Egypt. J. Plant Breed., 23(3):183-197 (2019)

BIOCHEMICAL AND MOLECULAR GENETIC MARKERS ASSOCIATED WITH SALT STRESS TOLERANCE IN EGYPTIAN BARLEY CULTIVARS

Samah. A. Mariey[1], Karima R. Ahmed[1], A.M.E. Agwa[1] , Mona A. Farid[2] and Ahmed M. Serag[3]

1-Barley Res. Dep., Field Crops Research Institute, Agricultural Res. Center, Giza-12619, Egypt
2- Department of Genetics, Faculty of Agriculture, Kafr elsheikh University, 33516, Egypt
3-Department of Genetics and Genetic Engineering, Faculty of Agriculture, Benha University

ABSTRACT

To identify salinity stress response and evaluate 15 Egyptian barley cultivars, two years field screening was carried out. During consecutive seasons 2015/2016 and 2016/2017 under two environments at Sakha farm (normal soil) and EL-Hamrowy farm as (saline soil). There were significant differences among all cultivars under study with respect to all traits. Moreover, the results revealed that the seed germination and seedling traits were decreased under salinity stress. Relative water content (RWC) significantly reduced under salinity stress for all cultivars. High proline content was recorded by Giza 136 (0.87 and 2.87mg/g) under control and salt stress respectively. Cultivars Giza 131, Giza 123 and Giza 136 had the best performance for grain yield under normal and salt conditions, and exhibited highly significant differences for all the salt tolerance indices. The SDS–PAGE revealed that the soluble protein accumulation increased in cultivars under control more that under salinity stress. 24 polymorphic bands were detected in all cultivars based on their gene expression in seedling under salinity and control with molecular weight ranging from 10 to 250 KDa. Seven SRAP combination primers were used to assess the genetic diversity among all cultivars. The primers showed high average percentage of polymorphic loci was 87.4 %. Highest PIC was related to primer me5+em5 was (0.94) indicating that this primer is highly informative. The dendrogram of SRAP markers had clustered all the Egyptian cultivars into four groups each group include the most closed cultivars together with genetic similarity coefficients (GSC) ranging from (0.64) to (0.92). The results of the present study showed that there were high genetic differences among Egyptian barley cultivars for salt tolerance which provide new information about the relationships among Egyptian barley cultivars which are useful for cultivar identification and for their utilization in further barley salt breeding programs

Key words*: Barley, SDS-PAGE, SRAP, Salt stress index, agronomical and physiological traits*

INTRODUCTION

Soil salinity is a main factor affects the growth and yield of plants in many areas in the world. In Egypt there are about two million feddans of the irrigated land adversely affected by the accumulation of salt. Salinity stress is a complex trait controlled by a large number of genes which make them elusive to selection for tolerant by conventional breeding programs **(Abo-Elenin *et al.*, 1981).** Barley (*Hordeum vulgare* L.) 2n=2x =14 is a crop with a great adaptation potential in many regions of the world It is an important crop. It is one of the most economic and important cereals grown under saline soils.

There are many tools for improving salt tolerance in barley such as morphological selection which was well-organized in breeding for salt stress (**Ahmed *et al.*, 2003**). Physiological markers are useful in selection different cultivars of barley for their salt tolerance during breeding programs (**Araus *et al*., 2008)**. Biochemical SDS-PAGE markers based on protein electrophoresis is used to understand the genetic basis of environmental stress in plants through changes in the patterns of proteins expressed. In barley (**Hellal *et al.*, 2017 and Samah *et al*., 2018**) used SDS-PAGE to identified the gene expression for salt tolerance in barley genotypes.

Conversely most of these tools were shortened for some stages of plant growth and might be exaggerated by environment stress (**Massood *et al.*, 2003**). Consequently, breeder looks for other tools to help them directly in evaluation the genetic variation among genotypes without environmental factors effects, such as molecular genetic markers. Molecular markers were used to evaluate genetic diversity through assessment of a theoretically unlimited number of polymorphic marker loci (**Nguyen *et al.*, 2004**).

Many molecular marker techniques were used to evaluate the extent of genetic diversity. Among these markers Sequence related amplified polymorphism (SRAP) adapted by **Li and Quiros (2001**). SRAP marker is a powerful technique for the assessment of genetic variability because it has shown a high degree of reproducibility and discriminatory power, as well as a high polymorphism rate in genetic studies. In barley, SRAP marker has been successfully used to evaluate the genetic diversity among the barley genotypes (**Yang *et al.*, 2008 and 2010 and Mariey *et al.*, 2017)**. Thus the objectives of present study were to investigate genetic diversity among 15 Egyptian barley cultivars for salinity tolerance using some agronomical, physiological traits, biochemical and SRAP markers in order to provide genetic information for future breeding program for salinity tolerant in barley.

MATERIALS AND METHODS

Field experiments:

Fifteen Egyptians barley cultivars were kindly provided by Sakha Barley Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt, were used in this study their names and pedigree shown in (Table 1).

These cultivars were planted in two environments (Sakha non-saline and EL-Hamrwy saline soil) during two season 2015/2016 and 2016/2017, planted in a randomized complete block design (RCBD) with three replicates using (plot area = 1.6 m2) for each plot. The measured traits were plant height, peduncle length, number of spikes m^2 and grain yield.

