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Genetic Diversity and Phenotypic Association with Salinity Tolerance in Egyptian Barley Cultivars Using SRAP markers

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Abstract: Lysimeter experiments were performed to evaluate the efficiency of sea water irrigation on plant growth and grain yield traits of 15 Egyptian barley cultivars. Irrigation treatments with saline water showed significant effects of irrigation on plant growth and yield and its components. Salt stress indices were calculated based on grain yield; data showed that MP, GMP and STI were more effective in identifying high yielding cultivars under study. The highest mean values for all studied traits under the treatments had detected in Giza 2000, Giza 131, Giza 136 and Giza 123 which they had a positive and highest values of first principle component analysis. Ten sequence related amplified polymorphism (SRAP) markers amplified 98 fragments and me5+em5 gave the highest polymorphism (100 %). The percentage of polymorphic loci of fifteen cultivars ranged from 71.43% to 91.64%. Shannon's information index ranged from 4.21 to 4.50. The dendrogram of SRAP markers had clustered all cultivars in to four groups each group include the most closed cultivars together according their response to salinity stress. Results showed that SRAP marker could be efficiently used to assess genetic variation among Egyptian barley and their ability for tolerance to salinity stress.
Keywords: *Hordeum vulgare,* lysimeter, sea water, phenotypic traits, principal components analysis, SRAP, PIC, UPGMA, cluster analysis.

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1. Introduction

Salinity of soils and irrigation water are the major problems affecting nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands (Bünemann et al., 2018; Larsen et al., 2017; Munns and Gilliham. 2015; Yihdego, 2017). Salinity affects agricultural production, water and nutrient uptake and metabolic activities in plant leading to drastic losses both in term of reduced economic yield and deteriorated quality of agricultural products (Egamberdieva et al., 2017; Geilfus, 2018; Rogers et al. 1995; Rouphael et al., 2018; Yousfi et al., 2007). Salinity is expected to increase in future, with serious implications for agriculture and food security (Fischer and Connor. 2018; McNeill et al., 2017; Misra, 2014; Saifullah et al., 2018; Zhang et al., 2018)

In the Mediterranean area, Egypt is one of the countries that had many salinity problems where over 33% of the cultivated land area is already saline (Eissa et al., 2016; Ghassemi et al. 1995; Hereher, 2013; Hussein et al., 2009; Khoudari, 2014; Masoud et al., 2018; Wassef and Schüttrumpf, 2016). The Nile water is not available in most of Egyptian lands, thus the use of saline water for irrigation could be alternative sources of water requirements for irrigation in agriculture (El-Desoky et al. 2007; Singh, 2018). Significant improvement in tolerance in crops is required against salinity to utilize saline water for crop production (Huang, 2018; Kibria et al., 2017).

Barley is considered a model crop for salinity tolerant in cereals due to its widely available genetic information (Hayes et al. 2002, Jamshidi and

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Javanmard, 2018; Munns et al., 2006) which help barley to grow under marginal environments and gave grain yield with low damage and high productivity (Maiti and Pratik, 2014; Munns et al. 2002; Wu et al., 2015; Zhang et al., 2017).

Despite of the salinity tolerance of barely (8 dSm^{-1}) (Hammami et al., 2016; Maas ,1997), barley breeders faced many challenges to improve salinity tolerance breeding programs because of the mechanism of tolerant to stress is controlled by many genes and their concurrent selection procedures particularly under stress conditions are difficult (Flowers , 2004). Large genetic pool is required to increase tolerance against salinity in barley plant and ensuring better productivity. These genetic resources can be use for development of salinity tolerant cultivars by ensuring protection against losses due to salt stress (Elakhdar et al., 2016; Shen et al., 2018). Like other crops, plant breeders applied various tools for improving salt tolerance in barley also (Bhusan et al., 2016). These include morphological screening which was more efficient in breeding for salt environments (Ahmed et al. 2003; Shahbaz and Ashraf, 2013).

Salt stress indices have been used in barley as a powerful tool for intended to ranking barley genotypes for salt tolerance (Bchini et al., 2011). Multivariate analysis was used in barley as a dominant tool for population grouping and screening of huge number of barley genotypes (Sharafi et al., 2004). However, most of these tools were limited for some stages of plant growth and might be affected by environment (Massood et al., 2003). Therefore, the plant breeders are looking for tools to help them to directly evaluate genetic variation among relatives without effect of environmental factors this tools called plant molecular breeding markers (Ahmad et al., 2017; Burg, 2018; Ismail and Horie, 2017). Many molecular markers were used to evaluate the level of barley genetic variability. Sequence related amplified polymorphism (SRAP) adapted by Li and Quiros, (2001) for a variety of purposes in different crops. 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 many genetic studies (Ahmed et al., 2017; Bhatt et al., 2017; Kok et al., 2018; Ni et al., 2018). In barley, SRAP marker has been successfully used to evaluate the genetic diversity among the barley genotypes or cultivars (Mariey et al., 2015; Yang et al., 2008; Yang et al., 2010). Thus the objective of the present study was to investigate the genetic diversity, using SRAP markers, among 15 Egyptian barley cultivars for salinity tolerance by treating with different irrigation treatments of sea water, in order to provide genetic information for future breeding program for salinity tolerant.

