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Research Article

Evaluation of Barley Genotypes Under Salinity Stress Conditions and their Molecular Characterization Using ISSR Markers

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Abstract:
The experiment was carried out in College of Education for Girls / Department of biology / University of Kufa 2023. The two genotypes of barley H9 and H20 were used derived from breeding and improvement programs in the fields of the Iraqi Atomic Energy Organization previously. The two sensitive varieties, Furat 6 and Furat 7, were used for the purpose of comparison. The increase in salinity at the 16 Ds. m-1 salt level led to a significant decrease in the germination rate for the two genotypes and the two local varieties used in the experiment, but there is a clear difference in the degree of effect, as it was The germination rate for the two genotypes H9 is 70% and H20 is 61%, while the germination rate was 24% in the Furat6 variety and decreased at its lowest levels to reach 15.5% in the Furat7 variety, which is considered the most affected by salinity. The results showed that the primers ISSR-UBC 818 and ISSR-UBC 840, ISSR-UBC 812 and ISSR-UBC 842, which were able to show a number of general bands in all the studied varieties, but failed to show distinctive bands.

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INTRODUCTION

Barley plants are characterized by their high adaptability (Hordeum vulgare L.) to a wide range of environments and breeding programs, genetic improvement, selection processes and expanded cultivation have helped in the production of thousands of commercial varieties (Von Bothmer, 1991., Jamil et al., 2011).

The barley crop is ranked fourth on the list of cereal crops in the world and comes in terms of economic importance, area and production after wheat crops wheat (Triticum ssp.), rice (Oryza sativa L.) and yellow corn (Zea mays L.) Martin[12].

Barley is one of the important crops in Asia, Africa, America and Europe, where the area cultivated with barley globally has reached approximately 47.01 million hectares. Barley is one of the very important small fodder grain crops, whose cultivation is successful in arid and semi-arid areas (Ceccarelli[6], 1994., FAOStat[9], 2018). Salinity causes a decline in the productivity of cultivated

crop species estimated at about 20% (Harris and Ashraf, 2005., Pirasteh-Anosheh, 2016). Salinity increases oxidative stress, as salt stress causes the production of free oxygen radicals (active), such as H2O2, OH, O2, which damage cytoplasmic membranes and lead to the death of plant cells Hernández[2].

The rapid progress in the sciences of molecular biology has created many means and methods that have been used in studies and evaluation of variations and relationships between genetic structures. One of the most important biotechnologies used is the polymerase chain reaction (PCR), due to its ease of use as well as its ability to replicate DNA In vitro and in very large quantities. Many modern biotechnologies based on this technology have emerged, including ISSR marker Cid-Contreras[7].

This Research Aims To: evaluation of salt tolerance in selected genotypes under salinity conditions at the germination stage in comparison with salt-sensitive varieties and determining the genetic variation in salt tolerance of selected varieties at the molecular level using the ISSR-PCR method.

MATERIALS AND METHODS

In this research, the two genotypes H9 and H20 were used derived from breeding and improvement programs in the fields of the Iraqi Atomic Energy Organization previously. The two sensitive varieties, Furat 6 and Furat 7, were used for the purpose of comparison. The research was carried out in College of Education for Girls/ Department of biology/University of Kufa 2023.

Samples were taken from the soil. Then it was dried, smoothed and sieved and certain proportions of saline and non-saline soil were mixed homogeneously to reach the salt level (0, 12, 16) dS. m-1 for the first experiment, as well as the salt level of 0 and 20 dS. m-1. The soil was placed in plastic pots. The seeds of the two genotypes and the two sensitive varieties were planted, with 8 seeds in one pot. The germination rate was measured for the first experiment at the three salt concentrations of 0, 12, and 16 dS.m-1. In the second experiment, the leaves of the two sensitive genotypes and varieties were taken from the salt level of 0 and 20. dS.m-1 for DNA extraction The DNA was extracted according to the Geneaid @ Genomic DNA Mini method with the Kit Plant extraction kit and the DNA concentration and purity were estimated by reading the sample's absorption of ultraviolet radiation with a UV spectrophotometer, which is used to measure optical density (O.D.) at the wavelengths 260 and 280 nanometers, then it were used in the ISSR-PCR program with 6 primers shown in Table (1).

