



THE PATTERN OF GENE EXPRESSION UNDER WATER STRESS USING CDNA-AFLP IN WHEAT (TRITICUM AESTIVUM L.)

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ABSTRACT: Drought is a major environmental factor that limits the productivity of crop and other products, around the world. Plants have developed various strategies to deal with this tension that one way is to change the pattern of gene expression, due to the activation of specific genes stress and generally rewriting the genetic activity in the cells. Methods cDNA-AFLD, is a powerful tool for the analysis of gene expression related to environmental stress that was used for analysis of differences between genes in wheat Tabassi (drought tolerant) and Taifun (drought-sensitive genotype), and drought stress condition matric potential of - 10. Results showed that more than 61% of the genes examined in both cultivars, were not affected by drought, over 15% of genes in both cultivars and Taifun Tabassi under drought stress showed a change in the number and intensity of expression, and less than 12% of genes, in particular, only one digit or Taifun Tabassi had a change of expression. In total, 1,268 pieces of cDNA-AFLP, and drought stress condition showed differences. In addition, some genes showed increased or decreased expression in response to drought.

Keywords: Drought, wheat, cDNA-AFLP, gene expression.

INTRODUCTION

Defining the problem

World's population at the beginning of the twenty-first century, are over 6 billion people, and the lack of food and starvation, has been plagued by more than 800 million people And over three billion people suffer from malnutrition. With the increasing world population, projected to be the world's population will reach 8 billion people on the border, 2020. Thus, the large number of population is the nutrient most important things politically, economically and socially, especially in developing countries. For these reasons, it is important, sources of genetic capacity of variety, and agricultural scientists and researchers are looking for new and effective ways to increase food quantity and quality. Among the crops, wheat is one of the most important sources of energy and protein supply for the world population. Wheat has the highest share of the country's total cultivated land and also, is important, as the main food of the people of our country, and the most important source of calories [14]. Wheat is among the oldest cultivated plants known by man, and probably has been the starting center for agriculture. Primary areas of domestication and cultivation of wheat has been reported in Mesopotamia, Iran, Syria and the West [19]. Wheat has the jet roots, the roots of the plants are expanded, the lateral and depth. Wheat varieties in terms of depth and spread their roots in the soil are different together so that, drought tolerant, has a denser and more extensive root system, than drought-sensitive varieties. Wheat stalks are Mashvarh and simple, and is mostly empty, and some figures are rounded stems, which are related to the effects of genes [23].

Dark wheat grains contain many species it is the most important crop species and bread wheat *Triticum aestivum* is called. Bread wheat (*Triticum aestivum*, L.) is undoubtedly among the few plants that are grown widely as a food source, plays a major role in agriculture is probably the beginning of the axis. Between wheat crops cultivation in the world's largest and account for roughly one-sixth of the world's total arable land is cultivated. In semi-arid climatic conditions vary, the products that they can be grown without irrigation is very limited in number. Due to wheat starch, protein and good baking properties of other grains are preferred. Although other grain bread can be made, but there is no grain wheat bread for human nutrition that be able to compete [23]. Product variety and quality of stored wheat, the staple food for more than a third of the world population has [2]. Wheat is one of the largest and most complex genome is among the crops. This plant genetic mixing between the three diploid species, there is a relative. Alohegzaploid wheat plant genomes and related AABBDD chromosome $2n = 6x = 42$ is shown. Figure 1 shows the evolution of bread wheat [9].

