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Biochemical and Molecular Depiction for Salt Stress Tolerance in Barley (*Hordeum vulgare* L.)

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Abstract

The problem of exacerbating high levels of salinity should be considered greatest challenges facing Egyptian agriculture, especially in light of the limited water allocated for irrigation. Based on this, the Egyptian state has taken effective strategic steps through specialized institutes and research centers to improve the degree of tolerance of field crops to salt stress, especially the barley crop. The aim of the present work has concentrated on unraveling the genetic and biochemical factors and genetic markers that might be responsible or linked to salinity tolerance in barley. Some agro-morphological and biochemical attributes were the most studied parameters under both conditions. Profile of ISSR primers was used for comparing the ten barley genotypes (5 parents besides the best 5 crosses) confirming salinity tolerance in all studied traits under both experiments. The final results revealed the importance of the three first parents and the highly five promising crosses were recorded highly rank of salinity tolerance in this regard. Molecular markers have succeeded in reaching molecular genetic differences using ISSR primers which can be a taxonomic basis for determining tolerant barley genotypes to salinity and the other sensitive accessions. Where, the polymorphism % closed to 100% within using the ISSR primers; SR-03, SR-12 and SR-21 besides, generating 30 unique bands through all ISSR primers. Thus, the greatest benefit of this study may have been achieved, which is the strategy for improving barley crop to confront the danger of salt stress, which has become a major environmental problem, especially in the northern Delta regions. Further, its direct obstruction of agricultural development paths in Egypt.

Kew words: - Barley, Salinity tolerance, Molecular Markers, Genetic Parameters, Biochemical attributes.

1. Introduction

The issue of food security and the priorities for preserving it are considered, the barley crop may be among these top priorities in our lives. This strategic crop is known that it is a plant species around it belonging to the Gramineae family and was one of the main food crops that a human used in their food where bread was made from. However, the erosion of the vast majority of agricultural lands due to salt stress may be a very serious factor in reducing the yields of field crops, especially barley. Given the great strategic importance of this crop, as it represents a bright future for contributing to the production of Egyptian bread, it was necessary to move forward with serious scientific and practical attempts with the aim of confronting the environmental stresses that threaten its productivity, especially salt stress. Despite this, the volume of scientific contributions in this regard is still very small and does not meet the purpose. This crisis requires more concerted in order under Egyptian conditions. This point in particular represents a maximum strategy for the Egyptian state, especially in light of the limited water allocated for irrigation. This is what we will address in some detail, reviewing the most important results of research and studies that discussed this serious crisis, while identifying the most important recommendations with the aim of producing highly productive barley lines that are tolerant of salt stress. The results of many scientists and researchers indicated a noticeable decline in the productivity of wheat and barley crops due to salt deposits in the soil prepared for agriculture **[1 , 2]**. The process of potassium diffusion had the greatest impact in determining the extent to which salt tolerance occurs by estimating the ability to spoil, based on the sodium chloride salt in the roots of six barley lines in reciprocal crosses **[3].** The strategy of decreasing pH values in some barley accessions under salinity conditions has succeeded in indicating a clear physiological sign, which is the closure of stomata based on this physiological matter. There is a decrease in especially in the seedling stage, which greatly helps to raise resistance to high degrees of salinity **[4 , 5].** Some genotypes of wheat and barley have shown excellent genetic mechanisms that provide evidence of tolerance to environmental stresses, especially high salinity and scarcity of irrigation water. This evidence has been characterized in barley entries and transferred through plant breeding

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methods for the wheat crop **[6].** Experiments on two Syrian barley varieties under salt stress also demonstrated a close relationship between the metabolic process and the carbon dioxide transfer system by closing the stomata. As well, the ideal gas exchange during these conditions **[7].** Promising and excellent results appeared regarding tolerance to salt stress in some barley genotypes under salinity and control experiments and these results were confirmed after analyzing the water soluble protein, molecular markers and QTLs for those promising barley accessions **[8 , 9].** Further, the mechanism of genetic correlation between quantitative traits useful to breeders has also proven to be highly effective in localizing the physiological traits responsible for salinity tolerance in these promising genotypes in this regard. Also, ten ISSR and SSR markers succeeded in identifying tolerant barley varieties over sensitive ones **[10].** Therefore**,** a large number of barley entries detected great genetic variability in varying degrees of salt tolerance, especially at the seedling stage **[11].** The physiological measurements, genetic parameters and salinity tolerance indices were a bright indication in proving the mechanisms of tolerance to environmental stress, especially salt, in some superior barley entries **[12].** Evaluating of salinity tolerance using tolerance indices parameters in some barley genotypes under field conditions was done by **[13].** Recent and repeated studies have significantly proven that barley accessions found under salinity conditions have an increased amount of proline, glycine betaine, and trehalose contents as indicators of tolerance. Further, increasing in both sodium and calcium levels due to the saline environment **[14].** New and useful information has emerged to detect and screen the genetic makeup of barley crop to should be obtain the most resistance to salinity, especially in the early life stages of the seedlings. These mechanisms have contributed to reaching the highest genetic characterization techniques for these lines in search of resistance genes **[15].** After this fruitful and thorough review of the highest positive papers in this context. All these intense scientific efforts crystallize the importance of genetic studies to improve barley crops to confront environmental stresses, and this is an ideal goal for this work. As a serious attempt to bridge the bread production gap in Egypt. Further, the Egyptian bread production crisis has become one of the largest crises affecting Egyptian food and national security. This imminent danger coincides with the boom in Egyptian barley productivity and the increasing hopes placed on its abundant production in order to fill this great gap in Egyptian bread productivity. This was the clear strategy in this investigation, which was a quick response to this imminent environmental threat represented by increasing degrees of salt stress, especially in the valley and delta regions affected by the highest levels of salt deposition due to limited irrigation water, especially in the summer of the Arab Republic of Egypt. Also, a large number of research and studies have been successfully completed in the past few years, the aim of which was to improve the quality and quantity of Egyptian barley. Further, raising the degree of its withstand, such as drought and salinity besides, its tolerance of the toxicity of heavy metals to safe levels through which it can the plant completes its life and gives the highest yield, and these efforts are considered constructive efforts in this regard. In this way, we will have come a long way in the path of genetic improvement of barley to tolerate salt stress through traditional and biotechnological plant breeding methods, which have yielded promising results by obtaining a group of barley hybrids that excel in this regard. Further, continuing to cultivate these hybrids for several segregation generations leads to obtaining barley lines that are genetically more stable. As well, collecting all the quantitative traits desired by the breeder, such as resistance to diseases and environmental stresses, especially salt stress. This is the strategic goal of this study and it must be achieved as soon as possible.

2. Materials and Methods

Materials:

This investigation included five barley cultivars divided into two groups. The first group contains two imported lines from ICARDA (line I and line II). While, the second barley group were 3 Egyptian cultivars namely; Giza 121, Giza 126 and Giza 2000, respectively. The five barley accessions were various response for salinity tolerance (Table, 1).

Table 1: Classification of the five Barley Genotypes used in a half diallel analysis.

Serial No.	Names of Genotypes	Origin	Drought tolerance
1 or (P1)	Line (1)	ICARDA	Tolerance
2 or (P2)	Line (2)	ICARDA	Tolerance
3 or (P3)	Giza 121	Egypt	Moderate
4 or (P4)	Giza 126	Egypt	Moderate
5 or (P5)	Giza 2000	Egypt	Moderate

Sowing:

The five barley parents were sown in three planting dates with 7 days interval in order to overcome the differences in flowering time among parents for crossing in season 2018. All entries (parents and their F1 crosses) were grown under normal and salinity conditions in a randomized complete block design with three replicates for each experiment in season 2019. The two experiments were grown in pots and the normal conditions means (normal irrigation or tap water). While, salinity treatment means (irrigate using 20.38% seawater performed from (Alexandria sea water and specifically from Al-Agami

Resort with EC 51.50 dsm^{-1} to be after dilution 10.50 dsm^{-1}) according to **[5].** The chemical analysis for the two kinds of water was shown in Table (2).

Table 2: Chemical analysis of Both Types of Water Irrigation (Tap water and Seawater) Using in This Study.

Characteristics	Normal Irrigation (Tap water)	Saline water (Seawater)
EC (dS/m)	1.53	10.50
pH (1:2.5)	7.11	8.13
Ca++	2.37	32.86
Mg++	1.64	29.14
Na+	8.94	78.37
K+	0.31	0.18
$CO3^{--}$	0.03	0.39
$HCO3^{-}$	1.78	3.96
Cl^{-}	12.48	55.28
$SO4^{-}$	1.19	49.36

Studied traits:

Fifty plant were taken from each genotype for the two experiments (normal and saline treatments) to evaluate the following traits:- 1):- **Plant height (cm):-** Length of the main culm was measured from the soil surface to the tip of the main panicle at maturity, **2):- Number of filled grains per panicle: -** Filled grains of the main panicle with separated and counted, **3):- 1000-grain weight (g):- It** was recorded as the weight of 1000 random filled grains per plant, **4):- Grain yield per plant (g):-** was recorded as the weight of grain yield of each individual plant, and adjusted to 14% moisture content, **5, 6 and 7):- Determination of Na+ uptake, K+ uptake and Na/K ratio,** 8):- **Osmotic adjustment**: - It was determined as follows: -

$$\frac{\text{O.P. x R.W.C.}}{100}(\text{Normal }) - \frac{\text{O.P x R.W.C.}}{100}(\text{drought})^{100};$$

Where: O.P= Osmotic pressure, R.W.C. = Relative water content, **9):- The proline content: -** was determined from a standard curve and calculated on a fresh basis is as follows: [(µg proline / ml C m1 toluence) / 115.5 µg / µ mole] / [(g sample/5)] = µ moles proline / g of fresh weight material.

The results related with proline content are average values at least 3-4 samples for each species, according to **[16]** and modified method by **[17], 10):- Glycine betaine contents:** It was carried out according to the method of **[18].**

Methods:-

Statistical analysis:-

All calculated data from all studied traits under the two experiments were analyzed using half diallel analysis by **[19] model** I, method II including heterosis over better-parent, general and specific combining ability effects where **GCA/SCA ratio: -** MSe of GCA-MS error term /Number of parent + 2/ MSe of SCA-MS error term, respectively.

Estimates of Genetic Parameters:

Variance components, heritability in broad sense, genetic coefficient of variability (GCV %) , phenotypic coefficient of variability (PCV %), D^z or the difference between the phenotypic coefficient of variation (PCV %) and genotypic coefficient of variation (GCV %), expected genetic advance in addition, genetic advance as percentage of mean were the most important measurements calculated for all studied traits under normal and salinity conditions in this study as follows:- The genetic coefficient of variability (GCV %) and phenotypic coefficient of variability (PCV %) were estimates according to the method suggested by **[20]** as follows:- Environmental Variance (σ^2e) = MS_e Genotypic Variance (G v) or (σ^2g) = $MS_g - MS_e / r$

Phenotypic Variance (Ph v) or (σ^2ph) = (σ^2e) + (σ^2g) or $MS_e + MS_g$

Where: MSe = Mean Square of error, MSg = Mean Square of genotypes, r =Number of replicates, X = Mean of Trait.

$$\textbf{Genetic coefficient of variability (GCV \%)} = \frac{\sqrt{Gv}}{X}\ x\ 100$$

$$\textbf{Phenotypic coefficient of variability (PCV \%)} = \frac{\sqrt{Ph\ v}}{X}\ x\ 100$$

Estimation of heritability in broad sense:

Broad sense heritability (h^2) expressed as the percentage of the ratio of the genotypic variance (g v) to the phenotypic variance (ph v) and was estimated on genotype mean basis as described by **[20 , 21].**

$H^2B = (\sigma^2g) / (\sigma^2ph)$ X 100, **D ²:** The difference between the phenotypic coefficient of variation (PCV %) and genotypic coefficient of variation (GCV %) or (PCV %) - (GCV %).

