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ASSESSMENT OF THE MOLECULAR BASIS OF SALT TOLERANCE IN BREAD WHEAT *Triticum aestivum* L.

By

AYA-TU-ALLAH EZZAT AWWAD MANCY
B.Sc.Agric. Sci. (Genetics), Faculty of Agriculture, Ain Shams University, 2015

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ABSTRACT

Aya-tu-Allah Ezzat Awwad Mancy: Assessment of The Molecular Basis of Salt Tolerance in Bread Wheat *Triticum aestivum* L. Unpublished M.Sc. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2022.

In most countries worldwide, including Egypt, bread wheat is essential among cereals crops. However, soil salinity is a global issue that has a negative impact on wheat growth, development, and productivity. Therefore, salt tolerance is an important feature that must be improved in wheat genotypes. Identifying informative and highly differential molecular markers is critical for developing salt-tolerant genotypes that could tolerate excessive salts in the soil. Twelve bread wheat recombinant inbred lines (RILs) derived from a cross between Shandaweel-1 and Giza-168, were evaluated for salinity tolerance. All genotypes were evaluated under two treatments including control (10 mM NaCl) and salt stress (102 mM NaCl). Some phenotypic traits including plant height, number of tillers/ plant and number of leaves/ plant were measured. The three phenotypic traits were positively correlated with salt tolerant trait index (STTI). The highest STTI values were shown in six salt-tolerant RILs namely RIL1 (96.82, 100 and 93.75), RIL5 (98.00, 100 and 100), RIL9 (96.65, 93.02 and 100), RIL10 (96.96, 100 and 93.75), RIL11 (94.73, 96 and 100) and RIL12 (96.08, 97.82 and 93.33), for plant height, number of tillers/plants and number of leaves/plants, respectively. The microsatellites analysis of wheat genotypes was carried out with 12 SSR primers generating total of 17 alleles with an average of 1.4 alleles and 44.4% polymorphism per primer. Out of the 12 microsatellites markers (SSRs) used to evaluate salt tolerance in wheat genotypes, three primers (wmc 432, gwm 88 and gwm 213) revealed genetic polymorphism between parental genotypes and among the studied RILs. Large variations could be observed for proline accumulation among the 12 wheat RILs and between their parents, and the results of estimation of proline content confirmed the results obtained on the morphological and the molecular

levels, where the highest amounts of proline in leaves were observed for the highest RILs group, the minimum increase was 710.52 µg.g- I.FW for RIL10 and the maximum increase was 1322.08 µg.g- I.FW for RIL12. Nevertheless, the lowest amounts of proline in leaves were observed for the lowest RILs group, and ranged from 232.9502 µg.g- I.FW for RIL6 to 506.2397 µg.g- I.FW for RIL4. These results indicate that there must be a relationship between proline accumulation and salt tolerance mechanisms in wheat. Due to their high performance under salt stress conditions, amplifying a polymorphic band within three SSRs primers associated with salt tolerance and accumulating the highest amounts of proline content under salt stress, six RILs out of the 12 studied could be considered as promising materials for improving bread wheat in breeding programs in the future.

Keywords: Bread wheat, RILs, Salinity tolerance, Pro content and Microsatellites.

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
APX	Ascorbate Peroxidase
CAT	Catalase
FAO	Food and Agriculture Organization
FW	Fresh Weight
ISSRs	Inter Simple Sequence Repeats
MAS	Marker Assisted Selection
MS	Molecular Size
OD	Optical Density
P1	Parent1
P2	Parent2
Pro	Proline
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RBD	Randomized Block Design
RFLP	Restriction Fragment Length Polymorphism
RILs	Recombinant Inbred Lines
ROS	Reactive Oxygen Species
SII	Salt Injury Index
SOD	Superoxide Dismutase
SSD	Single Seed Descent
SSRs	Simple Sequence Repeats
STTI	Salt Tolerance Trait Index
TAE	Tris Acetic EDTA
USDA	United States Department of Agriculture

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the king of cereals and considered as the principal source of food and is extensively grown, consumed and preferred in Egypt **(Kalhoro *et al.*, 2016).**

