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# Research Article
# Molecular Evaluation and Identification of Some Barley Hybrids Tolerant to Salt Stress

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## Abstract

**Background and Objective:** Barley is considering one of the most important cereal crops at the local and global levels. It is ranked second in terms of nutritional importance after wheat and its flour contributes significantly to bridging the large nutritional gap in the production of Egyptian bread. The aim of this study concentrated on knowing and testing the genetic behaviour responsible for salinity stress tolerance in barley as trying to improve barley crop and increase its ability for abiotic stress resistance under Egyptian conditions. **Materials and Methods:** Twenty-one crosses and ten parents of barley with different responses to salinity tolerance were evaluated in this investigation under normal and salinity conditions. Yield and its components and some physiological traits related to salt stress tolerance were the most important studied attributes evaluated in this regard under both conditions. Moreover, SSR markers were used to evaluate and identified associated markers for salinity tolerance in selected hybrids and comparing among the ten barley parents. **Results:** The final results confirmed that the three testers; Giza 123, Giza 126 and Giza 2000 besides; the crosses; Line 1XTester 1 (Giza 125XGiza 123), Line 2XTester 1 (Giza 133XGiza 123), Line 1XTester 2 (Giza 125XGiza 126), Line 2XTester 2 (Giza 133XGiza 126) and Line 1XTester 3 (Giza 125XGiza 2000) exhibited highly salinity tolerance under saline stress treatment compared with the control experiment. Among 15 analyzed barley entries, the chosen set of 11 markers amplified 20 alleles with an average of 1.81, with a range from 1-4 alleles. **Conclusion:** The results of SSR analysis and the data on valued agricultural trait loci determined the genetic distance among parents and their hybrids, which is of an unlimited rate for breeders.

Key words: Barley, salinity tolerance indices, lineXtester, physiological traits, PCASSR, hydraulic conductivity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Barley (*Hordeum vulgare* L.) is considered one of the most cereal crops that fall within the food security of humans and animals. It comes after wheat as it is a strong participant in the production of flour used in the manufacture of bread after the mixing process in certain proportions in light of the crisis of the bread industry in Egypt. Also, it attempts to bridge the food gap in this industry, but it is noticeable in light of the changing environmental and biological challenges and constraints that the area of this important crop has begun to shrink globally. This is remarkable as well as it is very dangerous at the local and global levels. The problem of high salinity levels in soil and irrigation water comes on top of these environmental pressures that reducing barley cultivation and then the production of bread under Egyptian conditions. It suffices to know that the high salinity of areas nearing the sea coast and higher level of salinity in irrigation water in field crops destroy approximately 40-50% of the final yield especially crops sensitive to salinity such as barley. So, many papers and studies were launched for genetic improvement in barley to salinity tolerance using traditional and modern plant breeding methods such as biotechnology. The following will be presented an important summary of papers results carried out in this regard. Combining ability of salinity tolerance based on NaCl-induced $K^+$ flux from roots of barley was determined by Chen *et al.*[1] through using 6 barley genotypes in half diallel cross and detected that the tolerant genotypes CM72 and Numar recorded highly significant GCA effects for salinity tolerance (low limit of $K^+$ lost under salinity stress). Decrease of hydraulic conductivity in salt-treated barley genotypes is very paramount for stomatal closure as well as lowered transpiration level is beneficial for salt tolerance at least at the seedling stage[2]. Nevo and Chen[3] revealed water stress and salinity tolerance in wild relatives for wheat and barley, salinity tolerance that has been extensively and virtually defined, evaluated and transferred to wheat entries with a demonstrated manifestation of bearing in experimental trials. The genetic improvement of salinity tolerance in barley was revealed by El-Mouhamady *et al.*[4] who recommended that the genotypes Giza 123, Giza 2000, Giza 123XGiza 125, Giza 123XGiza 126 and Giza 2000XGiza 125 exhibited highly salinity tolerance under saline treatment compared to the control experiment in all studied traits. A spring barley collection of 192 entries from a large geographical limit was studied by Long *et al.*[5] to classify Quantitative Trait Loci (QTLs) for salinity stress tolerance traits using an association mapping approach through using a thousand SNP marker set. Salinity tolerance indices were able to expose the most tolerant barley entries under salinity stress[6]. Salinity stress due to the biggest increase in the traits; MDA, proline content, Na and Ca concentrations of the roots under salinity treatment compared with the control experiment and leaves in 47 barley wild barley entries[7]. Mwando *et al.*[8] revealed new materials and important information to develop salinity tolerance at the germination stage in the future barley cultivars via genomic and marker-assisted selection. In addition, opening up many horizons for the genetically and functional characterization responsible for discovering tolerance genes for this matter. Recently, a massive number of molecular markers evidenced success in explaining the genetic variation of barley hybrids. Moreover, these markers are extremely effective in categorizing genes and selecting multi-genic traits and genes. Microsatellites (SSRs) are among the most important DNA markers and being used for investigation, genetic differentiation, genome and QTL mapping for different crops and barley[9-12]. It considered co-dominant, highly informative, multi-allelic, most used and reproducible markers[13]. Khatab and Mariey[14] and Mariey *et al.*[15] classify two barley groups; I: Salt tolerant (Giza 123, Rehan-03 and Saiko) and II: Sensitive (Line 1 and Line 2) appeared low viewing to identify salinity tolerance using 10 ISSR primers. While SSR primer HVM09 recorded a band with the molecular size of 125 bp which could be considered a positive molecular marker related to salinity tolerance in the same regard. The biggest variation for salinity tolerance between barley entries especially at the seedling stage was observed when[16] evaluated 280 barley genotypes for the ability of tolerance to this stress. Physiological studies and molecular markers analysis using 10-mer random primers may be very important stages linked to identify and classify salinity tolerance in various barley genotypes[17]. After the aforementioned fruitful information it could possible to briefly clarify the main objective of this investigation to calculating a set of stress indices using field data under non-saline and saline conditions and the coefficients of variation, it is possible to: (1) Determine barley salt tolerance, (2) Develop the most tolerant hybrid of barley under saline conditions and (3) To detect SSR identification of some barley hybrids tolerant to salt stress.

## MATERIALS AND METHODS

**Study area:** The present study was conducted at the farm of the National Research Center in Nubaria city, Beheira Governorate, the farm of El-Sirw city, Damietta Governorate, Department of Genetics and Cytology in National research Centre and Department of Genetics, Faculty of Agriculture, Kafrelsheikh University, Egypt during 2018/2019 and 2019/2020 seasons.

**Materials:** This investigation included ten Egyptian barley cultivars with the different responses for salt stress tolerance were shown in Table 1. The ten cultivars were sown in three planting dates with 7 days intervals to overcome the differences in flowering time among parents for crossing in season 2018/2019 as lineXtester analysis. All entries (ten parents and their 21 F1 crosses) were grown under normal and salinity conditions in a randomized complete block design with three replicates for each treatment in season 2019/2020. The two experiments were grown under field experiments conditions. Where, the normal experiment was conducted on the farm of the National Research Center in Nubaria city, Beheira Governorate. While, the salinity treatment was done in El-Sirw city, Damietta Governorate as a saline condition. All chemical analyses for the two experiments as shown in Table 2.

**Soil analysis:** Before conducting the two experiments, soil samples were taken from different sites of each experiment. Each sample was taken from a depth of 0-30 cm from each treatment. The chemical analysis was carried out for each soil extract 1:5 to estimate the soluble anions, cations and Total Dissolved Salts (TDS). The Electrical Conductivity (EC) was estimated in the extract of the soil saturate paste. The procedure for preparation and measurements of the soil extract was taken according to[18]. The methods of[19] for soil chemical analysis were followed. The description of the two soil experiments used in this investigation is shown in Table 2.

### Methods

**Studied traits:** Fifty plants were taken from each genotype for each experiment of (normal and saline treatments) to evaluate the following traits:

- Plant height (cm): Length of the main culm was measured from the soil surface to the tip of the main panicle at maturity
- Number of filled grains per panicle: Filled grains of the main panicle with separated and counted
- 1000-grain weight (g): It was recorded as the weight of 1000 random filled grains per plant
- Grain yield per plant (g): was recorded as the weight of grain yield of each plant and adjusted to 14% moisture content
- Determination of some physiological traits related to salinity tolerance such as $Na^+$ uptake, $K^+$ uptake and Na/K ratio

Shoot samples of each genotype were determined and performed after 45 days from the sowing of each experiment. Samples were weighed, dried for three days at 70°C, grounded and 1 g dried powder from each sample for all studied materials under normal and salt stress experiments and were taken for $Na^+$ and $K^+$ determination by flame photometer:

- Osmotic adjustment: It was determined by the formula of[20] as follows:

Table 1: Barley cultivars, type, no. of rows, pedigree salinity tolerance and released year used for lineXtester analysis

| Name | Type | Row | Pedigree | Salinity tolerance | Year of released |
|---|---|---|---|---|---|
| **Tester** | | | | | |
| Giza 123 | Hulled | Six | Giza 117/FAO 86 | Tolerance | 1988 |
| Giza 126 | Hulled | Six | BaladiBahteem/S D729-Por12762-BC | Tolerance | 1995 |
| Giza2000 | Hulled | Six | Giza117/Bahteem52//Giza118/FAO86/3/Baladi16/Gem | Tolerance | 2003 |
| **Lines** | | | | | |
| Giza 125 | Hulled | Six | Giza117/Bahteem52//Giza118/FAO86(sister line to G.124 | Moderate | 1995 |
| Giza 133 | Hulled | Six | ICB91-0343-0AP-0AP-0AP-281AP-0AP | Moderate | 2011 |
| Giza 134 | Hulled | Six | ICB91-0343-0AP-0AP-0AP-289AP-0AP | Moderate | 2011 |
| Giza 129 | Hulless | Six | DeirAlla 106/Cel//As46/Aths*2" | Sensitive | 2001 |
| Giza 130 | Hulless | Six | Comp.cross"229//Bco.Mr./DZ02391/3/DeirAlla 106 | Moderate | 2001 |
| Giza 131 | Hulless | Six | CM67B/CENTENO//CAMB/3/ROW906.73/4/GLORIABAR/COME-B/5/FALCON BAR/6/LINO | Moderate | 2001 |
| Giza 132 | Hulled | Six | Rihane-05//AS 46/Aths*2Athe/Lignee 686 | Sensitive | 2006 |

Table 2: Chemical analysis of both types of soils; normal (Nubaria) and saline (El-Sirw) using in this study

| Characteristics | Normal soil | Salinity soil |
|---|---|---|
| EC (dS $m^{-1}$) | 1.72 | 11.2 |
| pH (1:2.5) | 7.14 | 7.83 |
| $Ca^{++}$ | 3.52 | 5513-5722 |
| $Mg^{++}$ | 2.33 | 12.73 |
| $Na^{+}$ | 5.23 | 11.42 |
| $K^{+}$ | 0.55 | 56.22-61.55 |
| $CO_3^{-}$ | 0.08 | 0.37 |
| $HCO_3^{-}$ | 1.84 | 0.13 |
| $Cl^{-}$ | 15.77 | 1.41 |
| $SO_4^{-}$ | 1.64 | 39.83 |

EC: Electrical conductivity, TDS: Total dissolved salts, *Measure of soil saturation, **Measure of soil water extract 1:5

$$\text{Osmotic adjustment} = \frac{\text{OP} \times \text{RWC}}{100}(\text{normal}) - \frac{\text{OP} \times \text{RWC}}{100}(\text{drought})^{100}$$

where, OP is the osmotic pressure, RWC is the relative water content:

- Proline content: Was determined from a standard curve and calculated on a fresh basis is as follows: [(µg proline/mL C mL toluene)/115.5 µg $\mu^{-1}$ mole]/[(g sample/5)] = µ moles proline/g of fresh weight material. The results related to proline content are average values of at least 3-4 samples for each species, according to Abraham *et al.*[21] and the modified method by Kalsoom *et al.*[22]
- Glycine betaine contents: It was carried out according to the method of Senthilkumar *et al.*[23]

**Estimation of salinity stress tolerance indices:** All salinity tolerance indices were estimated for the ten barley parents and the best five F1 crosses recorded highly salt tolerance under saline experiment conditions compared to the control experiment in all studied attributes according to researchers[24-30].

