التباينات الأليلية لمورثات الديهيدرين في الجيل الطافر الثاني (M2) لبعض الطرز الوراثية من القمح القاسي

Allelic Variations of Dehydrin Genes in the Second Mutant Generation M2 of some Durum Wheat Genotypes

Dr. A. Shekhmous(1) **Dr. J. Saleh**(2-3) **Dr. M. Shaherli**(2-3) **Dr. S. Lawand**(2-3)

(1) National Commission for Biotechnology (NCBT) / Syria.

(2) Dep. of Field Crops, Faculty of Agriculture, Damascus University, Syria.

(3) The Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD).

الملخّص

نفذ البحث في مخابر قسم المحاصيل الحقلية، ومخبر التقانات الحيوية في كلية الزراعة بجامعة دمشق (سورية)، ومخابر الهيئة العامة للتقانة الحيوية في عام 2012، بهدف تحديد التباينات الأليلية لمورثات الديهيدرين ضمن طرز وراثية مختلفة من القمح القاسي هي دوما1، شام5، بحوث9، في الجيل الطافر الثاني (M2).

أظهرت دراسة تقييم التباين لمورثات الديهيدرين المسؤولة عن تحمل الجفاف اختلافاً واضحاً في هذه المورثات بين المعاملات المدروسة، إذ كانت التباينات الشكلية في الوزن الجزيئي بين نظائر الموقع الواحد كبيرةً أحياناً، وكانت على درجة عالية من التماثل في البعض الآخر، وأمكن تمييزها بسهولة على هلامة ميتافور أغاروز 4 %. أظهر تفاعل الـ PCR بالنسبة لمورثات الديهيدرين *Dhn1* و *Dhn3* و *Dhn6* و *Dhn10* نمطاً شكلياً واحداً، وُجد عند أغلب المعاملات المدروسة، وبالنسبة لمورثة الديهيدرين *Dhn12* فقد ظهر فيها نمط شكلي واحد عند معاملة واحدة، وهي المعاملة ذات التركيز المتوسط من DES لدى الصنف بحوث9.

وبالنسبة لمورثة الديهيدرين *Dhn2* فقد ظهر فيها سبعة أنماط شكلية، وظهرت في المورثات *Dhn4* و *Dhn8* و *Dhn11* خمسة أنماط شكلية، في حين ظهرت أربعة أنماط شكلية في مورثة الديهيدرين *Dhn7* ، وثلاثة أنماط شكلية في مورثة الديهيدرين *Dhn9*.

تفوقت مورثة *Dhn11* على باقي المورثات من حيث عدد الأنماط الشكلية التي أعطتها، والتي بلغت 46 نمطاً شكلياً لدى جميع المعاملات المدروسة، في حين أعطت المورثة *Dhn12* أقل عدد من الأنماط الشكلية، إذ بلغ نمطاً شكلياً واحداً، ولم تعط مورثة الديهيدرين *Dhn5* أي نمط شكلي في جميع المعاملات المدروسة. وامتلكت المعاملة (γ 25 Kr) أكبر عدد من الأنماط الشكلية في جميع مورثات الديهيدرين *Dhn* المدروسة في الصنف بحوث9 ، إذ بلغ عددها 18 نمطاً شكلياً، وامتلكت المعاملة (DES 0.05 %) أقل عدد من الأنماط الشكلية (10 أنماط) في الصنف دوما1.

الكلمات المفتاحية : القمح القاسي، التباينات الاليلية، الجيل الطافر الثاني، مورثات الديهيدرين.

©2017 The Arab Center for the Studies of Arid Zones and Dry Lands, All rights reserved. **ISSN:2305 - 5243 ; AIF(NSP)-316**

Abstract

This study was conducted at the Biotechnology Laboratory, Department of Agronomy, Faculty of Agriculture, Damascus University (Syria) and the laboratories. of National Commission for Biotechnology in the year 2012. The aim of the study was to detect the variations of Dehydrin in different genotypes of durum wheat in the second mutant generation (M2).

