CHARACTERIZATION OF SOME BARLEY GENOTYPES FOR SALT TOLERANCE USING MOLECULAR,BIOCHMICAL AND AGRONOMIC ANALYSIS

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Abstract

The present investigation was carried out to study the inheritance of salt tolerance in six parental barley genotypes (*Hordeum vulgare* L.), under sand culture screening experiment. These genotypes differ genetically in their salt tolerance potentiality, and their F1. The aim of the study to estimate general and specific combining ability (gca and sca) as well as heterosis for yield and its components and genetic components. Also to develop biochemical and molecular markers associated with salt tolerance for the six parental genotypes, under control and salt conditions. Four crosses exhibited significant positive heterosis, these were (Rihane -03 x Giza 123), (Rihane -03 x Line 2), (Rihane -03 x Line 4) and (Line 2 x Line 4). F1 hybrids had significant heterosis for four or more traits contributing to grain yield and it could be concluded that these crosses would be efficient for salt tolerance. Electrophoretic analysis showed 12 bands with polymorphic bands were detected in the six parents, banding patterns were different among all studied genotypes. Moreover, using molecular markers of random amplified polymorphic DNA (RAPD) using six primers, three (B8, D20 and Z7) out of them were more potential with the six parental barley genotypes and showed specific bands related to salt tolerance.

Key words: Barley, salt stress, Heterosis, SDS-protein, RAPD-PCR, Cluster analysis

INTRODUCTION

Abiotic stress in fact is the principal cause failure worldwide, dipping average yield for most major crops by more than 50% (Bray et al., 2000).A biotic stress causes losses worth hundreds of million dollars each year due to reduction in crop productivity and crop failure (Shilp and Narendra, 2005).Among abiotic stresses, soil salinity and water scarcity are the imperative factors declining plant production globally. Elevated levels of salinity limits water uptake and accumulation of sodium, which in turn cause severe biochemical and physiological abnormalities in plants (Munns and Tester, 2008). Today soil salinity becomes a menace for the ecosystems of arid and semi-arid regions having great intrinsic ecological values. To stop the land

degradation and desertification process, the understanding of salt tolerance, avoidance and adaptations of plant is necessary (Monteverdi et al., 2008).

Barley is the main crop grown in a large scale in the North Coastal Region of Egypt and also in the newly reclaimed lands with saline soils and shortage of fresh water. It is mainly used for animal forages and recently it is used as human food because of its nutritional and healthy values in many countries using hull-less barley. Barley area in the Nile Valley of Egypt has been gradually declined, especially in areas where soil and irrigation is feasible and can be grown with other strategic crops such as wheat. It can be grown in a wide range of environmental conditions and give satisfactory yields in areas that are not suitable for growing most of the others cereal due to problems of abiotic and biotic stresses (Mass,1986 and Katja et al., 2009).

One way to alleviate the problem of salinity is the breeding for salt tolerant genotypes that perform better than current sensitive varieties under moderate to high salinity stress. So for many of years breeding for salt tolerance has been an important task to increase crop productivity under salt stress and choice of parents for crossing is considered an important step in any plant breeding program aiming to an increase in the salinity tolerance of barley which could improve the profitability of some of more than one billion salt affected hectares present in the world (El-Fadly et al., 2007).

The analysis of genetic variation and relatedness in germplasm are of great value for genetic resources conservation and plant breeding programs to determine the best crosses among different genotypes. Over the years, the methods for assessing genetic diversity have ranged from classical strategies such as morphological analysis to biochemical and molecular techniques (Demissie et al., 1998). Molecular markers developed by analysis of proteins, isozymes and randomly amplified polymorphism DNA (RAPD) have shown excellent potential to assist selection of quantitative traits(Studer, 1992).

Sodium dodecyl sulphate polyacrylamid gel electrophoresis (SDS-PAGE) is most economical simple and extensively used biochemical technique for analysis of genetic structure of germplasm. Gel electrophoresis can directly equate variation in protein banding patterns to gene coding various proteins and proved to be useful in revealing polymorphic loci that encode isozymes or proteins (Masoje et al.,2001).The importance of protein profiling has long been acknowledged in plant abiotic stress studies and previous study has provided useful information on individual enzymes or transporters, measuring their stress-dependent change in quantity, activity, as well as modifications of structure protein, protein interaction and stress dependent protein movement (Kiegel et al., 2000).Salinity has been reported to cause either decrease or

increase in the level of soluble proteins, a complete loss of present protein and the synthesis of new protein in barely (Yildiz, 2007). Karimzadeh et al. (2006) observed changes in the electrophoresis pattern of water-soluble proteins from barley cultivars and pointed out accumulation of stress proteins in leaves when exposed to salinity.

