Original Research Article

Comprehensive selection criteria for some barley genotypes under different water stress treatments

ABSTRACT

Nowadays, water scarcity is a great danger to agriculture development, in semiarid and arid regions beside climate change risks. Egypt is suffering from scarce water resources for agriculture; it is of high priority to rationalize irrigation water use. During two seasons, 2019/2020 and 2020/2021 the experimental were carried out to investigate the effect of three different irrigation water treatments (I_1 at 45, I_2 at 60 and I_3 at 75% depletion of available soil moisture) on some photosynthetic, agronomical, grain quality parameters, water productivity for eight barley genotypes. Also, classify them on the SSR molecular level. The results showed that there were high genetic variations found among the eight genotypes with significant responses to irrigation water treatments. Rationalize irrigation water from I_1 to I_3 had a negative effect on all studied phenotypic traits, whereas had an appositive effect on leaf diffusive resistance and cured protein content and inducement all genotypes to flower early by average increasing (27.7, 12.7 and 7.08%) respectively. Giza 138, Giza 131, and Line 4 gave high mean performance values of the measured characters besides, attaining high WP values were 1.14, 1.08, and 0.89 kg grain/m^3 applied water I_1, I_2, and I_3 irrigation treatments, respectively. While, WUE values increased with increasing water availability. Water use efficiency values were 1.92, 1.71, and 1.34 kg grain/m^3 consumed water for I_1, I_2, and I_3 irrigation treatments, respectively. Twenty-six alleles were generated using ten SSR primers with a mean value of 2.6 alleles per locus. The Polymorphism Information Content (PIC) value of each SSRs marker ranged from 0.33 (Bmag 0387) to 0.47 (Bmac 0167) with an average value of 0.34. Cluster analysis clustered the eight barley genotypes into two major clusters divided according to their response to water stress tolerance. The genetic information about eight barley genotypes for water stress tolerance was established, for use them in breeding programs in Egypt.

Keywords: *Hordeum vulgar, agro- physiological, grain quality, water productivity and SSR markers.*

1. INTRODUCTION

Irrigated agriculture will face important challenges in the coming decades and must feed, in a climate change context, a growing population with less soil and water resources. For this reason, it will be increasingly important to use water as efficiently as possible **[1]**. Moreover, Egypt has long dry summers and short relatively winters and also, upsurge in the population in the past few decades at a rate of increase of nearly 2.5%. This, along with the rapid growth of human activities, has caused substantial changes in the environment, sometimes in damaging ways **[2]**. One of the great challenges facing Egypt is how to use

scarce resources in an equitable and sustainable way. In Egypt, more than 85% of the water withdrawn from the Nile is used for irrigated agriculture. The strategy of government's plans for sustainable agricultural development and improving new land processes are dependent on saving water. The following themes are essential for agricultural development **[3].**

Barley (*Hordeum vulgare* L.) is a major cereal crop that is well adapted to several a biotic stresses in dry areas, it was found to be moderately tolerant to drought stress, due to it is the limited amount of water that is available for irrigation **[4] and [3].** In Egypt barley production is constrained by biotic and a biotic stress besides a lack of suitable genetic variability, therefore, the barley breeders need to increase the genetic variability through selecting new genotypes with high yield potential and more adaptability to climate change **[5].** Thus, to accelerate barley breeding program, abundant genetic resources and reliable screening to identify the real water stress (drought) tolerant barley genotypes are needed.

Drought stress has a major negative impact on photosynthesis, reducing the rate of CO_2 assimilation due to stomata closure and reduced chlorophyll content and leaf photochemistry **[6].** Since, chlorophyll fluorescence (*Fv/Fm* ratio) and chlorophyll which measured as SPAD reading its often used as effective, reliable, and reproducible diagnostic tools for high-throughput assessments of plant germplasm for drought tolerance **[7].**

Drought stress can hinder the accumulation of various seed constituents, primarily starch and proteins Drought is a crucial environmental factor that impacts the quality traits of barley. Stress during the grain-filling stage may cause reduced grain-filling which has a great effect on barley plants. This involves mobilization and transport processes required for importing various constituents, and many biochemical processes for the synthesis of proteins, carbohydrates and lipids in the developing seeds. It may also accelerate cell death, and earlier attainment of harvest maturity **[8].**

Phenotypic evaluation using comprehensive methods considered an essential step in plant-breeding programs for breeders to utilize plant genetic resources preserved in worldwide seed collections across. Water stress affects morphological and physiological processes in plants resulting in photosynthetic inhibition and reducing plant growth and production **[6].** Barley breeders are working to recognize the performance, effect of water stress on morph-physiological and biochemical production parameters and the reaction of water deficit stress on genotype as a critical step in any breeding program used in selecting plants with suitable genes and higher adaptability to water shortage as well as screening tolerant genotypes **[7].**

Assessment of genetic diversity using molecular markers is one of the primary and important steps in breeding programs **[9] and [4].** DNA markers are powerful tools for assessing genetic variation, Simple sequence repeats (SSRs) markers that are based on the polymerase chain reaction (PCR) have possessions like high-level polymorphism, co-dominant inheritance, high reproducibility, locus specificity and random distribution on the genome, which all of these advantages make SSR markers as a superior marker for evaluation of genetic diversity, genetic relationship and phylogenetic development. Using SSRs technique as a powerful tool for genetic studies in barley breeding for water stress has been frequently confirmed in several investigations **[4] and [10].**

The present study aimed to investigate the genetic diversity of eight Egyptian barley genotypes using some relative importance of some agro-physiological, grain quality traits and classify them on the SSR molecular level in order to provide genetic information for the future breeding programs for water stress to increase the production in the newly reclaimed lands under different irrigation system in Egypt.

2. MATERIAL AND METHODS

2.1. Experimental site description

2.1.1. Location

An experiment was conducted during the two consecutive barley-growing seasons of 2019/2020 and 2020/2021, in the lysimeter setup of Soil Improvement and Conservation Research Department at Sakha Agricultural Research Station, Kafer El-Sheikh Governorate. The site is located in the middle North Nile Delta area of Egypt (30°57' N latitude, 31°07' E longitude with an elevation of about 6 m above mean sea level).

