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Allelic Variations of *LEA* and Dehydrin Genes in Chickpea Genotypes (*Cicer arietinum* L.)

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Abstract : Investigation was carried out at the Biotechnology Lab. Department of Agronomy, Faculty of Agriculture – Damascus University in the year 2016. The aim was to detect the allelic variations of *LEA* and Dehydrin genes in different genotypes of Chickpea (*Cicer arietinum*). Results of *LEA* and Dehydrin genes (responsible for drought tolerance) variation have shown a clear difference among the studied genotypes. 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% metaphor agarose gel. The PCR results for the Dehydrin genes *Dhn3* and *Dhn4* have shown a one morphological pattern in the most of the studied genotypes, while for the *Dhn1*and *Dhn2* only two patterns was found. *LEA1* showed three patterns, while the gene *LEA2* showed six patterns. The *LEA1* was superior in the number of polymorphic patterns, as the number of total patterns was 85 patterns in all genotypes, but on the other hand the *Dhn4* showed the lowest number of patterns with only 22 patterns. The genotypes (7, 8, 17) showed the largest number of patterns with 12 patterns, and the genotypes (19, 20) showed the lowest number with only 2 patterns.
Key Words: Chickpea, Alleles Variation, Dehydrin Gene, *LEA* Gene.

Introduction:

Chickpea (*Cicer arietinum* L.), one of the most important grain-legume crop, is grown in more than 45 countries, mostly in arid and semiarid zones[1]. It's the second important legume in the world with 14.7 million ha under cultivation and with 14.2 million tons produced annually[2]. Drought, cold and salinity are the major abiotic stresses affecting chickpea in order of importance[3]. It has been estimated that 70% of the crop yield loss can be attributed to abiotic stresses, especially drought[4]. Drought is a meteorological term and an environmental event, defined as a water stress due to lack or insufficient rainfall and/or inadequate water supply[5]. The seriousness of drought stress depends on its timing, duration and intensity[6]. Worldwide, 90% of chickpea is grown under rain fed conditions[1], where the terminal drought stress during the chickpea reproductive phase results heavy yield losses[7].

Chickpea cultivars have different response to drought stress and plant densities in dry conditions[8]. Plants respond and adapt to water deficit at both the cellular and molecular levels, for instance by the accumulation of osmolytes and proteins specifically involved in stress tolerance. An assortment of genes with diverse functions are induced or repressed by these stresses[9]. Most of their gene products may function in stress response and tolerance at the cellular level. Significantly, the introduction of many stress-inducible genes via

gene transfer resulted in improved plant stress tolerance[10]. The expression and interaction of these genes is complex and diverse, and every gene involved forms part of a coordinated response network. The speed and coordination of expression of these genes is vital for plant survival. The identification of differentially expressed genes between 2 genotypes differing in drought tolerance is an important indicator of drought-associated genes in chickpea[11].

The interface between the expression of stress responsive genes and plant physiological response to drought stress is critical for translating molecular genetics into advances in crop production under stress conditions[12]. A large number of genes have been described that respond to drought at the transcriptional level and the mechanisms of the molecular response to water stress in higher plants have been analyzed by studying the expression of genes responding to drought and other abiotic stresses[13, 14, 15, 16].

Late embryogenesis abundant proteins (LEA proteins) were first found in cotton (*Gossypium hirsutum*) seeds, accumulating late in embryogenesis[17]. These proteins are a diverse group of stress protection proteins which are classified into six groups. LEA proteins comprise the vast majority of stress-responsive proteins. Many reports have described LEA proteins induction in vegetative tissues of several plant species under water deficit conditions imposed by the environment or accumulated as part of a developmental program in desiccation tolerant structures or stages[18]. The expression profiles strongly supported a role for LEA proteins as protective molecules which enable the cells to survive protoplasmic water deficit[19]. An important role for at least some LEA proteins in cellular dehydration tolerance is indicated by their systematic expression at the onset of dehydration and the increase in stress tolerance observed upon over expression in different eukaryotic or prokaryotic hosts[20]. Several LEA genes or proteins, belonging to different groups, were induced during water-deficit stress in Arabidopsis[21] and maize[22], and played distinct roles in cells subjected to the stress. Group2 LEA proteins or dehydrins are highly hydrophilic, glycine-rich and boiling stable proteins which are the most frequently described so far[23].