2. Materials and Methods

2.1. Lysimeter Experiment and Plant Material

To study the effect of seawater irrigation on six agro-morphological traits of 15 Egyptian barley cultivars, two lysimeter experiments under natural condition were carried out. The experiment was done at Soil Improvement and Conservation Research Department, Sakha Research Station, Kafr Elsheikh, Egypt, during two successive winter growing seasons (2014-2015 and 2015-2016).

Table 1. Names and pedigree of 15 barley cultivars used in this study

No.	Name	Pedigree
1	Giza 123	Giza 117/FAO 86
2	Giza 124	Giza 117/Bahteem 52// Giza 118/FAO 86
3	Giza 125	Giza117 / Bahteem52// Giza118 /FAO86(sister line to G.124
4	Giza 126	BaladiBahteem/S D729-Por12762-BC.
5	Giza 127	W12291/B0gs//Hamal-02
6	Giza 128	W12291/4/11012-2170-22425/3/"Apam"/"B65"//"A16"
7	Giza 129	DeirAlla 106/Cel//As46/Aths*2"
8	Giza 130	Comp.cross"229//Bco.Mr./DZ02391/3/DeirAlla 106
9	Giza 131	CM67B/Centeno//CAMB/3/ROW906.73/4/Gloria-Bar/Come-B/5/Falcon-Bar/6/LINO
10	Giza 132	Rihane-05//AS 46/Aths*2Athe/ Lignee 686
11	Giza 133	ICB91-0343-0AP-0AP-0AP-281AP-0AP
12	Giza 134	ICB91-0343-0AP-0AP-0AP-289AP-0AP
13	Giza 135	ZARZA/Bermejo/4/DS4931//Gloria-Bar/COPAL/3/SEN/5/AYAROS
14	Giza 136	Plaisant/7/CLN-B/Ligee640/3/S.P-B//Gloria-Bar/COMEB/5/Falcon-Bar/6/Linocln-B/A/S.P/Lignee640/3/S.P-B//Gloria-Bar/COME B/5/Falcon-Bar/6/LINO
15	Giza 2000	Giza117/Bahteem52// Giza118/ FAO86 / 3/Baladi16/ Gem

Table 2. Physico-chemical properties of soil used in the experiment during two growing seasons

Chemical properties	2014/ 2015	2015/2016
pH	7.96	8.17
ECe (dSm^{-1})	3.77	4.14
Sodium Absorpation Ratio (SAR)	10.27	10.75
Soluble cations(meq100 ^{1} g soil)		
Ca^{++}	7.9	8.4 3
Mg^{++}	5.07	5.2 7
Na^{++}	26.2	28.17
K^{+}	0.4	0.7
Soluble anions meq100^{-1} g soil		
SO_4	17.67	18.34
Cl^{-}	18.9	20.73
HCO_3	3.0	3.5
CO_3	0.0	0.0
Physical properties		
Texture grade	Clayey	Clayey
Total $CaCo_3$ (%)	2.46	2.25
Bulk density (g/cm3)	1.21	1.27
Particle size distribution (%)		
Sand	19.27	19.58
Silt	28.33	29.54
Clay	52.4	50.88

The studied cultivars were kindly provided by Sakha Barley Research Department, Field Crops Research Institute, Agricultural Res. Center, Egypt. Names and pedigree of studied barley cultivars were shown in Table 1. The experiment was conducted using randomized complete block design (RCBD) arranged as a split plot with four irrigation treatment using sea water S0= tap water (control) (ECw= 0.6 dSm-1 equal 384 ppm) , S1= (ECw =5 dSm-1 equal 4000 ppm), S2= (ECw= 10 dSm-1 equal 8000 ppm), and S3= (ECw= 15 dSm-1 equal 12000 ppm) (Ayers and Westcot, 1985), as the main plot factor and cultivars as the subplot factor. Total 180 experimental plots (15 cultivars × 3 replications × 4 salinity treatments) were used in the experiment with net plot size (experimental unit) was 1.0 m^2 (5 rows × 0.2 m × 1 m). Egyptian barley cultivars were sown at 15th November (2014) and 25th November (2015) and harvested on 10th April (2015) and 30th April (2016). All local recommendations were uniformly followed to grow barley plants without any stress except irrigation treatments described above.

Days to heading the date of the appearance of 50% of spikes was recorded / each plot (when 1/3 of the spike exerted), at harvest maturity ten guarded plants were randomly taken from each plot to measure plant height (cm), number of spikes m^{-2}, number of grains $spike^{-1}$, 1000-grain weight and grain yield was determined using the full plot area (g/plot).

2.2. Properties of soil and irrigation saline water

Soil samples from different depth (0-15,15-30 and 30-45 cm) from each lysimeter plot were taken before planting and after harvesting in two seasons to determine chemical and physical soil properties following procedure described earlier (Black, 1965) as shown in Table (2). The used irrigation saline water was prepared by mixing sea water (48 dSm^{-1}) with tap water (0.6 dSm^{-1}) at proper ratios to get the required salinity levels (5, 10 and 15 dSm^{-1}). The chemical properties of the saline water (Table 3) were analyzed according Black (1965).