2.1.2. Soil characters

Soil samples were collected from all plots before conducting the experiment from three consecutive depths, 0-20, 20-40 and 40-60 cm, to determine some physical and chemical characteristics. Chemical properties of soil samples were analyzed according to **[11].** Particle size distribution was determined according to **[12]**. Soil moisture characteristics were monitored using Time Domain Reflect meter (TDR) probe. Sampling analysis valuesare presented in Table (1).

Table 1.Some chemical, physical properties and soil moisture constants of the experimental soil before sowing.

Chemical properties											
Soil depth (cm)	pH*	ECe (dSm^{-1})*	ESP(%)*	Soluble cation (Meq L^{-1})				Soluble anion (Meq L^{-1})			
				Na^+	K^+	Ca^{+2}	Mg^{+2}	CO_3^-	HCO_3^-	Cl^-	SO_4^-
0-20	8.12	4.95	13.86	33.7	0.9	10.4	5.9	-	3.5	25.6	21.8
20-40	8.26	5.63	14.71	38.3	1.2	11.8	6.8	-	4.5	28.8	24.8
40-60	8.45	6.27	15.42	42.6	1.5	13.3	7.5	-	6.5	31.6	27.2

Physical and soil moisture constants								
Soil depth (cm)	Particle size distribution (%)			Texture class	Soil moisture characteristics (%)			Bulk density ($Mg\ m^{-3}$)
	Sand	Silt	Clay		FC*	PWP*	AW*	
0-20	16.23	30.62	53.15	clay	41.88	21.36	20.32	1.23
20-40	15.31	31.18	53.51	clay	40.15	20.84	19.31	1.28
40-60	13.16	31.78	54.46	clay	36.58	18.12	18.46	1.52

**Where pH: was determined in soil water suspension (1:2.5), EC: was determined in saturated soil paste extract; ESP = exchangeable sodium percentage, FC = field capacity, PWP = permanent wilting point and AW = available soil water.*

2.1.3. Agro meteorological data

For the two growing seasons, the average monthly weather data of air temperature (°C), relative humidity (RH.,%), wind speed (WS, km day^{-1} at 2 m height), rainfall (mm $month^{-1}$), and pan evaporation (mm day^{-1}) were recorded from weather station of Sakha Agro-meteorological Station, Kafr EL-Sheikh Governorate, Egypt (Table 2).

Table 2. Meteorological data at the experimental area during the two growing seasons of barely 2019/2020 and 2020/2021.

Month	**Temperature (°C)**		**RH (%)**		**WS**	**Pan evap.**	**Rainfall**
	Max.	**Min.**	**Max.**	**Min.**			
2019/2020							
Nov	27.4	25.1	82.8	48.3	36.6	2.31	-
Dec	21.4	13.4	86.9	58.9	38.5	2.66	60.68
Jan	18.4	11.8	86.7	62.7	30.0	2.09	67.50
Feb	20.4	12.7	84.6	56.5	51.0	1.83	14.30

March	22.6	15.6	81.1	53.9	80.1	5.12	60.8
April	26.0	18.9	80.0	45.1	98.8	6.08	---
Seasonal	22.70	16.25	83.68	54.23	55.83	3.35	203.28
2020/2021							
Nov	25.0	17.5	86.6	56.8	46.9	2.28	18.35
Dec	22.9	13.7	87.7	55.7	44.9	2.49	18.78
Jan	21.0	13.5	86.7	59.5	39.2	2.57	14.05
Feb	21.5	12.5	87.5	55.9	58.3	3.56	---
March	23.8	15.2	83.8	49.8	83.4	4.48	5.4
April	27.6	19.4	74.6	45.8	95.0	7.28	---
Seasonal	23.63	15.30	84.48	53.92	61.28	3.78	56.58

*****Pan evap.: Pan evaporation (mm day^{-1}); WS: Wind velocity, km d^{-1} at 2 m height.*
Source: Meteorological Station at Sakha Agricultural Research Station 31°07' N latitude, 30° 57' E longitude with an elevation of about 6 meters above mean sea level, total rainfall for two seasons by lysimeter area (15.36 m^2) = 0.95 m.

2.2. Experimental design and tested treatments

The experiment was conducted using two factors (i.e. irrigation and barley genotypes). The Complete Randomized Design (CRD) with three replicationswas used to implement the lysimeter experiment. The first factor was assigned for three water stress treatments (I_1 = irrigation at 45% depletion of available soil moisture, I_2 = irrigation at 60% depletion of available soil moisture, and I_3 = irrigation at 75% depletion of available soil moisture). The second factor included eight barley genotypes.

2.3. Cultural practices

2.3.1. Barley genotypes

Eight barley genotypes provided by Barley Research Department, Field Crops Research Institute, Agricultural Research Center, Egypt, at Sakha,were used in this study. Names, rows, types and pedigrees of the selected genotypes are shown in Table(3).

Table 3. Name, row type and pedigree of eight barley cultivars used in the experimental site.

No.	Name	Row type		Pedigree
1	Giza 129	naked	Six rows	DeirAlla 106/Cel//As46/Aths*2"
2	Giza 131	naked	Six rows	CM67B/CENTENO//CAMB/3/ROW906.73/4/GLORIABAR/ COME-B/5/FALCON BAR/6/LINO
3	Giza 137	Hulled	Six rows	Giza 118 /4/Rhn-03/3/Mr25-//Att//Mari/Aths*3-02
4	Giza 138	Hulled	Six rows	Acsad1164/3/Mari/Aths*2//M-Att-73-337-1/5/Aths/ lignee686 /3/DeirAlla 106//Sv.Asa/ Attiki /4/Cen/Bglo."S")
5	Line 1	Hulled	Six rows	C .C 89/3/Alanda/Hamra//Alanda-01
6	Line2	Hulled	Six rows	BLLU/PETUNIA1//CABUYA/3/Alanda// Lignee527 / Arar
7	Line 3	Hulless	Six rows	Giza 117/GIZA 126
8	Line 4	Hulless	Six rows	Giza 123/5/Furat 1/4/M-Att-73-337-1/3/Mari/Aths*2//Attiki

Barley genotypes were sown on the 22nd and 25thof Nov 2019 and 2020, respectively and harvested on the 28th and 30thof April 2020 and 2021, respectively. All local recommendation was followed to grow barley plants without any stress, except for irrigation treatments.

