GENETIC STUDIES ON THE MOLECULAR BIOLOGY BASES OF SALINITY STRESS ON BARLEY (*Hordeum vulgare* L.)

By

Magdy Hamed Ahmed Bendary
B. Sc. Agriculture (Genetics), Ain Shams Univ., 1988

A thesis submitted in partial fulfillment

of

The requirements for the degree of

Master of Science

in

Agricultural Science

(Genetics)

Genetics Department

Faculty of Agriculture

Ain Shams University

2000

Approval Sheet

GENETIC STUDIES ON THE MOLECULAR BIOLOGY BASES OF SALINITY STRESS ON BARLEY (*Hordeum vulgare* L.)

By

MAGDY HAMED AHMED BENDARY

B. Sc. Agriculture (Genetics), Ain Shams Univ., 1988

This thesis for M. Sc. Degree has been approved by:

Prof. Dr. A. A. Abdelrahem **...……**

Prof. of Genetics, and Chairman of Agricultural Botany Dept. Fac. Agric., Suez Canal Univ.

Prof. Dr. S. A. Ibrahem **...……**

Prof. of Genetics, Fac. Agric., Ain Shams Univ.

Prof. Dr. Aly Zein El Abidin Abdelsalam (Supervisor) ..……..…..

Prof. and Chairman of the Dept. of Genetics, Fac. Agric., Ain Shams Univ. and Director of Ain Shams Center for Genetic Engineering and Biotechnology (ACGEB)

Date of Examination: 29 / 6 / 2000

GENETIC STUDIES ON THE MOLECULAR BIOLOGY BASES OF SALINITY STRESS ON BARLEY (*Hordeum vulgare* L.)

By

Magdy Hamed Ahmed Bendary
B. Sc. Agriculture (Genetics), Ain Shams Univ., 1988

Under the supervision of:

Prof. Dr. A. Z. E. Abdelsalam

Prof. and Chairman of the Dept. of Genetics, Fac. Agric., Ain Shams Univ. and Director of Ain Shams Center for Genetic Engineering and Biotechnology (ACGEB)

Prof. Dr. E. T. A. Kishk

Prof. of Plant Physiology, Desert Research Center

Dr. A. Bahieldin

Assoc. Prof. of Genetics, Fac. Agric., Ain Shams Univ.

LIST OF ABRRIVIATIONS

AFLP	: amplified fragment length polymorphism
bp	: base pair
BSA	: bulked segregant analysis
CC89	: composed cross 89
DM	: downy mildew
kDa	: kilodalton
MAS	: marker-assisted selection
NMR	: nuclear magnetic resonance
OA	: osmotic adjustment
PCR	: polymerase chain reaction
PEG	: polyethylene glycol
QTLs	: quantitative trait loci
RAPD	: random amplified polymorphic DNA
RFLP	: restriction fragment length polymorphism
RILs	: recombinant inbred lines
SDS-Protein	: sodium dodecyl sulphate-protein
TBA	: trait-based marker analysis
TE	: Tris- EDTA
ToMV	: tomato mosaic virus

ACKNOWLEGEMENT

The author wishes to express their deepest to Dr. A. Z. E. Abdelsalam, Prof. and Chairman of the Department. of Genetics, Faculty of Agriculture, Ain Shams University and Director of Ain Shams Center for Genetic Engineering and Biotechnology (ACGEB), Dr. E. T. A. Kishk, Professor of Pant Physiology, Plant Genetic Resources Department, Ecology and Dryland Division, Desert Research Center and Dr. A. Bahieldin, Associate Professor of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University for their supervision, suggesting the problem and constructive criticism throughout the course of this work and preparing the manuscript.

Thanks are due to all staff members of Ain Shams Center for Genetic Engineering and Biotechnology (ACGEB)

Acknowledgement is also extended to all members and the workers of Department of Genetics, Faculty of Agriculture, Ain Shams University and of Plant Genetic Resources Department, specially, Genetic and Cytology Unit, Ecology and Dryland Division, Desert Research Center (DRC).

I fell also obliged and grateful to my wife and my family for their continuous encouragement and for their kind help and patience.

ABSTRACT

Magdy Hamed Ahmed Bendary. Genetic Studies on the Molecular Biology Bases of Salinity Stress on Barley. Unpublished M. Sc. Thesis, Ain Shams Univ., Agric. Fac., Genetics Department, 2000

The aim of this study was to study some physiological, biochemical and molecular parameters underlying relative salt tolerance of five barley cultivars. Based on the results of a preliminary experiment involving yield and its attributes cultivar Giza 123 was chosen as the most tolerant and cultivar CC 89 as the most sensitive. The two selected cultivars were crossed and the F_{1s} were selfed to produce F_2 plants (83 individual plants), which were tested for their salt tolerance (8000 ppm) for grain number, plant height (cm) and tiller number. The concentrations of sodium, potassium, and calcium and their ratios in the two parents were found to be good indicators for physiological parameters. Proline content was increased in both cultivars under 8000 ppm salt stress with elevation varied between the two cultivars; as it reached 3.14 folds in the tolerant parent (G123), while it was 1.46 folds only in the sensitive line (CC89). The results of electrophoretic analysis for SDS-protein fraction indicated that the sensitive parent and F_2 sensitive group had one band with 32 kDa. Randomly amplified polymorphic DNA (RAPD) markers for salt tolerance were detected by the use of the bulked-segregant analysis (BSA). Each of primer P24 and P92 detected positive molecular marker with molecular weights of 700 bp and 550 bp, respectively, while P93 detected a negative molecular marker. Each of these three bands can be considered as the first published RAPD markers for salt tolerance in barley.

Key words: Barley, BSA, *Hordeum vulgare*, Salt stress, SDS-PAGE and RAPD.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Plants respond to abiotic stress actively to survive the stress by turning on some metabolic pathway or by modifying gene expression, the final aim being to survive the stress. This stress brings about a decrease in plant yield. Identification of the metabolic processes and the involved regulatory genes and active areas is indispensable in producing stress resistant plants.

Even species that are only distantly related have strong similarities in their molecular responses to abiotic stress. This makes it possible to extrapolate findings derived from only a few model plants to other species.

Although it is not a halophyte, barley tolerates high salt concentrations in the soil. Exposure of barley plants to high salt concentrations, however, alters some biochemical parameters of the cells. A plant growing in soil with high salt concentration must have a transmembrane transport system that can maintain a K^+/Na^+ ratio above the external ratio **(Cheeseman, 1988)**. In barley, there is evidence of NaCl – induced mechanism that extrudes Na+ based on a Na^+/H^+ antiport **(Garabarino and DuPont, 1988)**. Accumulation of proline has been observed in barley leaf segments treated with ABA and KCl or NaCl **(Pesci, 1989)**, and of glycine betaine in wheat plants subjected to salt stress **(McDonnell & Wyn Jones, 1988)**. To elucidate the effect of proline and glycine betaine accumulation on salt tolerance in barley, embryos were cultured in high salt conditions. **Lone *et al*. (1987)** observed that the exogenous addition of proline and glycine betaine to barley embryo cultures favours shoot lengthening. Barley mutant that accumulates a high level of proline has been isolated **(Kueh and Bright, 1981)**. However, the comparison of the effects of exogenous supplied proline with the enhanced levels of endogenous proline in the mutant line R5201 suggested that the increased proline accumulation in the mutant is too low to have a significant physiological effect **(Lone *et al.*, 1987)**.