Annals of Agric. Sci., Moshtohor **ISSN 1110-0419**
Vol. 60(1) (2022), 113 – 118 **https://assjm.journals.ekb.eg**

Identification of some Drought and Salinity Genes in four wheat cultivars (*Triticum aestivum*)

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Abstract

Four wheat cultivars, Gemmeiza10, Misr1, Sakha93, and Giza168 were used in this study as well as the hybrids resulted between all the studied four cultivars. Misr1 Sakha93 resulted hybrids were identified using salinity primers Na+/H+ antiporter gene amplification followed by sequencing while Giza168 Gemmeiza10 resulted hybrids were identified by drought primers DREB2 gene amplification followed by sequencing. The resulted sequences were compared with those available on NCBI website database through the BLAST bioinformatics tool. The obtained data showed that, the base size of Na+/H+ antiporter gene was 400 bp while the base size of DREB2 gene was 200 bp. the phylogenetic analysis showed that Na+/H+ antiporter gene obtained sequence was related to four accession numbers from gene bank. In addition, phylogenetic analysis exhibited an amount of genetic change on the root of clades 0.0020, where the Na+/H+ antiporter gene -based phylogeny tree included four clades. The clades grouping had low support (bootstrap value between 6-8%). Also, the obtained DREB2 gene sequences were aligned with six accession numbers in the NCBI website database as shown from the phylogeny tree. The phylogenetic tree deducted from the sequence comparison of DREB2 gene region showed that the length of branch that represents an amount of genetic change of 0.0020, where it's based phylogeny tree included four clades. The clades grouping had low support (bootstrap value between 8-12%).

Key words: Triticum aestivum, Na+/H+ antiporter gene, DREB2 gene, bioinformatics analysis.

Introduction

Bread wheat is a major food crop in most of the countries of the world which suffer from Saline soils, therefore increasing salinity tolerance in bread wheat is necessary **(Tuna *et al.*, 2008).** Drought continues to be an important challenge to agricultural researchers and plant breeders. It is assumed that by the year 2025, around 1.8 billion people will face absolute water shortage and 65% of the world's population will live under water-stressed environments. Tolerance to water stress is a complicated parameter in which crops' performance can be influenced by several characteristics **(Ingram and Bartels, 1996).** Hybridization in wheat will be considered in the light of the number of hybrids produced, the number of genes transferred to commercial cultivars and their use in worldwide agriculture. Barriers to wide hybridization and also progress made in overcoming such barriers will be considered. Areas requiring more research will be indicated **(STALKER, 1980).** In the last few years, many DREB cDNAs have been cloned and characterized in different economic plants, including rice (*Oryza sativa*) **(Dubouzet *et al.*, 2003; Tian *et al.*, 2005).** Although the complementary DNAs (cDNAs) for GmDREB2B;2/GmDREBa and GmDREB2C;2/GmDREBc have been isolated **(Li *et al.*, 2005).** Those for the GmDREB2A and GmDREB2D homologs have not. Therefore, we determined the cDNA sequences for these genes using the RACE method. Then, we amplified and cloned these coding regions from cDNA pools prepared from stress-treated soybean according to the deduced start and stop codons. All four homologous pairs shared high similarity with subtype 1 DREB2s of Arabidopsis in the N-terminal region, including the DNA-binding domain. Among them, the GmDREB2A homologs were most similar to DREB2A; they share the conserved motif CMIV-3 **(Nakano *et al.*, 2006)**. Na. and H. are the most common ions and they play primary roles in cell physiology: both are most important in cell bioenergetics and the concentration of protons within the cell is critical to the functioning of the cell and its proteins. Indeed, when the concentration of these ions becomes too high or too low they turn into potent stressors to all cells **(Padan *et al.*, 2000).**Hence, every cell has a very evident homeostatic mechanism for these ions. Proteins that play a primary role in this homeostatic mechanism are the Na./H. antiporters. These are membrane proteins that exchange Na. (or Li.) for H. They were discovered by P. Mitchell and colleagues **(West *et al.*, 1974).** DREBs contain a conserved apetala2/ethylene response factor (AP2/ERF) domain of approximately 60 amino acids **(Stockinger *et al.*, 1997)**. DREB genes form a large multigene family and can be classified into six small groups named as A-1 to A-6 **(Sakuma *et al.*, 2002).** The first isolated DREB family member was CRT/DRE-binding factor 1 (CBF1) which response to low temperature and water deficit from *Arabidopsis* **(Stockinger *et al.*, 1997)**. The aim of this study functional genomic analysis for abiotic stress tolerance in bread wheat (Triticum aestivum l.)

