UNIVERSITI PUTRA MALAYSIA

CHANGES IN ANTIOXIDANT ENZYMES AND GROWTH PARAMETERS IN RICE (Oryza sativa L.) GENOTYPES UNDER SUBMERGENCE

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By

REVANDY ISKANDAR MUDA DAMANIK

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

June 2013

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DEDICATED TO:

My MOTHER, BROTHERS, SISTER, BROTHER IN LAW, SISTER IN LAWS,

NEPHEWS (Nathan, Frans, Frederik) and

NIECES (Rinda, Felicia, Cornelia, Lika, Lina)

WHO ALWAYS HAVE SUPPORTING AND SHOWERING ME WITH THEIR

SPIRIT, LOVE AND PRAYER.

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

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June 2013

Chairperson : Professor Maziah Mahmood, PhD

Faculty : Biotechnology and Biomolecular Sciences

Submergence is one of the main abiotic stresses limitations for yield and quality production of many important crops. In order to better understand the physiological and biochemical responses by submergence stress in rice (*Oryza sativa* L.), this thesis was mainly examined to clarify the biochemical markers of contrasting rice genotypes/cultivar during cell level, germination and seedling stages, and discusses the basis of tolerance related genotypes/cultivar. For that reason, in order to recognize and also understand better for the biochemical mechanism of submergence tolerance, the objective of this study was to find out the activities of antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR), as well as for lipid peroxidation in rice plant subjected to different periods of submergence stress. The isoenzymic profile analysis of the antioxidant enzymes also examined in anoxia-stressed suspension culture to get further understanding of the mobilization of protein reserves as well as apperearance of new isoenzymes in submergence stress.

Until now, there have been some researchs and reports related to the influence of submergence stress on the growth of rice plants at seedling growth (Colmer and Pedersen, 2008; Das *et al.*, 2005; Ella *et al.*, 2003; Sarkar *et al.*, 2001), but only a small amount of research has been focused on the influence of submergence stress at early stage (a week after germination) of seedling. Plant growth rate and antioxidative defense responses of early seedlings in six selected Malaysian rice genotypes, MR84, MR185, MR219, MR219-4, MR220, and MR219-9, and FR13A cultivar (known as cultivated plant for submergence tolerant) at different submergence periods (4, 8, and 12 days) were examined. Eleven day old seedlings of hypoxia resistant Malaysian rice genotypes and tolerant rice cultivar (FR13A) were hydroponically grown in normoxic or hypoxic conditions inside the laboratorium. The result on chlorophyll content in rice seedlings suggested that photosynthesis was affected beginning as early as 4 d under submerged condition, except for FR13A cultivar. Activities of superoxide dismutase (SOD) increased linearly with the longer periods of submergence stress until up to 8 d submerged for FR13A cultivar, and MR84, MR185, MR219-4, and MR219-9 genotypes when compared with the control. In contrast, the result showed that catalase (CAT) activity was activated while ascorbate peroxidase (APX) activity was deactivated, and vice versa, in response for submergence conditions for FR13A cultivar and MR219-9 genotype. Additionally, submergence stress led to a significant increase in glutathione reductase (GR) activity for all genotypes started at 4 d to 8 d after being submerged, except for MR219 genotype. From the results obtained in this study, give strength to our hypothesis that detoxification of O_2^- to H_2O_2 was maintained at a stable level throughout the submergence stress until up to 8 days (specifically for MR219-4 and MR219-9 genotypes) and the role of GR might be

more efficient in cytotoxic H_2O_2 necessary for the observed protection from submergence stress.

The potential involvement of activated oxygen species by submergence stress was studied also focusing on two Malaysian rice genotypes, MR219-4 and MR219-9, and cultivar FR13A at the seedling stages. Fourteen-day-old seedlings of three rice cultivars were subjected to different submergence periods (4, 8 and 12 days) in greenhouse. Under 8 days of complete submergence, FR13A cultivar showed higher lipid peroxidation in terms of malondialdehyde (MDA) level and activities of antioxidative enzymes, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) when compared to the MR219-4 and MR219-9 genotypes. MR219-9 genotype showed higher SOD, APX and GR activities after 12 days of submergence. The levels of SOD activity indicated that detoxification of $O_2^{\cdot -}$ to H_2O_2 was maintained at a stable level throughout the submergence stress until up to 8 days and increased rapidly at 12 days of submergence. The results showed that tolerance to submergence is linked until 8 days submergence for MR219-4 genotype and FR13A cultivar. In addition, CAT activity has much higher affinity for scavenges H_2O_2 than APX, therefore ascorbate glutathione cycle might be more efficient to scavenge H_2O_2.

The consequence of oxygen deprivation (anoxia) on the antioxidant system in suspension culture of anoxia intolerant Malaysian rice genotypes and FR13A genotype cells was examined. The antioxidative enzymes were decreased for MR219-4 and MR219-9 genotypes for CAT and APX activities, and increased in FR13A cultivar starting at 20 days in suspension culture compared to that of control. CAT

and APX activities were maintained higher in oxygen depriviation conditions for all genotypes and cultivar suggested that anoxia stress in suspension cultures induced the level of H_2O_2 to toxic levels.