About 95% of wheat grown today is hexaploid and used for the preparation of bread and other baked products. It has total production of nine million tons in Egypt **(FAO, 2021);** and 775.83 million tons in the world, and its global production is predicted to hit 780.28 million tons by 2022**, (USDA, 2021).**

In Egypt, there is a significant gap between wheat agricultural output and its consumption. However, Egypt is the world's largest wheat importer; with wheat imports for the 2019/2020 marketing year were estimated at 12.5 million tons, increasing about 15% above the average of the last five years. As a result, it is critical to improve this crop in order to tackle this issue **(El-Rawy, 2020).**

The problem of salinization in the Nile Delta is one of the obstacles to narrow the gap between wheat demand and supply in Egypt. 33 percent of Egypt's agriculture area is salinized due to limited precipitation (25 mm yearly), drain water re-use, and politicians' restrictions on rice cultivation **(Elshafei *et al.*, 2019).**

Several scientists' findings suggest that high salt levels in soil inflict negative influence on plants through osmotic and ionic stress. **Brini and Masmoudi (2012)** findings reported that salt stress is main source in wheat genotypes to cause hormonal imbalance, fluctuation in nutrient uptake and overproduction of oxidizing agent **(Ilyas *et al.*, 2020).**

One of the most effective and feasible ways to minimize the detrimental effects of salinity on crop production is to enhance the salinity-tolerant ability **(Tao *et al.*, 2021).**

INTRODUCTION

The presence of genetic variation in wheat is the key to identify the contrasting parents for classical breeding **(Budak *et al.*, 2015).**

The single seed descent technique (SSD) in combination with *invitro* growth of embryos dissected from immature seeds can be used to decrease the breeding cycle. Starting with F_2, the SSD technique entails selecting one seed at random from each individual plant in each generation. All seeds from individual plants are gathered in F_6 and later generations, and the progeny of a single plant is treated as an SSD line**.** SSD lines are evaluated in field tests in the following stages of the breeding process. Pre-selection of lines should be done as soon as feasible, i.e. in the first field experiment targeted at seed multiplication, in order to shorten the breeding cycle and limit breeding materials **(Watson *et al.*, 2018).**

The overabundance of the amino acid proline (Pro), which acts as a compatible solute, an osmo-protectant, and plays a vital role in cytosolic enzymes and cellular organelles protection in a wide range of higher plant species, is well known as one of the most common responses to water deficit and saline environments. Furthermore, Pro is a nitrogen supply that can help with stress recovery and growth restoration. Salt-induced Pro buildup is generally a late reaction, manifesting only after cell damage has occurred, and increased levels of Pro persist even after stressed tissues have returned to normal osmotic conditions.**(Jiménez-Bremont *et al.*, 2006)**

Wheat genotypes, characteristics, and genes related to abiotic stress adaptability will enable breeders to adapt wheat to various environmental circumstances. As a result, breeding can be used along with stable molecular markers to improve the efficiency of selection for features that are difficult and expensive to characterize **(Mohamed and Elameen, 2019).**

INTRODUCTION

Due to high efficiency, reproducibility, easy-to-use, co-dominance and multi-allelic nature, high degree of polymorphism, relative abundance and good genome coverage, microsatellite markers are widely-used as molecular markers for fingerprinting germplasm to assess genetic diversity, pedigree analysis, evolutionary studies and genome mapping **(Mohammadi-Nejad *et al.*, 2008).**

Simple Sequence Repeats (SSRs) or microsatellites are still the most common type of markers used in wheat quantitative trait loci (QTL) research, whether used alone or in cooperation with other markers, and they span all 21 wheat chromosomes. SSR molecular markers are highly linked with wheat features related to salt tolerance and drought tolerance, according to several researchers **(Sobieh and Atta, 2013).**

The objectives of the present study were to:

i) Evaluate and screen some wheat recombinant inbred lines (RILs) for salinity tolerance, and to select lines for future breeding experiments.

ii) Understand the response generated following salt stress at the morphological, biochemical and molecular level.

iii) Validate microsatellite markers for salt tolerance in wheat by marker-trait association analysis on a diverse collection.

The information generated from the study can be utilized for wheat improvement.