Estimation of genetic advance:

The expected genetic advance (GA) and percentage of the mean (GAM) assuming selection of superior 5% of the genotypes was estimated in accordance with the methods illustrated by **[21]** as:

$$\textbf{(GA) = K X } (\sigma^2g) \textbf{ X } \sqrt{\text{Ph v}} / \text{Ph v}$$

Where K = Standardized selection differential at 5% selection intensity (K = 2.068).

The genetic advance as percentage of mean (GAM) was computed as:

$$\textbf{(GAM \%) = (GA) / X . 100}$$

Estimation of tolerance indices:-

All tolerance indices were estimated according to **[22-26]** as follows in table (10):- 1):- GYP: Is meaning the grain yield/plant for the control experiment, 2):- GYS: Is meaning the grain yield/plant for the salinity experiment, 3):- YSI: Is meaning yield stability index = YS/YP where: YS the average of yield under stress and YP= The average of yield under the control experiment, 4):- YI: Is meaning yield index (YS for each genotype/mean of YS for all genotypes), 5):- MP: Is means (Average yield for both trials): YS + YP/2 , 6):- STI: Is meaning salinity tolerance index (YP X YS/ (mean of YP) 2 , 7):- GMP: (YP X YS) 0.5 , 8):- YR: Is meaning yield reduction (1-YS/YP) , 9):- SSI: Is meaning salinity susceptibility index = DSI = (1-YS/YW)/D where YS = mean yield under salt stress, Yw = mean yield under control conditions, and D = environmental stress intensity= 1-(mean yield of all genotypes under stress/mean yield of all genotypes under irrigated conditions). Note: - Osmotic adjustment was conducted according to **[27].**

Molecular Characterization:-

Genomic DNA extraction and PCR conditions

Total genomic DNA of all samples was extracted from 10 green barley leaves using Qiagen DNeasy Plant Minikit following the protocol of the manufacturer (Qiagen Inc, Valencia, CA). The quality of the extracted DNA was assessed on agarose gel electrophoresis. PCR was performed using ten preselected ISSR primers based on their ability to generate reproducible and informative amplification patterns in table (10). Amplification reactions were carried out in Biometra T One Thermal Cycler (Analytik Jena, Jena, Germany). PCR amplification was performed in 25 µl reaction mix which contained 20-30 ng DNA template, 10 pmole of each primer, 2.5 µl of 2mM Thermo dNTPs, 5 µl of 5x Promega Green GoTaq Flexi Reaction Buffer, 2.5 µl of 25 mM Promega $MgCL_2$ and 0.125 µl of 5 U/µl Promega GoTaq Flexi DNA polymerase. The reaction was assembled on ice, amplification was performed at certain conditions as follows: an initial denaturing step at 94 ^{0}C for 5 min followed by 35 cycles at 94 ^{0}C for 30 sec, annealing at 50 ^{0}C for 1 min, an extension at 72 ^{0}C for 1 min and final extension at 72 ^{0}C for 7 mins. The PCR products were assessed on 1.6% agarose gel **[28-30].** The banding profile of ISSR were scored using Labimage program and the polymorphism percentage was estimated as follow :- Percent of polymorphism = (Number of polymorphic bands/Total Number of Bands) X 100.

Note:- Molecular Sizes of marker used in analyses were as follow: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500 and 3000 bp, Also: - T.B: Total bands, M.B: Monomorphic bands, P.B: Polymorphic bands, U.B or P.S.M: Unique bands or positive specific marker, P%: Polymorphism percentage and R.S (bp): Range size

Data Handling and cluster analysis (Phylogenetic Tree)

Data was scored for computer analysis on the basis of the presence or absence of the amplified products for each primer. Pairwise components of the ten barley genotypes based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficients according to **[31].** The similarity coefficients were then used to construct dendrograms, using the un weighted pair group method with arithmetic averages (UPGMA) employing the SAHN (Sequential, Agglomerative, Hierarchical and Nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (Applied Biostatistics Program).

Results

Before starting to present our results, it must be pointed out that the problem of increasing levels of salt stress in Egyptian soil is one of the greatest environmental challenges facing Egyptian agriculture. The simple reason for this, is the limited irrigation water especially in the summer, and this leads to an accumulation of salts level in the Egyptian soil layers. So that, this study should be carried out with great effectiveness, because it is not possible to wash these layers or reduce this salt level. Therefore, in this study we also hope to reach genotypes or a group of barley hybrids that have a high tolerance to environmental stresses,especially salt stress and a high yield, so that they will be a building block that can be invested in the future and worked on to produce barley lines and varieties that are tolerant to salt stress and have highly output at the same time.

Analysis of Variance:-

Results obtained in the table (3) revealed the importance of interaction test to the studied attributes and were observed the biggest rank of interaction for the two experiments. Also, results of general and specific combining ability parameters were similar with great form for the previous characters testing. Further, the percentage of GCA/SCA interaction for all traits under studying under both conditions were less than the unity. The importance of this test is also that it is used to determine the extent of differences between the varieties, hybrids, or lines under study. Further, these differences are required to be significant or highly significant differences in order for all measurements and genetic constants to be obtained. However, if the differences are not significant or highly significant, this model cannot be used under any circumstances. This procedure is used first before starting the study steps to know and ensure that all genotypes used are far apart from each other to the extent that ensures the occurrence of genetic differences in their hybrids.

Mean performance:-

Data obtained in all attributes measurement for both conditions detected the fruitful role of comparison method among all parameters under study. Also, this step will help the breeders for selecting the excellent barley materials tolerated to salinity stress besides, its high yielding. The highest and most desirable genotypes recorded highly salinity tolerance under both experiments for the studied attributes; (Number of filled grains/panicle, 1000-grain weight, grain yield/plant, K+ content, proline and glycine betaine contents) besides, the parents (1, 2 and 3) and the hybrids (P1 X P2, P1 X P3, P2 X P3, P2 X P4 and P3 X P4) in table (4), respectively. The same accessions were showed the optimum and ideal results for salinity tolerance in barley crop under normal and salinity conditions for the rest traits under studying namely; (plant height, Na+ content, Na/K ratio and osmotic adjustment). For example not limited, the mean values under both conditions were ranged from (81.06 to 112.83 and from 72.55 to 98.74 cm) for plant height trait, ranged from (24.83 to 66.03 and from 18.39 to 57.04 gm) for 1000-grain weight trait, ranged from (25.16 to 55.36 and from 17.62 to 47.22 gm) for grain yield/plant trait, ranged from (0.24 to 1.52) for osmotic adjustment trait and ranged from (14.09 to 81.09 and from 10.59 to 92.01) for proline content trait. Whereas, this decreasing in mean values of these traits under the stress treatment compared to the control means a significant and great physiological resistance to salinity and this is point will be clarified carefully at later in the discussion part. These results are a clear indication of the superiority of the new barley hybrids, starting from the first hybrid generation. In addition, this noticeable superiority in the results of all the traits under study under conditions of salt stress compared to the standard experiment also indicates that the superiority genes were already present in the parents involved in the hybridization program. Also, continuing to analyze and identify the mechanisms of salt stress tolerance in such promising genotypes is a logical matter and a constructive scientific step. Further, the rest of the genetic measurements will also be new evidence for identifying tolerance mechanisms in this regard. Based on this, the top priority for plant breeders involves collecting and capturing all the genes responsible for inheriting quantitative traits, which simply lead to increased yields under conditions of environmental stress and resistance to a very large number of diseases that reduce the final output of the barley crop. Further, this scientific strategy will be the most chance in the recent few years for enhancing all crops and increasing the ability of resistance and tolerance abiotic and biotic stresses. Also, this methods was successfully with great form for increasing the final output in rice, maize, sorghum, wheat and barley. Heterosis Over Better-Parent:-

Five crosses out of ten crosses exhibited highly moral positively values of heterosis over better-parent under both treatments for the attributes; number of filled grains/panicle, 1000-grain weight, grain yield/plant, proline and glycine betaine contents which confirmed that these barley accessions were revealed the biggest limit of salinity tolerance in this regard. These hybrids were (P1 X P2, P1 X P3, P2 X P3, P2 X P4 and P3 X P4), respectively. Also, these entries were showed significant and highly significant negatively percentages of heterosis over better-parent for plant height trait under normal and salinity treatments, table (5). While,, Na+ content, Na/K ratio and osmotic adjustment traits were not exhibited any significant percentages of this parameter for all studied genotypes under all conditions. On the other hand, significant and highly significant negatively values were detected in the crosses; (P1 X P4 and P1 X P5) for both experiments and the two crosses (P2 X P5 and P3 X P5) for salinity treatment only for K+ content trait. While, the hybrid; (P2 X P4) showed highly significant and positive percentage of heterosis over better-parent for K+ trait under salinity treatment only. Note that, the positive trend is the required direction for the genetic improvement of K+ trait. The mean values were for some traits under both conditions and ranged from (-1.81% to 30.72%, ranged from (2.08% to 12.79%) for plant height trait, ranged from (8.21% to -55.31%, from (13.83% to -57.64%) for number of filled grains/panicle trait and ranged from (10.78% to -39.45% and from 9.15% to -53.05%) for grain yield/plant trait and so on. These promising results, if any, indicate that these hybrids are a good and direct indication of the extent of genetic improvement achieved through traditional plant breeding programs, which are the first basis in any breeding process, improving yield components traits and tolerance to environmental stresses, especially salt stress in the Egyptian barley crop. Further, the process of hybridization between lines tolerant to salt stress with the more sensitive local varieties represents the actual nucleus for producing barley entries tolerant to salt stress besides, its high yield. Continuing to cultivate these promising genotypes for several segregation generations with simple selection ultimately leads to the production of barley lines that are tolerant to salt stress and are also high yield. Therefore, the strength of the hybrid in relation to the best parent is considered one of the most important genetic measurements obtained from the analysis of half

diallel analysis. This test succeeded in identifying the strongest parents that contain genes for the quantitative traits required to be inherited in the new hybrids so that they are high-yielding and tolerant to salt stress. It is worth noting that this test saves a lot of time in identifying the best and strongest parents. As well, screening them through simple selection starting from the second generation, where each plant represents an independent genetic makeup. This is a strategic and necessary scientific step before starting the process of breeding new barley lines by screening the largest possible number of them in order to identify the strongest parents included in the breeding program and improving the barley crop to salt stress tolerance under Egyptian conditions. Further, this genetic parameter was achieved the biggest indices for increasing salinity tolerance in the previous barley accessions which indicated its importance for enhancing this strategy crop of resisting biotic and abiotic stresses under Egyptian conditions.

General and Specific Combining Ability Effects:-

Data viewed in table (6) detected the importance of general combing ability effects for enhancing the ability of salinity tolerance in barley crop. The barley entries number (1, 2 and 3) were revealed highly moral positively values of GCA effects in the attributes; (Number of filled grains/panicle, 1000-grain weight, grain yield/plant, K^+ content, proline content and glycine betaine) for the two experiments. Further, the genotypes number (4 and 5) achieved the same results but in the reverse direction in the rest attributes. The hybrids; (P1 X P2, P1 X P3, P2 X P3, P2 X P4 and P3 X P4) recorded highly significant and positive values of SCA effects under both conditions for the characters; number of filled grains/panicle, 1000-grain weight, grain yield/plant, K^+ content, proline content and glycine betaine). While, the rest crosses under the same treatments were exhibited highly significant and negative values of SCA effects for the other traits; (plant height, Na+ content, Na/K ratio and osmotic adjustment) in table (7), respectively. These parameters indicated the fruitful role half diallel analysis and its importance for determining the relationship among all barley accessions under study. Further, this genetic relationship was the main step for choosing the promising barley entries for salt stress tolerance before starting the investigation. Also, the GCA effects can played an importance role for determining the best parents which were very similar among them for obtaining the highest barley hybrids for highly rank of yield and salinity stress tolerance under stress comparing to the control experiment. In addition, this parameter was very fruitful in the future of selecting and testing more barley genotypes for resistance abiotic and biotic stress under Egyptian conditions. Therefore, the previous results confirmed the essential and fruitful role of traditional plant breeding in enhancing and increasing the ability of salinity tolerance in barley crop besides, its ability of drought resistance in the future.

