SILICON UPTAKE ABILITY OF DIFFERENT RICE GENOTYPES AND THEIR TOLERANCE TO SALT STRESS WITH SILICON FERTILIZATION

By

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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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DEDICATED TO

My beloved husband and daughter;
My late parents;
My sister and brothers

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

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Chairman : Professor Mohamed Hanafi bin Musa, PhD
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Silicon (Si) has a wide range of beneficial impacts on rice (*Oryza sativa* L.) including mitigating salt stress. Planting of existing rice genotypes with Si fertilization is of great importance among the salt mitigation techniques. A series of experiments were carried out to investigate the Si uptake ability of popular *indica* type rice genotypes, Bw 367, At 362, Bg 359, Bg 360, Bg 94-1, and MR 297 and to explore their tolerance to salt stress with the provision of Si. Genotypic variation was observed in tissue Si concentration (µg/100 mg) ranging from 104.0 (Bg 94-1) to 151.0 (Bw 367) in the experiment conducted in nutrient solution using a split plot design. Silicon concentration of 2 mM was found as the adequate level for the highest Si accumulation and to improve plant growth. The different Si rates were tested with genotype Bw 367 in a pot experiment conducted in randomized complete block design. Basal application of Si at the rate of 100 kg SiO_2/ha recorded the highest tissue Si concentration of 395.3 µg/100 mg and the highest uptake (mg/pot) of 1912.5, 291.7 and 424.6 for K, Mg and P, respectively resulting in the maximum yield of 104.6 g/pot. The estimated Si rate for optimum yield was 115 kg SiO_2/ha. Two Si transporter genes, *OsLsi*2 and *OsLsi*6 were found in all the tested genotypes. The highest relative expression of *OsLsi*2 gene was observed in genotype Bw 367 (0.85) and the lowest in Bg 360 (0.38) which was similar to Bg 94-1(0.39). Consequently, Si content (µg/100 mg) in plant tissue followed the same trend with Bw 367 (153.07) and Bg 94-1 (105.05). Rice genotypes were tested in a split-split plot design with Si application revealed that Bw 367 and Bg 94-1 were tolerant and Bg 359, At 362, and MR 297 were moderately tolerant in the highest salinity level of 12 dS/m in solution culture. Accumulation of Si was comparatively higher in stressed plants (salinity level 12 dS/m), as indicated by genotypes Bw 367 and Bg 94-1 accumulating similar Si contents (about 228.00 µg/100 mg), where 3 and 16.4% reductions were observed in their shoot growth, respectively in contrast to the non-saline condition. Applied Si reduced the electrolyte leakage by 53% in Bg 94-1 and Na^+/K^+ ratio by 82% in Bw 367 even at a salinity level of 12 dS/m. Further, proline content and catalase activity were increased by 77 and 106%, respectively in Bw 367, which was statistically similar to Bg 94-1. Similar relative water content was observed in Si treated Bw 367, Bg

94-1 plants, and salinity resistant Pokkali plants which were about 70%. In conclusion, Si fertilization had promising effects on the amelioration of salt stress in *indica* rice genotypes which could accumulate more Si in saline conditions. Except for Bg 360, with Si fertilization, all tested genotypes could successfully be cultivated on marginal lands in saline or salinity-prone areas to keep sustainable rice production.

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

KEUPAYAAN PENGAMBILAN SILIKON BAGI GENOTIP PADI YANG BERBEZA DAN TOLERANSI TERHADAP TEKANAN GARAM DENGAN PEMBAJAAN SILIKON