In recent years, attention has increasingly focused on the DNA molecule as a source of informative polymorphisms, because each individual's DNA sequence is unique. DNA polymorphisms as DNA fingerprinting are becoming the technique of choice for laboratory assessment of cultivar identifications. Characterization of genotypes using DNA fingerprinting techniques provides quantitative estimates of genetic structure and the information required for a rational utilization of germplasm in breeding program (Jqbal et al., 2009)

Molecular markers have been proved to be powerful tools in the characterization and evaluation of genetic diversity within and among species and populations (Russell et al., 1997). Of these techniques the random amplified polymorphic DNA (RAPD) assay, which detects nucleotide sequence polymorphism by means of the polymerase chain reaction (PCR) and a single primer of arbitrary nucleotide sequence, have been developed and used in genetic and breeding studies in barley (Forster et al., 2000 and Jqbal et al., 2009).RAPD markers have proven to be a reliable marker system for genetic fingerprinting and also for determining the genetic relationships among germplasm collections. RAPD markers have the advantages of simplicity and the ability to detect relatively small amounts of genetic variation and also need no prior information (Williams et al.,1991). One frequently reported disadvantage associated with RAPD is unreproducibility that may arise if experimental conditions are not standardized carefully (Prenner et al., 1993). Despite this fact, RAPD markers have provided informative data consistent with other markers, especially at the intraspecific level (Lerceteau et al., 1997) and are effective for large-scale population genetic analysis. Albayrak and Gözük (1999) indicated that, RAPD-PCR can be used as a tool in the selection of commercially important traits such as resistance against diseases, drought and salinity present in wild barley lines

Therefore in this view, using six barley genotypes and their F1 offspring, the objectives of the present study were develop biochemical (protein) and molecular (RAPD-PCR) markers associated with salt tolerance in barley genotypes and to assess the level of genetic diversity relationship among them using RAPD molecular marker procedure. This relationship could be used by breeder in establishing strategies for selecting early generation materials in variety developmental programs.

MATERIALS AND METHODS

Plant Materials:

Six barley (*Hordeum vulgare* L.) genotypes differing in their tolerance to salinity were obtained from Barley Res. Department, Agricultural Research Center, Giza, Egypt, to be included in this study. The pedigrees of these genotypes are presented in Table 1. The six parental genotypes were crossed in all possible combinations in one way (using half diallel) to obtain a total of 15 single crosses to produce F_1 grains. Fifteen seeds of each of the 21 genotypes (15 hybrids and six parents) were grained in plastic dishes of 35 cm height and 55 cm diameter and capacity of 60 Kg washed sand. Plants were thinned to five plants per entry, per dish, after 40 days with three replications. Salt treatments of 10000 ppm along with unsalted control concentrations were used in this experiment using modified Hagland No.1 solution, as a nutrient solution, suggested by (Johnson et al., 1957).

Studied Characteristics

The growth measurements for the 21 genotypes were taken on five plants from three replications for statistical analysis and genetical parameter estimations of ten characters of such as: days to heading, plant height (cm), peduncle length (cm), spike length (cm), number of spikes per plant, number of tillers per plant, number of seeds per spike, one thousand-kernels weight (g), grain yield per plant (g) and biological yield per plant (g).

Table 1. The entry name, pedigree and degree of salt tolerance of the studied barley genotypes

Name	Pedigree	Degree of tolerance
Rihane-03	As46//Avt/Aths	Tolerant
Giza 123	Giza 117 /FAO86	Tolerant
Line 1	Gloria-Bar/Copal//shyri/Dc-B/3/Aloe/Rue CMB-93A-7631-1Y-1M-0Y	Sensitive
Line 2	Alger/Ceres//Sls/3/Erlapm/4/Wi2296/Espo ICB-92-1058-0AP-6AP-0AP	Tolerant
Line 3	Jlb70-01//Deirallal06//DL70//Pyo/3/Rm 1508/4/Arizona5908/Aths//Avt/attitkil/3/Ager ICB-1390-0412AP-0AP	Sensitive
Line 4	Cita"s"/4/Apmlrl/Manker/3/Maswi/Bo)/5/Copl"s"/6/Srs ICB-91-0476-0AP-0AP	Sensitive

Statistical analysis

Statistical analyses were conducted using the computer software MSTAT-C computer program according to (Snedecor and Cochran, 1969). Differences among means were compared using Duncan's New Multiple Range Test (Duncan, 1955), The heritability estimates were calculated according to Mather and Jinks, (1971).

Biochemical and molecular genetic analysis

Leaf samples from each entry were collected at 20 days old seedlings grown under control and saline conditions and placed directly in deep freezer at -80°C until they were used for biochemical and molecular analyses.

Soluble protein analysis using SDS-PAGE

Total protein of the six genotypes were analyzed using SDS– polyacrylamide gel electrophoresis according to the method of (Laemmli,1970) as modified by (Studier,1973).SDS-PAGE was used to compare among the six entries under salt treatments by their protein finger prints such as water soluble protein.