The dehydrins are a class of drought-induced proteins that lack a fixed three-dimensional structure. The dehydrin sequence is highly evolved and adapted to remain disordered under conditions of severe dehydration[24].

LEA and Dehydrin genes previously validated for their significance in stress responses in various model crops and other legumes, were amplified in chickpea and sequenced after purification using gene specific primer pairs[25]. Earlier studies in chickpea have also reported the induction of LEA and Dehydrin under drought stress[16, 26, 27]. Differences in water-stress tolerance among cultivars, or within a cultivar at various developmental stages, may result from differences in the expression of genes in signal-perception and transduction mechanisms[28]. It was important to develop a comprehensive understanding of LEA and Dehydrin expression at the RNA level in response to drought stress in phonological different stages. Using semi-quantitative method for evaluation of gene expression under stress condition has already been reported for some plants[29].

The main objective of this study was to detect allelic variations of *LEA* and Dehydrin genes in some of different genotypes of Chickpea (*Cicer arietinum*).

Experimental

Site and time of Study:

This study was conducted at the lab. of biotechnology laboratory affiliated to the Faculty of Agriculture - Damascus University, during the year 2016.

Plant material:

The study was conducted on 24 genotypes of Chickpea which were obtained from the General Commission for Scientific Agricultural Research (GCSAR), it were collected from different regions. (Table 1)

Table (1). Studied genotypes and their collecting sites.

Genotype	Origin	Province	Lon	Lat	Alt
1	SYR	Sweida	E36 50	N32 78	840
2	SYR	Ragga	E38 52	N35 65	390
3	SYR	Tartous	E36 15	N35 07	750
4	SYR	Edlib	E36 50	N35 59	260
5	SYR	Aleppo	E36 40	N36 42	370
6	SYR	Homs	E36 42	N34 44	465
7	SYR	Al Hasakah	E40 20	N36 56	520
8	SYR	Daraa	E36 02	N32 53	650
9	SYR	Hama	E36 42	N35 20	470
10	SYR	Damascus	E36 24	N33 51	1640
11	SYR	Dayr Az Zawr	E40 09	N35 20	230
12	SYR	AL Qunitara	E35 53	N33 10	949
13	SYR	Lattakia	E36 15	N35 63	915
14	JOR	AL Balga	E35 44	N32 03	947
15	TUN	Nabul	E10 29	N36 38	28
16	AFG	Baghlan	E68 51	N36 06	950
17	IRN	Kermanshah	E47 04	N34 19	1683
18	IND	Punjab	E47 45	N30 41	9
19	CYP	Nicosia	E33 05	N35 08	250
20	PAK	Punjab	E71 32	N32 35	220
21	DZA	Tlemcen	W01 21	N34 56	700
22	MAR	Centre	W07 41	N33 23	770
23	ESP	Valencia	W00 23	N39 29	27
24	RUS	-	-	-	-

DNA extraction:

DNA was extracted from fresh plantlets (2-3) weeks old, grown at 21°C under a 12/12 h day/night photoperiod by using CTAB method suggested by[30].

DNA quality was determined using 1% agarose gel and then quantified by spectrophotometer, and DNA concentration was adjusted to 60 ng μL^{-1} to be used in the PCR reactions.

LEA and Dehydrin genes primers were developed. The primer sequence (Designed by Primer Premier 3.0) is shown in (Table 2).

Six pairs of primers were selected depending on their chromosomal locations, and the primers were obtained from the Atomic Energy Commission of Syria, the details of selected primers are presented in (Table 2).

Table (2). Sequences of primers.