Results of dehydrin gene (responsible for drought tolerance) variation study showed a clear difference among the studied treatments. Variation in the molecular weight between loci per gene was very high in some cases, while it had a high degree of symmetry in other cases, and was easily distinguished on 4% agarose gel. The PCR results for the Dehydrin genes *Dhn1*, *Dhn3*, *Dhn6* and *Dhn10* showed a monomorphic pattern in most of the studied treatments, while for the *Dhn12* only one pattern was found in one treatment (medium concentration of DES in Bouhouth9). *Dhn2* showed seven patterns, while the genes *Dhn4*, *Dhn8* and *Dhn11* showed five patterns, and the *Dhn7* showed four patterns and finally *Dhn9* showed three patterns.

The *Dhn11* was superior in the number of polymorphic patterns, as the number of total patterns was 46 patterns in all treatments, but on the other hand the *Dhn12* showed the lowest number of patterns with only one pattern. The *Dhn5* didn't show any patterns in all cases, the treatment (γ 25 kr) in Bouhouth9 showed the largest number of patterns in the Dhn genes with 18 patterns, and the treatment (DES 0.05%) in Douma1 showed the lowest number with only 10 patterns.

Key Words: Durum wheat, Alleles variation, Second mutant generation, Dehydrin gene.

Introduction

Cereals are the most important crops on earth providing about 70% of food for the world's population. Wheat and rice constitute 50% of total cereals production (Lookhart and Bean, 2000), but wheat is unique among all cereal crops in terms of importance (Kazemi, 2009). It is grown and consumed as an essential food in many countries of the world, especially in areas suffering from drought problems. On the other hand, this crop provides about 22% of energy and of the daily energy in the countries 60%-19% of proteins to build the human body in developing countries, and about 40 of West Asia and North Africa (CIMMYT, 2009).

Araus (2004) Confirmed that the negative effect of climate changes is clearly and effectively obvious in rainfed areas, and statistics have showed a noticeable decrease in the cultivated area and productivity around the world (Aesawy, 2000), the international cultivated area of wheat was 218,480 MHa, and the international yield was 32646 Tones/Ha, otherwise the production was 713,182 Million Tones (FAO, 2014). In Syria, the cultivated area was 1,603 MHa, and the yield of this crop was 2252 Kg/Ha, and the production of it was 3.61 Million Tones (Syrian Ministry of Agriculture Statistics, 2012). .so it is necessary to improve the grain productivity by improving the methods of breeding and management of the crop (Araus *et al*., 2003) in addition to the use of biotechnology.

The use of molecular markers, which were developed to be used in the breeding programs, can reduce the difficulties of the introduction of desired traits in the genotype (Ramsay *et al*., 2000).

Drought resistance studies have been developed through breeding and crop improvement processes, and through molecular and genetic studies and gene transcription. Drought tolerance was increased through plant breeding process, due to the possibility of hybridization and selection processes which transfer the desirable traits from wild species to cultivated varieties (Acevedo and Fereres, 1993).

The Late Embrygenesis Abundant proteins (LEA) are present in many cellular types with varied concentrations, LEA proteins are defense proteins and they are observed under effect of different stresses like drought, saintly and coldness(Danyluk *et al*., 1994; Close, 1997; Choi *et al*., 2000) and the LEA were divided depending on the analytical studies conducted on cotton compared with other plant species, to: LEA D19 and LEA D11 (Dehydrins group) and LEA D7 and then the LEA D113 and LEA D95 groups were added later (Galau *et al*., 1993).

Dehydrins were detected as proteins in both Eukaryotic and prokaryotic, and different studies showed their presence of Dehydrins in dried algae (Velten and Oliver, 2001), especially in wheat and barley plants and their presence was associated with low heat stress (Galiba *et al*., 1995).

Dubcovsky *et al*. (1995) detected the Dehydrin genes in diploid wheat on chromosomes 4A, 5A and 6A in *Triticum monococcum*, and on 5D in *Triticum tauchii*. Limin *et al*. (1997) found that the genes family Wcs 120 in Hexaploid wheat (bread wheat) (*Triticum aestivum*) is similar to the gene *Dhn5* in barley, and located on the long arm of the chromosomes of the sixth Group.