Soil samples:

Soil samples were taken before land preparation in two depth from the soil surface; i.e. 0-15 cm and 15-30 cm. The chemical analysis of experimental sites in the first and second seasons, respectively were presented (Table 2), were analyzed according to **Black *et al.* (1965).**

Table (1): Name, and row type and pedigree of 19 barley cultivars used in the field experimental

No.	Name	Type	Row	Pedigree	Year of released
1	Giza 123	Hulled	Six	Giza 117/FAO 86	1988
2	Giza 124	Hulled	Six	Giza 117/Bahteem 52// Giza 118/FAO 86	1995
3	Giza 125	Hulled	Six	Giza117 / Bahteem52// Giza118 /FAO86(sister line to G.124	1995
4	Giza 126	Hulled	Six	Baladi Bahteem/S D729-Por12762-BC.	1995
5	Giza 127	Hulled	Two	W12291/B0gs//Hamal-02	1996
6	Giza 128	Hulled	Two	W12291/4/11012-2170-22425/3/"Apam"/"B65"//"A16"	1996
7	Giza 129	Hulless	six	Deir Alla 106/Cel//As46/Aths*2"	2001
8	Giza 130	Hulless	six	Comp.cross"229//Bco.Mr./DZ02391/3/Deir Alla 106	2001
9	Giza 131	Hulless	six	CM67B/CENTENO//CAMB/3/ROW906.73/4/GLORIABAR/ COME-B/5/FALCON BAR/6/LINO	2001
10	Giza 132	Hulled	Six	Rihane-05//AS 46/Aths*2Athe/ Lignee 686	2006
11	Giza 133	Hulled	Six	ICB91-0343-0AP-0AP-0AP-281AP-0AP	2011
12	Giza 134	Hulled	Six	ICB91-0343-0AP-0AP-0AP-289AP-0AP	2011
13	Giza 135	Hulless	six	ZARZA/BERMEJO/4/DS4931//GLORIABAR/COPAL/3/SEN/ 5/AYAROS	2011
14	Giza 136	Hulless	six	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	2011
15	Giza 2000	Hulled	Six	Giza117/Bahteem52// Giza118/ FAO86 / 3/Baladi16/ Gem	2003

Table (2): Chemical properties of soil samples from the field experiments site during, 2015/16 and 2016/17.

Chemical properties	2015/ 2016		2016/2017	
	Sakha	El Hamrowy	Sakha	El Hamrawy
pH	8.1	8.3	8	8.4
ECe ($dsm-^{1}$)	4.0	10.5	4.7	11.7
$CaCO_3$ %	0	0.73	0	0.88
Soil Paste	8.6	27.6	8.5	26.3
Sodium Absorpation Ratio (SAR)	-	12.45	-	14.77
Soluble cations $meq100^{1}$ g soil				
Ca^{++}	4.9	8.8	4.8	10.7
Mg^{++}	3.5	16.5	5.9	14.7
Na^{++}	15.6	55.5	14.9	65.6
K^{+}	0.2	0.75	0.5	0.6
Soluble anions $meq100^{-1}$ g soil				
SO_4	18.2	76	7.1	٧٨
Cl^{-}	11.2	٢٠,١	10.3	21.9
HCO_3	5.5	5.5	5.3	5.6
CO_3	-	0.73	-	0.81

Laboratory experiments:

Germination growth conditions:

Germination was carried out at growth chamber of plant breeding and biotechnology laboratory, Barley Dep., Sakha Station. Fifteen Egyptian barley cultivars were grown in incubator was (20-25 ^{o}C, relative humidity of 55-60% and 16 hours light period) under two levels of electrical conductivities ECw (C (control) tap water 0.6 dSm^{-1} and S= 10 dSm^{-1} arranged in a factorial design with 3 replications as completely randomized design (CRD), to study the effect of salinity stress on germination percentage and vigorous seedling.

Physiological traits:

Relative water content (RWC) was calculated as described by **Sumithra *et al.* (2006**). Proline content was determined according to **Bates *et al.* (1973**).

Biochemical makers (SDS-protein electrophoresis*):*

Young fresh leaves were ground in sucrose 20% and centrifuged at 10000 rpm for 10 min. SDS-PAGE Gel Electrophoresis was carried out according to **Laemmli (1970**).

DNA Extraction and SRAP – PCR Amplification

Genomic DNA was extracted from leaves using CTAB method according **Doyle and Doyle (1990)** DNA concentration was measured using Nanoodrop (ND-1000 Spectrophotometer). PCR cycling was carried out as the following program; initial denaturation at 94 °C for 4 min, followed by five cycles comprising for 1-min denaturation at 94 °C, 1-min annealing at 35 °C, and 30 s of elongation at 72 °C. In the following 30 cycles, denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and elongation at 72 °C for 30 s were carried out, ending with an elongation step for 10 min at 72 °C. Seven SRAP primer combinations were used their names and sequencing are listed in (Table 3). The PCR products were separated by electrophoresis using 1.2% agarose gel in 1 x TAE buffer against 100 bp DNA Ladder as a size marker. Bands were detected with ethidium bromide staining and visualized under UV light, then photographed on Gel Documentation.

Table (3): Seven SRAP primer combinations their names and sequencing

Name	primer sequences	Name	primer sequences
me2	F: TGAGTCCAAACCGGAGC	em3	R:GACTGCGTACGAATTAAT
me4	F:TGAGTCCAAACCGGAGC	em5	R: GACTGCGTACGAATTTGC
me4	F:TGAGTCCAAACCGGAGC	em6	R:GACTGCGTACGAATTGAC
me5	F:GAGTCCAAACCGGAAG	em4	R:GACTGCGTACGAATTTGC
me5	F:GAGTCCAAACCGGAAG	em5	R: GACTGCGTACGAATTTGC
me6	F: TGA GTC CAA ACC GGA CA	em3	R:GACTGCGTACGAATTAAT
me6	F: TGA GTC CAA ACC GGA CA	em6	R:GACTGCGTACGAATTGAC

Data analysis:

Agronomical traits analysis:

Data was subjected to statistical analysis according to the methods of variance analysis using least significant differences (LSD) for the comparison among means (**Snedecor and Cochran, 1989**). Barteletts test of homogeneity was adopted indicating no statistical evidence for heterogeneity of error for all trials (**Bartlett, 1937**). Thus, combined analysis over the two seasons in all trials were performed to estimate the significant differences among cultivars

Salt tolerance indices:

Estimation of Salt Tolerance Indices for each cultivars were calculated using the following formulas: Yield stability index (YSI) = Ys/Yp according to **Bouslama and Schapaugh, 1984**, Stress tolerance index (STI) = $Yp \times Ys/\bar{Y}p2$ as suggested by **Fernandez, 1992**, Stress susceptibility index (SSI) = $(1 - Ys/Yp)/ (1 - \bar{Y}s/\bar{Y}p)$ as suggested by **Fischer and Maurer, 1978** and Tolerance index (TOL) = $Yp - Ys$ according to **Rosielle and Hamblin, 1981**, Where Ys and Yp are the yields of varieties evaluated under saline (stress) and non-saline (non-stress) conditions and $\bar{Y}s$ and $\bar{Y}p$ are the mean yields of all varieties evaluated under stress and non-stress conditions, respectively.