Genetic Parameters and Salinity Tolerance indices:-

Values of (H^2b) were good in all attributes under normal and salinity experiments. While, Na+ content exhibited medium data in this regard for the two treatments. On the other hand, the percentages of GCV % and PCV % were appeared low in plant height trait, medium in attributes; number of filled grains/panicle, 1000-grain weight, grain yield/plant and glycine betaine content under both conditions. While, another traits under study namely; Na+ content, K+ content, Na/K ratio, osmotic adjustment and proline content were recorded high values for GCV% and PCV % under both conditions. While, the values of (D^Z) were medium in K+ content trait and high in the rest physiological attributes for the salinity treatment besides the control experiment. Results assessment of expected genetic advance (GA) based on 5% selection confirmed that the highest values were observed in all traits under study except plant height and Na+ content traits under both experiments. Appreciates of genetic advance as percentage of mean (GAM %), all traits exhibited high values under both treatment. While, plant height trait only was recorded the lowest rank under normal and salinity conditions, table (8). Data shown in table (9) detected that the barley accessions; (P1, P2, P3, P1 X P2, P1 X P3, P1 X P5, P2 X P3, P2 X P4 and P3 X P4) for the parameters; (YSI, MP and GMP) recorded highly data for salinity tolerance indices test in this investigation which confirmed that these barley entries were highly tolerance for salinity. The same results were observed for (YI and STI). After reviewing these results, it can be briefly said that all of these measurements have confirmed the vital and fruitful role of plant breeding, especially traditional breeding methods in improving the tolerance of crops, especially barley to salt stress. This became clear through the data obtained for all the traits under study, especially the grain yield trait under salt stress conditions compared to standard experiment. The rest of the estimated genetic measurements have already proven the extent of the genetic superiority enjoyed by the salt-stress-tolerant barley hybrids compared to their original parents. Based on these impressive results, it was necessary, in the first place to clarify the importance of continuing to cultivate barley hybrids that excel in all yield traits and attributes indicating tolerance to salt stress for several segregation generations with simple selection in each generation separately. The major aim in this work is reaching to highly limit of stable percentage of these promising barley genotypes so that we ultimately obtain barley lines that are tolerant to salt stress and have high yield. As well, their genetic stability under various agricultural environments. This is the fruitful achievement hoped for from these various genetic experiments and measurements. Further, these previous parameters were very importance and achieved a fruitful function for enhancing the program of barley breeding for resistance all biotic and abiotic stress in many areas of Egypt. This is the greatest goal in this work. Also, the strategy of improving field crops has succeeded to confront the conditions that are largely opposite in

developing countries, which depends on a large part of its annihilation and national income on agricultural production, especially in light of the limited irrigation water and the spread of arid lands.

Table 3 : Mean Squares of the half diallel analysis for all studied traits under the control and salinity conditions.

S.O.V	D.F	1		2		3		4		5	
		N	S	N	S	N	S	N	S	N	S
Reps	2	1.38	1.16	2.25	1.65	1.07	1.11	2.57	3.18	0.67	0.59
Genotypes	14	8.56**	11.03**	38.07**	23.0**	17.33**	8.12**	17.83**	24.03**	5.39**	7.16**
GCA	4	234.46**	215.06**	163.77**	182.0**	316.23**	240.0**	60.73**	109.04**	413.88**	296.43**
SCA	10	190.02**	87.55**	55.14**	111.32**	190.64**	158.33**	37.44**	78.22**	276.55**	181.33**
Error	28	1.12	0.67	1.55	1.83	0.37	0.26	0.46	0.87	1.38	1.62
Error term		0.37	0.22	0.51	0.61	0.12	0.08	0.15	0.29	0.46	0.54
GCA/SCA		0.17	0.35	0.42	0.23	0.23	0.21	0.23	0.19	0.21	0.23

Table (3):- Cont.

S.O.V	D.F	6		7		8	9		10	
		N	S	N	S		N	S	N	S
Reps	2	0.78	0.39	1.02	0.52	1.58	1.66	1.29	1.55	2.07
Genotypes	14	23.76**	15.0**	14.91**	10.05**	16.37**	67.80**	58.02**	18.42**	21.08**
GCA	4	94.66**	83.57**	271.34**	204.05**	39.88**	72.11**	54.03**	104.35**	110.79**
SCA	10	45.22**	61.45**	67.22**	150.84**	28.07**	40.16**	19.68**	58.39**	69.26**
Error	28	0.94	0.51	0.78	0.23	1.07	1.42	1.05	0.93	0.74
Error term		0.31	0.17	0.26	0.07	0.35	0.47	0.35	0.31	0.24
GCA/SCA		**0.30**	**0.19**	**0.57**	**0.19**	**0.20**	**0.25**	**0.39**	**0.25**	**0.22**

Table 4 :- Mean performances of all studied traits in all barley genotypes tested under the control and salinity conditions.

Entries	1		2		3		4		5		6		7		8	9		10	
	N	S	N	S	N	S	N	S	N	S	N	S	N	S		N	S	N	S
P1	**92.14**	**84.60**	**68.14**	**52.63**	**54.31**	**48.73**	**43.75**	**38.16**	**0.14**	**0.09**	**2.78**	**3.14**	**0.05**	**0.02**	**0.46**	**54.13**	**62.18**	**43.12**	**58.15**
P2	**85.34**	**79.16**	**59.02**	**50.12**	**45.22**	**39.06**	**46.0**	**41.28**	**0.12**	**0.07**	**2.40**	**2.58**	**0.05**	**0.02**	**0.55**	**47.04**	**55.16**	**39.60**	**44.50**
P3	**103.78**	**92.50**	**48.33**	**41.79**	**43.04**	**38.94**	**44.95**	**40.37**	**0.17**	**0.11**	**2.32**	**2.48**	**0.07**	**0.04**	**0.39**	**39.40**	**44.18**	**41.08**	**49.04**
P4	100.18	90.02	32.82	24.06	31.69	25.92	36.42	27.05	0.22	0.29	1.45	1.32	0.15	0.21	1.17	21.33	19.74	26.31	19.05
P5	104.72	87.54	37.0	29.11	34.17	28.77	31.45	24.59	0.28	0.32	1.18	1.07	0.23	0.29	1.05	18.92	17.03	21.54	17.45
P1 X P2	**83.43**	**76.02**	**78.10**	**59.91**	**62.80**	**55.43**	**55.36**	**46.44**	**0.06**	**0.04**	**3.21**	**3.78**	**0.01**	**0.01**	**0.32**	**69.56**	**72.85**	**55.49**	**74.03**
P1 X P3	**87.32**	**81.48**	**75.22**	**64.03**	**66.03**	**57.04**	**50.03**	**44.24**	**0.12**	**0.07**	**3.78**	**4.06**	**0.03**	**0.01**	**0.28**	**73.38**	**81.19**	**84.34**	**92.11**
P1 X P4	95.88	86.36	30.45	22.29	28.37	22.60	29.78	22.36	0.19	0.32	1.38	1.29	0.13	0.24	1.25	16.33	13.12	25.31	18.01
P1 X P5	99.51	89.04	34.78	25.58	33.05	27.16	26.49	21.39	0.36	0.39	1.12	1.04	0.32	0.37	1.13	14.09	11.32	19.41	12.64
P2 X P3	**81.06**	**72.55**	**63.87**	**58.12**	**52.44**	**46.77**	**54.89**	**47.22**	**0.08**	**0.05**	**2.98**	**3.11**	**0.02**	**0.01**	**0.24**	**81.98**	**92.01**	**51.96**	**62.55**
P2 X P4	**83.79**	**75.80**	**70.58**	**62.33**	**51.24**	**44.84**	**51.66**	**45.06**	**0.09**	**0.03**	**3.65**	**4.02**	**0.02**	**0.007**	**0.40**	**68.37**	**78.14**	**57.72**	**94.47**
P2 X P5	111.56	97.80	29.88	23.24	30.08	19.84	28.14	19.38	0.42	0.48	1.16	1.02	0.32	0.47	1.18	18.11	16.28	16.07	13.48
P3 X P4	**89.64**	**80.15**	**59.65**	**48.17**	**49.57**	**42.13**	**49.80**	**46.22**	**0.14**	**0.06**	**2.69**	**3.19**	**0.05**	**0.018**	**0.34**	**48.06**	**52.78**	**62.77**	**86.14**
P3 X P5	108.74	96.41	36.21	26.57	26.38	20.34	30.09	21.53	0.29	0.38	1.07	1.03	0.27	0.36	1.14	15.18	10.59	20.63	13.89
P4 X P5	112.83	98.74	28.94	21.03	24.38	18.39	25.16	17.62	0.43	0.52	1.10	1.05	0.39	0.49	1.52	17.11	14.74	20.08	15.44
LSD at 0.05	1.46	1.13	1.72	1.87	0.84	0.70	0.94	1.29	1.63	1.76	1.34	0.99	1.22	0.66	1.43	1.65	1.42	1.33	1.19
LSD at 0.01	2.13	1.64	2.50	2.724	1.22	1.02	1.36	1.87	2.36	2.56	1.95	1.43	1.77	0.96	2.08	2.40	2.06	1.94	1.73

Table 5 : Estimates of Heterosis over better-parent for the 10 barley crosses obtained from half diallel analysis in all studied traits under both conditions.

Entries	1		2		3		4		5	
	N	S	N	S	N	S	N	S	N	S
P1 X P2	-2.23*	-10.14**	14.61**	13.83**	15.63**	13.74**	20.34**	12.50**	-50.0 NS	-42.85 NS
P1 X P3	-5.23**	-3.68**	10.39**	21.66**	21.57**	17.05**	11.30**	9.58**	-14.28 NS	-22.22 NS
P1 X P4	4.05**	2.08**	-55.31**	-57.64**	-47.76**	-53.62**	-31.93**	-41.40**	35.71 NS	255.55 NS
P1 X P5	7.99**	5.24**	-48.95**	-51.39**	-39.14**	-44.26**	-39.45**	-43.94**	157.14 NS	333.33 NS
P2 X P3	-5.01**	-8.35**	8.21**	15.96**	15.96**	19.73**	19.32**	14.38**	-33.33 NS	-28.57 NS
P2 X P4	-1.81*	-4.24**	19.58**	24.36**	13.31**	14.79**	12.30**	9.15**	-25.0 NS	-57.14 NS
P2 X P5	30.72**	23.54**	-49.37**	-53.63**	-33.48**	-49.20**	-38.82**	-53.05**	250.0 NS	585.71 NS
P3 X P4	-10.52**	-10.96**	23.42**	15.26**	15.17**	8.19**	10.78**	14.49**	-17.64 NS	-45.45 NS
P3 X P5	4.77**	10.13**	-25.07**	-36.42**	-38.70**	-47.76**	-33.05**	-46.66**	70.58 NS	245.45 NS
P4 X P5	12.62**	12.79**	-21.78**	-27.75**	-28.65**	-36.07**	-30.91**	-34.86**	95.45 NS	79.31 NS
LSD at 0.05	1.46	1.13	1.72	1.87	0.84	0.70	0.94	1.29	1.63	1.76
LSD at 0.01	2.13	1.64	2.50	2.724	1.22	1.02	1.36	1.87	2.36	2.56

Table 5 :- Cont

Entries	6		7		8	9		10	
	N	S	N	S		N	S	N	S
P1 X P2	15.46 NS	20.38 NS	-80.0 NS	-50.0NS	-30.43 NS	28.50**	17.15**	28.*68***	27.30**
P1 X P3	35.97 NS	29.29 NS	-40.0NS	-50.0NS	-28.20 NS	35.56**	30.57**	95.59**	58.40**
P1 X P4	-50.35*	-58.91**	-13.33 NS	1100.0NS	171.73 NS	-69.83**	-78.89**	-41.30**	-69.02**
P1 X P5	-59.71**	-66.87**	540.0 NS	1750.0NS	145.65 NS	-73.97**	-81.79**	-54.98**	-78.26**
P2 X P3	24.16 NS	20.54 NS	-60.0NS	-50.0NS	-38.46 NS	74.27**	66.80**	26.48**	27.54**
P2 X P4	52.08 NS	55.81**	-60.0 NS	-65.0 NS	-27.27 NS	45.34**	41.66**	45.75**	112.29**
P2 X P5	-51.66 NS	-60.46**	540.0NS	2250.0NS	114.54 NS	-61.50**	-70.48**	-59.41**	-69.70**
P3 X P4	15.94 NS	28.62 NS	-28.57 NS	-55.0 NS	-12.82 NS	21.97**	19.46**	52.79**	75.65**
P3 X P5	-53.87 NS	-58.46 **	285.71 NS	800.0 NS	192.30 NS	-61.47**	-76.02**	-49.78**	-71.67**
P4 X P5	-24.13 NS	-20.45 NS	160.0NS	133.33 NS	44.76 NS	-19.78**	-25.32**	-23.67**	-18.95**
LSD at 0.05	1.34	0.99	1.22	0.66	1.43	1.65	1.42	1.33	1.19
LSD at 0.01	1.95	1.43	1.77	0.96	2.08	2.40	2.06	1.94	1.73

Table 6 :- Estimates of General Combining Ability Effects for the 5- parent of Barley Entries for all studied traits under the control and salinity conditions.