Genes	Annealing Temperature C°	Forward Primers	Reverse Primers
LEA1	50	GGGCCATACCCTTAACCT	ACAGACAACCGAAGCAAC
LEA2	52	CCACGACCAAAGTTACAGAGC	GCCTTATCTTCTATGTTGCCAATC
Dhn1	50	AACTACCTGGGTTGTGGG	TGGTGGCACTGGAGATG
Dhn2	51	TCCTCTCTCCCGAATTCTTG	AAAGTGGTGTTGGGATGACC
Dhn3	50	CCCACTCAGTAACAACATCC	TGCTCCTGTGGTAGCTGAGA
Dhn4	65	CGCGGATCCGAACACTGCGTTTGCTGGCTTTGA TG	GCTGATGGCGATGAATGAACACTG

Polymerase chain reaction (PCR) was performed for 25 µl containing 60 ng DNA, 12.5 µl of GoTaq Green Master Mix (Promega) and 2µl of Forward and Reverse primers (0.25 µM), and 6.5 distilled water. The amplifications were carried out using APOLLO Thermo cycler (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 (depending on the primer table 2), extension for 1 min at 72 °C with a final extension for 10 min at 72 °C. Amplified PCR products were separated using 4% metaphor agarose, and

then the gels were stained by 5µl ethidium bromide (50 µg µL^{-1}) 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 µg/µl) in the buffer solution in which the samples were stored.

DNA of chickpeas were analyzed using 6 pairs of primers *LEA1, LEA2, Dhn1, Dhn2, Dhn3 , Dhn4*.

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

Morphological differences at a molecular weight between one locus alleles were high in some genotypes, while the others were at a high degree of agreement, and can be easily recognized at 4% metaphor agarose gel. Polymerase chain reaction (PCR) for genes (*Dhn3, Dhn4*) showed one morphological pattern (A) in most of the studied genotypes. Which is the medium concentration of genotype19 (Table 3).

Table (3). Morphological patterns of polymorphic results of PCR-reaction and the discovered alleles in the genes (*Dhn3, Dhn*4) within genotypes.

	Genotypes																							
Genes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Dhn3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	-	A	A	A	A
Dhn4	-	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	-	A	-	A	A	A

Three morphological patterns (A, B, C) of *LEA* gene (*LEA1*) were shown in the genotypes (Table 4, Fig.1), and those patterns have been varied in appearance between the genotypes. These patterns were not detected in genotypes (19, 20), two patterns were shown in the genotypes (6, 16, 17), and three different pattern (different in the molecular weight) were shown in the genotypes (7, 8), while one pattern was shown in the rest of genotypes.

Table (4). Morphological patterns of polymorphic results of PCR-reaction and the discovered alleles in the genes (*LEA1*) within genotypes.

	Genotypes																							
Gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
LEA1	-	-	-	-	-	-	A	A	-	-	-	-	-	-	-	A	A	-	-	-	-	-	-	-
	-	-	-	-	-	B	B	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	-	-	C	C	C	C

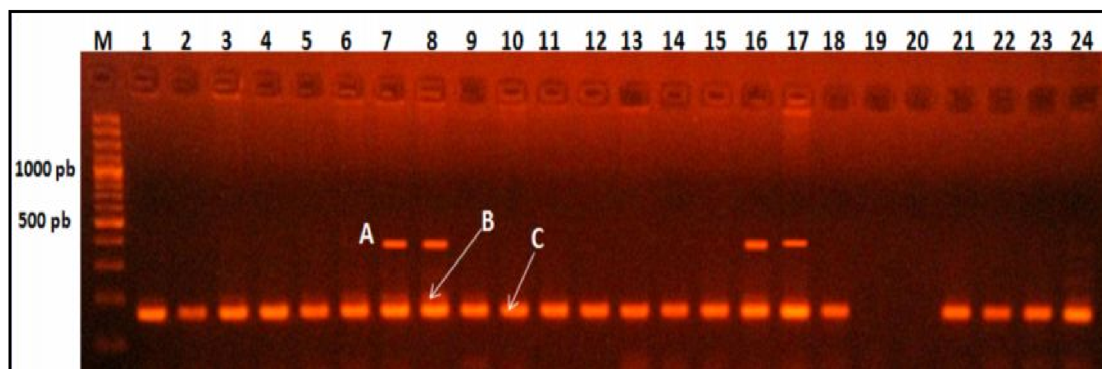

Fig. (1) Agarose gel 2% and the discovered morphological patterns for *LEA1* gene within the genotypes.

