ALLELIC VARIATIONS OF SOS, HVNHX AND SDO GENES IN SOME BARLEY (*Hordeum Vulgare* L.) GENOTYPES

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ABSTRACT

This study has been carried out at the Biotechnology Lab., Department of Field Crops, Faculty of Agriculture, Damascus University during the growing season 2019 - 2020, in order to detect the variations of SOS, HVNHX and SDO genes in different barley genotypes. Clear variations in the SOS, HVNHX and SDO genes, which are responsible for salinity tolerance were found among the investigated genotypes. It has been found that the variation in the amplicon size between loci per gene was very high in some cases, while there was a high degree of symmetry in other cases, and could be easily distinguished on 2% Agarose gel. The PCR results for the SOS genes (SOS1, SOS2, SOS3), HVNHX genes (HVNHX1, HVNHX2, HVNHX3) and SDO genes (Cu/Zn SODII, Cu/Zn SODI, CAT, GRI , APXIII) have shown only one morphological pattern in most of the studied genotypes, while revealed two patterns for the SOS3 gene, but the rest of genes (HVNHX1, HVNHX2, HVNHX3 Cu/Zn SODI, CAT) exhibited only one morphological pattern. The SOS3 was superior in the number of polymorphic patterns, as the number of total patterns was 14 in all the studied genotypes, but the Cu/Zn SODI showed the least number of polymorphic patterns with only 1 pattern, while the largest number (7 patterns) was detected in the genotype (H9), but the two genotypes Fourat9 and Fourat7 showed only one polymorphic pattern.

Key words: barley, alleles variation, SOS, HVNHX, Cu/Zn SODI, CAT.

التباين الأليلي لمورثات SOS و HVNHX و SOD في بعض طرز الشعير الشعير (*Hordeum vulgare* L.)

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المستخلص

أجريت الدراسة في مختبر التقانات الحيوية في قسم المحاصيل الحقلية – كلية الزراعة – جامعة دمشق، خلال الموسم الزراعي 2019–2020، بهدف الكشف عن التباينات الأليلية لمورثات *SOS* و *HVNHX* و *SDO* في الطرز الوراثية المدورسة من الشعير. أظهرت نتائج الدراسة تبايناً واضحاً في مورثات *SOS* و *HVNHX* و *SDO* المسؤولة عن تحمل الملوحة في الطرز الوراثية المدروسة. وقد اختلفت الانماط الناتجة بطول المورثة وذلك بين نظائر الموقع الواحد، حيث كانت كبيرة أحياناً، بينما كانت على درجةٍ عاليةٍ من التماثل في البعض الآخر، وأمكن تميزها بسهولة على هلامة ميتافور آغاروز 2%. أظهرت نتائج PCR لمورثات *SOS* (SOS1, SOS2, SOS3) ومورثات *HVNHX* (*HVNHX1* و *HVNHX2* و*HVNHX3*) ومورثات *SDO* (*SODII Cu / Zn* و*SODI Cu / Zn*) و *CAT* و *GRI* و *APXIII* نمطًا شكلياً واحدًا فقط في معظم الطرز الوراثية المدروسة. وأظهرت النتائج وجود نمطين للمورثة *SOS3*، بينما أظهرت نمطاً شكلياً واحد في باقي المورثات. تفوقت المورثة *SOS3* بعدد الأنماط الشكلية، حيث كان عدد الأنماط الكلية نحو 14 في جميع الطرز الوراثية المدروسة، في حين أعطت المورثة *Cu/Zn SODI* نمطاً شكلياً واحداً فقط، كما أظهرت النتائج تفوق الطراز الوراثي *H9* بعدد الأنماط التي أعطتها (7 أنماط)، في حين أعطى الطرازان فرات9 وفرات7 فقط نمطاً واحداً.

الكلمات المفتاحية: الشعير، تباين الأليلات، الأنزيمات المضادة للأكسدة.

