UNIVERSITI PUTRA MALAYSIA

THE FUNCTIONAL STUDY OF ORYZACYSTATIN-1 PROTEASE INHIBITOR UNDER SALINITY STRESS IN MALAYSIAN RICE CULTIVAR

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LIST OF ABBREVIATION

µL	Microlitre
BLAST	Basic Local Alignment Search Tools
Bp	Base pair
cDNA	Complement Deoxyribonucleic Acid
Cl^+	Calcium ion
dH_2O	Distilled water
DNA	Deoxyribonucleic Acid
DNTP	Deoxynucleotide triphosphate
G	Gram
Hr	Hour
$MgCl_2$	Magnesium Chloride
mL	Mililitre
MR	Malaysia Rice
mRNA	Messenger Ribonucleic Acid
Na^+	Sodium ion
NaCl	Sadium Hydroxide
OC-1	Oryzacystatin-1
PCR	Polymerase Chain Reaction
qPCR	Real-time PCR
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
TAE	Tris-Acetate EDTA
T_m	Melting Temperature
UBQ5	Ubiquitin5

ABSTRACT

Phytocystatin is a protein that encodes cysteine proteinase inhibitor. It is well known for its antipathogenic properties and responsible in plant stress tolerance. Proline is an amino acid that acts as osmoprotectant in plant. Previously, both phytocystatin and proline show response in plant abiotic stresses and have become a potential strategy in combating abiotic stress. In this study, we reported the isolation and characterization of Oryzacystatin-1 (*OC*-1) gene from Malaysian cultivar of *Oryza sativa* L., the gene expression profiling by using RT-PCR and the accumulation of proline in stress-induced rice plantlets of MR211, MR220 and MR219. Three Malaysian rice (*Oryza sativa* L.) cultivars , MR 211, MR 220 and MR 219 were tested in pot culture under different salinity concentration (0, 4, 8, 12 dS m^{-1}) and five different duration of salinity treatment (0, 3, 6, 9, 24 hr) for molecular and biochemical response. All the tests were conducted in randomized complete block design with three replicates in a culture room. The result showed that, the *OC*-1 gene expression level and proline content were influenced by salinity stress. A cDNA fragment encoding phytocystatin, *OC*-1 was also isolated. Sequence analysis shows that *OC*-1 contain all common motifs found in phytocystatin such as a single G residue, QVVAG sequence and PW dipeptide motif. The presence of several cystatin superfamily was proven through Protein BLAST confirming the *OC*-1 gene isolation earlier. Then, phylogeny studies have shown that the *OC*-1 gene is closely related to phytocystatin from *Zea mays* and *Coix lacryma-jobi*.

ABSTRAK

Phytocystatin adalah protein yang mengekod perencat proteinase sisteina. Phytocystatin terkenal dengan sifat antipatogen dan terlibat pada toleransi pokok terhadap tekanan. Prolin merupakan asid amino yang bertindak sebagai pelindung osmotik di dalam pokok. Sebelum ini, kedua-dua phytocystatin dan prolin telah menunjukkan tindak balas terhadap tekanan abiotik di dalam pokok dan telah menjadi satu strategi yang berpotensi dalam memerangi tekanan abiotik. Di dalam kajian ini, kami melaporkan pengasingan dan pencirian Oryzacystatin-1 gen daripada *Oryza sativa* L. bagi kultivar Malaysia, profil ekspresi gen dengan menggunakan RT-PCR serta pengumpulan prolin didalam pokok padi MR 211, MR 220 dan MR 219 yang telah diberikan tekanan kemasinan. Tiga kultivar padi Malaysia telah diuji didalam bekas kultur dengan menggunakan tahap kemasinan yang berbeza (0, 4, 8, 12 dS m^{-1}) serta lima kadar rawatan yang berbeza (0, 3, 6, 9, 24 jam) bagi tujuan tindak balas molekular dan biokimia. Kesemua ujian tersebut telah dijalankan secara rawak didalam reka bentuk lengkap dengan tiga replikasi didalam bilik kultur. Hasilnya, tingkatan ekspresi Oryzacystatin-1 gen dan kandungan prolin didalam pokok di pengaruhi oleh tekanan kemasinan. Analisa jujukan menunjukkan *OC*-1 mempunyai kesemua motif yang biasa dijumpai pada phytocystatin, seperti G residu, urutan QVVAG dan motif dipeptid PW. Hadirnya beberapa keluarga cystatin yang dibuktikan melalui BLAST protein, mengesahkan gen yang telah di asingkan adalah *OC*-1. Seterusnya, kajian filogeni telah menunjukkan *OC*-1 gen berkait rapat dengan phytocystatin daripada *Zea mays* dan *Coix lacryma-jobi*.

