MARKER-ASSISTED SELECTION FOR SALT STRESS AND QUALITY TRAITS IN SWEET SORGHUM (*Sorghum bicolor*)

BY

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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

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Date of Examination 24/ 07/ 2005

ACKNOWLEDGMENT

I wish to thank **Allah** who supported me with the strength and patience to complete this work.

The author wishes to express his deep gratitude and sincere appreciation to **Prof. Dr. Mohamed A. Rashed**, Professor and Head of Genetics Dept., Faculty of Agriculture, Ain Shams University for continuous supervision, kind encouragement, sincere help criticism and precious advices during the progress of thesis work and the preparation and writing of the manuscript.

Sincere appreciation is due to Dr. **Ahmed Fahmy Hussein Abo-Doma**, Associate Prof. of Genetics, Faculty of Agric., Ain Shams University, for his supervision, providing SSR primers and his efforts in writing and reviewing the manuscript.

Thanks are due to Prof. Dr. **Hamam A. ELrashedy,** Prof. of plant breeding, Sugar Crops Res. Inst.; Agric. Res. Center for his supervision and his efforts in writing and reviewing the manuscript.

Deep thanks to Prof. Dr. **Fatthy Mohamed Abdel-Tawab** Prof. of Genetics, Fac. of Agric. Ain Shams University, for his useful suggestions, continuous guidance throughout the course of this investigation.

Thanks are due to Prof. Dr. **Ahmed Abo-Doh,** Director of Sugar Crops Res. Inst. for his great help and encouragement.

Great thanks we due to all the staff members of **Molecular pathology & Biotechnology Lab.,** Dept. Pests & Diseases, Sugar Crops Res. Inst., Agricultural Research Center for their great help and encouragement during this work.

Many thanks are extended to all the **staff members of Molecular Genetics Lab.**, Faculty of Agric., Ain Shams University, for their great help and encouragement.

Finally, I am indebted as gift to **my wife and my children** for their great help and patience during this work and to **my mother** for here continuous encouragement and praying for me.

ABSTRACT

Khaled Adly Mohamed Khaled. Marker-Assisted Selection for Salinity Stress and Quality traits in Sweet Sorghum [*Sorghum bicolor* (L.) Moench], Department of Genetics, Faculty of Agriculture, Ain Shams University, 2005.

Eleven sorghum cultivars were screened to assess their salt tolerance based on some yield-related traits. Two sorghum cultivars were selected in which one of them was the most salt-sensitive (Atlas) and the other was the most salt-tolerant (Rex) according to their performance. Cross pollination was conducted between the two contrasting cultivars, Rex and Atlas to produce F_1 seeds. In order to study the nature of salt tolerance inheritance and segregation in F_2, self pollination was performed for F_1 plants to produce F_2 seeds. The F_2 seedlings were exposed to salinity stress (6000 ppm NaCl). The two extreme groups of F_2 individuals (salt tolerant and salt sensitive) were selected. The results showed that the effects of salt stress varied across the yield related traits in all cultivars, also yield related traits (total root length, root dry weight, shoot fresh weight, shoot dry weight, shoot\root ratio, total chlorophylls and plant height) were decreased by salt stress. Bulked segregant analysis was used to analyze DNA extracts with 20 random primers using RAPD-PCR and 15 pairs of SSR primers for the two contrasting parents, their F_1 and the two extreme F_2 groups (bulks of plants from each of tolerant and sensitive groups). SSR results were confirmed using the same primers with a limited subset of individual F_2 plants. Out of the 20 random primers, four primers gave polymorphism and developed molecular markers for salinity tolerance. These bands exhibited five positive molecular markers and three negative molecular markers. Three pairs of primers out of the fifteen SSR pairs produced scorable markers. Two positive molecular markers and two negative markers were detected.

The results indicated that RAPD and the SSR markers could be considered as reliable markers for the molecular detection of salinity tolerance in sweet sorghum [*Sorghum bicolor* (L.) Moench].