**Molecular characterization:** DNA was extracted from fresh leaves of the fifteen barley genotypes using the cetyltrimethylammonium bromide method as described by researchers[31]. Extracted DNA was used in a Polymerase Chain Reaction (PCR) using eleven SSR primers, Table 3 to identify SSR primers that could differentiate among the parental cultivars and their hybrids to study and genetic identity assessment for hybridity testing for salinity. Mixture PCR reaction contained 5 pmol of both forward and reverse primers, 0.2 mM of each dNTPs, 10 mM 10x pcr buffer, 0.5 unit Taq polymerase and 80 ng of DNA template then completed $dH_2O_2$ up to 25 µL. PCR reactions were performed for initial denaturation at 95°C for 5 min, followed by 35 cycles at 94°C for 1 min, annealing temperatures based on primer Tm (55-60°C) for 30 sec and extension at 72°C for 30 sec then final extension at 72°C for 7 min. PCR products were assessed on 2% agarose gel compared with 100 bp DNA ladder. The fifteen barley genotypes were the ten barley parents and the highest five F1 crosses recorded a high level of salinity tolerance in all studied traits under saline treatment compared to the normal experiment and the order of barley samples in PCR analysis were as follow; 1: Giza 123, 2: Giza 125, 3:Giza 126, 4: Giza 133, 5: Giza 2000, 6: Giza 132, 7: Giza 131, 8: Giza 134, 9: Giza 130, 10: Giza 129, 11: Giza 125XGiza 2000, 12: Giza 133XGiza 126, 13: Giza 125XGiza 126, 14: Giza 125XGiza 123 and 15: Giza 133XGiza 123, respectively.

**Data analysis:** All calculated data from all studied traits under both treatments were analyzed through the formula of Kumari *et al.*[32], heterosis and both types of combining ability effects (GCA and SCA) were calculated by previous studies[33,34]. GCA/SCA ratio: - MSe of GCA-MS error term/Number of parent +2/MSe of SCA-MS error term. Multivariable analyses and Principal Component Analysis (PCA) were calculated using the formula of Darwish *et al.*[35]. Cultivars were clustered using the unweighted pair group method using arithmetic average as outlined by Mareiy *et al.*[36]. Data were prepared for final analysis through the presence or absence of the total fragments for each primer. Pairwise components of the fifteen barley genotypes based on the presence or absence of unique and shared polymorphic products, were used to determine distance coefficients according to Rauch[37]. The distance coefficients were then used to construct dendrograms, using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA).

## RESULTS AND DISCUSSION

**Analysis of variance:** All barley entries presented in Table 4 were revealed highly significant differences under normal and salinity conditions for all studied traits. These results included the impact of salinity stress in ten agro-morphological traits compared to the normal conditions. Further, highly significant variations were obtained between all lineXtester analysis components for all studied attributes under both experiments namely; genotypes, parents, lines, testers, Line Vs Crosses and lineXtester, respectively.

Table 3: Name, sequences, chromosome location and expected bands of the selected SSR primers

| Primer name | Sequence | Chromosome location | Expected band size |
|---|---|---|---|
| Bmac0063 | F: AAACATGCACGTCACCTAC | 1H | 125 |
| | R: ACAACCACTGCATGAACAT | | |
| Bmag0770 | F: AAGCTCTTTCTTGTATTCGTG | 1 H | 260 |
| | R: GTCCATACTCTTTAACATCCG | | |
| Bmag0742 | F: AAACAGAGGGTTTTAGTAATGG | 2H | 148 |
| | R: AGTGAGATGGCAGTACATAGG | | |
| Bmag 0125 | F: AATTAGCGAGAACAAAATCAC | 2H | 138 |
| | R: AGATAACGATGCACCACC | | |
| Bmac0067 | F: AACGTACGAGCTCTTTTTCTA R:ATGCCAACTGCTTGTTTAG | 3H | 171 |
| Bmac0209 | F: CTAGCAACTTCCCAACCGAC | 3H | 176 |
| | R: ATGCCTGTGTGTGGACCAT | | |
| Bmac0084 | F: CTTGTGCCCTTTGATGCAC | 4H | 173 |
| | R: CATAACTTGAGGATGTGTGTGACA | | |
| Bmac0096 | F: GCTATGGCGTACTATGTATGGTTG | 5H | 173 |
| | R: TCACGATGAGGTATGATCAAAGA | | |
| Bmag0173 | F: CATTTTTGTTGGTGACGG R: ATAATGGCGGGAGAGACA | 6H | 150 |
| Bmac0064 | F: CTGCAGGTTTCAGGAAGG | 7H | 155 |
| | R: AGATGCCCGCAAAGAGTT | | |
| Bmag0135 | F: ACGAAAGAGTTACAACGGATA | 7H | 161 |
| | R: GTTTACCACAGATCTACAGGTG | | |

Table 4: Mean squares of LineXTester analysis in barley entries for all studied traits under normal and salinity conditions

| | | Plant height (cm) | | Number of filled grains/spike | | 1000-grain weight (g) | | Grain yield/plant (g) | | $Na^+$ content (ppm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SOV | DF | N | S | N | S | N | S | N | S | N | S |
| Rep. | 2 | 12.56 | 10.08 | 14.67 | 7.45 | 6.19 | 4.23 | 11.16 | 9.23 | 23.15 | 18.14 |
| Genotypes | 30 | 44.05** | 27.98** | 36.76** | 31.09** | 63.12** | 55.03** | 126.05** | 78.13** | 105.39** | 111.04** |
| Parents | 9 | 117.83** | 45.23** | 37.72** | 28.19** | 17.38** | 11.48** | 59.13** | 18.02** | 24.17** | 19.04** |
| Crosses | 20 | 198.77** | 81.09** | 55.13** | 46.23** | 26.04** | 19.37** | 83.76** | 41.32** | 33.25** | 41.28** |
| P vs. C | 1 | 116.45** | 106.34** | 67.33** | 81.15** | 14.32** | 11.07** | 49.0** | 53.02** | 10.38** | 8.53** |
| Lines (L) | 6 | 125.44** | 161.18** | 67.59** | 107.33** | 80.04** | 51.97** | 28.36** | 17.45** | 93.76** | 70.12** |
| Testers (T) | 2 | 234.77** | 188.57** | 113.55** | 152.19** | 126.59** | 74.02** | 43.15** | 31.04** | 157.33** | 106.01** |
| LXT | 12 | 24.39** | 16.08** | 72.84** | 52.10** | 160.04** | 183.15** | 110.31** | 119.26** | 35.64** | 29.20** |
| Error | 60 | 1.56 | 1.28 | 2.34 | 1.45 | 0.94 | 1.04 | 1.47 | 1.15 | 1.06 | 0.86 |

| | | $K^+$ content (ppm) | | Na/K ratio | | | Proline content | | Glycine betaine content | |
|---|---|---|---|---|---|---|---|---|---|---|
| SOV | DF | N | S | N | S | Osmotic adjustment | N | S | N | S |
| Rep. | 2 | 3.95 | 4.06 | 2.11 | 1.15 | 0.66 | 35.22 | 24.94 | 28.44 | 13.14 |
| Genotypes | 30 | 11.27** | 5.58** | 14.72** | 18.03** | 46.32** | 22.18** | 15.04** | 56.14** | 38.77** |
| Parents | 9 | 134.23** | 122.50** | 73.23** | 39.47** | 128.37** | 107.84** | 113.25** | 63.18** | 52.48** |
| Crosses | 20 | 207.11** | 151.34** | 115.07** | 77.14** | 141.06** | 121.30** | 2.41.57** | 92.04** | 103.18** |
| P vs. C | 1 | 136.22** | 125.84** | 104.56** | 35.94** | 37.05** | 13.84** | 28.07** | 20.17** | 8.13** |
| Lines (L) | 6 | 12.88** | 49.07** | 187.05** | 255.13** | 79.12** | 119.23** | 258.11** | 149.78** | 207.05** |
| Testers (T) | 2 | 57.62** | 71.03** | 233.84** | 308.11** | 83.02** | 226.83** | 293.09** | 172.10** | 255.98** |
| LXT | 12 | 193.17** | 175.0** | 118.93** | 127.03** | 15.08** | 18.32** | 26.04** | 143.15** | 131.23** |
| Error | 60 | 1.25 | 1.19 | 1.86 | 1.45 | 0.34 | 1.11 | 0.75 | 0.96 | 1.03 |

GCA/SCA ratio: MSe of GCA-MS error term/Number of parent+2/MSe of SCA-MS error term, *Significant at 5%,**Significant at 1%, NS: Not significant

**Mean performance:** Results viewed in Table 5 detected that the three barley testers; Giza 123, Giza 126 and Giza 2000 besides; the crosses; Line 1XTester 1 (Giza 125XGiza 123), Line 2XTester 1 (Giza 133XGiza 123), Line 1XTester 2 (Giza 125XGiza 126), Line 2XTester 2 (Giza 133XGiza 126) and Line 1XTester 3 (Giza 125XGiza 2000) recorded highly mean values of several filled grains/spike, 1000-grain weight and grain yield/plant traits under normal and salinity conditions. Further, the rest barley genotypes showed low to medium averages in this regard. While these promising genotypes recorded the lowest mean performance of plant height trait under both experiments compared to the other entries under testing. This confirms that the aforementioned superior barley genotypes showed great tolerance to salt stress. Also, their losing rate for yield and its components traits was not significant under the saline treatment compared to the standard experiment. While the rate of loss was very large for the rest of the barley entries under the same conditions. Also, the eight promising barley