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INTRODUCTION

One of the major challenges that mankind faces are the ability to feed the ever-growing population, especially in the face of increased stresses due to climate change and reduced availability of arable land (19, 32). Drought and salinity are the two major abiotic stresses, affecting agricultural production and threat plant biodiversity in arid and semi-arid environments (4). Soil salinity imposes an agricultural and economic burden that may be alleviated by identifying the components of salinity tolerance in barley, a major crop and the most salt tolerant cereal. Barley is one of the most adaptive cultivated cereal crops under stressful conditions in the world, which can produce even under harsh environmental circumstances. Barley (*Hordeum vulgare* L.) is one of the oldest cereal crops known to be cultivated since about 10,000 years in a region located between the Nile (Egypt) and Tigris Rivers (Iraq), also including Southern Turkey, Lebanon, Jordan, and Syria (26). Barley is classified as the fourth most important world cereal crop taking into account both quantity produced and cultivated area, after bread wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.) (14). It is mainly used as food and animal fodder, as well as for malting purposes. It provides an excellent source for genome mapping and genetic studies (31). Barley inherently exhibits a higher level of abiotic stress tolerance than many other crops (25, 29), which offers the possibility to extend its future production to areas suffering from climate change. Hordeum spp. are grown in the Mediterranean region due to high tolerance against heat, drought, and salinity compared to other small grains (41). Barley is considered as an important cereal crop in several developing countries, including Syria, but unfortunately it is often exposed to severe drought and salinity stresses that considerably cause a remarkable decline in the production capacity (6, 13). It has a natural tolerance to drought, salinity, and fungal diseases, thus making it a model organism in stress biology research. Indeed, a barley plant was shown to complete its life-cycle before using all the available soil water, even in high salt concentrations and defined as the most salt-tolerant cereal (26). One of the factors behind the natural tolerance of barley to abiotic stresses is early flowering, which ensures that pollination, seed development, and maturation occur in an optimum time period (37). Soil salinization is a limiting factor in crop production that affects at least 20% of irrigated lands, a number which is bound to increase due to poor irrigation practices and intrusions of groundwater caused by rising sea levels (17, 15). While many crops grow or yield poorly in saline soils, barley has been deemed the most salt-tolerant cereal crop (25). Although barley is a salt-tolerant field crop, its growth and development is severely affected by ionic and osmotic potential (ψs) in predominantly saline soils (20, 16). Salinity significantly reduces the production potential of most crops including barley also and can result in disruption of osmotic effects, ion-specific stress, ionic imbalance, and oxidative stress (35). The wealth of knowledge gathered on barley genetics, genomics, diversity, genetic transformation, and stress responses makes this crop a platform for dissecting tolerance mechanism that can be then exploited in other crops, particularly cereals. Furthermore, the relatively simple diploid genetics of barley and the tight relationship between the members of the Triticeae tribe facilitate the transfer of knowledge gained from barley research to other major cereals, for instance, bread wheat, durum wheat and rye (10). Barley germplasm show a great extent of variability in salinity stress tolerance (8, 10). Knowledge of the molecular basis of stress tolerance and adaptation is essential to develop crop cultivars with improved stress tolerance. Overexpression of Na^+ transporters (HvHKT2;1) were shown to contribute to the regulation of Na^+/K^+ homeostasis in barley during high salinity stress (22). In fact, K^+ retention ability and limitation of Na^+ uptake partially explains the tolerance of barley to ion toxicity and high salinity (2). Plants have developed efficient strategies to maintain ion concentration in the cytoplasm at low levels. Transporters such as Na^+/H^+ and K^+/H^+ antiporters (NHXs), sucrose transporters and amino acid transporters have important roles to keep this balance. A group of transporters including NHXs, high affinity K^+ transporters (HKTs), and salt overly sensitive1 (SOS1)