The relationship between the enzyme activity and isoenzyme of the SOD and peroxidase (POD) in cell suspension rice submitted to anoxia stress (oxygen depriviation) has been evaluated. Cell suspension culture of rice exhibited three kinds of SOD bands, identified as MnSOD, FeSOD and Cu/ZnSOD. A substantial improvement was identified in the activity of MnSOD isozymes since it has been prominent appeared for all genotypes and cultivar at different periods of stress. It is practical to take on that mitochondrial partition senses oxygen depletion condition faster than the rest of the cell (peroxisome, chloroplast, etc) partition because it is the most important consume of oxygen. The higher activity of POD and its isoenzymes activities for MR219-4 genotype and FR13A cultivar, maybe compensate for the decline in CAT activity. These results recommend that POD, SOD and CAT activities play necessary and synergy protection responsibility aligned with H_2O_2 toxic levels in rice suspension culture in anoxia condition. Induction of their isoenzymes/isoforms and activities of POD, CAT and SOD provide protection from oxidative damage, hence these parameters might be taken as selection criteria for study of submergent tolerance in rice.

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

PERUBAHAN DALAM ENZIM ANTIOKSIDAN DAN PARAMETER PERTUMBUHAN PADI (Oryza sativa L.) DALAM TEKANAN PERENDAMAN

Oleh

REVANDY ISKANDAR MUDA DAMANIK

Jun 2013

Pengerusi: Professor Maziah Mahmood, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Perendaman merupakan salah satu tegasan utama abiotik untuk menghadkan hasil dan kualiti tanaman yang penting. Dalam usaha untuk lebih memahami tindak balas fisiologi dan biokimia dalam tekanan perendaman pada padi (*Oryza sativa* L.), tesis ini terutamanya diperiksa untuk menjelaskan penanda biokimia genotip padi berbeza pada tahap sel, percambahan dan peringkat anak benih dan membincangkan asas toleransi genotip yang berkaitan. Oleh itu, dalam usaha untuk lebih memahami mekanisme biokimia toleransi perendaman, matlamat kajian ini adalah untuk menentukan aktiviti enzim antioksidan (superoxide dismutase, SOD; catalase, CAT; askorbat peroksidase, APX; glutathione reductase, GR), serta sebagai peroksidaan lipid dalam tanaman padi diserahkan kepada tekanan sederhana atau teruk semasa tekanan perendaman pendek atau jangka panjang. Analisis profil isoenzymic enzim antioksidan juga diperiksa dalam tekanan anoxia dalam suspensi kultur untuk

mendapatkan pemahaman lanjut dan mobilisasi rizab protein serta apperearance daripada isoenzim baru dalam tekanan perendaman.

Sehingga kini, telah ada beberapa laporan mengenai pengaruh tekanan perendaman pada pertumbuhan tanaman padi pada kadar pertumbuhan anak benih (Colmer dan Pedersen, 2008; Das et al, 2005; Ella et al, 2003;. Sarkar et al, 2001), tetapi sedikit penyelidikan tumpuan kepada pengaruh tekanan perendaman pada peringkat awal anak benih. Laju kadar pertumbuhan dan tindak balas pertahanan antioksida benih awal dalam pilihan enam beras genotip Malaysia, MR84, MR185, MR219, MR219-4, MR220 dan MR219-9, dan FR13A (kultivar toleran perendaman) pada tempoh perendaman yang berbeza (4, 8, dan 12 hari) telah diperiksa. Sebelas hari anak benih genotip padi Malaysia dan padi kultivar toleran (FR13A) telah secara hidroponik berkembang dalam keadaan normoxic atau hipoksia di dalam makmal. Hasil kajian terhadap kandungan klorofil dalam anak benih padi mencadangkan bahawa fotosintesis terjejas bermula seawal sebagai 4 d bawah keadaan tenggelam, kecuali bagi FR13A kultivar. Aktiviti superoxide dismutase (SOD) meningkat secara linear dengan tempoh perendaman sehingga 8 hari bagi kultivar FR13A, dan MR84, MR185, MR219-4, dan MR219-9 genotip berbanding dengan kawalan. Sebaliknya, hasilnya menunjukkan bahawa katalase (CAT) aktiviti telah diaktifkan manakala askorbat peroksidase (APX) aktiviti telah dinyahaktifkan, dan sebaliknya, sebagai tindak balas untuk keadaan terendam, untuk kultivar FR13A dab MR219-9 genotip.. Selain itu, tekanan perendaman membawa kepada peningkatan yang ketara dalam aktiviti glutation reductase (GR) bagi semua kultivar atau genotip pada 4 dan 8 hari perendaman, kecuali bagi MR219 genotip. Daripada keputusan yang diperolehi dalam kajian ini, memberi kekuatan kepada hipotesis kami bahawa detoksifikasi O_2^- untuk

H_2O_2 dikekalkan pada tahap yang stabil sepanjang tekanan perendaman sehingga 8 hari (MR219-4 dan MR219-9 genotip) dan peranan GR mungkin menjadi lebih cekap dalam H_2O_2 perlindungan sitotoksik perlu untuk diperhatikan daripada tekanan perendaman.