Werner-Fraczek and Close (1998) emphasized to the presence of Dehydrin genes on the arms of the chromosomes 4DS, 5BL and 6AL in *Triticum aestivum* L.cv *Chinese Springer* by using Cytogenetic stocks and Western blot techniques (Pan *et al*., 1994).

The main objective of this study was to detect variations of Dehydrin in the second mutant generation of some different genotypes of durum wheat.

Materials and methods

Site and time of Study: This study was conducted at the labs of biotechnology affiliated to the Faculty of Agriculture, Damascus University (Syria), and the laboratories of National commission for Biotechnology during thebyear 2012.

Plant material: The study was conducted on three genotypes of durum wheat ($Doma_1$, $Sham_5$, $Bhouth_9$) (Tab. 1**)** used in this study were obtained from the General Commission for Scientific Agricultural Research (GCSAR), and the Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD) were studied.

Table 1. Characterizations of the durum wheat genotypes used in this study.

	$Bhouth_9$	$Sham_5$	$Doma_1$
Zone	B , A	B	A - B
Yield (Kg.ha^{-1})	6914	1847	3350
Disease's loading	Resistant	Resistant	Resistance
Disease's resistance	Resistant	Resistant	Resistant – Median resistance
Heading date (days)	117	144	121
Maturity date (days)	163	181	165
Plant's height (cm)	79 – 64	56	78 - 66
Spike's length (cm)	8 – 7	8 - 6	10 - 8
Spike's Shape	pyramidal	pyramidal	Pyramidal
Spike's color	Creamy	Creamy	Creamy
Grain 'S Shape	Semi elongated - Oval	Oval	Semi elongated

Source: GCSAR (2009), ACSAD (2009).

Treatments: Artificial mutation program was executed during 2010/2011 by using different doses and concentrations of physical (Gamma ray) Shaherli (1992) and chemical (Ethylene amin EI, Dai etil sulfate DES) mutagenesis on three genotypes of durum wheat (Doma1, Sham5, Bhouth9).

During 2012, thirteen treatments were applied on the selected plants of the second mutant generation M2, depending on arain number and weight of grains of each genotype (Table 2).

Table 2. studied treatments.

Nº	Genotype	Treatment
1	$Doma_1$	Control
2	$Doma_1$	Medium dose of Gamma ray (γ 20 Kr)
3	$Doma_1$	Medium concentration of Ethylene Amine (EI 0.01 %)
4	$Doma_1$	High concentration of Ethylene Amine (EI 0.015 %)
5	$Doma_1$	Low concentration of Dai etil sulfate (DES 0.05 %)
6	$Doma_1$	High concentration of Dai etil sulfate (DES 0.02 %)
7	$Sham_5$	Control
8	$Sham_5$	Low dose of Gamma ray (γ 20 Kr)
9	$Sham_5$	Medium dose of Gamma ray (γ 20 Kr)
10	$Sham_5$	Low concentration of Ethylene Amine (EI 0.005 %)
11	$Bhouth_9$	Control
12	$Bhouth_9$	High dose of Gamma ray (γ 25 Kr)
13	$Bhouth_9$	Medium concentration of Dai etil sulfate (DES 0.01 %)

DNA extraction:

DNA was extracted from fresh plantlets (3-4 weeks old, grown at 27°C under a 12/12 h day/night photoperiod) by using CTAB method suggested by Murray and Thompson (1980).

DNA quality was determined using 1% agarose gel and then quantified by spectrophotometer. DNA concentration was adjusted to 50 ng μL^{-1} to be used in the SSR reactions.

Twelve SSR (Simple Sequence Repeat) markers were selected depending on their chromosomal locations (Choi *et. al*., 2000), and the markers were obtained from the Atomic Energy Commission of Syria, the details of selected SSR markers are presented in (Tab. 3).

Table 3. SSR marker, their sequences, and chromosomal location for the Dehydrine genes.

