1.33
	OM+CF+FF	3	5.93	0.33	10.84	0.78	7.13	0.56	9.33	1.17
	CF	3	1.72	0.45	5.04	0.23	2.86	0.80	7.52	1.61
	CF+FF	3	6.67	2.09	7.30	1.62	6.26	0.48	9.02	1.55
	Total	21	3.73	2.44	7.81	2.57	5.52	2.28	8.84	1.81
Fegrain	NF	3	14.40	0.32	13.04	0.74	15.92	2.57	13.34	1.94
	OM	3	20.09	1.79	19.77	1.18	19.09	0.95	31.40	4.57
	OM+FF	3	20.81	2.38	18.22	3.74	17.10	0.57	19.43	0.28
	OM+CF	3	18.98	1.55	19.32	1.13	17.47	1.11	17.29	3.54
	OM+CF+FF	3	16.85	1.44	18.04	1.41	18.05	1.24	18.97	0.53
	CF	3	16.65	1.25	14.04	0.47	14.85	0.54	15.16	3.34
	CF+FF	3	16.31	1.77	15.63	1.43	15.69	0.81	15.76	0.88
	Total	21	17.73	2.56	16.87	2.90	16.88	1.77	18.77	6.08
Mngrain	NF	3	25.68	3.28	46.30	6.34	40.74	11.58	54.67	17.41
	OM	3	23.24	2.33	35.88	2.69	59.00	4.76	45.75	2.34
	OM+FF	3	27.30	1.09	36.61	7.04	56.86	4.42	73.99	23.98
	OM+CF	3	23.94	0.53	36.96	2.53	64.75	10.15	85.88	4.23
	OM+CF+FF	3	25.72	3.68	37.18	2.14	77.95	1.10	120.03	18.40
	CF	3	30.86	2.37	41.69	2.29	41.28	3.01	117.18	32.05
	CF+FF	3	36.75	7.55	39.52	14.53	42.60	2.70	110.81	7.35
	Total	21	27.64	5.43	39.16	6.71	54.74	14.41	86.90	32.48
ZnGrain	NF	3	29.35	0.68	34.43	1.06	32.73	3.55	49.89	17.41
	OM	3	28.53	0.60	38.32	4.16	34.27	3.00	52.41	7.95
	OM+FF	3	29.90	2.98	36.36	6.91	35.12	1.08	58.72	2.58
	OM+CF	3	19.82	1.73	31.74	1.55	30.65	3.80	51.42	9.09
	OM+CF+FF	3	25.77	2.86	37.53	2.74	34.17	3.32	61.83	7.42
	CF	3	23.44	1.86	32.04	1.01	26.88	1.72	47.90	10.86
	CF+FF	3	31.97	8.07	31.39	6.03	31.60	1.37	53.27	6.24
	Total	21	26.97	4.99	34.55	4.35	32.20	3.54	53.63	9.42

Effect of Agricultural practices and water management on Soil micronutrient availability

