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ZINC UPTAKE, UTILIZATION AND THEIR EXPRESSION IN UPLAND RICE

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By

GOLNAZ SHARIFIANPOUR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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To the pillars of my life: God, my parents, my brother and sister and my husband Without you, my life would fall apart.
I might not know where the life's road will take me, but walking with you.

God, through this journey has given me strength.

Mother and father, you have given me so much, thanks for your faith in me, and for teaching me that I should never surrender.

Amir and Behnaz, you always told me "move forward" I think I reached to the first step. Thanks for inspiring my love for transportation.

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Chairman: Professor Zaharah Abdul Rahman

Faculty: Agriculture

Zinc deficiency is one of the most common micronutrient disorders in rice, for both lowland varieties grown under flooded conditions and upland varieties under upland condition. The concentration of Zn in acidic soil is very low; as soils of Malaysia are acidic so Zn monitoring in Malaysia is vital. Owing to the important role of Zn element in human health and wellness, and on the other hand, lack of Zn causes much serious illness, monitoring Zn in upland rice, as a nutrient factor is essential. In addition, the industry has to identify and breed for nutrient efficient varieties. Zinc is one of the important elements of protein transport in plants so it is vital to identify zinc as a main plant protein transporter. The main goal of this project was to evaluate Zn uptake and metabolism in upland rice, in order to formulate correct fertilizer recommendations. Zn-efficient upland rice was identified in the first study where the effect of different Zn concentration on root characteristics of 7 upland rice landraces were evaluated. To increase Zn content in rice in order to supply adequate Zn in Malaysian diet, the effect of different compost and different sources of Zn on two upland rice landraces identified from study 1 was evaluated in a glasshouse study. The expression of *OsZIP1*, *OsZIP3*, *OsZIP4*, *OsZIP5* and *OsZIP8* involved in Zn uptake by the two selected upland rice genotypes in the leaves and roots were also elucidated. Results revealed that, soluble forms of Zn are readily available to plants and the uptake of Zn is linear with concentration in the nutrient solution. In addition,, plants grown in solution treated with Zn accumulate a great proportion of this metal in the roots. Other root parameters (length, average diameter, surface area, volume, and number of root) did not show any significant differences in 0 to 20 mg Zn L^{-1}, but they decreased significantly at 30 mg Zn L^{-1}. This shows that Zn in high concentration causes root growth disorder. At 30 mg Zn L^{-1} young plants died, possibly due to toxic effect of Zn. All root parameters increased significantly during four weeks of observation. Bertih variety had the most Zn uptake (0.0328 mg $plant^{-1}$) and Nabawan had the lowest Zn uptake

(0.0138 mg plant^{-1}) during the four weeks. It seems that the Zn content of plants varies considerably, reflecting different factors affecting the Zn uptake by the landraces tested. All types of composts showed positive effects on Zn concentrations and uptake in all parts of rice. Vermicompost is the most effective compost among these three composts. It has been found that although chicken compost has more Zn concentration, it seems that because of high concentration of other elements (such as P which have interactions with Zn), plant could not absorb Zn properly. On the other hand, because of high N in chicken compost, and increasing the dry weight of plant under this treatment, Zn uptake did not show a significant difference with Zn uptake in plants grown in vermicompost treatment. Application of Zn-amended organic composts increased the percentage distribution of Zn in grain more than the application of $ZnSO_4$ or Zn-EDTA alone. In addition,, the results of analysis of the best soil treatment (Vermicompost+$ZnSO_4$) compared to control for Zn content in different fractions showed application of vermicompost caused a significant increase in the content of water soluble plus exchangeable, organic complexed fraction of soil Zn. Results of molecular study in roots and shoot showed an over expression of *OsZIP4*, *OsZIP1*, *OsZIP8*, *OsZIP5* under Zn deficient conditions , but not *OsZIP3* (*OsZIP3* is not expressed in roots and shoots). Generally, it can be concluded that rice variety detected with high Zn uptake could offer a sustainable and cost-effective way to overcome Zn deficiency problems and uptake of Zn by all parts of rice illustrated an increase in the presence of vermicompost+inorganic Zn sources ($ZnSO_4$ and Zn-EDTA) which was the most effective method for Zn fortification in rice plants. Also, application of organic matter under upland condition influenced favourably the transformation of applied Zn in soil fractions which are available to the nutrition of rice plants. From molecular aspect, as *OsZIPs* are Zn transporter that functions in Zn uptake and distribution; Zinc homeostasis is important to the proper growth and development of rice.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat untuk ijazah Doktor Falsafah