Entries	1		2		3		4		5	
	N	S	N	S	N	S	N	S	N	S
P1	-23.67**	-15.96**	13.56**	11.07**	45.06**	62.03**	4.11**	6.08**	-6.22**	-8.43**
P2	-43.57**	-37.02**	9.06**	12.06**	10.04**	14.73**	7.03**	5.13**	-1.68**	-2.07**
P3	-11.53**	-8.44**	27.11**	19.53**	31.93**	25.0**	9.25**	13.02**	-3.29**	-5.04**
P4	28.17**	40.05**	-32.85**	-21.72**	-12.52**	-54.61**	-8.25**	-7.92**	6.41**	12.89**
P5	50.60**	21.37**	-16.88**	-20.94**	-74.51**	-47.15**	-12.14**	-16.31**	4.78**	2.65**
LSD at 0.05 (gi)	1.28	1.34	1.68	1.47	2.23	1.68	0.65	0.43	0.38	0.24
LSD at 0.01 (gi)	1.53	1.48	2.08	1.89	2.49	1.83	0.79	0.61	0.49	0.37

Table 6 :- Cont.

Entries	6		7		8	9		10	
	N	S	N	S		N	S	N	S
P1	13.42**	11.33**	-3.68**	-1.94**	-3.86**	18.40**	22.31**	15.04**	9.27**
P2	25.87**	20.06**	-6.83**	-2.99**	-5.18**	13.07**	10.58**	23.70**	40.02**
P3	84.11**	54.57**	-9.28**	-14.07**	-8.32**	8.37**	14.08**	11.07**	17.66**
P4	-70.42**	-51.03**	8.46**	13.75**	9.42**	-26.52**	-29.15**	-25.73**	-46.13**
P5	-52.98	-34.93**	11.33**	5.25**	7.94**	-13.32**	-17.82**	-24.08**	-20.82**
LSD at 0.05 (gi)	1.63	1.38	0.89	0.38	0.94	1.25	1.09	1.16	0.74
LSD at 0.01 (gi)	2.18	1.82	1.05	0.65	1.26	1.78	1.83	1.57	1.19

Table 7 :- Estimates of Specific Combining Ability Effects for the 10 Barley crosses of all studied traits under both conditions.

Entries	1		2		3		4		5	
	N	S	N	S	N	S	N	S	N	S
P1 X P2	-4.64**	-6.11**	25.14**	61.03**	6.38**	4.19**	48.63**	36.05**	-4.22**	-9.12**
P1 X P3	-7.97**	-3.98**	40.78**	36.08**	9.77**	11.02**	14.93**	20.11**	-11.06**	-5.19**
P1 X P4	3.31**	9.11**	-13.20**	-14.08**	-24.30**	-3.15**	-21.34**	-11.88**	10.28**	8.46**
P1 X P5	17.64**	4.18**	-6.64**	-24.30**	-7.05**	-7.36**	-57.01**	-29.47**	9.81**	4.38**
P2 X P3	-12.55**	-15.94**	12.86**	5.38**	16.14**	5.99**	22.18**	10.79**	-7.0**	-10.06**
P2 X P4	-8.04**	-8.05**	10.04**	8.42**	30.0**	21.07**	40.0**	5.78**	-3.78**	-2.13**
P2 X P5	2.36**	12.37**	-15.69	-72.45**	-32.18**	-19.64**	-46.73**	-10.75**	5.87**	5.62**
P3 X P4	-10.11**	-5.03**	21.02**	17.55**	19.06**	7.88**	52.61**	39.37**	-14.03**	-1.96**
P3 X P5	14.92**	3.45**	-57.19**	-7.63**	-9.48**	-15.73**	-44.15**	-33.06**	6.55**	7.11**
P4 X P5	5.08**	10.0**	-17.12**	-10.0**	-8.34**	-4.27**	-9.12**	-26.94**	7.58**	2.89**
LSD at 0.05 (Sij)	1.32	1.58	1.39	1.12	1.72	1.54	1.71	1.42	0.73	0.55
LSD at 0.01 (Sij)	1.65	1.83	1.48	1.29	2.13	1.83	2.19	1.65	1.04	0.81

Table 7 : **Cont.**

Entries	6		7		8	9		10	
	N	S	N	S		N	S	N	S
P1 X P2	**4.77****	**2.88****	**-27.06****	**-4.35****	**-14.92****	**40.36****	**31.19****	**1.68****	**5.22****
P1 X P3	**6.83****	**10.07****	**-6.90****	**-9.11****	**-23.12****	**28.15****	**23.05****	**2.19****	**3.07****
P1 X P4	-20.30**	-11.86**	17.80**	3.91**	5.86**	-26.44**	-39.85**	-4.11**	-2.39**
P1 X P5	-9.45**	-14.27**	11.06**	5.23**	41.18**	-47.58**	-17.14**	-3.29**	-4.16**
P2 X P3	**13.92****	**8.90****	**-3.41****	**-5.08****	**-6.03****	**60.18****	**43.0****	**1.95****	**1.80****
P2 X P4	**11.09****	**18.70****	**-12.34****	**-1.78****	**-27.92****	**17.22****	**14.10****	**4.55****	**2.06****
P2 X P5	-7.55**	-17.66**	4.69**	4.93**	8.82**	-24.83**	-15.39**	-2.49**	-3.55**
P3 X P4	**3.79****	**5.03****	**-1.79****	**-3.59****	**-3.87****	**52.88****	**71.09****	**3.45****	**1.47****
P3 X P5	-0.89 NS	-0.39 NS	13.40**	6.0**	13.54**	-73.08**	-27.62**	-1.55**	-1.70**
P4 X P5	-2.21 NS	-1.40 NS	4.55**	3.84**	6.46**	-26.86**	-82.43**	-2.38**	-1.82**
LSD at 0.05 (Sij)	2.34	2.11	0.66	0.48	1.16	1.55	1.73	1.39	0.97
LSD at 0.01 (Sij)	2.77	2.53	0.79	0.63	1.42	1.92	2.08	1.46	1.34

Molecular Characterization:-

The best five barley crosses used in molecular markers technique were as follows; Cross 1 or Hybrid 1: (P1 X P2), Cross 2 or Hybrid 2: (P1 X P3), Cross 3 or Hybrid 3: (P2 X P3), Cross 4 or Hybrid 4: (P2 X P4) and Cross 5 or Hybrid 5: (P3 X P4), respectively. On this basis, the molecular markers step came as a very important step for coordination and interconnection with traditional genetics (plant breeding) to determine the mechanisms of tolerance to salt stress in the promising genotypes of the barley crop.

Profile of ISSR analysis

Ten ISSR markers in this investigation were very importance for determining all differences between barley entries. These ten primers showed 100 bands, (Table 10; Fig.1). In the same track, the highest number of polymorphic bands (10) and polymorphism % (100 %) were observed in primers SR-03, SR-12 and SR-21. Further, primers SR-01, SR-12 and SR-36 were recorded the highest rank of unique band (6, 7 and 4) for each one of them, respectively. Data generated in table (11) detected that the cross one (H1) was exhibited the highest number of amplified fragments (63) followed by the cross number 3 (H3) where it recorded (61) bands, followed by the cross number 2 (H2) where it showed (60) amplified fragments and then followed by the cross number 5 (H5) where it recorded (59) bands, respectively. While, the parent number one (P1) was recorded a little rank of fragments (42). While, the rest barley accessions generated various numbers of amplified fragments. In the same track, the primer SR-03 exhibited the highest number of amplicons (93). Also, the SR-08 marker recorded a little level of fragments (21) for ten barley entries. These data are a good and direct indication of the importance of these markers in determining salt stress tolerance mechanisms in promising high-yielding barley hybrids under Egyptian conditions. Further, these hybrids will represent high-yielding barley lines in the future and have a high ability of environmental stress tolerance, especially salt stress. The strategy of integrating traditional breeding programs and molecular markers has largely succeeded in developing and improving the tolerance of a large number of crops, such as rice, wheat, maize, sorghum, fava beans, soybeans, sugarcane, and sugar beets to environmental stresses such as heat, light, and water and salt stresses. As well, the resistance of these crops to a number of a large number of diseases.

Table 8 :- Estimates of some Genetic Parameters for all Studied Traits in Barley Genotypes under normal and salinity conditions.

Genetic Parameters	plant height (CM)		Number of filled grains/Spike		1000-grain weight (gm)		Grain yield/plant (gm)		Na+ content (ppm)		K+ content (ppm)		Na/K ratio		Osmotic adjustment	Proline Content		Glycine Betaine Content	
	N	S	N	S	N	S	N	S	N	S	N	S	N	S		N	S	N	S
Mean	95.99	85.87	50.19	40.59	42.18	35.73	40.26	33.52	0.207	0.214	2.151	2.278	0.14	0.171	0.761	40.19	42.75	39.02	44.73
Genotypic Variance	2.48	3.45	12.17	7.05	5.65	2.62	5.79	7.72	1.33	1.84	7.60	4.83	4.71	3.27	5.01	22.12	18.99	5.83	6.78
Environmental Variance	1.12	0.67	1.55	1.83	0.37	0.26	0.46	0.87	1.38	1.62	0.94	0.51	0.78	0.23	1.07	1.42	1.05	0.93	0.74
Phenotypic Variance	3.60	4.12	13.72	8.88	6.02	2.88	6.25	8.59	2.71	3.46	8.54	5.34	5.49	3.50	6.17	23.54	20.04	6.76	7.52
Heritability in Broad Sense	68.88	83.73	88.70	79.39	93.85	90.97	92.64	89.87	49.07	53.17	88.99	90.44	85.79	93.42	81.19	93.96	94.76	86.24	90.15
(GCV %)	1.64	2.16	6.95	6.54	5.63	4.53	5.97	8.28	557.12	633.86	128.16	96.47	1550.18	1057.49	294.12	11.70	10.19	6.18	5.82
(PCV %)	1.97	2.36	7.38	7.34	5.81	4.74	6.20	8.74	795.26	869.20	135.85	101.44	1673.62	1094.05	326.40	12.07	10.47	6.66	6.13
D ²	0.33	0.2	0.43	0.80	0.18	0.21	0.23	0.46	238.14	235.34	7.69	4.97	123.44	36.56	32.28	0.37	0.28	0.48	0.31
GA or (Expected genetic advance)	1.88	3.51	6.79	4.89	4.76	3.19	4.78	5.44	1.67	2.04	5.37	4.32	4.15	3.61	4.17	9.42	8.77	4.63	5.11
GAM or (Genetic advance as percentage of mean) %	1.95	4.09	13.52	12.04	11.28	8.92	11.87	16.22	806.76	953.27	249.65	189.64	2964.28	2111.11	547.96	23.43	20.51	11.86	11.42

Table 9 :- Estimation of the salinity tolerance indices parameters for all barley accessions especially for grain yield trait under both conditions.

