FUNCTIONAL GENOMICS STUDIES FOR DETERMINING LOCI ASSOCIATED WITH SALT TOLERANCE IN RICE (*Oryza sativa* L.)

BY

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B.Sc. Agric. Sc. (Genetics), Ain Shams University, 1993

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A thesis submitted in partial fulfillment

of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Agricultural Science

(Genetics)

Department of Genetics

Faculty of Agriculture, Ain Shams University

2005

Approval sheet

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ABSTRACT

AHMED MAHMOUD ABDELHAMID SHOKRY. Functional Genomics Studies for Determining Loci Associated with Salt Tolerance in Rice (*Oryza sativa* L.). Unpublished Doctor of Philosophy Dissertation, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2005.

Soil salinity is considered as one of the major and widespread abiotic stresses limiting rice production. Rice is one of three cereals produced annually at worldwide levels of approximately half a billion tons. It is also becoming evident that rice could play a major role as a model for cereal genomics due to the small size of the rice genome between 420 to 450 megabase pairs (Mbp) in which rice will has on average one gene approximately every 15 kilobase pairs (Kbp).

Five rice (*Oryza sativa* L.) cultivars were screened for salt tolerance in this study, i.e., Giza 159, Giza 177, Giza 178, Sakha 102 and Agami M1. Agami M1 was chosen as the most tolerant cultivar for salt stress.

Differential display was conducted for shoot tissues under control and salt concentration treatments at 10,000 ppm for one and ten hours. Out of 324 bands observed, 30 bands (9.26%) showed differential expression between treatments. They were classified into six patterns of expression.

The automated DNA sequencing reactions were conducted for some fragments. Computer analysis was done using Blast programs from National Center for Biotechnology Information (NCBI), USA to determine homologies and chromosome location for these fragments on the rice genome. Some cDNA fragments showed homology to some genes that play a role in salt tolerance mechanisms like ubiquitin and Ca^{2+}-transporting ATPase which

can be used for the isolation of salt related genes (full length) while, the other fragments can be used to discover new genes related to salt-stress response mechanisms.

Key words: Rice, Salt stress, mRNA, cDNA, Differential Display, Gene expression.

ACKNOWLEDGEMENTS

First and foremost, I'm indebted to ALLAH forever.

I would like to express my gratitude and deep thanks to **Prof. Dr. F. M. Abdel-Tawab,** Professor of Molecular Genetics, Genetics Dept., Fac. Agric., Ain Shams Univ. for his kind supervision, suggesting the problem, fruitful help, patriotic patience, energetic guidance, conclusive instructions, writing and reviewing the manuscript throughout the course of this study.

I feel deeply thankful to **Prof. Dr. A. Bahieldin,** Professor of Molecular Genetics, Genetics Dept., Fac. Agric., Ain Shams Univ. for his kind supervision, suggesting the problem, unlimited support, his encouragement from the very beginning up to writing and reviewing the manuscript.

Thanks are extended to **Prof. Dr. G. A. Gad El-Karim,** Deputy Director & Head of Research. Agric. Genet. Eng. Res. Inst. (AGERI), Agric. Res. Cent. (ARC), Giza, Egypt for his kind supervision, suggesting the problem, and unlimited support on both scientific and personal levels during the course of this study.

Special thanks are due to **Dr. Dina A. El-Khishin**, head of GPBI lab, AGERI, for providing the laboratory facilities for this study.

Special thanks are due to **Prof. Dr. Ismail Abd El-Monem**, Chief Researcher, Crop breeding institute, ARC and Project Coordinator in International Center for Agriculture in Dry Area (ICARDA), for providing green house facility for the screening part of this study.

Thanks are also due to all the **staff members** of the ESL lab and GPBI Lab, Genetic Engineering Research Institute (AGERI), Agric. Res. Center (ARC), for their great help with special gratitude to Miss Abla El-Awady for helping in sequencing part of the study.

Thanks are also extended to all the **staff members** of the Department of Genetics Fac. Agric., Ain Shams Univ. for their continued sport of this study, special gratitude to Dr. Sabah M. Hassan (my wife) for reviewing the manuscript and Mr. Sherif Idris

for helping in setting up the deferential display system in this study.

Special thanks are also extended to **my family** for their patience and encouragement during this work.

Last, but not least, I would like to dedicate this work to the soul of my father.

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I. INTRODUCTION

Rice is one of three cereals produced annually at worldwide level of approximately half a billion tons **(FAOSTAT, 2004)**. Unlike the other major cereals, more than 90% of rice is consumed by humans. Approximately, half of the world's population derives a significant proportion of their caloric intake from rice consumption. Given the predicted rise in the world's human population, it is likely that rice consumption, and therefore demand, will increase over the next several decades. As a limited acreage of intensively cultivated areas is available to increase rice cultivation, both higher yields and expansion of cultivation into salt-affected areas are prerequisites to meet the anticipated rising demand.

In addition to being an important cereal crop for human consumption, it is also becoming clear that rice could play a major role as a good model for cereal genomics. Rice has a genome size considerably smaller than the other major cereals, which is estimated at 420 to 450 megabase pairs (Mbp). Sorghum, maize, barley, and wheat have significantly larger genomes (1000, 3000, 5000, and 17000 Mbp, respectively). The small genome size of rice results in a higher gene density relative to the other cereals. Assuming a total of 30,000 genes in each of the cereal genomes, rice will have on average of one gene approximately every 15 kilobase pairs (Kbp). Maize and wheat will have one gene approximately every 100 and 500 Kbp, respectively. This higher gene density in rice makes it an attractive target for cereal gene discovery efforts and genome sequence analysis **(Goff, 1999)**.

Although genes in rice are present at a higher relative density than in other cereals, they are predicted to be arranged in a similar general order within the genome. Comparisons of the physical and

genetic maps of cereal genomes have led to concede that a significant amount of colinearity of gene order exists among the different cereal genomes **(Ahn *et al.*, 1993)**. Accordingly, the use of rice as a model for cereal comparative genomic analysis has been proposed and recently reviewed **(Havukkala, 1996)**.

Plants are frequently exposed to stresses, which are usually defined as external factors exerting disadvantageous influences on them **(Levitt, 1972)**. Water deficit, chilling and freezing, heat stress, salinity and oxygen deficiency are major stress factors restricting plant growth **(Boyer, 1982; Salisbury and Ross, 1989)**. Some of which (such as temperature) can become stressful in a few minutes; others may take days to weeks (soil water) or even months (mineral nutrients) to become stressful. Salinity can affect any process in the plant life cycle, so that tolerance will involve a complex interplay of characters. Many researchers investigated details of the physiology and biochemistry of salt tolerance and also looked at methods to screen overall plant performance that could be used in breeding programs. Plants, in general, are relatively tolerant during germination but become more sensitive during emergence and early seedling up to later stages of growth **(Azhar and McNeilly, 1989; Abdel-Tawab *et al.,* 1998a)**.

Differential display (DD), first described by **Liang and Pardee (1992),** is one of the methods for analyzing gene expression in eukaryotic cells and tissues. DD has been widely applied to study changes in mRNA expression induced by temporal developments, biotic and abiotic factors (**Liang and Pardee, 1992; liang *et al.*, 1992; McCarthy *et al.*, 1995; Hu *et al.*, 1996)**. This powerful technique simultaneously screens for both up-regulated and down-regulated transcripts in multiple cell populations under different developmental and environmental conditions.