PCR-RAPD analysis

Total genomic DNA was isolated using the method of (Dellaporta et al.,1983). PCR reactions were conducted using arbitrary 10-mer primers (Operon Technology, Inc., Alameda, CA, USA). Three out of eleven 10–bp oligonucleotide primers were screened for the ability to provide a suitable band pattern with various barley genotypes. The names and sequences of the primers that give clear bands are in Table 2. Agarose gel 1.2% was used for resolving the PCR products, using one Kb plus DNA ladder.

Table 2. The nucleotide sequences of the applied primers

Primer code	Sequences
B08	5 – GTCCACAGGG -3 '
D20	5 – ACCCGGTCAC-3 '
Z7	5 – CCAGGAGGAC-3 '

Estimates of nucleotide differences were calculated according to (Nei, 1987 and Nei and Miller, 1990).The amplified DNA fragments of the three primers in the six barley genotypes were used to calculate the nucleotide sequence divergence between each

pair of genotypes, The matrix of genetic distances was used to generate phylogenetic trees by means of un weighted pair–group method with arithmetical averages (UPGMA) and the use of (Neighbor-Join method with the use of computer software programs "Restsite" and " Tdraw " by (Nei and Miller, 1990).

RESULTS AND DISCUSSION

Sand culture conditions

Mean performance:

Mean performances of six parental barely genotypes and their 15 crosses in both control and salt stress as well as the combined analysis for all studied traits are presented in Table (3). Mean values for all traits of genotypes and their F1 were less under salt stress than the control. It could be concluded from results that the barley cultivars genotype Rihane –03 showed the most earliness for days to heading, and the Egyptian barley cultivar Giza 123 showed the higher mean values for spike length, no. of seeds per spike, biological yield and grain yield under the salt treatment. Therefore ,Rihane –03 and the Egyptian barley cultivar Giza 123 could be considered as salt tolerant genotypes and could be used in future breeding programs to increase yield ability under salt stress. These results are in a good agreement with (Ahmed et al., 1998 and Metwali, 2012) who concluded that Giza 123 can be considered as a source for salt tolerance.

Analyses of variance:

Mean squares for all traits under control and salinity conditions are recorded and presented in Table (4). The analysis of variance showed highly significant or significant differences among all genotypes for all traits under investigation at both environments and their combined data except for spike length and no. of seeds per spike under salt stress and also 1000-grain weight under control. Mean squares for parents vs. F_1 as an indication for average heterosis over all crosses were significant for all studied traits expect for no. of tillers per plant, no. of spikes per plant, 1000-grain weight and biological yield under the combined data. The interaction of genotypes with salinity treatment was highly significant for all traits except for no. of tiller per plant, spike length and no. of seeds per spike. The interaction of the parent with salinity treatment was highly significant and significant for days to heading, plant height, peduncle length, 1000-grain weight, biological yield and grain yield under combined data. These results are in a good agreement with (Ahmed, 2001) who found that the salinity reduced number of tillers, plant height, days to heading, no of spikes per plant, biological yield and grain yield

Heritability. Narrow sense heritability values were detected for all the studied traits which ranged from low as 8.43% to as high as 56.40% at levels of salinity. When

moderate to high narrow sense heritability occurred, phenotypic selection would be effective for improving high heritable traits. Similar results were obtained by (Abdel-Aty and Katta, 2002) under normal conditions.

Biochemical genetic studies

Electrophoresis analysis was carried out on water-soluble SDS-protein fraction for six parental barley genotypes, three tolerant (Rihane –03, Giza 123 and Line 2) and three sensitive (Line 1, Line 3 and Line 4) under control and salt are shown in Fig (1), The maximum numbers of bands were 12, which were not necessarily exhibited in all genotypes. The electrophoresis patterns revealed marked variations in the occurrence, distribution, intensity and density of the bands. The six parents were shown to exhibit some polymorphic bands with differential expression under salt stess. For instance, bands 7, 9, 10 and 11, with molecular weights 28.75, 18.05, 17.54 and 12.32 KDa, could be considered as a salt responsive revealing some degree of over expression. The sensitive parent Line 1 under salt treatment (Lane 6), was characterized by the appearance of a higher number of bands than its respective control (Lane 5). The genotype Line 4 also exhibited two bands no. 10 and 11 which were absent in this parent under control and found under salt with approximate molecular weights of 17.54 and 12.35 KDa, respectively. On the other hand, tolerant genotypes Rihane –03, Giza 123 and Line 2 under control were characterized by specific band no. 10 with relative molecular weight of 17.54 KDa which was absent in the sensitive genotypes Line 1 and Line 4 under control, and was lost in the sensitive genotype Line 3 (P5) under salt. This band could be considered as a positive marker for barley tolerant genotypes. These results confirmed the induction of some salt reactive bands after salt treatment, which may be interpreted as a differential expression on same proteins due to the effects of salt stress Salinity could have exerted strong inhibitory effects on gene expression in this set of sensitive barley genotypes. Those particular bands could be considered as negative molecular markers associated with salt tolerance in barley. This result agrees with (Katja et al., 2009 and Metwali, 2012).