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is the third top ranking crop planted in Malaysia that covers about 205,548 hectares area in Peninsular Malaysia (*Buku Perangkaan Pertanian*, 2007). The Malaysian Agriculture and Agro-based Industry Ministry, reported to target Malaysia to achieve a 100% self-sufficiency level (SSL) in paddy production by 2020 ("Self Sufficiency in Rice", 2015). To fulfill the current and future increasing demands, Malaysia needs to expand its rice area. It is predicted that in 2056, salinity problems would affect 100000 hectares of rice area. The continuous of salinity problem may result in reduction of rice area which would generate food shortage in domestic and global market (Selamat and Ismail, 2009).

Salinity is the biggest problem in rice production at all growth stages where it creates the osmotic effect and ion toxicity (Hakim *et al*., 2014). High salt concentration in the soil will lower the soil water potential and cause water to move from the plant root to the soil. While, high accumulation of Na^+ and Cl^- inside the cytoplasm of plant will inhibit many enzymatic processes (Jampeetong and Brix, 2009). In order to deal with the unfavorable growth condition, plant develops several mechanisms of biochemical and molecular adaptation by preserving the basic metabolic activities (Basu and Roycoudhury, 2014).

In recent years, several biotechnological approaches mainly in over-expression of phytocystatins gene have been successful studied for plant improvement. For instant, phytocystatin has been used to prevent from insect feeding in transformed plants (Christou *et al*., 2006; Kiggundu *et al*., 2010) and used to improve the yield of bio-engineered proteins such as vaccines and metabolic

enzymes (Pillay *et al*., 2012; Rivard *et al*., 2006). Cysteine protease (CP) is the main target of phytocystatins by controlling CP activities which regulated by developmental and environmental cues (Quain *et al*., 2014). Interestingly, studies have suggested that plant cystatin are also involved in responding to abiotic environment stress as they are highly expressed during harsh condition such as cold, drought, oxidant stress, wounds and salinity stress (Benchabane *et al*., 2010; Bangrak and Chotigeat, 2011; Pereira *et al*., 2011). However, the information of phytocystatin on Malaysian rice is very limited.

On the other hand, proline accumulation is a biochemical response in many plant species incited by salt stress. Proline as an osmoprotectant will preserve the osmotic stability and prevent damage in plant. However, its role as the plant defence system toward salt stress is controversial which proline are more recognized as a symptom of stress injury instead of being an indicator of stress tolerance (Htwe *et al*., 2011). Therefore, the clear explanation on molecular adaptation and biochemical mechanism that occur in plant as responds to salt stress are important in developing salt-tolerant plant that are tolerable to the saline environment.

OBJECTIVE

1. To isolate and characterize the Oryzacystatin-1 gene (*OC*-1) from Malaysian rice cultivars.
2. To study the *OC*-1 gene expression level in stress-induced rice plantlets of MR211, MR220 and MR219.
3. To analyze the proline content in stress-induced rice plantlets of MR211, MR220 and MR219.

REFERENCES

100 pc Self-Sufficiency in Rice Production by 2020. (2015, May 15). Retrieved from http://www.dailyexpress.com.my/news.cfm?NewsID=99775.