2.3. DNA Extraction and SRAP- PCR Amplification

Genomic DNA was isolated from fresh barley leaves using CTAB method according to Doyle and Doyle (1990). Purified total DNA was quantified and its quality verified by Nanodrop ND-100 P330 Spectrophotometer (IMPLN) Germany. Ten SRAP primer combinations were used. PCR cycling was carried out as the following program; initial denaturation at 94 °C for 4 min, followed by five cycles comprising of 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.

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Table 3. Chemical properties of tap water and different irrigation sea water dilutions S_1, S_2 and S_3

chemical properties	Control ($C=0.6\ dSm^{-1}$)	low salinity ($S_1=5\ dSm^{-1}$)	Moderated salinity ($S_2=10\ dSm^{-1}$)	High salinity ($S_3=15\ dSm^{-1}$)
pH	7.54	7.92	8.11	8.26
EC dSm^{-1}	0.6	5.0	10.0	15.0
Sodium Absorpation Ratio (SAR)	4.1	12.19	17.11	21.14
	Soluble cations (meq100 g^{-1} soil)			
Na^+	4.05	35.0	69.5	103.5
K^+	0.4	0.7	1.05	1.4
Ca^{++}	1.15	10.5	20.5	30
Mg^{++}	0.85	60	12.5	18
	Soluble anions (meq100 g^{-1} soil)			
Co_3^{--}	0	0	0	0
Hco_3^-	1.25	4.0	7.0	10.25
Cl^-	2.75	25.8	49.6	74.5
So_4^{--}	2.45	22.4	46.95	68.15

The PCR products were separated by electrophoresis using 2% agarose gel, using one Kb DNA Ladder. Bands were detected with ethidium bromide staining and visualized under UV light, then photographed on Gel Documentation system (UVITEC, UK).

2.4. Data analysis

2.4.1. Phenotypic traits analysis

The data from the two seasons were homogeneity and statistically analyzed as the randomized complete block design (RCBD) model. Salt tolerance indices of traits were calculated using the following equation mean productivity (MP) [Eq 1] as described by Hossain et al. (1990), Stress tolerance index (STI) following Fernandez (1992) [Eq 2], Stress susceptibility index (SSI) [Eq 3] following Fischer and Maurer (1978), Tolerance index (TOL) [Eq 4] according to Rosielle and Hamblin (1981)Geometric mean productivity (GMP) [Eq 5] according to Mardeh et al., (2006).

$$MP = (Yp + Ys)/2 \quad [1]$$

$$STI = Yp \times Ys/\bar{Y}p^2 \quad [2],$$

$$SSI = (1 - Ys/Yp)/ (1 - \bar{Y}s/\bar{Y}p) \quad [3],$$

$$TOL = Yp - Ys \quad [4],$$

$$GMP = \sqrt{Ys \times Yp} \quad [5]$$

Multivariate analysis including Principal component, biplot diagram and cluster analysis were analysed using Minitab v.12 (1996). Cultivars were clustered using unweighted pair-group method with arithmetical average (UPGMA) as described by Kovach (1995).

2.4.2. Molecular analysis

The amplified bands, from sequence related amplified polymorphism (SRAP), were scored as a binary data under the heading of total score-able 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).

Table 4. Analyses of variance of six traits of 15 Egyptian barley cultivars grown under different salinity levels during two growing seasons

Traits	Cultivar (C)	Salinity (SA)	Seasons (SE)	Interaction analysis			CV %
				C × SA	C× SE	C × SA × SE	
Heading data (days)	**	**	**	**	**	**	1.6
Plant height (cm)	**	**	**	**	**	**	2.7
No. grain spike $^{-1}$	**	**	**	ns	ns	ns	4.0
No. tillers m^{-2}	**	**	**	**	**	**	1.5
1000 grain weight (g $plot^{-1}$)	**	**	**	**	**	**	3.1
Grain yield (g $plot^{-1}$)	**	**	**	**	**	**	4.06

*significant, ** highly significant and ns non- significant

Table 5. Agro-morphological traits of Egyptian barley cultivars in response to different salinity levels (average of two growing seasons)