Table 5: Mean performances of all studied traits in all barley genotypes tested under the control and salinity conditions

| | Plant height (CM) | | Number of filled grains/spike | | 1000 grain weight (g) | | Grain yield/plant (g) | | $Na^+$ content (ppm) | | $K^+$ content (ppm) | | Na/K ratio | | | Proline content | | Glycine betaine content | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Entries | N | S | N | S | N | S | N | S | N | S | N | S | N | S | Osmotic adjustment | N | S | N | S |
| L1 | 102.07 | 99.18 | 31.18 | 22.05 | 28.93 | 22.06 | 23.15 | 18.94 | 0.32 | 0.45 | 1.65 | 1.41 | 0.19 | 0.31 | 1.07 | 22.03 | 17.41 | 25.86 | 21.03 |
| L2 | 104.16 | 96.05 | 26.84 | 18.33 | 33.14 | 25.60 | 30.05 | 22.84 | 0.26 | 0.38 | 1.12 | 0.96 | 0.23 | 0.39 | 1.33 | 32.07 | 24.55 | 17.22 | 14.78 |
| L3 | 107.13 | 101.14 | 34.55 | 27.28 | 38.02 | 26.19 | 28.39 | 15.33 | 0.27 | 0.31 | 1.27 | 1.13 | 0.21 | 0.27 | 1.03 | 27.49 | 13.12 | 31.09 | 24.12 |
| L4 | 103.98 | 97.13 | 29.65 | 22.48 | 30.09 | 21.14 | 31.74 | 24.13 | 0.29 | 0.36 | 1.73 | 1.48 | 0.16 | 0.24 | 1.42 | 18.42 | 15.76 | 27.81 | 20.32 |
| L5 | 105.18 | 100.02 | 32.41 | 25.84 | 26.12 | 18.44 | 24.38 | 17.45 | 0.34 | 0.41 | 1.15 | 1.06 | 0.29 | 0.38 | 1.55 | 37.06 | 31.00 | 21.44 | 15.42 |
| L6 | 103.04 | 97.16 | 28.73 | 20.13 | 35.13 | 29.03 | 26.25 | 17.62 | 0.26 | 0.35 | 1.73 | 1.52 | 0.15 | 0.23 | 1.92 | 26.81 | 23.15 | 32.16 | 27.68 |
| L7 | 108.32 | 102.38 | 35.46 | 19.86 | 27.08 | 19.33 | 25.43 | 19.77 | 0.35 | 0.44 | 1.36 | 1.09 | 0.25 | 0.40 | 1.18 | 20.41 | 12.77 | 29.01 | 13.64 |
| T1 | 89.11 | 85.03 | 44.08 | 35.43 | 50.39 | 42.03 | 43.07 | 36.11 | 0.20 | 0.13 | 2.15 | 2.26 | 0.09 | 0.05 | 0.83 | 53.18 | 58.03 | 43.67 | 49.11 |
| T2 | 93.15 | 89.03 | 51.17 | 38.71 | 58.81 | 45.76 | 48.12 | 41.05 | 0.18 | 0.08 | 2.49 | 2.64 | 0.07 | 0.03 | 0.59 | 45.78 | 47.14 | 38.04 | 43.48 |
| T3 | 88.44 | 81.05 | 49.38 | 41.03 | 44.13 | 40.01 | 38.96 | 32.04 | 0.21 | 0.11 | 2.05 | 2.31 | 0.10 | 0.04 | 0.72 | 65.13 | 69.11 | 51.19 | 54.39 |
| L1XT1 | 85.33 | 82.10 | 63.23 | 55.80 | 57.14 | 50.07 | 55.09 | 47.64 | 0.13 | 0.07 | 2.34 | 2.83 | 0.05 | 0.02 | 0.39 | 96.23 | 104.05 | 79.14 | 83.10 |
| L2XT1 | 87.15 | 84.20 | 68.17 | 48.39 | 62.03 | 53.71 | 59.32 | 44.13 | 0.15 | 0.10 | 2.94 | 3.22 | 0.05 | 0.03 | 0.52 | 87.33 | 92.14 | 68.73 | 74.83 |
| L3XT1 | 102.55 | 99.03 | 31.05 | 24.06 | 36.05 | 17.32 | 28.43 | 15.92 | 0.32 | 0.41 | 1.20 | 1.13 | 0.26 | 0.36 | 1.25 | 25.22 | 11.03 | 24.55 | 21.07 |
| L4XT1 | 105.67 | 101.23 | 25.42 | 19.02 | 40.11 | 28.14 | 34.05 | 28.21 | 0.42 | 0.55 | 2.03 | 1.78 | 0.20 | 0.30 | 1.36 | 17.61 | 14.32 | 20.18 | 12.05 |
| L5XT1 | 108.93 | 102.11 | 34.72 | 27.08 | 30.12 | 24.06 | 26.03 | 20.65 | 0.39 | 0.63 | 1.13 | 1.59 | 0.34 | 0.39 | 1.29 | 31.19 | 20.02 | 28.73 | 26.19 |
| L6XT1 | 99.60 | 95.13 | 30.29 | 14.62 | 34.00 | 20.38 | 23.17 | 14.72 | 0.28 | 0.34 | 1.89 | 1.32 | 0.14 | 0.25 | 1.11 | 28.37 | 25.54 | 30.07 | 23.76 |
| L7XT1 | 112.05 | 107.96 | 37.04 | 30.19 | 26.21 | 19.55 | 37.11 | 31.53 | 0.36 | 0.45 | 1.30 | 1.08 | 0.27 | 0.41 | 1.05 | 23.17 | 16.22 | 33.08 | 18.94 |
| L1XT2 | 90.04 | 82.17 | 76.38 | 69.02 | 64.19 | 57.12 | 56.17 | 47.08 | 0.12 | 0.05 | 2.35 | 2.51 | 0.05 | 0.01 | 0.28 | 74.23 | 77.05 | 103.55 | 108.32 |
| L2XT2 | 87.16 | 80.05 | 81.02 | 65.10 | 60.05 | 52.01 | 53.47 | 49.16 | 0.16 | 0.12 | 2.86 | 3.04 | 0.05 | 0.04 | 0.33 | 81.13 | 86.09 | 77.60 | 85.14 |
| L3XT2 | 103.16 | 76.87 | 29.81 | 26.09 | 33.60 | 27.81 | 26.43 | 19.12 | 0.35 | 0.44 | 1.18 | 1.05 | 0.29 | 0.41 | 1.94 | 22.04 | 11.02 | 27.41 | 19.32 |
| L4XT2 | 107.33 | 96.18 | 26.41 | 21.38 | 39.07 | 21.33 | 30.02 | 17.45 | 0.43 | 0.49 | 2.05 | 1.86 | 0.20 | 0.26 | 2.01 | 15.32 | 12.72 | 15.32 | 12.06 |
| L5XT2 | 102.39 | 94.06 | 30.04 | 12.15 | 28.02 | 25.31 | 20.15 | 14.92 | 0.48 | 0.37 | 1.27 | 1.28 | 0.37 | 0.28 | 1.96 | 33.14 | 27.08 | 20.13 | 12.40 |
| L6XT2 | 111.39 | 105.29 | 33.15 | 18.92 | 32.05 | 23.14 | 27.83 | 22.00 | 0.30 | 0.33 | 1.95 | 1.38 | 0.15 | 0.23 | 2.13 | 27.03 | 21.54 | 30.82 | 24.15 |
| L7XT2 | 104.13 | 98.55 | 38.05 | 27.13 | 25.31 | 15.84 | 34.85 | 24.60 | 0.37 | 0.61 | 1.27 | 1.13 | 0.29 | 0.53 | 1.88 | 25.13 | 16.49 | 32.16 | 17.43 |
| L1XT3 | 86.54 | 82.01 | 58.03 | 51.07 | 63.11 | 49.87 | 61.04 | 50.27 | 0.09 | 0.06 | 2.58 | 2.77 | 0.03 | 0.02 | 0.64 | 91.04 | 98.72 | 95.84 | 102.36 |
| L2XT3 | 106.19 | 75.81 | 29.08 | 14.38 | 30.44 | 22.25 | 33.14 | 22.05 | 0.28 | 0.34 | 1.29 | 1.26 | 0.21 | 0.26 | 2.34 | 28.10 | 34.07 | 31.15 | 30.02 |
| L3XT3 | 101.07 | 74.23 | 32.00 | 25.47 | 25.90 | 20.13 | 27.18 | 23.55 | 0.37 | 0.51 | 1.16 | 1.11 | 0.31 | 0.45 | 2.71 | 26.02 | 11.84 | 25.80 | 22.72 |
| L4XT3 | 109.04 | 102.36 | 24.70 | 16.32 | 38.83 | 32.18 | 35.70 | 29.40 | 0.44 | 0.61 | 2.07 | 1.53 | 0.21 | 0.39 | 1.14 | 30.16 | 24.12 | 24.60 | 17.32 |
| L5XT3 | 107.13 | 102.88 | 28.16 | 23.01 | 27.29 | 18.49 | 30.11 | 18.64 | 0.41 | 0.35 | 1.17 | 1.28 | 0.35 | 0.27 | 1.78 | 39.4 | 32.01 | 19.62 | 16.74 |
| L6XT3 | 104.86 | 100.03 | 34.06 | 30.05 | 40.12 | 29.08 | 28.14 | 20.07 | 0.31 | 0.42 | 1.64 | 1.29 | 0.18 | 0.32 | 1.29 | 22.18 | 19.32 | 27.06 | 12.68 |
| L7XT3 | 112.93 | 108.01 | 28.11 | 17.26 | 23.87 | 17.02 | 32.33 | 19.87 | 0.38 | 0.46 | 1.58 | 1.43 | 0.24 | 0.32 | 2.07 | 34.91 | 23.58 | 34.55 | 14.91 |
| LSD at 0.05 | 1.70 | 1.54 | 2.08 | 1.64 | 1.32 | 1.39 | 1.65 | 1.46 | 1.40 | 1.26 | 1.52 | 1.48 | 1.86 | 1.64 | 0.79 | 0.57 | 1.18 | 1.33 | 1.38 |
| LSD at 0.01 | 2.43 | 2.20 | 2.98 | 2.34 | 1.89 | 1.98 | 2.36 | 2.09 | 2.00 | 1.80 | 2.18 | 2.12 | 2.66 | 2.34 | 1.13 | 0.82 | 1.68 | 1.91 | 1.97 |

entries mentioned above recorded a low level of $Na^+$ content and highly averages of $K^+$ content under both treatments. Besides, their mean values of $Na^+/K^+$ ratio and osmotic adjustment traits were less than the other barley entries for the same conditions. Concerning proline and glycine betaine contents traits, the three barley testers and the highest five crosses mentioned above were recorded highly level in this regard under salinity treatment compared with the control experiment and were surpassed of the rest barley genotypes.

**Heterosis over better-parent:** Significant and highly significant negatively values of heterosis over better-parent were showed for plant height trait in the crosses; L1XT1, L1XT2, L2XT2 under normal and salinity conditions, L2XT1 and L1XT3 for the normal conditions only and L3XT2, L2XT3 and L3XT3 for salinity treatment only (Table 6). In the same negative direction, $Na^+$ content and $Na^+/K$ ratio traits were exhibited the same results in the crosses; L1XT1, L2XT1, L1XT2, L2XT2 and L1XT3 under both conditions except; the cross; L2XT2 where it was highly significant and negatively values of this parameter under the control treatment only in $Na^+/K$ ratio trait, respectively. In the same context, the promising five barley crosses namely; L1XT1, L2XT1, L1XT1, L2XT2 and L1XT3 recorded negative results of heterosis over better-parent but not significant for osmotic adjustment trait. On the other hand, the two crosses; L1XT1 and L2XT1 have exhibited highly significant and positive values of this genetic parameter for $K^+$ content trait under normal and salinity conditions. On the same track, the five barley promising crosses; L1XT1, L2XT1, L1XT2, L2XT2 and L1XT3 have detected significant and highly significant positive values of heterosis over better parent for the traits; the number of filled grains/spike, 1000-grain weight, grain yield/plant, proline and glycine betaine contents under both conditions except the cross; L2XT2 for 1000-grain weight trait under normal treatment only where it was not significant, respectively.

**General and specific combining ability effects:** Data revealed in Table 7 and associated with GCA effects confirmed that the five barley entries; Giza 123, Giza 125, Giza 126, Giza 133 and Giza 2000 were exhibited highly significant and positive values in the traits; the number of filled grains/spike, 1000-grain weight, grain yield/plant, $K^+$ content, proline and glycine betaine contents under both experiments. While, the same promising barley genotypes were showed highly significant and negatively values for plant height, $Na^+$ content, $Na^+/K^+$ ratio and osmotic adjustment traits under both treatments conditions. The barley crosses; (L1XT1, L2XT1, L1XT2, L2XT2 and L1XT3) exhibited highly significant and positive values of SCA effects for the two experiments in the traits; the number of filled grains/panicle, 1000-grain weight, grain yield/plant, $K^+$ content, proline and glycine betaine contents (Table 8). While the same five barley crosses mentioned above were recorded highly significant and negative values for SCA effects in the rest studied traits namely; plant height, $Na^+$ content, Na/K ratio and osmotic adjustment under normal and salinity conditions.

