UNIVERSITI PUTRA MALAYSIA

AGRONOMIC BIOFORTIFICATION OF RICE WITH SELENIUM

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By

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

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DEDICATION

Every challenging work needs self-efforts as well as guidance of elders especially those who were very close to our heart.

This is dedicated to:

My love, Yasser, who has been a great source of motivation and inspiration

And also to:

My parents for their endless love, support and encouragement

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

AGRONOMIC BIOFORTIFICATION OF RICE WITH SELENIUM

ABSTRACT

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Selenium (Se) is one of the most important micronutrient for humans and animals. There is a narrow range between its deficiency and toxicity. Se by itself does not commonly cause illness but it contribute to different disease such as heart disease, hypothyroidism, and a weakened immune system. Se concentration in Malaysian soils is very low. In most cases Se deficiency can be corrected by the application of Se as fertilizer into the soils, which is termed as agronomic biofortification. The main source of Se for most people in Asian countries is rice, but its concentration may be insufficient to maintain human health. Agronomic biofortification can be done to improve Se content in rice production. This study was undertaken to determine Se adsorption and desorption of Malaysian rice soils, to evaluate the Se efficiency of selected rice genotypes and to determine the response of Se application on rice. A field trial was carried out with five different selenium rates at 0, 5, 10, 20, 40 mg Se/kg fertilizer on MR219 rice variety, a popular variety used in Malaysia. The Se rates chosen did not affect the rice yield, the grain selenium concentration and the soil selenium concentration which means that for fortifying rice grain we should increase the level of Se. To obtain a better understanding of the extent to which added selenite would be retained by the soil, an investigation of selenite adsorption and desorption by rice growing soils was carried out. Sorption and desorption isotherms of different soils collected from Tg. Karang, Teluk Intan I, Teluk Intan II, Bagan Serai, Guar Cempedak h, Kangar, Jertih, Kota Bharu, Machang and UPM in Malaysia were determined by using a batch method. The Freudlich and Langmuir equation was well fitted to the obtained sorption and desorption isotherms. Adsorption data in this study revealed an L shape isotherm that could be explained by the high affinity of the soils for the Se sorption at low concentration which then decreased as the concentration increased. It is also concluded that selenium availability to plants is affected by soil pH, organic matter content and clay minerals. Sorption of selenite increases with decreasing pH and increasing organic matter. The result showed that the selenium desorption hysteresis in

different soils was due to irreversible Se inner sphere binding to the edge of the minerals. In the next experiment, six rice varieties collected from Penang (MR211, MR219, MR220, MR232, MR253, MR263) were studied at three levels (0, 20 and 200 μg Se/L) to investigate Se accumulation in roots and shoots. These six varieties showed significant effects on Se uptake in shoots and roots but different Se concentrations had no significant effects on root parameters. The results showed that Se accumulation in shoots in all the varieties is greater than Se accumulation in roots. With MR253 having the lowest amount. A greenhouse trial was conducted with 3 varieties from the solution culture study, soil chosen from the adsorption studies and Se rates of 0, 100, 300, 500 and 700 g ha^{-1}. The aim was to assess the Se concentration and the Se uptake in different part of the plants, especially in rice grain, but also to evaluate the effect of selenium on yield and some yield components. In this study, it was observed that except for rice yield, 1000 grain yield and total shoot dry weight other parameters such as leaf, culm and rice grains yield of rice varieties as well as total Se uptake and grain Se uptake were affected by different selenium rates. Among the different Se levels used, the level of 500 g ha^{-1} of Se would be the recommended rate in order to increase the Se concentration in grain. Between the Se concentration in grain and leaf, a positive correlation was observed. Also, there was a correlation between Se uptake and Se accumulation in the rice plant.

Abstrak tesis yang dikemukakan kedapa Senati Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

BIO-PNGUKUHAN PADI DENGAN SELENIUM MELALUI AMALAN AGRONOMI

ABSTRAK

Oleh

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Pengerusi: Professor Zaharah Abdul Rahman, PhD