Oleh

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Silikon (Si) mempunyai banyak faedah ke atas padi (*Oryza sativa* L.) termasuklah mengurangkan tekanan garam. Pembajaan Si merupakan teknik yang terbaik untuk mengurangkan tekanan garam di dalam penanaman genotip padi sedia ada. Satu siri kajian telah dijalankan untuk menilai keupayaan pengambilan Si ke atas genotip padi terkenal jenis *indica,* Bw 367, At 362, Bg 359, Bg 360, Bg 94-1, dan MR 297 dan toleransinya terhadap tekanan garam melalui pemberian Si. Variasi genotip telah diperhatikan dalam kepekatan Si di dalam tisu (µg/100 mg) di antara 104.0 (Bg 94-1) sehingga 151.0 (Bw 367). Kajian tersebut dijalankan dengan menggunakan larutan nutrient dalam rekabentuk 'split plot'. Kepekatan Si pada 2 mM didapati sebagai aras yang mencukupi untuk pengumpulan Si paling tinggi dalam meningkatkan pertumbuhan padi. Genotip Bw 367 telah diuji dengan pemberian Si pada kadar yang berbeza di dalam tanah dengan menggunakan rekabentuk blok secara rawak. Aplikasi Si pada tanah dengan kadar 100 kg SiO_2/ha mencatatkan kepekatan Si tertinggi di dalam tisu, 395.3 µg/100 mg dan pengambilan tertinggi (mg/pot) masing-masing 1,912.5, 291.7, dan 424.6 untuk K, Mg and P dengan memberikan hasil maksimum (104.6 g/pot). Anggaran kadar Si untuk hasil optimum ialah 115 kg SiO_2/ha. Dua gen pengangkut Si, *OsLsi*2 dan *OsLsi*6 telah ditemui dalam semua genotip yang diuji. Ekspresi relatif gen *OsLsi*2 dalam genotip adalah lebih tinggi dalam Bw 367 (0.85) berbanding Bg 360 (0.39) yang hampir sama dengan Bg 94-1(0.38)). Selanjutnya, kandungan Si (µg/100 mg) dalam tisu tumbuhan mengikuti trend yang sama dengan Bw 367 (153.07) dan Bg 94-1 (105.05). Selepas dirawat dengan Si dalam rekabentuk kajian 'split split plot' didapati genotip padi, Bw 367 dan Bg 94-1 adalah toleran, manakala Bg 359, At 362, dan MR297 adalah toleran sederhana di paras kemasinan tertinggi 12 dS/m dalam kultur larutan. Pengumpulan Si adalah lebih tinggi dalam tumbuhan yang tertekan (pada 12 dS/m), seperti yang ditunjukkan oleh genotip Bw 367 dan Bg 94-1 dengan kandungan Si yang hampir serupa (kira-kira 228.00 µg/100 mg), di mana pengurangan 3 dan 16.4% adalah diperhatikan dalam pertumbuhan pucuk, masing-masing berbeza dibandingkan dengan pertumbuhan biasa. Pada masa yang sama, Si yang ditambah pada paras 12 dS/m berjaya

mengurangkan kebocoran elektrolit (EL) sebanyak 53% dalam Bg 94-1 dan nisbah Na^+/K^+ sebanyak 82% dalam Bw 367. Seterusnya, kandungan prolin dan aktiviti katalase telah meningkat sebanyak 77 dan 106% masing-masing dalam Bw 367, yang secara statistik sama dengan Bg 94-1. Kandungan air relatif yang sama telah ditunjukkan pada genotip Bw 367, Bg 94-1 yang dirawat dengan Si berbanding genotip Pokkali yang resistan kemasinan iaitu kira-kira 70%. Sebagai kesimpulan, pembajaan Si mempunyai kesan yang baik terhadap pemulihan tekanan garam dalam genotip padi *indica*, di mana pengumpulan Si lebih banyak berlaku dalam keadaan kemasinan. Selain Bg 360, dengan pembajaan Si, semua genotip yang diuji boleh berjaya ditanam pada tanah marginal di kawasan yang terdedah kepada kemasinan atau cenderung kemasinan dalam mengekalkan pengeluaran beras yang mapan di negara ini.

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Declaration by Members of the Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
Bp	Base pairs
CAT	Catalase
cDNA	Complementary deoxyribonucleic acid
cm	Centimetre
DNA	Deoxyribonucleic acid
DNAase	Deoxyribonuclease
dNTPs	Deoxynucleotides
EC	Electrical conductivity
EL	Electrolyte leakage
g	Gram
kg	Kilogram
l	Litre
µl	Microliter
µg	Microgram
µm	Micrometre
meq	Milliequivalents
mg	Milligram
ml	Millilitre
mM	Millimolar
min	Minute
PCR	Polymerase chain reaction
ppm	Parts per million
RCBD	Randomized Complete Block Design
RPM	Rotation per minute

RNA	Ribonucleic acid
RNase	Ribonuclease
ROS	Reactive oxygen species
RT-PCR	Reverse transcriptase polymerase chain reaction
RWC	Relative water content
SAS	Statistical analysis system
SES	Standard evaluation system
%	Percentage
°C	Degree Celsius

CHAPTER 1

INTRODUCTION

1.1 Overview

Rice (*Oryza sativa* L.), a premier cereal crop feeds about half of the population around the world. It is occupied more than 165 million hectares of land worldwide. Approximately, 1.3 billion Asians heavily depend on rice (Cuong et al., 2017) and their per capita rice consumption is about 130 kg per annum, which provides a substantial amount of daily calorie requirement (Dhakal et al., 2019; Pee, 2014). However, rice production is restricted by different abiotic and biotic anxieties (Datta et al., 2017). Soil salinity, iron toxicity, flash floods, ill-drained condition, drought and soil acidity are the dominant abiotic stresses that affect rice production globally.