Then several reactions were carried out to amplify the DNA and the program shown in Table (2) was the optimal program. After the reaction ended, the products were passed through an agarose gel in the electrophoresis device for a period of time. 1:30 hours with a 1X TBE Buffer solution, dyeing the gel with ethidium bromide dye for 30 minutes and examining the gel using an ultraviolet (UV) device to view the DNA band and then estimate its concentration and purity, as it was photographed using a Polaroid Black-White Film Type 667 device.

RESULTS AND DISCUSSION

The results shown in Table (3) indicate that salinity led to a significant decrease in the germination rate in the barley varieties used in the experiment, but there is a large difference in the degree of decrease. The results indicated that the germination rate was very high in the Control treatment and reached 100% in the composition. The genotype was H9 and 95% in the genotype 20H, As for the two local varieties, Furat6 and Furat7, the germination rate was 86.5% for both varieties, but there was a clear decrease in the germination rate for both the genotypes and the two local varieties at the salt levels of 12 and 16 dS.m-1, as the level was 16 dS.m-1 is the most influential in reducing the germination rate compared to the level of 12 dS.m-1 The salt level of 12 dS.m-1 had a slight effect on the two genotypes H9 and 20H, as the germination rate was 74% and 65%, respectively.

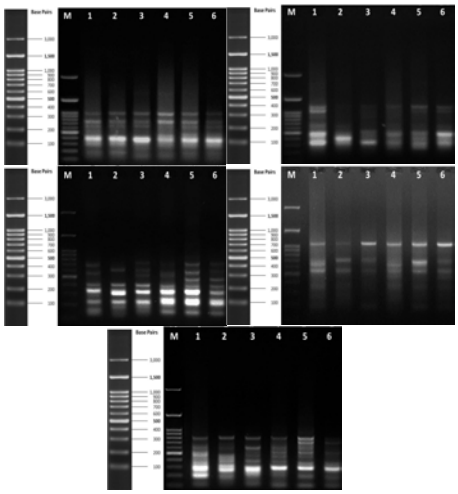

Fig. 1: PCR reaction products for 5 primers of barley genotypes on 0.8% agarose gel (V/cm, 1X TBE, h 1.30) imaged under U.V. after staining with ethidium bromide

Table 1: Names and sequences of ISSR primers used in this study.

Primer	Sequences (5' → 3')
ISSR-UBC 812	5' GA GA GA GA GA GA GA GAA 3'
ISSR-UBC 814	5' CT CT CT CT CT CT CT CTA 3'
ISSR-UBC 818	5' CA CA CA CA CA CA CA CAG 3'
ISSR-UBC 836	5' AG AG AG AG AG AG AG AG YA 3'
ISSR-UBC 840	5' GA GA GA GA GA GA GA GA YT 3'
ISSR-UBC 842	5'GA GA GA GA GA GA GA GA YG 3'

Table 2: PCR Programme for amplification.

Step	Temperature	Time
Initial Denaturation	94C°	5 min
No. of Cycles = 35 Cycles		
Denaturation	94C°	1 min
Annealing	50-55C°	30 sec
Extension	72C°	1 min
Final Extension	72C°	5 min

Table 3: Percentage of germination at different salt levels

	Germination %			
Varieties	**0**	**12**	**16**	**Mean**
H9	100	74	70	81
H20	94	65	61	73
Furat6	86.5	50	24	53.5
Furat7	86.5	50	15.5	50.7
Mean	91.75	59.75	42.63	
L.S.D.				
0.05	Salt= 11.49			
Genotype= 9.95				
Salt+Genotype= 5.75				

As for the two varieties Furat6 and Furat7, the percentage decreased. Germination rate is high, reaching 50% for both varieties. Table 3: Percentage of germination under salinity conditions for two genotypes and two sensitive varieties.