2.3.2. Lysimeter description

Lysimeters were divided into 3 groups, each group includes 24 lysimeters. Lysimeter has a cubic shape, with a surface area of 0.64 m^2 and a height of 0.6 m, and a filter (sand and gravel) of 0.1 m at the bottom. Each lysimeter was filled with 458.25 kg of the clay soilas shown in Figure (1).

Figure1. Lysimeter set used to conduct the experiment.

2.3.3. Measurements

2.3.3.1. Photosynthetic parameters

At the heading stage, Carbon dioxide rates (CO_2), and leaf diffusive resistance (LDR) were recorded using a portable porometer (steady-state porometer, LICOR, LI-1600, and Lincoln, NE, USA). The maximal photochemical efficiency of PSII was estimated by measuring the chlorophyll fluorescence as Fv/Fm ratio using an Optiscan OS-30P fluorometer (Opti-Science, Hudson, NH, USA). Total chlorophyll content was measured as a SPAD value determined using a chlorophyll meter (SPAD-502) Minolta Camera Co. ltd., Japan).

2.3.3.2. Agronomical parameters

At the heading stage, days to heading were recorded and at the harvest stage ten guarded plants were randomly taken from each plot to measure plant height (cm), number of tillers, number of grains $spike^{-1}$,1000 grain weight (g) and grain yield (g $lysimeter^{-1}$) and total grain yield were determined using the whole lysimeters(l) area (24X0.64= 15.36 m^2).

2.3.3.3. Grain composition traits

After harvest, grain samples were cleaned and grounded to fine powder to determine chemical composition, i.e. crude protein, fat%, ash content, crude fiber and total carbohydrates content, according to the procedures outlined by **[13].**

2.3.3.4. Soil-plant-water parameters

Applied irrigation water (AIW + rainfall), water productivity (WP), and water use effeciency (WUE) were calculated according to **[14].** It was calculated as follows:

Water use efficiency (WUE) = GY / WCU ($kg\ m^{-3}$)

Water productivity (WP) = GY / AIW ($kg\ m^{-3}$)

Where, AIW: Applied irrigation water ($m^3\ fed^{-1}$), GY: grain yield ($kg\ fed^{-1}$) and WCU: Total water consumptive use ($m^3\ fed^{-1}$).

2.3.3.5. Molecular markers

2.3.3.5.1. DNA Extraction and SSR - PCR Reaction

Genomic DNA was extracted from fresh leaves of eight barley genotypes according to the protocol of the Biosp in plant genomic DNA extraction Kit (Bio basic). Ten Microsatellite SSR primer pairs previously mapped and covered all seven barley chromosomes (Grain Genes database) were selected from the published genetic maps against eight Barley genotypes to identify their polymorphic markers. Polymerase chain reaction (PCR) amplification for SSR markers was prepared in a volume of 25 µl using 40 ng of genomic DNA, 2 µmoldNTPs, 25 m M of MgCl2, 10 pmol of each primer (forward and reverse), and a 0.5 µl of 5U of *Taq*polymerase and 12 µl of 10X PCR buffer. PCR was carried out as the following program; one cycle at 95 C for 5 min., then 35 cycles were performed as follows: 1 min. at 95 C for denaturation, 45 sec. at (45-55 C for annealing based on primer and 30 sec. at 72 C for extension, then incubated at 72 C for 7 min. Amplified products were separated using agarose gel electrophoresis (2%) in 0.5 x TBE buffer against 100 bp DNA Ladder.

2.3.4. Phenotypic data analysis

The data from the two seasons were statistically analyzed as the complete randomized design (CRD) model using the SPSS software. There is no significant interaction was found between year and treatment, thus, results were pooled across years **[15].** Fischer's protected least significant difference (LSD) at the 5% level of significance was used for treatment means. Pearson's correlation test was performed using the SPSS 22.0 version (SPPS Inc., Chicago, IL) to determine the relationship between every two studied traits.

2.3.5. SSR marker analysis

The amplified bands from SSR primers were scored as binary data under the heading of total scorable fragments which were determined for each genotype. The data were used to estimate the genetic similarity on the basis of a number of shared amplification products according to **[16].** Polymorphism information content (PIC) values were done to distinguish among genotypes for each primer according **[17].** Cluster analysis was performed to produce a dendrogram using an un-weighted pair-group method with arithmetical average (UPGMA) using the PAST program.

3. RESULTS AND DISCUSSION

3.1. Effects of different irrigation water requirements on the phenotypic traits

3.1.1. Analysis of variance (ANOVA)

The analysis of variance (ANOVA) of all studied traits including photosynthetic parameters

(CO_2, LDR, SPAD and *Fv/Fm*), agronomical traits (HD, PH, NT m^{-2}, NGS^{-1} and GY), grain quality traits (CPC, Ash and TCC) indicated a significant statistical effect ($P < 0.01$) by irrigation treatments (I), genotypes (G), and years (Y) as shown in (Tables 4 & 5). A significant two-way interaction between irrigation levels and genotypes (G X I) were observed for all studied traits expect number of grain $spike^{-1}$ (NGS^{-1}) were non-significant. While, the two-way interaction between years x irrigation levels (Y X I) and years x genotypes (Y X G) were non-significant across all traits. Likewise, the combined ANOVA indicated non-significant effect for three-ways interaction (G X I X Y) across all traits.

The results indicate that decreasing number of irrigation water (I_2 and I_3) caused a significant decrease in all measured traits, while caused a significant increasein LDR, HD and CPC as compared withthe traditional irrigation treatment (I_1). Also, significant differences were found among all the barley genotypes. Giza 138 was least effected by drought stress, whereas Line 1 gave the lowest number under water stress (Table 5).

Table 4. Effects of years, irrigation treatments, and barley genotypes on agronomical traitsand their interactions during two growing seasons.