have been shown to maintain intracellular ion and pH homeostasis, and also contribute to the regulation of a wide variety of physiological processes associated with growth and development (5). Transgenic barley lines overexpressing a sub family HKT transporter (HvHKT2;1) has been showed improved biomass production under salts tress (100 mM NaCl) probably through Na^+ exclusion or accumulation of excessive Na^+ in the leaves (22). Three main mechanisms of salinity tolerance have been proposed: (i) osmotic tolerance, i.e., "shoot ion-independent tolerance", (ii) ion exclusion from the shoot, and (iii) tissue tolerance (33, 36, 3). To determine the genetic basis for barley's salinity tolerance, several forward genetics studies have explored the impact of salinity on these three mechanisms. Osmotic stresses (drought and salinity) reduce assimilation rates, as they decrease stomatal conductance, disrupt photosynthetic pigments, reduce gas exchange, enhance production of reactive oxygen species, and lead to decreased plant growth and productivity (12). In general, breeding of stress-tolerant crops is the most efficient strategy to maintain productivity under conditions of environmental stress. It is thus necessary to screen the available genetic resources and understand their homeostatic mechanisms. Numerous studies have shown that salt stress leads to oxidative stress in plants as a result of increased reactive oxygen species (ROS), such as superoxide (O.-), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO^{-}), which are harmful to proteins and lipids involved in the cytoplasmic membrane and nucleic acids (27, 28). To reduce the harmful effects of ROS, plants have developed enzymatic and non-enzymatic antioxidant systems (1), such as SOD superoxide dismutase, this enzyme includes three types, according to attachments, of metal ions, such as Mn-SOD in mitochondria, Fe-SOD in chloroplasts, Cu/Zn-SOD in cytoplasm and chloroplasts (24, 23). Catalase (CAT), which breaks down H_2O_2 into water H_2O at different cell sites (11, 21), and glutathione reductase (GR) that catalyzes the reduction of glutathione disulfide (GSSG) to sulfohydryl (GSH) (40). The Salt Overly Sensitive (SOS) signaling pathway has a key role in exporting Na^+ through the Na^+/H^+ antiporter system. Cytosolic Ca^{2+} accumulation results in increased SOS3 accumulation, which binds to Ca^{2+} activating the protein kinase SOS2. The SOS3-SOS2 complex increases SOS1 expression and activates the SOS1 protein, a Na^+/H^+ antiporter that exports Na^+ from the cell (30, 34, 39). Generally, the discovery of new genes, the determination of expression patterns to abiotic stress and a better comprehension of their role in stress adaptation provide the basis for new strategies to improve the tolerance of cultures to stress (9, 7, 18).

MATERIAIS AND METHODS

Experimental site and time of study: This study was conducted at the laboratory of biotechnology affiliated to the Faculty of Agriculture, Damascus University, during the year 2019 - 2020.

Plant material: The investigation has been carried out on 14 Barley genotypes (H3, H5, H9, H17, H20, Araby Aswad, Improved Araby Abiad, Fourat4, Fourat5, Fourat6, Fourat7, Fourat9, Vulgare and Spontaneum), which were obtained from the General Commission for Scientific Agricultural Research (GCSAR).

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 (Murray and Thompson, 1980). DNA quality was determined using 1% agarose gel and then quantified by spectrophotometer, and DNA concentration was adjusted to 40 ng µL-1 to be used in the SSR reactions. SOS and HvNhx genes primers were developed. The primer sequence (Designed by Primer Premier 3.0) is shown in Table (16 selected depending on their chromosomal locations, and the primers were obtained from the Syrian Atomic Energy Commission, the details of selected SSR primers are presented in Table 1. Polymerase chain reaction (PCR) 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 (38). 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 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.

Table1. 11 pair of SSR primers and their sequences

Genes	Annealing Temp. (C°)	Forward Primers	Reverse Primers
Hv-NHX1	55	TGCATATCTACCAGTGCTTAT	GGTTCAAGACACA AGTTCAGT
Hv-NHX2	57.9	GGTTTTCGGCTTGCTGACTAA	CATTGGGCGCATGAACTTATC
Hv-NHX3	55	TGAGCCGAACATTACTGTGAT	ACGAGCTTACCTTTCAATACA
SOS1-1	51	GAAGAACTTTCGATGCAGGA	ATTTCCCAGAAATGATGCAA
SOS1-2	55.3	TGGACAGATTAGCAGCAACA	TTGGGTAGGAACAAGATCCA
SOS1-3	57	GCTCTAATAAAGCGCACAGC	CCAACAATTACTTGGGTTGCGGGCTTCA G AAACTGACAGA
Cu/Zn SODII	52	TTGCATTTCAACTGGACCAC	AAGCCACACCCATCCAGAC
Cu/Zn SODI	54	AGCTACTCTGCCACCAGCAT	GCTTCCATATCCAGTCCTTG
CAT	53	CTCCCACCTTAATGGCCTCT	CCTGTCATTGTGCGTTTCTC
GRI	54	AGCAAACTCCAAGGCAATGT	GAAATTGCTAGTCTATGCGTAC
APXIII	54	AGGACATTGGTCAGGTCCAG	CTTCTCCAGCCGATCAAAGA

