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IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES BY CDNA-AFLP TECHNIQUE IN RESPONSE TO SALINITY STRESS IN BREAD WHEAT

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Abstract

Wheat may be a quality cereal crop on the world, notably in an Asian country, with facile money price is sensitive to salt stress, which is a tangible associate example of dissent consequence on crop yields. The direct of this work was to review three wheat genotypes differing in their come to salinity stress and institution of genetic mechanisms entangled reciprocally to salt stress in grain filling stage for the first time at the molecular levels under mRNA populations by cDNA-AFLP technique. The institution of those genes within the future can offer an appropriate basis for the assembly of changed and saline tolerant cultivars. In our scrutiny, we tend to pick out many salt-stress-responsive transcripts derived fragments (TDF) that were seasoned by RT-PCR. Most of the transcripts single out here, utilizing the essential native alignment search tool (BLAST) output, were genes be a part of distinct occupation classes delineate to destructive metabolism, potency, cellular biogenesis, cell definition, a signal for specific sensors, organic phenomenon regulation, supermolecule debasement, and transfer. The impact demonstrates that cDNA-AFLP could be a constant technique the procedure of salt stress tolerance genes in *Triticum aestivum* would be very fruitful for the breeding and biotechnology of salt tolerance heterogeneity in alternative Poaceae families additionally as barley and rice.

Keywords: Wheat, Salinity Saline stress responses, *Triticum aestivum*

Introduction

Cereal crops such as wheat (*Triticum aestivum* L.) are among the most significant portions of providing daily food for humans. Because of the large harvested area (38.8%)was taken by wheat, compared to rice or maize (2-3%), and which is better cereal for preference to upgrading for salt stress (Simane and Struik, 1993).

Globally, the sphere will reach 9 billion by 2050, while agricultural intensify by only 1.8% (Food and agriculture organization of the united nations, 2018). At present, providing high-quality, necessary food to the people of the world is that the most acute crisis that people generally unit lining, and to rearrange for even cope with this crisis, the importance of food production is increasing day by day in extra property and adequate ways (Belaid *et al.*, 2005). The shooting up and function of crops are conditional on genetic, environmental, and interactions. Manifold climatic factors (rainfall, temperature, humidity, light, and wind), non-climate (food, gas, pests, disease, and weed competition), crop management, and agricultural inputs to shrink or intensify shooting up and plant growth are entangled (Allakhverdiev *et al.*, 2002). These stresses are commonly divided into two categories—biotic and abiotic stresses—depending on the nature of the trigger factor. Biotic stress is outlining as stress caused by other living organisms, as well as insects, bacteria, fungi, and weeds that affect plant development and productivity-carbonate. The second is generally linked with the climatic, edaphic, and physiographic components of the environment when they are limiting factors of plant growth and survival. Abiotic stresses, like salinity, drought, temperature, and extremes dangerous metals, are significant factors limiting crop productivity and property worldwide. Among these, salinity and nutrient deficiencies are significant problems, mostly in developing countries where the incomes of rural people depend on agriculture (Allakhverdiev *et al.*, 2002). In arid and semi-arid regions, water scarcity is a secondary factor in decreasing plant shooting up and grain yield. The first fruit of salinity on plant shooting up are the lack of uniformity in germination and the emergence of seeds so that the soil surface remains bare and without bushes. Salinity stress also causes perspiration, and photosynthesis through the osmotic mechanism of the fruit of ion toxicity also relates to the absorption of toxic ions and the alteration of physiological processes caused by toxicity, deficiency, or alteration in the mineral balance (Miranzadeh *et al.*, 2011). The return of plants to abiotic stresses such as salinity has been an essential topic in physiological studies (Sharafi *et al.*, 2019; Aslmarz *et al.*, 2019; Abdulkhaleq *et al.*, 2019), and now these studies have become more focused on molecular and reverse genetics (Orczyk *et al.*, 2017). Identifying new genes and determining their expression patterns in return to a variety of stresses and provide fruit strategies for plant breeding in Developed stress tolerance (Orczyk *et al.*, 2017).

The activity of all organisms is regulated by activating and deactivating gene expression (Fukumura *et al.*, 2003). Gene expression also related to the number of mRNA copies in a tissue that is directly related to the amount of gene expression (Fukumura *et al.*, 2003).