Genotypes	1	2	3	4	5	6	7	8	9
P1	43.75	38.16	0.87	1.13	40.95	1.029	40.85	0.13	0.77
P2	46.0	41.28	0.89	1.23	43.64	1.171	43.57	0.11	0.65
P3	44.95	40.37	0.89	1.20	42.66	1.119	42.59	0.11	0.65
P4	36.42	27.05	0.74	0.80	31.73	0.607	31.38	0.26	1.55
P5	31.45	24.59	0.78	0.73	28.02	0.477	27.80	0.22	1.31
P1 X P2	55.36	46.44	0.83	1.38	50.9	1.585	50.70	0.17	1.01
P1 X P3	50.03	44.24	0.88	1.31	47.13	1.365	47.04	0.12	0.71
P1 X P4	29.78	22.36	0.75	0.66	26.07	0.410	25.80	0.25	1.49
P1 X P5	26.49	21.39	0.80	0.63	23.94	0.349	23.80	0.20	1.19
P2 X P3	54.89	47.22	0.86	1.40	51.05	1.598	50.91	0.14	0.83
P2 X P4	51.66	45.06	0.87	1.34	48.36	1.435	48.24	0.13	0.77
P2 X P5	28.14	19.38	0.68	0.57	23.76	0.336	23.35	0.32	1.91
P3 X P4	49.80	46.22	0.92	1.37	48.01	1.419	47.97	0.08	0.47
P3 X P5	30.09	21.53	0.71	0.64	25.81	0.399	25.45	0.29	1.73
P4 X P5	25.16	17.62	0.70	0.52	21.39	0.273	21.05	0.30	1.79

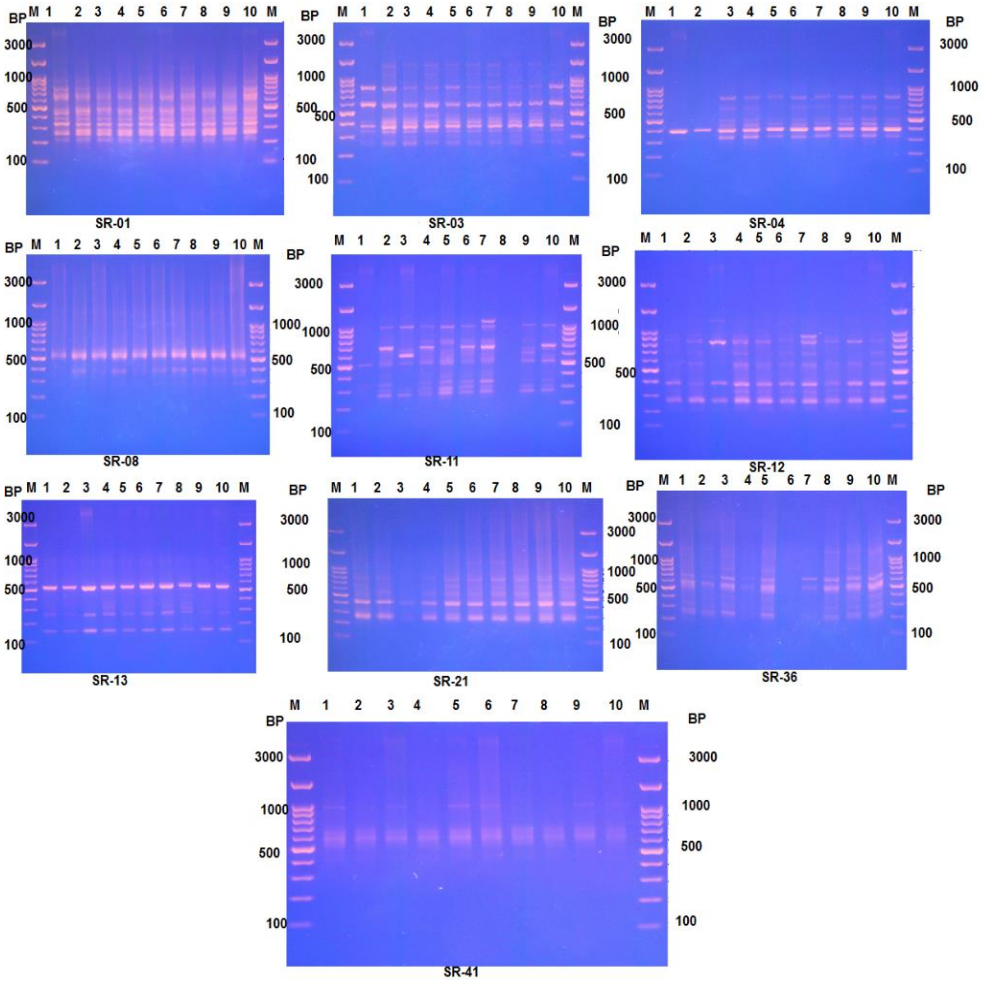

Fig 1. The inter-simple sequence repeat (ISSR) amplification pattern obtained in the ten *barley genotypes* namely 1: Parent 1 (Line one), 2: Parent 2 (Line two), 3: Parent 3 (Giza 121), 4: Parent 4 (Giza 126), 5: Parent 5 (Giza 2000), 6: Cross 1 or Hybrid 1: (P1 X P2), 7 :Cross 2 or Hybrid 2: (P1 X P3), 8: Cross 3 or Hybrid 3: (P2 X P3), 9: Cross 4 or Hybrid 4: (P2 X P4) and Cross 5 or Hybrid 5: (P3 X P4) using the ten ISSR primers namely; SR-01, SR-03, SR-04, SR-08, SR-11, SR-12, SR-13, SR-21, SR-36 and SR-41, respectively.

Table 10. Band variation and polymorphism percentage in the eight barley genotypes using 10 ISSR primers

No.	ISSR primers	T.B	M.B	P.B	U.B	P %	R.S (bp)	Sequence	Abbrev.	Mer
1	**SR-01**	10	1	9	**6**	**90.0%**	977-217	ACACACACACACACACC	(AC)8C	17
2	**SR-03**	10	0	**10**	0	**100.0%**	4409-260	ACACACACACACACACT	(AC)8T	17
3	**SR-04**	10	7	**3**	3	**30.0%**	851-343	ACACACACACACACACYA	(AC)8YA	18
4	**SR-08**	10	1	9	1	**90.0%**	528-289	AGAGAGAGAGAGGG	(AG)6GG	14
5	**SR-11**	10	1	9	1	**90.0%**	1246-310	AGAGAGAGAGAGAGAGT	(AG)8T	17
6	**SR-12**	10	0	10	**6**	**100.0%**	1274-261	AGAGAGAGAGAGAGAGYA	(AG)8YA	18
7	**SR-13**	10	1	9	3	**90.0%**	568-168	AGAGAGAGAGAGAGAGYC	(AG)8YC	18
8	**SR-21**	10	0	10	3	**100.0%**	1285-289	CACACACACACACACAT	(CA)8T	17
9	**SR-36**	10	3	7	**4**	70.0%	1245-211	GACAGACAGACAGACAWC	(GACA)4WC	18
10	**SR-41**	10	7	3	3	30.0%	1041-607	TCTCTCTCTCTCTCTCA	(TC)8A	17
Total		100	21	79	30	79.0%	4409-168			

T.B: Total bands, M.B: Monomorphic bands, P.B: Polymorphic bands, U.B or P.S.M: Unique bands or positive specific marker, P%: Polymorphism percentage and R.S (bp): Range size

Table 11. Total bands produced from each primer for 10 barley entries and all amplified fragments in each genotype.

Genotypes	Primers										
	SR-01	**SR-03**	**SR-04**	**SR-08**	**SR-11**	**SR-12**	**SR-13**	**SR-21**	**SR-36**	**SR-41**	*****
P1	**8**	**7**	**1**	**1**	**2**	**6**	**3**	**5**	**6**	**3**	**42**
P2	**9**	**10**	**1**	**2**	**8**	**7**	**3**	**5**	**8**	**3**	**56**
P3	**9**	**9**	**6**	**2**	**6**	**4**	**3**	**2**	**8**	**3**	**52**
P4	**8**	**12**	**6**	**2**	**8**	**7**	**5**	**5**	**2**	**2**	**57**
P5	**7**	**10**	**5**	**2**	**8**	**6**	**3**	**6**	**7**	**3**	**57**
H1	**9**	**9**	**6**	**2**	**9**	**7**	**5**	**6**	**7**	**3**	**63**
H2	**8**	**11**	**6**	**2**	**10**	**7**	**5**	**6**	**3**	**2**	**60**
H3	**9**	**9**	**6**	**2**	**8**	**7**	**6**	**5**	**7**	**2**	**61**
H4	**8**	**9**	**6**	**2**	**8**	**6**	**3**	**5**	**8**	**3**	**58**
H5	**10**	**7**	**6**	**4**	**7**	**7**	**3**	**5**	**7**	**3**	**59**
Total Bands	**85**	**93**	**49**	**21**	**74**	**64**	**39**	**50**	**63**	**27**	**565**

P: Parent, H: Hybrid.

Data observed in table (12) showed 30 unique markers or specific markers divided into (27) negative and (3) positive markers and distributed as follows six negative markers for **SR-01 primer** at molecular size 977 bp in the barley genotypes (P2, P3, H1, H2, H3 and H4), respectively. Also, three unique bands were showed for **SR-04 primer** where two positive markers at molecular size 412 bp were obtained in the parents (P1 & P2) and one negative markers was generated in (P5) at molecular size 680 bp, respectively. Further, one positive marker at molecular size of 528 bp in (P1) and 1 negative unique band (1246 bp) in (H1) were produced through the two primers; **SR-08 and SR-11**, respectively. In the same track, the primer **SR-12** was recorded six negative markers at molecular size of 1274 bp for the barley accessions; (P2, P4, H1, H2, H3 & H5), respectively. In the same context, three negative unique bands showed in **SR-13 marker** at molecular size of 448 bp for the barley entries; (P2, H1 & H2), respectively. Also, three negative markers were showed by the primer **SR-21** in the genotypes; (P5, H1 & H2) at molecular sizes of 1285 bp for (P5) and 318 bp for the other two barley genotypes; (H1 & H2), respectively. The primer **SR-36** generated four negative specific markers as follow; two negative markers for the genotypes (P5 & H1) at molecular size of 1245 bp and the other two negative markers for the barley accessions; (H3 & H5) at molecular size of 818 bp, respectively. Finally, three negative markers with size 1042 bp were generated in the barley accessions; (P4, H2 & H3) using **SR-41 primer,** respectively.

Table (12):- Mapping of positive (P) and negative specific markers for the 10 barley genotypes using 10 ISSR primers.