Soil	Treatments	N	Wc1	SD	Wc2	SD	Wc3	SD	Wc4	SD
Fe	NF	3	295.50	32.49	276.42	12.29	132.25	9.69	48.91	21.99
	OM	3	304.00	13.80	343.33	20.45	162.50	8.60	50.18	39.03
	OM+FF	3	292.92	39.41	341.67	28.41	148.42	3.83	37.97	8.79
	OM+CF	3	295.00	1.75	287.75	41.79	154.17	12.96	28.81	1.92
	OM+CF+FF	3	314.83	21.01	284.82	9.35	159.83	8.53	52.17	8.85
	CF	3	406.25	122.62	348.83	63.45	151.25	76.45	27.90	2.09
	CF+FF	3	294.17	35.23	275.98	13.41	134.92	14.91	30.95	1.84
	Total	21	314.67	58.94	308.40	42.36	149.05	27.76	39.56	17.90
Mn	NF	3	329.67	16.02	322.33	20.73	259.17	30.89	79.60	33.48
	OM	3	332.42	19.21	370.92	7.18	269.00	5.29	131.08	116.84
	OM+FF	3	355.25	14.42	342.17	8.00	280.92	20.51	101.00	61.06
	OM+CF	3	330.58	15.33	328.50	30.13	305.75	32.91	53.20	8.18
	OM+CF+FF	3	312.92	61.00	332.83	14.87	314.33	48.21	222.67	11.79
	CF	3	409.58	85.88	334.17	68.97	323.17	57.97	23.26	6.22
	CF+FF	3	362.83	24.00	359.00	8.05	311.00	51.06	36.39	2.00
	Total	21	347.61	46.91	341.42	30.42	294.76	40.40	92.46	78.09
Zn	NF	3	14.33	0.26	12.80	0.89	10.21	1.09	16.80	11.34
	OM	3	29.74	2.89	32.80	2.04	40.27	8.00	28.00	8.60
	OM+FF	3	27.42	4.34	26.43	1.40	25.60	2.15	27.76	1.36
	OM+CF	3	20.68	1.60	18.56	2.55	17.75	2.51	19.60	3.84
	OM+CF+FF	3	17.15	4.49	16.65	1.67	17.27	0.92	15.72	0.24
	CF	3	9.40	0.33	14.07	1.78	12.59	0.61	15.36	0.15
	CF+FF	3	11.26	1.10	15.04	0.67	13.47	0.48	14.97	0.23
	Total	21	18.57	7.74	19.48	7.16	19.60	10.24	19.74	7.20
Cu	NF	3	7.17	0.68	6.58	0.57	8.04	0.36	6.90	0.78
	OM	3	5.59	0.43	3.30	1.08	9.38	0.48	6.40	1.65
	OM+FF	3	4.78	0.91	3.35	0.90	9.69	0.34	7.18	0.30
	OM+CF	3	5.89	0.40	5.45	1.09	9.45	0.26	6.22	0.37
	OM+CF+FF	3	5.83	1.18	4.89	1.25	10.13	0.35	7.54	0.49
	CF	3	4.62	1.91	3.73	1.14	7.31	2.37	6.10	0.13
	CF+FF	3	6.21	0.31	6.50	0.82	9.70	0.35	6.92	0.30
	Total	21	5.73	1.16	4.83	1.58	9.10	1.26	6.75	0.80

Effect of Agricultural practices and water management on pH, Electric conductivity and Exchangeable Cations $\text{cmol}_c\ \text{kg}^{-1}$

