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ORIGINAL ARTICLE

Evaluation of Alteration of Stress Responsive protein in Bread Wheat land race under Terminal drought stress

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ABSTRACT
For evaluation and gene expression study of encoding stress responsive protein under terminal drought stress in tolerant and susceptible bread wheat, an experiment was undertaken in two parts based on factorial complete randomized-designed. Firs part of the experiment was done at greenhouse which leads to selection of two genotypes (susceptible and tolerant) in the second part; gene expression profile was evaluated by reverse Northern blot method. Specific primer was designed for wheat stress responsive protein gene. The results showed a significant increase in expression of Stress responsive protein gene in tolerant cultivar compared to the susceptible cultivar in drought stress condition. These genes can be categorized as gene in which induces resistance and drought tolerance in plants in stress condition.
Keywords: *drought, wheat bread, Northern reverse blot, protein stress response, Stress Responsive Protein*

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INTRODUCTION

The operation of the sciences, such as plant breeding, genetics, Agronomy, physiology makes significant progress for wheat in understanding and improving the response to the environmental stress. Although genomics techniques is for wheat, but a limited number of microarray profiling environmental stress have been carried out in different types of grains such as rice, barley, sorghum and maize. The results of these studies could significantly changes at the level of transcription (Including Dehydrin and antioxidants, Detox stimulators, transfer and other compatible method) and regulatory factors (Including transfer factor, kinase and hormones) the plant shows response to stress. Once you understand and detect changes within the plant cell Pathways messaging (signal transduction) various physical stresses in order to become a good biochemical response begins and each of them set a pure expression of stress response genes cause. All activities of these signals induced messaging, to adapt the plant and therefore it the tolerance [3]. Identify new genes and their expression pattern will in response to a variety of stresses to better understand their performance in adapting to a variety of stresses caused plants to be and effective ways to be created to improve stress tolerance in plants modified. To study the pattern of gene expression in mRNA level, there are many different ways. Generally can be divided into two categories PCR-based methods Real-time divided PCR-based methods such as hybridization of Such as Northern blot and reverse Northern blot (Large array or microarray) [1]. Nucleic acid and protein structures play an important role in protecting RNA, regulation of gene expression patterns in different levels of stress play.

MATERIALS AND METHODS

To select local genotypes were used of bread wheat in the grain of seed collections Seed and Plant Improvement Institute, Karaj. Genotypes in this study were introduced of 120 genotypes screening

trifoliate stage for drought tolerance as the most tolerant and the most susceptible genotypes. Drought stress was since the beginning of booting stage to later. From this point on, the normal field capacity moisture pots and vases choice has been set to stress the point between field capacity and permanent wilting point. After reaching the flowering stage sampling of leaf 2 was susceptible and tolerant. RNA was extracted using RNA X Plus kit was performed Sina gene. By cDNA synthesis kit was Revert Aid Reverse Transcriptase German Company Thermo Scientific. All samples were amplified. Primer is designed to blast the NCBI gene was performed on the website and nucleotide sequences were compared And the nucleotide sequence of the gene of wheat was 100% compliance, As a result was of the sequence wheat. Figure (1) shows nucleotide sequences match with stress response protein gene.

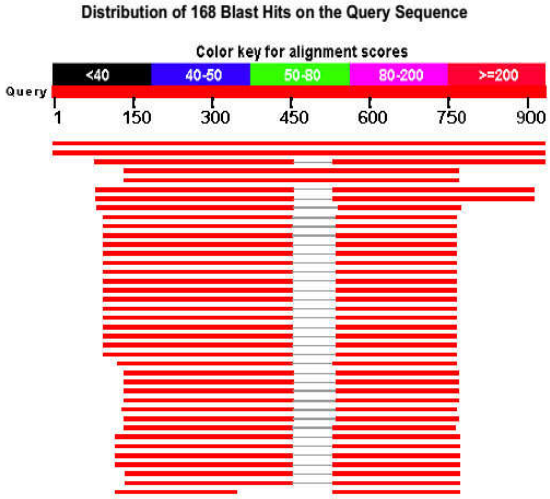

Figure (1) nucleotide sequences matched with stress response protein

Tubulin gene was used as a housekeeping gene. Primers were designed using software OLIGO5, Specifications have been recorded primers examined in Table 1.

Table 1 details the gene expression intensity spots in Northern blot analysis used in reverse

Gene name		'5	'3	Annealing Temp.	Length Base	Length piece	Access Number
TUBULIN	F:	GGGGCATAGGAGGAAAGCA		58	20	700	KJ40609.1
	R:	GGGGCATAGGAGGAAAGCA					
Stress Responsive Protein				59	20	248	JQ923471
	F:	CCAACTACGCCATGTCCGTG					
	R:	TCCACAGCACCGACTCGTCC					

To study the gene expression of the reverse blot method was used Northern. DNA array was developed for this purpose, The PCR products were denaturation and of each sample was about 0.5 micrograms of PCR product stains on the membrane. Next Total-cDNA Probes using the kit DIG labeling DNA was labeled dNTP. The hybridization was performed reaction. The appearance of stains substrate CDP-Star Company Roche with DIG High Prime DNA Labeling and Detection Starter Kit 11, the substrate is ready for use. XRAY film was on the membrane in the darkroom and spots appeared. After darkening spots were recognizable enough, the reaction was stopped painting. Scoring was done on the spot using software Totallab, All stains were on the spot α-tubulin by standard software and were analyzed the software SAS ver.9. Data with SAS V9.0 software factorial based on completely randomized design with two replications.