Biochemical and Molecular markers analysis:

The amplified bands from SRAP and SDS-PAGE were scored as a binary data under the heading of total scorable fragments which determined for each cultivar. The

data were used to estimate the genetic similarity on the basis of number of shared amplification products according to (**Nei and Li, 1979**). Polymorphism information content (PIC) values were done to distinguish between cultivars for each primer according (**Anderson *et al*. 1993**). Cluster analysis was performed to produce a denderogram using un-weighted pair-group method with arithmetical average (UPGMA) using PAST program adapted by **Hammer *et al*. (2001**).

RESULTS AND DISCUSSION

Field experiments screening analysis:

Analysis of variance of five traits for the 15 Egyptian barley cultivars showed a significant difference among all cultivars for all studied traits as shown in (Table 4). The interaction between environments and cultivars were significant for all traits and the interaction between seasons and cultivars were significant for peduncle length and No. of spikes m^{-2}, while the interaction for plant height, 1000 grain weight and grain yield were non- significant. Regarding the interaction among cultivars, location and seasons the data showed a significant for all traits. The results were agreement with **Magda *et al.* (2013) and Samah *et al.* (2016).**

Table (4): The analysis of variance of fifteen barley cultivars combined over the two 2015/2016 and 2016/2017 study seasons under two locations Sakah and EL-Hamrowy

Source of variation	D.F	Means of square				
		Plant height (cm)	Peduncle length (cm)	No..spikes m^{-2}	1000grain Weight (gm)	Grain yield (Ard fed^{-1})
Cultivars (C)	14	106.62**	34.66 **	53633 **	407.51 **	383085 **
Environments (E)	1	8935 **	33.19 **	380240 **	18.24 **	769622*
Seasons (S)	1	41033 **	1066 **	4471880**	73633 **	1.237 **
CX L	14	130.75 **	10,71 **	27759.2***	80.06 **	117780 ***
C X S	14	176.85 ns	38.97 **	34169 **	60.83 ns	314049 ns
C X SX L	14	87.478**	9.410**	18683. **	127.20***	117426. **

The mean performances of combined data analysis of the five studied traits for 15 cultivars under the two environments (Sakha as control and EL-Hamrwy as salt soil) were presented in (Table 5). Data showed that Giza 123, Giza 131, Giza 130, Giza 136 and Giza 2000 showed the high mean performance values for all studied characters under normal and salt stress, therefore, we could consider that these cultivars are highly salt tolerant. While, Giza 124, Giza 129 and Giza 132 gave the lowest mean values for most of studied characters. Thus we could consider them as sensitive salt cultivars. These results were in good harmony with **Ahmed *et al*. (2013) and Samah *et al.* (2013&2016).**

Laboratory Experiments Analysis:

Germination growth analysis*:*

The effect of salinity on seed germination percentage and vigorous seedling are shown in (Table 6). The results revealed that the seed germination and seedling traits were decreased under salinity stress. Moreover, Giza 131, Giza 123, Giza 125, Giza 128, Giza 2000, Giza 136 and Giza 135 had high germination percentage and high seedling traits values under both control and salt stress more than other cultivars. Similar results have also been reported by in barley **(Askari *et al.*, 2017; Hagh *et al.*, 2017 and Samah *et al.*, 2018**) They reported that the decrease in seed germination under salinity stress might be caused by the high osmotic pressure and by the toxic effect of high salt concentration on embryo growth.

Table (5): Combined means performance of the five traits under normal and saline conditions across 2015/2016 and 2016/2017 seasons

Cultivars	Plant height (cm)			Peduncle length (cm)			No. spikes (m^{-2})			1000-grain weight(g)			Grain yield (Ard fed^{-1})		
	N	S	R	N	S	R	N	S	R	N	S	R	N	S	R
Giza 123	104.9	86.0	18.0	29.5	25.7	12.9	669.2	487.5	27.2	59.6	47.1	21.0	19.4	11.4	41.2
Giza 124	97.4	73.7	24.3	24.2	20.7	14.5	422.5	304.0	28.0	55.3	39.4	28.8	16.5	8.3	49.7
Giza 125	97.3	75.1	22.8	30.0	22.2	26.0	454.2	250.8	44.8	54.4	41.7	23.3	15.0	10.0	33.3
Giza 126	102.5	75.1	26.7	25.2	18.5	26.6	482.5	233.8	51.5	59.4	41.6	30.0	16.6	7.7	53.6
Giza 127	100.8	82.1	18.6	24.7	22.5	8.9	514.2	338.3	34.2	57.4	44.5	22.5	16.7	9.0	46.1
Giza 128	100.0	70.2	29.8	26.2	22.8	13.0	513.5	229.5	55.3	59.4	43.2	27.3	18.0	6.2	65.6
Giza 129	102.5	81.8	20.2	20.3	17.5	13.8	495.0	234.2	52.7	50.4	36.4	27.8	14.8	7.0	52.7
Giza 130	101.2	73.4	27.5	26.2	23.4	10.7	466.7	374.2	19.8	56.3	46.4	17.6	20.0	10.8	46.0
Giza 131	103.2	91.1	11.7	30.3	26.2	13.5	693.8	472.5	31.9	61.1	48.7	20.3	17.8	11.2	37.1
Giza 132	95.3	65.9	30.8	20.6	16.2	21.4	349.2	247.5	29.1	50.9	33.5	34.2	14.8	5.8	60.8
Giza 133	106.2	81.0	23.7	24.8	22.5	9.3	468.3	205.8	56.1	56.0	47.0	16.1	15.2	7.0	53.9
Giza 134	98.5	78.0	20.8	29.7	21.8	26.6	570.0	288.3	49.4	54.6	38.3	29.9	14.8	10.1	31.8
Giza 135	100.2	83.5	16.7	28.2	24.8	12.1	557.5	311.7	44.1	57.5	37.5	34.8	15.6	6.2	60.3
Giza 136	102.7	85.0	17.2	26.8	25.4	5.2	640.0	413.2	35.4	59.5	43.0	27.7	18.4	11.2	39.1
Giza 2000	100.0	72.6	27.4	26.3	23.6	10.3	636.7	451.7	29.1	58.4	41.4	29.1	19.9	10.6	46.7
Average	100.9	78.3	22.4	26.2	22.3	15.0	528.9	322.9	39.2	56.7	42.0	26.0	16.9	8.5	47.9
L.S.D	2.80			0.48			24.29			1.12			0,45		