Ashraf, M. and Orooj, A. (2006). Salt Stress Effects on Growth, Ion Accumulation and Seed Oil Concentration in an Arid Zone Traditional Medicinal Plant Ajwain (*Trachyspermum ammi* L. Sprague). *Journal of Arid Environment*. 64 : 209-220.

Azooz, M., Shaddad, M.A. and Abdel-Latef, A.A. (2004). The Accumulation and Compartmentation of Proline in Relation to Salt Toleranc of Three Sorghum Cultivars. *Indian Journal of Plant Physiology*. 9 : 1-8.

Bangrak, P. and Chotigeat, W. (2011). Molecular Cloning and Biochemical Characterization of A Novel Cystatins from *Hevea* Rubber Latex. *Plant Physiology and Biochemistry*. 92(3) : 244-250.

Bates, L.S., Waldren, R.P. and Teare I.D. (1973). Rapid Determination of Free Proline for Water Stress Studies. *Plant Soil*. 39 : 205-207.

Basu, S. and Roychoudhury, A. (2014). Expression Profilling of Abiotic Stress-Inducible Genes in Response to Multiple Stresses in Rice (*Oryza_sativa* L.) Varieties with Contrasting Level of Stress Tolerance. *Journal of Biomedicine and Biotechnology*. : pp. 1-12.

Benchabane M., Schluter,, U., Vorster, J., Goulet, M.C. and Michaud, D. (2010). Plant cystatins. *Biochemie*. 92 : 1657-1666.

Botella, M.A., Xu, Y., Prabha, T.N., Zhao, Y., Narasimhan, M.L., Wilson, K.A., Nielssn, S.S., Bressan, R.A. and Hasegawa, P.M. (1996). Differential Expression of Soybean Cysteine Proteinase Inhibitor Genes during Development and in Response to Wounding and Methyl Jasmonate. *Plant Physiology*. 112 : 1201-1210.

Buku Perangkaan Pertanian. (2007). Putrajaya, Malaysia: Kementerian Pertanian dan Industri Asas Tani.

Bustin, S.A. (2000). Absolute Quantification of mRNA using Real-Time Reverse Transcription Polymerase Chain Reaction Assays. *Journal of Molecular Endocrinology*. 25 : 169-193.

Christoff, A.P. and Margis, R. (2014). The Diversity of Rice Photocystatins. *Mol Genet Genomics*. 289 : 1321-1330.

Christou, P., Capell, T., Kohli A., Gatehouse, J. and Gatehouse, A. (2006). Recent Development and Future Prospect in Insect and Pest Control in Transformed Crops. *Trends Plant Science*. 11 : 302-308.

Chan, S.N., Bakar, N.A., Mahmood, M., Ho, C.L. and Shaharuddin, N.A. (2014). Molecular Cloning and Characterization of Novel Phytocystatin Gene from Tumeric, *Curcuma longa*. *Journal of Biomedicine and Biotechnology*. 1: 1-9.

Chu, M.H., Liu, K.L., Wu, H.Y., Yeh, K.W. and Cheng, Y.S. (2011). Crystal Structure of Tarocystatin-Papain Complex: Implication from the Inhibition Property of Group 2 Phytocyatatins. *Planta*. 234 : 234-254.

Dager, J.C., Bhagwan, H. and Kumar, Y. (2004). Effect on Growth Performance and Biochemical Contents of *Salvadora persica* when Irrigated with Water of Different Salinity. Indian *Journal of Plant Physiology*. 9 : 234-238.

Diop, N.N., Kidric, M., Repellin, A., Gareil, M., Lameta, A.D., Phamthi, A.T. and Fodil, Y.Z. (2004). A Multicystatin is Induced by Drought Stress in Cowpea (*Vigna unguiculata* L. Walp.) Leave. *FEBS Lette.* 577 : 545-550.

Fasahat, P., Muhammad, K., Abdullah, A. and Ratnam, W. (2012). Proximate Nutritional Composition and Antioxidant Propertiest of *Oryza rufipogon*, A Wild Rice Collected from Malaysia Compared to Cultivated Rice, MR 219. *Australian Journal of Crop Science*. 6(11) : 1502-1507.