Cultivars	Heading data (days)				Plant height (cm)				No. spikes m^{-2}			
	C	S1	S2	S3	C	S1	S2	S3	C	S1	S2	S3
Giza 123	75.8	73.5	71.3	70.3	66.0	60.0	58.0	52.0	6.0	6.0	4.5	3.1
Giza 124	83.2	81.3	80.0	78.8	58.0	54.0	48.0	36.0	4.7	4.2	3.7	3.2
Giza 125	86.7	86.5	81.3	80.3	60.0	58.0	42.0	40.0	5.4	5.1	4.9	3.7
Giza 126	78.8	71.2	74.0	74.8	66.0	65.0	50.0	38.7	4.9	4.5	3.7	3.2
Giza 127	82.8	80.0	71.5	73.5	23.7	22.0	16.3	14.0	4.8	4.7	3.7	3.0
Giza 128	85.2	79.0	77.5	77.2	24.0	22.0	21.3	16.3	5.4	5.0	4.1	3.0
Giza 129	84.5	82.7	81.7	82.7	60.0	58.0	48.0	36.0	5.1	4.7	3.9	3.1
Giza 130	82.5	74.7	72.7	77.3	72.0	66.0	60.0	52.0	5.2	4.8	4.5	3.6
Giza 131	76.8	73.8	71.3	72.3	72.0	66.0	60.0	54.0	6.3	5.4	4.0	3.6
Giza 132	87.8	86.5	83.7	85.3	58.0	54.0	48.0	33.0	4.7	4.0	3.7	2.2
Giza 133	87.2	85.3	81.3	81.3	70.0	60.0	51.0	39.0	4.9	4.6	4.0	3.3
Giza 134	86	82.7	82.0	81	60.0	55.0	49.0	39.0	5.8	4.8	4.3	3.5
Giza 135	85.3	82.8	810	80.3	62.0	56.0	49.0	45.0	5.3	4.8	4.3	3.6
Giza 136	84.5	80.5	78.3	74.5	71.0	66.0	58.0	50.0	6.5	6.2	5.3	3.6
Giza 2000	78.0	76.0	74.2	72.7	72.0	72.0	60.0	54.0	5.9	4.5	3.9	3.2
average	**83.0**	**79.4**	**77.5**	**77.5**	**59.7**	**55.6**	**47.9**	**39.9**	**5.3**	**5.0**	**4.2**	**3.2**
LSD 0.05	**3.04**	**1.83**	**2.87**	**2.75**	**3.4**	**2.8**	**4.5**	**4.2**	**0.3**	**0.3**	**0.3**	**0.2**
CV %	**1.41**	**1.56**	**2.4**	**2.19**	**3.7**	**2.5**	**4.6**	**7.3**	**1.3**	**5.8**	**4.2**	**4.0**

Cultivars	No. Grain $spike^{-1}$				1000-grain weight (g)				grain yield (g $plot^{-1}$)			
	C	S1	S2	S3	C	S1	S2	S3	C	S1	S2	S3
Giza 123	189.4	183.5	157.8	135.6	103.3	101.0	94.3	90.0	245.3	240.3	194.8	149.2
Giza 124	146.0	135.5	94.9	41.0	109.2	105.8	91.5	90.0	179.8	179.5	171.0	120.0
Giza 125	155.2	152.1	108.6	70.9	103.3	100.0	95.0	87.5	237.3	236	194.7	138.3
Giza 126	139.1	131.9	105.5	61.1	97.5	95.8	93.3	88.3	209.3	207.7	1950	138.8
Giza 127	136.2	133.2	92.5	61.1	103.3	100.0	94.2	80.7	205.3	200.7	175.7	139.2
Giza 128	126.7	122.7	94.5	66.0	105.0	100.0	94.2	82.5	233.8	234.3	214.3	135.3
Giza 129	134.9	128.6	90.4	31.6	98.3	95.0	85.7	83.2	198.3	198.0	1650	119.3
Giza 130	133.9	130.0	69.3	75.0	103.3	100.0	90.8	87.8	210.0	189.3	158.3	153.3
Giza 131	186.5	180.3	150.7	131.3	110.0	100.0	93.3	86.7	296.3	293.3	230.0	178.3
Giza 132	123.7	122.6	82.3	35.0	94.2	90.0	81.7	74.2	177.7	172.0	130.3	117.7
Giza 133	143.6	139.5	99.1	60.0	106.7	100.8	94.2	86.7	191.7	191.3	145.5	133.5
Giza 134	139.6	135.4	101.1	71.9	105.8	95.0	87.5	78.3	228.3	218.3	196.0	153.0
Giza 135	141.5	140.9	107.5	79.8	103.3	92.5	87.5	85.0	2230	222.7	153.3	151.7
Giza 136	189.0	183.3	151.4	131.9	110	108.2	95.1	90.8	236.7	235.0	191.0	166.3
Giza 2000	181.8	180.9	142.4	125.0	107.5	102.5	950	75.8	236.3	235.3	153.3	127.3
average	**151.2**	**146.7**	**109.9**	**78.5**	**103.9**	**98.9**	**92.2**	**84.5**	**227.8**	**209.5**	**177.9**	**141.6**
LSD 0.05	**1.5**	**13.0**	**5.7**	**5.6**	**4.4**	**1.83**	**5.21**	**2.75**	**20.9**	**7.03**	**6.8**	**6.59**
CV %	**1.3**	**1.6**	**3.7**	**5.5**	**2.27**	**1.56**	**3.7**	**2.19**	**6.9**	**2.19**	**2.5**	**3.0**

C, Control (0.6 dSm^{-1}); S_1, 5 dSm^{-1}; S_2, 10dSm^{-1}; S_3, 15 dSm^{-1}.

Polymorphism information content (PIC) values were used to distinguish between cultivars for each primer (Anderson et al., 1993). Cluster analysis was performed to produce a dendrogram using un-weighted pair-group method with arithmetical average (UPGMA) (Hammer et al., 2001).

3. Results and Discussion

3.1. Agro-Morphological Traits

Combined analysis of variance showed significant differences among all cultivars for days to heading (HD), plant height (PH) number of spikes m^{-2} (SM^{-2}), number of grains $spike^{-1}$ (GS^{-1}), 1000 grain weight (g) and grain yield (GY g/plot) under all different concentrations of irrigation of sea water during the two growing seasons 2014-15 and 2105-16 (Table 4). Interaction between cultivars and salinity treatments and interaction between cultivar with seasons showed highly significant differences in all traits except GS^{-1} indicated that cultivars performance changed over different salinity levels and over the seasons.