RESUITS 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. DNA concentrations were between 0.26-0.45 ng μl^{-1} in the buffer solution in which the samples were stored. DNA of barley were analyzed using 11 pairs of SSR primers (SOS1, SOS2, SOS3, HvNHX1, HvNHX2, HvNHX3, Cu/Zn SODII, Cu/Zn SODI, CAT, GRI and APXIII). Results showed differences among DNA amplified fragments for one locus in the studied genotypes, and these differences reflect genetic variation at the level of one locus, as it has been showed the presence of different alleles on the same locus. Morphological differences at an amplicon size (bp) 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 2% agarose gel. The polymerase chain reaction (PCR) for the genes (HvNHX1, HvNHX2, HvNHX3) showed one morphological pattern (AA) in most of the investigated genotypes. It has been noted that morphological pattern (A) appeared with the gene HvNHX1 in the genotypes Fourat4, Fourat5, Fourat6, improved Araby Abiad, vulgare, Spontaneum, H3, H5, H9, H17 and H20, while this pattern was not detected in the barley genotypes Fourat7, Fourat9, and Araby Aswad. One morphological pattern (A) was observed in the gene HvNHX2, this pattern occurred in the barley genotypes Fourat4, Fourat9, Araby Aswad, improved Araby Abiad vulgare, Spontaneum, H3, H5, H9, H17, and H20, while it was not detected in the barley genotypes Fourat5, Fourat6 and Fourat7 (Table 2). Also morphological pattern (A) was observed in the gene HvNHX3, this pattern was found in the barley genotypes Fourat4, Fourat5, Fourat6, Fourat7, Vulgare, Spontaneum and Araby Aswad, while it was not detected in the barley genotypes Fourat9, improved Araby Abiad, H5 and H20.

Table 2. Morphological patterns of polymorphic results of PCR-reaction and the alleles discovered in the genes (HvNHX1, HvNHX2, HvNHX3) within genotypes

Genes	Genotypes														No. of Alleles
	Fourat4	Fourat5	Fourat6	Fourat7	Fourat9	Araby Aswad	Improved Araby Abiad	Vulgare	Spontaneum	H3	H5	H9	H17	H20	
HvNHX1	AA	AA	AA	--	--	--	AA	AA	AA	AA	AA	AA	AA	AA	11
HvNHX2	AA	--	--	--	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	11
HvNHX3	AA	AA	AA	AA	--	AA	--	AA	AA	AA	--	AA	AA	--	10

Two morphological patterns (A, B) of SOS gene (SOS3) occurred in the genotypes Fourat5, H3, H9 and Spontaneum (Table 3, Fig.1), and those patterns varied in appearance among the investigated genotypes. These patterns were not detected in the genotypes Fourat4, Fourat7, Fourat9 and H20. The patterns A was shown in the genotypes Fourat5, Spontaneum, H3 and H9, while the pattern B was shown in Fourat5, Fourat6, Spontaneum, improved Araby Abiad, Vulgare, Araby Aswad, H3, H5, H9, and H17, while it was not detected in the rest of the studied genotypes.

Table 3. Morphological patterns of polymorphic results of PCR-reaction and the alleles discovered in the genes (SOS3) within genotypes

Genes	Genotypes														
	Fourat4	Fourat5	Fourat6	Fourat7	Fourat9	Spontaneum	Improved ArabyAbiad	Vulgare	Araby Aswad	H3	H5	H9	H17	H20	No. of Alleles
SOS3	--	AA	--	--	--	AA	--	--	--	AA	--	AA	--	--	4
	--	BB	BB	--	--	BB	BB	BB	BB	BB	BB	BB	BB	--	10

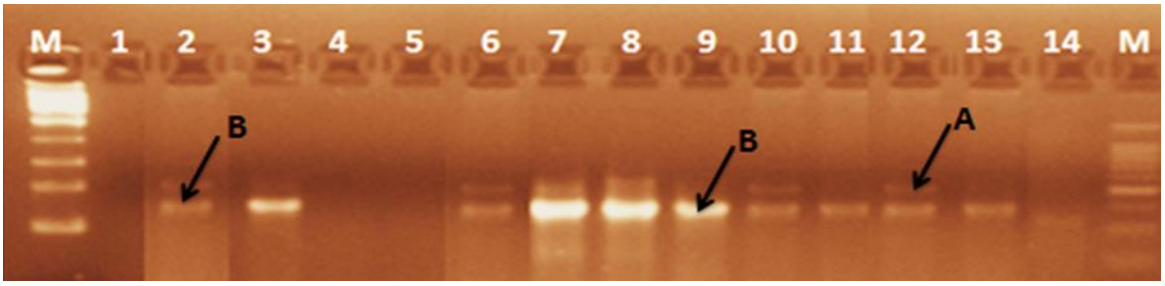

Fig 1. Agarose gel (2%) and discovering morphological patterns for *SOS3* gene within barley genotypes.