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Selenium (Se) ialah salah satu nutrient mikro yang penting diperlukan oleh manusia dan haiwan. Julat kekurangan dan kelebihan untuk nutrien ini adalah kecil. Pada kebiasaannya, kekurangan Se secara sendirinya tidak akan menyebabkan penyakit, tetapi ia akan menyumbang kepada penyakit yang berbeza seperti penyakit jantung, hypotiriodism, dan juga sistem imun yang lemah. Kepekatan Se dalam tanah di Malaysia adalah rendah. Pada kebiasaanya, kekurangan Se boleh dibetulkan dengan aplikasi Se sebagai baja ke dalam tanah, juga dikenali sebagai bio-pengukuhan secara amalan agronomi. Punca utama Se bagi kebanyakan penduduk dari Negara Asia adalah melalui nasi, tetapi kepekatannya dalam nasi mungkin tidak mencukupi untuk mengekalkan kesihatan. Bio-pengukuhan melalui amalan agronomi boleh dijalankan untuk menambahbaik kandungan Se dalam pengeluaran padi. Kajian ini telah dijalankan untuk menentukan penyerapan dan penyahserapan Se dalam tanah padi di Malaysia, di samping menilai keberkesanan Se pada genotip padi terpilih dan akhirnya menentukan tindak balas aplikasi Se pada padi. Satu kajian penanaman telah dijalankan ke atas varieti padi MR 219, satu varieti padi yang popular di Malaysia, dengan menggunakan lima kadar Se yang berbeza pada 0, 5, 10, 20, 40 mg Se/kg tanah. Kadar Se yang digunakan tidak mempengaruhi hasil pengeluaran padi, kepekatan Se dalam bijirin dan juga kandungan Se dalam tanah, menunjukkan bahawa untuk mengukuhkan kandungan Se dalam bijirin padi, paras Se patut dipertingkatkan. Untuk memperoleh pemahaman yang lebih mendalam mengenai tahap pengekalan selenite yang ditambah ke dalam tanah, satu kajian telah dijalankan untuk mengkaji penyerapan and penyahserapan selenite oleh tanah yang digunakan untuk tanaman padi. Isoterma penyerapan dan penyahserapan oleh tanah yang berlainan yang dikumpul dari Tg. Karang, Teluk Intan I, Teluk Intan II, Bagan Serai, Guar Cempedak, Kangar, Jertih, Kota Bharu, Machang dan UPM di Malaysia telah ditentukan. Isoterma penyerapan dan penyahserapan yang diperolehi menunjukkan kaitan yang baik dengan

menggunakan persamaan Freudich dan Langmuir. Data penyerapan dari kajian ini menunjukkan isoterma berbentuk L dan boleh diterangkan melalui affiniti tanah tersebut untuk menyerap Se pada kepekatan yang rendah yang kemudiannya akan berkurangan apabila kepekatan Se meningkat. Ini juga menunjukkan bahawa ketersediaan Se kepada tumbuhan adalah dipengaruhi oleh pH tanah, kandungan bahan organik dan jenis mineral lempung dalam tanah. Serapan selenite meningkat dengan menurunnya pH dan peningkatan kandungan bahan organik. Keputusan menunjukkan bahawa histeresis penyahserapan dalam tanah yang berlainan adalah disebabkan oleh pengikatan sfera dalaman Se kepada keliling mineral secara tidak berbalik. Dalam eksperimen berikutnya, enam varieti padi yang dikumpul dari Pulau Pinang (MR211, MR219, MR220, MR232, MR253, MR263) telah dikaji dengan tiga kadar (0, 20 dan 200 µg Se/L) untuk menyiasat pengumpulan Se dalam akar dan pucuk. Keenam-enam varieti ini menunjukkan reaksi yang jelas melalui penyerapan Se pada pucuk dan akar, tetapi kepekatan Se yang berlainan menunjukkan tiada kesan yang jelas pada parameter akar. Keputusan menunjukkan bahawa pengumpulan Se dalam pucuk kesemua varieti tersebut adalah lebih ketara daripada pengumpulan Se dalam akar. MR253 mencatatkan nilai yang paling rendah. Satu percubaan rumah kaca telah dijalankan dengan menggunakan tiga varieti dipilih daripada kajian kultura larutan, jenis tanah yang dipilih daripada kajian peyerapan dengan kadar Se 0, 100, 300, 500 dan 700 g ha^{-1}. Tujuan kajian ini adalah untuk menilai kepekatan Se dan juga pengambilan Se pada bahagian yang berlainan tanaman padi, terutamanya pada bijirin, dan juga menilai kesan Se ke atas hasil dan sesetengah komponen hasil. Dalam kajian ini, permerhatian telah menunjukkan bahawa selain hasil padi, hasil 1000 bijirin dan jumlah berat kering pucuk, parameter yang lain seperti daun, batang dan hasil bijirin untuk kesemua varieti padi dan juga jumlah pengambilan Se dan pengambilan Se oleh bijirin adalah dipengaruhi oleh kadar selenium yang berlainan. Antara kadar Se yang digunakan, Se pada kadar 500 g ha^{-1} adalah kadar yang dicadangkan untuk meningkatkan kepekatan Se dalam bijirin. Korelasi positif dilihat diantara kepekatan Se dalam bijirin dan daun dan diantara pengambilan Se dan pengumpulan Se dalam padi.