Table 6. The respective reduction values in traits of barley cultivars irrigated with different levels of water salinity (average of two growing seasons).

Cultivars	Days to heading			Plant height (cm)			No. spike m^{-2}			No. grains $spike^{-1}$			1000-grain weight (g)			grain yield (g $plot^{-1}$)		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
Giza 123	2.1	3.3	2.1	2.2	8.7	12.9	2.0	20.6	39.2	9.1	12.1	21.2	0.0	25.0	48.3	3.1	16.7	28.4
Giza 124	2.3	3.8	5.3	3.1	16.2	17.6	0.2	4.9	32.1	6.9	17.2	37.9	10.6	21.3	31.9	7.2	35.0	71.9
Giza 125	0.2	6.2	7.4	3.2	8.0	15.3	0.5	18.0	41.7	3.3	30.0	33.3	5.6	9.3	31.5	2.0	30.0	54.3
Giza 126	9.6	6.1	5.1	1.7	4.3	9.4	0.8	6.8	33.7	1.5	24.2	41.4	8.2	24.5	34.7	5.2	24.2	56.1
Giza 127	3.4	13.6	11.2	3.2	8.8	21.9	2.2	14.4	32.2	7.2	31.2	40.9	2.1	22.9	37.5	2.2	32.1	55.1
Giza 128	7.3	9.0	9.4	4.8	10.3	21.4	0.2	8.3	42.1	8.3	11.3	32.1	7.4	24.1	44.4	3.2	25.4	47.9
Giza 129	3.0	5.9	7.3	3.4	12.8	15.4	0.2	16.8	39.8	3.3	20.0	40.0	7.8	23.5	39.2	4.7	33.0	76.6
Giza 130	9.5	11.9	6.3	3.2	12.1	15.0	9.9	24.6	27.0	8.3	16.7	27.8	7.7	13.5	30.8	2.9	48.2	44.0
Giza 131	3.9	7.2	5.9	9.1	15.2	21.2	1.0	22.4	39.8	8.3	16.7	25.0	14.3	36.5	42.9	3.3	19.2	29.6
Giza 132	1.5	4.7	2.8	4.5	13.3	21.2	3.2	26.7	33.8	6.9	17.2	43.1	14.9	21.3	53.2	0.9	33.5	71.7
Giza 133	2.2	6.8	6.8	5.5	11.7	18.7	0.2	24.1	30.4	14.3	27.1	44.3	6.1	18.4	32.7	2.9	31.0	58.2
Giza 134	3.8	4.7	5.8	10.2	17.3	26.0	4.4	14.1	33.0	8.3	18.3	35.0	17.2	25.9	39.7	3.0	27.6	48.5
Giza 135	2.9	5.0	5.9	10.5	15.3	17.7	0.1	31.3	32.0	9.7	21.0	27.4	9.4	18.9	32.1	0.4	24.0	43.6
Giza 136	4.7	7.3	11.8	1.6	13.5	17.5	0.7	19.3	29.7	7.0	18.3	29.6	4.6	18.5	44.6	3.0	19.9	30.2
Giza 2000	2.6	4.9	6.8	4.7	11.6	29.5	0.4	35.1	46.1	0.0	16.7	25.0	23.7	33.9	45.8	0.5	21.7	31.2
Average %	3.9	6.7	6.7	4.7	11.9	18.7	1.7	19.2	35.5	6.8	19.9	33.6	9.3	22.5	39.3	3.0	28.1	49.8

C, Control (0.6 dSm^{-1}); S_1, 5 dSm^{-1}; S_2, 10dSm^{-1}; S_3, 15 dSm^{-1}.

Salinity stress affects most of traits in barley (Bchini et al., 2011). Similarly, means of days to heading decreased as salinity level increased (Oraby et al., 2005). On the basis of mean performances, the results showed that all studied traits were decreased with increasing the concentrations of sea water from 5 to15 dSm^{-1} for all cultivars compare with control as shown in Table 5. Results clearly indicated that all cultivars were differed significantly in days to heading DH. The earliness cultivar among the fifteen cultivars was Giza 123 under control and all the saline water concentrations with values (75.8, 73.5, 71.3 and 70.3 days) respectively. The latest cultivar was Giza 132 under control and all the salinity treatments. For plant height PH, tallest cultivar was Giza 136 among all the cultivars under control and sea water concentrations recorded (110.0, 108.2, 95.1 and 90.8cm). Data in Table 5 showed that Giza131 had the highest number of spikes m^{-2} when it was irrigated with different salinity concentration (0.6, 5,10 and 15 dSm^{-1}) of sea water with values of 296.7,293.3, 230.0, 178.8 spikes m^{-2}, respectively.

Results indicated that the highest number of grains $spike^{-1}$ was produced by Giza 2000 under all salinity treatments. Giza 136 gave the highest values of 1000 grain weight with values (6.5, 6.2, 5.3 and 3.6 g) after irrigated with three salinity treatments. Concerning, grain yield data showed the highest yield production was produced by Giza 123 under control condition (189.4 g/plot), low salinity (183.5 g/plot), moderated salinity (157.8 g/plot) and high salinity (135.6 g/plot) treatments. In the current study, the results revealed wide response variations among the cultivars for saline water stress.