Dehydrin gene	Annealing temperature c°	(3́-5) Forward Primers	(3́-5) Reverse Primers
Dhn1	64	GACGAGGGATGGCCACAAGACTGA	AGTAACGCATGGCTGCGGATGCTA
Dhn2	61	CCAGCCGACCAGGGACGACCACAA	TTTCGAGCCATCGTACGCAAAGGATG
Dhn3	62	AGGCAACCAAGATCAACACCACCTG	GCGGAAGTTTTACTGCATCTCCATC
Dhn4	64	CGGCAGCGCAAGATGGAGTACCAG	CCCCTCCAACAGCCAAGTGAGCTA
Dhn5	67	AAATGACTGGCATGGGGAGGCATA	CTCCACCAACGAAAGTGAGCTAGG
Dhn6	64	TGACGTCGTGGCACACACCCTC	ACCAGGCCATGTCACAGTACTGC
Dhn7	65	GTCATTTCCAGCCGACGAGGAAGG	CGGGTCCATACAAGAAGCCATATT
Dhn8	61	TCATGGAGGATGAGAGGAGCACCCA	GGCTTTGAGTAGTGGCCTGGAGGTA
Dhn9	68	ATGGAGTTCCAAGGGCAGCAGGAC	AGGCTTCGACGCGTAGCTATGCAA
Dhn10	64	GCCAAGAGGCAGCAAGATGGAATACC	TCGGCTTATTGCTCCACCTCCGCTCA
Dhn11	61	AAGAGTTGAAGCACCGTCGAGGG	CGTACATGGTCAAAGAACCGTGT
Dhn12	58	GATGATCCAGCAGCAACTCA	TCAGCTCGAGCTTGACGACT

Polymerase chain reaction (PCR) reactions were performed in a total volume of 25 µl containing 200-250 ng DNA, 12.5 µl of GoTaq Green Master Mix (Promega) and 0.25 µM of each primer. The amplifications were carried out using APOLLO Thermocycler (USA). PCR amplification procedure was performed by an initial denaturation step of 5 min at 94 °C followed by 30 cycles of three steps: denaturation for 1 min at 94°C, annealing for 1 min at 58 or 60 °C (depending on the primer), extension for 1 min at 72 °C with a final extension for 10 min at 72 °C. Amplified PCR products were separated using 8% non-denaturing polyacrylamide gel, and then the gels were stained by ethidium bromide and visualized under UV light. 50bp and 100bp DNA Ladder was used as a molecular size standard.

Results and discussion

The ratio between the studied DNA extracted samples at photo waves with a length of 260/280 nm using spectrophotometer showed values between 1.821-1.964, indicating a high quality of DNA, the DNA concentrations were between (0.26-0.45 ug/ul) in the buffer solution in which the samples were stored.

DNA of wheat under investigated treatments were analyzed using 12 SSR primers characterized Dehydrin gene loci *Dhn1*, *Dhn2*, *Dhn3*, *Dhn4*, *Dhn5*, *Dhn6*, *Dhn7*, *Dhn8*, *Dhn9*, *Dhn10*, *Dhn11*, *Dhn12*.

The results showed differences among DNA amplified fragments for one locus in the studied treatments, and these differences reflect genetic variation at the level of one locus, as it has showed presence of different alleles on the same locus.

Morphological differences at a molecular weight between one locus alleles were high in some treatments, while the others were at a high degree of agreement, and can be easily recognized at 4% metaphore agarose gel. Polymerase chain reaction (PCR) for Dehydrin genes (*Dhn1*, *Dhn3*, *Dhn6*, *Dhn10*) showed one morphological pattern (A) in most of the studied treatments. For Dehydrin gene (*Dhn12*), it showed one morphological pattern (A) in one treatment which is the medium concentration of DES in Bhouth9 (tab. 4).

Table 4. Morphological patterns of polymorphics results of PCR-reaction and the discovered alleles in the genes (*Dhn1*, *Dhn3*, *Dhn6*, *Dhn10*,*Dhn12*) within treatments Of the studied wheat varieties.