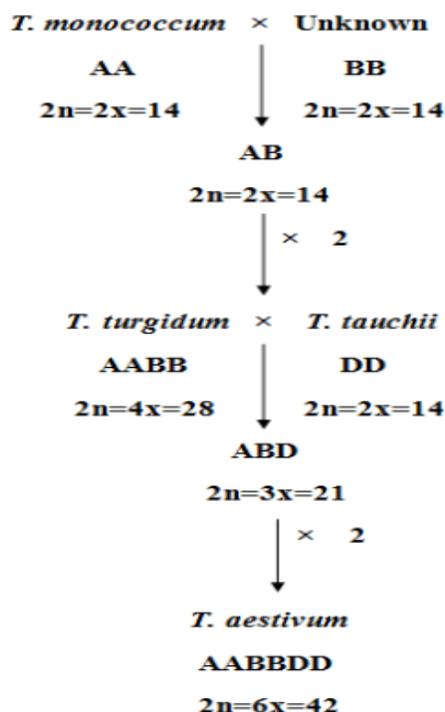


Figure 1. Evolution of Bread Wheat

Most of the wheat producing regions are facing water shortages during the growing season. And one type of land (continuous, terminal and temporary) occurs during the growing season, it's important to deal with stress in these areas to show good [16]. Given that land is a defining feature of the geography of Iran and unchangeable natural phenomenon, there is no escape, so we have to deal with it. Water stress is the most significant environmental stress in agriculture worldwide and improving yield under drought is a major goal of plant breeding [20]. In agriculture, drought tolerance, ability to produce a crop economy with minimal reduction in stress conditions relative to non-stress conditions is considered [11]. To produce improved genotypes and drought tolerance is necessary to have an understanding of the genetic basis of drought tolerance. Drought tolerance is a complex trait that depends on the cause of action and reaction between the different morphological, physiological, biochemical characteristics [21]. Finding genes that control mechanisms and ways to achieve it paves tolerant varieties. Among the environmental stresses, drought is one of the most important obstacles to the development of agriculture. The tension in many areas outside of cultivation or production has led to a decline in performance [30].

Drought stress occurs when the rate of evaporation and transpiration is greater than the rate of water absorption. In terms of terminology, drought is an extended period of time in which to plant available water than usual needs of the region. Drought, from an agricultural perspective, is the lack of adequate soil moisture for plant needs at a specific time [22]. From a practical point of view, it is more drought tolerant plant with the capacity to live in conditions with water restrictions related to the final product depends on the plant [24]. Plants grown greatly affected by environmental stresses such as water deficit, salinity, and temperature are high. Molecular and biochemical responses of plants to non-live stress tolerance, growth, and morphological characteristics of plant stress occurred in the period in which it is dependent [17]. Differences in gene expression in all stages of growth and influence of environmental conditions occur. Lack of water in seed development and function depends on its tolerance and plant growth stage where the stress occurs [5]. Identification of new genes and their expression pattern in response to various stresses, will be to obtain a better understanding of their function in plants adapt to a variety of stresses and develops strategies to improve stress tolerance in plants improvement [8].

Recently, a number of other genes in response to drought stress in different plant species have been cloned and characterized [18]. The first goal in the development of transgenic plants such as tobacco, single genes work and are responsible for changing a single metabolite that can cause tolerance to drought or salinity (water) are. Self-induced stress proteins with specific functions such as key enzymes in the biosynthesis of proteins and water thoroughly Asmvlayt, causing venom enzymes and transport proteins are first targeted transgenic plants. Transmission capacities under different types of genes lead to increasing induced by non-live stress tolerance in transgenic plants [4, 25, 29].

Differences happen in gene expression in all stages of life, from birth to death. Identification of the gene expression, the knowledge and the understanding of the genetic and molecular mechanisms of a biological system will help us. Two decades ago, based on molecular techniques to study gene expression analysis of a gene such as Norton spotting techniques. But nowadays the techniques that can be used in different genes present in the test sample to detect.

One of these methods is the cDNA-AFLP technique. Although conventional methods such microarray standard tools for analyzing genome are expressed but its application is limited to sequence the entire genome of a species or a large collection of transcript sequences of the known are available in their [26]. cDNA-AFLP technique by Bechem [3], introduced a repeatable method suitable to detect transcripts with distinct presentation [15]. The main advantage of this method is no need for pre- genome sequence data [28].

Background and Research

Drought, salinity and temperature are the major abiotic stresses and plants respond to environmental stresses via the physiology and cellular and molecular processes that lead to the regulation of metabolic and structural changes are to respond. Figure 2 charts the stress tolerance of a plant adaptive response to abiotic stress tolerance of the show.

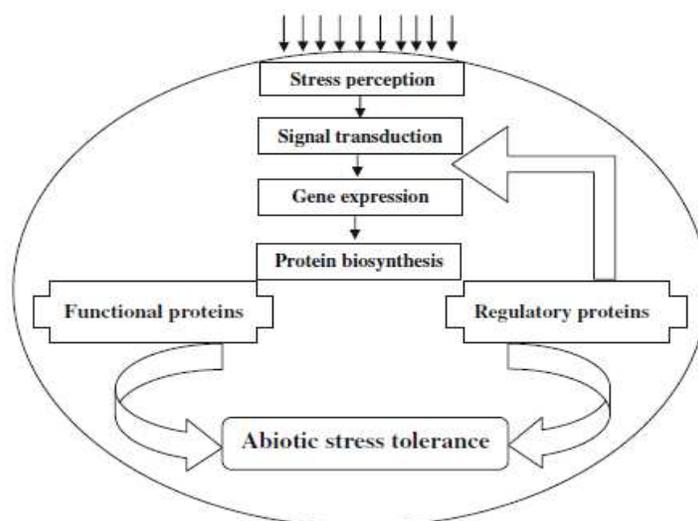


Figure 2. Diagram of the stress tolerance of a plant adaptive response to abiotic stress tolerance [1].