**Tolerance indices:** Data presented in Table 9 revealed that the genotypes; Line 1, testers (1, 2 and 3) and the crosses number (1, 4 and 5) for (YSI) parameter and the three barley testers besides, the promising five crosses for (MP and GMP) parameters recorded the highest mean values for salinity tolerance indices test in this study for grain yield trait. These results confirmed that these barley genotypes considered highly salinity tolerance under stress treatment compared to the control conditions. On the same track, the three barley testers and the promising five crosses for the (YI) parameter besides the same genetic materials except tester 1 and 3 for (STI) were exhibited mean values higher than the unity. This means that these barley entries were given highly salinity tolerance under salinity stress compared to the standard experiment and this result was not achieved in the rest of barley genotypes, respectively. On the other hand, all barley entries for the parameter (YR) and the genotypes; (Line 1), testers (1, 2 and 3) and crosses number (1, 4 and 5) for the parameter SSI were exhibited mean values lower than one which confirmed that these superior barley entries were revealed highly tolerance for salinity stress in this regard.

**Principal component analysis (PCA):** Principal Component Analysis (PCA) based on ten agro-physiological traits were used to distinguish all genotypes into groups (Fig. 1). Where the selection of genotypes could be based on the values of both first and second principal component analysis illustrated in Table 10 that the first and second principal components analysis (PCA1 and PCA2) covered about 85.71% of the total variation. Where, the first PCA1 axis, showed 80.54% of the total variation influenced by six traits (plant height, number of filled grains/spike, 1000 grain weight, grain yield/plant, proline and glycine Betaine contents). While, the second PCA2 axis represents 5.17% of the total variability due to K content, Na content, Na/K ratio and osmotic adjustment. Tolerant genotypes to salinity stress could be defined according to the values of PCA1. Results in Table 10distinguished that the three barley testers; Giza 123, Giza 126 and Giza 2000 besides; the crosses; Line 1XTester 1 (Giza 125XGiza 123), Line 2XTester 1 (Giza 133XGiza 123), Line 1XTester 2 (Giza 125XGiza 126), Line 2XTester 2 (Giza 133XGiza 126) and Line 1XTester 3

Table 6: Estimates of heterosis over better-parent for the 21 barley crosses obtained from LineXtester analysis in all studied traits under normal and salinity conditions

| | Plant height (CM) | | Number of filled grains/spike | | 1000 grain weight (g) | | Grain yield/plant (g) | | $Na^+$ content (ppm) | | $K^+$ content (ppm) | | Na/K ratio | | Osmotic adjustment | Proline content | | Glycine betaine content | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Crosses | N | S | N | S | N | S | N | S | N | S | N | S | N | S | | N | S | N | S |
| L1XT1 | -4.24** | -3.44** | 43.44** | 57.49** | 13.39** | 19.12** | 27.90** | 31.93** | -35.0** | -46.15** | 8.38** | 25.22** | -44.44** | -60.0** | -53.01$^{NS}$ | 80.95** | 79.30** | 81.22** | 69.21** |
| L2XT1 | -2.19* | -0.97$^{NS}$ | 54.65** | 36.57** | 23.09** | 27.78** | 37.72** | 22.20** | -25.0** | -23.07** | 36.74** | 42.47** | -44.44** | -40.0** | -37.34$^{NS}$ | 64.21** | 58.77** | 57.38** | 52.37** |
| L3XT1 | 15.08** | 16.46** | -29.55** | -32.09** | -28.45** | -58.79** | -33.99** | -55.91** | 60.0$^{NS}$ | 215.38$^{NS}$ | -44.18$^{NS}$ | -50.0$^{NS}$ | 188.88$^{NS}$ | 620.0$^{NS}$ | 50.60$^{NS}$ | -52.57** | -80.99** | -43.78** | -57.09** |
| L4XT1 | 18.58** | 19.05** | -42.33** | -46.31** | -20.40** | -33.04** | -20.94** | -21.87** | 110.0$^{NS}$ | 323.07$^{NS}$ | -5.58$^{NS}$ | -21.23$^{NS}$ | 122.22$^{NS}$ | 500.0$^{NS}$ | 63.85$^{NS}$ | -66.88** | -75.32** | -53.78** | -75.46** |
| L5XT1 | 22.24** | 20.08** | -21.23** | -23.56** | -40.22** | -42.75** | -39.56** | -42.81** | 95.0$^{NS}$ | 384.61$^{NS}$ | -47.44$^{NS}$ | -29.64$^{NS}$ | 277.77$^{NS}$ | 680.0$^{NS}$ | 55.42$^{NS}$ | -41.35** | -65.50** | -34.21** | -46.67** |
| L6XT1 | 11.77** | 11.87** | -31.28** | -58.73** | -32.52** | -51.51** | -46.20** | -59.23** | 40.0$^{NS}$ | 161.53$^{NS}$ | -12.09$^{NS}$ | -41.59$^{NS}$ | 55.55$^{NS}$ | 400.0$^{NS}$ | 33.73$^{NS}$ | -46.65** | -55.98** | -31.14** | -51.61** |
| L7XT1 | 25.74** | 26.96** | -15.97** | -14.78** | -47.98** | -53.48** | -13.83** | -12.68** | 80.0$^{NS}$ | 246.15$^{NS}$ | -39.53$^{NS}$ | -52.21$^{NS}$ | 200.0$^{NS}$ | 720.0$^{NS}$ | 26.50$^{NS}$ | -56.43** | -72.04 | -24.25** | -61.43** |
| L1XT2 | -3.33** | -7.70** | 49.26** | 78.30** | 9.14** | 24.82** | 16.72** | 14.68** | -33.33** | -37.50** | -5.62$^{NS}$ | -4.92$^{NS}$ | -28.57** | -66.66** | -52.54$^{NS}$ | 62.14** | 63.44** | 172.21** | 149.12** |
| L2XT2 | -6.43** | -10.08** | 58.33** | 68.17** | 2.10$^{NS}$ | 13.65** | 11.11** | 19.75** | -11.11** | -50.0** | 14.85$^{NS}$ | 15.15$^{NS}$ | -28.57** | 33.33$^{NS}$ | -44.06$^{NS}$ | 77.21** | 82.62** | 103.99** | 95.81** |
| L3XT2 | 10.74** | -13.65** | -41.74** | -32.60** | -42.86** | -39.22** | -45.07** | -53.42** | 94.44$^{NS}$ | 450.0$^{NS}$ | -52.61$^{NS}$ | -60.22* | 314.28$^{NS}$ | 1266.66$^{NS}$ | 228.81** | -51.85** | -76.62** | -27.94** | -55.56** |
| L4XT2 | 15.22** | 8.03** | -48.38** | -44.76** | -33.56** | -53.38** | -37.61** | -57.49** | 138.88$^{NS}$ | 512.50$^{NS}$ | -17.67$^{NS}$ | -29.54$^{NS}$ | 185.71$^{NS}$ | 766.66$^{NS}$ | 240.67** | -66.53** | -73.01** | -59.72** | -72.26** |
| L5XT2 | 9.91** | 5.64** | -41.29** | -68.61** | -52.35** | -44.68** | -58.12** | -63.65** | 166.66$^{NS}$ | 362.50$^{NS}$ | -48.99$^{NS}$ | -51.51$^{NS}$ | 428.57$^{NS}$ | 833.33$^{NS}$ | 232.20** | -27.61** | -42.55** | -47.08** | -71.48** |
| L6XT2 | 19.58** | 18.26** | -35.21** | -51.12** | -45.50** | -49.43** | -42.16** | -46.40** | 66.66$^{NS}$ | 312.50$^{NS}$ | -21.68$^{NS}$ | -47.72$^{NS}$ | 114.28$^{NS}$ | 666.66$^{NS}$ | 261.01** | -40.95** | -54.30** | -18.98** | -44.45** |
| L7XT2 | 11.78** | 10.69** | -25.64** | -29.91** | -56.96** | -65.38** | -27.57** | -40.07** | 105.55$^{NS}$ | 662.50$^{NS}$ | -48.99$^{NS}$ | -57.19* | 314.28$^{NS}$ | 1666.66$^{NS}$ | 8.47$^{NS}$ | -45.10** | -65.01** | -15.45** | -59.91** |
| L1XT3 | -2.14* | 1.18$^{NS}$ | 17.51** | 24.46** | 43.0** | 24.64** | 56.67** | 56.89** | -57.14** | -45.45** | 25.85$^{NS}$ | 19.91$^{NS}$ | -70.0** | -50.0** | -11.11$^{NS}$ | 39.78** | 42.84** | 87.22** | 88.19** |
| L2XT3 | 20.07** | -6.46** | -41.10** | -64.95** | -31.02** | -44.38** | -14.93** | -31.17** | 33.33$^{NS}$ | 209.09$^{NS}$ | -37.07$^{NS}$ | -45.45$^{NS}$ | 110.0$^{NS}$ | 550.0$^{NS}$ | 225.0** | -56.85** | -50.70** | -39.14** | -44.80** |
| L3XT3 | 14.28** | -8.41** | -35.19** | -37.92** | -41.30** | -49.68** | -30.23** | -26.49** | 76.19$^{NS}$ | 363.63$^{NS}$ | -43.41$^{NS}$ | -51.94$^{NS}$ | 210.0$^{NS}$ | 1025.0$^{NS}$ | 276.38** | -60.04** | -82.86** | -49.59** | -58.22** |
| L4XT3 | 23.29** | 26.29** | -49.97** | -60.22** | -12.0** | -19.57** | -8.36** | -8.23** | 109.52$^{NS}$ | 454.54$^{NS}$ | 0.97$^{NS}$ | -33.76$^{NS}$ | 110.0$^{NS}$ | 875.0$^{NS}$ | 58.33$^{NS}$ | -53.69** | -65.09** | -51.94** | -68.15** |
| L5XT3 | 21.13** | 26.93** | -42.97** | -43.91** | -38.15** | -53.78** | -22.71** | -41.82** | 95.23$^{NS}$ | 218.18$^{NS}$ | -42.92$^{NS}$ | -44.58$^{NS}$ | 250.0$^{NS}$ | 575.0$^{NS}$ | 147.22* | -39.50** | -53.68** | -61.67** | -69.22** |
| L6XT3 | 18.56** | 23.41** | -31.02** | -26.76** | -9.08** | -27.31** | -27.77** | -37.35** | 47.61$^{NS}$ | 281.81$^{NS}$ | -20.0$^{NS}$ | -44.15$^{NS}$ | 80.0$^{NS}$ | 700.0$^{NS}$ | 79.16$^{NS}$ | -65.94** | -72.04** | -47.13** | -76.68** |
| L7XT3 | 27.69** | 33.26** | -43.07** | -57.93** | -45.90** | -57.46** | -17.01** | -37.98** | 80.95$^{NS}$ | 318.18$^{NS}$ | -22.92$^{NS}$ | -38.09$^{NS}$ | 140.0$^{NS}$ | 700.0$^{NS}$ | 187.50** | -46.39** | -65.88** | -32.50** | -72.58** |
| LSD at 0.05 | 1.7 | 1.54 | 2.08 | 1.64 | 1.32 | 1.39 | 1.65 | 1.46 | 1.4 | 1.26 | 1.52 | 1.48 | 1.86 | 1.64 | 0.79 | 0.57 | 1.18 | 1.33 | 1.38 |
| LSD at 0.01 | 2.43 | 2.2 | 2.98 | 2.34 | 1.89 | 1.98 | 2.36 | 2.09 | 2 | 1.8 | 2.18 | 2.12 | 2.66 | 2.34 | 1.13 | 0.82 | 1.68 | 1.91 | 1.97 |