Treatments / dehydrin genes	13	12	11	10	9	8	7	6	5	4	3	2	1
Dhn1	A	A	A	A	A	A	A	A	A	A	A	A	---
Dhn3	A	A	---	A	A	A	A	A	---	A	A	A	---
Dhn6	A	A	A	A	A	A	A	A	A	A	A	A	---
Dhn10	A	A	A	A	A	A	A	A	A	A	A	A	---
Dhn12	A	---	---	---	---	---	---	---	---	---	---	---	---

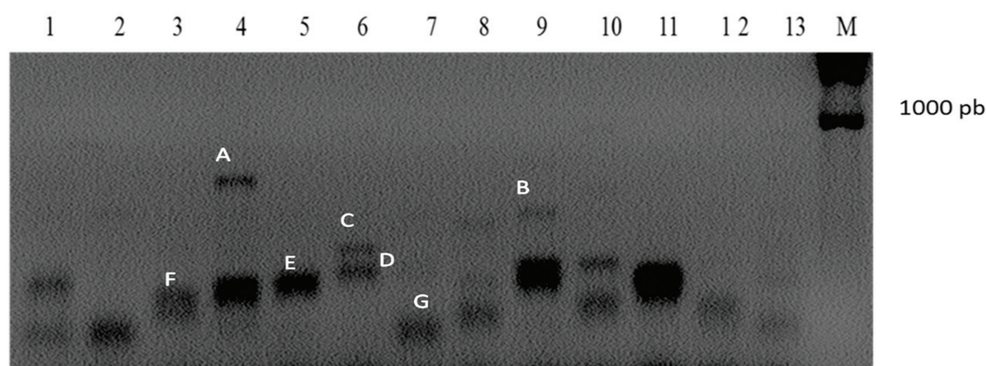

Fig. 1. Metaphore agarose gel 4% and the discovered morphological patterns for Dehydrin gene (Dhn2) within the treatments of the studied wheat varieties.

Seven morphological patterns (A,B,C,D,E,F,G) of Dehydrin gene (Dhn2) were shown in the treatments (Table 5, Fig.1), and those patterns have been varied in appearance between the treatments. Two patterns were shown in the treatments (1, 4, 6, 9, 10), while one different pattern (different in the molecular weight) was shown in the treatments (2, 3, 5, 7, 8, 11, 12, 13).

Table 5. Morphological patterns of polymorphics results of PCR-reaction and the discovered alleles in the genes (*Dhn2*) within treatments Of the studied wheat varieties.

Treatments / Dehydrin genes	13	12	11	10	9	8	7	6	5	4	3	2	1
Dhn2	---	---	---	---	---	---	---	---	---	A	---	---	---
	---	---	---	---	B	---	---	---	---	---	---	---	---
	---	---	---	---	---	---	---	C	---	---	---	---	---
	---	---	D	D	D	---	---	D	---	---	---	---	---
	---	---	---	---	---	---	---	---	E	E	---	---	E
	---	F	---	F	---	F	---	---	---	---	F	---	---
	G	---	---	---	---	---	G	---	---	---	---	G	G

For the *Dhn4*, variations of the morphological patterns resulted from the PCR were high, as five patterns (A,B,C,D,E) were observed. These patterns were not detected in treatment 8 (low-dose of Gamma Ray γ in $Cham_5$), and four patterns were observed in two treatments (9.12), and three patterns in treatments (1, 4, 6, 7, 10, 11.13), and in treatment (2) two patterns were detected, and one pattern was observed in two treatments (3, 5). (Table 6).

Table 6. Morphological patterns of polymorphics results of PCR-reaction and the discovered alleles in the genes (Dhn4) within treatments Of the studied wheat varieties.

Treatments / Dehydrin genes	13	12	11	10	9	8	7	6	5	4	3	2	1
Dhn4	**A**	**A**	**A**	**A**	**A**	**---**	**A**	**A**	**---**	**A**	**---**	**A**	**A**
	B	**B**	**B**	**B**	**B**	**---**	**B**	**B**	**---**	**---**	**---**	**---**	**B**
	---	**---**	**---**	**---**	**C**	**---**	**---**	**---**	**---**	**C**	**---**	**---**	**---**
	D	**D**	**D**	**D**	**D**	**---**	**D**	**D**	**D**	**D**	**D**	**D**	**D**
	---	**E**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**

No morphological patterns in Dehydrin gene (*Dhn5*) were observed in all treatments and control after applying the Polymerase chain reaction (PCR).

