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Comprehensive evaluation and economic analysis in some barley genotypes under soil salinity

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Abstract. *Mariey SA, El-Bialy MA, Khedr RA, Mohamed EN, Meleha AMI, Khatab IA. 2023. Comprehensive evaluation and economic analysis in some barley genotypes under soil salinity. Asian J Agric 7: 20-33.* Soil salinity is one of the abiotic stresses that cause a significant reduction in barley production. Understanding the phenotypic and genetic diversity among Barley genotypes is necessary to improve barley salt tolerance. Herne comprehensive sets of morph-physiological, grain quality traits and Simple Sequence Repeat (SSR) markers combined with economic analysis were done to determine the phenotypic and genetic variation of eight barley genotypes under salinity stress during seasons 2019/2020 and 2020/2021. High genetic variation was observed among studied genotypes for all measured traits. Salinization caused a significant increase in (Sodium content, soluble carbohydrate content, and crude protein content %) in sensitive genotypes (Giza 132 and line 1). SSRs markers generated clear patterns with high polymorphism with 31 alleles by an average of 2.07 alleles per locus. Out of 15 SSR markers, nine (Bmac 0209, Bmag 0011, Bmag 125, Bmac 0871, Bmag 770, Bmac 701, Bmag 0387, Bmac316, and Bmag 0009) were highly useful in distinguishing tolerant and sensitive Barley genotypes. Soil salinity decreased the benefit-cost ratio for Giza 123,136 and 137, which appear beneficial as salt-tolerant cultivars. Those cultivars had low reductions for almost studied traits and had the highest grain yield production due to increasing the farmer's income under salt affect area.

Keywords: Association analysis, barley, economic statement, parameters, phenotypic, salinity, SSR

INTRODUCTION

Crop production is mainly affected by many abiotic stresses; soil salinity is a major global climatic factor that restricts crop yields and food production worldwide (Bakari et al. 2018; Younis et al. 2020). Salt tolerance is a complex trait that uses different methods and parameters to assess plant germplasm for salt tolerance. Soil salinity is one of the chief abiotic environmental stress that causes the greatest yield losses of 20% in crop production in arid, semi-arid, coastal regions and humid and sub-humid landscapes (Mwando et al. 2020). It is estimated that around 6% of the world's total land area is affected by soil salinity, through 20% of arable land and 33% of irrigated land (Hossain 2019). Furthermore, salinity-affected areas expected to increase to be more than 50% of the world's total arable land by 2050 (Saade et al. 2016).

In Egypt, soil salinization regions were found on about 60% of the cultivated lands of the Northern Delta region, 20% of the Southern Delta and middle region, and 25% of Upper Egypt regions (FAO 2008). About 18.9 billion m^3 of wastewater was cleared directly into the Nile or into agricultural drains to be reused back to the River Nile (EEAA 2016). Salinization disturbs plants in numerous ways: water stress, nutritional disorders, and ion toxicity affecting photosynthesis. Plants' photosynthesis is one of the greatest basic biochemical and physiological processes of plant growth and productivity (Isayenkov and Maathuis 2019).

Salinity decreases the ability of plants to take up water by depressing soil water potential leading to water deficits. It disturbs all plants' growth stages through the salt-specific influence of high sodium (Na^+) and chloride (Cl^-) concentrations in tissues due to ion toxicity. The principal cause of ion-specific damage was Na^+, which greatly affects enzyme activation and protein synthesis. Salinity stress affects plant physiological mechanisms, changing plant growth, mineral dissemination, membrane insecurity, and diminished photosynthetic productivity (Acosta-Motos et al. 2017).

The best solution to the salinity problem is to use salt-tolerant species. Barley (*Hordeum vulgare* L.) consider the most salt-tolerant species among cereal. It ranks the fourth important cereal crop as a major food source and feeds many animals and people living in complicated areas. Due to its resilience and relatively stable yields, under both highly productive areas and subsistence low-input agricultural systems compared with other grains crops (FAOSTAT 2019). It is an excellent model crop for studies of mechanisms and inheritance of salinity tolerance which using in developing tools to improve salt tolerance in cereals (Zhu et al. 2020). A major objective of crop

breeding programs is to produce new varieties with improving responses to salt tolerance and to get high yields through evaluating diverse genotypes to increase their effectiveness (Allel et al. 2019; Al-Ashkar et al. 2020; Akhter et al. 2021; Mariey et al. 2022a,b).

Barley breeders are working to understand the behavior and efficiency of salinity on barley plants. Several researchers in barley have studied the effect of salinity stress on agro-physiological and biochemical traits (Mariey et al. 2017; Mariey et al. 2018; Mariey et al. 2019; Rajeswari et al. 2019; El Sabagh et al. 2019; Sorkhi 2020; Zeeshan et al. 2020; Akhter et al. 2021; Dell'Aversana et al. 2021; Mariey et al. 2022a,b) which they reported that salinity stress harmfully affects practically all stages of plant growth and development.

DNA based markers are required methods that were not influenced by environmental conditions. Therefore, it is consistently used for polymorphism, fingerprinting, genetic diversity detection, and genetic analyses (Grover and Sharma 2016). Among several types of DNA molecular markers applied in different genetic studies, microsatellites or Simple Sequence Repeats (SSRs) were still actually used in breeding programs. Those markers provide important information about a species, as they have a high polymorphism rate, co-dominant inheritance, high reproducibility, locus specificity, and random distribution on the genome (Brbaklic et al. 2021). Moreover, SSRs are powerful markers that are excellent for assessing genetic diversity and crop improvement for salinity stress tolerance (Mariey et al. 2016; Khatab et al. 2021; Mehta et al. 2021; Mariey et al. 2022a).

Hence, successful salinity tolerance breeding programs must adopt a strategy to identify salt-tolerant genotypes by identifying comprehensive donating characteristics such as physiological, morphological, and biochemical traits. Therefore, metabolic pathways with molecular markers recognize their contribution to salt tolerance to improve and increase crop yields in saline areas to produce enough food for the increasing global population (Mwando et al. 2020; Khatab et al. 2021). So, the association of molecular markers with phenotypic evaluation is an important factor in investigating tolerance's genetic role by predicting the genomic regions that affect plant response. Those will be useful as a comprehensive evaluation of breeding programs for environmental stress (Sallam et al. 2018; Khatab et al. 2021; Mariey et al. 2021; Mariey et al. 2022b).

Even though increasing crop production per unit under saline areas is necessary, an increase in the farmer's income is the most important goal because of the non-linear between crop yield and the price of products. Moreover, soil salinity affected negatively on crop growth and productivity, which leads to lower revenues, profitability, and land values, so knowing the crop that should be grown in saline lands is an important part of the on-farm decision makers (NDSU 2015; Hammami et al. 2020).

Thus, the present study aimed to comprehensively evaluate relative importance traits such as morph-physiological, grain composition parameters, and SSR analysis combined with economic analysis to accurately determine the phenotypic and genetic diversity of eight Barley genotypes. This study would also establish specific markers traits associated with salt tolerance to categorize Barley genotypes to use them in salinity breeding programs and to increase the production in the saline area in Egypt.

MATERIALS AND METHODS

Barley genotypes

Eight Egyptian barley genotypes kindly provided by Sakha Barley Research Department, Field Crops Research Institute, Agricultural Research Center, Egypt, are used in this study. First, those barley genotypes were observed to know their response to salinity stress as a first step to using them in the barley breeding program for salinity. Next, through crossing between them as a second step, names, rows-type, pedigree, salinity response, and years were shown in (Table 1).

Field experiments

Field experiments site

Two environmental field experiments were conducted under saline-sodic and normal conditions during two growing seasons, 2019/20 and 2020/21, at El Karada Water Requirements Research Station farm, Water Management Research Institute, National Water Research Center, in Kafr El-Sheikh Gov, Egypt. That site is located at 31° 05' 36.28" N, 30° 56' 53.56" E, with an elevation of 6 meters above mean sea level.