ISSR Primers	MS(bp)	Parent 1	Parent 2	Parent 3	Parent 4	Parent 5	Hybrid 1	Hybrid 2	Hybrid 3	Hybrid 4	Hybrid 5	(P or N) Marker
SR-01	977	+	-	+	+	+	+	+	+	+	+	Negative (P2)
	977	+	+	-	+	+	+	+	+	+	+	Negative (P3)
	977	+	+	+	+	+	-	+	+	+	+	Negative (H1)
	977	+	+	+	+	+	+	-	+	+	+	Negative (H2)
	977	+	+	+	+	+	+	+	-	+	+	Negative (H3)
	977	+	+	+	+	+	+	+	+	-	+	Negative (H4)
SR-04	412	+	-	-	-	-	-	-	-	-	-	Positive (P1)
	412	-	+	-	-	-	-	-	-	-	-	Positive (P2)
	680	+	+	+	+	-	+	+	+	+	+	Negative (P5)
SR-08	528	+	-	-	-	-	-	-	-	-	-	Positive (P1)
SR-11	1246	+	+	+	+	+	-	+	+	+	+	Negative (H1)
SR-12	1274	+	-	+	+	+	+	+	+	+	+	Negative (P2)
	1274	+	+	+	-	+	+	+	+	+	+	Negative (P4)
	1274	+	+	+	+	+	-	+	+	+	+	Negative (H1)
	1274	+	+	+	+	+	+	-	+	+	+	Negative (H2)
	1274	+	+	+	+	+	+	+	-	+	+	Negative (H3)
	1274	+	+	+	+	+	+	+	+	+	-	Negative (H5)
SR-13	448	+	+	+	-	+	+	+	+	+	+	Negative (P2)
	448	+	+	+	+	+	-	+	+	+	+	Negative (H1)
	448	+	+	+	+	+	+	-	+	+	+	Negative (H2)
SR-21	1285	+	+	+	+	-	+	+	+	+	+	Negative (P5)
	318	+	+	+	+	+	-	+	+	+	+	Negative (H1)
	318	+	+	+	+	+	+	-	+	+	+	Negative (H2)
SR-36	1245	+	+	+	+	-	+	+	+	+	+	Negative (P5)
	1245	+	+	+	+	+	-	+	+	+	+	Negative (H1)
	818	+	+	+	+	+	+	+	-	+	+	Negative (H3)
	818	+	+	+	+	+	+	+	+	+	-	Negative (H5)
SR-41	1042	+	+	+	-	+	+	+	+	+	+	Negative (P4)
	1042	+	+	+	+	+	+	-	+	+	+	Negative (H2)
	1042	+	+	+	+	+	+	+	-	+	+	Negative (H3)
Range	**1274-318**											
Total		2	3	1	3	3	6	5	4	1	**2**	**27 (Negative) + 3 (Positive)**

P: Positive, N: Negative, MS: Molecular Size

Similar Indices and Cluster Analysis:

Data observed in table (13) exhibited 45 relationships between 10 barley accessions revealed about similar. The values were 0.500 to 0913 with an average of (0.706). The highest level of was (0.913) within (P5 & H4) and the lowest limit was (0.500) within (P1 & P3). Other high and optimum means generated in this regard for example among (P4 & H1) (0.800), (P5 & H1) 0.857), (H1 & H2) (0.846), (H1 & H3) (0.875), (H1 & H4) (0.841), (H1 & H5) (0.800), (H2 & H3) (0.815) and (H3 & H4) (0.868). These results confirmed the fruitful role of correlated indices of all barley entries and its role of salinity tolerance in the future through the steps of traditional and modern techniques of genetics. Data of phylogenetic tree (Fig, 2) revealed the result of 10 barley entries were contained two main cluster where the first one was (P1) only. While, the second cluster had two sub-cluster. The first one was (P3) only and the second sub-cluster divided into two sub-sub-cluster. Where, the first one were the barley genotypes; (P4 & H2) only. While, the second sub-sub-cluster were included the rest barley accessions. These findings confirmed the essential and vital role of this analysis for showed all relationships among the ten barley accessions which exhibited highly rank of salinity tolerance under Egyptian conditions. Also, these results will help the breeders for screening the promising accessions that achieved high similar besides, all attributes under study for the two experiments.

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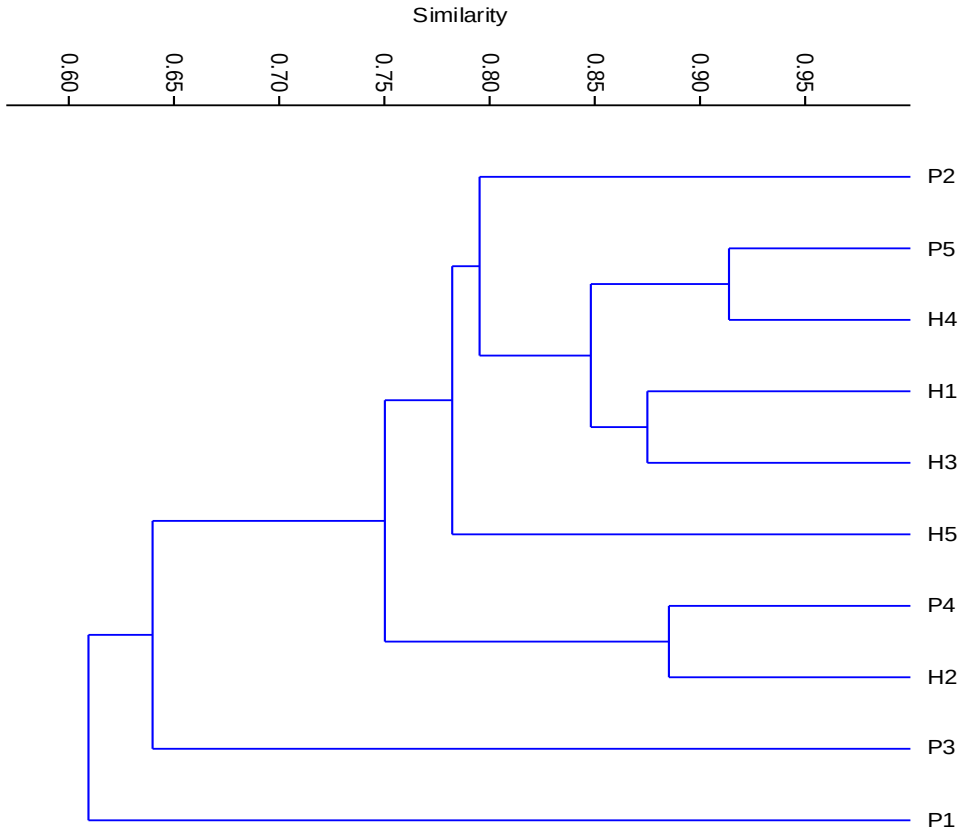
Table 13. Genetic similarity % in the ten barley genotypes using 10 ISSR Primers.

Genetic Similar	P1	P2	P3	P4	P5	H1	H2	H3	H4	H5
P1	1.0									
P2	0.709	1.0								
P3	**0.500**	0.629	1.0							
P4	0.548	0.692	0.537	1.0						
P5	0.627	0.803	0.688	0.723	1.0					
H1	0.629	0.825	0.661	**0.800**	**0.857**	1.0				
H2	0.523	0.738	0.582	0.885	0.716	**0.846**	1.0			
H3	0.622	0.765	0.656	0.796	0.825	**0.875**	**0.815**	1.0		
H4	0.610	0.786	0.728	0.707	**0.913**	**0.841**	0.727	**0.868**	1.0	
H5	0.714	0.774	0.634	0.723	0.750	**0.800**	0.716	0.796	0.790	1.0



UPGMA

Nei-Li's similarity coefficient

Fig. 2: Dendrogram representing the genetic relationship between the ten barley genotypes using UPGMA cluster analysis of Nei-Li's similarity coefficient generated from the 10 ISSR prime

3. Discussion:-

Ancient sciences, such as plant breeding or traditional genetics, are still among the most important scientific methods and means that light the way for the public of researchers with the aim of improving field crops. Despite their age, these methods have been able to develop degrees of tolerance and resistance of various crops to environmental and biotic stresses. In this regard, we address the science of plant breeding in developing a group of promising barley hybrids that represent the original materials in manufacturing the barley accessions resistant of salinity, especially in the valley and delta region. The problem of increasing degrees of salt stress has become one of the environmental challenges that hinder agricultural development paths, especially in countries whose economies depend primarily on agricultural output, such as the Arab Republic of Egypt. If we look carefully at the causes of this serious environmental challenge, we find that the only reason for high soil salinity is water stress, especially the scarcity of water sources necessary for agriculture and the washing of the soil content of accumulated salts, which cause toxicity to a large number of field crops sensitive to salt stress, such as barley crop which be the strategy food crops in the present time, especially given the inability of wheat to fully meet the critical needs of the bread industry. Therefore, the strategy of launching this investigation was very effective in producing barley accessions that are highly resistant and tolerant to salt stress and have high yield at the same time **[2 , 8].** Based on this crisis, it was necessary for scientists and researchers to quickly develop new barley lines that would confront the high salt stress in Egyptian soil, which began to increase in frequency, especially in the past few years. The logical protocol in this regard is to first select the ideal barley genotypes resistant to salinity besides their final output, with aim of crossing it with other sensitive entries and lines to produce new promising hybrids classified tolerant. Also, it will represent actual nucleus for the production of future barley varieties that are excellent in terms of stress tolerance salty and high yield as well and this is the greatest goal in this study which will be discussed in some detail by explaining the total results presented previously **[9 , 10].** Therefore, the previous primers have actually succeeded in providing the highest polymorphism percentage among the ten barley genotypes (The five parents and the five best hybrids in terms of tolerance to salt stress and also for the rest of the traits under study). Therefore, these primers succeeded in identifying molecular genetic differences between previous genotypes and could be considered as classification tools for promising barley accessions at the molecular level in terms of tolerance to salt stress. Further, this clearly confirms the importance of molecular markers in understanding and expressing genetic action or molecular genetic responses and also genetic differences in the plant community in table (11), **[53].** Data viewed in table (3) confirmed that each of additive and dominance effects considering an importance chance for enhancing the ability of stress tolerance under both conditions and were impacted for increasing the ability of salinity withstand in barley accessions. Decreasing limit of general and specific combining ability effects than the unit in all studied traits under both conditions would be reflected an importance function of Dominance effect in inheriting and improving all traits for salinity tolerance. Further, the optimum method of plant breeding using for genetic improvement these materials for salinity tolerance was selection method by bulk method not pedigree method **[8 , 15 , 32- 35].** After listing the most important results shown in table (4) indicated the superior barley materials which have already succeeded in achieving a significant and striking tolerance for salinity stress under saline treatment compared to the control experiment. It was found that the mechanism of excellence centered in giving promising mean values for a group of physiological traits besides some agro-morphological traits. On top of the physiological characters comes decreasing the limit of Na+ content in leaves comes in first followed by increasing the high level of K+ content followed by decreasing of the percentage of Na/K ratio in the leaves followed by decreasing the limit of osmotic adjustment and then followed by producing the highly rank of biochemical traits content. Where, salinity-tolerant genotypes can by the root system reducing the level of sodium entry and increasing potassium content responsible for positive functions that are very important to cells and metabolism processes. As well as, the genotypes tolerant to salinity can modify the osmotic pressure by reducing and convert it from high pressure to low osmotic pressure (osmotic adjustment) by inserting some components. These components would do this purpose in order to preserve the water level and prevent water from leaving the inside of the cell to the outside besides preventing from reaching to the plasma stage. In this context, it was also found that the hereditary structures that are tolerance and resistant to this environmental stress can form compounds and organic acids that would achieve the cause of endurance and resistance such as proline and glycine betaine contents in leaves with high level. As well as, they try to maintain a high yield under salinity stress compared to natural conditions **[9 , 10 , 13 , 14 , 36-39, 40, 41]** in table (5). Data cleared in table (6) may be pointed to the fruitful interaction and inheriting the same attributes under study besides, enhancing salinity withstand in barley genotypes. Also, data observed in table (7) indicated the importance of diallel analysis in enhancing the same genetic function for raising salinity tolerance efficiency in improved and superior crosses of all studied traits in this investigation **[3 , 35 , 42 , 43 , 44].** There is no doubt that estimating the genetic components has the greatest credit for determining the efficiency degree of the simple selection process that follows each segregation generation. This stage determines the quality of traits required for highly genetic improvement to salinity tolerance in barley genotypes table (8). Therefore, the science of plant breeding to face adverse environmental conditions played this successfully role in hunting genes responsible for inheriting the quantitative traits desired in this regard. Results obtained in table (8) confirmed that the greatest part of phenotypic variance was genotypic variation indicated that environmental factor was weak for controlling the previous traits in this context. High values of heritability in broad sense for all traits studied except Na+ content under both conditions means that the biggest role of additive gene action for enhancing salinity tolerance in these