For the *LEA2*, variations of the morphological patterns resulted from the PCR were high, as seven patterns (A, B, C, D, E, F, G) were observed. These patterns were not detected in genotypes (19, 20, 24). One pattern was observed in three genotypes (4, 18, 22, 23), three patterns in genotype (14), while it were four patterns in genotypes(16, 21), and five patterns were observed in the rest genotypes. (Table5, Fig2).

Table (5). Morphological patterns of polymorphic results of PCR-reaction and the discovered alleles in the gene (*Dhn4*) within genotypes.

	Genotypes																							
Gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
LEA2	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A	A	A	-	-	-	A	-	-	-
	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B	B	B	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-
	D	D	D	-	D	D	D	D	D	D	D	D	D	-	D	-	D	-	-	-	-	-	-	-
	E	E	E	-	E	E	E	E	E	E	E	E	E	-	E	E	E	-	-	-	E	-	-	-
	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	-	-	F	F	F	-

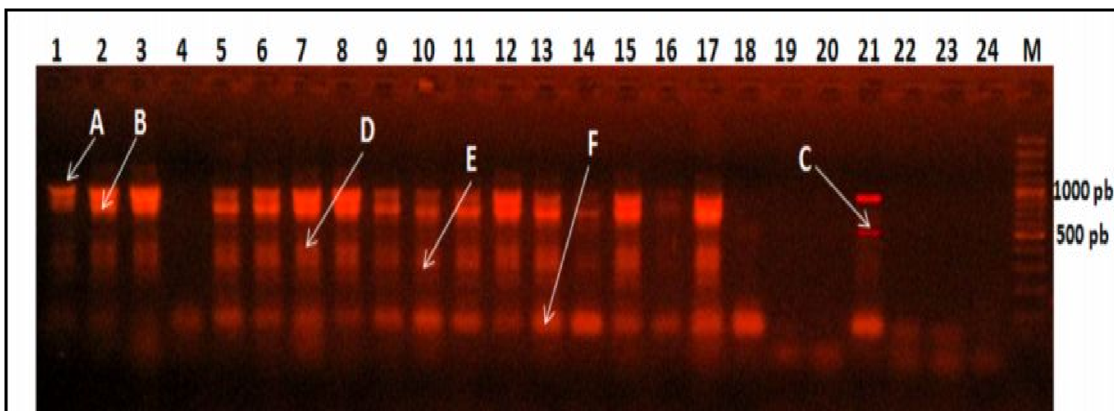

Fig. (2) Agarose gel 2% and the discovered morphological patterns for *LEA2*gene within the genotypes.

For the *Dhn1*, PCR results showed 2 morphological patterns (A, B) (Table 6). All genotypes had one pattern.

Table (6). Morphological patterns of polymorphic results of PCR-reaction and the discovered alleles in the genes (*Dhn*1)) within genotypes

	Genotypes																							
Gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Dhn1	A	-	A	A	-	-	A	-	-	A	-	-	A	-	-	-	A	-	-	-	-	-	-	A
	-	B	-	-	B	B	-	B	B	-	B	B	-	B	B	B	-	B	B	B	B	B	B	-

Two morphological patterns (A, B) were observed in *Dhn1*, these patterns were not detected in genotypes (14, 19, 20), two of them were observed in genotypes (17, 18, 21, 24), while one pattern show up in the rest of genotypes (Table 7, Fig 3).