Table 1. Name, row type, salinity response pedigree, and released years of eight barley cultivars used in the field experimental

Name	Row type	Pedigree	Salinity response	Released year
Giza 123	Hulled	Giza 117/FAO 86	Tolerance	1998
Giza132	Hulled	Rihane-05//AS 46/Aths*2Athe/ Lignee 686	Sensitive	2006
Giza 136	Hulless	Plaisant/7/cln-b/ligee640/3/s.p-b//gloriaar/ come b/5/falconbar/6/linocln-b/a/s.p-/lignee640/3/s.p-b//gloria-bar/come b/5/falconbar/6/lino	Tolerance	2011
Giza 137	Hulled	Giza 118 /4/Rhn-03/3/Mr25-//Att//Mari/Aths*3-02	Un-know	2017
Giza 138	Hulled	Acsad1164/3/Mari/Aths*2//M-Att-73-337-1/5/Aths/ lignee686 /3/Deir Alla 106//Sv.Asa/ Attiki /4/Cen/Bglo."S")	Un-know	2017
Promising Line1	Hulled	C .C 89/3/Alanda/Hamra//Alanda-01	Un-know	-
Promising Line2	Hulled	Giza 118/3/Alanda/Hamra//Alanda-01	Un-know	-
Promising Line3	Hulled	Giza 124/6/Alanda//Lignee527/Arar/5/Ager//Api/CM67/3/ Cel/WI2269//Ore/4/ Hamra-01	Un-know	-

Field experiments design

Barley genotypes were planted in a Randomized Complete Block Design (RCBD) with three replicates; each plot was devoted to one genotype, which was planted in four rows 2 m long, spread out with 20 cm among rows (plot area= 1.6 m^2).

Sowing was done at a rate of 210 kg ha^{-1} using the broadcasting method on 23 and 25 November 2019, the 2020 seasons. Harvesting on 8 and 10 May 2020, the 2021 seasons. The normal cultural practices for growing barley were applied as recommended according to the Ministry of Agriculture's recommends.

Field experiments of site soil samples

Soil samples were taken before land preparation from the experimental site to a depth of 30 cm from the soil surface to perform physical and chemical analyses, as presented in Table 2.

Field experiments of site crop evapotranspiration

Crop evapotranspiration under normal soil (ETc, mm/day). Etc is evaporation from perfectly managed fields, large, well-irrigated that give full production under available climatic conditions is given by the equation below.

$$ETc = kc \times ET0 \quad (1)$$

Where: Kc is crop coefficient, and ETo is Reference crop evapotranspiration (mm/day^{-1})

Crop evapotranspiration under saline soil conditions (Etc adj). Etc adj is the evapotranspiration from crops cultivated under environmental management conditions that deviate from the standard circumstances.

$$ETc\ adj = ks \times kc \times ET0 \quad (2)$$

Where, ks is the water stress coefficient measured phenotypic characters affected by salinity stress. Under both control and saline condition, the following phenotypic characters were measured for each genotype in the two growing seasons; the types of traits, unite, abbreviation, and methodology reference of each trait are shown in Table 3.

Table 2. The average of physical and chemical properties and soil classification for soil samples from the field experiments sites during two growing seasons 2019/2020 and 2020/2021

Soil analysis		Normal site	Saline site
Physical analysis			
Soil texture	Coarse sand (%)	1.56	2.04
	Fine sand (%)	18.94	18
	Silt (%)	28.15	27.6
	Clay (%)	51.35	52.36
	Texture	Clayey	Clayey
Soil moisture	Bulk density (g cm^{-3})	1.21	1.15
Constants	Field capacity (%)	40	39.9
	Wilting point (%)	21.7	20.5
Chemical analysis			
EC(dSm-1)		2.76	10.7
PH		7.6	7.8
Sodium Absorpation Ratio SAR		2.63	17.84
Exchangeable soduim percentage ESP		10.11	45.50
Soluble cations	Ca++	0.33	7.82
meq100 1 g soil	Mg++	0.15	28.58
	Na+	1.29	76.1
	K+	0.1	2.05
Soluble anions	$CaCO^{3-}$	0	0
meq100-1 g soil	HCO^{3-}	13.2	59.9
	Cl^-	23.35	33
	SO^{4--}	4.95	14.1
Soil classification		Non-saline	Saline-sodic soils

Table 3. The names, units, abbreviations, and methodology references of all traits

Types of traits	Traits	Unite	Abb.	Methodology reference
Physiological traits	Chlorophyll a	µg mL^{-1}	Ch. a	(Moran 1982)
	Chlorophyll b	µg mL^{-1}	Ch. b	(Moran 1982)
	Relative water content	%	RWC	(Gonzalez and Gonzalez-Vilar 2001)
	Sodium Na content	mg g^{-1}Dw	Na	(Chapman and Pratt 1978)
	Potassium K content	mg g^{-1}Dw	K	(Chapman and Pratt 1978)
	Soluble carbohydrate content	mg g^{-1}Dw	**SCC**	(Naguib 1962)
Agro-morphological traits	Days to heading	days	HD	Days from sowing to 50% flowering
	Plant height	cm	PH	length of plant from soil to tip spike
	Number of grains $spike^{-1}$	grains	NGS	Number of grains each spike
	Number of tillers m^{-2}	tillers	NT	Number of tillers per m^2
	Thousand kernel weight	g	TKW	Weight 1000 grain
	Grain yield	t ha^{-1}	GY	Weight grain per plot
Grain composition traits	Crude protein content,	%	CPC	(Peter and Young 1980)
	Total ash content	%	TAC	(AOAC 2000)
	Total starch content	%	TSC	(Duis et al. 1956)
	Crude fiber content	%	CFC	(AOAC 2000)

Physiological traits

All the physiological traits done at the heading stage of barley leaves from the top of ten plants from each plot were randomLy taken to determine the physiological traits as follows: Photosynthetic pigments (chlorophyll a (Chl a) and chlorophyll b (Chl b)) were determined with approximate ratios of 1:100 (w/v) for fresh leaves. While N, N-dimethylformamide and determined spectrophotometrically at two wavelengths (664 and 647), according to Moran (1982).

$$Cha = 12.64\ A664\text{-}2.99A647 \quad (3)$$
$$Chb = \text{-}5.6\ A664 + 23.26\ A647 \quad (4)$$

Where: A664: the absorbance at wavelength 664; A647: the absorbance at wavelength 647

Relative Water Content (RWC%) was measured according to (Gonzalez and Gonzalez-Vilar 2001). Fresh leaves were cut into small pieces and weighed as Fresh Weight (FW), then sodden for 24 h in distilled water and weighed again to obtain the Turgid Weight (TW). Then, they were dried and weighed to obtain DW then using the equation.

$$RWC = (FW - DW/TW - DW) \times 100 \quad (5)$$

Where: FW is fresh weight, DW is dry weight, and TW is the turgor weight of leaf samples.

Sodium (Na) and potassium (K) content (mg g-1DW) were measured on the dry sample using standard flame photometer procedure (Model PFP7 Flame photometer, Jenway, Bibby Scientific Ltd, UK) as described by (Chapman and Pratt 1978) and soluble carbohydrate content SCC ($mgg^{-1}DW$) was measured on dry sample according to (Naguib 1962).

Agro-morphological traits

Six agro-morphological traits were estimated to study the effect of salinity on morphological traits and yield and its compound traits. (i) Days to Heading (HD): measured as the number of days to flowering when 50% of the spikes in a plot had extruded anthers. (ii) Plant Height (PH): was measured on a random sample of five plants in each plot as the length from the soil surface to the tip of the spike at harvest time. (iii) Number of Grains per Spike (NGS^{-1}): was measured on a random sample of ten spikes in each plot as calculated by the mean of grain number. (iv) Number of Tillers per m^2 (NT m^2): was measured on a random sample of ten spikes in each plot as calculated the mean of tillers number for each plot. (v) Thousand Kernel Weight (TKW): was measured by weighing 1000 kernels randomLy from each genotype grain yield. (vi) The Grain Yield (GY): was determined by harvesting the yield of the central area (1.6 m^2) of the plot and then transformed to the unit of ($t\ ha^{-1}$)

Grain composition traits

Harvested grains of the eight Barley genotypes from two seasons were composed and bulked per each genotype and grounded to a fine powder to measure: (i) Crude Protein Content (CPC%), the grain nitrogen percentage was determined using the micro-Kjeldahl method as described by Peter and Young (1980). (ii) Total Ash Contents (TAC%) were measured according to (AOAC 2000). (iii) Total Starch Content (TSC%) which was determined by the sulfuric phenol method (Duis et al. 1956). (iv) Crude Fibers Content (CFC %) was determined according to (AOAC 2000).

DNA extraction and SSR-PCR Reaction

DNA extraction

Total genomic DNA was extracted from young fresh leaves of eight barley genotypes CTAB protocol according to Doyle and Doyle (1990). DNA quality and concentration were estimated using NanoDrop Spectrophotometer (ND-1000, Thermo-Scientific).