For the Dhn7, PCR results showed 4 morphological patterns (A,B,C,D) (Table 7). All these patterns appeared in treatment (1), while 2 patterns appeared in treatments (2 and 3), but no patterns were discovered in the other treatment.

Table 7. Morphological patterns of polymorphic results of PCR-reaction and the discovered alleles in the genes (*Dhn7*)) within treatments Of the studied wheat varieties.

Treatments / Dehydrin genes	13	12	11	10	9	8	7	6	5	4	3	2	1
Dhn7	---	---	---	---	---	---	---	---	---	---	---	---	A
	---	---	---	---	---	---	---	---	---	---	---	---	B
	---	---	---	---	---	---	---	---	---	---	C	C	C
	---	---	---	---	---	---	---	---	---	---	D	D	D

Five morphological patterns (A,B,C,D,E) were observed in Dhn8, three of them were observed in treatments (4.12), and two patterns in treatments (6, 10), and one pattern was observed in the other treatments (Table 8).

Table 8. Morphological patterns of polymorphics results of PCR-reaction and the discovered alleles in the genes (*Dhn8*)) within treatments Of the studied wheat varieties

Treatments / Dehydrin genes	13	12	11	10	9	8	7	6	5	4	3	2	1
Dhn8	---	---	---	A	---	---	---	---	---	---	---	---	---
	---	---	---	---	---	---	---	---	---	B	---	---	---
	---	C	---	---	---	---	---	C	---	---	---	---	---
	---	D	---	---	---	---	---	---	---	D	---	---	---
	E	E	E	E	E	E	E	E	E	E	E	E	E

For the Dhn9, PCR results showed 3 morphological patterns (A,B,C), where two patterns appeared in treatments (12, 13), and one pattern was observed in the other treatments (Table 9).

Table 9. Morphological patterns of polymorphics results of PCR-reaction and the discovered alleles in the genes (*Dhn9*) within treatments Of the studied wheat varieties.

Treatments / Dehydrin genes	13	12	11	10	9	8	7	6	5	4	3	2	1
Dhn9	A	A	---	---	---	---	---	---	---	---	---	---	---
	B	B	B	---	B	---	---	B	B	---	B	B	B
	---	---	---	---	---	---	---	---	---	C	---	---	---

For the Dhn11, PCR results showed 5 morphological patterns (A,B,C,D,E). All the patterns appeared in treatments (8, 11), 4 patterns appeared in treatments (6, 7, 9, 10, 12, 13), and 3 patterns in treatment (5), and two patterns in treatments (1, 2, 3, 4) (Table 10).

Table 10. Morphological patterns of polymorphics results of PCR-reaction and the discovered alleles in the genes (*Dhn11*)) within treatments Of the studied wheat varieties

Treatments / Dehydrin genes	13	12	11	10	9	8	7	6	5	4	3	2	1
Dhn11	A	A	A	A	A	A	A	A	---	---	A	A	A
	---	---	B	B	---	B	---	---	---	---	---	---	---
	C	C	C	C	C	C	C	C	C	C	---	C	---
	D	D	D	---	D	D	D	D	D	---	---	---	---
	E	E	E	E	E	E	E	E	E	E	E	E	E

PCR- reaction allowed detecting the morphological variations of DNA fragments for the genetic loci of the studied Dehydrins, and these variations were caused by differences in molecular weight of these fragments, which reflects the differences in the number of nucleotide from which it was formed.

The different morphological patterns of DNA fragments resulted from PCR- reaction reflects different allele numbers of each gene within the studied plants, and the genetic differences for each locus. It also give an indication about the mutations which a locus may exposed to. The more alleles, the more mutations happened, and that affect the gene structure and changes in molecular weight.

