

UNIVERSITI PUTRA MALAYSIA

ENHANCEMENT OF GROWTH, YIELD AND LODGING RESISTANCE OF RICE VARIETY MR219 THROUGH SILICON AND PACLOBUTRAZOL APPLICATION

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

ENHANCEMENT OF GROWTH, YIELD AND LODGING RESISTANCE OF RICE VARIETY MR219 THROUGH SILICON AND PACLOBUTRAZOL APPLICATION

By

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April 2017

Chairman: Assoc. Prof. Uma Rani Sinniah, PhD Faculty: Agriculture

Lodging which is the displacement of plant from its vertical position is a factor affecting rice yield. It could be minimized by increasing the strength of the culm. Silicon (Si) is associated with sturdiness and rigidity whereas paclobutrazol (PBZ) inhibits gibberellin synthesis thus halting internode elongation. To date, no comprehensive research has been carried out in Malaysia particularly on the role of Si in rice. Thus, Si and PBZ were utilized to reveal its effect on agronomic traits and lodging resistance in MR219. Silicon was applied at 0, 2, 4 and 6 g per pot at 56 DAS as topdressing on soil surface in the first screenhouse experiment. Number of tillers, spikelets per panicle, percentages of filled spikelets and effective tillers, weight per panicle, hardness, flag leaf area, chlorophyll content, Si and lignin content increased as the rate of Si increased. Silicon application of 4 g/pot increased and improved yield performance and lodging resistance. The next experiment resolved the best method and stage of Si application whereby plants treated with 4 g/pot of Si as topdressing on soil surface at reproductive stage improved yield and fiber contents. Number of tillers per pot, spikelets per panicle, percentage of filled spikelets, weight per panicle and Si content were highest in application at reproductive stage as topdressing followed by soil incorporation. Meanwhile, chlorophyll a content of topdressed and soil incorporated and flag leaf area of plants treated at reproductive stage as topdressing was the highest. Fiber contents of plants treated at reproductive and maturity stages as topdressing and soil incorporation were significantly higher. The third experiment combined Si with PBZ and tested six treatments: untreated, Si applied at 4 g and 6 g, PBZ applied at 400 mg/l, Si applied at 4 g and 6 g with PBZ 400mg/l. Silicon was applied as topdressing whereas PBZ as foliar at 57 DAS. Application of PBZ reduced plant height, culm length and flag leaf area but increased chlorophyll content. Percentage of filled spikeletes and weight per panicle of plants treated with Si or PBZ were significantly higher as compared to plants treated with combination of two factors. Silicon treated plants had significantly higher lignin and Si content. Cinnamyl

alcohol dehydrogenase was up-regulated 3.4 fold in Si treated plants compared to others. Acidified phloroglucinol staining of leaf sample proved the presence of lignin with varying intensity. Hardness and brittleness was highest in plants treated with Si or PBZ. Scanning electron micrographs showed prominent deposition of trichomes, silica bodies, dumb-belled shaped or ladder like structures whereas X-ray spectra of leaf surface attest silicification of silica cells was more intensive in Si treated plants. In conclusion, Si enhanced yield components, reinforced cell structure and improved overall growth of rice plant. Farmers could use crop residues, such as rice husk ash, straw or rice hull as a cheaper alternative of Si source at the start of reproductive stage to maximize output. Amino acids such as glutamine and histidine, and Si solubilizers could be incorporated to increase Si uptake in rice plant.



