UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF CAUSAL AGENT OF BROWN SPOT AND USE OF SILICON AND MANGANESE FOR DISEASE MANAGEMENT IN RICE

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By

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Chairman : Assoc. Prof. Wong Mui Yun, PhD
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Brown spot is one of the diseases that became a new threat to rice production and was reported to reduce the yield up to 45% under severe infection. However, pathological related information on this disease is lacking in Malaysia. This study focused on identification of the causal agent through morphological and molecular methods, as well as determination of the effects of silicon (Si) and manganese (Mn) on disease severity and PR-proteins gene expression. A total of 15 isolates were isolated, identified and screened with pathogenicity test before characterized culturally. Two isolates of *Bipolaris oryae* and 13 isolates of *Exserohilum rostratum* were identified based on morphology and internal transcribe spacer (ITS) region of rDNA. AS.P2 (Gene bank accession number KT831962) as one of the most virulent isolates in pathogenicity test was further studied for cultural characterization. Highest spore production was recorded in T2 (12 h flourescent light + 12 h complete darkness) and T4 (24 h darkness) cultured on corn meal agar (CMA) with no significant difference between them (21.67×10^6 spores/mL and 21.50×10^6 spores/mL, respectively). The lowest spore production was shown by cultures grown on malt extract agar (MEA) (0.83×10^6 spores/mL). Two sources of Si, namely calcium silicate ($CaSiO_3$) and black rice husk ash (BRHA) were tested in combination with Mn to determine a cheaper source with the same effectiveness. Results showed significantly higher value of brown spot index (BSI) for T1 (control) where no Si and Mn were applied with 41.14%. Addition of Si and Mn increased 38.65 to 51.25% for grain yield production, if compared to T1 (control). BRHA showed good performance comparable to $CaSiO_3$ in tiller number, percentage of filled grains, spikelet number per panicle, panicle length and 1000 grains weight. Plants treated with Mn showed differential expression of β-1,3-glucanase and chitinase with β-1,3-glucanase showed the highest expression at 2 DAI and chitinase at 1 DAI. Similarly, plants treated with Si showed differential expression of β-1,3-glucanase and chitinase with the highest expression recorded at 3 DAI and 1 DAI, respectively. This study found *E. rostratum* a new causal agent for rice brown spot disease in Malaysia

and BRHA has the potential to be a cheaper source of Si to be applied in agriculture sector compared to $CaSiO_3$.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PENCIRIAN AGEN PENYEBAB BINTIK PERANG DAN PENGGUNAAN SILIKON DAN MANGAN UNTUK PENGURUSAN PENYAKIT TERSEBUT PADA PADI

Oleh

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Bintik perang merupakan salah satu penyakit yang memberi ancaman kepada penghasilan padi dan dilaporkan mampu menurunkan 45% hasil pada tahap infeksi yang teruk. Walau bagaimanapun, maklumat berkaitan patologi penyakit ini masih kurang di Malaysia. Kajian ini memfokuskan kepada pengenalpastian agen penyebab melalui kaedah morfologi dan molekular, serta menentukan kesan silikon (Si) dan mangan (Mn) terhadap kadar keterukan penyakit dan ekspresi gen protein PR. Sejumlah 15 pencilan telah diasing, dikenalpasti dan disaringkan melalui ujian kepatogenan sebelum dicirikan secara kultur. Dua pencilan *Bipolaris oryae* dan 13 pencilan *Exserohilum rostratum* berjaya dikenalpasti berdasarkan morfologi dan jujukan DNA kawasan ITS dalam gen rDNA. AS.P2 (nombor aksesi Gen Bank, KT831962) sebagai salah satu pencilan yang getir dalam ujian kepatogenan, dipilih untuk kajian pencirian kultur. Penghasilan spora tertinggi dicatatkan oleh T2 (12 jam berpendarfluor + 12 jam gelap) dan T4 (24 jam gelap) yang telah dikulturkan di atas 'corn meal agar' (CMA) dan tiada perbezaan signifikan ditunjukkan (21.67×10^6 spora/mL and 21.50×10^6 spora/mL, masing-masing). Penghasilan spora terendah ditunjukkan oleh pencilan di atas 'malt extract agar' (MEA) (0.83×10^6 sporea/mL). Dua sumber silikon, iaitu kalsium silikat ($CaSiO_3$) dan abu hitam sekam padi (BRHA) telah dikombinasikan dengan Mn untuk menentukan sumber yang lebih murah dengan keberkesanan yg sama. Hasil kajian menunjukkan nilai indeks bintik perang (BSI) lebih tinggi dan signifikan pada T1 (kawalan) dimana tiada Si dan Mn digunakan, 41.14%. Penggunaan Si dan Mn meningatkan 38.65 ke 51.25% hasil padi, berbanding T1 (kawalan). BRHA menunjukkan pretasi yang setanding dengan $CaSiO_3$ berdasarkan bilangan tiller, peratusan biji padi penuh, bilangan spikelet per panicle, panjang panicle dan berat 1000 biji padi. Pokok padi yang dirawat dengan Mn menunjukkan perbezaan ekspresi β-1,3-glukanase and kitinase dimana β-1,3-glukanase menunjukkan ekspresi tertinggi pada 2 DAI dan kitinase pada 1 DAI. Begitu juga dengan pokok yang dirawat dengan Si, ia menunjukkan ekspresi tertinggi