It can be noticed from (Tab.11) that the superiority of Dehydrin gene (*Dhn11*) compared to the other genes depending on the morphological patterns. It gave 46 morphological patterns for all studied treatments, while the gene (*Dhn12*) gave the lowest number of morphological patterns and it gave only one morphological pattern. The gene (*Dhn5*) didn't give any morphological pattern in the studied treatments. The treatment (ɣ 25 Kr) in the variety Bhouth9 had the highest number of morphological patterns in all studied Dehydrin genes which counted 18 morphological patterns, and the treatment (DES 0.05 %) in the genotype Doma1gave the lowest number of morphological patterns which was only 10 .

It was also noticed, the compared superiority of treatments (ɣ 20 Kr), (EI 0.015 %) and (DES 0.02 %)over to the control in Doma1due to having the highest number of genetic loci for Dehydrin (14,15,16 genetic loci respectively), and the two treatments (ɣ 20 Kr) and (EI 0.005 %) surpassed over the control in Sham5 by having the highest number of genetic loci which counted (15,16 genetic loci), respectively. For Bhouth9 the two treatments(ɣ 25 Kr) and (DES 0.01 %) superposed over the control by having 16,18 genetic loci respectively, indicating a positive relation between the variety bearing of dehydration and number of genetic loci. This result is related to the genotype of studied varieties and under different doses of the ray.

Conclusions:

From the above results, it can be concluded:

- Eleven Dehydrin genes proved were detected responsible for drought tolerance, and there was a dehydrin gene was not detected in any of the treatments and in the control plants.
- Dhn11 gave the highest number of morphological patterns (45 patterns), while Dhn12 gave the lowest number (1 pattern).

Recommendations:

1. Detecting the Sequencing of Dehydrin genes and isolating the genes responsible for drought tolerance in the studied wheat genotypes.
2. Studying the variations of gene expression of dehydrins in other wheat genotypes during the late stages of the plant's growth and within different stages of plant life.
3. Studying the variation in gene expression of dehydrins at the level of RNA, by using modern technologies such as Real Time-PCR.

Table11. Number of morphological patterns of Dehydrin genes and for the treatments of studied wheat varieties.

Nº		13	12	11	10	9	8	7	6	5	4	3	2	1
Treatments / Dehydrin genes	Gens	(% DES 0.01)	(γ 25 Kr)	Control Bhouth9	(% EI 0.005)	(γ 20 Kr)	(γ 15 Kr)	Control Sham5	(% DES 0.02)	(% DES 0.05)	(% EI 0.015)	(% EI 0.01)	(γ 20 Kr)	Control Doma1
Dhn1	**12**	1	1	1	1	1	1	1	1	1	1	1	1	0
Dhn2	**18**	1	1	1	2	2	1	1	2	1	2	1	1	2
Dhn3	**10**	1	1	0	1	1	1	1	1	0	1	1	1	0
Dhn4	**33**	3	4	3	3	4	0	3	3	1	3	1	2	3
Dhn5	**0**	0	0	0	0	0	0	0	0	0	0	0	0	0
Dhn6	**12**	1	1	1	1	1	1	1	1	1	1	1	1	0
Dhn7	**8**	0	0	0	0	0	0	0	0	0	0	2	2	4
Dhn8	**19**	1	3	1	2	1	1	1	2	1	3	1	1	1
Dhn9	**12**	2	2	1	0	1	0	0	1	1	1	1	1	1
Dhn10	**12**	1	1	1	1	1	1	1	1	1	1	1	1	0
Dhn11	**46**	4	4	5	4	4	5	4	4	3	2	2	3	2
Dhn12	**1**	1	0	0	0	0	0	0	0	0	0	0	0	0
		16	**18**	**14**	**15**	**16**	**11**	**13**	**16**	**10**	**15**	**12**	**14**	**13**