N: normal, S:salinity , R : redaction percentage and L.S.D : least significant differences

Physiological Parameters analysis:

The Relative water content (RWC) significantly reduced under salinity stress for all cultivars High means values of RWC were recorded under control and found in Giza 136 with 78.6% low values of RWC were recorded under salinity which found in for Giza 132 (20.0%) Parallel results were reported by **Kamboj *et al.* (2015**) **and Samah *et al.* (2018)** .

High values of proline content were found in Giza 136 (0.87 and 2.87mg/g) under control and salt stress respectively, followed by Giza 123 with values of 0.82, and 2.11 mg/g. It could be concluded that the proline accumulation was increased in all tolerant cultivars such as (Giza 123,128, 131,136 and 2000) due to salinity stress. These results were in agreement**), Behrouz *et al.* (2015) and Samah *et al.* (2016 & 2018)**, they confirmed that the accumulation of proline during barley experience to salinity stress showed high degree of tolerance to salinity.

Estimation of Salt Tolerance Indices

A two-year mean value of screening methods for characterizing salt tolerance indices are presented in (Table 7). High tolerance index (TOL) values were found in (Giza 131, Giza 123 and Giza 136 cultivars), whereas lowest TOL values was found in the Giza 124, and Giza 129. According to stress susceptibility index (SSI), the cultivars Giza 127, Giza 132 and Giza 126 had the highest values, while Giza 124, and Giza 132 had the lowest values, which were considered as salt sensitive cultivars and had poor yield stability in both stress and non-stress conditions. Based on ranking of mean productivity (MP) and stress tolerance index (STI), the cultivars Giza 131, Giza 123 and Giza 136 had the highest values. The highest YSI was achieved by the Giza 124, and Giza 132. Therefore, the cultivars Giza 131, Giza 123 and Giza 136 had the best performance for grain yield under normal and salt stress conditions.

Table (6): Means performance of the seedling and physiological traits under normal and saline conditions during two cropping seasons 2016 and 2017

Cultivars	G.P %		Vigorous Seedling Traits												physiological parameters			
			Length (cm)				Fresh weight(mg)				Dry weight (mg)							
	N	S	Shoot		Roots		Shoot		Roots		Shoot		Roots		RWC%		Proline	
			N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S
Giza123	100	83	15.67	13.67	5.67	5.00	1.56	1.01	0.96	0.41	0.71	0.58	0.45	0.31	59.70	43.60	0.82	2.11
Giza124	90	73	13.67	13.67	5.33	2.00	0.75	0.26	0.41	0.23	0.27	0.15	0.21	0.06	52.20	39.70	0.37	0.80
Giza125	100	80	15.00	10.33	6.33	4.00	1.08	0.83	0.54	0.23	0.88	0.66	0.49	0.31	62.50	45.60	0.46	1.15
Giza126	93	82	12.67	9.67	5.33	2.67	1.01	0.59	0.48	0.25	0.78	0.54	0.33	0.15	67.40	53.10	0.49	1.48
Giza127	95	83	14.00	8.67	5.67	3.00	1.22	0.64	0.62	0.24	0.67	0.51	0.26	0.23	61.30	55.00	0.33	0.82
Giza 128	100	93	12.00	8.00	6.33	3.67	0.94	0.62	0.49	0.27	0.77	0.37	0.25	0.18	56.80	53.30	0.63	2.13
Giza 129	93	82	12.33	7.67	6.67	3.00	0.73	0.46	0.36	0.12	0.73	0.45	0.27	0.21	61.00	57.40	0.45	0.82
Giza130	95	82	13.67	8.67	6.50	3.67	0.86	0.56	0.62	0.25	0.86	0.56	0.27	0.21	66.20	56.20	0.41	0.91
Giza131	100	88	15.33	10.00	5.67	3.67	1.55	0.99	0.95	0.39	0.91	0.79	0.28	0.15	71.50	58.20	1.43	2.43
Giza 132	93	78	15.33	10.67	5.33	1.67	0.66	0.47	0.32	0.14	0.74	0.45	0.24	0.20	60.70	49.90	0.34	0.80
Giza 133	98	79	15.00	5.50	6.50	2.00	0.91	0.73	0.46	0.23	0.57	0.43	0.25	0.16	68.40	51.70	0.73	0.94
Giza 134	97	80	13.67	12.50	5.33	3.33	0.95	0.98	0.35	0.17	0.78	0.49	0.25	0.14	57.80	50.30	0.48	1.23
Giza135	100	85	14.67	6.67	5.67	2.33	0.98	0.74	0.38	0.14	0.58	0.42	0.35	0.32	50.60	47.10	0.75	0.95
Giza136	100	88	15.67	9.50	7.00	4.00	1.68	0.86	0.92	0.26	0.86	0.47	0.43	0.30	78.60	65.20	0.87	2.87
Giza2000	100	86	15.67	11.00	7.67	5.00	0.92	0.58	0.55	0.24	0.73	0.45	0.44	0.28	73.80	53.20	0.59	1.99
Average	96	82	14.29	9.75	6.07	3.27	1.05	0.69	0.56	0.24	0.72	0.49	0.32	0.21	63.23	51.97	0.61	1.43
LSD SXC	1.06		1.05		0.39		7.40		0.05		0.01		0.23		3.02		1.56	

N: normal, S: salt stress, G.P: Germination Percentage, RWC: Relative Water Content

Table (7): Salt tolerance indices of the 15 barley cultivars under normal and saline condition across the two seasons 2015/2016 and 2016/2017.