Materials and Methods

Source of the wheat (Triticum aestivum)

The Wheat cultivars used in this study were obtained from wheat Research Department, Field Crops Research Institute, Agricultural Research Center (ARC), Ministry of Agricultural, Cairo, Egypt.

The hybridization to obtain F2

The hybridization and reverse hybridization were done between:

1-Drought Tolerance

Giza 168 ♂ **X** **Gemmiza 10** ♀

Giza 168 ♀ **X** **Gemmiza 10** ♂

2-Tolerance of salinity

Misr 1 ♂ **X** **Sakha 93** ♀

Misr 1 ♀ **X** **Sakha 93** ♂

Field F2

Wheat genotypes-hybrids cultured on Hoagland growth medium **(Hoagland and Snyder, 1933),** F1 hybridization product was field-grown to obtain F2 (the second filial generation) wase obtained F1(first filial generation) genotypes, which used for RNA extraction to be studied genetically.

Genomic RNA isolation and cDNA Synthesis

RNA samples were prepared with a plant RNA purification kit (Qiagen, Germany) at 0, 1, and 4 days after inoculation. The extracted RNA was stored at −80 °C and used for library construction.

cDNA Synthesis was done using Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit.

PCR Amplification of cDNA

Primers used in PCR to salinity and Drought

Na+/H+ antiporter gene:

5'> CTACCTATTCTTCACCAGCAC <3'forward

5'>AGCAGCATTGACAGCATATAC <3'reverse primers.

DREB2 gene:

5'>TGAAACCATCAAGAAGTGGAAG <3'forward

5'> GCAGTAGGGAATGAACCAAG <3'reverse Primers.

The PCR reaction was performed for the cDNA in 25 µl volumes by mixing 30 ng cDNA with 2µl of primer (10 p mole/µl) and master max (Takara, Japan) and PCR water. The PCR reaction continued with denaturation for 5min. at 94 °C, followed by 35 cycles of 30 sec. denaturation at 94°C, 30 sec. annealing temperatures at 56°C, and 1min. extension at 72°C. The 35 thermal cycles were followed by a final extension of 5 min. at 72°C. Following amplification, PCR products were electrophoresed on a 0.9% agarose gel.

Bioinformatics

The current genes sequences were registered at DNA database under accession number. Sequences were compared with those available in the Gene Bank database using Blast. The Phylogenetic tree was constructed through two Bioinformatics Processes. In the first process, the nucleotide sequences of the recovered genes phenotypes and their homologs sequences, from the DNA database, were aligned. In the second process, the aligned sequences were submitted to the MEGA 7 software (http://www.megasoftware.net/) for drawing the phylogenetic tree. The Phylogenetic tree was constructed by applying the algorithms maximum likelihood in MEGA software.

Results and Discussion

The hybridization done to obtain F2 between

Cultivars of wheat (Triticum aestivum) we were choosing four wheat Cultivars Gemmeiza10, Misr1, Sakha93 and Giza168 to this study and were done the hybridization between

1. Misr1 and Sakha93
2. Giza168 and Gemmeiza10

The results of this study suggest that wheat genotype Sakha 93 can be selected to grow under salinity levels of irrigation water **(Maha *et al.*, 2017).** F1 hybridization product was field-grown to obtain F2 for RNA extraction to be identified genetically.

PCR Amplification using Na+/H+ antiporter and DREB2 Primer

RNA samples were prepared with a plant RNA purification kit (Qiagen, Germany). The extracted RNA was used for library construction cDNA. The product of the cDNA synthesis used in PCR. The cDNA was amplified by PCR using the Na+/H+ antiporter and DREB2 Primers were used. Na+/H+ antiporter gene sequencing was made for Tolerance of salinity hybridization and DREB2 gene was made for Tolerance of Drought hybridization.