Parameters	Agronomical traits				
	HD	**PH**	**TM**	**NGS^{-1}**	**GY**
Year					
2019/2020	80.7	85.1	86. 2	10.95	318.6
2020/2021	81.6	84.3	88.2	9.95	321.2
Irrigation treatments					
I_1	77.4	93.9	103.0	11.8	389.0
I_2	81.3	84.3	84.2	10.7	324.9
I_3	83.3	74.1	74.2	8.8	246.2
Barley genotypes					
Giza 129	82.7	74.7	76.3	8.1	250.1
Giza 131	80.7	87.7	87.4	10.8	325.9
Giza 137	81.0	89.3	91.3	10.5	321.8
Giza 138	78.7	90.7	97.3	11.8	354.6
Line 1	82.7	76.3	76.3	8.8	285.8
Line 2	82.3	79.3	77.3	9.2	315.9
Line 3	81.3	81.0	92.3	10.1	314.8
Line 4	79.7	82.7	88.7	11. 7	320.6
Analysis of variance F test					
Years	**	**	**	**	**
Genotypes	**	**	**	**	**
Irrigation	**	**	**	**	**
LDS 0.05					
Years	0.33	0.33	0.51	0.34	0.39
Genotypes	0.66	0.66	1.1	0.67	0.79
Irrigation	0.41	0.40	0.62	0.42	0.48
G X I	**	**	**	NS	**
G X Y	Ns	Ns	Ns	NS	Ns
I X Y	Ns	Ns	Ns	NS	Ns
GX I X Y	NS	NS	NS	NS	NS

*Which Ns, * and ** non-significant and significant at the 0.05 and 0.01 levels of probability, respectively, HD: days to heading, PH: plant height, TM: no of tillers m^2, no. of grain spike (NGS^{-1}), GY: grain yield (g $plot^{-1}$).*

Table 5.Effect of years, irrigation treatments, and barley genotypes on photosynthetic, and grain quality, irrigation and their interactions during two growing seasons.

Parameters	Photosynthetic parameters				Grain quality parameters		
	CO_2	LDR	SPAD	Fv/Fm	CPC%	Ash	TCC
Year							
2019/2020	57.3	20.8	46.3	0.606	11.6	3.2	78.1
2020/2021	58.5	21.5	45.9	0.591	11.2	3.5	77.6
Irrigation treatments							
I_1	60.8	18.5	46.9	0.713	10.6	3.6	79.6
I_2	60.0	20.8	42.3	0.684	12.0	3.3	78.2
I_3	52.3	24.6	33.5	0.401	12.7	2.9	76.9
Barley genotypes							
Giza 129	45.2	17.6	32.8	0.421	10.3	2.2	76.4
Giza 131	69.0	23.5	44.3	0.607	12.5	3.5	78.8
Giza 137	53.1	21.1	45.5	0.653	11.7	3.4	77.8
Giza 138	69.5	24.4	48.3	0.735	12.6	3.6	79.7
Line 1	42.6	17.1	42.5	0.457	11.0	2.9	75.6
Line 2	56.7	18.6	33.1	0.530	11.3	3.3	76.8
Line 3	66,1	23.4	41.4	0.583	11.7	3.1	77.3
Line 4	58.1	20.9	44.1	0.617	11.5	3.4	78.1
Analysis of variance F test							
Years	**	*	**	*	*	**	**
Genotypes	**	*	**	*	*	**	**
Irrigation	**	*	**	*	*	**	**
LDS 0.05							
Years	1.76	6.49	5.01	0.004	0.08	0.02	0.09
Genotypes	3.53	1.29	1.01	0.014	0.17	0.05	0.19
Irrigation	2.16	7.91	6.22	0.008	0.13	0.03	0.12
Interaction							
G X I	**	**	**	**	**	**	**
G X Y	Ns	Ns	Ns	Ns	Ns	Ns	Ns
I X Y	Ns	Ns	Ns	Ns	Ns	Ns	Ns
GX I X Y	NS	NS	NS	NS	NS	NS	NS

*Which Ns, * and ** non-significant and significant at the 0.05 and 0.01 levels of probability, respectively, CO2: Carbon dioxide rates, LDR: leaf diffusive resistance, Fv/Fm: Chlorophyll fluorescence, SPAD:Total chlorophyll content, CPC: crude protein content, AC: ash content, TCC: total carbohydrate content.*

3.1.2. The phenotypic mean performances

The average of mean performances and relative changes of all measured characters under three water irrigation treatments (I_1, I_2 and I_3) during two growing seasons, were calculated to investigate the phenotypic diversity of eight barely genotypes in order to define their response to water stress tolerance were presented in (Table 4,5 & Figure 2)

3.1.2.1. Agronomical traits

Reducing irrigation water by different levels (I_1= irrigation at 45% depletion of available soil moisture, I_2= irrigation at 60% depletion of available soil moisture, and I_3= irrigation at 75% depletion of available soil moisture) caused a significant decrease in PH, NG S^{-1}, TM, and GY with average reduction of 21.08, 27.08, 25.42 and 44.62% for the 75% depletion of available soil moisture (I_3) treatment as compared with irrigation at I_1: 45% depletion of available soil moisture (Table 4). Whereas, decreasing irrigation levels persuaded all barley genotypes to early flowering by an average 4.79 and 7.08% for the 60 and 75% depletion of available soil moisture (I_2 and I_3) treatments, respectively as compared

with irrigation at 45% depletion of available soil moisture (I_1) respectively. The results showed that the Egyptian barley cultivar Giza 138 had the highest average values of 90.7 cm, 97.3 t m^{-2}, 11.8 grain and 354.6 g/plot for HD, PH, TM, NGS^{-1} and GY traits, respectively. However, Line 1 and Giza 129 had the lowest average values for HD, PH, TM, NGS^{-1} and GY traits.

The results in (Figure 2 A&B) showed that the interaction between irrigation treatments and barley genotypes had a highly significant positive effect on HD (Fig 2.A) and negative effect GY (Fig 2. B) in both seasons. The results showed also that, I_1 with Giza 138 treatment gave the maximum values of HD and GY compared with I_2 and I_3 treatments in the two growing seasons.

3.1.2.2. Photosynthetic parameters

Water stress caused a significant decrease in Carbon dioxide rates (CO_2) as shown in) by average values (60.8, 60.0 and 58.8 rates) under (I_1, I_2 and I_3), respectively, with an average reduction (13.98%) when irrigation at 75% depletion of available soil moisture (I_3) as a compare by irrigation at 45% depletion of available soil moisture (I_1). The barley genotype Giza 138 recorded the highest CO_2 rate with an average value (69.5 rates), while the lowest CO_2 rate was recorded by barley genotype Line 1 (42.9 rates).