Table 7. Salt tolerance indices of the barley cultivars under different salinity levels (average of two growing seasons)

Cultivars	**Stress Susceptibility Index (SSI)**			**Tolerance index (TOL)**			**Stress Tolerance Index (STI)**			**Mean productivity (MP)**			**Geometric Mean Productivity (GMP)**		
	S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
Giza 123	1.1	0.7	0.6	5.9	38	53.8	1.5	1.3	1.1	186.5	170.4	162.5	186.4	169.3	160.3
Giza 124	2.4	1.3	1.5	10.5	51.1	105	0.9	0.6	0.3	140.8	120.5	93.5	140.7	117.7	77.4
Giza 125	0.7	1.1	1.1	3.1	46.6	84.3	1.0	0.7	0.5	153.7	131.9	113.1	153.6	129.8	104.9
Giza 126	1.0	0.9	1.2	7.2	33.6	78	0.8	0.6	0.4	135.5	122.3	100.1	135.5	121.1	92.2
Giza 127	0.7	1.2	1.1	3.0	43.7	75.1	0.8	0.5	0.4	134.7	114.4	98.7	134.7	112.2	91.2
Giza 128	1.1	0.9	1.0	4.0	32.2	60.7	0.7	0.5	0.4	124.7	110.6	96.4	124.7	109.4	91.4
Giza 129	1.6	1.2	1.6	6.3	44.5	103.3	0.8	0.5	0.2	131.8	112.7	83.3	131.7	110.4	65.3
Giza 130	1.0	1.8	0.9	3.9	64.6	58.9	0.8	0.4	0.4	132.0	101.6	104.5	131.9	96.3	100.2
Giza 131	1.1	0.7	0.6	6.2	35.8	55.2	1.5	1.2	1.1	183.4	168.6	158.9	183.4	167.6	156.5
Giza 132	1.3	1.2	1.5	1.1	41.4	88.7	0.7	0.4	0.2	123.2	103.0	79.4	123.1	100.9	65.8
Giza 133	1.0	1.1	1.2	4.1	44.5	83.6	0.9	0.6	0.4	141.6	121.4	101.8	141.5	119.3	92.8
Giza 134	1.0	1.0	1.0	4.2	38.5	67.7	0.8	0.6	0.4	137.5	120.4	105.8	137.5	118.8	100.2
Giza 135	0.1	0.9	0.9	0.6	34	61.7	0.9	0.7	0.5	141.2	124.5	110.7	141.2	123.3	106.3
Giza 136	1.0	0.6	0.6	5.7	31.2	57.1	1.5	1.3	1.1	186.2	173.4	160.5	186.1	172.7	157.9
Giza 2000	0.2	0.8	0.6	0.9	39.4	56.8	1.4	1.1	1.0	181.4	39.4	153.4	181.3	160.9	150.7

S_1, 5 dSm^{-1}; S_2, 10dSm^{-1}; S_3, 15 dSm^{-1}.

Table 8: Correlation coefficients between salt stress indices and grain yield of 15 barley cultivars under different salinity levels during two growing season

Salinity levels	Traits	GY	SSI	TOL	STI	MP
S1 (5 dSm^{-1}) (low salinity)	SSI	-0.186				
	TOL	0.041	0.965**			
	STI	0.99**	-0.119	0.114		
	MP	0.99**	-0.131	0.098	0.995**	
	GMP	0.99**	-0.131	0.098	0.995**	1.000**
S2 (10dSm^{1}) (moderated salinity)	SSI	-0.858**				
	TOL	-0.583**	0.908*			
	STI	0.986*	-0.776*	-0.465		
	MP	0.440*	-0.396	-0.297	0.457	
	GMP	0.994**	-0.801*	-0.494	0.994	0.431**
S3 (15 dSm^{-1}) (high salinity)	SSI	-0957**				
	TOL	-0.828**	0.938**			
	STI	0.982**	-0.894*	-0.725*		
	MP	0.985**	-0.901*	-0.723*	0.995*	
	GMP	0.966**	-0.939*	-0.785*	0.990*	0.995**

GMP, Geometric mean productivity; GY, Grain yield; MP, Mean productivity; SSI, Stress susceptibility index; STI, Stress tolerance index; TOL, Tolerance index.

Barely cultivars (Giza 131, Giza 136, Giza 123 and Giza 2000) had the highest values of all traits, potentially salt tolerant. However, Giza 132, Giza 129 and Giza 124 had lowest values of these traits, those cultivars were salinity sensitive. Similar genotypic variations were reported earlier (EL-Doskoy et al., 2007; El-Sodany, 2004; Hammami et al., 2016). They concluded that exposure of barely plants to saline water irrigation caused significant reduction in plant height, spike length, number of grains per spike, grain weight per spike, straw and grain yields. Moreover, based on stability parameters, barley genotypes with more salinity tolerance were more phenotypically stable under saline water irrigation (Askari et al., 2017).