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

PENAMBAHBAIKAN PERTUMBUHAN, HASIL DAN KETAHANAN REBAH PADI MR219 MELALUI PENGAPLIKASIAN SILIKON DAN PAKLOBUTRAZOL

Oleh

DEIVASEENO A/P DORAIRAJ

April 2017

Pengerusi: Prof. Madya Uma Rani Sinniah, PhD Fakulti: Pertanian

Rebah yang merupakan anjakan pokok daripada kedudukan menegak ialah satu faktor yang mengehadkan hasil padi. Ini dapat dikurangkan dengan meningkatkan ketahanan batang padi. Silikon (Si) dikaitkan dengan kekukuhan dan ketegaran manakala paklobutrazol (PBZ) menghalang sintesis giberelin lalu menganggu pemanjangan ruas. Sehingga ke hari ini, tidak ada sebarang penyelidikan komprehensif yang dijalankan di Malaysia terutamanya peranan Si dalam padi. Maka, Si dan PBZ digunakan untuk mendedahkan kesannya terhadap ciri-ciri agronomik dan ketahanan rebah MR 219. Aplikasi Si dilakukan secara taburan pada permukaan dengan kadar 0, 2, 4 dan 6 g setiap bekas pada 56 HLT dalam eksperimen pertama. Bilangan anak pokok, spikelet setiap tangkai, peratus spikelet berisi dan anak tangkai efektif, berat setangkai, kekerasan batang, luas daun pengasuh, kandungan klorofil, si dan lignin meningkat seiring dengan kadar Si. Aplikasi Si pada kadar 4 g/bekas meningkatkan prestasi hasil dan ketahanan rebah. Eksperimen seterusnya menentukan kaedah dan peringkat terbaik bagi aplikasi Si di mana hasil aplikasi Si pada kadar 4 g di peringkat reproduktif secara penaburan atas tanah meningkatkan hasil dan kandungan serat. Bilangan anak pokok setiap bekas, spikelet setiap tangkai, peratus spikelet berisi, berat tangkai dan kandungan Si adalah paling tinggi dalam aplikasi pada peringkat reproduktif secara tabur atas tanah diikuti dengan campuran tanah. Sementara itu, kandungan klorofil a dalam pokok yang ditabur Si atas tanah dan dicampur dalam tanah serta luas daun pengasuh adalah tertinggi pada pokok yang diaplikasikan dengan Si pada peringkat reproduktif secara tabur atas tanah. Kandungan serat dalam pokok yang diaplikasikan Si pada peringkat reproduktif dan matang secara kaedah tabur atas tanah dan campur dalam tanah adalah lebih tinggi. Eksperimen ketiga mengabungkan Si dengan PBZdan menguji enam rawatan: tiada rawatan, aplikasi Si kadar 4 g dan 6 g, PBZ 400 mg/l, aplikasi Si kadar 4 g dan 6 g dengan PBZ 400mg/l. Si diaplikasi secara tabur atas tanah manakala PBZ secara semburan pada 57 HLT. Aplikasi PBZ mengurangkan tinggi pokok, panjang batang padi dan luas daun pengasuh tetapi meningkatkan kandungan klorofil. Peratus spikelet berisi dan berat setiap tangkai didapati lebih tinggi dan signifikan pada pokok yang diaplikasikan Si atau PBZ berbanding dengan pokok yang diberikan kombinasi kedua-dua rawatan. Pokok-pokok yang diaplikasikan dengan Si mempunyai kandungan lignin dan Si yang lebih tinggi. Sinamyl-alkohol dehidrogenase, mengalami peningkatan sebanyak 3.4 kali pada pokok yang diaplikasi Si berbanding yang lain. Pewarnaan floroglusinol berasid pada sampel daun menunjukkan kehadiran lignin dengan keamatan yang berbeza. Kekerasan dan kerapuhan batang adalah lebih tinggi dalam pokok yang dirawat dengan Si atau PBZ. Mikrograf imbasan elektron menunjukkan kehadiran trikom, "silika bodies", "dumbbelled shaped" atau struktur-struktur seperti tetingkat yang jelas manakala analisis spektra sinar-X pada permukaan daun menampakkan silifikasi sel-sel silika yang lebih tinggi dalam pokok yang diaplikasi Si. Kesimpulannya, Si menambahbaik komponen hasil, menguatkan struktur sel dan meningkatkan penumbesaran pokok padi secara keseluruhannya. Para petani boleh menggunakan hampas tanaman seperti abu sekam padi, jerami atau sekam sebagai sumber alternatif Si yang lebih murah pada awal peringkat reproduktif untuk memaksimumkan hasil. Amino asid seperti glutamin dan histidin, serta pelarut Si boleh digunakan untuk meningkatkan pengangkutan Si dalam pokok padi.

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I certify that a Thesis Examination Committee has met on 19 April 2017 to conduct the final examination of Deivaseeno a/p Dorairaj on her thesis entitled "Enhancement of Growth, Yield and Lodging Resistance of Rice Variety MR219 through Silicon and Paclobutrazol Application" 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 Doctor of Philosophy.