PENYERAPAN ZING, PENGGUNAANNYA DAN PENYATAANNYA DALAM PENANAMAN PADI HUMA

Oleh

GOLNAZ SHARIFIANPOUR

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Pengerusi: Professor Zaharah Abdul Rahman, PhD

Fakulti: Pertanian

Kekurangan zink merupakan satu daripada gangguan mikronutrien yang paling biasa dalam padi, yang ditanam dalam sawah atau secara padi huma. Kepekatan kandungan Zn dalam tanah berasid sangat rendah; oleh itu penggunaan unsur Zn di Malaysia adalah penting. Disebabkan peranan penting unsur Zn dalam kesejahteraan dan kesihatan manusia, kekurangan unsur Zn akan menyebabkan penyakit yang serius, oleh itu penggunaan unsur Zn bagi padi huma, sebagai faktor nutrien amatlaht penting. Zink adalah salah satu elemen penting dalam penyerapan protein di dalam tumbuhan, oleh itu adalah penting untuk mengenal pasti zink sebagai penyerap utama protein tumbuh-tumbuhan. Matlamat utama projek ini ialah untuk menilai penyerapan dan penggunaan Zn dan metabolisme di dalam padi huma , dan dapat meyediakan baja yang betul. Zn dalam padi huma telah dikenal pasti dalam kajian pertama yang menjelaskan kesan kepekatan Zn yang berbeza pada sifat akar bagi 7 jenis padi huma. Untuk meningkatkan kandungan Zn dalam padi sekali gus membekalkan Zn yang mencukupi dalam diet penduduk Malaysia , kesan baja yang berbeza dan sumber Zn yang berbeza pada dua jenis padi huma yang dikenal pasti daripada kajian 1 telah dinilai dalam rumah kaca. Penyataan *OsZIP1* , *OsZIP3* , *OsZIP4* , *OsZIP5* dan *OsZIP8* terlibat dalam penggunaan Zn, oleh dua genotip padi huma dipilih dan dikaji dalam daun dan akar. Hasil kajian menunjukkan bahawa, larutan Zn sedia ada pada tumbuhan dan penggunaan Zn adalah linear dengan kepekatan dalam larutan nutrien. Di samping itu, tanaman yang ditanam dalam larutan dirawat oleh Zn mengumpul sebahagian besar unsur logam ini dalam akar. Parameter akar lain (panjang , diameter purata , kawasan permukaan , isi padu, dan tip akar) tidak menunjukkan sebarang perbezaan yang signifikan dalam 0 hingga 20 mg L^{-1} Zn, tetapi mereka menurun secara signifikan pada 30 mg Zn L^{-1}. Ini menunjukkan bahawa Zn dalam kepekatan tinggi menyebabkan gangguan terhadap pertumbuhan akar. Pada 30 mg Zn L^{-1} anak pokok akan