The percentages of salinity reduction in all cultivars for all studied traits (Table 6) were increased with the increase concentration of sea water irrigation (Table 6). The Respective reduction values in days to heading under different irrigation of sea water treatments were 3.9, 6.7 and 6.7%, respectively. Meanwhile, the respective reduction values in plant height under (5, 10 and 15 dSm^{-1}) were 4.7, 11.9 and 18.7% respectively, and the lowest cultivar reduction under S_3 was Giza 126 (9.4%). Moreover, the average respective reduction values in number of spikes m^{-2} were 1.7, 19.2 and 35.5%, under (5,10 and 15 dSm^{-1}) irrigation with saline water respectively, and the lowest cultivar reduction was detect by Giza 130 (2.0%). The reduction in number of grains $spike^{-1}$ under (5, 10 and 15 dSm^{-1}) irrigation of sea water was 0.8, 19.9 and 33.6% respectively, and Giza 123 had the lowest reduction among the cultivars was (31.2%). The reduction in 1000-grain weight for studied cultivars was increased with increasing salinity level of irrigation water.

The magnitudes of reduction of grain yield were 3.0% under irrigation by sea water concentration (5dSm^{-1}), 28.1% when irrigated by sea water concentration (10 dSm^{-1}) and 49.8% reduction when irrigated by 15 dSm^{-1}sea water concentration. The lowest cultivars reduction was observed by Giza 123 was (36.4%), while the highest cultivar reduction was detect by Giza 129 was 76.7%. These results are in agreement with the previous reports (Abd El Wahed et al., 2015; Allel et al., 2106; Hussian et al., 2009) who reported that grain yield reduction could be attributed to the reduction plant growth and yield components. Ayers et al., (1952) reported that if the reduction in yield was 50% this was considered as an uneconomic yield. In our study reduction in Giza 123, Giza 131, Giza136 and Giza 200 cultivars were less than 50%, so theses cultivars can be grown successfully up to EC15 dSm^{-1} of irrigation of sea water and had economic yield.

3.2. Effect of Sea Water Irrigation on Salt Stress Indices in Barley Cultivars

Responses of 15 Egyptian barley cultivars for saline water tolerant which were evaluated by using a set of stress indices including stress susceptibility index (SSI), tolerance index (TOL), salt tolerance index (STI), mean productivity (MP) and geometric mean productivity (GMP) were calculated on the basis of grain yield of barley cultivars over the two seasons (2014-15 and 2015-16) are presented in Table 7.

Table 9. Principal component analysis for morphological traits and salinity stress indices of barley cultivars

Cultivars/ Traits	PCA1	PCA2	PCA3	PCA4	PCA5
Salt Stress Indices					
Stress susceptibility index (SSI)	-4.57	-0.01	-0.16	0.09	0.05
Tolerance index (TOL)	-0.09	-1.22	0.47	0.06	0.45
Stress tolerance index (STI)	4.61	0.02	-0.17	0.09	0.05
Mean productivity (MP)	2.51	0.96	0.04	0.04	-0.04
Geometric mean productivity (GMP)	2.01	-1.26	-0.10	0.05	-0.17
Agronomic Traits					
Days to Heading (day)	0.60	-0.60	0.09	-0.07	-0.24
Plant height (cm)	0.77	-0.45	0.19	-0.11	-0.10
No grains spike $^{-1}$ (g)	-2.25	0.02	0.14	-0.18	-0.06
No spikes m^{-2}	4.34	-0.19	-0.13	-0.22	-0.69
1000-grain weight (g)	-4.43	0.01	-0.17	0.07	0.04
Grain yield (g plot^{-1})	0.14	1.58	-0.28	0.05	-0.24
Cultivars					
Giza 123	6.41	-1.37	-0.20	-1.33	-0.49
Giza 124	-2.64	4.88	0.15	-1.38	0.64
Giza 125	1.55	0.76	1.15	0.27	-1.12
Giza 126	-1.39	0.67	0.87	-0.98	1.30
Giza 127	-3.01	-1.31	-0.73	-2.51	-1.40
Giza 128	-2.94	-3.91	1.71	-3.35	-0.29
Giza 129	-3.95	1.70	0.74	-0.37	1.20
Giza 130	-2.27	0.56	0.29	2.42	-3.69
Giza 131	6.17	-0.49	1.59	-0.35	0.57
Giza 132	-4.78	1.62	-2.22	1.67	1.18
Giza 133	-1.78	1.12	-0.67	2.23	1.00
Giza 134	-0.74	-2.03	1.78	0.66	0.15
Giza 135	-0.37	-3.10	0.15	2.78	0.65
Giza 136	7.17	-0.16	1.40	1.30	0.47
Giza 2000	3.59	-0.76	-6.03	-1.04	-0.16
Eigen value	15.566	4.745	3.972	3.363	1.721
Variance%	47.171	14.378	12.037	10.190	5.214
Cumulative variance (%)	47.17	61.52	73.64	83.84	89.01

PCA, principal component analysis.

The results showed the highest values of MP, GMP and STI were observed by Giza 131, Giza 123, Giza 136 and Giza 2000 after irrigated by sea water. Data (Table 8) showed that grain yield had a significant and positive correlation with MP, GMP and STI under all sea water irrigation concentration.