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TABLE OF CONTENTS

Page

ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	viii
LIST OF TABLES	xiv
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xviii

CHAPTER

0

1	INTR	ODUCTI	ON	1
2	LITE	RATURE	REVIEW	5
	2.1	Origin	of Rice	
	2.2	Taxono	omic Classification	5 5 5
	2.3	Morpho	ology	5
	2.4		and Development Stages of Rice Plant	6
		2.4.1	Vegetative Stage	6
		2.4.2	Reproductive Stage	6
		2.4.3	Ripening Stage	7
	2.5	Rice C	rop Establishment Practices	7
	2.6	Lodgin	g	8
		2.6.1	Types of Lodging	9
		2.6.2	Causal Factors of Lodging	9
		2.6.3	Effects of Lodging	10
		2.6.4	Lodging Resistance Traits	11
	2.7	Silicon		13
		2.7.1	Chemistry and Mechanism of Silicon Uptake by	13
			Plants	
		2.7.2	Silicon in Soils	14
		2.7.3	Role of Silicon	15
	2.8	Plant G	browth Regulators	18
		2.8.1	Paclobutrazol	18
		2.8.2	Role of Paclobutrazol	18
	2.9	Lignin		20
		2.9.1	Lignin Biosynthesis	20
		2.9.2	Lignin Composition and Structure	20
3	OPTI	MIZATI	ON OF RATE OF SILICON TO IMPROVE	22
	YIEL	D, GROV	VTH AND LODGING RESISTANCE OF MR 219	
	3.1	Introdu	ction	22
	3.2	Materia	als and Methods	23
		3.2.1	Treatment and Experimental Design	23
		3.2.2	Crop Establishment	23
		3.2.3	Cultural Practices	24

	3.2.4	Growth Parameters	24
	3.2.5	Flag Leaf Area and Chlorophyll Content	24
	3.2.6	Yield and Yield Components	24
	3.2.7	Quantification of Silicon	25
		3.2.7.1 Oxidization Technique	25
		3.2.7.2 Colorimetric Determination	25
		3.2.7.3 Inductively Coupled Plasma-Optical	25
		Emission Spectrometry (ICP-OES)	
	3.2.8	Macronutrients Analysis	25
	3.2.9	Lodging Resistance	26
	3.2.10	Thioglycolic Acid Lignin Quantification	26
	3.2.11		20
3.3	Results	-	27
5.5	3.3.1	Plant Growth Assessment	27
	3.3.2	Flag Leaf Area and Chlorophyll Content	27
	3.3.3		27
	3.3.4	Lodging Resistance	31
	3.3.5	Macronutrient Analysis	32
	3.3.6	Silicon Quantification	33
2.4	3.3.7	Thioglycolic Acid Lignin Quantification (TGA)	35
3.4	Discus		36
3.5	Conclu	Ision	39
			10
		METHOD AND STAGE OF SILICON	40
		N ON GROWTH, YIELD AND LODGING	
		IN MR 219	10
4.1	Introdu		40
4.2		als and Methods	41
	4.2.1	Treatment and Experimental Design	41
	4.2.2	Crop Establishment	41
	4.2.3	Cultural Practices	41
	4.2.4	Growth Parameters	42
	4.2.5	Flag Leaf Area, Chlorophyll Content and	42
		Photosynthetic Rate	
	4.2.6	Yield and Yield Components	42
	4.2.7	Lodging Resistance	42
	4.2.8	Thioglycolic Acid Lignin Quantification	43
	4.2.9	Fiber Content	43
		4.2.9.1 Neutral Detergent Fiber (NDF)	43
		4.2.9.2 Acid Detergent Fiber (ADF)	43
		4.2.9.3 Acid Detergent Lignin	43
	4.2.10	X-Ray Fluorescence (XRF) Analysis	44
	4.2.11	Statistical Analyses	44
4.3	Results	3	44
	4.3.1	Plant Growth Assessment	44
	4.3.2	Flag Leaf Area, Chlorophyll Content and	45
		Photosynthetic Rate	
	4.3.3	Yield and Yield Components	48
	4.3.4	Lodging Resistance	52
	4.3.5	Fiber Analysis	53
		4.3.5.1 Thioglycolic Acid Lignin	53
			55