*Significant at 5%,**Significant at 1% and $^{NS}$: Not significant

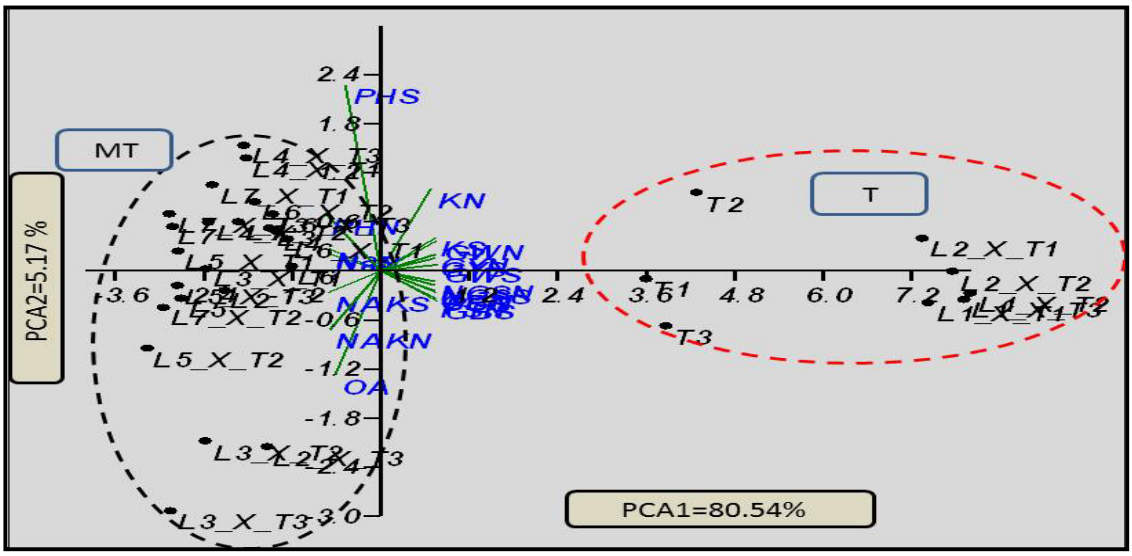


Fig. 1: Bi-plot based on 10 agro-physiological traits for 31 Egyptian barley genotypes

Table 7: Estimates of general combining ability effects of the 10- barley entries for all studied traits under normal and salinity conditions

| | Plant height (CM) | | Number of filled grains/spike | | 1000-grain weight (g) | | Grain yield/plant (g) | | $Na^+$ content (ppm) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parents | N | S | N | S | N | S | N | S | N | S |
| **Lines** | | | | | | | | | | |
| L1 | -10.64** | -2.87** | 2.49** | 3.48** | 6.22** | 2.39** | 1.57** | 3.09** | -2.36** | -4.26** |
| L2 | -8.12** | -5.11** | 3.18** | 5.03** | 4.03** | 5.04** | 3.48** | 1.47** | -1.48** | -1.98** |
| L3 | 4.97** | 10.0** | -1.21$^{NS}$ | -2.41** | -1.57** | -4.23** | -2.37** | -6.32** | 0.34$^{NS}$ | 0.75** |
| L4 | 11.03** | 6.07** | -0.66$^{NS}$ | -7.34** | -3.22** | -3.07** | -8.98** | -4.17** | 9.98** | 3.04** |
| L5 | 8.17** | 4.76** | -15.45** | -13.02** | -7.39** | -1.79** | -8.73** | -2.96** | 0.68$^{NS}$ | 1.46** |
| L6 | 2.66** | 5.33** | -11.04** | -1.79** | -2.14** | -0.95* | -7.75** | -8.47** | 4.90** | 0.55** |
| L7 | 18.19** | 9.63** | -4.34** | -5.10** | -8.43** | -12.67** | -7.16** | -3.05** | 2.88** | 11.14** |
| LSD at 0.05 (gi) | 3.56 | 2.71 | 1.82 | 1.06 | 1.19 | 1.27 | 0.35 | 0.72 | 0.88 | 0.28 |
| LSD at 0.01 (gi) | 4.15 | 3.12 | 2.34 | 1.65 | 1.48 | 1.51 | 0.44 | 0.89 | 1.04 | 0.42 |
| **Testers** | | | | | | | | | | |
| T1 | -8.34** | -11.03** | 7.23** | 6.63** | 5.92** | 4.11** | 12.58** | 9.05** | -2.49** | -1.49** |
| T2 | -11.79** | -7.55** | 13.58** | 4.34** | 3.70** | 1.55** | 7.08** | 6.45** | -3.71** | -5.43** |
| T3 | -6.13** | -9.23** | 6.22** | 10.18** | 2.88** | 9.62** | 10.28** | 4.91** | -8.74** | -3.78** |
| LSD at 0.05 (gi) | 1.42 | 1.26 | 2.39 | 1.17 | 0.96 | 0.81 | 0.49 | 0.37 | 0.74 | 0.54 |
| LSD at 0.01 (gi) | 1.59 | 1.38 | 2.48 | 1.32 | 1.33 | 1.06 | 0.68 | 0.52 | 1.02 | 0.68 |

| | $K^+$ content (ppm) | | Na/K ratio | | | Proline content | | Glycine betaine content | |
|---|---|---|---|---|---|---|---|---|---|
| Parents | N | S | N | S | Osmotic adjustment | N | S | N | S |
| **Lines** | | | | | | | | | |
| L1 | 6.07** | 2.17** | -5.04** | -3.23** | -6.15** | 2.96** | 7.29** | 6.48** | 1.29** |
| L2 | 3.91** | 4.01** | -2.90** | -1.18** | -3.04** | 4.03** | 1.99** | 3.89** | 3.11** |
| L3 | -0.22$^{NS}$ | -3.12** | 3.14** | 0.36$^{NS}$ | 7.11** | -7.01** | -6.0** | -2.08** | -4.05** |
| L4 | -5.34** | -2.11** | 0.08$^{NS}$ | 0.55$^{NS}$ | 2.34** | -3.15** | -8.56** | -8.59** | -2.87** |
| L5 | -3.0** | -1.82** | 6.11** | 9.02** | 5.71** | -5.08** | -3.81** | -0.08$^{NS}$ | -5.01** |
| L6 | -6.57** | -4.95** | 1.69** | 1.02** | 1.43** | -1.75** | -1.77* | -13.42** | -0.06$^{NS}$ |
| L7 | -1.46** | -6.86** | 2.22** | 2.54** | 1.96** | -15.45** | -8.07** | -8.65** | -5.69** |
| LSD at 0.05 (gi) | 0.38 | 0.53 | 0.28 | 0.74 | 0.46 | 1.49 | 1.56 | 1.19 | 1.03 |
| LSD at 0.01 (gi) | 0.61 | 0.79 | 0.41 | 0.91 | 0.69 | 1.63 | 1.94 | 1.35 | 1.23 |
| **Testers** | | | | | | | | | |
| T1 | 1.97** | 3.12** | -2.15** | -5.28** | -3.45** | 6.28** | 10.47** | 7.12** | 2.85** |
| T2 | 2.88** | 5.19** | -1.67** | -1.47** | -1.89** | 8.15** | 5.19** | 10.09** | 6.07** |
| T3 | 1.76* | 4.37** | -1.48** | -2.33** | -4.02** | 11.02** | 3.27** | 5.24** | 4.36** |
| LSD at 0.05 (gi) | 1.66 | 1.35 | 1.04 | 1.02 | 0.56 | 2.88 | 2.44 | 1.55 | 1.27 |
| LSD at 0.01 (gi) | 1.82 | 1.53 | 1.29 | 1.17 | 0.71 | 3.25 | 3.19 | 1.92 | 1.39 |

*Significant at 5%, **Significant at 1% and NS: Not significant, N: Normal conditions and S: Salinity conditions

Table 8: Estimates of specific combining ability effects for the 21 barley crosses of all studied traits under the control and salinity conditions

| | Plant height (CM) | | Number of filled grains/spike | | 1000 grain weight (g) | | Grain yield/plant (g) | | $Na^+$ content (ppm) | | $K^+$ content (ppm) | | Na/K ratio | | | Proline content | | Glycine betaine content | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Crosses | N | S | N | S | N | S | N | S | N | S | N | S | N | S | Osmotic adjustment | N | S | N | S |
| L1XT1 | -88.34** | -135.67** | 65.12** | 49.18** | 26.03** | 19.71** | 22.05** | 13.02** | -134.16** | -98.75** | 86.67** | 119.33** | -185.39** | -79.02** | -44.76** | 142.39** | 83.55** | 30.18** | 125.67** |
| L2XT1 | -129.45** | -52.84** | 33.21** | 41.07** | 105.43** | 110.17** | 45.08** | 78.22** | -76.04** | -72.18** | 31.04** | 45.90** | -96.18** | -132.11** | -62.08** | 52.83** | 39.77** | 72.55** | 46.11** |
| L3XT1 | 13.96** | 9.63** | -12.05** | -25.03** | -42.19** | -31.09** | -20.05** | -9.34** | 3.72** | 6.52** | -6.18** | -27.55** | 12.55** | 32.06** | 82.56** | -9.83** | -6.73** | -6.93** | -8.95** |
| L4XT1 | 17.82** | 6.25** | -7.19** | -15.86** | -28.04** | -4.26** | -13.05** | -7.28** | 7.93** | 8.23** | -14.65** | -10.08** | 22.14** | 17.49** | 15.07** | -17.95** | -28.23** | -11.04** | -6.13** |
| L5XT1 | 9.85** | 13.62** | -8.33** | -33.18** | -10.04** | -8.15** | -10.04** | -12.15** | 2.14** | 25.34** | -5.49** | -13.47** | 16.15** | 7.12** | 13.04** | -23.40** | -4.16** | -4.15** | -22.17** |
| L6XT1 | 27.23** | 3.81** | -10.03** | -17.22** | -7.22** | -19.43** | -23.05** | -4.22** | 5.08** | 22.48** | -28.30** | -9.75** | 8.79** | 3.68** | 37.11** | -8.67** | -16.58** | -23.11** | -19.16** |
| L7XT1 | 18.09** | 4.58** | -5.19** | -8.42** | -9.16** | -23.18** | -15.07** | -6.02** | 32.60** | 13.08** | -15.72** | -26.29** | 10.62** | 19.27** | 3.95** | -10.35** | -12.09** | -15.60** | -32.88** |
| L1XT2 | -94.33** | -112.0** | 35.95** | 12.08** | 83.17** | 27.04** | 55.87** | 29.02** | -55.19** | -91.33** | 90.88** | 125.34** | -48.56** | -80.50** | -59.13** | 123.45** | 96.52** | 54.0** | 112.03** |
| L2XT2 | -71.06** | -127.0** | 128.32** | 96.0** | 60.17** | 37.86** | 47.55** | 53.60** | -38.17** | -77.22** | 115.60** | 70.62** | -70.38** | -112.16** | -38.21** | 117.87** | 61.05** | 38.82** | 107.94** |
| L3XT2 | 6.32** | 28.40** | -3.85** | -1.96** | -4.29** | -11.15** | -7.82** | -12.03** | 6.33** | 1.89** | -36.94** | -19.84** | 4.58** | 12.09** | 72.06** | -26.34** | -3.66** | -2.99** | -14.06** |
| L4XT2 | 16.48** | 5.32** | -6.81** | -5.12** | -7.23** | -21.06** | -2.87** | -9.88** | 12.05** | 9.38** | -2.98** | -42.16** | 13.05** | 7.41** | 4.85** | -19.72** | -1.98** | -14.70** | -9.44** |
| L5XT2 | 4.19** | 11.02** | -5.23** | -8.06** | -18.04** | -27.12** | -7.54** | -25.07** | 8.12** | 11.32** | -7.42** | -16.84** | 11.71** | 40.09** | 12.18** | 7.08** | -11.02** | -8.52** | -2.54** |
| L6XT2 | 8.15** | 6.19** | -4.93** | -7.94** | -12.75** | -8.44** | -10.05** | -2.89** | 3.11** | 26.14** | -27.17** | -12.74** | 21.48** | 16.55** | 7.12** | -13.42** | -8.47** | -4.25** | -3.78** |
| L7XT2 | 24.11** | 17.61** | -10.02** | -3.77** | -6.95** | -14.25** | -8.07** | -20.14** | 9.17** | 12.83** | -11.09** | -9.03** | 1.98** | 3.72** | 3.18** | -21.05** | -5.17** | -16.72** | -10.22** |
| L1XT3 | -42.79** | -122.19** | 86.23** | 92.11** | 122.38** | 105.87** | 37.12** | 38.56** | -22.45** | -81.33** | 50.02** | 61.19** | -69.80** | -117.0** | -107.83** | 173.80** | 75.19** | 55.28** | 22.88** |
| L2XT3 | 11.06** | 15.93** | -7.88** | -11.05** | -1.42** | -2.55** | -5.60** | -7.92** | 4.04** | 2.13** | -13.81** | -17.24** | 27.33** | 14.05** | 11.07** | -6.12** | -41.03** | -14.83** | -5.39** |
| L3XT3 | 5.17** | 4.13** | -1.83** | -4.87** | -2.67** | -11.09** | -7.44** | -9.13** | 11.03** | 11.63** | -20.13** | -12.63** | 23.49** | 8.57** | 6.15** | -24.15** | -7.13** | -25.17** | -13.92** |
| L4XT3 | 10.04** | 10.06** | -21.07** | -4.97** | -3.48** | -5.11** | -2.55** | -7.14** | 17.09** | 3.59** | -9.37** | -7.41** | 16.08** | 21.04** | 13.79** | -10.03** | -21.05** | -4.17** | -14.83** |
| L5XT3 | 7.44** | 15.08** | -15.66** | -1.94** | -14.03** | -6.80** | -11.03** | -2.48** | 3.97** | 10.56** | -2.90** | -5.04** | 7.38** | 19.28** | 5.42** | -7.31** | -12.83** | -10.09** | -7.23** |
| L6XT3 | 12.55** | 7.38** | -3.19** | -12.56** | -4.72** | -19.02** | -5.12** | -6.49** | 8.33** | 1.77** | -5.93** | -4.88** | 15.89** | 6.39** | 9.14** | -13.42** | -5.31** | -2.74** | 3.11**- |
| L7XT3 | 233.51** | 390.69** | -225.57** | -128.49** | -224.95** | -87.95** | -58.32** | -70.24** | 191.30** | 253.92** | -166.13** | -187.43** | 257.09** | 291.98** | 15.32** | -405.66** | -170.64** | -85.82** | -240.82** |
| LSD at 0.05 (SIJ) | 1.83 | 1.65 | 1.42 | 1.39 | 1.12 | 1.72 | 1.54 | 1.71 | 1.53 | 1.28 | 0.76 | 1.41 | 1.25 | 0.82 | 1.73 | 1.63 | 1.32 | 2.39 | 1.91 |
| LSD at 0.01 (SIJ) | 2.14 | 1.81 | 1.65 | 1.48 | 1.29 | 2.13 | 1.83 | 2.19 | 1.84 | 1.69 | 1.02 | 1.72 | 1.61 | 1.33 | 2.19 | 1.85 | 1.72 | 2.68 | 2.33 |