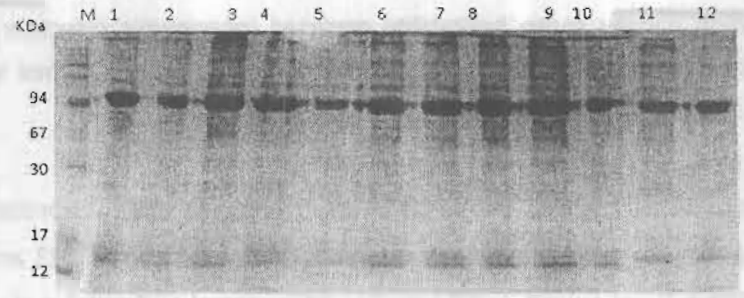


Fig 1. SDS-PAGE profiles of barley leaf protein (Water-Soluble) for six parental genotypes under control and salt stress. Lanes 1 and 2: Rihan-03 under control and salt treatment, respectively, Lanes 3 and 4: Giza 123 under control and salt treatment, respectively, Lanes 5 and 6: line1 under control and salt treatment, respectively, Lanes 7 and 8: line 2 under control and salt treatment, respectively, Lanes 9 and 10: line 3 under control and salt treatment respectively, Lanes 11and 12: line 4 under control and salt treatment, respectively.

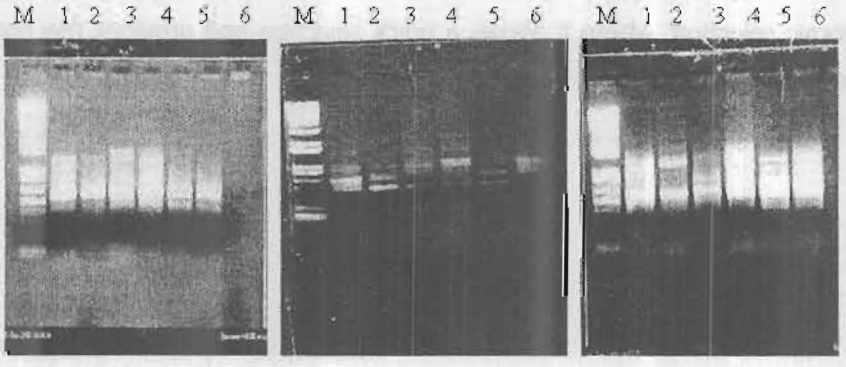
Molecular genetic markers.

Genomic DNA of the barley genotypes were extracted and were used in performing randomly amplified polymorphic DNA (RAPD). Three arbitrary oligonucleotide primers were uesd, Base sequence and number of fragments amplified using these different primers showed that: the number of amplified fragments differed from one genotype to another indicating that all barley genotypes are not always identical in their DNA ability to be amplified and these primers have amplified 23 PCR bands (Table 5) A maximum of 8 fragments were amplified with primer Z7 and minimum of 3 fragments were amplified with primer B8 These results agree with (Adrian et al., 2010 Baum et al., 2000).The results of RAPD analysis using Primer B8 produced eight amplified fragments and it was the lowest polymorphic primer since it produced just three polymorphic fragments (37.5%). The largest polymorphic fragment with 4000 bp molecular size was found only in salt sensitive parent Line 1 (P3) Fig(2a). This fragment may be related to salt sensitivity. Second polymorphic fragment had molecular size of 3400 bp and was not detected in sensitive parent Line4 (P6). This fragment may also be related to salt sensitivity. The fragment with molecular size 100bp was found in each of Rihane –03 (P1) and Line1 (P3).The total number of amplified fragments developed by using D20 primer was seven. This primer developed five polymorphic fragments Table (5) and Fig (2b). It was clearly noticed that the amplified fragment with molecular size 2000 bp is a positive marker in the three tolerant parents Rihane –03, Giza 123 and Line2 and not detected in any sensitive

parents. This fragment could be considered as a positively linked marker with salt tolerance. There were two monomorphic fragments with molecular size of 1650 bp and 500 bp, which were found in all parents. The fragment with molecular size 1000 bp was found in all parents excepts in the sensitive parent Line 1 (P3). While the fragment with molecular size 650 bp was found in all parents except in the tolerant parent (P2) Giza 123. On the other hand, fragment with molecular size of 100 bp was found in all parents except in the two sensitive parents Line1 (P3) and Line 4 (P6).Fig (2c) represents the amplified fragment patterns of primer Z7. This primer gave eight amplified fragments, they all 100% were polymorphic as shown in Table (5). There were three amplified fragments with molecular size of 2000 bp, 1650 bp and 1600 bp, detected only in the tolerant parent Line 2 (P4). The amplified fragment with molecular size 500bp was detected only in the salt tolerant parents (P1) Rihane–03, (P2) Giza 123 and (P3) Line 2, while it was not detected in the sensitive parents. This fragment can be considered as a linked marker to salt tolerance. On the other hand, the amplified fragment with molecular size of 650 bp was presented in all parents except for the two sensitive parents, Line1 (P3) and Line 4 (P6).

