بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

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MONA MAGHRABY

MOLECULAR EVALUATION OF SOME SALINITY TOLERANT GENOTYPES OF SORGHUM USING RT-PCR

By

NOURHAN AIMAN HANAFI ABDEL-AZEEM

B.Sc. Agri. Sci., (Biotechnology), Fac. Agri., Ain Shams University, 2013

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ABSTRACT

Nourhan Aiman Hanafi Abdel-Azeem: Molecular Evaluation of Some Salinity Tolerant Genotypes of Sorghum Using RT-PCR, Unpublished Master thesis, Department of Biotechnology, Arid Land Agriculture graduate studies and Research Institute, Faculty of Agriculture, Ain Shams University, 2020.

Twenty sorghum (Sorghum bicolor L. {Monech}) genotypes were assessed for fingerprint using 10 ISSR primers. All tested ISSR primers yielded amplified products and generated 152 alleles (average of 15.2 bands/primer). Out of the 10 used primers, eight primers obtained 24 unique cultivar markers. However, molecular characterization revealed polymorphism percentage of a 100% for ISSR markers, indicating a high level of a polymorphism among the studied sorghum genotypes. On the other hand, cluster analysis using UPGMA analysis classified the 20 sorghum genotypes into two main groups. The cluster analysis showed a high genetic variation among the studied sorghum genotypes and the diversity of these genotypes was in an agreement with their source pedigree. The results of principle coordinate analysis (PCoA) were closely in line with those of the cluster analysis.

Eight salt tolerance-related traits were recorded after 30 days of salt stress treatment (10,000 ppm) such as fresh and dry weights, fresh and dry plant height, % germination, lux, numbers of leaves and roots traits, which performed from the previously distinguished genotypes such as A2 and B5 tolerant genotypes as well as B8 and A9 sensitive genotypes. Averages of salt tolerance related traits values were statically analyzed using ANOVA and LSD test. ANOVA one way test showed that there were significant differences among genotypes averages, whereas P value was less than 0.05 ($P < \alpha$ 0.05). Therefore, the alternative hypothesis was accepted and the null hypothesis was rejected, as $H\alpha > H_o$ and H_o: $\mu1 = \mu2 = \mu3 ... = \mu k$, which means that salt is negatively affected on salt tolerance-related traits in the studied genotypes. The results of

LSD test showed that A2 was the most tolerant genotype; while B8 was the most salt sensitive one as well as B5 and A9 were moderate in salt tolerance and sensitivity, respectively.

Moreover, RT-PCR was used to evaluate the gene expression of *SbBADH1* & *SbBADH2* salt tolerance related genes at various times of salt treatment. The expression of *SbBADH1* gene showed that there were some changes in the expression for the four tested genotypes. While, *SbBADH2* gene showed a different behavior than *SbBADH1* gene. However, A2 was the most tolerant genotype, while B8 was the most sensitive one to the salt stress treatment which agreed with the results of the previously recorded salt tolerance-related traits. Finally, these results could be used by breeders to develop new breeding protocols for salt tolerance improvement in sorghum.

Keywords: *Sorghum bicolor*, Salinity, DNA, RNA, ISSR, UPGMA, PCoA, Polymorphism, ANOVA, LSD, RT-PCR, *SbBADH*.

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LIST OF ABBREVIATIONS

AGERI	Agricultural Genetic Engineering Research Institute
ANOVA	Analysis of Variance
ARC	Agricultural Research Center
cDNA	Complementary DNA
CMO	Choline Mono Oxygenase
CTAB	Cetyl Trimethylammonium Bromide
D.W	Dry weight
df	Degrees of freedom
dH_2O	distilled water
DNA	Deoxyribonucleic Acid
F.P	Forward primer
F.W	Fresh weight
FCRI	Field Crops Research Institute
GB	Glycine Betaine
H_o	The null hypothesis
$H\alpha$	The alternative hypothesis
ISSR	Inter Simple Sequence Repeats
K	The number of treatments or independent comparison
MSB^2	Mean square of sum of squares between groups
MSW^2	Mean square of sum of squares within groups
N	Total number of observations or total sample size
NaOH	Sodium Hydroxide
P.H	Plant height
PCoA	Principle Coordinate Analysis
PCR	Polymerase Chain Reaction
PTBL	Plant Transformation Biopharmaceuticals Laboratory
qPCR	quantitative Polymerase Chain Reaction
R.P	Reverse primer
RAPD	Random Amplified of Polymorphic DNA