Key words:

Sorghum bicolor, salt stress, molecular markers, RAPD-PCR., SSR-PCR, marker- assisted selection

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I. INTRODUCTION

Because of its juicy and sweet stalks, sweet sorghum [*Sorghum bicolor* (L.) Moench] has been used to produce concentrated syrup for food use. Interest in sweet sorghum has been especially important in areas of the world undergoing shortage of sugar. Sweet sorghum has also been widely used for forage and silage production for animal feed. Under both natural and agricultural conditions, plants are frequently exposed to stress, which is usually defined as an external factor exerting a disadvantageous influence on plant growth and development (**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 environmental factors (such as temperature) can become stressful in a few minutes; others may take days to weeks (soil water deficient) 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 are generally, relatively tolerant during germination but become more sensitive during emergence and early seedling stages of growth (**Azhar and McNeilly, 1989; Abdel-Tawab *et al.*, 1998b**).

Molecular markers offer specific advantages in assessment of genetic diversity and in trait-specific crop improvement. Use of molecular markers in the applied breeding programs can facilitate appropriate choice of parents involved for crosses.

The detection of molecular markers linked to many genes of interest has been widely achieved .In this concern, RAPD as a simple and rapid procedure has gained a worldwide acceptance and application (**Paran *et al.*, 1991**). Moreover, RAPD-PCR markers were detected for salt stress in wheat and maize, as reported by **Bahieldin *et al.*, (1994); Abdel-Tawab *et al.*, (2002)** and in sorghum **(Rashed *et al.*, 2001).** DNA simple sequence repeats (SSR or microsatellites) are numerous and highly polymorphic in plants (**Morgante and Olivieri 1993; Saghai Maroof *et al.*, 1994; Wang *et al.*, 1994; Rongwen *et al.*; 1995 and Yang *et al.*, 1995**). The high information content of SSR makes them excellent genetic markers for many types of investigations, including marker-assisted selection. **Michelmore *et al.* (1991)** developed the bulked segregant analysis of F_2 plants as a simpler alternative to isogenic line analysis where the highest and lowest extremes of the F_2 population are bulked for the development of RAPD and SSR molecular markers needed for QTLs-assisted selection. Most of the detected linkages between marker loci and quantitative traits were shown to be highly significant (**Edwards *et al.*, 1987**).

The objectives of this study were:

1- To screen the responses of eleven sorghum cultivars (*Sorghum biocolor*) under salt stress treatments with respects to the performances of some yield-related traits.

2- To select the most tolerant and the most sensitive cultivars to salt stress to be hybridized in order to obtain (F_l) plants, followed by selfing to obtain (F_2) plants.

3- To study the effect of salt stress on the two contrasting cultivars and their F_l and F_2 generations based on their performance some yield- related traits.

4- To develop some molecular markers (RAPD and SSR) associated with salt tolerance in sorghum using bulked segregant analysis (BSA).

5- To confirm the SSR results of bulked segregant analysis on a limited subset of F_2 individual plants.

II. REVIEW OF LITERATURE

1- Salt tolerance assessment

The concept of stress tolerance is defined as, the plant's fitness to cope with an unfavorable environment (**Levitt, 1972; 1980**). On the basis of their water requirements and adaptation to environmental stresses, plants are commonly characterized as xerophytes (drought-tolerant), halophytes (salt-tolerant) and glycophytes (stress-sensitive) (**Barbour *et al.*, 1987; Salisbury and Ross, 1989**).

Water salinity is environmental stress factor that inhibits the growth and yield of glycophytic crop plants in many regions of the world **(Epstein 1985, and Cheesman 1988)**. The precise mechanisms by which excessive salinity inhibits the growth of crop plants are still not fully understood, but may include osmotic effects as well as the direct toxicity caused by certain ions (**Cheesman 1988).**

Very similar mechanisms in stress tolerance have been observed in different plant species, supporting the theory that stress tolerance mechanisms are ubiquitous and that the advantages of halophytes may result from the more efficient performance of a few basic biochemical pathways (**Leopold *et al.*, 1992; Donoghue, 1994 and Bohnert *et al.*, 1995**).

Plant growth models may provide a method to integrate the complexities of plant responses to salt stress with the relevant environmental variables that interact with the measurement of tolerance. Models responsive to salt stress would provide insights for breeders and aid in developing more practical research on the physiological mechanisms of plant salt tolerance (**Shannon, 1996**).