mati , mungkin disebabkan oleh kesan toksik Zn . Semua parameter akar meningkat secara signifikan dalam empat minggu pemerhatian. Variati Bertih menyerap Zn yang paling banyak (0.0328 mg pokok^{-1}) dan Nabawan menyerap Zn yang paling rendah (0.0138 mg pokok $^{-1}$) dalam tempoh empat minggu. Ini menunujukkan bahawa kandungan Zn tanaman berbeza dengan ketara , mencerminkan faktor yang berbeza kesan kepada penyerapan Zn oleh jenis padi huma yang diuji. Semua jenis kompos menunjukkan kesan positif ke atas kepekatan Zn dan penyerapan di semua bahagian padi. Vermikompos adalah yang paling berkesan berbanding dengan ketiga-tiga kompos. Kajian telah mendapati bahawa walaupun kompos tahi ayam mempunyai lebih kepekatan Zn, ia juga mempunyai kepekatan unsur lain yang tinggi (seperti P yang akan berinteraksi dengan Zn). Erti kata lain tanaman tidak dapat menyerap Zn dengan betul disebabkan N yang tinggi dalam kompos tahi ayam, yang akan meningkatkan berat kering tanaman di bawah rawatan ini. Penyerapan Zn tidak menunjukkan perbezaan yang signifikan dengan penyerapan Zn dalam tumbuhan yang ditanam dalam rawatan vermikompos . Penggunaan kompos organik + Zn meningkat peratusan Zn dalam bijirin lebih daripada penggunaan $ZnSO_4$ atau Zn - EDTA bersendirian. Di samping itu, , keputusan analisis rawatan vermikompos + $ZnSO_4$ adalah terbaik berbanding rawatan kawalan dan menunjukkan peningkatan yang signifikan dalam kandungan water soluble dan extractable Zndari rawatan vermikompos + $ZnSO_4$. Hasil kajian akar dan pucuk padi menunjukkan lebih ketara penyataan *OsZIP4* , *OsZIP1* , *OsZIP8* , *OsZIP5* di bawah keadaan kekurangan Zn, tetapi *OsZIP3* (*OsZIP3* tidak dinyatakan dengan ketara dalam akar dan pucuk). Secara umumnya, kesimpulan bahawa jenis padi huma yang dikesan menyerap Zn yang tinggi boleh menawarkan cara yang mampan dan kos efektif untuk mengatasi masalah kekurangan Zn dan penyerapan Zn oleh semua bahagian pokok padi seperti yang digambarkan dalam peningkatan sumber vermikompos+Zn bukan organik ($ZnSO_4$ dan Zn-EDTA) merupakan kaedah yang paling berkesan untuk memperkayakan Zn dalam penanaman padi. Selain itu, pemupokan bahan organik yang dicampur dengan Zn boleh digunakan sebagai sumber Zn untuk padi huma.. Daripada aspek molekul, seperti *OsZIPs* ,Zn adalah pengangkut yang berfungsi dalam penyerapan Zn; Zink homeostasis adalah penting untuk pertumbuhan yang betul dan perkembangan tanaman padi.

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I certify that a Thesis Examination Committee has met on (19 January 2015) to conduct the final examination of Golnaz Sharifianpour on her thesis entitled "Comparison of the Effect of Blogging and Pen-and-Paper on the Essay Writing Performance of Iranian Graduate Students" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Degree of Doctor of Philosophy

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LIST OF ABBREVIATIONS

AF	Available factor
AMOX	Amorphous sesquoxide bound form
CRYOX	Crystalline sesquioxide bound form
CTAB	Cetyltrimethyl ammonium bromide
DAT	Day after transplanting
DEPC	Diethyl pyrocarbonate
DMA	Deoxymugineic acid synthase
DTPA	Diethylene triamine pentaacetic acid
EDTA	Ethylen ediaminete traacetic acid
MnOX	Manganese oxide bound
NA	Nicotianamine
NAAT	Nicotianamine aminotransferase
NAS	Nicotianamine synthase
OC	Organically complexed
OM	Organic matter
QRT-PCR	Quantitative real time polymerase chain reaction
Res	Residual
UPM	University Putra Malaysia
V	Variety
WSEX	Water soluble exchangeable
YSL	Yellow stripe-like transporter
ZIP	Zinc and iron regulated transporter like protein

CHAPTER ONE

INTRODUCTION

Zinc (Zn) is an essential micronutrient for normal growth and development of all living organisms, including humans and plants (Broadley *et al.*, 2007; Wong *et al.*, 1999; Hodges, 1991). In humans, Zn acts as a co-factor for the activity of more than 200 enzymes and is required for many biological processes such as normal development and function of the immune system, neuro-sensory functions (Meunier *et al.*, 2005), reproductive health, and brain functions. Similarly, in plants, Zn plays multiple roles in basic biochemical processes such as enzyme catalysis or activation, protein synthesis, carbohydrate and auxin metabolism, chlorophyll production, pollen formation, cytochrome and nucleotide synthesis, maintenance of membrane integrity, and energy dissipation (Alloway, 2009; Singh and Singh, 1981).