Table 5 .Estimates of polymorphism for the scored amplified fragments developed from the three primers.

Primer	Fragment			% Polymorphism
	Monomorphic	Polymorphic	Total	
B8	5	3	8	37.50
D20	2	5	7	71.43
Z7	0	8	8	100.00
Total	7	16	30	65.22



primer (B8) Fig(2a) **primer (D20 Fig(2b)** **primer (Z7) Fig(2c)**

Fig (3a, b and c): RAPD banding patterns of three primers B8, D20 and Z7 for six barley parental genotypes. M DNA ladder (1kb plus molecular weight). Lane 1 (Rihane0-3), Lane 2 (Giza 123), Lane 3 (line1), Lane 4 (line2), Lane 5 (line3) and Lane 6 (line4).

Our results are also in parallel with those of (Bahieldien et al. 1994) who identified a 24-mer synthetic primer specific for amplifying a conserved sequence (600bp) for induced salt tolerance and Metwali (2012) who showed that all barley genotypes are not always identical in their DNA ability to be amplified and the total of amplified bands is 352 PCR bands. On the contrary, primers P18, P86 and P24 were able to generate positive marker, P92 was able to generate negative marker and 93 was able to generate positive and negative marker for salt tolerance.

This phenomenon was supported with the highly substitution rate between Line3 and Line1. This high rate indicates that the divergence process was already exist. These relationships will be supported by the phylogenetic analysis.

The phylogenetic tree presented in Fig. (3) Was developed using the UPGMA method and the detected fragments from the RAPD technique. RAPD analysis seems to be one of the powerful tools for detecting polymorphism and could discriminate among all the six parental barley genotypes. The phylogenic tree succeeded in clustering together the three tolerant parents P1, P2 and P4. It grouped first Rihane-03 (P1) and the most related parent P5. This cluster was grouped with the tolerant parent Giza 123 (P2). The tree grouped these three parents with P4. The tree also grouped together with the two sensitive parents Line 1 (P3) and Line3 (P5) in one group. The polymorpic analysis revealed that the tolerant parent P4 was the oldest one and diverged first from its ancestor then the two sensitive parents diverged. The other tolerant parent P2 (Giza,123) diverged from this lineage then the other two tolerant parents.

As a conclusion the use of molecular markers will be good alternative to the agronomic selection, where it allows a quick selection and provides the breeder with the genetic markers for salt stress.

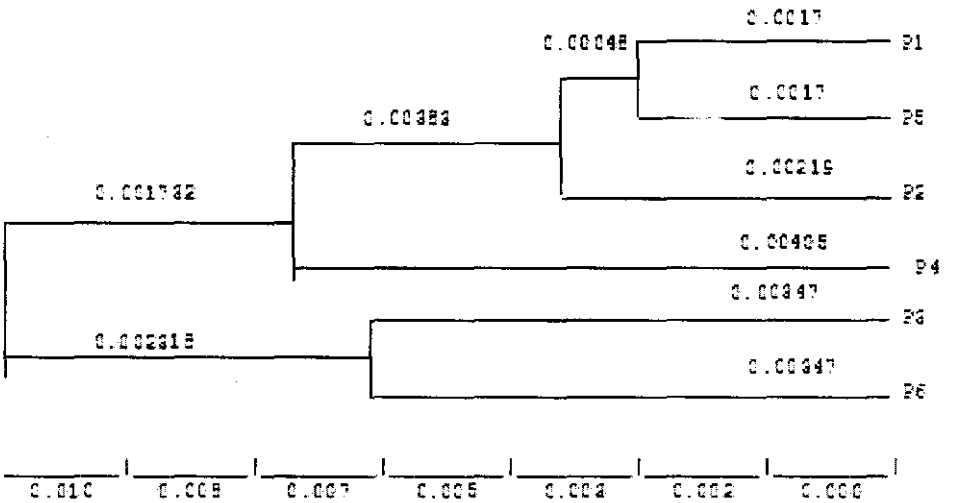


Fig 3. UPGMA dendrogram of six parental barley genotypes using three primer P1 Rihane 0-3 (t) ,P4 Line 2 (t) and P2 Giza 123 (t) P5 Line 3 (s),P3 Line 1 (s)and P6 Line 4 (s)

Table 3. Mean performances of ten yield-related traits of six barley genotypes and their 15 F1 hybrids under control, salt treatment and their combined data in sand culture experiment.