(1: Fourat4, 2: Fourat5, 3: Fourat6, 4: Fourat7, 5: Fourat9, 6: Spontaneum, 7: Araby Aswad, Improved, 8: Vulgare, 9: Araby Abiad,10: H3, 11: H5, 12: H9, 13: H17, 14: H20).

Regarding the ***Cu/Zn SOD1*** one pattern was observed in the genotype H9, while it was not detected in the remaining studied barley genotypes. (Table, 4). On the other hand, one morphological pattern (A) was observed in *CAT*, this pattern was showed in two genotypes (H9, H17), while it was not detected in the rest studied barley genotypes. (Table 5).

Table 4. Morphological patterns of polymorphic results of PCR-reaction and the alleles discovered in the gene (Cu/Zn SOD1) within genotypes

Genes	Genotypes														
	Fourat4	Fourat5	Fourat6	Fourat7	Fourat9	Spontaneum	Improved ArabyAbiad	Vulgare	Araby Aswad	H3	H5	H9	H17	H20	No. of Alleles
Cu/Zn SOD1	--	--	--	--	--	--	--	--	--	--	--	AA	--	--	1

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

Genes	Genotypes														
	Fourat4	Fourat5	Fourat6	Fourat7	Fourat9	Spontaneum	Improved ArabyAbiad	Vulgare	Araby Aswad	H3	H5	H9	H17	H20	No. of Alleles
CAT	--	--	--	--	--	--	--	--	--	--	--	AA	AA	--	2

For the Cu/Zn SOD11, *GR*1, APX 111, SOS1, SOS2, PCR results did not detect any morphological patterns in all the investigated barley genotypes. PCR-reaction allowed detecting the morphological variations of DNA fragments for the genetic loci of the studied *SOS3*, *HvNHX*, *Cu/Zn SOD1*, *CAT* genes, and these variations were caused by differences in amplicon size (bp) 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 genotypes, and the genetic differences for each locus. It can be noticed that the superior *SOS* gene (*SOS3*) compared to the other genes depending on the morphological patterns, gave 14 morphological patterns for all the studied barley genotypes, while the gene (*Cu/Zn SOD1*) gave the lowest number of morphological patterns (1) (Table, 6). The genotype H9 had the highest number of morphological patterns among all the other studied genotypes which (7), followed with the genotypes Araby Aswad ,H3 and H17) (5 morphological patterns), while the genotypes Fourat7 and Fourat9 gave the lowest number of morphological patterns (only 1).

Table 6. Number of morphological patterns of *SOS* , HvNHX, Cu/Zn SOD1, Cu/Zn SOD11, *GR*1, APX 111 and CAT genes for the studied genotypes

Gene	Genotypes														
		Fourat4	Fourat5	Fourat6	Fourat7	Fourat9	Spontaneum	Improved ArabvAbiad Vulgare		Araby Aswad H3	H5	H9	H17	H20
SOS1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
SOS2	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
SOS3	**14**	**0**	**2**	1	0	**0**	1	**1**	1	2	2	**1**	**2**	**1**	**0**
HvNHX1	11	1	1	1	0	0	0	1	1	1	1	1	1	1	1
HvNHX2	11	1	0	0	0	1	1	1	1	1	1	1	1	1	1
HvNHX3	10	1	1	1	1	0	1	0	1	1	1	0	1	1	0
Cu/Zn SOD11	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Cu/Zn SOD1	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**
CAT	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0**
GR1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
APX 111	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Total	49	3	4	3	1	1	3	3	4	5	5	3	7	5	2

Conclusions: from the above results it could be concluded that six *SOS*, *HvNHX*, *Cu/Zn SOD1and CAT* genes proved to be responsible for salinity tolerance. *SOS3* gave the highest number of morphological patterns (14 patterns), while *Cu/Zn SOD1* gave the lowest number (1 patterns).

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