traits. While, the medium limit of heritability observed in Na+ content only indicated the efficiency of dominance effect for controlling salinity tolerance in this attribute. While, the second group of traits which exhibited high gap among PCV% and GCV% confirmed that environmental variance had a great and a significant impact for inheriting these traits under both experiments. High values of genetic advance (GA) showed in most traits under both conditions confirmed the fruitful step for both types of gene action for increasing besides, enhancing salinity tolerance in barley entries. This case may be revealed a little function of single plant selection for inceasing genetic improvement in these two traits in the previous barley materials **[15 , 43- 46].** Results of salinity tolerance indices test in table (9) demonstrated that these previous genotypes were not only tolerant to salinity but were also promising and superior to the point where they became the true nucleus of high-yielding barley varieties and tolerated for a high level of seawater salinity. Because it has already succeeded in reducing the rate of losing and decreasing in the final yield under the level of salinity compared to the natural conditions. Moreover, it has already given a good output indicates that these crosses must continue to be cultivated for several segregation generations with the simple selection after each generation. Ultimately reaching to lines classified as highly environmentally and genetically stability, high-yielding under salinity conditions and this is the desired goal of this study **[13 , 15 , 42 , 43 , 47].** In the same context, plant breeding by mutations had effective success in improving the tolerance of a large number of strategic crops to abiotic stress such as wheat **[48].** Further, a large number of studies have also been launched on the tolerance of crops such as rice to heavy metals toxicity **[49]** besides, also enhancing some quality traits in rice **[50].** In the end, plant breeding research also had a great role in promoting field crops to resist various diseases, especially in lupins **[51]** and potato **[52].** Molecular genetics, using molecular markers has achieved a major scientific leap in the field of identifying genetic differences between varieties and different genotypes at the molecular level. Further, identifying plant lines that contain genes for tolerance to environmental stresses, such as water and salt stress. This effective technology has also achieved great progress in the field of improving various field crops with the aim of increasing their ability to resist biotic and abiotic stresses besides, obtaining the highest output from it. On this basis, the use of molecular markers is an effective tool in the field of genetic engineering and genetic improvement in plants using genetic modification pathways **[41 , 46 , 48 , 53].** This is what called for the use of the ten ISSR primers in this investigation with the aim of identifying the molecular basic that is cause salinity tolerance in the promising barley hybrids which achieved excellent results in terms of tolerance and also in terms of yield and its components traits. For unique markers, SR-01, SR-12 and SR-36 primers have been able to achieve great success by distinguishing among the ten promising barley genotypes through generating 30 distinct genetic markers or specific markers where they produced the highest rank of unique bands (6, 7, 4) for each one of them in table (10), respectively. These markers are considering a good indication for identifying promising genotypes that are tolerant to salt stress in barley hybrids, which after several segregation generations of cultivation may reach the highest rate of stable and genetic stability to be barley lines that are highly tolerant to salt stress and besides, its high yield **[48 , 53].** Based on these results, it can be said that specific markers has had a great role in the fields of plant breeding, especially those related to methods for improving crops to confront environmental stresses such as water and salt stress besides, high toxicity of heavy metals. However, in this investigation, 31 unique bands played a major role in identifying barley genotypes that are tolerant to salt stress, so that these markers can be easily tracked in the future and also used to transfer salt stress tolerance genes to other more sensitive varieties and lines. Thus, this aims to produce barley accessions that have a highly output as well, tolerance to any degree of salt stress, table (10), **[48 , 53].** Further, data obtained in table (11) referred to SR-01, SR-03, SR-11 and SR-12 primers succeeded of exhibited highly number of amplified fragments among the ten barley genotypes where the bands number were (85, 93, 74 , 64) for each one of them, respectively. These results actually confirmed that the four aforementioned ISSR primers had a very high genetic ability to differentiate between the different barley accessions of at the molecular level. Therefore, this indicates its superior scientific ability for scientific use in plant breeding programs and the search for genes that tolerate environmental stresses in field crops in general and in barley in particular **[1, 2, 5].** Looking more closely at this ISSR markers technique, it was observed that this method has achieved great success and is highly effective in improving the degree of tolerance of plants, especially field crops, to environmental and biotic stresses. Where, it had a large share in raising the degree of tolerance of rice to salt stress **[53],** increasing the resistance of the lupine crop to diseases **[51],** and the tolerance of barley to water and salt stress **[2, 8, 32]**, increasing the degree of salinity and drought tolerance in wheat **[1 , 5],** research To a greater degree in the extent of genetic diversity of soybean accessions **[44]** and other various crops. There is no doubt that this successful amount of results that have been presented so far is a vital and effective reflection of the integration of improving salt stress tolerance of some barley genotypes using traditional plant breeding methods using simple hybridization programs and also modern ones using biotechnology methods through the successful use of molecular markers (ISSR primers), which have already succeeded in finding molecular genetic differences that distinguish genotypes that are tolerant to salt stress from those that are sensitive to this serious environmental challenge. Therefore, this successful path in barley breeding may ultimately lead to the production of promising barley lines in terms of tolerance and high yield as well **[1, 2, 5].** In this same context, this scientific strategy of integrating the sciences of plant breeding and modern molecular genetics has led to the production of barley varieties with high tolerance to environmental stresses such as water and salt stress, as well, resistance to a large segment of diseases. Further, these cultivars have become accessible to farmers in Egypt and achieve high productivity despite the increasing level of salinity in Egyptian soil **[1, 2, 5].** Also, the importance thing in this regard is determine the limit of

environmental and genetic compatibility between ten barley genotypes under study (the parents and the five best hybrids in terms of drought resistance and high yields for the two conditions in table (12) and Fig.(2). This study was largely expressive of the extent of closeness or similar and also genetic divergence between barley accessions, because this trend is of great importance in determining which of them will be environmentally and genetically compatible for cultivation and growth together, unlike those hybrids that are far apart from their parents, and it is not recommended to cultivate them together in the future. For example, but not limited to, the results confirmed that the parent number five was very similar to the hybrid number four with similar % (0.913) and were fell together in the same cluster. This was the highest percentage of closeness and genetic compatibility in this regard, which shows that they will be compatible with each other for cultivation and growth together, as this hybrid (P4) reaches genetic stability in the future. Further, the presence of many examples of genetic compatibility in table (14) that indicate this matter. Also, the first and third barley hybrids had a compatibility rate that reached to (0.875 %) of similar and were in the same cluster that they were compatible with each other. This confirms that they will later be genetically compatible in all genetic and physiological aspects in terms of tolerance to salinity stress besides, its high yield, table (13) and Fig. (2), **[1, 2, 5, 53].** The problem of environmental stresses, especially water and salt stress, are serious environmental challenges that must be at the top of the list of countries' priorities in dealing with them, or at least in trying to contain them and reduce their destructive effects on the national economy represented by the agricultural output of field crops, especially barley. This is what was called for by the urgent and necessary need to launch this specialized study on improving tolerance to salt stress in the barley crop especially in the valley and delta regions within the scope of Egyptian agriculture. Accordingly, the aspects of this study were varied, covering the yield components, physiological, and molecular genetic aspects in an attempt to determine the genetic evidence that characterizes the genotypes that are tolerant to salt stress in the barley crop. Therefore, it has already succeeded in developing five barley hybrids that are tolerant to salt stress and have high yield under high salinity conditions using salty seawater compared to the standard experiment. Also, its tolerance to high salinity was confirmed after evaluating a significant number of genetic and physiological tests and salinity stress tolerance indices, all of which confirmed its tolerance to salt stress. This was presented and explained in detail previously. This is considered a good integration of traditional plant genetics and biotechnology in improving barley crop to salt stress tolerance. The previous results would be revealed that the traditional plant breeding and molecular markers using ISSR primers technique were played an importance role for enhancing the ability of salinity stress tolerance in barley accessions under study. Thus, this complementary among the previous two genetics methods was very fruitful in improving a large number of Egyptian crops to tolerate and resistant abiotic and biotic stress under Egyptian conditions. Also, the continuous of this strategy in barley crop will solve the problem of salinity tolerance in the region which contain a highly rank of salt and heavy metals such as the north of delta and the regions which nearing from the sea. These successes in the field of improving the barley crop to confront environmental stresses, especially salt stress, are an important bridge to collect quantitative traits containing genes for high yield and to confront salt stress in genetic compositions that are promising for the barley crop. As well, hybrids, which are barley varieties in the future, after they reach genetic consistency and stability. Environmental. Egyptian universities and research centers have played a pivotal role in this regard through importing wild lines and varieties of barley from their original areas of origin and wild pastures, with the aim of transferring salt stress tolerance genes to local entries and varieties sensitive to this environmental factor within the National Hybridization Program at the Field Crops Research Institute. Accordingly, a large genetic base was created for plant breeders, especially barley breeders, through which they can choose the strongest and most suitable genetic combinations to enter into the hybridization program.

4. Conclusion

The strategy of this study centered on a serious attempt to genetically improve tolerance to salt stress in some promising barley genotypes under salt stress condition compared to natural conditions. The most important results obtained can be summarized in the following points: - 1):-Developing some barley hybrids that are tolerant to salt stress, after confirming this scientific fact through physiological evidence of salt stress tolerance. 2:- All results of yield components and physiological traits demonstrated the tolerance of barley hybrids under stress conditions compared to the standard experiment. 3):- The results of ISSR markers demonstrated the presence of genetic evidence at the molecular level in the five tolerant barley hybrids, and this represents a major shift in this regard.

Conflict of interest

There is no conflict of interest.

5. References

[1] A. A. El-Mouhamady, H. M. Abdel-Rahman, A. A. Rizkalla, M. A. El-Metwally, Assessment of Water Stress Tolerance in Wheat Genotypes Based on Half Diallel Analysis and DNA Fingerprinting. Pak. J. of Biol. Sci, (3) (2019) 103-116.

[2] I. A. Khatab, A. A. El-Mouhamady, S. A. Mariey, M. M. El-Hawary, M. A. F. Habouh, Molecular evaluation and identification of some barley hybrids tolerant to salt stress. Pak J Biol Sci, (24) (2021 a) 997-1014. DOI: 10.3923/pjbs.2021.997.1014.