In recent years, having studied methods for gene expression, has attracted the attention of some researchers, to compare patterns of gene expression in plants of tolerant and sensitive to environmental stresses living or non-living, or between different environment. Gao et al [13] The effect of drought in rice cultivars using a cDNA-AFLP found that more than 90% of the genes expressed in both genotypes to drought stress were not effective, about 8% of stress genes in two genotypes were induced less than 1% have been expressed in the tolerant and sensitive is under drought stress.

Chen et al [7] studied the pattern of gene expression in response to salinity in wheat using cDNA-AFLP. The results showed that, 29/1 cent pieces in particular salinity tolerant cultivar and 61/1 of salt stress on susceptible parts specifically expressed and reported that the difference in the number of genes expressed suggests that tolerance to salt stress is associated with genetic predisposition figures.

Feng and Stewart [10], the expression pattern of 1626 genes under drought stress in two cotton varieties were evaluated using the same method.

Their results showed that only 29% of the genes were induced by water scarcity and drought have different effects on the expression of most genes is species.

Sequencing of 148 genes differentially expressed in response to drought stress in two species showed that 101 genes have similarity to other known genes.

These genes include genes, signal transduction and cell communication, metabolism, transcription factors and genes associated with drought.

Wang et al [27], using analysis of cDNA-AFLP, examined differences in gene expression pattern 54912 interaction of plants with the fungus *Puccinia striiformis*, stripe rust virulence factors and observed that after inoculation with 2/4 percent differences in gene expression pattern emerged. After sequencing, it was found that 40% of 186 pieces has a specific function and most of these genes are involved in pathways (4/5%), the response of defense (9/5 Percentage), the metabolism of (5%) and energy (13%).

Gnshan and colleagues [12], studied the effect of temperature-sensitive spring and autumn leaves and spikes of two wheat cultivars tolerant Using cDNA-AFLP technique and found that showed the leaf and the ear, May 16, and a 38 percent increase in the expression of genes 6 and 3 percent decrease in the expression of genes. campalans et al [6], using cDNA-AFLP differential gene expression under drought stress *Prunus amygdalus* examined. The results showed that the cloned high similarity to known sequences in the databases showed that it is possible encoding: transmitter amino acids/peptides, cysteine Prvtyytaz, fundamental expression of the proline-rich proteins, enzymes LPA-AT and If lmw HSP.

MATERIALS AND METHODS

In this study gene expression under drought stress in two wheat cultivars Tabassi, as Iranian varieties tolerant to drought and Taifun as European varieties were susceptible to drought. In the experiment for growing plants in pots, the composite sample of soil surface horizons (deep plowing) and mixing them complete control of the farm, the specific weight of soil sample was added to the pot. According to the University farm soil salinity control, soil samples were washed into the pot until the electrical conductivity of the water as it exits the electrical conductivity of the irrigation water. After reaching the optimum soil moisture pot, wheat seeds were planted in pots. Tested at two levels of matric potential drought 1 - load (stress) , 10 - once intense stress tests. For detailed exercise stress levels based on quantitative criteria , the volumetric soil moisture content by using the method of disc pressure tested the intake of 100 , 350 , 1000, 2000 , 3000 , 5000 , 7000 , 10000 , 12000 , 15000 cm as the soil moisture curve (Figure 3) were plotted. And calculate the amount of water needed for each pot and the pots were watered.