Genotype	Days to heading (days)			Plant height (cm)			Peduncle length (cm)			No. of tillers per plant			No. of spike per plant		
	Cont.	Salt	Mean	Cont.	Salt	mean	Cont.	Salt	mean	Cont.	Salt	Mean	Cont.	Salt	Mean
Rihane-03 (P1)	78.17	63.73	70.95	54.00	30.70	42.35	12.87	8.27	10.57	5.93	2.67	4.30	4.90	1.27	3.08
Giza 123 (P2)	75.60	69.70	72.65	62.87	41.33	52.10	10.70	9.87	10.28	5.53	2.53	4.03	5.13	1.93	3.53
Line 1 (P3)	74.83	71.67	73.25	73.00	40.17	56.58	15.73	10.67	13.20	6.40	2.40	4.40	5.40	2.43	3.92
Line 2 (P4)	72.37	71.43	71.90	66.43	44.57	55.50	15.43	11.67	13.55	6.80	2.33	4.57	5.47	1.37	3.42
Line 3 (P5)	74.60	70.33	72.47	62.53	38.27	50.40	16.40	10.87	13.63	5.47	4.40	4.93	5.13	2.57	3.85
Line 4 (P6)	72.20	69.13	70.67	67.73	42.20	54.97	14.93	10.67	12.80	7.27	2.80	5.03	6.17	1.93	4.05
P1 x P2	73.37	74.33	73.85	69.23	44.63	56.93	14.93	9.43	12.18	4.33	2.27	3.30	3.80	1.53	2.67
P1 X P3	72.93	73.30	73.12	65.87	39.73	52.80	14.17	9.33	11.75	6.33	3.40	4.87	5.77	1.77	3.77
P1 X P4	73.73	65.17	69.45	63.70	35.63	49.67	13.83	11.50	12.67	4.87	2.40	3.63	4.00	1.60	2.80
P1 XP5	74.33	74.70	74.52	62.27	35.13	48.70	14.93	10.00	12.47	9.87	3.47	6.67	5.03	1.63	3.33
P1 X P6	71.37	69.23	70.30	68.77	39.57	54.17	15.73	9.70	12.72	8.20	3.27	5.73	6.60	1.90	4.25
P2 X P3	75.67	69.90	72.78	63.27	26.80	45.03	12.57	8.77	10.67	4.93	1.47	3.20	4.00	1.10	2.55
P2 X P4	71.20	69.83	70.52	66.87	36.67	51.77	13.77	7.27	10.52	5.87	1.93	3.90	4.70	1.13	2.92
P2 X P5	74.77	70.07	72.42	68.67	35.50	52.08	15.60	8.77	12.18	5.93	1.80	3.87	4.87	1.43	3.15
P2 X P6	75.17	72.93	74.05	63.07	35.77	49.42	12.97	6.73	9.85	6.00	1.67	3.83	4.93	1.33	3.13
P3 X P4	75.33	71.53	73.43	63.33	40.27	51.80	14.47	9.00	11.73	7.00	3.53	5.27	6.57	2.07	4.32
P3 X P5	73.27	72.33	72.80	64.97	39.87	52.42	15.83	10.73	13.28	5.47	2.87	4.17	4.67	1.80	3.23
P3 X P6	70.80	71.03	70.92	59.23	39.67	49.45	14.87	8.83	11.85	6.67	3.00	4.83	6.30	1.70	4.00
P4 X P5	71.40	70.07	70.73	64.93	43.93	54.43	15.20	10.80	13.00	6.00	2.73	4.37	4.97	2.23	3.60
P4 X P6	74.33	71.13	72.73	71.80	44.60	58.20	16.93	9.80	13.37	8.40	3.60	6.00	6.73	2.20	4.47
P5 X P6	75.00	71.53	73.27	53.30	41.13	47.22	16.60	11.37	13.98	7.13	3.40	5.27	5.27	2.37	3.82
L.S.D 0.05	2.72	3.23	2.86	4.87	4.55	4.39	1.58	1.59	1.36	2.07	1.38	1.31	1.05	0.43	0.69
0.01	3.63	4.32	3.79	6.52	6.09	5.82	2.12	2.14	1.80	2.77	1.84	1.73	1.41	0.58	0.92
C.V %	2.23 %	2.77 %	2.51 %	4.57 %	7.09 %	5.51 %	6.53 %	9.98 %	8.06 %	19.59 %	30.27 %	23.5 %	12.17 %	14.8 2%	14.11%