4

G

xi

		5 5 5 6
Discuss Conclus NED E WTH,	X-Ray Fluorescence Analysis ion sion	5 5
Discuss Conclus NED E WTH,	sion	5
Conclus NED El)WTH,	sion	
NED E WTH,		6
WTH,	FFECT OF SILICON AND PACLOBUTRAZOL	
	riber of billeon in billeber in belobe	e
ntrodua	, YIELD AND LODGING RESISTANCE OF MR 21	
		6
	lls and Methods	6
5.2.1	Treatment and Experimental Design	6
5.2.2	Crop Establishment	6
5.2.3	Cultural Practices	6
5.2.4		6
5.2.5		6
5.2.6	Yield and Yield Components	6
5.2.7	Quantification of Silicon	6
	5.2.7.1 Oxidization Technique	6
	5.2.7.2 Colorimetric Determination	6
5.2.8	Macronutrients Analysis	6
5.2.9	Lodging Resistance	6
5.2.10		6
5.2.11	Scanning Electron Microscopy	6
5.2.12	Histochemical Staining	6
5.2.1 <mark>3</mark>	Gene Expression Analysis	(
	5.2.13.1 Total RNA Extraction	6
	5.2.13.2 RNA Quantification	6
	5.2.13.3 DNase Treatment	6
	5.2.13.4 Primer Design	6
	5.2.13.5 Reverse Transcription	6
	5.2.13.6 Real Time Polymerase Chain Reaction	6
5.2.10	Statistical Analyses	6
Results		6
5.3.1	Plant Growth Assessment	6
5.3.2	Flag Leaf Area and Chlorophyll Content	7
5.3.3	Yield and Yield Components	7
5.3.4	Silicon Content	7
5.3.5	Macronutrients Analysis	7
5.3.6	Lodging Resistance	7
5.3.7	Lignin Content	7
5.3.8		-
5.3.9		8
5.3.10		8
		9
		9
Jonera		
RY, C	ONCLUSION AND RECOMMENDATIONS FOR	9
5.3 5.3 Di Co	3.9 3.10 scuss onclus	3.9 SEM Observations

APPENDICES BIODATA OF STUDENT LIST OF PUBLICATIONS

118 147 148



Table	I	Page
3.1.	Effect of different rates of silicon application on growth parameters of rice MR219	27
3.2.	Effect of different rates of silicon application on chlorophyll content of rice MR219	28
3.3	Effect of different rates of silicon application on yield components of rice MR219	31
3.4.	Bending parameters corresponding to lodging resistance in response to different rates of silicon	32
3.5	Main and interaction effects of type of sample and rate of silicon application on macronutrients	33
3.6.	Macronutrient content of aboveground and leaf samples of rice MR219	33
4.1.	Effect of method and stage of silicon application on height and culm length of rice MR219	45
4.2.	Main and interaction effects of method and stage of silicon application on leaf area, chlorophyll content and photosynthetic rate of rice MR219	46
4.3	Main and interaction effects of method and stage of silicon application on yield components of rice MR219	49
4.4	Main and interaction effects of method and stage of silicon application on lodging resistance of rice MR219	53
4.5	Main and interaction effects of method and stage of silicon application on thioglycolic acid lignin content of rice MR219	54
4.6	Main and interaction effects of method and stage of silicon application on fiber content of rice MR219	55
4.7	Main and interaction effects of method and stage of silicon application on silicon content of rice MR219	57
5.1	Detailed information of genes and primers used in this study	68
5.2.	Effect of silicon and paclobutrazol on internode length and panicle length of rice MR219	71
5.3	Effect of silicon and paclobutrazol on chlorophyll content and flag leaf area of rice MR219	72

LIST OF TABLES

C

- 5.4 Effect of silicon and paclobutrazol on percentage of effective tillers, 74 weight/panicle and number of spikelets/panicle of rice MR219
- 5.5 Effect of silicon and paclobutrazol on percentage of filled spikelets 75 and weight of 100 grains of rice MR219
- 5.6 Effect of silicon and paclobutrazol on silicon content in leaf and stem 76 samples of rice MR219