In the same trend, decreasing water irrigation significantly reduced both chlorophyll fluorescence CF (*Fv/Fm ratio*) and total chlorophyll content (SPAD reading) in all the eight Barley genotypes as the results showed in (Table 5) with average reduction (43.7 and 28.5%) when irrigation at 75% depletion of available soil moisture (I_3) as a compare by irrigation at 45% depletion of available soil moisture (I_1), respectively. Results in Table (5) showed that the Egyptian barley cultivar Giza 138 had the highest values of TCC (SPAD) and CF (*Fv/Fm ratio*) with an average values (48.3 SPAD and 0.735), respectively. On other hand, the Line1 had the lowest (SPAD) and CF (*Fv/Fmratio*) with average values (33.1 SPAD and 0.457) respectively.

Even though, water stress increased leaf diffusive resistanceLDR rates in all genotypes as shown in (Table 4) by an average increasing (24.7%) when irrigation at 75% depletion of available soil moisture (I_3) as a comparison by irrigation at 45% depletion of available soil moisture (I_1). The Egyptian barley cultivars Giza 138 and Giza 131 had the highest LDR average values (24.2 and 22.5 rates), however, Line 1 and Giza 129 had the lowest LDR with average values (17.1 and 17.6), respectively.

The results in (Figure 2, C&D) revealed that the interaction between irrigation treatments and barley cultivars had a highly significant positive effect on LDR (Fig2, C) and a negative effect on CO_2 (Fig 2, D) in both seasons. The results showed that I_1 with Giza 138 treatments gave the maximum values of CO_2 and LDR rate under (I_1, I_2 and I_3) across the two growing seasons.

3.1.2. 3. Grain quality characteristics

The effect of water stress on grain protein content, ash%, and total carbohydrate is displayed in (Table 5). Grain protein was increased by increasing water stress whereas grain protein increased from 10.63 to 12.67% by increasing water stress from I_1 to I_3 in a combined analysis. The maximum, ash% and carbohydrate observed at (I_1) irrigation was (3.48% and 78.34%), respectively. While, the minimum ash% and carbohydrate content (2.94% and 75.91%) was recorded at (I_3) irrigation, respectively in the combined analysis.

The results in Figure (2, E&F) showed that the interaction between irrigation treatments and barley genotypes had a highly significant positive effect on CPC (Fig 2, E) and a negative effect on TCC (Fig 2, F) in both seasons. The results showed that I_1 with Giza 138 treatment gave the maximum values of CPC and TCC rate under (I_1, I_2 and I_3) across the two growing seasons.

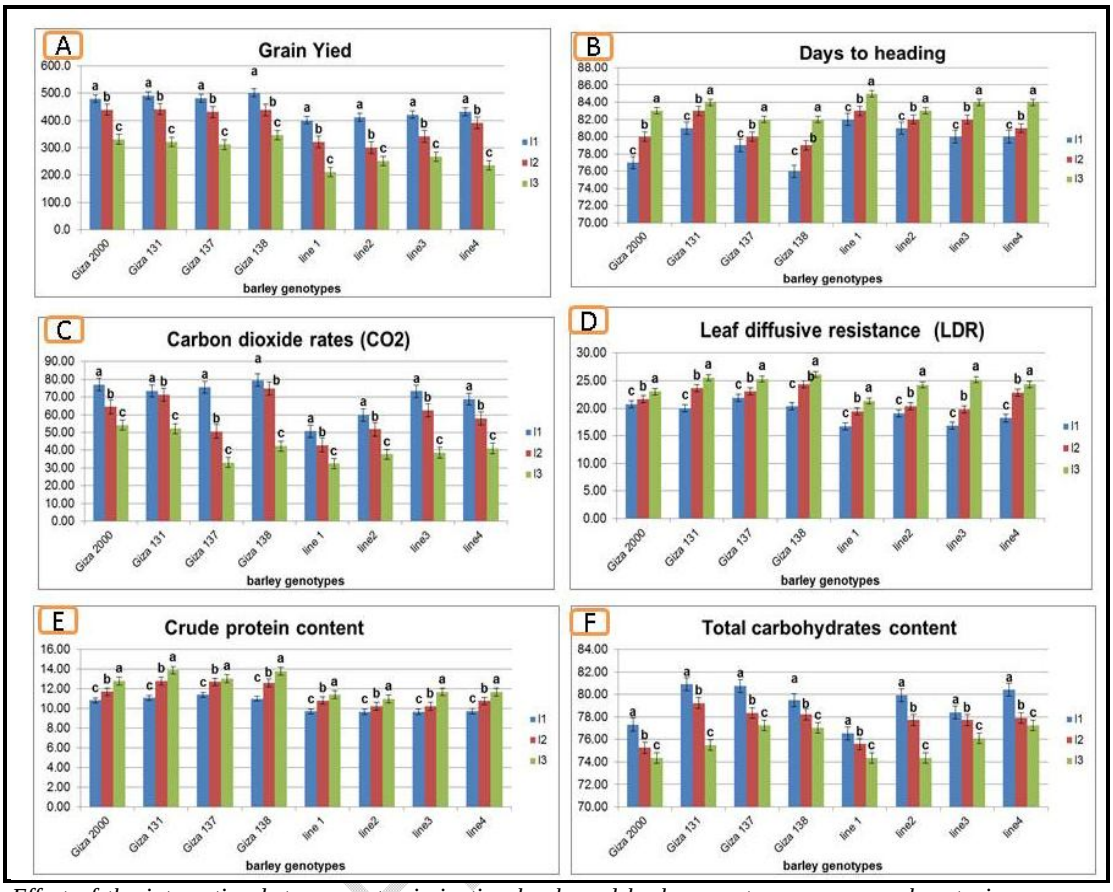

Figure 2: Effect of the interaction between water irrigation levels and barley genotypes on some phenotypic studied traits (combined analysis of two seasons).