Microsatellite primer

Fifteen Microsatellite SSR primer pairs were previously mapped and covered. All seven barely chromosomes (Grain Genes database) were selected from the published genetic maps according to (Varshney et al. 2007) against eight Barley genotypes to identify their polymorphic markers were shown in (Table 4).

Table 4. The name, sequence, and chromosome location of 15 SSR primers

Primer name	Sequence		Chromosome location
Bmag770	F:AAGCTCTTTCTTGTATTCGTG	R:GTCCATACTCTTTAACATCCG	1H
Bmac0213	F: ATGGATGCAAGACCAAAC	R:CTATGAGAGGTAGAGCAGCC	1H
Bmag749	F:CGGATTCTTGAGTAGTCTCTG	R:GATCTGTTTTTTGTAGAACATGC	2H
Bmag0125	F: AATTAGCGAGAACAAAATCAC	R:AGATAACGATGCACCAC	2H
Bmac0209	F:CTAGCAACTTCCCAACCGAC	R:ATGCCTGTGTGTGGACCAT	3H
EBmac0871	F:TGCCTCTGTTGTGTTATTGT	R: CCCCAAGTGAACATTGAC	3H
EBmac0701	F:ATGATGAGAACTCTTCACCC	R:TGGCACTAAAGCAAAAGAC	4H
HVMLOH1A	F:CCTCCCCTCTGATATGATAA	R:GTACAGACGGTTTAATTGTCC	4H
Bmac0113	F:TCAAAAGCCGGTCTAATGCT	R:GTGCAAAGAAAATGCACAGATA	5H
Bmag0387	F:CGATGACCATTGTATTGAAG	R:CTCATGTTGATGTGTGGTTAG	5H
Bmac0316	F':ATGGTAGAGGTCCCAACTG	R :ATCACTGCTGTGCCTAGC	6H
Bmag0009	F:AAGTGAAGCAAGCAAACAAACA	R:ATCCTTCCATATTTTGATTAGGCA	6H
Bmag0011	F:ACAAAAAACACCGCAAAGAAGA	R:GCTAGTACCTAGATGACCCCC	7H
Bmag0135	FACGAAAGAGTTACAACGGATA	R:GTTTACCACAGATCTACAGGTG	7H
EBmac0603	F:ACCGAAACTAAATGAACTACTTCG	R:TGCAAACTGTGCTATTAAGGG	7H

PCR-SSR reaction

SSR reaction was carried out in 25 µL using 20 ng/µL of genomic DNA templates. Next, 2.5 µL of 1X PCR buffer containing (15 mM MgCl, 0.5 µL of 15 mM dNTP mixture (2.5 mM of each), 1.25 µL of 5 u/µL of *Taq* DNA polymerase, and 0.25 µL of 10 µM forward and reverse primers) and18.5 ddH2O for amplification.

PCR amplification

PCR was carried out as the following PCR program; initial denaturation at 94°C for 5 min for one cycle, denaturation at 95°C for 1 min for 35 cycles, and annealing temperature (45-55°C) are specific for each primer for 30 sec and final extension at 72°C for 7 min with final holding at 4°C.

Gel electrophoresis

Amplified products were separated using agarose gel electrophoresis (2%) in 0.5 x TBE buffer against 100 bp DNA Ladder.

Economic impact statement

The economic productivity of eight barley genotypes was measured through Total Seasonal Return (TSR) ($/ha), Total Costs (TC) ($/ha), Net Return (NR) ($/ha), and Benefit-Cost Ratio (BCR) as affected by soil salinity were expressed in productive crop units of kg/m^3, and price of barley grains (1.5 $ per 1 Kg) for each treatment were done according to (Cimmyt 1988).

Data analysis

Phenotypic variation data analysis

A test for homogeneity of variance was used to compare variances over two years before determining the cogency of combined analysis according to the Bartlett test (Bartlett 1937).

All the data were statistically exposed to ANOVA in a two-way Randomized Complete Block Design (RCBD) to conclude the effects of genotypes, salinity, and their interaction on the studied traits, performed using SPSS-16.0 statistical software package (SPSS Inc. Chicago, IL, USA). The means of the different phenotypic parameters were compared by the Least Significant Difference (LSD). Correlation analysis using Pearson's parametric correlation test was performed using SPSS 22.0 (SPPS Inc. Chicago, IL) to determine the relationship between the two traits studied. GGE-biplots were used to study genotype-by-environment interaction using the Principal Component Analysis (PCA) to display the two-way data in the bi-plot graph. These were performed using a computer program Minitab v. 19 (Minitab Inc Coventry, UK), according to Sally et al. (1986). ClustVis, a web tool for visualizing the clustering of multivariate data, was used to construct heatmaps (Metsalu and Vilo 2015).

Molecular markers data analysis

The amplified bands from SSR were scored as binary data under the heading of total scorable fragments, which were determined for each genotype. The data were used to calculate allele frequencies, allele number, and genetic similarity according to (Varshney et al. 2007). Polymorphism Information Content (PIC) values were estimated according to (Nei and Li 1979). The marker efficiency of the 15 SSR primers, including diversity index (DI), Effective Multiplex Ratio (EMR), Resolving power (Rp), discriminating power, and Marker Index (MI) values, were calculated according to (Amiryousefi et al. 2018). Un-weighted Pair-Group Method with Arithmetical (UPGMA) cluster analysis was performed to produce a dendrogram on Jaccard's similarity coefficient using the PAST (Paleontological Statistics) software package (Hammer et al. 2001).

RESULTS AND DISCUSSION

Effect of salinity on the experimental soil

The variability of biophysical environments on experimental sites

The field experiments were conducted in two contrasting biophysical environments. The soil of the first environment is classified as non-saline soil, containing salts that provide an ECe of 2.76 dSm^{-1} and ESP of 10.11% and are very diverse, from soil rich in clay and poor in organic matter. While the second soil of the other environment is classified as Saline-Sodic soil, which contains salts that provide an ECe of 10.7 dSm^{-1} and ESP of more than 15, i.e.(45.5%), which leaching is difficult for the reason that the clay colloids are dispersed as shown in Table 2.

The changeability of soil salinity on crop evapotranspiration (ETc and Etc adj)

Crop growth season has been divided into four growth stages: initial, development, mid-season, and late season, as shown in Figure 1. The water needs of barley were calculated during the growth period by multiplying the evaporation and crop coefficient for each of the four stages of growth. The results showed the accumulative values of ETc were 170.30 and 141.35 mm for the two types of abovementioned soils, respectively distributed as follows: 16.94, 35.68, 89.93 and 27.75 mm and 14.06, 29.62, 74.64 and 23.03 at various stages of growth respectively. Additionally, the ETc was 17% higher in non-saline soil than in saline-sodic soil.

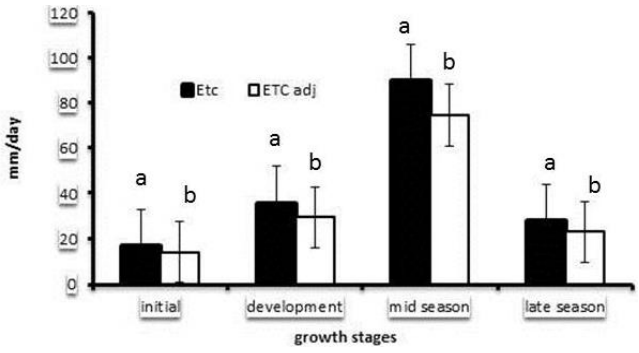

Figure 1. The effect of soil salinity on crop evapotranspiration (ETc and Etc adj)

Influence of salinity stress on the phenotypic traits among barley genotypes

Analysis of variance

A combined analysis of variance for the sixteen different phenotypic traits with homogeneous variance across the two seasons was measured on the eight Barley genotypes presented in Table 5. According to the Fisher variance comparison test for all measured phenotypic parameters, highly significant differences in treatments (T) (between normal and salinity stress) were found. ANOVA analysis revealed highly significant differences among genotypes (G) in all studied traits. The genotype × treatment interaction significantly differed among all studied traits, except total starch content TSC was non-significant.

The means performance of barley genotypes due to salinity stress

Physiological traits

Salinity stress caused a significant decrease in photosynthetic pigments Cha (Chlorophyll a) contents in all the eight Barley genotypes compared to its content under normal conditions by average reduction values (44.07%). The highest Cha content was found in Giza 137, with a value of 11.99 µg mL^{-1} under normal conditions, and Giza 123 had the maximum value (8.71 µg mL^{-1}) under salinity conditions. While Line 1 had the lowest Cha content under normal soil (8.47 µg mL^{-1}), and Line 3 recorded the lowest one (3.38 µg mL^{-1}) with an average value (5.71) under the salt condition as shown in (Figure 2A, Table 6).