77

- 5.7 Effect of silicon and paclobutrazol on macronutrients in leaf and aboveground plant samples of rice MR219
- 5.8. Bending resistance of MR219 in relation to application of silicon and 78 paclobutrazol
- 5.9. Effect of silicon and paclobutrazol on thioglycolic acid lignin content 79 in leaf and stem plant samples of rice MR219

LIST OF FIGURES

Figure		Page
3.1.	Effect of silicon on flag leaf area	28
3.2.	Relationship between total chlorophyll content and flag leaf area in response to application of silicon	29
3.3.	Effect of silicon on total number of tillers and number of tillers per hill	30
3.4.	Relationship between number of spikelets per panicle and weight per panicle in response to application of silicon	31
3.5.	Relationship between hardness and stiffness of culms in response to application of silicon	32
3.6.	Colorimetric content of silicon in stem and leaf samples of rice MR219	34
3.7.	Silicon content as quantified by ICP in stem and leaf samples of rice MR219	35
3.8.	Thioglycolic acid lignin content in stem samples of rice MR219	36
4.1	Effect of combination of silicon treatments on flag leaf area	47
4.2	Effect of combination of silicon treatments on photosynthetic rate	48
4.3.	Effect of combination of silicon treatments on total number of tillers	50
4.4.	Effect of combination of silicon treatments on number of tillers per hill	50
4.5.	Effect of combination of silicon treatments on weight of 100 grains	51
4.6.	Effect of combination of silicon treatments on weight per panicle	52
4.7.	Effect of combination of silicon treatments on neutral detergent fiber content	55
5.1.	Effect of silicon and paclobutrazol on height of rice MR219	70
5.2.	Effect of silicon and paclobutrazol on culm length of rice MR219	70
5.3	Relationship between total chlorophyll content and flag leaf area of rice MR219	72

5.4.	Effect of silicon and paclobutrazol on number of tillers and number of tillers per hill of rice MR219	73
5.5.	Relationship between spikelets per panicle and weight per panicle of rice MR219	75
5.6.	Relationship between hardness and brittleness of culm of rice MR219	78
5.7.	mRNA transcript abundance of cinnamyl alcohol dehydrogenase of rice culm MR219	80
5.8.	Scanning electron micrographs of silicon mapping and distribution of rice MR219 leaves	81
5.9.	SEM and the respective EDX spectra of leaf adaxial surface of rice MR219	83
5.10.	SEM and the respective EDX spectra of leaf abaxial surface of rice MR219	85
5.11.	Parenchyma cells of longitunidal section of stem stained with phloroglucinol at 40X	88
5.12.	Phloroglucinol staining showing lignin deposition in leaf cross section	89

(C)

LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADL	Acid detergent lignin
Al	Aluminium
AID	autoclaved induced digestion
bp	Base pair
С	Control
CAD	cinnamyl alcohol dehydrogenase
cDNA	Complementary Deoxyribonucleic acid
cm	Centimeter
0	Degree
°C	Degree Celcius
DNA	Deoxyribonucleic acid
EDX	Energy dispersive X-ray
GA	Gibberellin
g	Gram
g/l	Gram/litre
G	Guaiacyl
GC	Guanine - cytosine content
н	p-hydroxyphenyl
hr	Hours
ICP	Inductively Coupled Plasma
IRRI	International Rice Research Institute
KADA	Kemubu Agricultural Development Authority
KETARA	Northern Integrated Agricultural Development
	Area
kg	Kilogram
kg ha-1	Kilogram per hectare
kPa	Kilo Pascal
Mn	Manganese
mRNA	Messenger Ribonucleic Acid
μΙ	Microlitre
mg	Miligram
ml	Millilitre
mm	Millimetre
mM	Milimolar
min	Minutes
MADA	Muda Agricultural Development Authority

nm	Nanometer
NDF	Neutral detergent fiber
NPK	Nitrogen, phosphorus and potassium
Ν	Normality
OD	Optical density
PBZ	Paclobutrazol
PBLS	Barat Laut Selangor Integrated Agricultural Development Area
%	Percentage
Р	Phosphorus
PCR	Polymerase Chain Reaction
qPCR	Quantitative polymerase Chain Reaction
SEM	Scanning electron Microscopy
Si	Silicon
S	syringyl
TGA	Thioglycolic acid lignin
ton/ha	Tonnes per hectare
w/w	Weight/weight
XRF	X-Ray Fluorescence
Zn	Zinc

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CHAPTER I

INTRODUCTION

Oryza sativa, rice is a tropical grain that serves as the most important staple food for a large part of the human population especially Asians. This major food crop is relatively cheap as compared to other crops, nutritious and fulfils one's calorie intake. According to IRRI (1995), more than a billion people depend on rice cultivation for livelihoods. In most of the developing countries such as Malaysia and Thailand, rice which is heavily regulated and subsidized is equated to food security and political stability.