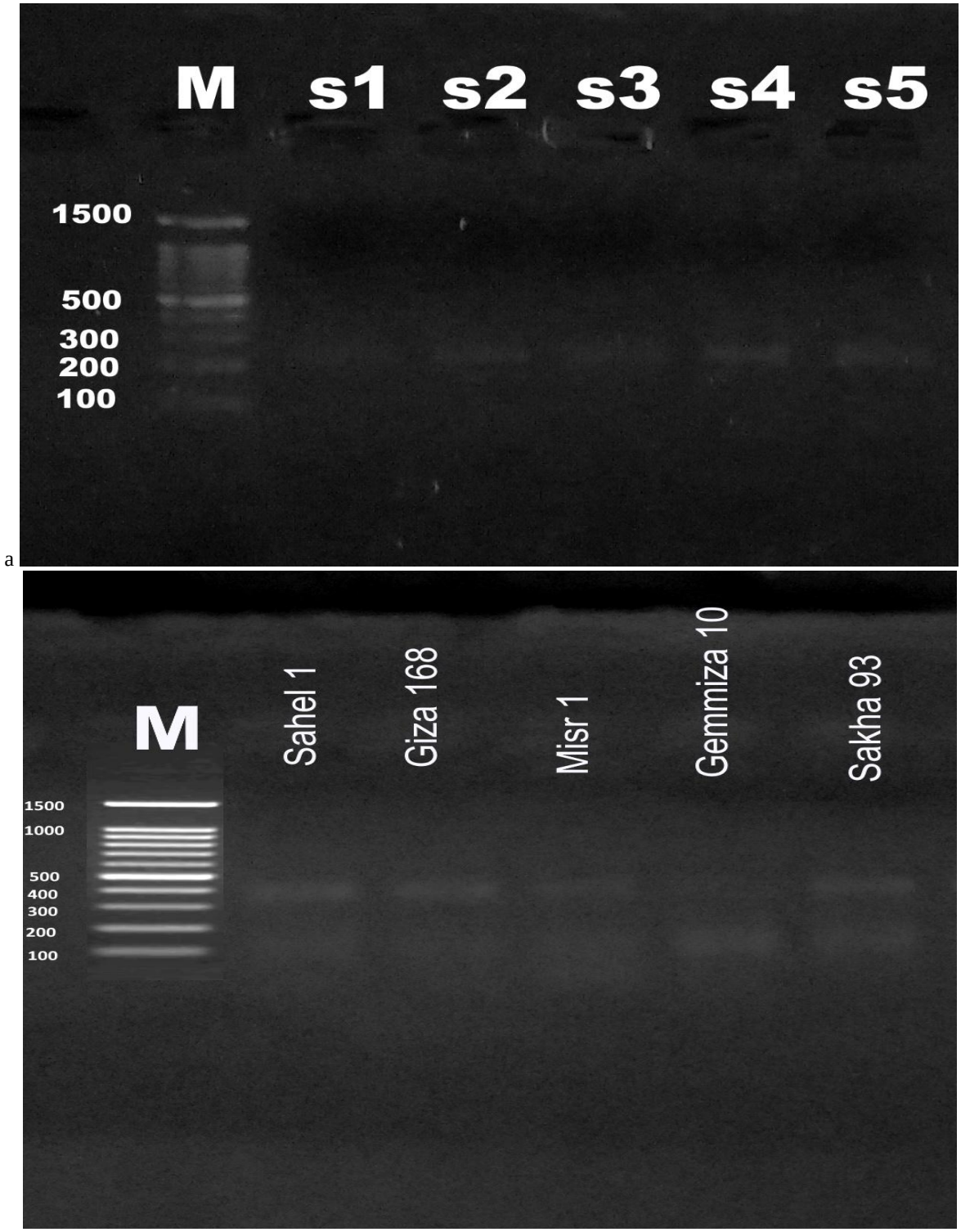

Figure. 1 a PCR product for DREB2 gene. b PCR product for Na+/H+ antiporter gene

RNA Sequencing and Phylogenetic tree for Na+/H+ antiporter gene sequences

Na+/H+ antiporter gene sequences of the isolate the base size of Na+/H+ antiporter gene was 400 bp. The DNA sequences of this isolate showed 100% identity to Na+/H+ antiporter gene. After sequencing the current Na+/H+ antiporter gene sequences was registered at DNA database under accession number MZ645750. The result obtained was compared in the gene bank (NCBI). The Sequences of accession number MZ645750 and four accession numbers from gene bank were aligned and phylogeny tree was submitted to the MEGA 7 software Analyses of Phylogenetic for the Na+/H+ antiporter sequences observed the relationship between The Sequences of accession number MZ645750 and four accession

