UNIVERSITI PUTRA MALAYSIA

ALLEVIATION OF SALINITY STRESS IN RICE GENOTYPES USING GROWTH REGULATORS

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FP 2015 74

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By

KHADIJA MOHAMED MISRATIA

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

February 2015

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DEDICATION

Prophet Muhammad

(Peace Be Upon Him)

The Greatest Social Reformer .This thesis is also dedicated to the memory of my late father; to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time. And invaluable teachers in all realms of my studies.

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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

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February 2015

Chairman: Professor. Mohd Razi Bin Ismail ,PhD

Faculty: Agriculture

Salinity is a major problem of rice especially the salt sensitive cultivars in the granary areas. This problem consequently reduces the potential acreage for the production of the crop. To solve this problem, the present study was conducted to determine how growth regulators (GA_3 and kinetin) could be explored to alleviate salinity stress in different rice cultivars. Under laboratory condition, exogenous applications of 150 ppm GA_3 and 15 ppm kinetin on six rice cultivars (MR185, MR211, MR219, MR220, MR232 and Pokkali as check) were used to alleviate salinity stress of the crop at different salinity levels (0, 50, 100, 150 and 200 Mm NaCl). It was found that higher salinity levels (150 and 200 mM) reduced seed germination, shoot length, root length, vigour index, fresh weight, dry weight, relative water content, soluble sugar, soluble protein, free proline, K^+, Ca^{2+}, Mg^{2+} in rice seedlings. However, these parameters were mildly affected in plants grown at lower salinity levels (50 and 100 mM NaCl). Application of 150 ppm GA_3 alleviated salinity stress in all the cultivars and thus improved all the traits measured. With application of 150ppm GA_3, Pokkali and MR219 tolerated salinity stress better than the rest tested cultivars. Application of 150ppm GA_3 was chosen for its better performance and used for improvement of morphological, physiological and yield traits of Pokkali and MR219 under glass house condition at different salinity levels (0, 50, 100, 150 and 200 mM NaCl). All the studied morphological traits (plant height, tillers plant^{-1}, leaves plant^{-1}, leaf length, plant fresh and dry weight) and physiological attributes (chlorophyll *a*, *b*, and total chlorophyll contents, photosynthetic rates, stomatal conductance, transpiration rate,) were severely affected at higher salinity levels (150 and 200 mM). Application of 150ppm GA_3 consistently improved the salinity tolerance of the two cultivars morphologically and physiologically at mild salinity levels (0-100 mM NaCl). Higher salinity levels (150 and 200 mM) severely and significantly ($P \leq 0.05$) affected the tested cultivars and it resulted in tiller sterility. Beyond this level, application of 150ppm GA_3 could not alleviate the salinity stress imposed. However, at mild salinity levels (50 and 100mM NaCl), there was production of panicle but panicle length, filled grains per panicle, weight of filled grains, seed index, harvest index, and grain yield per pot were all

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significantly reduced ($P \leq 0.05$) with increase in the number and weight of unfilled grains and spikelets. Finally, 150 ppm GA_3 was used to improve biochemical and ionic changes, enzymatic activities and expression profile of *OsLEA* gene in MR219 and Pokkali under moderate salinity (100 mM). Under this salinity level, there was significant increase in concentrations of soluble sugar, soluble proteins and free proline in both MR219 and Pokkali cultivars at $P \leq 0.05$. With GA_3 application, salinity stress was less alleviated and biochemical changes were less stabilized. For the ionic contents, there was high Na^+ content while K^+, Ca^{2+} and Mg^{2+} were low in concentration in the two cultivars in the control. However, with the application of GA_3 at moderate salinity(100mM NaCl), there was decrease in Na^+ level while K^+, Ca^{2+} and Mg^{2+} increased. For enzymatic activities, peroxides, Catalase and Ascorbate peroxides were significantly ($P \leq 0.05$) high in the control. Under salinity stress, GA_3 treatment improved the enzymatic activities above the control (zero GA_3 application) at $P \leq 0.05$. In Semi quantitative analysis for *OsLEA* gene expression, there was no distinction between the gene bands of the control and the salinity stressed plants in MR219. However, when GA_3 was applied to the plants, there was clear distinction between the gene bands of control and salinity stressed plants. For expression of *OsLEA* genes in Pokkali, the were band differences among the control plants and plants treated with GA_3. The expression level of *OsLEA* gene in Pokkali was also different. Based on the results of the present investigations, it may be concluded that MR219 (indigenous) and Pokkali (exotic) rice cultivars can be produced in moderately saline soils with the application of 150 ppm GA_3.