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APPROVAL

I certify that a Thesis Examination Committee has met on 10 March 2015 to conduct the final examination of Nafiseh Alifar on his thesis entitled "Agronomic Biofortification of Rice with Selenium" 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 relevant degree of Doctor of Philosophy.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

Se	Selenium
SeO_3^{2-}	Selenite
SeO_4^{2-}	Selenate
SeO_2	Selenide
Na_2SeO_3	Sodium Selenite
Na_2SeO_4	Sodium Selenate
H_2SeO_4	Selenic acid
H_2SeO_3	Selenic acid
SeF_4	Selenium tetrafluoride
$SeCl_2$	Selenium chloride
H_3Po_4	Phosphoric acid
$Fe(oH)_3$	Iron(III) oxide-hydroxide
Fe_2O_3	Iron(III) oxide
$CaSo_4.2H_2O$	Gypsum
PO_4^{3-}	Phosphate
SeM	Selene methionine
GPX	Glutathione Peroxidase
TRXR	Thioredoxin Reductase
PRI	The European reference intake
RDI	Recommended dietary intakes
NPC	Nutritional Prevention of Cancer
DMRT	Duncan Multiple Range Test
EC	Electrical Conductivity
MOP	Muriate of potash
K2O	Potassium oxide
HF	Hydrogen fluoride
$HCIO^{4-}$	Perchloric acid
HNO^{3-}	Nitric acid
NH_4OAC	Ammonium Acetate
ICP-EOS	Inductively coupled plasma optical emission spectroscopy
DNMRT	Duncan New Multiple Range Test
$\mu g\ g^{-1}$	micro gram per gram
$mg\ g^{-1}$	milligram per gram
$mg\ kg^{-1}$	milligram per kilogram
$kg\ ha^{-1}$	Kilogram per hectare
$mmol\ m^{3-}$	mill mole per cubic metre
H	hours
μM	Micro Molar
mM	Milli molar
$Mg\ Se\ L^{-1}$	Milligram selenium per liter
$Pg\ ml^{-1}$	Picogram per millilitre
$\mu g\ m^{1-}$	microgram per metre
$Mg\ ha^{-1}$	megagram per hectare

W:v	Weight per volume
RCBD	Randomized complete block design
CEC	Cation Exchange Capacity
ANOVA	Analysis of variance
XRD	X-Ray Diffraction
HI	Hysteresis index
OM	Organic matter
°C	Centigrade

CHAPTER ONE

INTRODUCTION

Selenium (Se) is a trace element needed in small amounts by humans and animals for the normal function of a number of selenium dependent antioxidative enzymes, such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR). However, this element can be toxic in larger amounts (>400 µg day^{-1}) (Rotruck *et al.*, 1973; Hartikainen, 2005; Fordyce, 2013). The beneficial and the toxic effects of selenium depend on the amount consumed and on its chemical form (Vadhanavikit *et al.*, 1993; Fairweather-Tait, 1997). Selenium (Se) deficiency occurs in both humans and animals (Reilly, 1996; Rayman, 2000). It is recorded as a risk factor for certain human cancers, and conditions related to low immunity and oxidative stress (Combs, 2001). For farm animals the common Se-deficiency related conditions are white muscle disease, exudative diathesis, and liver necrosis(Reilly, 1996). Selenium goes into the food chain through plants that accumulate Se from the soil (Palmgren *et al.*, 2008). There are Se deficient crops and soils in many areas of the world, containing less than 0.6 and 0.1 µg Se g-1 respectively(Gupta and Gupta, 2000). In nearly all European countries, such as Germany and Austria, the Se supply is below the recommended daily intake (Reilly, 1998).

As stated by a Danish survey, the estimated 5th percentile and the mean dietary intake of Se were 23 and 43 µg day_{-1}, respectively (Larsen *et al.*, 2007). The selenium intake from food of plant origin amounted to a modest 1/5 of this intake. While the recommended dietary intake of Se is 50 and 40 µg day_{-1} for men and women, respectively (Alexander and Council, 2005).