In the case of Chb (Chlorophyll b) content, salinity decreased Chb content in all genotypes by average reduction values (54.46%). Giza 137 had the highest Chb content with a value (of 5.03µg mL^{-1}) under the normal condition with an average value (of 4.02), and Giza 136 had the highest Chb (2.83 µg mL^{-1}) under the salinity condition. However, Line 3 had the lowest Chb content (3.18 µg mL^{-1}) under normal conditions, while Line 1 gave the lowest Chb content (1.59µg mL^{-1}) under salt stress, as shown in (Fig2B, Table 6). Similar results were obtained by (Lin et al. 2016; Zeeshan et al. 2020; Akhter et al. 2021). They claimed that both chlorophyll a and b contents of barley plant decreased in response to salinity stress due to the rise of chlorophyllase enzyme, which converts chlorophyll into chlorophyllide and python under saline conditions.

Salinity stress significantly reduced RWC by 5.23 % in all eight Barley genotypes. Results (Figure 2C, Table 6) showed that high RWC was found in Giza 137 under normal and salt stress (84.25 and 80.84%), respectively. On the other hand, line 1 had the lowest RWC percent (62.17 and 60.08%) under both normal and salinity stress, respectively. Similar results were obtained with (Dell'Aversana et al. 2021), who reported that RWC is an important trait, reflecting the ability of plants to adjust their osmotic potential to preserve turgor pressure under salinity stress. Thus, the genotype with a high RWC percentage can tolerate salinity stress. In this study, Giza 137 recorded the highest RWC percentage while Line1 had the lowest percentage.

Salinity stress caused increasing in Na content in all Barley genotypes under salinity conditions as compared to the normal condition with 43.87%. However, these increases differed greatly among the genotypes (Table 6, Figure 3D). Line 1 had the highest Na content with values (of 0.99 and 2.023 mgg^{-1}DW) under normal and salinity conditions, respectively. While Giza 138 had the lowest Na content (0.736 mgg^{-1} DW) under normal treatment and Giza 123 (1.26 mgg^{-1}DW) under salinity treatment.

In the case of K content, salinity significantly reduced K content by (26.71%), in all genotypes (Table 6, Figure 2E). Giza 136 had high K content (2.63 and 2.15 mg g^{-1} DW) under normal and salinity treatment, respectively. While Line 1 had the lowest K content (1.79 and 1.14 mg g^{-1}DW) under normal and salinity treatments, respectively. The results agree with (Mahlooji et al. 2018; Isayenkov and Maathuis 2019; Narimani et al. 2020; Sorkhi 2020; Zhu et al. 2020). They found that the accumulation of toxic Na^+ in cytoplasm motivates stomatal closure, which causes a strong imbalance between entered light and energy utilization, decreases the photosynthetic rate and lead to the formation of Reactive Oxygen Species (ROS). Excessive Cl^- and Na^+ uptake leads to Ca^{2+} and K^+ deficiency and other nutrient imbalances. Tolerant genotypes of barley may avoid Na^+ accumulation in aboveground parts, which facilitates the photosynthetic process and leads to higher grain yield and has minimum Na content and maximum K content.

Salinity significantly increased Soluble Carbohydrate Content (SCC) by 6.79% among all studied genotypes (Table 5, Figure 2F). Giza 132 had the maximum SCC under normal and salinity stress (0.40 and 0.44 mgg^{-1} DW). While the minimum SCC (0.31 and 0.33 mgg^{-1} DW) was found in line 3 under normal and saline conditions, respectively. Similar results were observed in barley, where water deficit is the first effect of salinity stress on plants, and the accumulation of soluble sugars in leaves is a way for osmotic equipoise when plants are exposed to salt stress (Narimani et al. 2021).

Agro-morphological traits

Salinity stress quickens days to heading (HD) with high significant differences among Barley genotypes, through average values of (87.29 and 80.5 days) under salinity and normal condition, respectively, as shown in (Figure 3A, Table 6). The results exhibited that Giza 136 was the earliest cultivar at normal and salinity conditions (77.91 and 81.13 days), respectively. While Line 1 was the latest Barley genotype with values (84.47 and 91.80 days) under normal and salt stress, respectively. The same results were comforted by (Saade et al. 2016; Mariey et al. 2017; Allel et al. 2019; Mariey et al. 2022b), who identified that there is the genetic basis for earlier flowering in Barley genotypes associated with higher salinity tolerance.

Table 5. The analysis of variance for physiological, agro-morphological, and grain quality traits of eight Barley genotypes combined over the two seasons

Trait	Genotypes (G)	Treatments (T)	GXT	LSD
Physiological traits				
Chlorophyll a	15.06**	224.5**	3.86*	0.001
Chlorophyll b	1.406**	39.54**	0.283*	0.061
Relative water content	92.05**	0.300**	0.800*	0.115
Sodium Na+ content	0.023**	0.166**	0.017*	0.203
Potassium K+ content	0.316**	2.075**	0.112*	0.081
Soluble Carbohydrate Content	0.004**	0.0162**	0.0013*	0,004
Agro-morphological traits				
Days to Heading	55.015**	351.7**	19.142**	0.502
Plant Height	2789**	7268**	134.1*	0.678
Number of grains spike^{-1}	1440**	1816.7**	97.55**	0.553
Number of tillers m	11918**	376518**	3414.8*	0.319
Thousand kernel weight	4.309**	13.44**	0.457**	0.153
Grain Yield	3.93**	75.33**	0.177*	0.167
Grain chemical composition				
Crude protein content	0.504**	40.16**	1.534**	0.20
Total Ash content	0.1237**	4.057**	0.078*	0.001
Total starch content	7.56**	229.6*	1.27 NS	1.32
Crude fiber content	2.1104**	14,85**	0.2118*	1.07

Note: Ns, * and ** are non-significant and significant at the 0.05 and 0.01 levels of probability, respectively

Table 6. Minimum (Min.), maximum (Max.), an average of each trait scored on the Barley genotypes under normal and salinity stress

Traits	Normal condition			Salinity stress condition		
	Min.	Max.	average	Min.	Max.	average
Physiological traits						
Chlorophyll a	8.47	11.99	10.04	3.38	8.71	5.71
Chlorophyll b	3.18	5.03	4.02	1.68	2.83	2.20
Relative water content	62.17	84.25	75.78	60.08	80.84	71.05
Sodium Na content	0.74	0.99	0.93	1.33	2.023	1.52
Potassium K content	1.79	2.63	2.14	1.14	2.15	1.75
Soluble carbohydrate content	0.31	0.40	0.36	0.35	0.44	0.40
Agro-morphological traits						
Days to Heading	77.91	84.47	80.51	81.13	91.80	87.29
Plant height	99.80	117.13	105.70	71.80	91.47	81.09
Number of grains spike-1	42.13	96.13	68.13	36.47	72.47	55.83
Number of tillers m-2	286.80	466.80	369.47	140.33	233.47	192.34
Thousand kernel weight	8.46	11.99	10.04	3.41	7.81	5.59
Grain yield	3.94	5.97	5.11	1.36	3.82	2.55
Grain chemical composition						
Crude protein content	10.21	12.43	11.55	12.52	14.39	13.38
Total Ash content	2.38	3.02	2.78	1.88	2.19	2.26
Total starch content	60.41	64.39	62.34	55.39	62.34	57.88
Crude fiber content	4.97	6.87	5.73	3.97	5.73	4.72

Salinity stress caused a significant decrease in the plant height PH among all genotypes, as shown in (Table 6, Figure 3B). The Egyptian cultivar Giza 137 had the tallest genotypes with 117.13 and 91.47 cm values under both normal and saline conditions, respectively. Conversely, Line 1 displays the shortest genotypes under normal and salt conditions with values of 99.8 cm and 71.8 cm, respectively. Salt stress harmfully affected the Number of Grain Spike-1, (NGS) by an average reduction of 17.26%, as shown (Table 6, Figure 3C). The Egyptian cultivar Giza 137 had the highest NGS under normal and salt-stressed conditions, with 96.13 and 72.47 NGS values. At the same time, the lowest NGS was shown by line 1 with average values of (68.13 and 55.83) under normal and salt-stressed conditions, respectively. Soil salt stress reduced the Number of Tillers m^{-2} (NT) for all genotypes once compared with normal conditions. Results showed that Giza 123 and Giza 137 had the highest NT under normal and salt-stressed conditions. In contrast, the lowest NT was shown by line 1 under the normal condition with average values of (394.47 NTm^{-2}), and Giza 138 with average values of (192.39 NTm^{-2}) under salt-stressed conditions (Table 6, Figure 3D). Soil salinity decreased in Thousand Kernels Weight (TKW) in all genotypes (Table 6, Figure 2E). The maximum TWK was produced by Giza 137 under normal and salinity stress with values (of 11.99 and 7.81 g), respectively. While the minimum TWK was exhibited by line 1 and Giza 132 with average (10.04 g and 5.59 g), respectively, under normal and salinity.