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Different molecular ways are introduced to research the differential expression of genes that may be named, serial analysis of gene expression (sage) ،cDNA microarray ، reverse transcription-polymerase chain reaction (RT-PCR),

suppression subtractive hybridization (SSH) ‹cDNA amplified fragment length polymorphism (cDNA-AFLP). Each of those methods has advantages and disadvantages, and, based on the aim, the number of necessary data regarding the gene sequences offered and, therefore, the sequences in question square measure used furthermore because of the financial potentialities (Morozova and Marra, 2008).

Some methods, like microarray, despite their high potency in evaluating the expression of thousands of genes in an exceedingly single trial, ar related to the necessity for necessary info regarding the sequence of obtainable target genes and also the price and inability to discover low concentrations of transcripts. Some organisms and plenty of analysis laboratories, particularly in countries with low economic power, do not seem to be applicable (Kivioja *et al.*, 2005). In addition to methods such as RT-PCR, although it has the speed and sensitivity to ferret out gene expression changes. The cDNA-AFLP method is one of the most fruit methods for transcriptome breakdown in the establishment of transcripts responsive to stress (Bachem *et al.*, 1996). In this way, it is feasible to quickly identify the transcripts entangled in stress tolerance without having to know the target sequence available (Lee *et al.*, 2004). Az well, due to the high sensitivity of the cDNA-AFLP method, genes whose transcript sizes are too small can also be investigated (Lee *et al.*, 2004).

Materials and Methods

Plant material and salinity treatment

Three contrastive genotypes of spring Iranian bread wheat include Aflac, Sirvan, Arg, with three levels of salinity (Control, 14 dS/m Cl and 21 dS/m Cl) in factorial experiment in main plots subsamples were conducted in completely randomized design with three replication in an exceedingly vase experiment under natural conditions at Ramin Agricultural University. In each pot, ten seeds were sown on based on seed efficiency, and half a dozen of them were maintained when emergence. Irrigation was accomplished up to the dewatering stage with potable water and through the growing season with saline water. Operations were accomplished, including fertilization, weeding, and irrigation regularly. Due to the interaction and intensifying fruit s of contrastive environmental stresses, the project tried to avoid any biological or abnormal stresses to plants before and after the growing season. At the milky stage, green leaves of the genotypes were harvested for sampling and used in later stages of the study.

RNA preparation and cDNA synthesis

Total RNA was extracted from fresh leaves victimization (RNX-Plus Solution) by the manufacturer's instructions. The RNA extracted was treated at 37°C with a QIAGEN DNase kit to avoid possible DNA contamination. The concentration of RNA was determined by spectrophotometry victimization NanoDrop 8000 spectrophotometer (Thermo Scientific). The RNA integrity was determined by running 2µL of total RNA in a formamide denaturing gel. For cDNA synthesis, 20µL of total RNA were used initially for the first-strand synthesis with QIAGEN synthesis cDNA kit, followed by second-strand synthesis victimization Vivantis double-strand cDNA synthesis kit following the manufacturer's instructions.

cDNA-AFLP reaction

cDNA-AFLP was carried out victimization the About 20µL of double-strand cDNA were digested by EcoR I and Tru9 I adapters. The preselective amplification mixture was prepared by adding 2µL of 10-fold diluted DNA from the restriction-ligation reaction, 3µL of AFLP preservative primer pairs, and 15 of AFLP Amplification Core mix. The preselective amplification was carried out in a Veriti™ thermal cycler Techne (TC-512 Gradient) programmed 94°C for 30 s, followed by 25 cycles at 56°C for 1 min, 56°C for 30s, and 72°C for 1 min, with an incubation step at 72C for 5 min.

Selective amplification was accomplished victimization a touchdown program in a Veriti™ thermal cycler Techne (TC-512 Gradient) programmed at 94 °C for 45 s, followed by 36 cycles at 65 °C for 30 s, 72 °C for 60 s, and 13 cycles at 94 °C for 30 s, 65 °C for 30 s, (–0.7 °C per cycle) 72 °C for 1 min and finally 94 °C for 30 s followed by 13 cycles, 56 °C for 30 s, 72 °C for 60 s, with a subsequent hold for 10 min at 72 °C. For high-throughput analysis of contrastively expressed fragments, the PCR products of the selective amplification were separated on a 6 % polyacrylamide gel.