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

PENGURANGAN STRES KEMASINAN PADA GENOTIP PADI DENGAN MENGGUNAKAN PENGAWALATUR PERTUMBUHAN

Oleh

KHADIJA MOHAMED MISRATIA

Februari 2015

Pengerusi: Professor Mohd Razi Bin Ismail ,PhD

Fakulti: Pertanian

Kemasinan merupakan masalah utama kepada tanaman padi terutamanya bagi kultivar yang sensitif terhadap kemasinan di kawasan jelapang padi. Masalah ini sekaligus mengurangkan keluasan potensi bagi pengeluaran tanaman padi. Untuk menyelesaikan masalah ini, kajian ini telah dijalankan untuk menentukan bagaimana pengawalatur pertumbuhan (GA_3 dan kinetin) boleh diterokai untuk mengurangkan ketegasan terhadap kemasinan dalam kultivar padi yang berbeza. Di dalam kaijan makmal, 150 ppm GA_3 dan 15 ppm kinetin telah digunakan dan diberikan secara luaran kepada enam kultivar padi (MR185,MR211, MR219,MR220,MR232 dan Pokkali sebagai cek) untuk mengurangkan tekanan kemasinan tanaman pada tahap kemasinan yang berbeza (0,50 , 100, 150 dan 200 Mm NaCl).

Didapati bahawa tahap kemasinan yang lebih tinggi (150 dan 200 mM) mengurangkan percambahan benih, panjang pucuk, panjang akar, indeks tenaga, berat basah, berat kering, kandungan air relatif, gula yang terlarut, protein yang terlarut, kandungan prolin yang bebas, K^+, $Ca^{+2,}$ Mg^{+2} dalam benih padi. Walau bagaimanapun, parameter-parameter ini sedikit terjejas dalam tumbuhan yang ditanam pada tahap kemasinan yang lebih rendah (50 dan 100 mM NaCl). Penggunaan 150 ppm GA_3 telah mengurangkan tegasan kemasinan dalam semua kultivar dan seterusnya memperbaiki ciri-ciri yang diukur. Dengan pengunaan 150ppm GA_3, Pokkali dan MR219 dapat bertahan terhadap tegasan kemasinan yang lebih baik berbanding kultivar lain yang telah diuji.

Penggunaan 150ppm GA_3 telah dipilih kerana menunjukkan prestasi yang lebih baik dan digunakan untuk penambahbaikan morfologi, fisiologi dan hasil ciri-ciri Pokkali dan MR219 di dalam rumah kaca pada tahap kemasinan yang berbeza (0, 50, 100, 150 dan 200 mM NaCl). Semua ciri-ciri morfologi yang dikaji (ketinggian tumbuhan, tangkai per tumbuhan^{-1}, daun per tumbuhan^{-1}, panjang daun, berat segar tanaman dan berat kering tanaman) dan sifat-sifat fisiologi (klorofil *a*, *b*, dan jumlah kandungan

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klorofil, kadar fotosintesis, kealiran stomata, kadar transpirasi) terjejas teruk pada tahap kemasinan yang lebih tinggi (150 dan 200 mM). Penggunaan 150ppm GA_3 menunjukkan peningkatan terhadap toleransi kemasinan yang konsisten di dalam kedua-dua kultivar secara morfologi dan fisiologi pada tahap kemasinan yang sederhana (0-100 mM NaCl). Tahap kemasinan yang lebih tinggi (150 dan 200 mM) menjejaskan dan memberi kesan yang ketara ($P \leq 0.05$) kepada kultivar-kultivar yang diuji dan menyebabkan anak padi yang tidak subur. Tahap kemasinan yang lebih tinggi dengan pemakaian 150ppm GA_3 tidak boleh mengurangkan ketegasanan kemasinan yang telah dikenakan.

Walau bagaimanapun, pada tahap kemasinan yang sederhana (50 dan 100mm NaCl), terdapat pengeluaran tangkai tetapi panjang tangkai, bijirin penuh setiap tangkai, berat bijirin penuh, indeks benih, indeks penuaian, dan hasil bijirin setiap pasu telah berkurang dengan ketara ($P \leq 0.05$) dengan peningkatan dalam bilangan dan berat bijirin yang tidak dipenuhi dan bilangan spikelet.