3.1.2.4. Irrigation parameters

Results in Table 6a show that both applied irrigation water (AIW) and water consumptive use (WCU) values increased with applying water during short period (i.e. I_1 treatment, since irrigation occur when 45% of the available water depletes). The 2-year average AIW values were 2248, 1982, and 1818 m^3/fed for I_1, I_2, and I_3 irrigation treatments, respectively. Results show also that more water was consumed with increasing water availability. The 2-year average WCU values were 1332, 1250, and 1208 m^3/fed for I I_1, I_2, and I_3 irrigation treatments, respectively. Results agree with **[18].**

Results in Table 6b show that, WUE and WP values increased with increasing water availability. Water use efficiency values were 1.92, 1.71, and 1.34 kg grain/m^3 consumed water for I_1, I_2, and I_3 irrigation treatments, respectively. While, water productivity values were 1.14, 1.08, and 0.89 kg grain/m^3 applied water I_1, I_2, and I_3 irrigation treatments, respectively. Results revealed also that, the highest values of WUE (1.84 kg grain/m^3 consumed water) and WP (1.15 kg grain/m^3 applied water) were recorded for Giza 138 genotype, while the lowest values of WUE (1.3 kg grain/m^3 consumed water) and WP (0.81 kg grain/m^3 applied water) were recorded for Giza 129 genotype. Results agree with **[19] and [18].**

Table 6a. Effect of irrigation treatments on amounts of applied irrigation water (AIW) and water consumptive use (WCU) during the two growing seasons.

Treatment	2019/2020	2020/2021	2-year average

	AIW (m^3/fed)	WCU (m^3/fed)	AIW (m^3/fed)	WCU (m^3/fed)	AIW (m^3/fed)	WCU (m^3/fed)
I_1	2323.1	1397.8	2172.2	1266.6	2248	1332
I_2	2014.7	1273.1	1949.1	1227.2	1982	1250
I_3	1850.6	1227.2	1785.0	1187.8	1818	1208
Average	2062.8	1299.4	1968.8	1227.2	2016	1263

Table 6b: Effect of irrigation treatments on water use efficiency (WUE) and water productivity (WP) of barley genotypes.

Parameter	GY (g/Lysimeter)	GY(kg/fed)	WUE (kg/m^3)	WP (kg/m^3)
Year				
2019/2020	318.6	2090.8	1.61	1.01
2020/2021	321.2	2107.9	1.72	1.07
Irrigation				
I_1	389.0	2552.8	1.92	1.14
I_2	324.9	2132.2	1.71	1.08
I_3	246.2	1615.7	1.34	0.89
Genotypes				
Giza 129	250.1	1641.3	1.30	0.81
Giza 131	325.9	2138.7	1.69	1.06
Giza 137	321.8	2111.8	1.67	1.05
Giza 138	354.6	2327.1	1.84	1.15
Line 1	285.8	1875.6	1.48	0.93
Line 2	315.9	2073.1	1.64	1.03
Line 3	314.8	2065.9	1.64	1.02
Line 4	320.6	2103.9	1.67	1.04

3.1.3. Pearson Correlation coefficients

Pearson correlation coefficient among all studied phenotypic traits through the three water irrigation treatments was done to understand the relationships among all studied traits; Data indicated clearly that the Pearson correlation coefficients between grain yield and all phenotypic traits were highly positive and significantly correlated. While days to heading hadhigh negative and significantlycorrelated with all phenotypic traits as shown in (Figure 3).

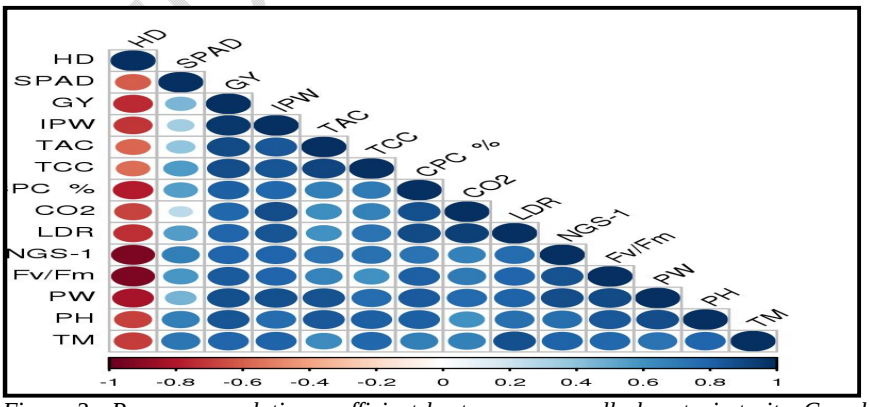

Figure 3. Pearson correlation coefficient heatmap among all phenotypic traits. Correlation key and the scale reads, red circle indicted negative correlation, blue circle indicted positive correlation, smaller circle indicted lesser significance; bigger circle indicted greater significance. The size of the circle is relative to the correlation

coefficients, CO2: Carbon dioxide rates, LDR: leaf diffusive resistance, Fv/Fm: Chlorophyll fluorescence, SPAD: Total chlorophyll content, HD: days to heading, PH: plant height, TM: no of tillers m^2, NGS^{-1}, no. of grain spike , GY: grain yield, CPC: crude protein content, AC: ash content, TCC: total carbohydrate content

3.1.4. Molecular Markers data analysis

3.1.4.1. SSR marker analysis

Ten SSR primer pairs were screened to differentiate eight Egyptian barley genotypes for water stress tolerance in and to detect polymorphic markers used as a marker assisted selection to accelerate barley breeding programs. Out of ten primers, two primers showed monomorphic fragment profiles as one markers were Bmag 0 213 (1H) and Bmag 0853 (7H). On the other hand, primers Bmac 0096 (5H, Figure 4), and EBmac 0755(7H) generate polymorphic with two bands. Four primers produced three markers Bmag 0125 (2H, Figure4), (GBM1045 (3H, Figure4), EBmag 0701 (4H) and Bmag 0378 (5H, Figure 4). The remained two SSR primer Bmac0018 (6H) and Bmac 0167 (7H) produced four bands.