numbers from (NCBI). The result showed that the present specimens were grouped in Phylum Na+/H+ antiporter gene (Fig. 1). The phylogenetic tree deducted from the sequence comparison of Na+/H+ antiporter gene region showed that the length of branch that represents an amount genetic change of 0.0020, and the Na+/H+ antiporter gene -based phylogeny tree included four clades. The clades grouping had low support (bootstrap value between 6-8%) and showed that the present specimen was placed in Na+/H+ antiporter gene ID: MZ645750 number(1)in phylogenetic tree neighbor to numbers (2)in phylogenetic tree And showed number(3,4,5)in phylogenetic tree distant on number(1)in phylogenetic tree.

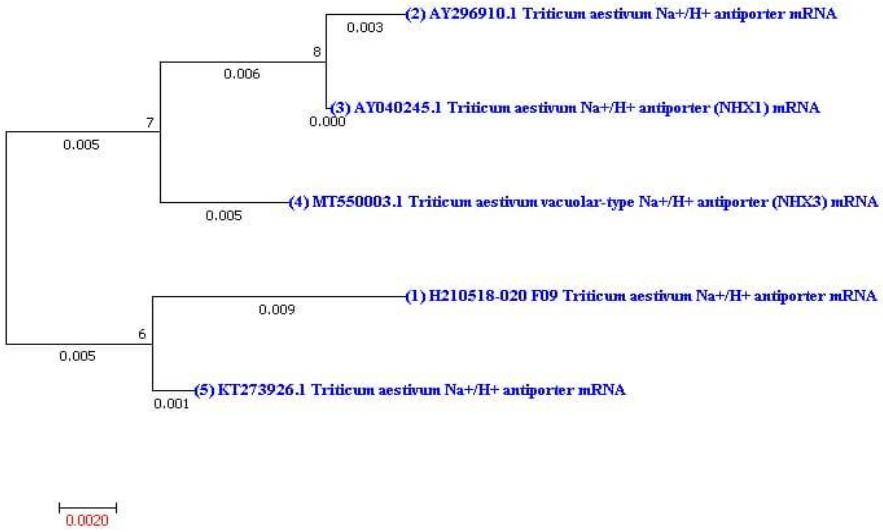

Figure.1 Relationship between the Sequences of accession number MZ645750 and four Accession numbers from gene bank. The phylogenetic tree was constructed by applying the algorithms maximum likelihood

RNA Sequencing and Phylogenetic tree for DREB2gene sequences

DREB2gene sequences of the isolate the base size of DREB2gene was 200 bp. The DNA sequences of this isolate showed 100% identity to DREB2gene.After sequencing the current DREB2gene sequences was registered at DNA database under accession number MZ645751. The result obtained was compared in the gene bank (NCBI). The Sequences of accession number MZ645751 and six accession numbers from gene bank were aligned and phylogeny tree was submitted to the MEGA 7 software Analyses of Phylogenetic for the DREB2gene sequences observed the relationship between The Sequences of accession number MZ645751and six accession numbers from (NCBI) .The result showed that the present specimens were grouped in Phylum DREB2gene (Fig. 2). The phylogenetic tree deducted from the sequence comparison of DREB2gene region showed that the length of branch that represents an amount genetic change of 0.0020, and the DREB2gene -based phylogeny tree included four clades. The clades grouping had low support (bootstrap value between 8-12%) and showed that the present specimen was placed in DREB2gene ID: MZ645751number (1) in phylogenetic tree neighbor to the other accession number was compared in phylogenetic tree.