Despite the dietary selenium intake which is sufficient to saturate plasma GPx, it is too low to cause any possible cancer defensive effect, which may occur when selenium is supplemented as selenised yeast at 200 µg day_{-1} (Clark *et al.*, 1996). Scandinavian and Nothern European countries are among the low selenium regions. Miller and Byers (1937) classified plants according to their ability to absorb selenium from the soil and convert the selenium to soluble forms. Documentation of human selenium toxicity is rare, but cases include contamination from consumption of home-grown produce (Rosenfeld and Beath, 1964) and from well water (Beath, 1962; Brogden *et al.*, 1979).

The detection of selenium (Se) in water and soil has gained considerable interest in recent years. The Se content in these two types of environmental samples represents the combination of naturally occurring forms as well as the forms of Se put back into the environment by human activity. The Se content of plants represents the amount of the element extracted from the soil and water sources, and the organic forms in the plants are quite available to the animal world(Palmer *et al.*, 1998).

Plants do not need Se, but they can absorb it from soil solution and recycle it to ingesting animals. Selenium is taken up by plants and incorporated into amino acids and proteins (Shrift, 1973). The selenium accumulation levels in plants depend on the pH value, the amount of available selenium, the $CaCO_3$ content and the salinity of the soil, as well as on plant species (Selim and Sparks, 2010). The plant based food's selenium content differs meaningfully between different areas of the world, depending on soil selenium content and plant species (Larsen *et al.*, 2007). The main source of Se for the majority of people living in the Asian countries is rice, which is consumed by high proportion of Asian population, as its staple diet, but concentrations may be insufficient to maintain human health (Cao *et al.*, 2001; Chen *et al.*, 2002). Recently, the demand for specialty and high quality rice has increased remarkably (Bouman *et al.*, 2005). According to new research findings, most of the rice in the global market fails to provide the daily minimum requirement of Se for an average adult (Williams et al. 2009). Several solutions have been tested to raise those low Se values in cereal crop growing on Se deficient soils, including mineral supplements for human or livestock and the application of Se into soil crop systems, called agronomic biofortification (Eurola *et al.*, 1991; Lyons *et al.*, 2004). The best example is the use of Se fertilizers in depleted soils, which has been extensively practiced with success since 1984 in Finland (Eurola *et al.*, 1991) and also in New Zealand and in China (Gissel-Nielsen *et al.*, 1984).

In comparison with direct supplementation, agronomic biofortification has many beneficial effects, since inorganic Se absorbed by the plant is transformed into an organic form having a higher bioavailability(Hartikainen, 2005; Premarathna, 2005). Application of Se fertilizer to rice fields is cost-effective and an easy way to increase grain Se concentration. Biofortification also provides a conceivable remedy of reaching undernourished rural populations who may have limited access to commercially marketed fortified foods and supplements (Bouis *et al.*, 2011). Therefore, precise application rates and the effective factor in Se adsorption by soils must be considered to maximize the uptake by rice grain.

The ideal type of Se fertilizer and the best fertilizer management strategy for Se deficient paddy cultivation has not yet been identified. Also, there is documentation that in comparison with selenate (SeO_4^{2-}), selenite (SeO_3^{2-}) is not absorbed and transported easily (Arvy, 1993; de Souza *et al.*, 2000). Chen et al. (2002) reported that application of foliar SeO_4^{2-} fertilizer was 30% more effective than foliar SeO_3^{2-} fertilizer. Unfortunately, no studies have been reported about the selenite absorption in different rice genotypes in Malaysia.

1.1 Justification

One of the ways to increase grain Se concentration is by application of Se fertilizer to rice fields. However, the application of fertilizers containing Se is complicated because too low or too high Se concentrations can be harmful for both human beings and animals. In order to eschew Se toxicity and deficiency, it is vital to monitor and optimize Se concentrations in rice. Plant uptake of Se depends on the availability of Se in soils (Wang and Gao, 2001; Martinez *et al.*, 2009) The Se concentration in acidic soil is very low. Since the soils of Malaysia are acidic, Se monitoring is vital.

Rice Se content can be increased by different ways such as by foliar application which is not cost beneficial and by permanent spraying which also leads to environmental Se pollution. Therefore, biofortification will be used. Monitoring Selenium in rice, as the main source of this nutrient is very essential. There is no known scientific research on Se biofortification in Malaysian rice varieties.

1.2 Objectives

1. To increase the Se content of rice in order to supply an adequate amount of Se in Malaysian diet
2. To assess the status of soil Se availability in some rice growing areas
3. To test the efficacy of Se accumulation in some rice genotypes under glasshouse and field conditions
4. To determine the best Se accumulating rice variety and
5. To determine the selenium rate to be applied in fertilizer in order to raise the selenium content

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