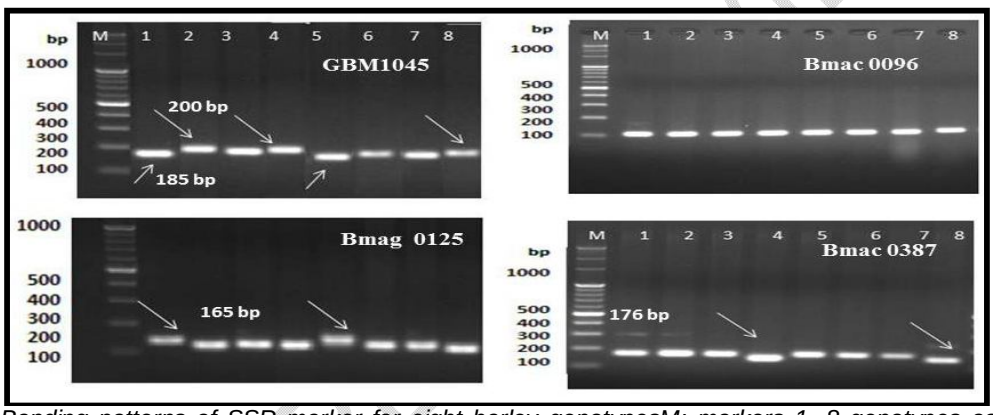

Figure 4.Banding patterns of SSR marker for eight barley genotypesM: markers 1- 8 genotypes as shown in Table (1).

Twenty-six alleles were amplified as a result of fingerprinting ten SSR primers ranging from one to four alleles with a mean value of 2.6 alleles per locus as shown in (Table 7). The Polymorphism Information Content (PIC) value of each SSRs marker ranged from 0.37 (Bmag 0387) to 0.47 (Bmac0167) with an average value of 0.34 (Table 7). The SSR (Bmac 0167 and GBM 1045) primer generates high marker efficiency indices such as the number of alleles (NA), number of polymorphism bands (NPB), percentage of polymorphism (PP%), polymorphism information content (PIC), effective multiplex ratio (EMR), and marker index MI values. The primers with a high value of PIC were sufficient to differentiate all of the studied genotypes.

Table 7.The marker efficiency indices of multiplexing sets of the used 10 SSR primers.

	name	Sequence	L. Ch	NA	NPP	PIC	PPP%	DI	MI	EMR
1	Bmac0213	F. ATGGATGCAAGACCAAAC R:CTATGAGAGGTAGAGCAGCC:	1H	1	0	0.00	0.0	0.0	0.0	1.0
2	Bmag0125	F:AATTAGCGAGAACAAAATCAC R:AGATAACGATGCACCAC	2 H	3	2	0.37	66.7	0.36	0.45	1.6
3	Bmag0853	F:ACAAGTATCCTGCAAACCTAA R: CGACCTTCTTAATGGTTAGTG	3H	1	0	0.00	0.00	0.0	0.0	1.0
4	GBM1045	F- TACACGCACTGAAAAGACGG R- CTCGCTGCTGAGTTTGTCTG	3H	3	3	0.45	100	0.57	0.57	1.24
5	EBmac0701	F:ATGATGAGAACTCTTCACCC R:TGGCACTAAAGCAAAAGAC	4 H	3	3	0.46	100	0.62	0.46	2.0
6	Bmag0387	F:CGATGACCATTGTATTGAAG R:CTCATGTTGATGTGTGGTTAG	5H	3	2	0.33	0.39	0.57	0.47	1.12
7	Bmac00	F:GCTATGGCGTACTATGTATGGTTG	5H	2	2	0.46	100	0.55	0.62	1.3

	96	R: TCACGATGAGGTATGATCAAAGA								
8	Bmac 0018	F: GTCCTTTACGCATGAACCGT R ACATACGCCAGACTCGTGTG	6H	4	3	0.44	0.75	0.57	0.57	1.75
9	EBmac0 755	F:AGCCTTGTGTATCAGGACA R:CTGCTGGTGTTCTCTAAAAGT	7H	2	1	0.41	0.50	0.38	0.38	0.75
10	Bmac01 67	F: CATTTCCACTTCAAAATATCC R: CCAAAGTTTGAGTGCAGAC	7H	4	4	0.47	100	0.58	0.62	2.0
	Average			2.6	2.0	0.34 3	468	4.48	0.386	1.36
	Total			26	20	3.43	46.8	0.448	3.86	13.6

Which L. Ch: location on chromosome, NA: number of alleles, NPB: number of polymorphic bands, PPP%: polymorphism (%); PIC: polymorphism information content; DI diversity index; ratio MI: marker index and EMR: effective multiplex

3.1.4.2. Cluster analysis

Genetic relationships among eight barley genotypes based on ten SSR primers, data were presented in a UPGMA cluster dendrogram (Figure 5). All genotypes clearly grouped into main two clusters according to the Jaccard similarity index. The first cluster (I) is divided into two clusters, the first one consisted of three tolerant (T) cultivars (Giza 131, Giza 138 and Line 4) and the second sub-cluster includes the moderate tolerance (MT), (Giza 137 and Line 3). Whereas the second cluster divided into two clusters the first one consisted of sensitive cultivars (S) such as (Giza 129 and Line 1) and the other cluster was included moderate water stress sensitive cultivars (MS) such as (Line 2).

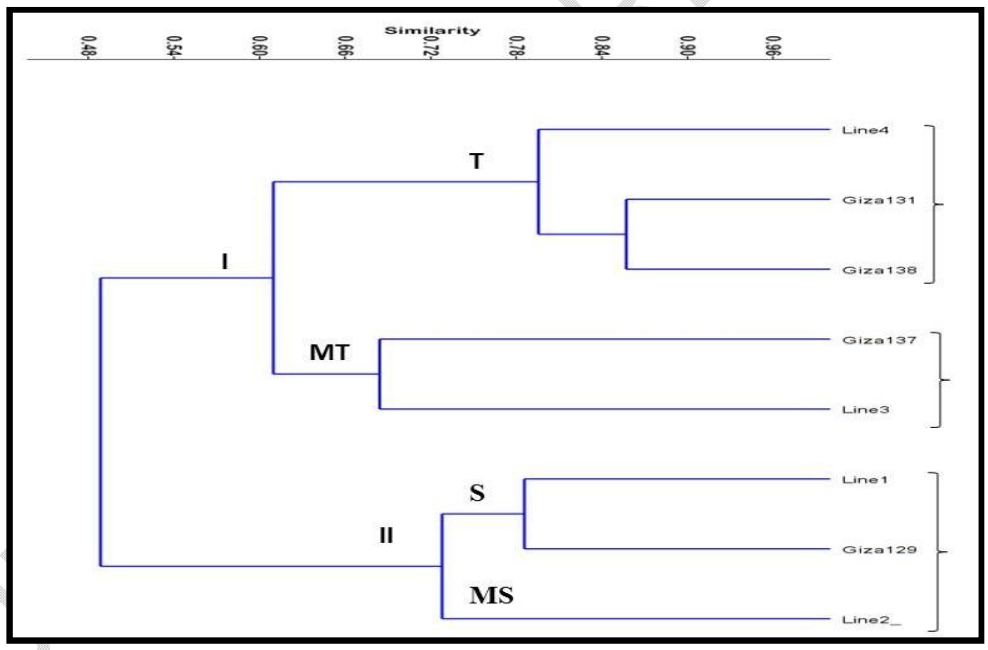

Figure 5.UPGMA Cluster analysis, among the eight barley genotypes based on ten SSR markers due their response to water stress.