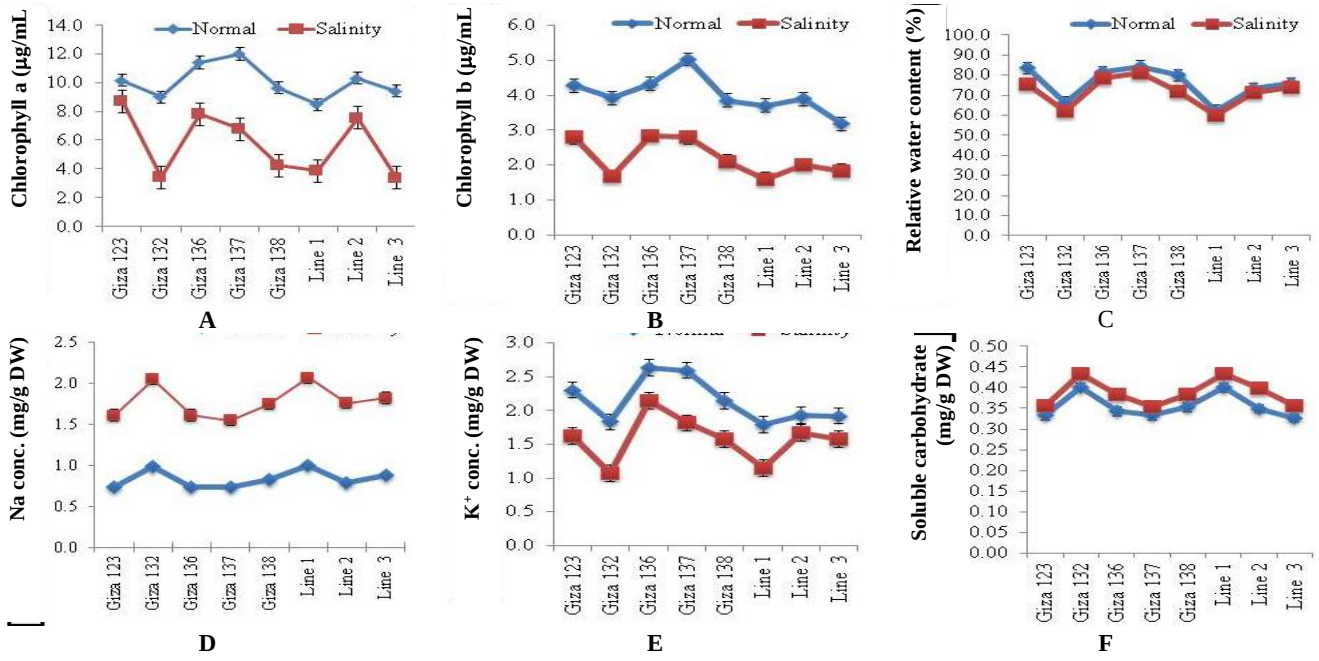

Figure 2. Effect of salinity stress on physiological traits in the eight barley genotype

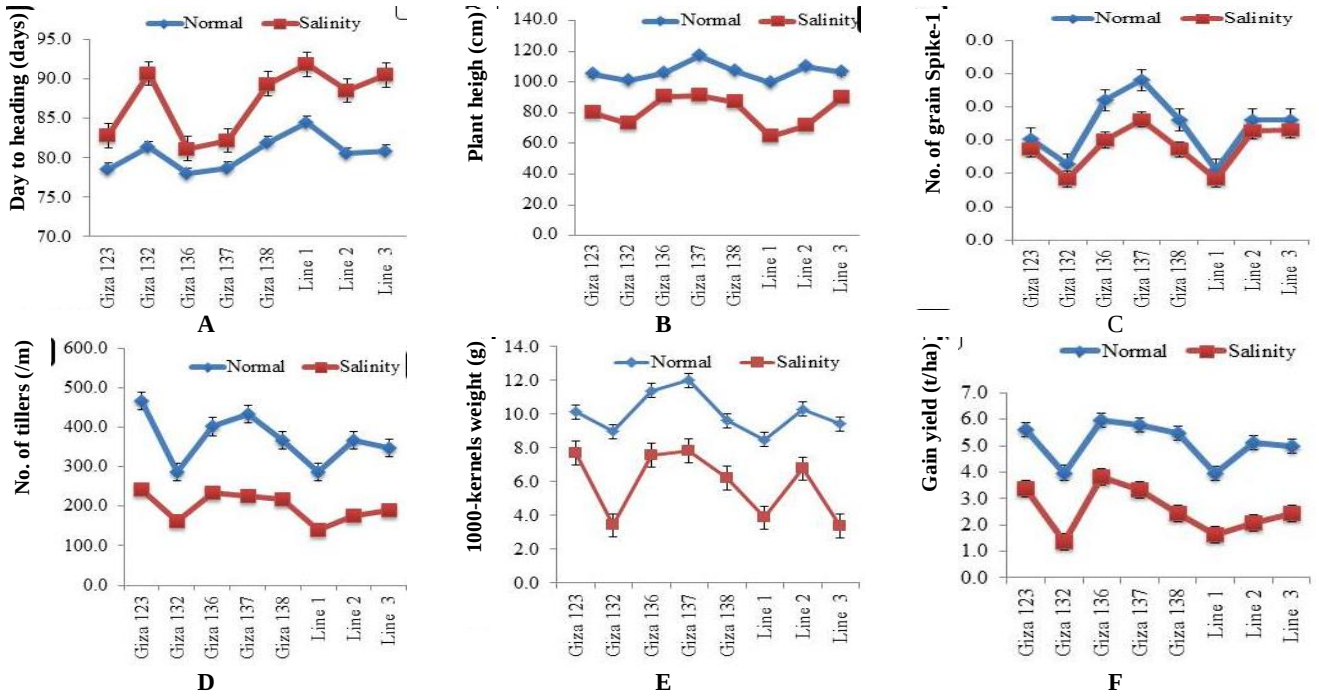

Figure 3. Effect of salinity stress on agro-morphological traits in the eight Barley genotype

In addition, salt stress dangerously affected grain yield GY (t ha^{-1}) by a high average reduction of 51.19%. Salinity caused a decrease in GY for all genotypes under study with an average (5.11 and 2.55 t ha^{-1}) under normal and salinity stress, respectively, as presented (Table 6, Figure 3F). Results showed that Giza 136, Giza 137, and Giza 123 had the maximum GY values (5.97, 5.79, and 5.59 t ha$^{-1)}$ under normal conditions and (3.82, 3.37, and 3.36 t ha^{-1}) under stress conditions. However, line 2 and Giza 132 had the minimum values under normal and stress conditions. Similar results have been observed by (Mariey et al. 2018; El Sabagh et al. 2019; Mariey et al. 2019; Rajeswari et al. 2019; Zeeshan et al. 2020; Akhter et al. 2021; Dell'Aversana 2021; Mariey et al. 2022a,b), they confirmed that the reduction in grain yield was due to reduction in yield components. Grain yield is a final product of the action and interaction of many environmental, agronomical, and physiological characteristics.

Grain composition traits

Salt stress significantly increased Grain Protein Content (GPC) in all genotypes under salinity stress more than in normal conditions (Table 6, Figure 4A). For example, under salinity stress, Giza 132 had the maximum GPC (14.39 %) in line 3, and line 1 had the minimum GPC (12.83%). Under normal conditions, Giza 137 had the maximum GPC with values (of 12.43%), while line 1 had the minimum GPC (10.21%) with an average (of 11.55%). Our results are in good harmony with those (Saleh-Amal et al. 2017; Nadeem et al. 2020), who reported that salinity increases CPC. This high protein might play an important role in increasing the osmotic pressure of the cytoplasm under salt stress and reducing other grain compositions.