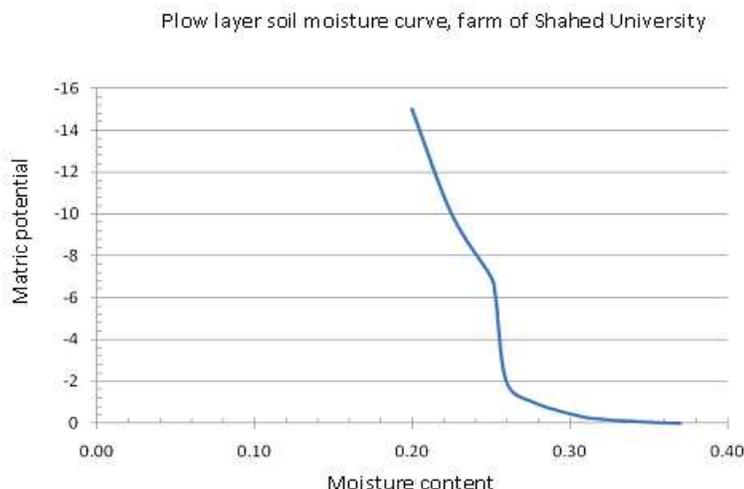


Figure 3. Plow layer soil moisture curve, farm of Shahed University

In order to check the identity of the nucleotide sequences obtained in this study from the Center for Biotechnology Information NCBI bioinformatics tools were used. Using BLAST software Hemoloso sequence of each TDF with other genes in the NCBI database revealed biotechnology. Potential yield components were identified and the studies of other researchers, potential genes involved in drought tolerance were identified. For analysis of genes associated with drought tolerance in wheat, different patterns of gene expression in two varieties under drought stress and non-stress Tabassi and Taifun were compared using cDNA-AFLP. 32 pairs of primer combinations were used for selective amplification in Table 1 are shown.

Table 1. Combinations of primers used in polymerase chain reactions

P_{NN}				E_{NN}				Starter	
P-CT	P-AT	P-GC	P-GA	E-AT	E-AG	E-GA	E-GC		
P18-M17	P14-M17	P20-M17	P19-M17	E14-M17	E13-M17	E19-M17	E20-M17	M-CG	M _{EN}
P18-M18	P14-M18	P20-M18	P19-M18	E14-M18	E13-M18	E19-M18	E20-M18	M-CT	
P18-M22	P14-M22	P20-M22	P19-M22	E14-M22	E13-M22	E19-M22	E20-M22	M-GT	
P18-M14	P14-M14	P20-M14	P19-M14	E14-M14	E13-M14	E19-M14	E20-M14	M-AT	

RESULTS AND DISCUSSION

In this study, different patterns of gene expression in two varieties Tabassi (drought tolerant) and Taifun (drought-sensitive) under stress and non-stress conditions was investigated using cDNA-AFLP. 32 pairs of primer combinations were used for selective amplification. Expression of the results showed that the number of spare parts TDF increased expression under drought conditions in most of the varieties Tabassi, Taifun figure is probably due to the greater sensitivity of this figure is higher than Tabassi is under stress.

The data obtained show that the number of genes expressed in the tolerant varieties under drought conditions compared to control conditions significantly reduced the number of genes expressed in the susceptible cultivar, showed a slight decrease. It may prove, in order to save energy and increase tolerance to drought tolerant varieties of reduced expressed genes. the difference in the number of expressed genes suggests that drought tolerance is associated with genetic predisposition figures. To review the relevant TDF drought tolerance, 15 pieces specifically Tabassi only cultivar were expressed under drought stress, were sent for sequencing. In order to study and evaluate the performance of similar nucleotide sequences obtained in this study from the Center for Biotechnology Information NCBI bioinformatics tools were used. Potential yield components were identified and the studies of other researchers, potential genes involved in drought tolerance were identified.

Table 2. Data from TDF homology search of the NCBI database

TDF	PLANT	ACCESSION	E-VALUE	FUNCTION
1	<i>Triticum aestivum</i>	AK330371.1	1e-176	kanadaptin-like
2	<i>Triticum aestivum</i>	AY568302.1	5e-134	WHPA8
4	<i>Brachypodium distachyon</i>	Xp-0035792o5.1	2e-04	PB1-Uncharacterized protein 2
5	<i>Hordeom vulgare</i>	AK372467.1	5e-57	Integral membrane protein DUF106
10	<i>Hordeom vulgare</i>	BAJ95658.1	8e-57	glutamyl-tRNA synthetase
13	<i>Triticum aestivum</i>	BT009064.1	8e-20	ING1
15	<i>Hordeom vulgare</i>	BAJ95084.1	1e-16	lon protease

According to Table (2) determined that some of TDF sequencing showed similarity with genes that are involved in the drought tolerance. And given that the TDF specifically expressed in the figure Tabassi under drought conditions, it can be concluded that the isolated parts of the figure is probably Tabassi under drought stress are shown a series of genes associated with drought tolerance in wheat. These results can be used in future studies using molecular markers for screening, identifying and cloning genes for drought tolerance and better understanding of the mechanisms of drought tolerance.

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