Penglibatan potensi spesies oksigen yang diaktifkan oleh tekanan perendaman telah dikaji juga memberi tumpuan kepada dua beras genotip Malaysia, MR219-4 dan MR219-9, dan FR13A kultivar di peringkat anak benih. Benih tiga kultivar beras yang berusia empat belas hari telah tertakluk kepada tempoh perendaman yang berbeza (4, 8 dan 12 hari) di dalam rumah kaca. Di bawah 8 hari tenggelam, kultivar FR13A menunjukkan peroksidaan lipid yang lebih tinggi dari segi tahap malondialdehyde (MDA) dan aktiviti-aktiviti enzim antioksida, superoxide dismutase (SOD), katalase (CAT), peroxidase ascorbate (APX), dan glutation reductase (GR) apabila dibandingkan kepada genotip MR219-4 dan MR219-9. MR219-9 menunjukkan genotip SOD yang lebih tinggi, APX dan aktiviti GR selepas 12 hari tenggelam. Tahap aktiviti SOD menunjukkan bahawa detoksifikasi O_2 menjadi H_2O_2 telah dikekalkan pada tahap yang stabil sepanjang tekanan perendaman sehingga 8 hari dan meningkat pesat pada 12 hari perendaman. Keputusan menunjukkan bahawa toleransi untuk tenggelam dalam beras dikaitkan sehingga 8 hari tenggelam pada genotip MR219-4 dan kultivar FR13A. Di samping itu, aktiviti CAT mempunyai pertalian yang lebih tinggi untuk mengurangkan H_2O_2 daripada APX, itu askorbat kitaran glutation mungkin lebih berkesan untuk mengurangkan H_2O_2.

Kesan kekurangan oksigen (anoxia) pada sistem antioksidan dalam kultur suspensi anoxia pada beras genotip Malaysia dan sel-sel genotip FR13A diperiksa. Enzim

antioksidan telah menurun pada MR219-4 dan MR219-9 genotip untuk CAT dan aktiviti APX, dan meningkat pada kultivar FR13A bermula pada 20 hari dalam suspensi kultur berbanding dengan kultur kawalan. Aktiviti CAT dan APX dikekalkan lebih tinggi dalam keadaan kekurangan oksigen untuk semua genotip dan kultivar mencadangkan bahawa tekanan anoxia dalam suspensi kultur mendorong tahap H_2O_2 ke tahap toksik.

Hubungan antara aktiviti enzim dan isoenzim superoxide dismutase (SOD) dan peroxidase (POD) dalam suspensi kultur sel diserahkan kepada tekanan anoxia (oksigen depriviation) telah dinilai. Suspensi kultur beras memberikan tiga jenis band SOD, dikenalpasti sebagai MnSOD, FeSOD dan Cu / ZnSOD. Satu peningkatan yang ketara telah dikesan dalam aktiviti isozymes MnSOD sejak ia telah terkemuka muncul untuk semua genotip dan kultivar pada tempoh yang berbeza tekanan. Ia adalah munasabah untuk menganggap bahawa mitokondria kekurangan oksigen lebih cepat daripada seluruh sel kerana ia adalah mengambil yang paling penting oksigen. Aktiviti yang lebih tinggi POD dan isoenzim aktiviti untuk genotip MR219-4 dan kultivar FR13A, mungkin mengimbangi penurunan dalam aktiviti CAT. Keputusan ini menunjukkan bahawa aktiviti POD, CAT dan SOD memainkan peranan penting perlindungan dan sinergi terhadap H_2O_2 tahap toksik dalam suspensi kultur padi dalam keadaan anoxia. Induksi isoenzim/isoforms dan aktiviti POD, CAT dan SOD menyediakan perlindungan daripada kerosakan oksidatif, maka parameter ini mungkin diambil sebagai kriteria pemilihan untuk kajian toleransi perendaman dalam beras.

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May GOD bless all of you

Revandy Iskandar Muda Damanik, June 2013.

Trust in the LORD with all thine heart;
and lean not unto thine own understanding.
Proverbs 3:5

I certify that a Thesis Examination Committee has met on 14 June 2013 to conduct the final examination of Revandy Iskandar Muda Damanik on his thesis entitled "Changes in Antioxidant Enzymes and Growth Parameters in Rice (Oryza sativa L.) Genotypes under Submergence" 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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Date: 16 August 2013

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

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

REVANDY ISKANDAR MUDA DAMANIK

Date: 14 June 2013

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