



UNIVERSITI PUTRA MALAYSIA

**AGRONOMIC AND BIOCHEMICAL EXPRESSION OF SALT STRESS
IN IRANIAN RICE CULTIVARS**

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**DOCTOR OF PHILOSOPHY
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RICE CULTIVARS**

By

MOHAMMAD REZA MOMAYEZI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for Doctor of Philosophy
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DEDICATION

*Anyone who has never made a mistake has never tried
anything new.*

Albert Einstein (1879-1955)

**THIS THESIS IS DEDICATED TO THE ALMIGHTY ALLAH
WHO
GAVE ME THE STRENGTH AND PATIENCE TO COMPLETE THIS STUDY**

AND ALSO TO

YOU

**MY FATHER AND MOTHER,
MY WIFE AND MY CHILDREN**

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



AGRONOMIC AND BIOCHEMICAL EXPRESSION OF SALT STRESS IN IRANIAN RICE CULTIVARS

By

MOHAMMAD REZA MOMAYEZI

July 2010

Chairman : Professor Zaharah Abdul Rahman, PhD

Faculty : Agriculture

Salinity stress is a complex effect causing an external osmotic potential to prevent water uptake, an inhibitory specific-ion effect such as Na^+ , Cl^- on plant metabolism and nutrient imbalance in stressed plants. Therefore tolerance of a plant to salinity stress should contain the ability to overcome the osmotic challenge and alleviate ion toxicity. Plants show different responses to salt stress at the molecular, cellular and whole plant levels. The capacity to tolerate salinity can be a key factor in rice productivity. It is generally accepted that proline as a common osmolytes was accumulated under salinity condition to conserve osmotic stability and to prevent damage however the role of proline in osmotic adjustment is still being debated.

Mechanisms avoidance and tolerance against salinity stress support this idea that these mechanisms constitute a significant aspect of salt tolerant in rice. Therefore our study explored the defense mechanism of rice at different growth stages (germination, seedling and reproductive stages) that contribute to salt tolerance in rice. The concentration and composition of solutes in the soil solution are usually very different. Thus this study was carried out to investigate the rice response to salt composition and concentration under a saline soil solution. Therefore the general



hypothesis here is that amino acids accumulation as a defense mechanism may be affected by changing in the salt compositions and concentrations.

Eleven widely grown rice cultivars in Iran were selected for this study. These are Pouya, Shafag, Neda, Kadous, Tabesh, Tarom-e-Hashemi, Sahel, Khazar, Shirodi, Fajr and Nemat and the relationship between the physiological and biochemical parameters; root characteristics, shoot height, dry weight, water content percentage and free amino acids status as affected by salt concentrations and composition were determined at the different rice growth stages. The root of rice seedlings were scanned by WINRHIZO system. Proline content was measured by acid - ninhydrin method. The elements (K, Ca, Mg and Na) were measured by dry ashing method and inductively coupled argon-plasma emission spectrometry (ICP trace analyzer). The free amino acids were separately determined in shoot and root by HPLC system. Quality of suberin in the lamella of roots was determined by staining of free-hand section. The sections were examined using a light microscope (Olympus bx51, Olympus and Melville, NY, USA).

At germination stage, the results revealed that (1) the mean germination time (MGT) was positively affected by increasing salt concentration. Conversely, a negative relationship between germination index (GI) and salt concentration was recorded; (2) a significant difference between germination parameters and salt compositions was observed and (3) there was non-significant correlation between proline and water content.

The analyzed data at seedling and reproductive stages showed that (1) the root length, root surface area, shoot height and dry weight of root and shoot as the



physiological parameters were reduced by increasing salinity levels, (2) the relationship between the root surface area and salt compositions was not significantly ($p \leq 0.01$) observed; (3) the interaction between salt concentration and composition was mostly significant ($p \leq 0.01$); (4) a significant reduction in K^+/Na^+ , Ca^{2+}/Na^+ and $Ca^{2+}+Mg^{2+}/Na^+$ ratios in seedlings when salinity level was increased was recorded; (5) the free amino acids in root did not show significant differences. However free amino acids in shoot were significantly ($p \leq 0.05$) increased as salt concentration increased; (6) Aspartic acid can be susceptible and reliable indicator for osmotic adjustment in the rice shoot, at least in these rice genotypes (7) salt composition intensify suberin development and (8) yield components were affected by salt concentration more than salt composition.

It can be concluded that (A) asparagine as a compatible solute was involved in osmotic adjustment; (B) these rice cultivars were not able to compartmentalize Na^+ , K^+ and Ca^{2+} in their seedling segments (C) Fajr as a salt tolerant cultivar regulated suberization more than that observed in salt sensitive Khazar and (D) the anion associated with Na were more effective at seedling stage than at germination or reproductive stages.



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

**EKSPRESI AGRONOMI DAN BIOKIMIA TEKANAN GARAM
KEATAS KALTIVAR PADI IRAN**

Oleh

MOHAMMAD REZA MOMAYEZI

Julai 2010

Pengerusi : Profesor Zaharah Abdul Rahman, PhD

Fakulti : Pertanian

Tekanan garam memberi kesan yang kompleks, seperti, menyebabkan tekanan osmotik luar yang menahan penyerapan air, kesan menyekat ion-spesifik seperti Na^+ dan Cl^- ke atas metabolisma tanaman dan ketidak-seimbangan nutrien dalam tanaman yang menerima tekanan. Oleh itu tanaman yang boleh menahan tekanan garam perlu mempunyai sifat-sifat yang boleh mengatasi cabaran osmotik dan ketoksikan ion. Tanaman menunjukkan berbagai tindakbalas terhadap tekanan garam samada di tahap molekul, sel dan keseluruhan tanaman. Keupayaan menahan kemasinan boleh menjadi satu faktor penting dalam pengeluaran tanaman padi. Tindakbalas biokimia yang biasa oleh sel tanaman terhadap tekanan osmotik ialah dalam meng sintesis solut organik yang tertentu (osmolytes), yang akan berkumpul dengan tingginya dalam sitoplasma. Fakta biasa yang diketahui ialah pengumpulan prolin sebagai osmolyte yang dikumpul didalam keadaan bergaram untuk mengekalkan kestabilan osmotik dan menghalang kerosakan sel. Tetapi, peranan prolin untuk mengekal keadaan osmotik masih diperdebatkan.