Akhir sekali, 150 ppm GA_3 telah digunakan untuk meningkatkan perubahan biokimia dan ionik, aktiviti enzim dan profil ekspresi gen *OsLEA* dalam MR219 dan Pokkali dengan kemasinan sederhana (100 mM). Di tahap kemasinan ini, terdapat peningkatan yang ketara dalam kepekatan gula terlarut, protein terlarut dan proline yang bebas bagi kedua-dua kultivar iaitu MR219 dan Pokkali pada $P \leq 0.05$.

Dengan pemakaian GA_3, ketegasan kemasinan dapat dikurangkan dan perubahan biokimia yang kurang stabil. Untuk kandungan ion, kandungan Na^+ yang tinggi manakala kandungan K^+, Ca^{+2} dan Mg^{+2} adalah rendah bagi dua kultivar dalam rawatan kawalan. Walau bagaimanapun, dengan penggunaan GA_3 pada tahap kemasinan yang sederhana (100mm NaCl), terdapat penurunan dalam tahap Na^+ manakala K^+, Ca^{+2} dan Mg^{+2} meningkat. Untuk aktiviti enzim, peroksidase, katalase dan peroksidase askorbat menunjukkan kandungan ($P \leq 0.05$) yang sangat tinggi dalam kemasinan sederhana (100mm NaCl). Rawatan GA_3 meningkatkan aktiviti enzim atas kemasinan yang sederhana (tanpa pemakaian GA_3) pada ($P \leq 0.05$).

Dalam analisis kuantitatif untuk Semi ungkapan *OsLEA* gen, tidak ada perbezaan antara kumpulan-kumpulan gen kawalan dan kemasinan untuk kultivar MR219. Walau bagaimanapun, apabila GA_3 telah digunakan pada tumbuhan, terdapat perbezaan yang jelas antara kumpulan gen kawalan dan tumbuhan yang tegas terhadap kemasinan.

Untuk ekspresi gen *OsLEA* dalam Pokkali, terdapat perbezaan di dalam band antara tumbuhan kawalan dan tumbuh-tumbuhan dirawat dengan GA_3. Tahap ungkapan *OsLEA* gen dalam Pokkali juga berbeza. Berdasarkan keputusan penyiasatan ini, ia boleh disimpulkan bahawa MR219 (asli) dan Pokkali (eksotik) kultivar beras boleh dihasilkan dalam tanah sederhana masin dengan penggunaan 150 ppm GA_3

ACKNOWLEDGEMENTS

All praise and thanks to Allah the exalted, for the grace bestowed upon me to start and complete my research . I express my heartfelt appreciation to my indefatigable supervisor, Prof. Dr. Mohd Razi Bin Ismail, for his invaluable guidance and advices, endless support, patience and encouragement throughout the duration of this study and also for his critical, constructive criticism and helpful suggestion during the preparation of my thesis, my thanks also go to all my co-supervisors Prof. Dr. Adam B Puteh , and Prof. Dr. Mohamed Hanafi Bin Musa,for their support and advice during my research. I really enjoyed every of my moment with you all.

My incomparable to all of my family members, I thank you for giving me the liberty to pursue my wish; I thank you for the discomfort you had to go through to give me the best. My appreciation goes to my siblings, Ali and Abdullah. I have lived with, share my and there, moment of sorrow and happiness together. I thank you for your prayers, encouragements, and motivational messages and for keeping in touch.

Thanks to all my colleagues who have contributed in one way or the other to this success and for expanding my horizon of knowledge especially my true good friend, Mr. Abdeladim Gazwi , Rabha Mohammed, Mahbod, Parisa AZ:Z, Nurul-Idayu, Nurul Amalina, NorAzrin,Wan Nor afzan, Afifah Abdul Razak, Noor Asma, Zulkerami,Yusuff, Ibrahim, Ishaq.

In last, but not least, I am also indebted to the (Higher Education Commission, Libya) for financial support for my PhD and (Higher Education Commission, Malaysia).