Table 3. cont.,

Genotype	Spike length (cm)			No. of seeds Per spike			1000 grain weight (g)			Biological yield (g)			Grain yield (g)		
	Cont.	Salt	Mean	Cont.	Salt	Mean	Cont.	Salt	Mean	Cont.	salt	mean	Cont.	Salt	Mean
Rihane-03 (P1)	7.07	4.93	6.00	51.80	30.53	41.17	23.17	12.49	17.83	11.252	2.683	6.968	0.792	0.260	0.526
Giza 123 (P2)	7.67	6.07	6.87	62.93	46.80	54.87	34.00	25.09	29.54	20.299	7.630	13.964	4.582	1.420	3.001
Line1 (P3)	6.20	3.87	5.03	52.80	30.00	41.40	30.73	9.99	20.36	28.323	5.806	17.065	3.515	0.658	2.087
Line 2 (P4)	6.27	4.73	5.50	49.60	28.80	39.20	34.13	31.97	33.05	20.967	3.419	12.193	3.538	0.699	2.119
Line 3 (P5)	5.60	4.93	5.27	47.20	33.60	40.40	22.46	20.41	21.44	15.802	5.596	10.699	2.595	0.871	1.733
Line 4 (P6)	7.07	5.40	6.23	54.00	37.20	45.60	28.94	11.31	20.13	17.500	2.773	10.136	2.938	0.517	1.728
P1 x P2	7.87	5.00	6.43	60.00	40.80	50.40	34.41	26.55	30.48	19.742	3.601	11.672	4.479	0.515	2.497
P1 X P3	7.80	4.73	6.27	62.27	33.20	47.73	26.91	20.22	23.56	19.604	4.185	11.894	2.820	0.827	1.823
P1 X P4	7.20	4.53	5.87	58.40	32.00	45.20	30.13	13.82	21.98	17.198	4.846	11.022	3.023	0.731	1.877
P1 XP5	6.13	4.33	5.23	56.80	32.00	44.40	27.18	15.01	21.09	21.106	4.779	12.943	1.867	0.482	1.175
P1 X P6	7.93	5.20	6.57	60.33	39.60	49.97	32.31	21.37	26.84	21.454	5.874	13.664	2.650	1.211	1.931
P2 X P3	7.47	3.40	5.43	58.00	24.47	41.23	33.14	13.80	23.47	16.529	1.649	9.089	3.094	0.582	1.838
P2 X P4	6.53	4.40	5.47	49.60	30.00	39.80	36.95	28.44	32.70	21.903	3.182	12.542	4.806	0.693	2.750
P2 X P5	7.53	5.60	6.57	58.73	40.40	49.57	31.39	31.56	31.47	22.296	3.436	12.866	4.543	1.404	2.973
P2 X P6	7.13	4.07	5.60	63.20	30.80	47.00	25.77	19.72	22.74	21.011	3.462	12.237	2.997	0.884	1.941
P3 X P4	6.80	4.47	5.63	59.00	26.27	42.63	24.16	16.46	20.31	14.736	4.948	9.842	3.457	0.560	2.009
P3 X P5	6.80	5.20	6.00	54.00	37.60	45.80	32.11	20.96	26.54	18.709	4.141	11.425	2.341	0.743	1.542
P3 X P6	7.20	5.33	6.27	59.20	39.93	49.57	32.54	17.62	25.08	15.928	5.554	10.741	4.150	0.720	2.435
P4 X P5	6.60	4.87	5.73	44.80	32.40	38.60	31.09	23.41	27.25	18.029	4.895	11.462	3.874	1.214	2.544
P4 X P6	7.87	6.13	7.00	60.20	44.00	52.10	27.31	22.65	24.98	30.509	6.839	18.674	4.957	1.286	3.121
P5 X P6	7.00	4.93	5.97	55.60	32.80	44.20	25.27	15.03	20.15	26.105	5.319	15.712	3.189	0.692	1.941
L.S.D 0.05 0.01	1.11 1.48	1.58 2.12	0.97 1.28	8.82 11.80	12.09 16.18	7.69 10.17	8.19 10.95	5.67 7.59	5.62 7.43	4.44 5.95	1.34 1.79	3.26 4.32	1.08 1.44	0.37 0.50	0.75 0.99
C.V %	9.55%	19.72%	13.99%	9.52%	21.27%	14.35%	16.69%	17.27%	17.27%	13.50%	18.02%	16.28%	19.52%	27.84%	23.48%

Table 4. Mean squares estimates of ten yield-related traits of six barley genotypes and their 15 F1 hybrids under control, salt treatment and their combined data in sand culture experiment.