*Significant at 5%, **Significant at 1% and NS: Not significant

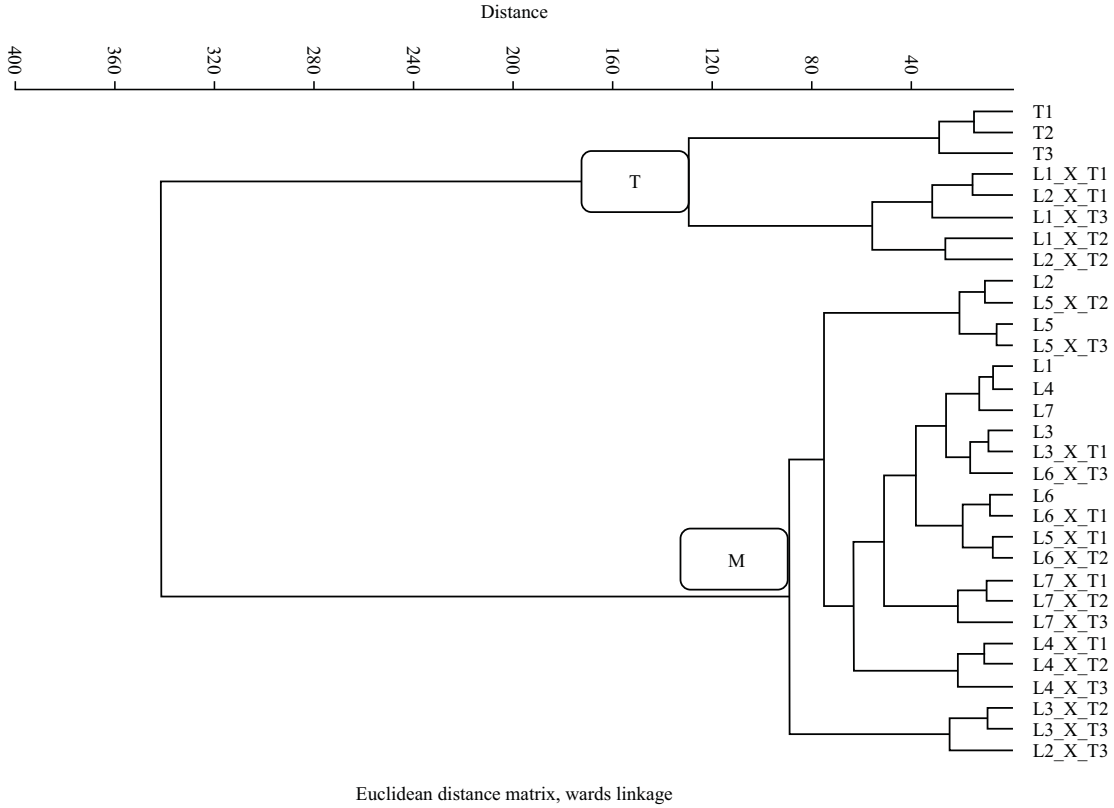


Fig. 2: Cluster analysis based on 10 agro–physiological traits for 31 Egyptian barley genotypes

Table 9: Estimation of salinity tolerance indices for the ten barley parents and the highest five crosses especially for grain yield/plant trait under both treatments

| Genotypes | GYP | GYS | YSI | YI | MP | STI | GMP | YR | SSI |
|---|---|---|---|---|---|---|---|---|---|
| Line 1 | 23.15 | 18.94 | 0.81 | 0.59 | 21.04 | 0.26 | 20.96 | 0.19 | 0.86 |
| Line 2 | 30.05 | 22.84 | 0.76 | 0.72 | 26.44 | 0.42 | 26.19 | 0.24 | 1.09 |
| Line 3 | 28.39 | 15.33 | 0.53 | 0.48 | 21.86 | 0.26 | 20.86 | 0.47 | 2.13 |
| Line 4 | 31.74 | 24.13 | 0.76 | 0.76 | 27.93 | 0.47 | 27.67 | 0.24 | 1.09 |
| Line 5 | 24.38 | 17.45 | 0.71 | 0.55 | 20.91 | 0.26 | 20.62 | 0.29 | 1.31 |
| Line 6 | 26.25 | 17.62 | 0.67 | 0.55 | 21.93 | 0.28 | 21.50 | 0.27 | 1.22 |
| Line 7 | 25.43 | 19.77 | 0.77 | 0.62 | 22.60 | 0.30 | 22.42 | 0.23 | 1.04 |
| Tester 1 | 43.07 | 36.11 | 0.83 | 1.14 | 39.59 | 0.95 | 39.43 | 0.17 | 0.77 |
| Tester 2 | 48.12 | 41.05 | 0.85 | 1.29 | 44.58 | 1.21 | 44.44 | 0.15 | 0.68 |
| Tester 3 | 38.96 | 32.04 | 0.82 | 1.01 | 35.50 | 0.76 | 35.33 | 0.18 | 0.81 |
| Cross 1 | 55.09 | 47.64 | 0.86 | 1.50 | 51.36 | 1.61 | 51.22 | 0.14 | 0.63 |
| Cross 2 | 59.32 | 44.13 | 0.74 | 1.39 | 51.72 | 1.61 | 51.16 | 0.26 | 1.18 |
| Cross 3 | 56.17 | 42.08 | 0.74 | 1.33 | 49.12 | 1.45 | 48.61 | 0.26 | 1.18 |
| Cross 4 | 53.47 | 45.16 | 0.84 | 1.42 | 49.31 | 1.48 | 49.13 | 0.16 | 0.72 |
| Cross 5 | 61.04 | 50.27 | 0.82 | 1.58 | 55.65 | 1.88 | 55.39 | 0.18 | 0.81 |

Line 1: Giza 125, Line 2: Giza 133, Line 3: Giza 134, line 4: Giza 129, Line 5: Giza 130, Line 6: Giza 131, Line 7: Giza 132, Tester 1: Giza 123, Tester 2: Giza 126, Tester 3: Giza 2000 , Cross 1 : Giza 125XGiza 123, Cross 2: Giza 133XGiza 123, Cross 3: Giza 125XGiza 126, Cross 4: Giza 133XGiza 126 and Cross 5: Giza 125XGiza 2000, respectively

(Giza 125XGiza 2000) which were positive and get high values of PCA1 could be more tolerant genotypes and suitable for both saline and normal conditions.

**Cluster and biplot analysis:** Biplot analysis identifies the greater genotypes for both stress and non-stress conditions. Genotypes exposed to Biplot analysis, are compared for assessing relationships between all the attributes at once. Biplot and cluster analysis using (Euclidean distance matrix and words Linkage) based on their ten agro-physiological traits and PCA values had clustered all 31 barley genotypes; (tester, lines and their crossing) into two major groups (Fig. 1 and 2). The First group (T) includes all the tolerant genotypes which had higher grain yield and higher values of