Zinc deficiency in cereal plants is a well-known problem that causes reduced agricultural productivity all over the world (Fageria *et al.*, 2002). In addition,, it causes widespread Zn deficiency in humans, especially in developing countries where diets are cereal-based and poor in animal and fish products (Frossard *et al.*, 2000). If humans are Zn-deficient, some of the consequences are stunted growth of children (Brown *et al.*, 2004), poor immune function resulting in greater susceptibility of children and adults to common infectious diseases, such as diarrhoea and pneumonia (Walker *et al.*, 2008) and possibly an increased rate of premature births for Zn-deficient mothers (Hess and King, 2009). Human nutrition research has suggested the following approaches to mitigate human Zn deficiency: dietary diversification, i.e. increasing consumption of red meat, fruits, and vegetables that are high in bioavailable Zn (Gibson and Anderson, 2009), supplementation, i.e. use of Zn-containing vitamin pills or drops (Haider and Bhutta, 2009; Hess and King, 2009), fortification, i.e. addition of Zn to a staple food during commercial processing prior to marketing to consumers (Brown *et al.*, 2010).

Rice is one of the most important staple foods for the increasing world population, especially in Asia. Recently, the demand for specialty and high quality rice has increased remarkably (Bouman *et al.*, 2005). In general, rice production and sufficiency is the main concern of all Asian countries currently facing the ever-growing population and climatic uncertainties. Because of water constraints, rice production in many countries is now undergoing important changes from traditional high water-consuming lowland (paddy) rice cultivation to a promising new cultivation method of "upland rice". Upland rice is grown as a dry field crop in irrigated but non-flooded and non-puddled fertile soils (Bouman *et al.*, 2005).

Upland rice cultivation in Malaysia is practiced mostly by the rustic communities living especially in Sabah and Sarawak. Certain upland rice varieties have desirable characteristics, particularly in terms of their fragrance, colors, sizes, and shapes. These qualities contribute to their popularity among the farmers and health-conscious consumers as an organic food. The yield average of the upland rice is minor than lowland rice, and its ranges being from 0.46 to 1.1 tonnes ha^{-1} (Sheng *et al.*, 2008).

Biofortification may be an even more important approach for rice than for other crops because rice is not usually ground into flour before selling it to the consumer, making fortification should be more attractive option for rice than for wheat or legumes. Biofortification, is increasing Zn content of a staple food crop during the growth period of the plant, through either agronomic or genetic enrichment, which biofortification of staple crops offers specific advantages that complements the other approaches (Bouis *et al.*, 2011). a) many of the poorest people in the world who cannot afford appropriate dietary diversification live in areas beyond the reach of supplementation programs, but may benefit from increased nutritional value of their staple food; b) higher cost-effectiveness, since there is very little recurrent cost in reaching additional people once genetically-biofortified nutritious varieties have been developed understanding of Zn behavior in soil solution and plants especially Zn uptake by root and shoot under upland condition help us to know how to increase Zn content in grain and help for rice biofortification.

Upland rice needs to have a deeper root and a higher root length and density than lowland rice cultivars because of the limited water availability under aerobic as compared to flooded conditions. The primary source of Zn for rice plants is through root uptake. Thus, the Zn availability in the rhizosphere must be increased, to boost Zn uptake by roots. Under nutrient-deficient conditions, plants tend to alter their root size and morphology for efficient nutrient acquisition (Welch and Shuman, 1995).

Moreover, Zn application methods and sources are aimed at improving Zn availability for rice uptake. Most common method of Zn fertilization is through soil application. Selection of appropriate Zn sources for soil application can be an alternative strategy to improve plant availability of Zn. In one hand, Zn fertilizers with good solubility (such as Zn-EDTA and $ZnSO_4$) generally resulted in greater Zn transport to the roots compared with insoluble ZnO or fritted Zn (Giordano and Mortvedt, 1973). The greater soil transport of Zn increased the possibility of Zn being intercepted by the fast growing roots, which might have been associated with a greater effect of banding Zn-EDTA than fritted Zn. On the other hand, composts are useful sources of nutrients including N and P and their application improves micronutrient availability by changing soil chemical, physical and biological properties (Eghball *et al.*, 2004). In agriculture, compost application is the most common input of organic matter (OM) to soil. It also increases soil fertility and water retention as well as chemical speciation (Soumare *et al.*, 2003). In addition, composts contain a number of metals at varying concentrations, depending on the source, and commonly Zn. For instance vermicompost, whether used as soil additives or as components of greenhouse bedding plant-container media, have improved seed germination, enhanced seedling growth and development, and increased overall plant productivity (Atiyeh *et al.*, 2002).