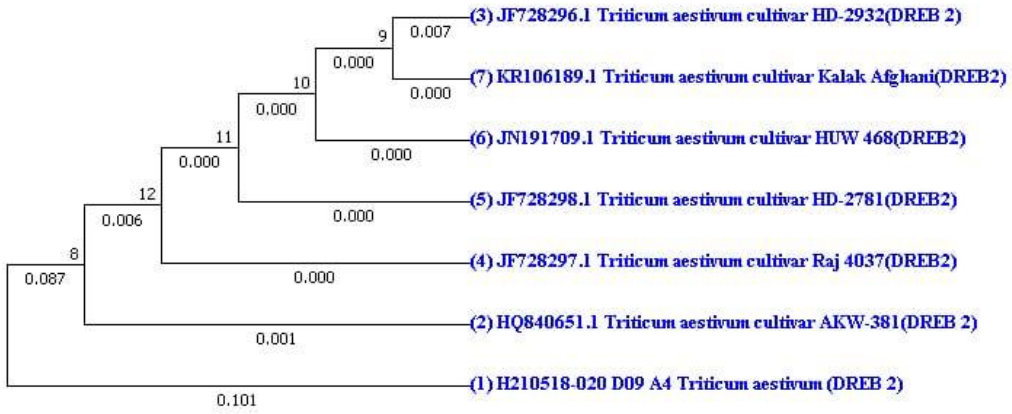

Figure.2 Relationship between The Sequences of accession number MZ645751 and six Accession numbers from gene bank. The phylogenetic tree was constructed by applying the algorithms maximum likelihood

References

Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003). *OsDREB* genes in rice, *Oryza sativa L.*, encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J. 33: 751-763.

Hoagland, D.R.; Snyder, W.C. (1933). Nutrition of strawberry plant under controlled conditions. (a) Effects of deficiencies of boron and certain other elements, (b) susceptibility to injury from sodium salts. Proceedings of the American Society for Horticultural Science. 30: 288–294.

Ingram, J. and D. Bartels, (1996). "The molecular basis of dehydration tolerance in plants," *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 47, no. 1, pp. 377–403.

Maha, A. Gadallah, Sanaa, I. Milad, Mabrook, Y. M., Amira, Y. Abo Yossef and Gouda M. A (2017). Evaluation of Some Egyptian Bread Wheat (Triticum aestivum) Cultivars under Salinity Stress. ALEXANDRIA SCIENCE EXCHANGE JOURNAL, VOL.38, No. 2. APRIL-JUNE 2017.

Nakano T, Suzuki K, Fujimura T, Shinshi H (2006). Genome-wide analysis of the ERF gene family in Arabidopsis and rice. Plant Physiol 140: 411–432.

Padan, E., T. Krulwich, in: G. Storz, R. Hengge-Aronis. (2000). (Eds.), Bacterial Stress Responses, ASM Press, Washington, DC, pp. 117-130.

Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi- Shinozaki K (2002). DNA-binding specificity of the ERF/AP2 domain of *Arabidopsis* DREBs, transcription factors involved in dehydration and cold-inducible gene expression. Biochem. Biophys. Res. Commun. 290: 998-1009.

Stalker, H. T., (1980). Utilization of wild species for crop improvement. Adv. Agron. 33: 11 l-147.

Stockinger EJ, Gilmour SJ, Thomashow MF (1997). *Arabidopsis thaliana* CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc. Natl. Acad. Sci. USA, 94: 1035-1040.

Tian XH, Li XP, Zhou HL, Zhang JS, Gong ZZ, Chen SY (2005). *OsDREB4* genes in rice encode AP2-containing proteins that bind specifically to the dehydration- responsive element. J. Integr. Plant Biol. 47: 467-476.

Tuna L., C. Kaya, D. Higgs, B.Murillo-Amador, S.Aydemir & A. R.Girgin. (2008). Resistance gene stacking in wheat breeding programs and further genetic analysis. Silicon improves salinity tolerance in wheat plants. Environ. Exp. Bot., 62:10–16.

West, L.C. and P. Mitchell, (1974). Biochem. J. 144 87-90.

XP, L. I., Tian AG, Luo GZ, Gong ZZ, Zhang JS, Chen SY (2005). Soybean DRE-binding transcription factors that is responsive to abiotic stresses. Theor Appl Genet 110: 1355–1362.