Table 10: Principal component analysis for the ten agro-physiological traits of 31 barley genotypes

| Traits/genotypes | PCA1 | PCA2 | PCA3 |
|---|---|---|---|
| **Agronomical traits** | | | |
| Plant height (CM) | 13.471 | -2.2984 | 0.049515 |
| Number of filled grains/spike | 1.6825 | -0.95248 | 0.39623 |
| 1000-grain weight (g) | 1.6966 | -0.50369 | 0.18808 |
| Grain yield/plant (g) | 1.0574 | -0.36937 | 0.089815 |
| Physiological traits | | | |
| $K^+$ content (ppm) | -4.6286 | 1.2635 | -0.07637 |
| $Na^+$ content (ppm) | -4.8753 | 1.3466 | -0.08047 |
| Na/K ratio | -4.8942 | 1.3452 | -0.08011 |
| Osmotic adjustment | -4.679 | 1.401 | -0.08084 |
| Proline content | 1.5012 | -2.295 | -0.82311 |
| Glycine betaine content | 1.2594 | -1.9982 | 0.66596 |
| **Genotypes or parents** | | | |
| Giza 125 L1 | -1.933 | 0.60231 | -0.32963 |
| Giza 133 L2 | -2.1212 | -0.23578 | -0.03763 |
| Giza 134 L3 | -1.5142 | 0.52557 | -0.07853 |
| Giza 129 L4 | -1.4143 | 0.4849 | -0.76605 |
| Giza 130 L5 | -2.7109 | -0.33266 | 0.60215 |
| Giza 131 L6 | -1.2086 | 0.058849 | -1.2318 |
| Giza 132 L7 | -2.8261 | 0.54467 | 0.54657 |
| Giza123 T1 | 3.5951 | -0.09331 | -1.1525 |
| Giza126 T2 | 4.2814 | 0.96138 | -1.2424 |
| Giza 2000 T3 | 3.8649 | -0.66925 | -0.81457 |
| **Crossing** | | | |
| L1_X_T1 | 7.4183 | -0.38792 | 0.48885 |
| L2_X_T1 | 7.3407 | 0.40076 | -0.09735 |
| L3_X_T1 | -2.3801 | 0.027831 | 0.21893 |
| L4_X_T1 | -1.8254 | 1.3822 | -0.27532 |
| L5_X_T1 | -2.7478 | 0.24381 | 1.5816 |
| L6_X_T1 | -1.2724 | 0.39163 | -1.3414 |
| L7_X_T1 | -2.2871 | 1.0557 | 1.7473 |
| L1_X_T2 | 7.9925 | -0.26964 | 1.2517 |
| L2_X_T2 | 7.7513 | -0.0024 | 0.75829 |
| L3_X_T2 | -2.3823 | -2.0751 | -0.03376 |
| L4_X_T2 | -2.342 | 0.59587 | -1.0155 |
| L5_X_T2 | -3.1682 | -0.94167 | -0.16411 |
| L6_X_T2 | -1.711 | 0.84688 | -0.87654 |
| L7_X_T2 | -2.9513 | -0.44698 | 1.6226 |
| L1_X_T3 | 7.904 | -0.3465 | 0.32341 |
| L2_X_T3 | -1.5404 | -2.147 | -1.0377 |
| L3_X_T3 | -2.8567 | -2.9322 | 0.21404 |
| L4_X_T3 | -1.8598 | 1.5356 | 0.44981 |
| L5_X_T3 | -2.7594 | -0.17593 | 0.52263 |
| L6_X_T3 | -1.4666 | 0.69737 | -0.16756 |
| L7_X_T3 | -2.8692 | 0.70101 | 0.33451 |
| Eigenvalue | 15.30 | 0.98 | 0.75 |
| Variance (%) | 80.54 | 5.17 | 3.96 |

PCA1 under normal and saline stress conditions. So, we could consider them as a tolerant cultivars such as (Giza 123, Giza 126, Giza 2000, Line 1XTester 1 (Giza 125XGiza 123), Line 2XTester 1 (Giza 133XGiza 123), Line 1XTester 2 (Giza 125XGiza 126), Line 2XTester 2 (Giza 133XGiza 126) and Line 1XTester 3 (Giza 125XGiza 2000). While the second group (M) included the moderated tolerant genotypes in this regard.

**Molecule marker analysis:** Eleven SSR primer pairs previously mapped and covered all seventh chromosomes (Grain Genes database) were used in this study (Fig. 3a-d). These primers were screened against selected fifteen barley genotypes in an attempt to detect polymorphic markers. Out of these eleven primers, five primers showed monomorphic fragment profiles as one allele were Bmac0063 (1H), Bmag0125 (2H), Bmac 0209 (3H), Bmag0084 (4H Fig. 3c) and Bmac135(7H). Four primers produced two alleles were Bmac 0742 (2H), Bmac 0067 (3H Fig. 3d), Bmac0096 (5H) and Bmac 0173 (6H Fig. 3b), one primer produced three markers were Bmac 0064 (7H) and the SSR primer Bmag 770 (1H Fig. 3a) produced four alleles. Twenty alleles were produced as a result of fingerprinting 11 SSR primers ranging from one to four alleles per locus with a mean value of 1.81 alleles per locus as shown in Table 11. The outstanding six primer pairs (Bmac 0742, Bmac 0067, Bmac0096, Bmac 0173, Bmac 0064 and Bmag 770) generated clear fragment patterns with high polymorphism (100%) which were used to evaluate the genetic diversity of the selected 15 barley genotypes. The PIC (Polymorphism Information Content) value of each SSRs marker measure the marker ranged from 0.46 (Bmac0067 Fig. 3d) to 0.85 (Bmag 770 Fig. 3a) (Table 11). Furthermore, indicators of locus diversity (polymorphism information content-PIC) were calculated and Not considering the monomorphic loci. The primers which recorded a high rank of PIC were considered an ideal way to compare and distinguish among all barley genotypes under study. s The highest number of the fragment was developed by the primer (Bmag 770, 1H Fig. 3a) showed four fragments, which amplified specific allele with molecular size 260 bp found in all tolerance genotypes namely; (Giza 123, Giza 126, Giza 2000, Giza 125XGiza 123, Giza 133XGiza 123, Giza 125XGiza 126, Giza 133XGiza 126 and Giza 125XGiza 2000).

Molecular genetics in particular molecular parameters is considering the fruitful method in the field of plant genetics responsible for finding out the causes and molecular genetic evidence that attributes and causes barley tolerance to salinity. In addition, its main role in distinguishing between different entries and crosses at the molecular level. As well as, determining the degree of convergence and genetic separation (Genetic distance) between these genotypes under study through estimating phylogenetic tree or cluster analysis. Therefore, the goal of conducting molecular markers is the molecular description for the ten barley parents besides, the best and strongest five hybrids with high tolerance to salinity stress based on all results obtained from all studied traits

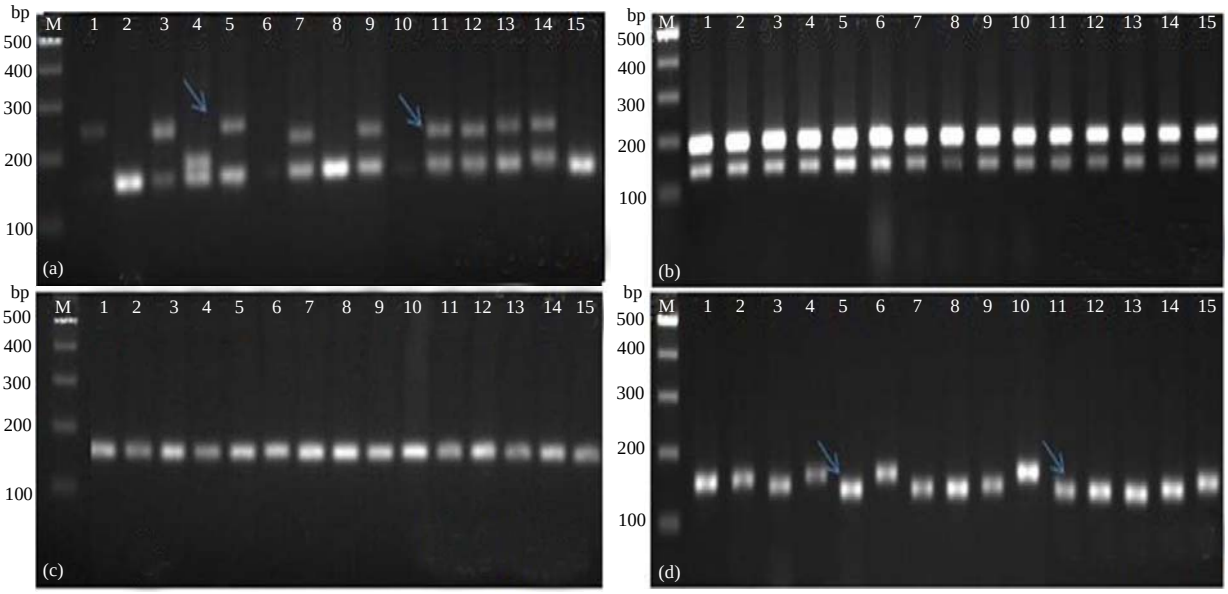


Fig. 3(a-d): Amplification results of the SSR primers

(a) Bmag 0770, (b) Bmag0173, (c) Bmac 0084 and (d) Bmac0067 in studied barley genotypes. M: Marker, 1: Giza 123, 2: Giza 125, 3: Giza 126, 4: Giza 133, 5: Giza 2000, 6: Giza 132, 7: Giza 131, 8: Giza 134, 9: Giza 130, 10: Giza 129, 11: Giza 125XGiza 2000, 12: Giza 133XGiza 126, 13: Giza 125XGiza 126, 14: Giza 125XGiza 123 and 15: Giza 133XGiza 123, respectively

Table 11: List of multiplexing sets of the used SSR primers motifs, no. of alleles, no of polymorphic bands, polymorphism information contents (PIC) and polymorphism (%)

| Primer name | Motif | No. of alleles | No. of polymorphic bands | PIC | Polymorphism (%) |
|---|---|---|---|---|---|
| Bmac0063 | (AC)14 | 1 | 0 | 0.0 | 0.0 |
| Bmag0770 | (GT)13,(AG)19 | 4 | 4 | 0.85 | 100 |
| Bmag0742 | (TC)29 | 2 | 2 | 0.47 | 100 |
| Bmag 0125 | (AG)19 | 1 | 0 | 0.0 | 0.0 |
| Bmac0067 | (AC)18 | 2 | 2 | 0.46 | 100 |
| Bmac0209 | (AC)13 | 1 | 0 | 0.0 | 0.0 |
| Bmac0084 | (AC)13 | 1 | 0 | 0.0 | 0.0 |
| Bmac0096 | (AT)6(AC)16 | 2 | 2 | 0.48 | 100 |
| Bmag0173 | (CT)29 | 2 | 2 | 0.34 | 100 |
| Bmac0064 | (AC)21 | 3 | 3 | 0.39 | 100 |
| Bmag0135 | (AG)10GG(AG)12 | 1 | 0 | 0.0 | 0.0 |
| Average | | 1.81 | 1.36 | | 54.54 |
| Total | | 20 | 15 | | |

under normal and salinity conditions. The UPGMA cluster divided into three main clusters (named as T, MT and S) (Fig. 4). Accordingly, after all the physiological and molecular genetics results mentioned above, can be considered the following four barley crosses namely; cross 1 (Giza 125XGiza 123), cross 3 (Giza 125XGiza 126), cross 4 (Giza 133XGiza 126) and cross 5 (Giza 125XGiza 2000) besides, the parents Giza 123, Giza 126, Giza 131 and Giza 2000 the most tolerant genotypes which grouped in the first cluster (T). Meanwhile, the second cluster (MT) include moderately tolerant barley genotypes namely; Giza 125, Giza 130, Giza 133 and Giza 134 besides, the cross 2 (Giza 133XGiza 123). The remained cluster (S) includes the sensitive barley genotypes; Giza 129 and Giza132 in diverged form as shown in Fig. 4.