S.O.V	d.f	Days to heading (days)			Plant height (cm)			Peduncle length (cm)			No. of tillers per plant			No. of spike per plant		
		Cont.	Salt	Combined	Cont.	Salt	Combined	Cont.	Salt	Combined	Cont.	Salt	Combined	Cont.	Salt	Combined
Genotypes	20	10.189**	20.061**	11.763**	72.903**	62.398*	93.378**	6.836**	5.322**	8.922**	5.017**	1.629**	4.714**	2.164**	0.551**	1.846**
Parents	5	14.711**	25.433**	6.109	121.918**	69.431*	165.299**	13.892**	4.064	13.755**	0.587	0.849**	0.792	1.551	1.805	0.879
F_1S	14	8.157*	16.578**	14.405**	60.572**	63.520**	74.100**	4.591**	5.465**	7.766**	2.861**	0.448**	2.298**	6.564**	1.665	6.416**
Parents vs.F_1	1	16.027	41.963	2.800*	0.462*	11.497	3.665*	2.986	9.61	0.941*	57.351	22.063	58.148	-56.370	-21.315	-57.299
Salinity	1			323.841**			20805.435**			779.023**			417.654**			381.686**
Gen x san.	20			18.487**			41.923**			3.236**			1.932			0.869**
P x san.	5			34.036**			26.050*			4.200			0.644			2.476
F_1 x san.	14			10.330**			49.994**			2.290**			1.011**			1.813
P vs. F_1 x san.	1			54.940			8.294			11.66			21.266			-20.382

* and ** indicate significant at $P \leq 0.05$ and $P \leq 0.01$ respectively

Table 4. cont.,

S.O. V df		Spike length (cm)			No. of see ds Per spike			1000 grain weight (g)			Biological yield (g)			Grain yield (g)		
		Cont.	Salt	Combined	Cont.	Salt	Combined	Cont.	Salt	Combined	Cont.	Salt	Combined	Cont.	Salt	Combined
Genotypes	20	1.258**	1.341	1.807**	82.389**	102.234	127.630**	49.921	122.231**	124.640**	60.238**	6.444**	41.528**	3.224**	0.309**	2.308**
Parents	5	1.700**	1.596	2.791**	87.800**	136.718	205.345**	78.617	233.134**	222.053**	98.989**	12.011**	71.853**	4.859**	0.460**	3.878**
F_1s	14	0.916	1.317	1.521	69.466	97.216	100.830*	42.049	88.044**	94.518**	49.148**	4.878**	33.111**	2.651**	0.269**	1.753**
Parents vs.F_1	1	3.836	0.397**	0.891*	236.256	0.066**	114.255	16.649	46.334	59.283	21.743	0.533*	7.741	3.071	0.114**	2.228*
Salinity	1			148.526**.			14804.838**			3038.112**			7516.085**			202.443**
Gen xSan.	20			0.792			56.994			47.513**			25.155**			1.225**
P x San.	5			0.505			19.173			89.697**			39.147**			1.441**
F_1 x San.	14			0.711			65.852			35.565			20.915**			1.666**
P vs. F_1 x San.	1			3.361			122.087			3.752			14.555			-6.029

* and ** indicate significant at $P \leq 0.05$ and $P \leq 0.01$ respectively

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توصيف بعض التراكيب الوراثية من الشعير لتحمل الملوحة باستخدام الأدلة الجزيئية والمحصولية

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أجريت هذه التجربه بهدف دراسة وراثة تحمل ستة أصناف من الشعير للملوحة من خلال تجربة تحت ظروف الصوبة الرملية حيث يوجد اختلاف وراثى بين الأباء فى درجة تحملها للملوحة، وكذلك أجرى البحث بغرض دراسة و معرفة القدرة العامة و الخاصة على الائتلاف، قوة الهجين، الارتباط بين الصفات محل الدراسة ، دراسة النظام الوراثى الذى يحكم الصفات المدروسة و درجة التوريث بين هذه الآباء الستة تحت الملوحة و الكنترول ، بالإضافة لدراسة بعض الصفات البيوكيمائية و الجزيئية للآباء الستة لتحديد أدلة جزيئية مرتبطة بالملوحة – أظهرت دراسة قوة الهجين وجود أربع هجن أعطت قوة هجين عالية المعنوية و هى هجين ريحان –٣ x جيزة ١٢٣ ، ريحان –٣ x السلالة ٢ ، ريحان –٣ x السلالة ٤ و السلالة ٢ x السلالة ٤ حيث أوضحت النتائج أنها هجن مبشرة ذات أهمية فى تحملها للملوحة. أظهر التفريد الكهربى للبروتينات الذائبة فى الماء باستخدام تقنية SDS-PAGE وجود ١٢حزمة كحد أقصى داخل الأباء الستة حيث اظهرت الاباء الستة تباين فى الحزم الموجودة. استخدمت تقنية التكبير العشوائي باستخدام PCR لإيجاد بعض الدلائل المميزة المرتبطة بتحمل الملوحة لستة أباء وقد تـــم استخدام ستة بوادىء عشوائية ثلاثة منهم وهم (B8 , D20, Z7) كانوا اكثر مقدرة على التمييز بين الآباء لصفة تحمل الملوحة.