Livestock and poultry manure can be an alternative source of fertilizer in organic farming where the use of anthropogenic chemicals is prohibited (Wong *et al.*, 1999; Hodges, 1991). In addition, oil palm wastes were reported to have many characteristics that are superior to peat in growing media (Lim and Ratnalingam, 1980). Zinc added to soil as fertilizer may become rapidly unavailable for upland rice uptake (Johnson-Beebout *et al.*, 2009), therefore, screening or selecting of upland rice genotypes with high efficient Zn acquisition in poor Zn conditions is an alternative to alleviate Zn deficiency and decrease the utilization of Zn fertilizer.

Rice acquire Zn from rhizosphere and the absorbed Zn must be transported into aerial parts as well as to the intracellular compartments of the cell where it is required for the Zn-dependent processes. It is necessary to understand the molecular mechanism through which plants mobilize, take up, translocate, and store Zn. Several members of the Zn-regulated like protein (*ZIP*) gene family (Guerinot, 2000) have been characterized and shown to be involved in Zn uptake and transport in plants (Connolly *et al.*, 2002). In rice, several *ZIP*s have been reported e.g. *OsIRT1*, *OsIRT2*, *OsZIP1*, *OsZIP3*, *OsZIP4*, *OsZIP5*, *OsZIP7*, and *OsZIP8* (Lee, Jeong *et al.*, 2010; Lee, Kim *et al.*, 2010; Yang *et al.*, 2009; Ishimaru *et al.*, 2007; Ishimaru *et al.*, 2006; Ishimaru *et al.*, 2005; Ramesh *et al.*, 2003).

It is commonly found that Zn-efficient genotypes are able to acquire faster and more Zn from the soil and fertilizers and also able to utilize Zn internally more efficiently. To determine the Zn-efficient genotype, it has been generally accepted that root morphology and Zn transporters in roots and shoots are key factors related to Zn acquisition of plant. With this supportive background information and keeping these points in view, the present investigations on Zn was undertaken with the following objectives to evaluate Zn uptake and metabolism in upland rice.

General objective was:

To elucidate the differences between efficient and non-efficient genotype in Zn uptake

The specific objectives were:

1) To identify Zn-efficient upland rice
2) To evaluate the effect of different Zn concentrations on root characteristics of 7 upland rice landraces
3) To evaluate the effect of different compost and different sources of Zn on two upland rice landraces in glasshouse
4) To increase Zn content in rice in order to supply adequate Zn in Malaysian diet
5) To elucidate the expression of *OsZIP1, OsZIP3, OsZIP4, OsZIP5* and *OsZIP8* involved in Zn uptake by 2 selected upland rice landraces in the leaves and roots.

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LIST OF PUBLICATIONS

Published

1. Upland rice root parameters and their relationship on utilizing different levels of applied Zinc; published in *International Journal Of Scientific Research In Environmental Sciences* (IJSRES) 23 of November 2013 Available online at http://www.ijsrpub.com/ijsres ISSN: 2322-4983; ©2013 IJSRPUB pages 372-376

2. Elucidating the expression of Zinc transporters involved in Zinc uptake by upland Rice landraces in Malaysia; Published by American-Eurasian Network for Scientific Information in *journal of* Advances in Environmental Biology (AEB) 24 December 2013 Pages: 4854-4857

3. Effect of Different Rates of Zinc on Root Morphological Traits among Different Upland Rice Landraces in Malaysia; International Journal of Agriculture and Forestry 2014, 4(3): 255-260 DOI: 10.5923/j.ijaf.20140403.18

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