Reference

- Acevedo, E. and E. Fereres. 1993. Resistance to abiotic stresses. In ; Plant Breeding, ed. M.D. Haward, N.O. Bosemark, I. Romagosa, London; Chapman and Hall : 406-421.
- Aesawy, A. M. 2000. Periodicity and prediction of annual surface air temperature over eastern Mediterranean. Bulgaria Journal of Meteorology and Hydrology, 11(1-2): 36-54.
- Araus, J, J. Bort, P. Steduto, D. Villegas, and C. Royo. 2003. Breeding cereals for Mediterranean conditions: Ecophysiological clues for biotechnology application. Annals of Applied Biology, 142(2): 129-141.
- Araus, J. L. 2004. The problems of sustainable water use in the Mediterranean and research requirements for agriculture. Ann. Appl. Biol., 144: 259-272.
- Choi, D.W, M. C. Koag, and T.J. Close. 2000. Map location of Dhn gene determine by gene – specific PCR. Theor. Appl Genet. 101:350-354.
- CIMMYT (International Maize and Wheat Improvement Center). 2009. Drought Tolerance Wheat and Enhanced Quality Project, MTP:66-71.
- Close,T. J:1997. Dehydrins: A commonatity in response of plants to dehydration and two temperatures physiol plant 100:291-296.
- Danyluk J, M. Houde, T.E. Rassar qnd F. Sarhan .1994. Differential expression of agene encoding and acidic Dehydrin

in chilling sensitive and freezing tolerant gramineae species f E 135 Letl. ; 344(1): 20-24.

- Dubcovsky, J., C. Luo, and J. Dvorak. 1995. Linkaye relationships among stress-induced genes in wheat. Theor Appl Genet.19:795-801.
- FAO .2014. Agricultural statistics of the Food and Agriculture Organization of the United Nations, Rome.
- Galau, G. A, H. Y-C. Wang and D. W. Hughes. 1993. Cotton LEA 5 and LEA 14 encode a typical late embryogenesis – abundant proteins. Plant Physiol. 101: 695-696.
- Galiba, G., A. Quarris, J. Sutka, A. Morgounov and J. W. snap. 1995. RFLP mapping of the vernalization (Vrn1) and frost resistance (Fr1) genes on chromosome 5A of Wheat. Theor apple Genet, 90: 1174-1179.
- Kazemi Arbat, H. 2009. Especial Farming, Cereals (First Volume). Iran University Press. 318.
- Limin, A. E., J. Danvluk, L. P. Chauvin, D. B. Fowler, and F. Sarhan. 1997. Chromosome mapping of louc- temperature induced wcs 120 family genes and regulation of cold-tolerance expression in wheat. Mol. Gen. Genet 253.720-727.
- Lookhart, G. and S. Bean. 2000. Cereal Proteins: Composition for Their Major Fractions and Methods for Identification. In: Kulp K. and J. G. Ponte Jr (Eds.) Handbook of Cereal Science and Technology(2nd Edition). Marcel Dekkar Inc., New York, USA: 363-383.
- Murray, M. G. and W. F. Thompson. 1980. Rapid isolation of high molecular weight DNA. Nucleic Acids Res 8: 4321-4325.
- Pan, A., P. M. Hayes, F. Chen, T. H. H. Chen, T. Blake, S. Wright, I. Karsai, and Z. Bedo. 1994. Genetic analysis of components of winter hardinessin barley (Hordeum Volgare L.) Theor Apple Genet 89: 900-910.
- Ramsay, L., M. Macaulay, S. Deglilvanissevich, K. Maclean, L. Carsle, J. Fuller, K.J. Edwards, S. Tuvesson, M. Morgante, A. Massari, E. Maestri, N. Marmiroli, T. Sjakste, M. Ganal, W. Powell, and R. Waugh. 2000. A simple sequence repeat- based linkage map of barley. Genetics 156:1997-2005.
- Shaherli, M. 1992. Having primary germblasm of spring barley by using chemical mutagens and gamma radiation with benzoin acid. KHAKOV.
- Syrian Ministry of Agriculture Statistics. 2012.
- Velten, J. and M.J. Oliver. 2001. Tr 288, are hydrine with a dehydrin twist. Plant Mol/ Biol.; 45(6): 713-722.
- Werner-Fraczek, J. E., and T.J. Close. 1998. Genetic Studies of triticeae dehydrins: assignment of seed protein and a regulatory factor to map positions. Theor. Appl. Genet. 97: 220-226.

Nº Ref: 496