However, salinity stress reduced total ash content TAC in all eight Barley genotypes, as displayed in (Table 5, Figure 4B). Giza 137 and Giza 123 had the same highest TAC under normal and salinity conditions with values of (3.02 and 2.38 $mgg^{-1}Dw$), respectively. Results showed that Line 1 had the lowest TAC content under normal and salinity with an average (of 2.78 and 2.19%), respectively.

Moreover, salt stress reduced Total Starch Content (TSC) by an average reduction of 7.82% in all the studied genotypes (Table 6, Figure 4C). The maximum value of TSC was found in Giza137 with values (of 64.39%) under normal, and Giza 136 was (59.37%) under stress conditions. While line1 had the minimum values of TSC under normal and salinity stress.

In addition, salinity stress decreased crude fiber content CFC in all eight Barley genotypes with an average decrease (5.73 and 4.62%) under normal, and Salinity conditions, respectively (Table 6, Figure 4D). Giza 136 and Giza 137 both had the maximum value of CFC. At the same time, Giza 132 and line 1 recorded the minimum values of CFC under normal and salinity, respectively.

Pearson correlation analysis

The Pearson correlation between all sixteen studied parameters under two different soil salinity levels over two seasons is displayed in Figure 5. Under normal conditions, GY displayed a highly significant and significant positive correlation with Cha, PH, NGS, NT, CPC, RWC, K^+, and TSC. While it had a significant negative correlation with Na^+, SCC, and HD**.** However, under salinity stress, GY had a highly positive correlation with Cha, K^+, RWC, PH, NGS, and NT, while GY had a significant negative correlation with Na^+, SCC, and HD. The phenotypic correlation between normal and salinity stress conditions for the same trait was exposed in (Figure 6, yellow cells.). A strong positive correlation ($|r|> 0.7$) was found in six traits (Cha, RWC, Na^+, NGS, NT, and GY, and a medium positive correlation ($|r|> 0.5$) was found in six traits (Cha, K+, SCC, HD, PH, and NT) while (TAC, TSC, and CFC) exhibit negative correlation.

Genotypic diversity among eight Barley genotypes due to salinity stress

SSR marker efficiency indices analysis

Thirty-one alleles were generated from 15 SSR primers using studied eight Barley genotypes with an average of 2.07 alleles per locus (Table 7). Five SSR primers revealed monomorphic fragment profiles as one marker were Bmac 0213 (1H), Bmag749(2H), HVMLOHIA (4H), Bmac 0113 (5H), and Bmag0135 (7H). Five SSR primers produced two fragments, Bmac 0209 (3H), Bmac 0871 (3H), Bmag 0009 (6H), Bmag 0011 (7H), and EBmac 0603 (7H). Four SSR primers produced three bands, Bmag 125(2H), EBmac 0701(4H), Bmag 0387 (5H), and Bmac 316 (6H), besides the SSR primer Bmag 770 (1H) primer produced four fragments.

Table 7. The marker efficiency indices of multiplexing sets of the used 15 SSR primers

Primer name	Motifs	Size band	NA	NPB	PIC	PP%	DI	EMR	DR	PR	MI
Bmag770	(GT)13 (AG)19	158	4	4	0.85	100	0.49	2.6	0.57	2.75	0.087
Bmac0213	(AC)23	168	1	0	0	0	0	1.0	0	0	0
Bmag749	(AG)11	166	1	0	0	0	0	1.0	0	0	0
Bmag0125	(AG)19	134	3	2	0.37	66.7	0.46	1.12	0.86	1.75	0.021
Bmac0209	(AC)13	176	2	2	0.47	100	0.48	1.25	0.62	1.0	0.036
EBmac0871	(TG)13	180	2	1	0.51	50	0.49	1.13	0.70	1.75	0.034
EBmac0701	(AC) 23	149	3	3	0.48	100	0.38	2.5	0.44	1.50	0.035
HVMLOH1A	(GA)6	175	1	0	0	0	0	1.0	0	0	0
Bmac0113	(AT)7 (AC)18	187	1	0	0	0	0	1.0	0	0	0
Bmag0387	(AG)16	150	3	2	0.32	66.7	0.38	2.25	0.44	0.50	0.035
Bmac0316	(AC) 19	135	3	3	0.44	100	0.37	1.5	0.45	1.0	0.034
Bmag0009	(AG)13	172	2	2	0.81	100	0.22	1.75	0.24	0.50	0.029
Bmag0011	(AG) 25	147	2	1	0.56	50	0.37	0.75	0.46	0.50	0.035
Bmag0135	(AG)10 (GG) 12	152	1	0	0	0	0	1.0	0	0	0
EBmac0603	(CA) 10	149	2	2	0.52	100	0.31	1.63	0.35	0.75	0.023
Total			31.0	22.0	5.33	833.4	3.91	21.48	5.13	12.0	0.37
Average			2.07	1.47	0.36	55.56	0,26	1.34	0.34	0.80	0.02

Note: NA: Number of Alleles, NPB: Number of Polymorphic Bands, PP%: Percentage of Polymorphism (%), PIC: polymorphism Information Content, DI: Diversity Index, EMR: Effective Multiplex Ratio, GR: Discriminating Power, MI: Marker Index, RP: Resolving Power

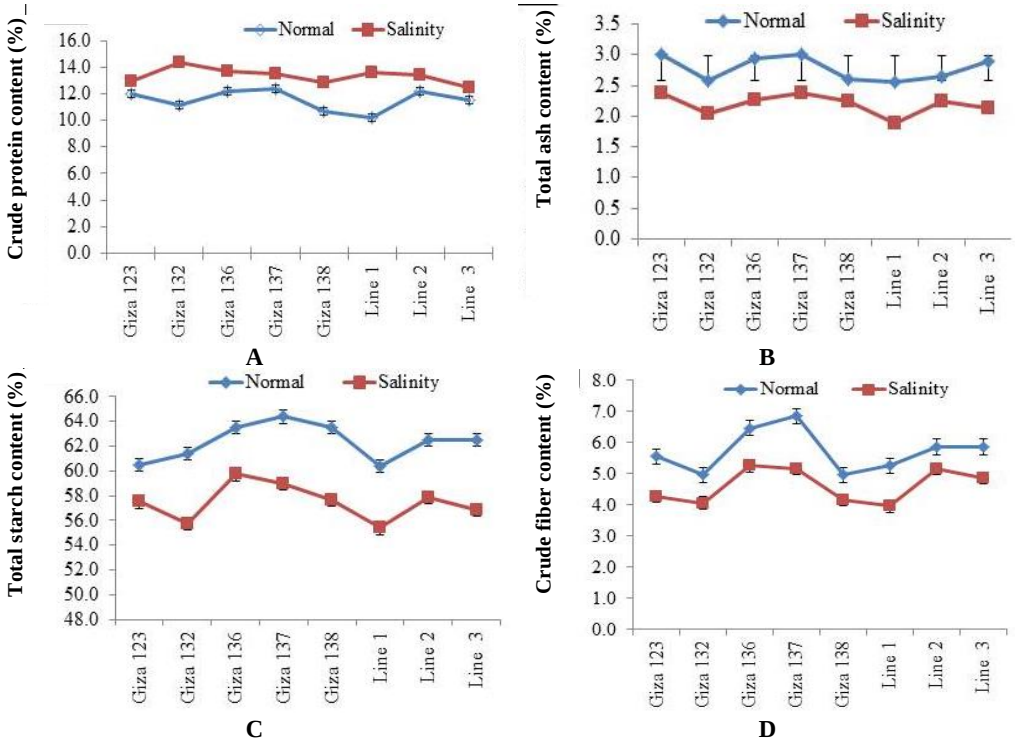

Figure 4. Effect of salinity stress on grain quality traits in the eight Barley genotype