Gaddour, K., Carbajosa, J.V., Lara, P., Lamoneda, I.I., Diaz, I. and Carbonero, P.A. (2001) Constitutive Cystatin-Encoding Gene from Barley (*Icy*) Responds Differentially to Abiotic Stimuli. *Plant Molecular Biology*. 45 : 599-608.

Gnanamanickam, S.S. (2009). Rice and Its Importance to Human Life. *Progress in Biological Control*. 8 : 1-11.

Grudkowska, M. and Zagdanska, B. (2004). Multifunctional Role of Plant Cysteine Proteinases. *Acta Biochimica Polonica*. 51(3) : 609-624.

Gupta, B. and Huang, B. (2014). Mechanism of Salinity Tolerance in Plants: Physiological, Biochemical, and Molecular Characterization. *Journal of Biomedicine and Biotechnology.* : 1-18.

Habib, H. and Fazili, K.J. (2007). Plant Protease Inhibitor: A Defense Strategy in Plants. *Academic Journal*. 2(3) : 68-85.

Hakim, M.A., Juraimi, A.S., Hanafi, M.M., Ismail, M.R., Selamat A., Rafii,M.Y. and Latif, M.A. (2014). Biochemical and Anatomical Changes and Yield Reduction in Rice (*Oryza sativa* L.) under Varied Salinity Regimes. *Journal of Biomedicine and Biotechnology.* : 1-11.

Hakim, M.A., Juraimi, A.S., Begum, M., Hanafi, M.M., Ismail, M.R. and Selamat, A. (2010). Effect of Salt Stress on Germination and Early Seedling Growth of Rice. *African Journal of Biotechnology*. 9(13) : 1911-1918.

Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J. and Ahmad, A. (2012). Role of ProlineUnder Changing Environments. *Plant Signaling & Behaviour.* 7 : 1-11.

Htwe, N.N., Maziah, M., Ling, H.C., Zaman, F.Q. and Zain, A.M. (2011). Response of Some Selected Malaysian Rice Genotypes to Callus Induction under In Vitro Salt Stress. *African Journal of Biotechnology*. 10(3) : 350-362.

International Rice Genome. (2005). The Map-Based Sequence Of The Rice Genome. *Nature*..436 : 793-800.

Jain, M. (2009). Genome-Wide Identification of Novel Internal Control Genes for Normalization of Gene Expression During Various Stages of Development in Rice. *Plant Science*. 176(5) : 702-706.

Jamil, M., Bashir, S., Anwar, S., Bibi, S., Bangash, A., F. Ullah, and E.S. Rha. (2012). Effect of Salinity on Physiological and Biochemical Characteristics of Different Varieties of Rice. *Pakistan Journal Botany*, vol. 44, pp. 7-13.

Jampeetong, A. and Brix, H. (2009). Effects oF NaCl Salinity on Growth, Morphology, Photosynthesis and Proline Accumulation of *Salvinia natans*. *Aquatic Botany*. 91 : 181-186.

Keyster, M., Adams, R., Klein, A. and Ludidi, N. (2013). Nitric Oxide (NO) Regulates the Expression of Single Domain Cystatins in Glycine max (soybean). *Plants Omics Journal*. 6(3) : 183-192.

Kidric, M., Kos, J. and Sabotic, J. (2014). Proteases and Their Endogenous Inhibitors in the Plant Response to Abiotic Stress. *Botanica Serbica*. 38(1) : 139-158.

Kiggundu, A., Muchwezi, J., Vyver, C.V.D., Viljoen, A., Vorster, J., Schluter, U., Kunert K. and Michaud, D. (2010). Deleteriuos Effects of Plant Cystatins Against the Banana Weevil *Cosmopolites sordidus*. *Archives of Insect Biochemistry and Physiology*. 73 : 87-105.

Kondo, H., Abe, K., Niashimura, I., Watanabe, H., Emorit, Y. and Soichi, A. (1990). Two Distinct Cystatin Species in Rice Seeds with Different Specificities Against Cysteine Proteinases. *The Journal of Biological Chemistry*. 265(26) : 15832-15837.