Isolation, reamplification and sequencing of transcript-derived fragments

The polymorphic transcript-derived fragments (TDFs) based on their presence, absence, or contrastive intensity were cut from the gel, with maximum care to avoid any contaminating fragment(s). DNA was purified victimization Vivantis gel extraction kit according to the manufacturer's instructions. Extracted target bands were used as a template for reamplification victimization the same primers and program for selective amplification.

Sequence analysis

The outcome sequences were analyzed for homologs victimization BLAST Network Service of National Center for Biotechnology Information (NCBI, Bethesda, MD, USA). Each TDF sequence was compared against all sequences in the non-redundant databases victimization the BLASTX program (Sayari *et al.*, 2005), which compares translated nucleotide sequences with protein sequences.

Real-time PCR analysis

After sequence and analysis of the obtained fragments, specific primers were designed for a single out gene. Primer Quest Tool (IDT software) was accustomed to style this primer, and primers designed victimization the oligo analysis tool for constant software packages were tested for dimmer primer, binding temperature, GC content, and at last, appropriate primers to perform. Real-time polymerase chain reaction at the start primers were tested in a very PCR reaction with four temperatures gradient, and also, the best temperature was elect for a real-time polymerase chain reaction.

Real-time PCR reaction victimization ABIs 1st step plus real-time PCR system and amplicon Denmark x PCR master mix kit by following the manufacturer's instructions. Applied math analyses were accomplished, victimization the t-test to match mean values.

Uncovering of differentially expressed transcripts

To isolate differentially expressed transcripts, we accomplish cDNA-AFLP analysis on total RNA samples from leaves grown under normal and salinity stress conditions. cDNA-AFLP analysis can reveal adjust the expression of any gene as long as that it takes the restriction sites that have been nominated for analysis. Selective amplification with eight primer combinations showed a total of 274 TDFs, 64 of which were contrastively expressed. Of the 274 TDFs, 13 were up-regulated and four down-regulated. A total of 64 contrastively expressed. TDFs move in size from 300 to 600 bp were excised from the gel, reamplified and refined for direct sequencing, that yielded twenty-four cDNA fragments that gave rise to useable sequence information. Sequencing of many cDNAs falling, in all probability because of a combination of the PCR merchandise and these fragments, were not further additional analyzed.

Functional classifications of contrastively expressed TDFs

After sequencing sixty-four chosen TDFs, reliable sequences were made by twenty-four of them. Every sequence was single out by similarity search mistreatment the essential native alignment search tool (BLASN) program against the Gen Bank non-redundant (nr) public sequence information (NCBI). Sequences were classified into functional teams that supported their similarity with notable proteins.

The sequence comparison of the 64 TDFs against the nr database revealed that 62 % (18 TDFs) of them had homology with genes with known functions, whereas for 23.7 % (2 TDFs) there were not hits and 14.5 % (4 TDFs) had homology with proteins with unknown function (Table 1). The TDFs with the known or putative function was sub-tied to the NCBI database and are presented in Table 1 with Gen Bank Accession numbers. The up- and down-regulated genes are also categorized into these functional groups (Table 1). Fig. 1 shows the percentages of durum wheat genes assigned to contrastive functional categories. Approximately 15.26 % of TDFs are entangled in gene expression regulation and a further 8.94 % in signal for specific sensors. Other relevant groups of contrastively expressed TDFs include catabolism (12.26 %), potency catabolism (13.15 %), hypothetical proteins (9.21 %), protein debasement (4.82 %), cellular biogenesis (19.21 %), and cellular biogenesis (9.26 %), (17.1%) general metabolism.