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I certify that a Thesis Examination Committee has met on 9 February 2015 to conduct the final examination of Khadija Mohamed Misratia on her thesis entitled "Alleviation of Salinity Stress in Rice Genotypes using Growth Regulators" 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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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 members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

%	Percentage
ANOVA	Analysis of molecular variance
ANOVA	Analysis of variance
cm	Centimetre
DNA	Deoxyrobose nucleic acid
DNase	Deoxribonuclease I
dNTPs	Deoxribonucleotide triphosphate
EDTA	Ethylene diamine tetracetate
G	Gram
HCL	Hydrochloric acid
H_2O_2	Hydrogen perioxide
IRRI	International Rice Research Institute
L	Liter
M	Molar
Mg	Milligram
Min	Minute
Ml	Millilitter
NaCl	Sodium Chloride
^0C	Degree celcius
PCR	Polymerase chain reaction
PVP	polyvinylpolypyrrolidone
Rpm	Rotation per minute
TBE	Tris-borate-EDTA
Tm	Melting temperature
UV	Ultraviolet
µg	Microgram
µl	Microliter
ppm	part per million
mM	Mill mole
GA_3	Gibberellic Acid
KIN	kinetin
CRD	Completely Randomized Design
RCBD	Randomized Complete Block Design
SAS	Statistical Analyses System
SEM	Scanning Electron Microscopy
FGP	Final germination percentage
GI	Germination index
MGT	Mean germination time
SVI	Seedling vigor index
TDM	Total dry matter
F.W	Fresh weight
SDW	Shoot dry weight
RDW	Root dry weight
RWC	Relative water content
TSP	Triple supper phosphate
MOP	Muriate of potash

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APX	Ascorbate peroxidase
POX	Peroxidases
CAT	Catalase
Kg	Kilogram
g	gram
DAT	Days after transplanting

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CHAPTER 1

INTRODUCTION

1.1 Overview

Salinity has been found as an important abiotic limiting factor for world crop production. Salinity is affecting about 953 million hectares with 8 percent of the land surface Singh (2009). Currently, approximately 6% of the world's land area, which is equivalent to 800 million hectares, is affected by either salinity or sodicity (FAO, 2008). In addition, salinity affects 20% of the world's irrigated land, which accounts for one-third of the world food production (Chinnusamy *et al.*, 2005). It has been estimated that salinity is affecting 3 hectares of additional arable land each minute world wide (FAO, 2008). This progressive loss of arable land has potentially serious consequences for the expanding global population, which is steadily increasing towards seven billion, and set to increase by a further 50% by 2050 (FAO, 2009).

Rice (*Oryza sativa* L.) is one of the staple cereal crops in the world, feeding over two billion peoples. With unit increase in world population, the consumption of rice is also increasing. Among the constraints, the abiotic stress especially soil salinity is the main factor its area and production worldwide (Gao *et al.*, 2007). Rice is considered to be moderately sensitive to salinity. Salinity affects rice from seed sowing to harvesting (Castillo *et al.*, 2003). The soil salinity reduces osmotic potential of the soil solution due to sodium toxicity and ultimately limits the plant growth as well as grain yield (Castillo *et al.*, 2003).

The salt susceptible rice cultivars accumulate low K^+/Na^+ ratio in the leaves which causes high reduction in grain yield (Asch *et al.*, 2000), with desiccation symptoms (Buu and Lang, 2004). However, tolerant cultivars accumulate less Na^+ and more proline as well as K^+ in their body. There is an antagonistic relationship between Na^+ and K^+ contents in plants under saline condition. Alternatively, researchers have been working towards screening of salt-tolerant rice cultivars. However, none of those efforts yet show success (Ashraf *et al.*, 2010). During the last decade, developing salt tolerant plants through modern biotechnology has been accorded very high research priority in plant biotechnology research and development. Recently, transgenic technology has been perceived as a viable option for generating plants with innate ability to tolerate different level of salts (Wang *et al.*, 2003).

In salt stress condition, the plant responses differ greatly depending on the level of salinity, distribution of salts in the root zone (Sonneveld and De Kreij, 1999; Dong *et al.*, 2008; Bazihizina *et al.*, 2009). High concentrations of salts in the soil make it difficult for roots to absorb and results ion toxicity in the plant (Munns and Tester, 2008). When roots were subjected to expose in salinity, the water use efficiency decreased consequently (Bazihizina *etal.*,2009), several physiological processes i.e., photosynthesis and respiration reduced (Chen *et al.*, 2008). High salinity also causes

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various nutritional disorders by decreasing the uptake of cations, such as K^+ and Ca^{+2} (Asch *et al.*, 2000). When salt concentration increases inside the plants, the salt starts to Accumulate inside the older leaves and eventually they die (Munns, 2002). Therefore, understanding the mechanisms of tolerance to high soil concentration of NaCl is essential to improve crop towards salt tolerance. The mechanisms of $Na^+$and K^+ transport in plants under salt stress has been extensively researched and reviewed (Apse and Blumwald, 2007) and (Shabala and Cuin, 2008) .