3.1.4.3. Genetic similarity coefficient matrix

Genetic similarity coefficient matrix (Table 8) was established by simple matching coefficient using the data generated by the ten expressing primers. These primers enabled us to study the genetic diversity among all the Egyptian barley genotypes for water stress tolerance. The genetic similarity coefficient matrix showed more relation and close in most tolerant cultivars and they are more diverged than other sensitive genotypes. The genetic similarity ranged from low similarity GSC=(0.37) were found between Giza138 and Line 1

which proposes that these were the least-related genotypes to high similarity GCS=(0.87) were found between Giza 138 and Line 4, also the high GSC=(0.85) was observed between Giza 131 and Giza 138 , indicating that it was a very close relationship among these barley genotypes.

Table 8.Genetic similarity coefficient matrix for ten SSR primers studied eighty Egyptian barley cultivars for water stress conditions.

Cultivar	Giza 129	Giza 137	Giza 138	Giza 131	Line 1	Line 2	Line 3	Line 4
Giza 129	1.00							
Giza 137	0.39	1.00						
Giza 138	0.39	0.73	1.00					
Giza 131	0.44	0.80	0.85	1.00				
Line 1	0.80	0.47	0.37	0.47	1.00			
Line 2	0.53	0.69	0.68	0.79	0.75	1.00		
Line 3	0.60	0.67	0.77	0.67	0.52	0.80	1.00	
Line 4	0.56	0.56	0.87	0.74	0.47	0.69	0.75	1.00

4. DISCUSSION

Water stress negatively affects photosynthesis parameters, in this study, different irrigation water treatments caused a significant reduction inCO_2 concentration among eight barley genotypes, in which CO_2 and H_2O inside the chloroplast of plant cells were responsible for creating sugars and O_2 the presence of light, so the decline in CO2 conductance will follow be stomatal closing **[7].** Also different irrigation water treatments caused a significant reduction in chlorophyll content as a result of photo-oxidation caused by reactive oxygen species (ROS) and reduced the chlorophyll fluorescence *Fv/Fm* ratio which is considered one of the sensitive indicators of the severity of drought stress. *Fv/Fm* values were reduced in all genotypes under water stress conditions, but tolerant genotypes Giza 138. Giza 131 and Line 1 conserved higher CO_2, SPAD reading and *Fv/Fm* **[6].** However, water deficit caused a significant increase in leaf diffusive resistanceby average increase (24.7%) among all genotypes which LDR has been widely used as an indicator of stomata response to environmental conditions and water stress in many plant species **[20].**

In this regard, water stress during the grain-filling period reduced grain yield by decreasing the number of tillers m^{-2} and 1000 grain weight, as it is a more critical phase and results in substantial yield losses. **[9]; [4] and 7]** they reported thatwater stress treatments imposed at different growth stages reduced significantly the grain yield and yield components. While, water deficit accelerated the flowering in dehydration-avoidant plants under (I_2 and I_3) irrigated treatment.

About the effect of water stress on grain quality parameters**, [21]** found that Barley grain protein contents were unaffected at different water stress, but ash content and total starch content displayed a gradual decline with the increase in water **[8]** reported that water deficit affected protein, carbohydrate, lipid and ash contents, with an increase in the protein content and a reduction in the carbohydrate, oil and ash contents in wheat.In general, Giza 138 was the highest in grain protein (12.57%) and the higher ash (3.46%) while Giza 131 produced the highest carbohydrate content (78.78%).

Results indicate that the highest values of WUE (1.84 kg grain/m^3 consumed water) and WP (1.15 kg grain/m^3 applied water) were recorded for Giza 138 genotype, while the lowest values of WUE (1.3 kg grain/m^3 consumed water) and WP (0.81 kg grain/m^3 applied water) were recorded for Giza 129 genotype. Results agree with **[3]; [19] and [18].**

SSR markers were used in this study to screen eight barley genotypes for water stress tolerance. Twenty-six alleles were produced as a result of fingerprinting ten SSR primers ranging from one to four alleles per locus with an average value of 2.6 alleles per

locus. UPGMA methods were used to represent the relationship among all eight genotypes which classified them into two separate major groups. The first group was divided into two clusters including tolerant and moderate genotypes, whereas, the second group was divided in two clusters consisting of sensitive and moderate water stress sensitivity. Several studies have been using SSR markers in order to study the genetic diversity in barley for water stress) **[4] and [10]** they reported that the SSRs technique could consider as a powerful tool for genetic studies in barley breeding for drought stress. In the current study, SSRs analysis showed that this technique was informative in a range of barley germplasm which agreed with our morphological, physiological and grain quality data, which provided us useful information on the level of polymorphism and diversity in the eight barley genotypes tolerant to water stress.These results showed a clear cut to differentiate the studied barley of genotypes and selection criteria for chosen character, which could be of unlimited value in barley breeding programs for developing water stress tolerant.

5. CONCLUSION

High genetic differences among eight Egyptian barley genotypes irrigated by different water treatments based on a comprehensive set selection morphological, physiological and grain quality coupled with SSR markers were established in this study. Therefore, these genetic modifications among barley genotypes could be more powerful to assess genetic relationships and classify the eight barley genotypes for their ability for water stress tolerance in breeding programs to produce suitable genotypes at water stress conditions in order to increase the crop production and rationalize water to the newly reclaimed land.

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