Cultivars	Yield under normal	Yield under stress	Tolerance index	Stress sensitive index	Stress tolerance index	Mean product	Yield stability index
Giza 123	19.4	11.4	4.53	1.02	0.84	4.56	0.34
Giza 124	16.5	8.3	1.30	0.62	0.34	2.58	0.60
Giza 125	15.0	10.0	2.34	0.95	0.29	2.60	0.38
Giza 126	16.6	7.7	3.33	1.12	0.31	2.91	0.27
Giza 127	16.7	9.0	3.39	1.14	0.29	2.87	0.26
Giza 128	18.0	6.2	2.76	1.07	0.26	2.58	0.30
Giza 129	14.8	7.0	1.70	0.88	0.20	2.12	0.43
Giza 130	20.0	10.8	2.13	1.07	0.16	2.02	0.31
Giza 131	17.8	11.2	4.64	1.04	0.83	4.55	0.32
Giza 132	14.8	5.8	1.98	0.84	0.32	2.62	0.45
Giza 133	15.2	7.0	2.99	1.10	0.27	2.68	0.28
Giza 134	14.8	10.1	2.63	1.10	0.20	2.35	0.28
Giza 135	15.6	6.2	2.38	1.13	0.15	2.06	0.27
Giza 136	18.4	11.2	4.41	1.03	0.79	4.42	0.33
Giza 2000	19.9	10.6	2.07	0.93	0.26	2.41	0.40

Data in (Table 8) showed that grain yield had a positive and significant correlation with MP and STI indices. Therefore, the selection based on high values of MP and STI indices will lead to select cultivars with high yield under normal and saline conditions, as we found from our results in (Table 7) that the varieties Giza 123, Giza 131 and Giza 136 produced the highest yield under normal and saline conditions. From these results we could consider that the cultivars with low fluctuations under different stress environments can be considered as salt sensitive varieties so in our case the SSI and TOL can be used to screen salt sensitive cultivars

as they are strongly associated with YSI. In contrast, salt tolerant varieties should have acceptable yield performance under stress and high yield performance under non-stress environments. Thus, the mean productivity (MP) and stress tolerance index (STI) indices can be considered as tools for screening salt tolerant varieties as they are not associated with YSI. Theses result is consistent with the findings of (**Ravari *et al.*, 2016 and Samah *et al.*, 2017**). They reported that MP and STI were established to be the better salt stress indices than others indices for selecting cultivars with high yield under stress conditions, while TOL and SSI will be more useful indices for selection of sensitive cultivars under salinity stress.

Table (8): Correlation coefficients between salt tolerance indices and grain yield of 15 barley cultivars across two seasons 2015/2016 and 2016/2017.

Salt indices	Grain Yield under normal	Grain Yield Under stress	Tolerance index	Stress sensitive index	Stress tolerance index	Mean product
Yield under stress	0.117**					
Tolerance Index	0.75ns	0.234ns				
Stress sensitive index	0.23ns	0.221ns	0.19**			
Stress tolerance Index	0.10**	0.113**	0.147**	0.24ns		
Mean product	0.08**	o.130**	0.117**	0.241ns	0.042**	
Yield stability index	0.242ns	0.231ns	0.19**	0.114*	0.242 ns	0.340 ns

Ns, * and ** non-significant and significant at the 5% and 1% levels of probability, respectively.

Biochemical fingerprinting of total soluble protein SDS-PAGE:

To identify proteins involved in salt stress response in 15 Egyptians barley cultivars, SDS–PAGE profile was done and revealed that the total soluble protein accumulation increased under control than salinity stress. Banding pattern of total protein was shown in (Table 9 and Fig 1). Twenty-four polymorphic bands were detected in all cultivars based on their gene expression under control and salinity with molecular weight ranging from 10 to 250 KDa. The results found some Levels of proteins with molecular weights of 75, 15 and 10 KDa polymorphic were common bands under control and salt treatments for all cultivars.

Likewise, the results indicated that the salt stress led to increase in the number of some new polypeptides in barley seedling under salt stress compared with control, such as the protein with molecular weight 150 KDa and 100 KDa were found in under salinity stress ,while not found under control in Giza 135 ,besides other two protein with molecular weight 45and 25 KDa were found under salinity stress and not found under control in Giza 133.

Moreover, there was another protein with molecular weight 37 KDa was found in all cultivars under control and salt stress expect for Giza 134 and Giza 2000.

The resulted also indicted the there were some proteins induce only under control but not fond under salt treatments such as, protein with molecular weight 100 KDa in Giza 136 and Giza 133, and protein with molecular weight 20 KDa were found in Giza134 and Giza 136.

Table (9): Molecular weight (MW) KDa of SDS- PAGE total proteins of fifteen Egyptian Barley cultivars their under control and salt stress for each of them. (+) means presence and (-) means absence of band

MW KDa	Treatments	Giza 123	Giza 124	Giza 125	Giza 126	Giza 127	Giza 128	Giza 129	Giza 130	Giza 131	Giza 132	Giza 133	Giza 134	Giza 135	Giza 136	Giza 2000
250	control	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
	salinity	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+
150	control	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
	salinity	-	-	-	-	-	+	+	+	-	-	-	+	-	-	+
100	control	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+
	salinity	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+
75	control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	salinity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
50	control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	salinity	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
45	control	-	+	-	+	-	+	+	+	+	+	-	+	+	+	+
	salinity	-	+	-	+	-	+	+	-	+	+	+	-	+	+	+
37	control	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-
	salinity	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-
30	control	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+
	salinity	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
25	control	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-
	salinity	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-
20	control	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-
	salinity	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
15	control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	salinity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	salinity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Total	24	18	20	16	18	18	20	23	21	20	21	14	18	20	18	17

These results confirmed that the effects of salinity stress on barley might be changing their gene expression and protein accumulation during a biotic stress. Some proteins were expressed to salinity stress as a lower level in stress compared with control, some proteins remained without changed in stress gave the initial increase in total soluble proteins during salt stress was due to the expression of new stress proteins, but the decrease was due to a severe decrease in photosynthesis, these results were in agreement with (**El-Hamamsy and Behairy, 2015; Hellal *et al*,. 2017 and Samah *et al.*, 2108).** They used SDS-PAGE method to screen the total soluble protein for salinity tolerance analysis in barley and they found high differences parents of protein accumulation in barley genotypes.