		N	Wc1	SD	Wc2	SD	Wc3	SD	Wc4	SD
pH	NF	3	5.94	0.43	5.89	0.17	5.45	0.20	5.78	0.54
	OM	3	6.66	0.15	7.02	0.18	6.81	0.23	6.04	0.35
	OM+FF	3	6.87	0.22	6.96	0.08	6.62	0.25	6.65	0.28
	OM+CF	3	6.51	0.25	6.65	0.25	6.37	0.25	6.00	0.27
	OM+CF+FF	3	6.39	0.28	6.33	0.06	6.18	0.35	6.03	0.30
	CF	3	6.11	0.22	6.14	0.24	5.36	0.46	5.32	0.15
	CF+FF	3	5.57	0.45	5.70	0.26	5.32	0.12	5.20	0.13
	Total	21	6.29	0.49	6.38	0.51	6.02	0.64	5.86	0.53
EC	NF	3	31.82	6.04	31.62	3.03	32.58	2.10	18.87	6.47
	OM	3	105.27	5.91	105.75	12.47	118.87	3.77	98.75	4.74
	OM+FF	3	118.32	1.76	115.30	2.40	120.73	3.02	74.60	9.90
	OM+CF	3	83.27	8.69	86.05	9.54	95.03	9.83	98.88	6.66
	OM+CF+FF	3	83.23	8.58	90.43	3.99	118.18	4.99	99.62	3.52
	CF	3	34.97	4.92	37.92	2.26	44.32	2.50	46.68	7.75
	CF+FF	3	36.99	1.92	42.52	7.61	42.80	1.40	45.97	3.19
	Total	21	70.55	34.36	72.80	33.39	81.79	38.42	69.05	31.18
Ca	NF	3	5.33	0.15	5.11	0.20	5.24	0.06	5.22	0.13
	OM	3	7.58	0.10	8.36	0.31	8.30	0.26	7.28	0.95
	OM+FF	3	7.70	0.50	7.75	0.02	7.77	0.49	7.91	0.27
	OM+CF	3	6.55	0.46	6.88	0.13	7.05	0.67	6.88	0.15
	OM+CF+FF	3	6.67	0.27	6.71	0.57	6.98	0.11	6.53	0.35
	CF	3	5.29	0.34	5.63	0.01	5.29	0.01	5.24	0.12
	CF+FF	3	4.80	0.43	5.30	0.05	5.14	0.12	5.25	0.23
	Total	21	6.28	1.13	6.53	1.20	6.54	1.27	6.39	1.10
K	NF	3	0.73	0.04	0.75	0.10	0.26	0.12	0.40	0.19
	OM	3	14.19	3.82	9.04	3.53	10.83	6.33	14.25	5.20
	OM+FF	2	10.32	4.07	8.80	2.23	5.23	2.17	7.11	2.37
	OM+CF	3	17.85	1.45	17.49	0.65	19.10	0.96	18.62	2.52
	OM+CF+FF	3	19.13	1.37	18.10	0.23	20.59	0.54	20.01	0.70
	CF	3	0.80	0.06	0.97	0.19	0.53	0.24	0.49	0.14
	CF+FF	3	0.83	0.14	0.65	0.08	0.17	0.09	0.39	0.13
	Total	20	9.06	8.23	7.97	7.37	8.10	8.70	9.17	8.59
Mg	NF	3	2.15	0.06	1.92	0.08	1.96	0.03	1.88	0.11
	OM	3	3.71	0.06	3.92	0.11	3.88	0.07	3.31	0.54
	OM+FF	3	3.71	0.25	3.70	0.13	3.56	0.36	3.30	0.25
	OM+CF	3	3.15	0.19	3.13	0.01	3.11	0.36	2.85	0.05
	OM+CF+FF	3	3.06	0.14	3.00	0.16	3.16	0.11	2.80	0.11
	CF	3	2.03	0.13	2.06	0.04	1.94	0.04	1.54	0.10
	CF+FF	3	1.87	0.13	1.99	0.01	1.89	0.05	1.66	0.20
	Total	21	2.81	0.76	2.82	0.80	2.78	0.81	2.51	0.76
Na	NF	3	2.32	0.13	2.42	0.07	2.35	0.18	1.98	0.25
	OM	3	4.04	0.09	3.99	0.26	3.91	0.14	4.58	0.54
	OM+FF	3	3.76	0.43	4.47	0.84	4.24	0.73	3.84	0.23
	OM+CF	3	4.37	0.20	3.73	0.08	3.18	0.10	3.42	0.30
	OM+CF+FF	3	4.02	0.19	3.74	0.48	4.28	0.68	3.43	0.44
	CF	3	2.61	0.07	2.88	0.03	2.92	0.09	3.61	0.12
	CF+FF	3	2.64	0.18	2.94	0.12	2.81	0.08	3.25	0.48
	Total	21	3.40	0.82	3.45	0.76	3.38	0.79	3.52	0.75

APPENDIX 2. Evaluation of genotypes grain nutrient contents based in pericarp[1] color

Concentration in nutrient in two grains types grouped by color (White pericarp and red pigmented pericarp rich in Anthocyanin)

Color		Fe	Zn	Cu	Mn	Ca	K	Mg	Na	P	Al
White	Mean	13.75	32.73	1.50	24.66	140.93	1211.96	1527.35	102.62	3945.95	1.91
	SD	1.93	4.55	0.80	4.65	22.49	179.14	161.67	14.47	434.38	0.60
Red	Mean	13.75	34.34	1.67	24.32	146.02	1170.36	1510.82	102.53	4058.80	1.74
	SD	1.19	6.84	0.71	8.42	23.42	86.11	93.64	12.05	213.02	0.64
Total	Mean	13.75	32.94	1.53	24.62	141.59	1206.54	1525.19	102.61	3960.67	1.89
	SD	1.84	4.84	0.78	5.16	22.41	169.82	153.81	14.05	412.37	0.61
	N	46	46	46	46	46	46	46	46	46	46

Morphology and yield parameters related to the genotypes used in the experiment are available in:

https://www.gene.affrc.go.jp/databases-core_collections_wr_en.php
https://www.gene.affrc.go.jp/databases-core_collections_jr_en.php

List of Publications

Assessment of the influence of water management on yield component and morphological behavior of rice at post-heading stage. Juan Damian Marques Fong , Tsugiyuki Masunaga and Kuniaki Sato. Paddy Water Environment , pages 1-10 http://dx.doi.org/10.1007/s10333-015-0491-1

Control of Micronutrients Availability in Soil and Concentration in Rice Grain through Field Water Management. Juan Damian Marques Fong , Tsugiyuki Masunaga and Kuniaki Sato . Journal of Agricultural Science Vol. 7, 5: 163-174. http://dx.doi.org/10.5539/jas.v7n5p163