Table (7). Morphological patterns of polymorphic results of PCR-reaction and the discovered alleles in the genes (*Dhn8*)) within genotypes

	Genotypes																							
Gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Dhn2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	A	-	-	A	-	-	A
	B	B	B	B	B	B	B	B	B	B	B	B	B	-	B	B	B	B	-	-	B	B	B	B

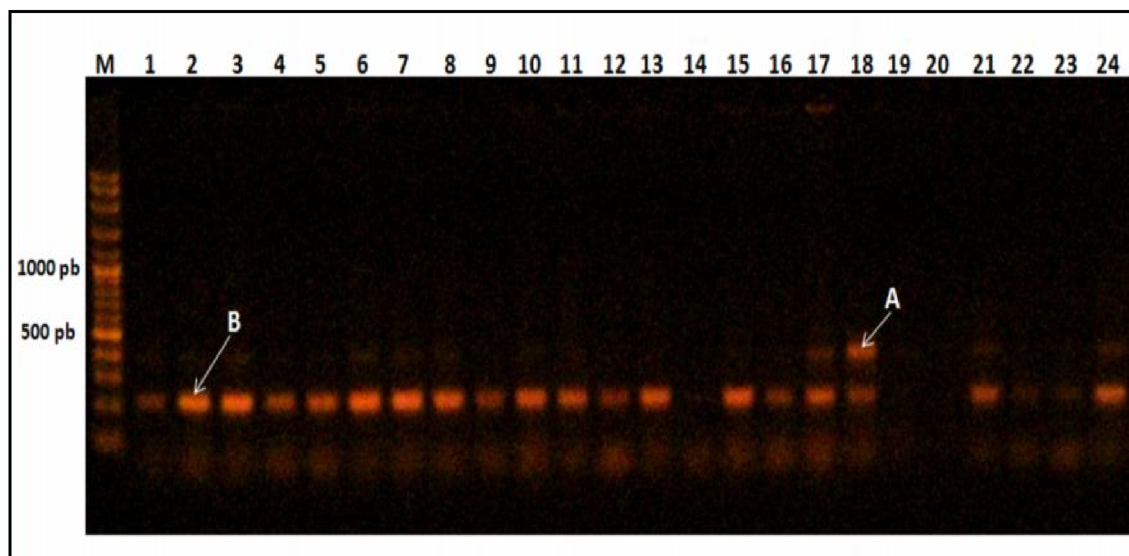

Fig. (3) Agarose gel 2% and the discovered morphological patterns for *Dhn2* gene within the genotypes.

PCR- reaction allowed detecting the morphological variations of DNA fragments for the genetic loci of the studied *LEA* and Dehydrin genes, 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 can be noticed from (Table 8) that the superior *LEA* gene (*LEA2*) compared to the other genes depending on the morphological patterns, gave 85 morphological patterns for all studied genotypes, while the gene (*Dhn4*) gave the lowest number of morphological patterns (22 morphological patterns). The genotypes (7, 8, 17) had the highest number of morphological patterns in all studied genotypes which counted 12 morphological patterns, and the genotypes (19, 20) gave the lowest number of morphological patterns which was only 2 patterns.

Table(8). Number of morphological patterns of *LEA* and Dehydrin genes for the studied genotypes

		Genotypes																							
Gene		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
LEA1	29	1	1	1	1	1	2	3	3	1	1	1	1	1	1	1	2	2	1	0	0	1	1	1	1
LEA2	85	5	5	5	1	5	5	5	5	5	5	5	5	5	3	5	4	5	1	0	0	4	1	1	0
Dhn1	24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Dhn2	25	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	2	2	0	0	2	1	1	2
Dhn3	23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
Dhn4	22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1
Total		10	10	10	6	10	11	12	12	10	10	10	10	10	7	10	10	12	7	2	2	9	6	6	6

Conclusions:

From the above results it could be concluded:

1. Six *LEA and* Dehydrin genes proved to be responsible for drought tolerance.
2. *LEA2* gave the highest number of morphological patterns (85 patterns), while Dhn4 gave the lowest number (22 patterns).

Recommendations:

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

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