Molecular marker analysis:

Amplification results of SRAP-PCR marker analysis:

Presently, many techniques of DNA based molecular markers such as RAPD, RFLP, SSR and SRAP etc., are available which detect polymorphism at the DNA level.

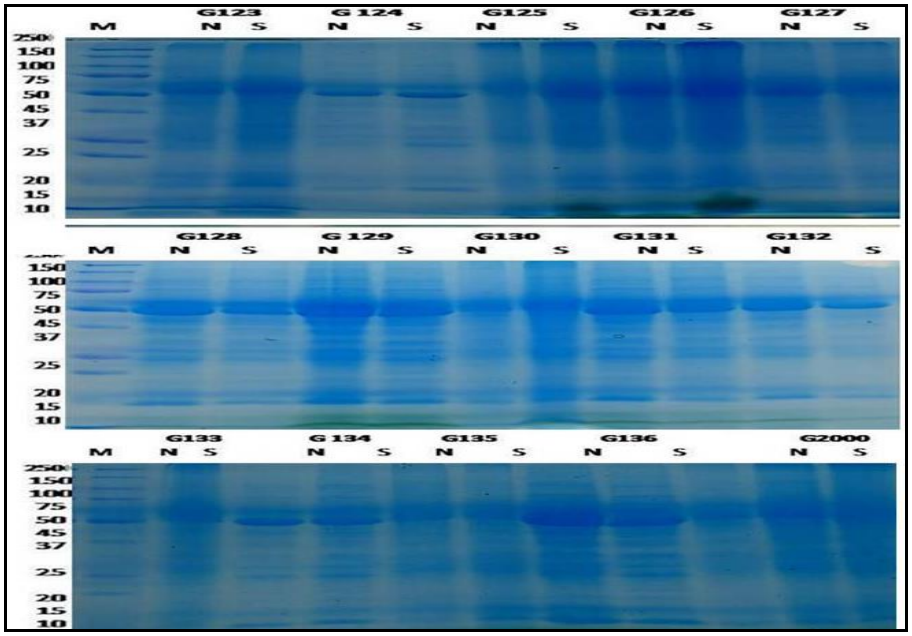

Fig. (1): Gel Electrophoresis of SDS-PAGE of total soluble proteins of 15 barley Egyptian cultivars under two ECw (N: normal and S: salinity treatment) and. M = molecular weight marker

The present study used SRAP techniques to assess genetic polymorphism among 15 barley cultivars for salt tolerance. Data in Table 10 showed that the total fragments were 69 bands. The band number for each pair of primers was 46 band ranged from six bands in (me4+em5, Fig 2 B) to twelve bands in (me5+ em5 Fig 2A) with an average (6.6%) per primer combination. The percentage of polymorphism for each primer combination varied from 33.3% (me3+em4) to 100% (me5+em5 Fig 2A) with average 61.4%.

Polymorphic Information Content (PIC) values were evaluated to assess the genetic diversity for seven selected primers were ranged from lowest PIC was 0.35 % related to primer combination me4+em5 to highest PIC was 0.96%, which was related to primer combination me5+em5.Thus the primer combination me5+em5 was highly informative and could be useful primer set to confirm the genetic differences among barley cultivars for salt tolerant.

UPGMA Cluster analysis and Genetic Similarity:

Cluster analysis shaped a dendrogam among the 15 Egyptian barley cultivars based on seven SRAP fragments using Jaccard's genetic similarity coefficient and outlined by the Un-weighted Pair-Group Method (UPGMA) (Fig3). The dendrogram of SRAP markers had clustered all the Egyptian cultivars into four groups, each group include the closest cultivars together. Group I consisted of the salt tolerant Egyptian barley cultivars (Giza 123, Giza 131, Giza 136 and Giza 2000). Group II consisted of salt moderated tolerant Egyptian barley cultivar (Giza 126, Giza 130, Giza 133, Giza 127 and Giza 135). However, Group III consisted of salt sensitive moderated Egyptian barley cultivar (Giza 125, Giza 134 and Giza 128). Group IV consisted of salt sensitive Egyptian barley cultivar (Giza 129, Giza 132, and Giza 124), indicting the close relationship within each of pair of barley cultivars.

The genetic similarity is an important index for estimation of the genetic differentiation among Egyptian barley cultivars using Jaccard's similarity coefficients (Table 11).The genetic similarity coefficient (GSC) ranged from low similarity (0.6٤) (between Giza12٦ and Giza١٣٤) which proposes that these were the least-related cultivars to high similarity (0.92) between(Giza١٢٦ and Giza13٠).

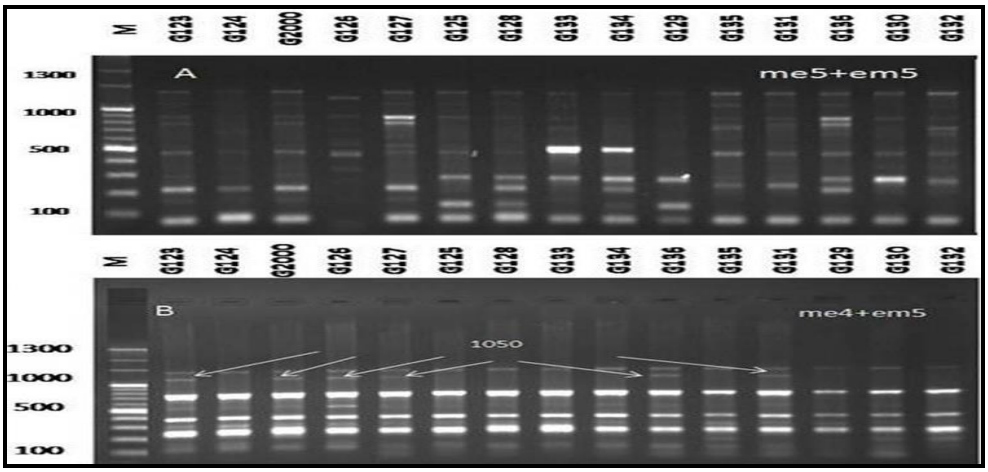

Fig. (2): Amplification results of the primers combination (A) me5+em5, (B) me 4+ em5 in 19 Egyptian barley cultivars.