The depressive effect of salinity on seed germination and plant growth could be related to decline in endogenous levels of hormones (Debez *et al.*, 2001). Salinity could be relieved through application of phytohormones by regulating plant growth and development. In many reports it is concluded that application of hormones such as GA_3 and Kinetin had beneficial impact in alleviating the adverse effects of salt tress (Xiong *et al.*, 2002). Gibberellin is also a main focus of some plant scientists as plant treatment against salt stress (Hisamatsu *et al.*, 2000). Prakash and Prathapasenan(1990) also reported that Gibberellic acid (GA_3) is helpful to enhance rice growth under saline conditions by improving seed germination, leaf expansion, stem elongation and flowering (Magome *et al.*, 2004). There is also evidence that GA_3 can significantly relieve NaCl-induced growth inhibition in rice (Wen *et al.*, 2010) which could also be monitored through expression level of *OsLEA* gene. In plants, a group of very hydrophilic proteins, known as Late Embryogenesis Abundant (LEA), accumulates at high levels both during the last stage of seed maturation and during water deficit in vegetative organs, suggesting a protective role during water limitation (Battaglia *et al.*, 2008). The LEA proteins play important roles in normal seed development and plant response to environmental stress , such as dehydration, salinity, osmotic and low temperature (Battaglia *et al.*, 2008). However, still the precise function of LEA proteins in plant development and stress response remains to be clarified. Hence this study was conducted to assess the salinity tolerance of various rice cultivars in relation to alleviative role of plant hormones, enzymes and expression level of *OsLEA* gene involved in salinity tolerance.

1.2 Problem statement/significance of the study

Salinity is a major problem over a vast area in South and South-East Asia. A large majority of salt-affected soils in Malaysia occur in the coastal regions. Sea-water intrusion is the main cause of soil salinisation. However, although sea-water is the origin of salinity, four main factors influence the formation of coastal saline soils, their spatial distribution, the degree of salinity, and the potential for future soil salinisation : Low-elevation coastal landform,tidal inundation,underground seepage and over-drainage of adjacent peatland. Abiotic stress especially salinity has spent billions of dollars annually. The researchers has taken much efforts to develop salt tolerant rice cultivars over the decades through different approaches including breeding and genetic engineering techniques as well as screening of available crop. Apart from these strategies, salinity effect on plants could be alleviated through plant growth regulators. The exogenous application of plant growth regulators in salt stress condition has gained considerable attention towards profitable crop production. Many researchers were busy to ameliorate the adverse effect of salinity on horticultural crops, but very little work

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has been done on rice especially the expression level of *OsLEA* gene. Looking the economic importance of rice in developing countries and soil salinity as a productivity decreasing factor, this research was carried out to explore the salt tolerant cultivars, expression level of *OsLEA* genes and salinity relieving role of plant growth regulators for sustainable rice production.

1.3 Objectives of the study

1. To enhance salt tolerance of different rice cultivars using GA_3 and kinetin and determine the best concentration of GA_3 or kinetin.

2. To improve morphological and physiological traits of salt tolerant rice under salinity stress using GA_3.

3. To explore the potential of GA_3 on enhancement of ion accumulation and improvement of bio-chemical changes in rice cultivars under salinity stress.

4. To determine the influence of GA_3 treatment on enzymatic activities of rice cultivars under salinity stress.

5. To use semi- quantitative RT-PCR to determine *OsLEA* gene expression in rice cultivars under salinity stress.

6. To find out how GA_3 treatment could improve yield and yield components of salt tolerant cultivars at different salinity levels.

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LIST OF PUBLICATIONS

PUBLISHED AS FULL PAPERS

Khadija, M., Mohd Razi Ismail, M., Robiul Islam, M., Oad, F.C., Mohaned Hanafi, M., and Adam Putehi, A. (2015). Interactive effects of gibberellic acid (GA_3) and salt stress on growth, biochemical parameters and ion accumulation of two rice (*Oryza* sativa L.) varieties differing in salt tolerance. Journal of *Food, Agriculture & Environment* Vol.13 (1) : 6 6 - 7 0 .

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ACCEPTED

Khadija, M., Mohd Razi Ismail, M., Oad, F.C., Mohaned Hanafi, M., and Adam Putehi, A. (2013). Effect of Various Salt Concentrations and Salinity Alleviating Role of Gibberellic Acid (GA_3) On Ionic Accumulation And Yield Of Rice Cultivars. 16-07-2013.

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