Mekanisma penghindaran dan ketahanan terhadap tekanan garam mendukung idea ini yang mengatakan mekanisma ini amat mempengaruhi ketahanan tanaman padi terhadap garam. Dengan itu, kajian kami meneliti mekanisma ketahanan tanaman padi pada beberapa tahap pertumbuhan (tahap percambahan biji benih, tahap “seedling” dan tahap reproduktif) terhadap ketahanan garam. Kepekatan dan komposisi solut dalam larutan tanah biasanya amat berlainan. Dengan itu, kajian ini dijalankan untuk mengkaji tindakbalas tanaman padi terhadap komposisi dan kepekatan garam dalam larutan tanah bergaram. Hipotesis umum dalam keadaan ini ialah asid amino akan berkumpul sebagai mekanisma untuk pertahanan mungkin akan dipengaruhi oleh komposisi dan kepekatan garam.

Untuk kajian ini, 11 kultivar padi yang biasa ditanam di Iran telah dipilih, iaitu Pouya, Shafag, Neda, Kadous, Tabesh, Tarom-e-Hashemi, Sahel, Khazar, Shirodi, Fajr dan Nemat telah dipilih. Kajian kaitan diantara parameter fisiologi dan biokimia; ciri-ciri akar, ketinggian pokok, berat kering, kandungan peratus air dan kandungan prolin telah ditentukan pada tahap pertumbuhan padi yang berlainan. Akar anak padi telah diimbis menggunakan system WINRHIZO. Kandungan prolin ditentukan mengikut pencirian oleh acid – ninhydrin method. Elemen-elemen (K, Ca, Mg dan Na) ditentukan dengan kaedah pengabuan kering dan pengukuran dengan menggunakan alat Inductively Coupled Argon-Plasma emission spectrometry (ICP trace analyzer). Asid amino bebas ditentukan berasingan didalam daun dan akar dengan system HPLC. Kualiti suberin dalam sel lamella akar ditentukan dengan mewarnakan keratin- tangan tisu akar seperti yang dihuraikan. Keratan yang diwarna diperiksa menggunakan mikroskop cahaya (Olympus bx51, Olympus and Melville, NY, USA).



Pada tahap percambahan benih, keputusan menunjukkan iaitu (1) min masa percambahan (MGT) di pengaruhi secara positif oleh peningkatan kepekatan garam. Sebaliknya, kaitan negatif dicatitkan diantara indeks percambahan (GI) dan kepekatan garam; (2) perbezaan bermakna diantara parameter percambahan dan komposisi garam diperolehi, dan (3) didapati korelasi tak bermakna diantara kandungan prolin dan kandungan air.

Data yang dianalisis di tahap anak benih dan di tahap reproduktif menunjukkan (1) panjang akar, luas permukaan akar, ketinggian pokok dan berat kering pokok dan akar sebagai parameter fisiologi menurun dengan meningkatnya kandungan garam, (2) Pertalian diantara luas permukaan akar dan komposisi garam adalah tidak bererti pada $P \leq 0.01$; (3) Interaksi diantara kepekatan dan komposisi garam kebanyakannya amat bermakna ($P \leq 0.01$); (4) Didapati penurunan ketara dalam nisbah K^+/Na^+ , Ca^{2+}/Na^+ dan $Ca^{2+} + Mg^{2+}/Na^+$ dalam anak padi apabila kadar garam meningkat; (5) Amino asid bebas dalam akar tidak menunjukkan perbezaan yang bererti. Tetapi kandungan amino asid bebas dalam pokok meningkat dengan bererti ($P \leq 0.05$) apabila kepekatan garam meningkat; (6) asid aspartic mudah dipengaruhi dan boleh menjadi petunjuk sebagai asid amino yang dikeluarkan untuk menyesuaikan keadaan osmotik dalam pokok padi (sekurang-kurangnya kultivar padi yang diguna dalam kajian ini); (7) komposisi garam meningkatkan pembentukan suberin, dan (8) didapati kepekatan garam lebih mempengaruhi komponen hasil daripada komposisi garam.

Dengan itu rumusan bolehlah dibuat bahawa (A) asparagine merupakan solut yang dikeluarkan untuk menyesuaikan keadaan osmotik; (B) kultivar padi yang digunakan

tidak mampu membendung pengambilan unsur Na^+ ; (C) Fajr sebagai kultivar tahan-garam, membentuk lebih banyak suberin daripada Khazar yang sensitif-garam, dan (D) anion bersekutu dengan Na^+ adalah lebih berkesan pada tahap anak-benih berbanding dengan tahap percambahan biji benih dan tahap reproduktif.

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My family is the reason and purpose of my being, so my accomplishment is for my family too.



I certify that a Thesis Examination Committee has met on 21 June, 2010 to conduct the final examination of Mohammad Reza Momayezi on his Doctor of Philosophy thesis entitled “Agronomic And Biochemical Expression Of Salt Stress On Iranian Rice Cultivars” in accordance with 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 degree of Doctor of Philosophy (PhD).

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Date: 12 August 2010



DECLARATION

I hereby 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 or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

Mohammad Reza Momayezi

Date: 21 June 2010



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