Table (10): List of used SRAP primers names, sequences, no. of total fragment, No. of polymorphic bands, Polymorphism % and polymorphism information contents (PIC).

No.	Name	Total fragment	Number of polymorphic fragments	Percentage of polymorphic fragments	Polymorphic information content PIC
1	me2+em3	10	5	50.0	0.51
2	me4+em5	6	2	33.3	0.35
3	me4+em6	7	3	42.9	0.43
4	me5+em4	12	7	58.3	0.61
5	me5+em5	13	13	100.0	0.96
6	me6+em3	10	٨	80.0	0.89
7	me6+em6	11	8	72.7	0.73
Average		9.8	6.6	61.4	0.63
Total		69	٤٦		

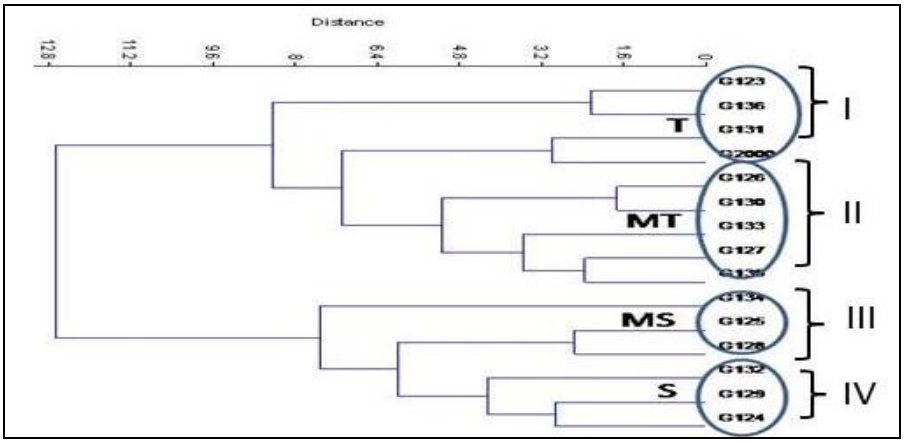

Fig. (3): Cluster analysis (UPGMA) based on genetic similarity estimates from the SRAP marker analysis.

Table (11): Genetic distance estimates for 15 barley cultivars based on seven SRAP primers markers analysis.

Cultivars	G123	G124	G125	G126	G127	G128	G2000	G132	G133	G134	G135	G136	G129	G130
G124	0.67													
G125	0.69	0.78												
G126	0.76	0.68	0.72											
G127	0.80	0.74	0.80	0.84										
G128	0.72	0.74	0.85	0.73	0.79									
G2000	0.75	0.67	0.71	0.74	0.78	0.72								
G132	0.64	0.76	0.74	0.69	0.71	0.76	0.68							
G133	0.82	0.72	0.74	0.79	0.85	0.78	0.73	0.74						
G134	0.67	0.62	0.71	0.64	0.69	0.71	0.65	0.69	0.72					
G135	0.83	0.73	0.73	0.76	0.86	0.73	0.79	0.71	0.81	0.65				
G136	0.87	0.67	0.66	0.72	0.76	0.70	0.73	0.66	0.80	0.69	0.77			
G129	0.69	0.79	0.83	0.70	0.79	0.84	0.76	0.81	0.73	0.67	0.72	0.69		
G130	0.77	0.73	0.77	0.92	0.89	0.76	0.79	0.71	0.81	0.67	0.83	0.73	0.74	
G131	0.73	0.64	0.68	0.74	0.78	0.66	0.79	0.70	0.71	0.65	0.83	0.66	0.67	0.77

Genetic diversity among the 15 cultivars using SRAP markers:

Results in Table 12, showed genetic diversity among 15 Egyptian barley cultivars using SRAP markers. Percentage of polymorphic loci ranged from 70.4% (Giza 124) to 80.5% for Giza 136). Polymorphic information content (PIC) values, used to measure the genetic diversity were ranged from 0.71 to 0. 85 with an average 0.736.

Genetic diversity indices include Simpson index, Shannon's diversity index and Berger- Parker index were an important indices to estimate the levels of genetic diversity among the 15 Egyptian barley cultivars were shown in Table 12. The obtained Simpson index ranged from 0.9800 for Giza 124 to 0.9825 for Giza 136 with an average (0.9805). About Shannon's information index ranged from 3.9120 (Giza 124) to 4.0435 (Giza 136) with average (3.3988). About Berger-Parker index the values ranged from 0.0177 (Giza 136) to 0.0200 (Giza 124). Moreover, the changes of these indices were consistent with the percentage of polymorphic loci.