Kondo, H., Emori, Y., Abe, K., Suzuki, K. and Arai, S. (1989). Cloning and Sequence Analysis of the Genomic DNA Fragment Encoding Oryzacystatin. *Gene*. 81 : 259-265.

Leo, F.D., Volpicella, M., Licculli, F., Liuni, S., Gallerani, R. and Ceci, L.R. (2002). Plant-PIs: A Database of Plant Protease Inhibitors and Their Gene. *Oxford University Press*. 30(1) : 347-348.

Lutts, S., Kinet, J.M. and Bouharmont, J. (1996). Effects of Various Salts and of Mannitol on Ion and Proline Accumulation in Relation to Osmotic Adjustment in Rice (*Oryza sativa* L.) Callus Caltures. *Journal Plant Physiology*. 149 : 186-195.

Massonneau, A., Condamine, P., Wisniewski, J.P., Zivy, M. and Rogowsky, P.M. (2005). Maize Cystatins Respond to Developmental Cues, Cold Stress and Drought. *Biochim Biophys Acta*. 1729 : 186-199.

Moradi, F. and Ismail, A.M. (2007). Responses of Photosynthesis, Chloophyll Fluorescence and ROS-Scavenging Systems to Salt Stress during Seedling and Reproductive Stages in Rice. *Annals of Botany*. 99(6) : 1161-1173.

MARDI. (n.d). Retrieved May 16, 2015, from

http://www.mardi.gov.my/c/document_library/get_file?uuid=f20ceb36-8d77-424d-b73e-fafb0ddf23c0&groupId=10138

MARDI. (n.d). Retrieved May 16, 2015, from

http://www.mardi.gov.my/c/document_library/get_file?uuid=29d5002c-afd5-4892-b118-838f828e0006&groupId=10138

Munns, R. (2005). Genes and Slat Tolerance: Bringing Them Together. (2005). *New Phytologist*. 167(3) : 645-663.

Paddy Statistic of Malaysia 2013 (1st Edition), (2014). Department of Agriculture, Peninsular Malaysia.

Pereira, K.R.B., Botelho-Junior S. and Domingues, D.P. (2011). Passion Fruits Flowers: Kunitz Trypsin Inhibitors and Cystatin Differentially Accumulate in Developing Buds and Floral Tissues. *Phytochemistry*. 72(16) : 1955-1961.

Pernas, M., Monge, R.S., and Salcedo, G. (2000). Biotic and Abiotic Stress Can Induce Cystatin Expression in Chestnut. *FEBS Letter*, vol. 467, pp. 206-210.

Pillay, P., Kibido, T., dePlessis, M., Vyver, C., Beyeni, G., Vorster, B.J., Kunert, K.J. and Schluter, U. (2012). Use of Transformed Oryzacystatin-1-Expressing Plant Enhances Recombinant Protein Production. *Application of Biochemistry and Biotechnology*. 68 : 1608-1620.

Quain, M.D., Makgopa, M.E., Marquez-Garcıa, B., Comadira, G., Fernandez-Garcia, N., Olmos, E., Schnaubelt D., Kunert, K.J. and Foyer, C.H. (2014). Ectopic Phytocystatin Expression Leads to Enhanced Drought Stress Tolerance in Soybean (*Glycine max*) and *Arabidopsis thaliana* Through Effects on Strigolactone Pathways and Can Also Result in Improved Seed Traits. *Plant Biotechnology Journal*. 12 : 903-913.

Rambaut, A. (2013, August 12). How to read a phylogenetic tree. Retrieved June 20, 2015, from http://epidemic.bio.ed.ac.uk/how_to_read_a_phylogeny

Ramezani, E., Sepaniou, M.G., and Badi, H.A.N. (2011). The Effect of Salinity on The Growth, Morphology and Physiology of *Echium amoenum* Fisch.&Mey. *African Journal of Biotechnology*. 10(44) : 8765-8773.