The four genotypes used responded similarly to increasing salinity concentration, as they were all in the direction of decrease with increasing concentration. The increase in salinity at the 16 dS.m-1 salt level led to a significant decrease in the germination rate for the two genotypes and the two local varieties used in the experiment, but there is a clear difference in the degree of effect, as it was The germination rate for the two genotypes H9 is 70% and H20 is 61%, while the germination rate was 24% in the Furat6 variety and decreased at its lowest levels to reach 15.5% in the Furat7 variety, which is considered the most affected by salinity. The high degree of salinity led to a decrease in the germination percentage for all Genotypes: The reason for this may be attributed to decrease water absorption by the seeds due to the high osmotic pressure of the soil solution (Pearson[14]). The results showed that there was a difference between the two genotypes and the local varieties in the degree of tolerance to salinity, as the H9 genotype was the most tolerant, while the Furat7 variety was the most sensitive, especially at the salt level of 16 dS.m-1.

The adverse effects of salinity on plant growth are by increasing the osmotic potential and the toxic effects of sodium (Na+) and chloride (Cl-). Subsequently, the uptake of water and nutrients by plant decreases and the function of nutrients, which are of chemical similarity to Na+and Cl-is interrupted by these ions (Munns[13]). Evaluating crop response under stress is a useful and promising tool for the development of tolerant crop genotypes. Accordingly, it is important to develop a suitable screening method for properly evaluating the tolerance of crop plants such as barley under salinity stress. Such a method can be used for the development of barley fields as well as the barley genotypes with higher salinity tolerance. This was confirmed by the ISSR results indicating the presence of genetic variation between the genotypes selected for the salt-tolerant trait and the salt-sensitive varieties.

To investigate genetic variation using the ISSR method. The primers were separated into groups based on the efficiency and discriminating ability of the primer, as the primer ISSR-UBC 814 failed to produce any general bands or distinctive bands for any of the studied varieties and under several salt concentrations despite changing the temperature at different levels and repeating the reaction several times. This may be the reason for this result. Due to the absence of sites complementary to the sequences of these primers on the genome of the studied species, that is, the absence of linkage regions between the primer and the DNA of the species under

study.

The primer ISSR-UBC 812 was able to show 3 general bands with sizes of 410, 700 and 1000 base pairs among the studied taxa, while ISSR-UBC 818 produced one general band with sizes of 260 base pairs and the primer ISSR-UBC 840 produced general bands with sizes of 490 and 700 and 1000 base pairs and the primer ISSR-UBC 842 produced a general band with a size of 580 base pairs, meaning that these primers failed to produce special bands that represent genetic variation between the two genotypes and the two sensitive types. The primer ISSR-UBC 836 showed two general bands with a size of 240 and 310 base pairs.

In addition to a distinct band with a size of 100 base pairs in the genotypes H9 and H20 and its absence in the same two genotypes in normal soil and in the two salt-sensitive varieties Furat6 and Furat7, this is an indication that this band may indicate the genetic expression of salt-tolerant genes that disappeared in the same genotypes. In normal soil, that is, these genes did not appear in the absence of salinity, but rather appeared clearly when it was high.

The method of analyzing the results of the study was based on the presence or absence of bands that result from the duplication of certain pieces of the genome of the species and genotypes used, as well as the sizes of those bands that depend on the number and complementary locations of the primer sequences on the template DNA strand, which differ from one primer to another (Al-Jubouri et al., 2009) as very small bands were neglected. This agrees with the results of Swoboda Bhalla[15] that the obtained duplicate bands, which vary in size depending on the primer design, are identical to what was reported by Dalmasso[8], who confirmed that the type of gene that A primer is designed to affect the size of the duplicated bundles. These primers have contributed to determining genetic variation between varieties through the absence and appearance of the double bundles, and their number and size.