## DISCUSSION

Salinity stresses an utmost observed problem worldwide that has made a thoughtful impact on agriculture. It has instigated deleterious effects on physiology and productivity as well as yield in most plants. Results of lineXtester analysis largely indicated the vital and active role of additive and additiveXadditive types of gene action responsible for

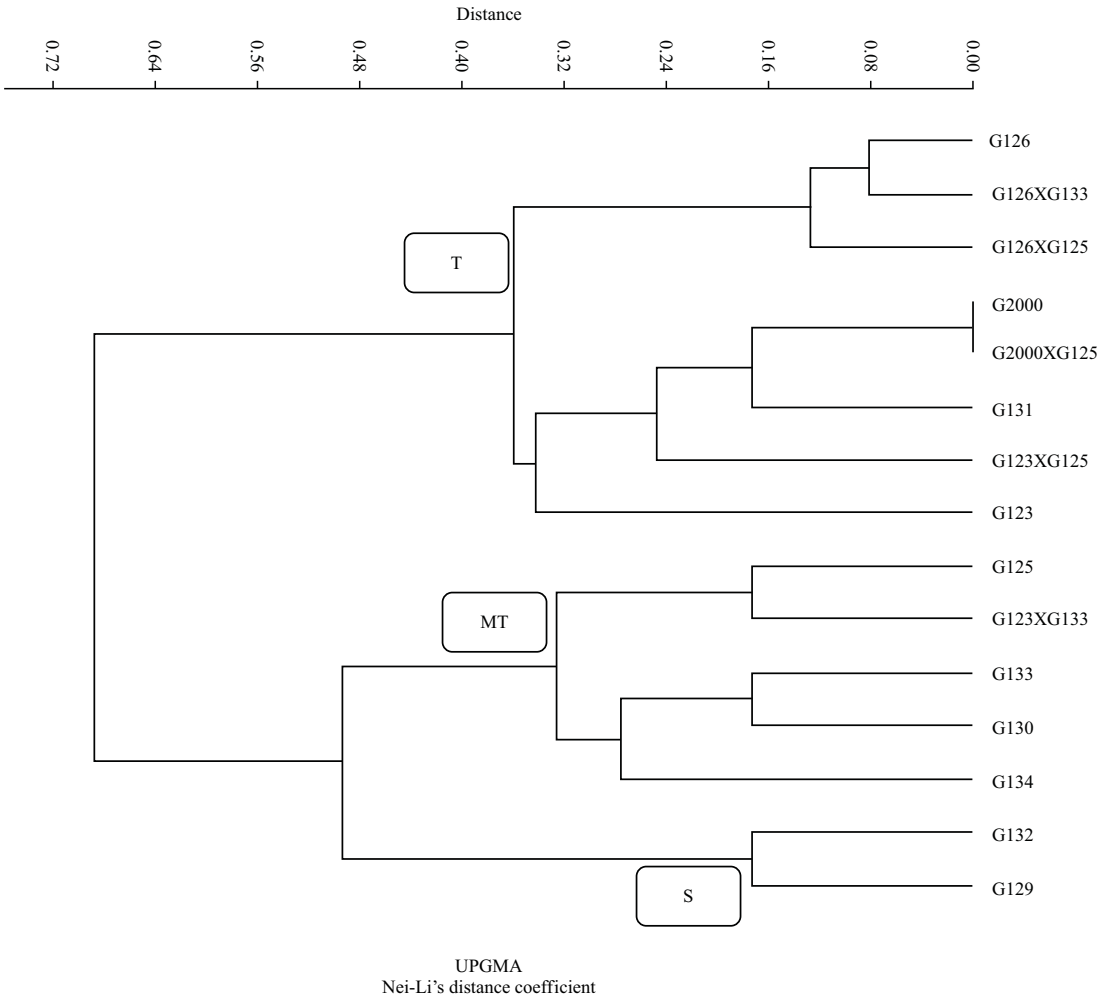


Fig. 4: Dendrogram representing the genetic relationship among the fifteen barley genotypes using UPGMA cluster analysis of Nei-Li's distance coefficient using 11 SSRs primers

inheriting and controlling a salinity-tolerant trait in barley genotypes. This is due to the importance of the three salt-tolerant barley varieties namely; Giza 123, Giza 126 and Giza 2000 which had the main role in producing 21 barley hybrids by independently hybridizing each of them with the rest of barley genotypes classified medium to sensitive tolerance to this dangerous environmental factor. The final result of 21 barley hybrids confirmed that most of them are considered tolerant or medium tolerance to salinity stress, Table 4[4,7,38-41]. The eight promising barley entries presented in Table 5 namely; Giza 123, Giza 126 and Giza 2000 besides; the crosses; Line 1XTester 1 (Giza 125XGiza 123), Line 2XTester 1 (Giza 133XGiza 123), Line 1XTester 2 (Giza 125XGiza 126), Line 2XTester 2 (Giza 133XGiza 126) and Line 1XTester 3 (Giza 125XGiza 2000) which exhibited higher mean values for yield and its components traits scored the first rank of salinity tolerance in this regard. Because these genetic materials were able to control the entering of sodium elements and also able to reduce its percentage and toxicity in cells. On the contrary, they allowed increasing the accumulation of potassium content, which is responsible for the resistance to salt stress and helps to build new cells. This reflects the ability to genetically control adventurous roots responsible for this process. In addition, controlling of opening and closing of stomata especially the upper ones besides, reducing the amount of water losing used in the transpiration process during photosynthesis under saline stress compared to the normal conditions. Further, after all of the above mentioned, it is noticed that the eight barley promising genotypes that tolerate salt stress have also succeeded in producing and excreting a large number of organic acids such as proline and glycine betaine contents under saline-stress level compared to normal treatment. Because research and studies have proven that these compounds have a close relationship with

resistance to water and salt stresses in the tolerant entries when mass-produced. While, sensitive genotypes cannot produce these compounds in large quantities,[4,38-41]. Data obtained in Table 6 and associated with heterosis over better-parent confirmed the importance of dominance and dominanceXdominance gene action beside the fruitful role of SCA effects for controlling, inheriting, enhancing and increasing the ability of salinity tolerance in the promising five barley crosses namely; L1XT1, L2XT1, L1XT2, L2XT2 and L1XT3, respectively. There is no doubt that the aforementioned five superior barley hybrids are the largest evidence for the transgressive segregation represented in the first generation. Therefore, the cultivation of these genotypes for several isolation generations with the simple selection of important traits such as salinity tolerance and high grain yield may ultimately lead to access to genetically stable, high yielding barley lines as well as tolerance to salt stress. These results were in agreement with those reported by[4,40,42]. Results of GCA effects obtained in Table 7 detected the biggest role of additive and additiveXadditive types of gene action responsible for controlling salinity stress tolerance in barley entries through studying the recent traits. This strongly indicates the success achieved in the genetic improvement of tolerance to high soil salinity in the previous superior genotypes. Also, continuing to cultivate these superior entries for a few segregation generations may eventually lead to obtaining barley lines classified as high genetic stability, highly salinity tolerance and good yield[4,40,42]. In the same track, results obtained in Table 8 and related to SCA effects confirmed the important role of dominance and dominanceXdominance types of gene action in controlling, activate and raising the ability of salinity tolerance in the superior five barley crosses in all attributes understudy in this investigation. Given that these five hybrids will serve as the nucleus for the production of barley varieties that are tolerant to salt stress, as a serious step for the genetic improvement of barley to confront environmental stresses[4,40,42]. Salinity tolerance indices are considering the most important indicators used to verify all field experimental results prove or not prove salinity tolerance trait in all tested genetic materials of barley, Table 9. Accordingly, all results of grain yield/plant trait evaluated under salinity conditions compared to the control experiment for the promising eight barley genotypes namely; the three testers; (Giza 123, Giza 126 and Giza 2000) and the five barley crosses; Line 1XTester 1 (Giza 125XGiza 123), Line 2XTester 1 (Giza 133XGiza 123), Line 1XTester 2 (Giza 125XGiza 126), Line 2XTester 2 (Giza 133XGiza 126) and Line 1XTester 3 (Giza 125XGiza 2000) which exhibited highly salinity tolerance based on all salinity tolerance indices parameters in this regard. Simply, because it was able to reduce the waste in the final yield under salinity stress compared to the rest of barley genotypes tested under the same conditions (YR). Also, most of them gave results lower than one of them (SSI) parameters which confirms reached these genetic materials to highly limit of salinity tolerance compared to the rest of all genotypes under the same conditions. Further, also, it was able to increase the production of some organic compounds such as proline and glycine betaine contents that cause salinity tolerance. This mechanism is not available in the rest of the genotypes which medium tolerance or even sensitivity to salt stress. This indicates the extent of the change and the physiological development to salinity stress tolerance in these promising barley genotypes[38,39]. Biplot and cluster analysis has been widely used for the description of genetic diversity and grouping based on agro-morphological and physiological traits which were considered as a helpful tool for breeders to design successful breeding programs for stress conditions. These results were in good harmony with[43-47] where they studied genetic diversity in barley using multivariable analysis to grouping barley genotypes based on phenotypic traits. The present study confined that parental barley (7 lines and 3 testers) and their F1 hybrids exposed to saline conditions were examined for morphological attributes, yield parameters, harvest index and confirmed via DNA markers. Based on SSR analysis, it could use this marker as a positive marker for salt stress tolerance[41,45,46,48,49]. Using direct field and other methods to assess barley to salt stress tolerance made it possible to identify promising with a set of positive traits for use in practical breeding and selection[50]. Among 15 analyzed barley entries, the chosen set of 11 markers amplified 20 alleles with an average of 1.81, with a range from 1 to 4 alleles. Our finding was comparable with the findings of[51] who detect an average PIC value of 0.58 in barley lines using 28 SSR markers. Our results are also similar to the mean PIC value of 0.57 obtained by[52]. Average mean PIC values were slightly higher than reported by[53,54] which ranging from 0.28 to 0.46. Microsatellite markers with higher values of Polymorphic Information Content (PIC) were considered as a powerful marker to classify and discriminate any genotypes. A locus with an assessed PIC value high than 0.50 is considered to be highly differentiated[49]. In our study, the PIC values of the SSR markers ranged between 0.39 and 0.85. The UPGMA results (Fig. 4) agreed with Bi-plot and agro–physiological traits analysis (Fig. 1 and 2). The three clusters were detected in the UPGMA plot showed a pattern correlated to the salinity stress in barley parents and their hybrids. In addition, barley genotypes could be divided into groups according to morphology and different target traits such as yield and DNA marker (SSR) to a tolerance

of abiotic stresses such as salinity[12]. The system of genetic variation assessment of barley genotypes with the SSR markers was established. Also, the results of SSR analysis and the data on valued agricultural trait loci determined the genetic distance among parents and their hybrids, which is of unlimited rate for breeders.

## CONCLUSION

This study succeeded in identifying the genetic behaviour associated with salinity tolerance in some promising barley entries included the ten parents and their F1 crosses obtained from lineXtester analysis. Results of all traits under study gave conclusive evidence that cannot be doubted that the genotypes; Giza 123, Giza 126 and Giza 2000 besides; the crosses; Line 1XTester 1 (Giza 125XGiza 123), Line 2XTester 1 (Giza 133XGiza 123), Line 1XTester 2 (Giza 125XGiza 126), Line 2XTester 2 (Giza 133XGiza 126) and Line 1XTester 3 (Giza 125XGiza 2000) were recorded the highest mean values under salinity treatment compared to the control experiment and considered highly tolerance to salinity stress in this regard. As well, these superior barley genotypes also gave positive results for salinity stress tolerance in all genetic parameters and salinity tolerance indices. Further, the molecular genetic markers using 11 SSR markers have already succeeded in determining the genetic basic responsible for salinity tolerance in 15 promising barley genotypes and superior to salinity tolerance (The ten parents besides, the best 5 F1 crosses with a high response for salt stress tolerance). This technique may be considered as a taxonomic basis at the molecular level to determine the semantics responsible for salinity tolerance in this track.

## SIGNIFICANCE STATEMENT

This study detected some barley genotypes with a high ability to salt stress tolerance under Egyptian conditions. Also, this result has been confirmed after studying some agro-morphological and physiological attributes related to salt stress tolerance in 31 barley genotypes under salinity stress conditions compared to the standard experiment. Based on these results, it can be concluded that the eight promising barley genotypes tolerant to salt stress especially the five F1 crosses mentioned above can be considered as a nucleus for producing high-yielding barley lines that are tolerated to salt stress. Further, this investigation will help the researchers to uncover the critical areas in molecular genetic markers which discovering 20 alleles generated from 11 SSR primers pairs (five of them were monomorphic and 15 fragments were polymorphic with 75.0% polymorphism). In addition, marker Bmag succeeded in producing four alleles with a specific marker with molecular size 260 bp found in all tolerance barley entries. Thus, it is possible to enter those tolerant materials in the genetic improvement program for salinity tolerance in a barley crop. This is a new theory and a successful scientific leap in this regard.

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