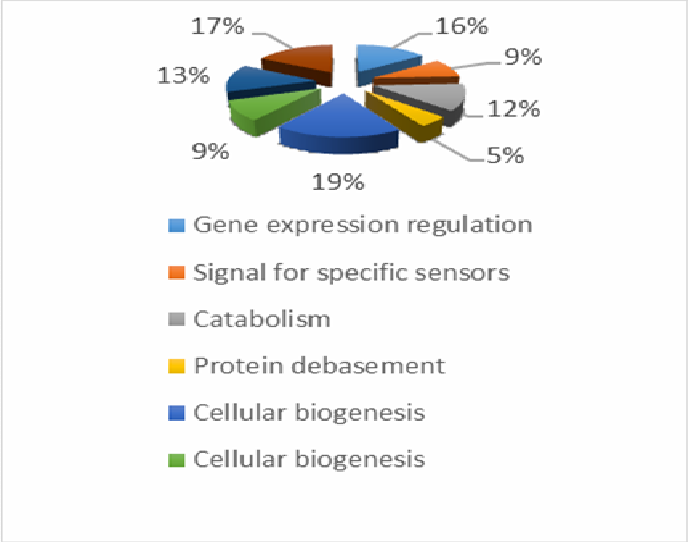

Fig. 1 : Distribution of differentially expressed genes under salt stress In *Triticum aestivum*

Validation of expression patterns by qRT-PCR analysis

To validate the reliability of the cDNA-AFLP for ferret out on of contrastively expressed genes and verification of the expression patterns observed in the cDNA-AFLP analysis, qRT-PCR was accomplish for ten TDFs belonging to contrastive functional categories, 13 up-regulated (TDFs 24, 22, 19, 18, 15, 14, 12, 11, 10, 9, 6, 4, 3, and 11) and 3 down-regulated (TDFs 23, 21, and 20) transcripts. These selected TDFs were studied during salinity stress conditions.

Quantitative outcomes of this method were compared wit Tubulin internal control gene according to data obtained from real-time and analysis by Rost software.

Results and Discussions

In this study, several salt-induced transcripts with eight primer combinations were single out by victimization the cDNA-AFLP technique. Databank search revealed similarities with wound, salt, and other biotic and abiotic stress responsive transcripts (Table 1). Most of the cDNA analyzed are homolog to EST isolated from other wheat cultivars submitted to biotic or abiotic ones. These data confirm that plants often use correlated ways in return to various environmental stresses including biotic and abiotic ones (Yen *et al.*, 2001). The roles of calcium, activated oxygen, ABA (abscissic acid), and ethylene in communicating the signaling network have been suggested in both kinds of stresses (Piao *et al.*, 1999; Bowler and Fluhr, 2000).

Table 1: Similarity of transcript-derived fragments (TDFs) expressed during the salt stress given to Three genotypes of bread wheat with known gene sequences

TDFs	Sequence similarity (number of TDFs)	Accession number	BLASTN score	Expression (14 dS/m Cl)	Expression (21 dS/m Cl)
TDF 2	Vitromax dehydration responsive factor 1 variant 1.2 type b (DRF1) mRNA, complete cds, alternatively spliced	FJ560500	1e-50	up	down
TDF 3	mRNA for predicted protein, complete cds, clone: NIASHv2009F03	AK362675	6e-22	up	up
TDF 4	strain DSM 15391, complete genome	CP009428	3e-16	up	up
TDF 6	Group 18S ribosomal RNA (LOC112936516), rRNA	XR_003238821	2e-17	down	up

TDF 7	chromosome 3B-specific BAC library, contig ctg1035b	FN564436	2e-12	up	up
TDF 8	mRNA for hypothetical protein, clone: RAFL09-30-H08	AK226957	6e-33	up	down
TDF 9	genome assembly, chromosome: 3	LR215054	3e-16	up	up
TDF 10	genome assembly, chromosome: 2	LR215053	2e-07	up	up
TDF 11	kinase with tetratricopeptide repeat domain-containing protein (BSK8), mRNA	NM_123491	2e-08	up	down
TDF 12	plasma membrane-associated cation-binding protein 1 (LOC106436799), mRNA	XM_013877747	4e-40	down	up
TDF 17	sulfatepermease 1 (sfp1), transcript variant X5, mRNA	XM_008650050	2e-33	up	up
TDF 18	spontaneumcalcineurin B-like protein 3 (CBL3) gene, complete cds	HQ696012	5e-09	up	up
TDF 19	spontaneum high-affinity K+ transporter 4 gene, complete sequence	HQ696002	4e-21	up	down
TDF 20	partial mRNA for td2IL1 protein	FR820609	5e-33	down	down
TDF 21	Dehydrin mRNA, clone pTd27e	AM180929	7e-21	down	down
TDF 22	Triticum aestivum NAC transcription factor NTL5 (NTL5) mRNA, complete cds	HM027574	2e-17	down	up
TDF 23	Zea mays chromosome 4 PCR sequence AGI.1227 genomic sequence	GQ845075	3e-11	down	down
TDF 24	Arabidopsis thaliana chromosome 2 sequence	CP002685	5e-09	up	down