RESULTS AND DISCUSSION

After gene amplification by PCR amplification of gene segments of the desired size were observed on the gel.

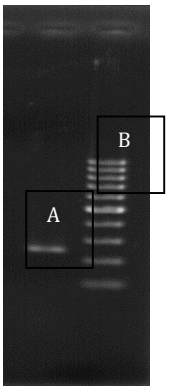

Figure 2: The gene fragment amplified by PCR, A: Ladder 100 bp fermentase, B: Stress Responsive Protein

After obtaining the sufficient amount of gene products, the products of the spots on the membrane and the membrane appeared with the labeled RNA. Image (3) show photos of large arrays Northern blot membranes prepared from both susceptible and tolerant genotypes in both repeat which has been under drought stress.

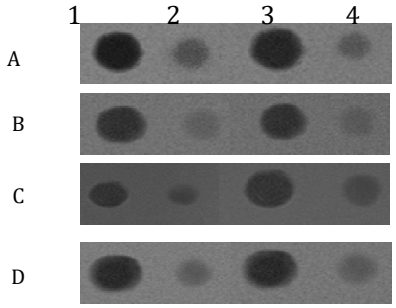

Fig 3: The intensity of gene spots in the reverse dot Northern blot Agency. A: sensitive genotypes under drought stress B: sensitive genotypes under normal conditions C: tolerant of drought conditions D: tolerant under normal conditions.1: Stress Responsive Protein Gene first replications 2: House gene, Tubulin first replications, 3. Stress Responsive Protein gene second replications, 4: Gene house Tubulin second replications.

Fig 4 shows the expression of stress response genes in relation to tubulin protein which represents is tolerant, the average increase in the protein expression which is equal to 11.8 and the role of this gene are highlighted in stress tolerance. The difference between normal and stress tolerant genotypes are more susceptible genotypes and the role of this gene show in the resistant variety.
The average sensitive cultivar is 2.8. No significant difference between levels of stress, susceptible and control cultivar. And have not seen an increase in these cultivars. Fig 4 shows the expression of the protein Stress Responsive in wheat in stress and control.

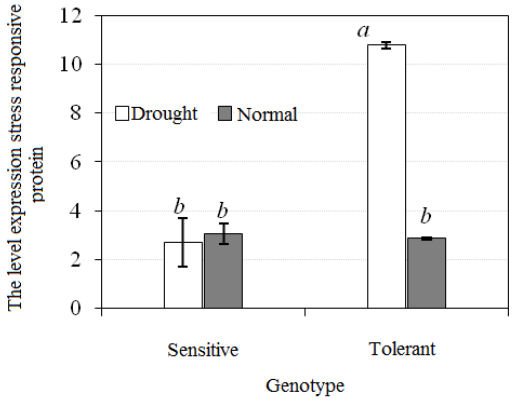

Fig 4: Shows the protein expression Stress Responsive the two local varieties of wheat in both normal and stress significant difference was observed in tolerant genotype sensitive genotype.

Summary data (mean square) analysis of variance related to stress response protein (Stress Responsive Protein) in Table 2 below. In this table, the numbers are obtained of each gene after gene normalization housekeeper (tubulin).

Table 2: Summary analysis of variance examines the changes in gene expression under drought stress in susceptible and resistant bread wheat

S.O.V	DF	Average of squares
		Stress Responsive Protein
Cultivar	1	32.182**
Drought	1	28.614**
Cultivar* Drought	1	34.375**
Error	4	0.609
CV	%	16.1

** Significant at 1 percent

The variance analysis of gene expression at cultivars level of the land and cultivars land is significant at 1%. Analysis and comparison of gene expression in response to drought stress tolerant and sensitive wheat in the Transcriptome and proteome level what could have valuable information for the breeding program. Total proteins called stress protein responses which are expressed in cells under stress, the role of these proteins varied and the tension expressed in the plant. These proteins are involved generally in response to environmental stresses induced pathways. As a general rule, the responses to biotic and abiotic stresses induced by a series of proteins that protect the cells are carried out. Abiotic stresses such as heat, cold, drought, salinity, poor diet, ozone, heavy metals, visible radiation, toxic chemicals, oxidative stress caused by biotic and abiotic factors of the most significant threats in the field of agriculture [2]. The results showed that the protein essential role in plant resistance to drought.

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