The absence of bands in the primer ISSR-UBC 814 and their appearance in the form of a smear indicates that they did not find complementary sites on the DNA, that is, the absence of sites complementary to the sequences of those primers in the DNA of the barley varieties used. This case is common and it agrees with the findings of Yassin (2011) During his study on date palm, several primers were unable to show any replication products and were identical to what Zakaria[3] mentioned about the failure of primer A13 to replicate DNA in all the studied genotypes of wheat plants.

The results showed that the primers ISSR-UBC 818 and ISSR-UBC 840, ISSR-UBC 812 and ISSR-UBC 842, which were able to show a number of general bands in all the studied varieties, but failed to show distinctive bands. This may be a result of the absence of a genetic mutation indicating genetic polymorphism, meaning that the primers did not give complete results when Using these results are consistent with other results reached by Al-Jubouri[1] and thus the primers were associated with complementary regions of the Conserved Sequence (DNA).

It is worth noting that these primers did not show any variation between the species studied, and these general bands indicate the presence of a piece of DNA shared between the studied varieties, while the primer ISSR-UBC 836 was able to show a discriminating ability between the studied varieties and gave clear replication products in the genotypes H9 and H20 under salinity conditions, as it showed two general bands with a size of 240 and 310 base pairs in the tolerant and sensitive barley varieties. For salinity, while one distinct band with a size of 100 base pairs appeared in the genotypes of salt-tolerant barley 9H and H20 under salinity conditions, and it was lost in the same two genotypes and in the local varieties Furat6 and Furat7 in the absence of salinity, as this band can be considered a positive indicator that may be responsible for genetic variation. For the trait of salt tolerance in the studied barley genotypes, which indicates that this band is related to the tolerance of these two genotypes, as showing this band in the two genotypes under salinity conditions only may lead to the stimulation of salt-tolerant genes, and thus show the gene expression of these genes and this As a result of the interaction between genetics and environment (AL-Mishhadani[4]).

By reviewing and discussing the results, it can be said that the need to produce salt-tolerant varieties capable of growing and producing in conditions of high salinity is an urgent necessity as a result of the increase in saline lands and lands affected by salinity. These salt-tolerant varieties are able to grow and produce when the percentage of salts in the soil is high, especially sodium ions. The results of the study confirmed that the two salt-tolerant genotypes showed a higher germination rate than the control varieties Furat6 and Furat7 under conditions of salt stress.

Through this study, we conclude that it is possible to perform a PCR reaction with a number of primers and using ISSR indicators to obtain distinctive bands that appear in a certain type and not in other types to find the

genetic fingerprint of that type (Wang[16]) and that the reason for the discrepancy between barley varieties is the difference in The duplication products of the PCR reaction may be due to the difference in the sequence of the primers, as the difference in one nitrogenous base leads to a difference in the sequence of the primers used or to the difference in the target regions in the DNA. This difference in the percentage of divergent bands may be due to the difference in the arrangement of the nitrogenous bases.

In the genome of the studied genotypes, or because of the genetic origin from which the genotypes descended, which is affected by the genetic origin from which they arose, or the difference in the design of the primers used in the ISSR-PCR reaction, as it is very common within the target gene (Ali[5]), the 100 base pair bands can be considered in this primer, ISSR-UBC 836 emerged as a good and positive indicator for the salt-tolerant genotypes 9H and H20 and it may be responsible for the salt-tolerant character of these two genotypes. This variation cannot be considered a definitive indicator, especially since ISSR-PCR depends on a specific short sequence of the primer compared to the genome of There are many sequences and thus a higher percentage of variations can be obtained if primers with sequences different from these studied sequences are used. The results also show that the salt-sensitive varieties were similar in the number of bands and their locations and differed with the 9H and H20 genotypes, which showed a clear difference in salinity conditions. These results similar to (Shoman *et al.,* 2001) to determine genetic variation in barley plants and other results of (Al-Faqi[2]).

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