β-1,3-glukanase dan kitinase pada 3 DAI dan 1 DAI, masing-masing. Kajian ini menemukan *E. rostratum* sebagai agen penyebab baru penyakit bintik perang padi di Malaysia dan BRHA mempunyai potensi untuk dijadikan sumber Si yang lebih murah untuk di aplikasikan dalam sektor pertanian berbanding $CaSiO_3$.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ATP	Adenosine Tri-phosphate
Bp	Base pair
BRHA	Black rice husk ash
BSI	Brown spot index
CA	Czapex-doc agar
$CaSiO_3$	Calcium silicate
cDNA	Complementary Deoxyribonucleic acid
CMA	Corn meal agar
$cmolckg^{-1}$	Centimoles per kilogram
DAI	Day after inoculation
DAP	Day after planting
DOA	Department of Agriculture
FOA	Food and Agriculture Organization of the United Nations
g	Gravitational force
IRRI	International Rice Research Institute
K	Potassium
LKPP	Lembaga Kemajuan Perusahaan Pertanian
MEA	Malt extract agar
Mn	Manganese
N	Nitrogen
NPK	Nitrogen, Phosphorus, Potassium
OCM	Organic clay muck
P	phosphorus
PDA	Potato dextrose agar
PR protein	Pathogeneisis related protein
RHA	Rice husk ash
rpm	Rotation per minute
RT-PCR	Reverse transcriptase Polymerase Chain Reaction
S	Silicon
spore/mL	Spore per milliliter
V	Volt
w/v	Weight/Volume

CHAPTER 1

INTRODUCTION

Rice supplying 20% of energy to the consumers, containing high thiamine, riboflavin and niacin, covers 17 countries in Asia and Asia Pacific, 9 in North and South America and 8 from Africa (FAO, 2004). In Asia, approximately 640 million tons of rice was produced per year, covering 90% of worlds total rice production (IRRI, 2008).

The infection caused by pests and diseases may become the main factors preventing from getting good quality and high quantity of rice yield. The common disease caused by both fungal and bacteria are bacterial leaf streak, sheath blight, false smut, blast, brown spot and stem rot whereas green leaf hopper, leaf folder, stem borer, plant hopper and black bug are the insects that often infected rice plants (Rice Knowledge Bank, IRRI). Brown spot is one the diseases that causing seedling blight and it may damage the foliage and rice panicle when grown under unfavorable and low nutrient content of soil (Marchetti and Peterson, 1984). Gray center or light reddish brown lesions surrounded by a dark reddish brown margin with bright yellow halo can be observed as symptom of brown spot disease (Ou, 1985).

Low level of calcium, potassium, magnesium, manganese, iron, and silicon may enhance the occurrence of brown spot disease on the grains (Webster and Gunnel, 1992). Combination of plant physiological disorders, process of disease development and mechanism of disease resistant was regarded as the factors enhance the spread of the disease. Brown spot disease may reduce 12 to 30% of rice weight and 18 to 22% of filled grains per panicles, depending on the resistant variety being used (Prabhu and Lopes, 1980). In the Philippines, brown spot disease was described as a minor disease, however, the occurrence was repeatedly observed (Estrada, 1984).

Silicon (Si) is one of the important element that alleviate both biological and physiological stresses has been applied to control brown spot disease of which low Si available for plant uptake (Datnoff *et al.,* 1997). However, the importance of this element in agronomy and horticulture are not very well applied (Heckman, 2013) and plant physiologist always ignored Si as the deficiency or toxicity of this element did not show any symptoms (Ma *et al.,* 2006).

The availability of certain micronutrients in soil may be reduced due to water shortage as they mainly dependent on the water uptake by the plants (Allaway, 1968; Gardner *et al.,* 1985; Weil and Holah, 1989). Manganese is one of the micronutrients that may reduce or increase brown spot disease in rice plant (Kaur *et al.,* 1979). It helps in production of lignin and soluble phenolic compounds as well as inhibiting lytic enzyme produced by the pathogen

(Graham and Webb, 1991). Plant with low level of manganese (Mn) showed amino acids, lignin and phenolic compounds reduction, all involves in resistant mechanism against pathogen attack (Brown *et al.,* 1984; Burnell, 1988).

Good quality rice should be grown as it will determine the price of the rice (Soave *et al.,* 1984). Thus, a good source of fertilizer should be implemented so that farmers will not be burdened by new practices to control brown spot disease and at the same time they can produce a very good and high production. This study investigated whether the uses of Si and Mn as a new cultural practice could solve the problems. In addition, new source of Si which is cheaper and affordable to the farmers were accounted, and therefore RHA was tested in this study. The main objectives were:

i. To isolate and identify brown spot fungal pathogen *via* morphological and molecular methods.
ii. To determine the effects of Si and Mn on disease incidence and rice yield under glasshouse conditions.
iii. To study the effect of Si and Mn on gene expression of PR-proteins.

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Mahmad Toher, A.S., Mior Ahmad, Z.A. and Wong, M.Y. 2016. First report of Exserohilum rostratum as pathogen of rice brown spot in Malaysia. *Disease Notes. The American Phytopathological Society* 100:226.

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