Up=up-regulated, Down=down-regulated

The contrastive salt-induced cDNA fragments single out here belong to five contrastive classes:

cDNA fragments related to salt and drought stress-responsive sequences. Three cDNA fragments are closely related to EST induced by salt stress in *Triticum turgidum*, such as the TdDRF1 gene and Arabidopsis thaliana, such as TaCHP, TaSnRK2, and TaHKT in *Triticum aestivum*. On the other hand, the fragment, which seems to be expressed in return to a variety of abiotic stresses (salt, drought, temperature), may encode HKT transfers (High-affinity K^+ Transferer), which mediate Na^+- specific transfer or Na^+-K^+ co-transfer (Chandima *et al.*, 2014). Salt tolerance of plants could depend on HKT, which mediates Na+-specific transfer or Na+-K+ transfer and play a key role in the regulation of Na^+ homeostasis (Hazzouri *et al.*, 2018). It is feasible that the variation in copy number may heartstrings salt tolerance of wheat, and that allelic variation in gene sequence may also help heartstrings salt tolerance (Chandima *et al.*, 2014).

The databank search also suggests that the cDNA fragment encodes an NADP glyceraldehydes dehydrogenase. This enzyme, entangled in membrane structure, shares common domains with several other dehydrogenases. It also has common domains with the δ1pyrroline-5-carboxylate synthetase enzyme (P5CS) responsible for proline synthesis during salt and drought return in plants (Huang *et al.*, 2008; Chandima *et al.*, 2014). Moreover, transgenic potato plants expressing glyceraldehydes phosphate dehydrogenase showed improved tolerance to salt stress (Sayari *et al.*, 2005).

Various environmental stress factors together with drought, high salinity, and heat, etc. adversely have an effect on wheat production in an exceedingly vital manner (Lopes *et al.*, 2014). Vitromax dehydration responsive factor 1 fragments are closely related to EST induced by salt stress in *Triticum turgidum* subsp. durum. Dehydration-responsive element-binding (DREB1A) factors, a category of transcription factors (TF) play a vital role in combating drought stress (Kumer *et al.*, 2016). it is renowned that DREB1A specifically interacts with the dehydration responsive parts (DRE/CRT), causing expression of genes concerned in environmental stress tolerance in plants (Nakashima *et al.*, 2000). Despite its essential to the interaction in plants, the structural and sensible aspects of DREB1A TF in wheat keep unanswered. Prior research appears that wheat DREBs (DREB1 and DREB2) were isolated using numerous ways as well as yeast two-hybrid screens however no in-depth structural models were reported (Nakashima *et al.*, 2000).

Conclusion

Contrastive cDNA fragments induced by salt stress in wheat leaves. Most of them are related to EST entangled in several abiotic and biotic stresses and a number of them showed homologies with known proteins entangled in contrastive stress return. These data suggest that these genes may have a role in salt stress definition in wheat plants. Our outcomes corroborate previous data suggesting that stress due to changes in osmotic potential may occur during a variety of applied stresses such as low temperature, pathogen infection, drought, and salinity (Lsayenkov *et al.*, 2019). This is owing to the storage of large amounts of osmotically active substances in storage tissues (Isayenkov, 2012). It was also suggested that some genes associated with the return to pathogens (including chitinase) are entangled in normal developmental processes in healthy tobacco plants (Kumar *et al.*, 2015). The genes induced here by salinity can be further

investigated to understand the regulation of their expression. Their overexpression in new varieties may improve salt stress tolerance in potato or other plants. However, because return of plants to abiotic stresses is polygenic, it involves a cascade of cellular changes necessary to render the plants stress tolerant. These single out genes may be conjointly used as probes to assess salinity or osmotic stress reaction in contrastive potato genotypes. They'll facilitate in understanding osmotic stress tolerance in solanaceous species. Any characterization of stress come back in plants could also be envisaged victimization these genes.

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