[3] Z. Chen, S. Shabala, N. Mendham, I. Newman, G. Zhang, M. Zhou, Combining ability of salinity tolerance on the basis of naclinduced K+ flux from roots of barley. Crop Sci, (48) (2008) 1382–1388.
[4] L. Vysotskaya, P. E. Hedley, G. Sharipova, D. Veselov, G. Kudoyarova, J. Morris, H. G. Jones, Effect of salinity on water relations of wild barley plants differing in salt tolerance. AoB Plants, (2010) (6). https://doi.org/ 10.1093/ aobpla/plq006.
[5] I. A. Khatab, A. A. El-Mouhamady, S. A. Mariey, Comprehensive Selection Criteria for High-Yielding Bread Wheat (Triticum aestivum L.) Hybrids under Salinity Stress. Egyptian Journal of botany, (3) (2021 b) 709-730. DOI: 10.21608/EJBO.2021.65333.1637.
[6] E. Nevo, G. Chen, Drought and salt tolerance in Wild relatives for wheat and barley improvement. Plant Cell Environ, (4) (2010) 670-685.
[7] H. M. Kalaji, B.K. Govindjee, J. Koscielniakan, K.Z. Gołaszewskae, Effects of salt stress on photosystem II efficiency and CO2 assimilation of two Syrian barley landraces. Environmental and Experimental Botany, (73) (2011) 64-72. https://doi.org/10.1016/j.envex.
[8] A. A. El-Mouhamady, Kh. A. Amer, A.Y. Ragab, Development of Salinity Tolerance in Some Genotypes of Barley Using Line X Tester Analysis and Some Techniques of Biotechnology. J of Appl Sci Res, (2) (2012 a) 972-982.
[9] N. Long, V.O. Dolstra, M. Malosetti, B.Kilian, A. Graner, R.G.F. Visser, D. Linden, Association mapping of salt tolerance in barley (Hordeum vulgare L.). Theoretical and Applied Genetics, (126) (2013) 2335-2351. https://doi.org/ 10.1007/s00122-013-2139-0.
[10] I. A. Khatab, S. A. Mareiy, Development of Agronomical and Molecular Genetic Markers Associated with Salt Stress Tolerance in Some Barley Genotypes. Current Research Journal of Biological Sciences, (5) (2013) 198-204.
[11] F.F.B. Abu-El-lail, K.A. Hamam, K.A. Kheiralla, M.Z. El-Hifny, SALINITY TOLERANCE IN 280 GENOTYPES OF TWO-ROWS BARLEY. Egypt. J. Plant Breed, (2) (2014) 331-345.
[12] A. M. E. Abdel-Hamid, Physiological and Molecular Markers for Salt Tolerance In four Barley Cultivars. European Scientific Journal, (3) (2014) 252-272.
[13] A. Jamshidi, H.R. Javanmard, Evaluation of barley (Hordeum vulgare L.) genotypes for salinity tolerance under field conditions using the stress indices. Ain Shams Engin. J, (9) (2018) 2093-2099. http://dx.doi.org/10.1016/ j.asej.2017.02.006.
[14] F. Ebrahim, A. Arzani, M. Rahimmalek, D. Sun, J. Peng, Salinity Tolerance of Wild Barley Hordeum Vulgare SSP. Plant Breeding, (2019):1–13.
[15] E. Mwando, Y. Han, T. T. Angessa, G. Zhou, C. B. Hill , X. G. Zhang, C. Li, Genome-Wide Association Study of Salinity Tolerance During Germination in Barley (Hordeum vulgare L). Frontiers in Plant Science, (11) (2020) 1-15.
[16] F. P., Chinard, Photometric Estimation of Proline and Ornithine. J. Biol Chem, (1) (1952) 91-5.
[17] L. S. Bates, R. P. Waldren, I. D. Teare, Rapid determination of free proline for water-stress studies. Plant Soil, (39) (1973) 205–207.
[18] C. M. Grieve, S.R. Grattan, Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil, (70) (1983) 303-307.
[19] J. B. Griffing, Concept of general and specific combining ability in relation to diallel crossing systems. Aust J of Biol Sci, (9) (1956) 463-493.
[20] G. W. Burton, E. M. Devane, Estimating heritability in tall fescue (Festuca arundinacea) from replicated clonal material. Agro J, (45) (1953) 478-481. doi:10.2134/agronj1953.00021962004500100005x.
[21] H. W., Johnson, H.F. Robinson, R.E. Comstock, Estimates of genetic and environmental variability in soybeans. Agro J, (47) (1955) 314-318. http://dx.doi.org/10.2134/agronj1955.00021962004700070009x.
[22] R. A. Fischer, R. Maurer, Drought resistance in spring wheat cultivars. I Grain yield response. Aust J Agric Res, (29) (1978) 897–907.
[23] M. Bouslama, W. T. Schapaugh, Stress tolerance in soybean. Part 1: evaluation of three screening techniques for heat and drought tolerance. Crop Sci, (24) (1984) 933-937.
[24] G. C. J., Fernandez, Effective selection criteria for assessing plant stress tolerance. In: Proc of on the Sym Taiw, (25) (1992) 257-270.
[25] P. Gavuzzi, F. Rizza, M. Palumbo, R. G. Campaline, G. L. Ricciardi, B. Borghi, Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. Can J Plant Sci, (77) (1997) 523-531.
[26] S. A. Golestani, M. T. Assad, Evaluation of four screening techniques for drought resistance and their relationship to yield reduction ratio in wheat. Euphyt-ica, (103) (1998) 293-299.
[27] M. M. Jones, N. C. N.C. Turner, Osmotic adjustment in leaves of sorghum in response to water deficits. Plant Physiol, (61) (1978) 122–126.
[28] J. Sambrook, K. F. Fritsch, T. Maniatis, Molecular cloning. Second edition (cold spring Harbor New York), (1989).
[29] E. Zietkiewicz, A. Rafalski, D. Labuda, Genome Fingerprinting by Simple Sequence Repeats (SSR)-Anchored Polymerase Chain Reaction Amplification. Geno, (20) (1994) 176-183.
[30] G. Gezahegn, T. Kassahun, B. Endashaw, Inter Simple Sequence Repeat (ISSR) analysis of wild and cultivated rice species from Ethiopia. Afri J of Biot, (9) (2010) 5048-5059.
[31] P. Jaccard, Nouvelles Researchers Sur La IstributionLorale. Bull Soc Vaud Sci Nat, (44) (19080 223-270.
[32] W. A. Ramadan, H. M. Abdel-Rahman, A. A. El-Mouhamady, M. A. F. Habouh, K. A. Aboud, Molecular Genetic Studies on Some Barley Entries for Drought Tolerance. Int. J. of Pharm Tech Res, (12) (2016) 265-285.
[33] R. M. Esmail, A. A. Abdel Sattar, N. S. Abdel-samea, A. A. El-Mouhamady, E. M. Abdelgany, F B. Fathallaha, Assessment of genetic parameters and drought tolerance indices in maize diallel crosses. Res. J. Pharm. Biol. Chem. Sci, (6) (2016) 2409-2428.

[34] A. A. El-Mouhamady, Z. M . El-Ashary, F. I. Mohamed, T.A. Elewa, K. A. Aboud, Study The Effect of Water stress Conditions on Some Genotypes of bread Wheat (Triticum aestivum L.) Based on Morphological, Physiological Traits and DNA Fingerprinting. RJPBCS, (4) (2016) 2065-2077.
[35] S. E. I. Eldessouky, S. A. A. Heiba, A. A. El-Mouhamady, Y. M Abdel-Tawab, DNA Fingerprinting and Half Diallel sAnalysis of Some Rice Genotypes under Water Deficit Conditions. Research Journal of Pharmaceutical, Biological and Chemical Sciences, (4) (2016) 985-997.
[36] A. A. El-Mouhamady, M. A. F. Habouh, E. Naif, Elicitation of Novel Sunflower Lines Tolerant to Water Deficit Conditions Through Mutation Breeding. Asian J. of Plant Sci, (3) (2022) 360-378 DOI: 10.3923/ajps.2022.360.378.
[37] A. A. El-Mouhamady, E. Naif, M. M. El-Hawary, Stability Analysis and Molecular Description of Some Promising Sorghum Lines Tolerant to Salt Stress. Pak. J. of Biol. Sci, (12) (2021 b) 1278-1296. DOI: 10.3923/pjbs.2021.1278.1296.
[38] M. A. Al-Kordy, H. M. Ibrahim, A. A. El-Mouhamady, H. M. Abdel-Rahman, Genetic Stability Analysis and Molecular Depiction in Elite Entries of Rice (Oryza Sativa L.). Bulletin of the National Research Centre, (85) (2019) 1-15.
[39] A. A. El-Mouhamady, E. H. El-Seidy, K. A. Aboud, Identification of a Molecular Markers Linked to Drought Tolerance in Some Genotypes of Barley. Aust. J. of Basic and Applied Sci, (5) (2012 b) 196-204.
[40] A. A. El-Mouhamady, M. G. Abdul Aziz, M. A. F. Habouh, Determination of Genetic Parameters associated with Salt Stress Tolerance in Canola Based on SCoT Markers and Protein Pattern Analysis. Asian J. Plant Sci, (20) (2021 a) 534-554. DOI: 10.3923/ajps.2021.534.554.
[41] A. A. El-Mouhamady, M. R. Rady, E. H. El-Seidy, Assessment of Genetic Variability for Six Lines of Wheat Using Physiological Traits and Molecular Markers Technique under Normal Irrigation and Water Stress Conditions. World Applied Sciences Journal, (4) (2014) 506-516.
[42] I. A. Khatab, A. A. El-Mouhamady, S. A. Mariey, T. A. Elewa, Assessment of Water Deficiency Tolerance Indices and their Relation with ISSR Markers in Barley (Hordeum vulgare L.). Cur Sci Int, (1) (2019) 83-100.
[43] R. S. Tawfik, A. A. El-Mouhamady, Molecular Genetic Studies on Abiotic Stress Resistance in Sorghum Entries through Using Half Diallel Analysis and Inter-Simple Sequence Repeat (ISSR) Markers. Bull. of the Nati. Res. Cent; (117) (2019) 1-17.
[44] A. A. El-Mouhamady, M. A. El-Metwally, Appreciation of Genetic Parameters and Molecular Characterization in Some Promising Accessions of Soybean (Glycine max L.). Pak. J. of Biol. Sci, (4) (2020) 425-438.
[45] I. A. Khatab, A. A. El-Mouhamady, H. M. Abdel-Rahman, M. A. Farid, I. S. El-Demardash, Agro-morphological and Molecular Characterization of Sorghum (Sorghum vulgare L.) for Water Stress Tolerance. Int. J. Curr. Res. Biosci. Plant Biol, (1) (2017) 37-55.
[46] A. A. Abdel Sattar, A. A. El-Mouhamady, Genetic Analysis and Molecular Markers for Yield and its Components Traits in Faba Bean (Vicia FabaL.). Aust. J. of Basic and Applied Sci, (7) (2012) 458-466.
[47] C. Zhonghua, S. Shabala, N. Mendham, I. Newman, G. Zhang, M. Zho, Combining Ability of Salinity Tolerance on the Basis of NaCl-Induced K+ Flux from Roots of Barley. Crop Sci, (48) (2008) 1382–1388.
[48] S. A. A. Heiba, S. E. I. Eldessouky , A. A. El-Mouhamady , I. S. El-Demardash, A. A. Abdel-Raheem, Use of RAPD and ISSR Assays for the Detection of Mutation Changes in Wheat (Triticum aestivium L.) DNA Induced by Ethyl-Methane Sulphonate (EMS). Int. J. of Chem Tech Res, (3) (2016) 42-49.
[49] S. A. A. Heiba, A. A. El-Mouhamady, S. E. I. Eldessouky, H. B. M. Ali, T. A. Elewa, Study the genetic variations related to the resistance of heavy metals toxicity in some rice genotypes using RAPD markers. Int. J Cur Micobl. Ap Sci, (1) (2016) 174-89.
[50] A. A. El-Mouhamady, A. M. El-Ekhtyar, I. S. El-Demardash, Molecular Markers Linked to Some traits in Rice. J. of Appl. Sci. Res, (5) (2012 c) 2689-2699.
[51] A. H. Zian, I. S. El-Demardash, A. A. El-Mouhamady, E. El-Barougy, Studies the Resistance of Lupine for Fusarium oxysporum F. sp Lupini) Through Molecular Genetic Technique. World Applied Sciences Journal, (8) (2013) 1064-1069.
[52] I. A. Khatab, A. A. El-Mouhamady, In vitro Screening and Molecular Genetic Markers Associated with Fungal Pathogenic Toxin Filtrate Tolerance in Potato. Egypt. J. Chem, (6) (2022) 477-487.
[53] A. A. El-Mouhamady, Biochemical and Molecular Characterization of Some Rice Accessions Tolerated To Salt Stress. Egypt. J. Chem, (66) (2023) 881-891.