Rice production systems are distinctive as they are grown in a wide range of ecological and climatic conditions, such as rainfed lowlands, uplands, mangroves and deepwater areas. The lowland rice agriculture is now responsible for 86% of the total world rice crop and the yields are typically in the range of 2.0 - 3.5 ton/ha (Ladha et al., 1997). In Malaysia, rice is the third most important crop, after rubber and oil palm. Rice is mainly grown in the eight granaries in Peninsular Malaysia namely, MADA, KADA, KETARA, PBLS, Krian, Seberang Perak, Seberang Perai and Kemasin. The total area dedicated to rice planting in Malaysia is 674 928 ha of which two third is in Peninsular Malaysia whereas one third of the area is in Sabah and Sarawak (Ramli et al., 2012). For a population of 30 million, rice production is only 70% sufficient and the demand will continue to increase as the population increases. It is suffice to state that in Malaysia, rice production is not parallel to population increment.

Reliance on neighbouring countries such as Thailand and Vietnam for 30% of rice importation is not a wise solution. What would befall this nation if major rice exporters refuse to sell this prized grain due to shortage, economical or political grounds? To address the crisis, Malaysia needs to produce at least 2.5 million metric tonnes of paddy annually or increase the yield to 10 ton/ha from the current average of 4.5 ton/ha to meet the demand. The National Food Security Policy aims to increase its domestic production to at least 85% by 2015 which will then reduce importation of rice by 15% (Mohd Rashid and Mohd Dainuri, 2013). This remains a distant vision as of 2016, since our production is yet to even hit 75%. Lack of arable land, improper nutrient management practices, unchanged agronomic practices, failure of farmers to adopt and adapt to new technological inputs, biotic and abiotic stresses had collectively led to the failure to reach potential rice yield.

Lodging, a yield limiting factor that is caused by both biotic and abiotic factors, is defined as the displacement of the stem from its upright position (Fageria et al., 2006). Lodging can be bending of the stem, breaking of the stem and also the inability of the root structure to support the plant. Lodging is a complex phenomenon that interferes with water and nutrient uptake, reduces light interception, provides favourable environment for leaf disease, increases harvesting cost and losses and decreases grain yield (Kono, 1995; Tripathi et al., 2003). IRRI (1995) has reported a yield loss of 1

ton/ha which accounts for 20% of the total rice production whereas in Southern India there was a loss of 26 kg/ha due to lodging (Duwaryi et al., 2000). MADA granary which covers an area of 98 860 ha is the largest granary in Malaysia producing 25% of the total national rice production (MOA, 2008). A loss of 41% of yield due to lodging has been reported in this granary. As direct seeding is the main mode of rice establishment in Malaysia, the occurrence of lodging is more pronounced due to weed infestation, weaker and shallow root system.

Several studies had shown that silicon (Si), a beneficial element, can prevent lodging, increase photosynthesis, decrease susceptibility to disease and insect damage, prevent lodging, and alleviate water and various mineral stresses (Korndorfer and Lepsch, 2001; Epstein, 1999; Ma, 2004; Wu et al., 2006). Silicon could increase thickness of culm wall and increase size vascular bundles (Shimoyama, 1958; Wu et al., 2006). Highly abundant it may be but Si rarely exists in pure form. It is usually found in various forms of silicon dioxide (silica) or silicates. For instance, it exists as alumina silicate in clay soils where rice is grown. This element is widely used in the field of plant pathology as an alleviator of biotic stresses. Silicon applied as silicon dioxide, SiO_2 in fields are absorbed and appears to accumulate in leaves and promote mechanical strengthening. As rice is a Si accumulator, it is vastly utilized in China and Japan to increase productivity. Despite its significance, there is a glaring void and lack of researches on the effect of Si in growth, yield and lodging resistance in Malaysia specifically.