Phenotypic traits under normal conditions

Phenotypic traits under salinity conditions

traits	Cha	Chb	RWC	K+	Na+	SCC	HD	PH	NGS	NT	TKW	GY	CPC	TAC	TSC	CFC
Cha	.711*	.789**	.731*	.883(**)	-.882(**)	-0.47	-.780(*)	.787(*)	.905(**)	.770(*)	0.27	.887(**)	.852(**)	0.00	.787(*)	0.24
Chb	0.651	0.67	.759*	.830(**)	-.625(*)	0.07	-.663(*)	.628(*)	0.54	.634(*)	0.09	0.54	0.55	0.36	0.41	0.07
RWC	0.52	0.561	.750(*)	.872(**)	-.747(*)	-0.37	-.784(*)	.727(*)	0.62	.841(**)	0.05	.694(*)	0.53	-0.03	0.31	0.35
K+	0.66	0.422	0.176	0.51	-.775(*)	-0.31	-.710(*)	.896(**)	.733(*)	.750(*)	0.04	.767(*)	0.61	0.19	0.51	0.40
Na+	-.857(**)	-0.615	-.867(**)	-.833(*)	.912(**)	.707(*)	.923(**)		-.819(**)	-.938(**)	-0.57	-.984(**)	-.822(**)	0.39	-0.59	-0.14
SCC	-0.55	-0.512	-.755(*)	-0.619	.796(*)	0.52	0.60	-0.45	-.647(*)	-.649(*)	-0.45	-.776(*)	-0.57	.759(*)	-0.44	-0.21
HD	-0.58	-0.483	-.714(*)	-.762(*)	0.697	0.457	0.63	-.623(*)	-.702(*)	-.978(**)	-0.45	-.869(**)	-.710(*)	0.35	-0.39	0.03
PH	0.67	0.316	.722(*)	0.687	-0.631	-0.685	-0.527	0.66	.712(*)	.680(*)	0.28	.810(**)	0.50	-0.06	0.53	0.55
NGS	0.773*	0.31	0.439	0.485	-.790(*)	-.795(*)	-.717(*)	0.469	.915(**)	.672(*)	0.21	.870(**)	.728(*)	-0.24	.897(**)	0.45
NT	0.606	0.404	0.407	0.531	-0.674	-0.476	-0.661	0.407	0.385	.810(*)	0.45	.888(**)	.728(*)	-0.36	0.34	0.02
TKW	-0.01	-0.223	0.057	-0.108	-0.445	-0.627	-0.423	0.174	0.072	0.446	0.70	0.53	0.47	-.635(*)	0.21	-0.29
GY	.859(**)	0.594	.748(*)	.862(**)	-.905(**)	-0.658	-.836(**)	.808(*)	.749(*)	.883(**)	0.309	.920(**)	.824(**)	-0.40	.664(*)	0.22
CPC	0.50	0.131	0.019	0.361	-0.251	-0.237	0.041	0.491	0.646	-0.055	-0.027	0.376	0.30	-0.16	.652(*)	-0.15
TAC	0.424	0.317	0.434	0.388	-0.583	-0.436	-0.62	0.246	0.18	0.693	0.415	0.538	.709(*)	-0.13	-0.13	-0.05
TSC	0.14	0.39	0.65	0.286	-0.314	-0.132	-0.596	0.043	0.018	0.615	-0.026	0.196	0.162	-0.123	-0.34	0.42
CFC	0.31	0.193	0.298	0.09	-0.479	-0.463	-0.659	-0.117	0.245	0.624	0.36	0.419	0.608	-0.387	0.042	-0.61

Figure 5. Pearson correlations between the sixteen phenotypic studied traits. Correlations under control conditions are above the diagonal, and correlations under saline conditions are below the diagonal. A heatmap colors these correlations: green indicates significant positive correlations, and red shows significant negative correlations. Within trait correlations between control and saline conditions are positioned on the diagonal alignment (yellow cells). All correlations are significant ($p < 0.01$(**) and $p < 0.05$(*), the abbreviation of traits were shown in Table 3

The Polymorphism Information Content (PIC) value of each SSRs marker ranged from 0.37 (Bmac 0009) to 0.85 (Bmag770), with an average value of 0.36. The outstanding six primer pairs (Bmag 770, Bmac 0209, EBmac 0701, Bmac 0316, Bmag0009, and EBmac 0603) generated clear fragment patterns with high polymorphism (100%). The SSR (Bmag 770) primer generates high marker efficiency indices such as Number of Alleles (NA), Number of Polymorphism Bands (NPB), Percentage of Polymorphism (PP%), polymorphism information content (PIC), effective multiplex ratio (EMR), resolving power (RP), discriminating power (DP) and marker index (MI) values were (4, 4,100 %, 0.85, 2.61, 2.75, 0.86 and 0.087) respectively.

Genetic similarity and cluster analysis

Genetic relationships among eight Barley genotypes based on fifteen SSR primers data were presented in a UPGMA cluster dendrogram (Figure 6). All genotypes are clearly grouped into three groups. The genetic similarity ranged from low genetic similarity GS 0.34 % between (Line 2 and Line 1) to 0.97 % between (Giza 137 and Giza 123) according to the Jaccard similarity index. Most tolerant and moderate genotypes were clustered in group I and II. On the other hand, the sensitive genotypes Giza 132 and Line1 were located in the III group with GS 0.72 % between them. High results of UPGMA cluster analysis were in agreement with field estimation, which indicated that these genotypes were closely related to each other. In general, this is imitated from their response to salt stress performance during the field evolution.

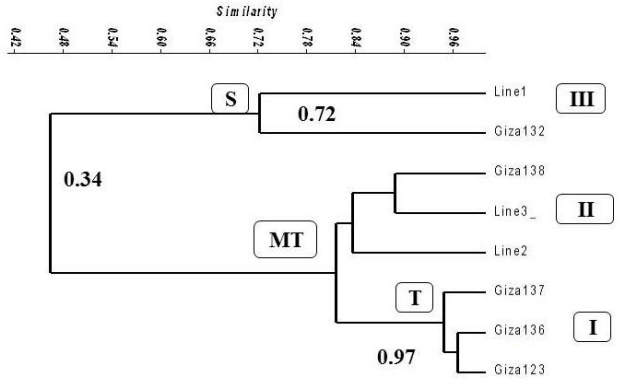

Figure 6. Genetic similarity and UPGMA cluster analysis among the eight Barley genotypes based on SSR markers due to their response to salinity stress. Our results were in agreement with several investigations having studied barley for salinity using SSR markers, such as (El-Akhdar et al. 2016; Mariey et al. 2016; Khatab et al. 2021; Mehta et al. 2021; Mariey et al. 2022a). They used SSR markers to investigate genetic diversity and genetic relationships among Barley genotypes for salt stress conditions, and they reported that the SSRs technique could consider a powerful tool for genetic studies in barley breeding for salinity stress

Relations between genotypes concerning phenotypic and genotypic data under salinity environment (GE)

Bi-plot analysis

Bi-plot was used to study the differences and). PCA-Biplot analysis was presented in a horizontal axis using 16 phenotypic traits and 15 SSR primers to design the relationship trend and categorize barley genotypes under soil salinity stress. The first and two principal components accounted for 97 % (PCA1= 83.5 % + PCA2 =13.5 %) of the total variability, which was graphically displayed in (Figure 7). The eight barley genotypes were divided into three groups. The salt-sensitive genotypes (Line 1 and 132) group was influenced by Na^+ and TSC located in the down left side (negative) of the horizontal axis according to their negative effect correlations with most other traits. Tolerant genotypes (Giza 123, Giza 137, and Giza 136) are more influenced by the molecular primers, and almost of phenotypic traits located on the right side (positive) of the horizontal axis according to their positive effect correlation with them under salinity stress. The modreted salt tolerance genotypes was influenced by HD which locted up left side (negative) of the horizontal axis according to their negative effect correlations with most all traits, but they gave modreted means performace for most of traits.

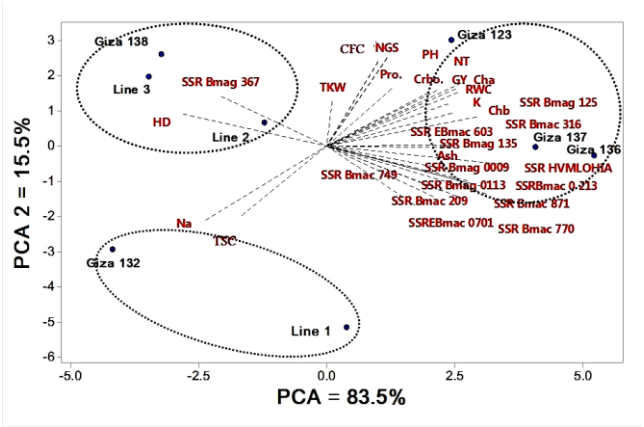

Figure 7. PCA biplot cluster tree illustrates the genetic distance between six-barley based on the analysis of 15 phenotypic traits and genotypic data using 15 SSR primers, all the abbreviation of traits (Tables 3 and 5)

Cluster heatmap

Multivariate heatmap clusters using Euclidean distance and average linkage by R software displayed the interaction between the phenotypic data cluster and molecular data clusters, as shown in (Figure 8). Row dendrograms show that the eight barley genotypes were clustered into two main clusters; the first cluster includes the salinity tolerant genotypes (Giza 123, Giza 136, and Giza 137). The second cluster is divided into two sub-clusters; the first sub consists of sensitive genotypes (Line 1 and Giza 132).