RNA Quality Countrol. (n.d.). In Biomedical Genomic. Retrieved May 16, 2015, from http://biomedicalgenomics.org/RNA_quality_control.html.

Rawlings, J.S., Rennebeck, G., Harrison, S.M., Xi, R. and Harrison, D.A. (2004). Two Drosophila Suppressors of Cytokine Signaling (SOCS) Differentially Regulate JAK and EGFR Pathway Activities. *BMC Cell Biol*. 5 : 38.

Razzaque, M.A., Talukder, N.M., Roy, T.S., Hasanuzzaman, M. and Bhadra, A.K. (2010). Salinity Stress Effets on Biochemical Changes in Leaves of Rice

Genotypes Differing in Slat Tolerance. *Recent Research in science and Technology*. 2(1) : 2076-5061.

Rivard, D., Anguenot, R., Brunelle, F., Le, V.Q., Vezina, L.P. and Michoud, D. (2006). An In-build Proteinase Inhibitor Syatem for the Protection of Recombinant Proteins Recovered from Transform Plants. *Plant Biotechnology Journal*. 4 : 359-368.

Ryan, C.A. (1990). Protease Inhibitor in Plant: Gene for Improving Defences against Insects and Pathogens. *Annual Review of Phytopathology*. 28 : 425-449.

Sambrook, J., Fritsch, E.F. and Maniatis, T. Molecular Cloning: A Laboratory Manual. *Cold Spring Harbor Laboratory*. ISBN: 0879693096.

Sarunyaporn, M., Kanyaratt, S. and Gopalan, S. (2013). High-Quality Reference Genes For Quantifying The Transcriptional Responses Of *Oryza Sativa* L. (Ssp. *Indica* And *Japonica*) To Abiotic Stress Conditions. *Chinese Science Bulletin.* 58(16) : 1919-1930.

Selamat, A. and Ismail, M.R. (2009). Deterministic Model Approaches in Identifying and Quantifying Technological Challenges in Rice Production and Research, and in Predicting Population and Comsumption in Malaysia. *Pertanika Journal of Tropical Agricultural Science*. 32(2) : 267-291.

Sultana, N., Ikeda, T. and Itoh, R. (2000). Effect of NaCl Salinity on Photosynthesis and Dry Matter Accumulation in Developing Rice Grains. *Environmental and Experimental Botany*. 42(3) : 211–220.

Takaiwa, F., Oono, K. and Suguira, M. (1984). The Complete Nucleotide Sequence of A Rice 17S rRNA Gene. *Nucleic Acid Research*. 12(13) : 5441-5448.

Talei, D., Valdiani, A., Maziah, M. and Mohsenkhah, M. (2013). Germination Response of MR 219 Rice Variety to Different Exposure Times and Periods of 2450 MHz microwave Frequency. *Journal of Biomedicine and Biotechnology*. : 1-7.

Yoshida, S., Forno, D.A., Cock, J.H. and Gomez, K.A. (1976). Laboratory Manual for Physiological Studies of Rice. *Philippines.* 83.

Zahra, S., Amin, B., Ali, V.S. M., Ali, Y. and Mehdi, Y. (2010). The Salicylic Acid Effect on the Tomato (*Lycopersicum esculentum* Mill.) Sugar, Protein and Proline Content under Salinity Stress (NaCl). *Journal of Biophysics and Structural Biology*. 2(3) : 35-41.

Zhang, X., Liu, S. and Takano, T. (2008). Two Cysteine Proteinase Inhibitors from *Arabidopsis thaliana*. AtCYSa and AtCYSb, Increasing the Salt, Drought, Oxidation and Cold Tolerance. *Plant Molecular Biology*. 68 : 131-143.

Zhao, P., Zhou, X.M., Zou, J., Wang, W., Wang, L., Peng, X.B. and Sun, M.X. (2014). Comprehensive Analysis of Cystatin Family Genes Suggests Their Putative Functions in Sexual Reproduction, Embryogenesis and Seed Formation. *Journal of Experimental Botany Advance*.