Soil salinization has become the main consideration affecting both crop quality and quantity of the glycophytic plants. A million hectares of paddy land are abandoned or marginal in production as a result of soil salinity (Reddy et al., 2017). In Sri Lankan context, salinity-prone paddy land extent is being increased along the coastal belt as well as inland (Perera et al., 2018). The coastal area of Sri Lanka is 22% of the country's total land area and nearly 0.112 million ha of land are affected by the soil salinity. Further, in this coastal belt, abandoned unproductive land has been increased (Perera et al., 2018).

Rice is a glycophyte plant that is more susceptible to salt stress during the seedling stage and reproductive stages (Ali et al., 2014). Osmotic stress, nutrient imbalance and ion toxicity are the main negative effects of salt stress. An excessive amount of sodium ions present in soil interferes with the absorption of other nutrients particularly potassium (K). Salt susceptible rice varieties accumulate a high amount of Na^+, a low amount of proline and K^+ in the plant tissues. Other than the nutrient imbalance in the plant, soil salinity imparts the physiological process including photosynthesis and respiration. Poor seed germination, stunted growth, chlorosis, the formation of sterile spikelets, partially filled grains and lower yields are the other negative effects of salinity imposed on rice plants.

Rice is a silicon (Si) accumulator. It accumulates Si up to 10% of its dry weight of shoot. The beneficial effects of Si are mostly attributed to the high accumulation of Si in plant shoots. Moreover, the Si accumulation in rice plants differs greatly among genotypes, which is attributed to the differences in the roots' Si uptake (Ma et al., 2007). The expression level of Si transporter genes *OsLsi*1, *OsLsi*2 and *OsLsi*6 determine the uptake ability of rice plant.

Second to oxygen, Si is the most abundant element present in the soil. However, the arable lands are having an insufficient level of plant available Si (Nwajiaku et al., 2018). Further, over a long period, growing of rice genotypes with high yield potential depletes available Si in the soil. Silicon enhances the growth of the plant and improves the plant's defence system against multiple biotic and abiotic stresses, including salinity (Javaid et al., 2019), metal toxicities (Huang et al., 2018) and drought (Guntzer et al., 2012).

Many research findings have shown the promising results of exogenous applied Si on amelioration of salt stress in many plants, including rice. Silicon in saline soil promotes rice plant growth and reduces the reduction of grain yield. Silicon-nutrition improves the nutrient uptake and keeps the leaves more erect to prevent mutual shading. Enhanced potassium and reduced sodium uptake resulted in a high K^+/Na^+ ratio in rice plant tissues. The stomatal conductance and leaf transpiration rate were also improved due to the deposition of Si in guard cells of stomata thereby controlling the osmotic stress in the rice plant.

During the last decades, the development of salt-tolerant rice genotypes and screening of rice genotypes for salt tolerance have been accorded top research priority in food security. Using growth regulators, such as gibberellic acid (Rodriguez et al., 2006) and microbes (Zhang et al., 2018) are other possible alternatives for rectifying salt stress in rice. However, applied Si will be more advantageous over the other salt amelioration approaches, as it has a multifaceted role in growth of rice plant and combating against biotic and abiotic stresses as well.

1.2 Problem statement

Food security has become an imperative and important factor worldwide due to the increasing global population, decreasing arable land and dramatic climate change. Global food production has been badly affected by environmental damage due to unsatisfactory agricultural activities and ever-increasing population pressure. Hence, world food production may soon become insufficient to feed world hunger. The ever-increasing demand imposed on agricultural land by burgeoning population has resulted land degradation thus, the rice cultivation has been shifted to the more marginal areas which demand heavier inputs for higher agricultural productivity.

According to the comprehensive review, the development of different rice varieties for salt-tolerance, management of soil nutrients and drainage improvement is the feasible approaches to enhance the production in salt-affected paddy lands. However, Si fertilization in salt affected paddy lands are more advantageous among them due to its many beneficial effects on growth and development of rice plant. Even though three Si transporter genes: *OsLsi*1, *OsLsi*2 *OsLsi*6 are involved in accumulation of Si in various parts of the rice plant, their relative expression differs among the genotypes resulting different Si uptakes. However, research findings on identification of *indica* rice varieties having high silicon uptake ability and their performance against salt stress are scanty.

1.3 Objectives

The overall objective was to improve the productivity of salt-affected paddy lands with Si nutrition.

Specific objectives were: (i) To study the Si uptake of lowland rice, (ii) To study the Si requirement for higher plant growth and grain yield, (iii) To screen rice varieties for the presence of, *OsLsi*2 and *OsLsi*6 genes and elucidate their expression involved in plants for Si uptake and accumulation and (iv) To study the rice genotypes tolerance to salt stress with Si fertilization at the seedling stage.

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