The second sub-cluster includes the moderated salinity divided into sub-sub clusters; moderated salinity (Line 3) and moderated tolerance (Giza 138 and Line 2). It also clearly demonstrates the effects of each field trait on the genotypes and the effects of each initiator molecule on the Egyptian barley genotypes. Column dendrograms show the 16 phenotypic traits and the 15 SSR primers.

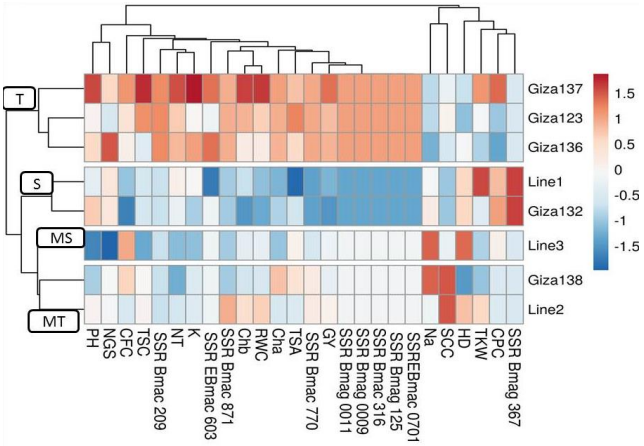

Figure 8. Multivariate heatmap illustrating the genetic diversity of eight Egyptian barley genotypes, based on the 15 SSR primers and 16 phenotypic using the module of a heatmap of ClustVis, all the abbreviation of traits (Table 3)

These results were in good harmony with (Al Lawati et al. 2021; Mariey et al. 2021; Pour-Aboughadareh et al. 2021; Mariey et al. 2022b) They used cluster analysis and principal PCA to classify the barley genotypes into genetic groups as an interaction between environment and genetics data.

The budgets for different barley genotypes under each soil type are shown in Tables 8-9. Although the results show that the total cost of producing different barley genotypes under saline-sodic soil conditions is lower than normal soil conditions, Giza 136 achieved the highest value of Net Return per hectare (NR) ($1780.46 & $927.40) under both normal and saline-sodic soil, respectively.

Table 8. Crop budgets under normal soil

Operation	Total costs ($)							
	Giza 123	**Giza 132**	**Giza 136**	**Giza 137**	**Giza 138**	**Line 1**	**Line 2**	**Line 3**
Grain Yield	2022.40	1408.00	2143.96	2016.00	1897.60	1312.00	1632.00	1525.86
Straw Yield	90.62	95.57	118.64	106.28	107.48	129.02	104.96	112.37
Total Revenue	**2113.020**	**1503.570**	**2262.600**	**2122.280**	**2005.030**	**1441.020**	**1736.960**	**1638.023**
Seeds	39.5	39.5	39.5	39.5	39.5	39.5	39.5	39.5
Land Preparation								
Plowing	25.41	25.41	25.41	25.41	25.41	25.41	25.41	25.41
Leveling	12.71	12.71	12.71	12.71	12.71	12.71	12.71	12.71
Fertilization								
Super Phosphate	7.62	7.62	7.62	7.62	7.62	7.62	7.62	7.62
N-Fertilizer	28.28	28.28	28.28	28.28	28.28	28.28	28.28	28.28
Labor	12.71	12.71	12.71	12.71	12.71	12.71	12.71	12.71
Weeds Control	8.26	8.26	8.26	8.26	8.26	8.26	8.26	8.26
Irrigation	25.41	25.41	25.41	25.41	25.41	25.41	25.41	25.41
Harvest								
Machine	27.45	27.45	27.45	27.45	27.45	27.45	27.45	27.45
Labor	8.89	8.89	8.89	8.89	8.89	8.89	8.89	8.89
Total Variable Costs	175.80	175.80	175.80	175.80	175.80	175.80	175.80	175.80
Fixed Costs								
Rent	285.89	285.89	285.89	285.89	285.89	285.89	285.89	285.89
Total Costs	**482.13**	**482.13**	**482.13**	**482.13**	**482.13**	**482.13**	**482.13**	**482.13**
Net Return	**1630.89**	**1021.43**	**1780.46**	**1640.14**	**1522.90**	**958.88**	**1254.82**	**1156.10**

Table 9. Crop budgets under saline soil

Operation	Total costs ($)							
	Giza 123	**Giza 132**	**Giza 136**	**Giza 137**	**Giza 138**	**Line 1**	**Line 2**	**Line 3**
Grain yield	1136.0	566.4	120.3	1145.6	850.67	608.0	705.43	780.8
Straw yield	85.85	109.08	101.33	96.06	83.21	102.82	55.20	36.91
Total revenue	**1221.85**	**675.84**	**1311.63**	**1241.66**	**933.88**	**710.82**	**760.63**	**817.71**
Seeds	47.40	47.40	47.40	47.40	47.40	47.40	47.40	47.40
Land preparation								
Plowing	25.41	25.41	25.41	25.41	25.41	25.41	25.41	25.41
Leveling	12.71	12.71	12.71	12.71	12.71	12.71	12.71	12.71
Fertilization								
Super phosphate	7.62	7.62	7.62	7.62	7.62	7.62	7.62	7.62
N-fertilizer	17.78	17.78	17.78	17.78	17.78	17.78	17.78	17.78
Labor	12.71	12.71	12.71	12.71	12.71	12.71	12.71	12.71
Weeds control	8.26	8.26	8.26	8.26	8.26	8.26	8.26	8.26
Irrigation	25.41	25.41	25.41	25.41	25.41	25.41	25.41	25.41
Harvest								
Machine	27.45	27.45	27.45	27.45	27.45	27.45	27.45	27.45
Labor	8.89	8.89	8.89	8.89	8.89	8.89	8.89	8.89
Total variable costs	193.64	193.64	193.64	193.64	193.64	193.64	193.64	193.64
Fixed costs								
Rent	190.60	190.60	190.60	190.60	190.60	190.60	190.60	190.60
Total costs	**384.24**	**384.24**	**384.24**	**384.24**	**384.24**	**384.24**	**384.24**	**384.24**
Net return	**837.61**	**291.24**	**927.40**	**857.43**	**549.64**	**326.58**	**376.39**	**433.47**

Note: Price of barley grains (0.32 $ per 1 Kg)

While the lowest value ($958.88&326.58) was obtained with the Barley genotype Line 1 under both normal and saline-sodic soil, respectively, these results are in great harmony with those obtained by (NDSU 2015; Hammami et al. 2020; Mariey et al. 2022a). They study the economic analysis of the effect of salinity stress on the crops, and they reported that in fields with moderately or so highly saline soils, salt-sensitive crops may no longer be viable as profitability is dramatically reduced.

In conclusion, high genetic differences among eight Egyptian barley genotypes under salinity stress conditions based on a comprehensive set of Agro-morph-physio-chemical parameters coupled with SSR markers and economic analyses were investigated in this study. Thus, these genetic differences among Egyptian Barley genotypes could be more efficient in assessing genetic relationships and classifying the eight for their ability to tolerate salinity stress in breeding programs to produce suitable cultivars at normal and salt stress condition. The results provide information about the ability of eight Barley genotypes for salinity tolerance which Giza 123,136 and 137 provided as salt tolerance genotypes, and two new lines (line 2 and line 3) beside Giza 138 provided as moderated salt tolerance genotypes could use as cultivar identification in further barley breeding programs for salt tolerant in Egypt.

A limited study uses agro-physiological and grain chemical composition with DNA markers under salinity stress for barley genotypes. Also, there is not much work for studying the **e**conomic analysis for Barley salt tolerance or do association among phenotypic parameters, genotypic markers, and economic analysis to improve barley genotypes salt tolerance and farmer income together.

As the results from our data, we could use the tolerance genotypes in future research as a good parent to get promising tolerant lines for salinity stress. Furthermore, using them in breeding programs for salinity and exploring the impact of salt tolerance genotypes on the economic value of the crop to increase farmer income in salt-affected areas.

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