Malaysia which has a hot and humid climate is predominantly covered by tropical soils that undergo chemical weathering and leaching. As rice is grown continuously without the need for rotation with at least two harvests a year on the same plot of land, depletion of available silicon is very much higher due to the process of desilicification. In a study carried out by Japanese researchers on paddy soils in tropical Asia, the available silicon dioxide in Malaysian soil is 10.4 mg/100 g dry soil which according to criterion adopted in Japan for silica fertilization is low (Kawaguchi and Kyuma, 1974). Based on that standard, values less than 10.5 mg/100 g dry soil are considered low whereas values in the region of 13 mg is considered positive. This remains the only research which gave an indication of Si status in Malaysian soil. Though it has been about four decades since then, to date no studies has been undertaken to reveal the potential benefits in terms of agronomic traits and lodging resistance with the application of Si.

Besides fertilizers, growth regulators are known to control many processes within plants. There are various reports on the effect of exogenously applied plant growth regulators on plant growth and development (Bevilaqua et al., 1993,1995; Pan and Zhao, 1994; Asborno et al., 1999). Paclobutrazol (PBZ) is a triazole fungicide that is often termed as a plant growth retardant as it is a known opponent of the plant hormone gibberellin. acts inhibiting gibberellin biosynthesis, It by reducing internodial growth to give stouter stems, increasing root growth, causing early fruitset and increasing seed set. Paclobutrazol is used to reduce shoot growth too and has been utilized in rice by Bambang (2012) who found that an application of 400 mg/l was optimum in reducing plant height and culm height, increased bending resistance and vield.

Lodging could be reduced by lowering the centre of gravity that is to reduce the plant height. Increasing the mechanical or physical strength of the rice culm is also another mean of addressing lodging. Cell wall which is a highly complex structure and primarily made up of cellulose, also contains hemicelluloses, lignin, polysaccharides and protein. The structural carbohydrates such as lignin, cellulose and hemicelluloses can be termed as the backbone for plants and play a more dominant role as it provides mechanical support hence an increase in these contents would further improve culm strength. In addition, the thickness of cell walls in the sclerenchyma and the number of vascular bundles are important factors that affect the stem mechanical strength of rice (Li et al., 2009).

Silicon, a non-essential nutrient as many biologists believe, is often excluded in nutrient solution and fertilization as it is often thought that the soil itself can sustain its supply. Unfortunately their conviction is wrong since the silica that occurs in soil is in an unavailable polymerized form and for its absorption by plants it has to be depolymerized and rendered soluble by means of biological or chemical reactions in soil. Plants absorb Si exclusively as monosilicic acid, also called orthosilicic acid, by diffusion and also by the influence of transpiration-induced root absorption known as mass flow (Elawad and Green, 1979).

It is believed that lodging can be reduced by optimum use of nitrogen, right amount of water, reducing the plant height, strengthening the culm and incorporation of plant growth regulators. Breeding is by far the most effective method to combat lodging but it takes about 12 to 15 years to develop a new variety. As such, farmers lose opportunities to grow better varieties earlier. Thus, the best method to overcome the problem is to manipulate physiological activities of plants by use of chemical for convenience, in this case it would be a plant nutrient, Si and plant growth retardant, PBZ.

Therefore, the overall strategy will be to incorporate Si which can enhance the mechanical strength of the rice culm with PBZ which has proven to decrease the length of internodes. The hypothesis is that a short plant with an increase in rigidity will help our cause in overcoming the problem of lodging. However, Si will remain the main focus of this study as its combination with PBZ is yet to be tested.

1.2 Objectives

The primary objective of this research is to look into the possible use of Si in increasing yield and lodging resistance.

The specific objectives of this research were:

1. To determine the best rate of Si application on yield, growth and lodging resistance of MR219.

2. To resolve the best method of Si application on yield, growth and lodging resistance of MR219.

3. To study effect of timing of Si application on yield, growth and lodging resistance of MR219.

4. To study the combined effect of Si and PBZ on yield, growth and lodging resistance of MR219.



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