Table (12): Genetic diversity among 19 barley cultivars using seven SRAP primer combinations

Cultivars	Total polymorphic band	Percentage of polymorphic bands	Polymorphic information content PIC	Simpson Index	Shannon's information index	Berger-Parker index
G123	51	71.8	0.72	0.9804	3.9320	0.0196
G124	50	70.4	0.71	0.9800	3.9120	0.0200
G125	57	80.2	0.82	0.9824	4.0430	0.0175
G126	56	78.8	0.79	0.9821	4.0250	0.0179
G127	55	77.4	0.78	0.9818	4.0070	0.0182
G128	53	73.2	0.75	0.9811	3.9700	0.0189
G2000	47	66.2	0.68	0.9787	3.8500	0.0213
G132	51	71.8	0.73	0.9804	3.9320	0.0196
G133	54	76.1	0.79	0.9815	3.9890	0.0185
G134	51	71.8	0.71	0.9804	3.9320	0.0196
G135	51	71.8	0.73	0.9804	3.9320	0.0196
G136	57	80.3	0.85	0.9825	4.0433	0.0175
G129	55	77.5	0.78	0.9818	4.0072	0.0182
G130	54	76.1	0.77	0.9815	3.9890	0.0185
G131	53	74.7	0.75	0.9811	3.9700	0.0189
Average	51.53	72.5	0.736	0.9805	3.9386	0.0195

In this study, SRAP marker gave 69 alleles which were amplified by seven primer combinations in 15 cultivars, it was higher in alleles number than other DNA markers in the genetic diversity in barley such RAPD **(Guasmi *et al.*, 2012**), SSR **(Varshney *et al.*, 2007)** and ESTs **(Salem *et al.*, *2*010).** The high polymorphic percentage (92%) and PIC value (0.96), together with a high genetic similarity (0.92) observed among 15 cultivars in this study suggests a high level of heterogeneity. The high polymorphism percentage in this study agree with those obtained by (**Yang *et al.*, 2008 and 2010); Said *et al.* (2015) and Mariey *et al.* (2017)** who used SRAP marker to evaluate the genetic diversity in barley and suggested that SRAP technology is useful for genetic diversity and relationship analyses, marker assisted selection and genetic map construction in barley. From the data, it is clear that there was a wide genetic diversity among 15 Egyptian barley cultivars based on the seven SRAP markers analysis. The association of molecular markers with phenotypic evaluation is one of important factors to understand and investigate the genetic role of tolerance by prediction the genomic regions that affect the plant's response (**Roy *et al.*, 2011**).In the present study, morphological and physiological characters analysis of fifteen Egyptian barley cultivars was used with molecular analyses (SRAP marker) to investigate the genetic relationships and classified the 15 Egyptian barley cultivars for their response to salt tolerance. SRAP marker was able to differentiate among different DNA of high and low performance in all agronomic traits evaluated. Dendrogram based on SRAP rather than agree with morphological characters distance. The SRAP data can be used in selecting diverse parents in breeding program and in maintaining genetic variation in the germplasm. The results provide new information about the relationships between Egyptian barley cultivars which are useful for cultivar identification and their utilization in further barley breeding programs for salt tolerant in Egypt.

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الدلائل الوراثية البيوكيميائية والجزيئية المرتبطة بتحمل الملوحة لأصناف الشعير المصرية

سماح عبدالله مرعى[١] , كريمة رشاد احمد[١] , امين محمد عجوة[١], منى على فريد[٢] و احمد محمد سراج[٣]

١. قسم بحوث الشعير معهد بحوث المحاصيل الحقلية ، مركز البحوث الزراعية ، مصر

٢. قسم الوراثة – كلية الزراعة –جامعة كفر الشيخ–

٣. قسم الوراثة والهندسة الوراثية – كلية الزراعة –جامعة بنها

لتحديد استجابة ١٥ صنفًا من الشعير المصري لظروف الإجهاد الملحى تم إجراء تجربتين خلال موسمين متتاليين ٢٠١٥/٢٠١٦ و ٢٠١٦/٢٠١٧ تحت موقعين في مزرعة سخا (التربة الطبيعية) ومزرعة الحمرواي (تربة مليحة) باستخدام قياس بعض الصفات المورفولوجية و الفسيولوجية ، قياس مؤشرات تحمل الملوحة ، وتحديد الدلائل البيوكيميائية ودراسة علاقات التتابع لتعدد الأشكال المكبرة(SRAP). أظهر تحليل التباين للصفات وجود فروق كبير بين التراكيب الوراثية لجميع الصفات. أظهرت النتائج أن معدل الإنبات و والصفات الخضرية انخفضت تحت الملوحة. انخفض محتوى الماء النسبي (RWC) بشكل ملحوظ تحت الملوحة لجميع الأصناف. تم الحصول على قيم عالية من محتوى البرولين في الجيزة ١٣٦ (٠.٨٧ و ٢.٨٧ mg / جم) تحت التربة الطبيعية و الملحية . أعصت كل من الأصناف الجيزة ١٣١ ، الجيزة ١٢٣ ، الجيزة ١٣٦ أعلى قيم لمحصول الحبوب تحت الظروف الطبيعية والملحية كما وجدت اختلافات كبيرة في جميع مؤشرات تحمل الملوحة. كشف تحليل SDS–PAGE أن تراكم البروتين القابل للذوبان يزداد في الأصناف تحت الكنترول أكثر تحت الملوحة. تم اكتشاف ٢١ حزمة متعددة الأشكال في جميع الأصناف بناءً على تعبير الجينات في االاصناف تحت الملوحة . الوزن الجزيئي يتراوح من ١٠ إلى ٢٥٠ كيلو دالتون. استُخدمت سبعة بدائل للـ SRAP لتقييم التنوع الوراثي بين جميع الأصناف. أظهرت النتائج SRAP أن متوسط النسبة المئوية للمواقع متعددة الأشكال لكل البادئات كانت ٨٧.٤٪. أعلى (PIC) ، كان موجود فى البريمر me5 em5 +كان (٠.٩٤) مشيرا إلى أن هذا البريمر هو غني بالمعلومات. وقد جمَّعت شجرة النسب الوراثية بناء على الدليل الجزئي SRAP جميع الأصناف المصرية في أربع مجموعات تضم كل مجموعة الأصناف الأكثر قرابة مع معامل التشابه الوراثي (GSC) التي تتراوح من (٠.٦٤) إلى (٠.٩٢). أظهرت نتائج الدراسة الحالية وجود فروق وراثية عالية بين أصناف الشعير المصرية لتحمل الملوحة والتي توفر معلومات جديدة عن العلاقات الوراثية بين أصناف الشعير المصرية والتي تفيد في تحديد الأصناف واستخدامها في برامج تربية لتحمل الشعير الإجهاد الملحى .