SSI was significantly negative correlated with grain yield under moderated and high salinity treatments (Table 8). Giza 2000, Giza 131, Giza 136 and Giza 123 with a lower SSI were identified as the most tolerant cultivars whereas Giza 124, Giza 129 and Giza 132, with the highest SSI were identified as the most tolerant cultivars sensitive (Table 7). TOL index had a positive correlation with grain yield under low salinity 5 dSm^{-1}, while had a negative correlation with and grain yield under moderated (10 dSm^{-1} and high salinity 15 dSm^{-1}), and had a significant and positive correlation with SSI under low, moderated and high salt stress (Table 8). This result is consistent with the findings of (Bchini et al., 2011; Ravari et al., 2016 and Taherian et al., 2017). They reported that Both of MP, GMP and STI were proved to be 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 tolerant cultivars under increased degree of stress. Similarly, the genotypes classification is closely similar based to mean MP, GMP and STI, they were positively correlated with respect to grain yield under stress and non-conditions (Naderi et al 1999). Consequently, GMP is a good indicator for evaluating genotype tolerance under salinity stress and possesses a similar level with STI. The higher STI values show the higher genotype tolerance and also yield potential under stress.

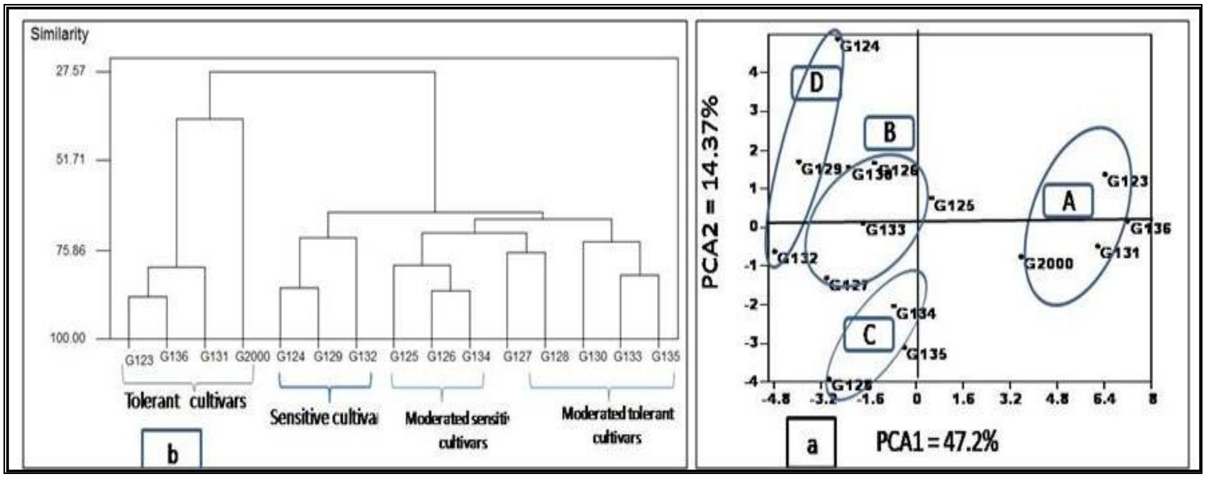

Fig.1. cluster analysis (b) and biplot diagram (a) based on agro– morphological traits and salt stress indices for 15 Egyptian barley cultivars under saline water stress.

3.3. Phenotypic Diversity in Barley Against Salinity Tolerance Based on Multivariate Analysis

In order to further investigate relationship among Egyptian barley cultivars principal component analysis (PCA) were performed (Table 9) using Nei's distance matrix, in order to further classification of barley cultivars into groups based on their morphological traits and stress tolerance indices. PCA was justifying about 61.56 % of total variance. The first PCA1 clarified 47.17 % of total variation prejudiced by days to heading, plant height, number spikes m^{-2} and grain yield with a positive and high values with STI, MP and GMP, while had negative and low values and with TOL and SSI. PCA1 identified as tolerance components.

Table 10. SRAP Primers combinations name, sequences, number of total fragment, number of polymorphic fragments, Percentage of polymorphic fragments polymorphic information content.

No.	name	primer sequences	NTF	NPF	PPF	PIC
1	me1+ em1	F: TGAGTCCAAACCGGATA R: GACTGCGTACGAATTAAT	7	4	57.1	0.60
2	me1+em2	F|:TGAGTCCAAACCGGATA R:GACTGCGTACGAATTTGC	9	5	55.6	0.56
3	me1+em3	F: TGAGTCCAAACCGGATA R: GACTGCGTACGAATTGAC	8	5	62.5	0.65
4	me2+em1	F: TGAGTCCAAACCGGAGC R:GACTGCGTACGAATTAAT	10	5	50.0	0.51
5	me2+em2	F:TGAGTCCAAACCGGAGC R:GACTGCGTACGAATTTGC	6	2	33.3	0.35
6	me4+em5	F:TGAGTCCAAACCGGAGC R:GACTGCGTACGAATTGAC	7	3	42.9	0.43
7	me5+em4	F:GAGTCCAAACCGGAAG R:GACTGCGTACGAATTAAT	12	7	58.3	0.61
8	me5+em5	F:GAGTCCAAACCGGAAG R:GACTGCGTACGAATTTGC	13	13	100.0	0.96
9	me5+em6	F: GAGTCCAAACCGGAAG R: GACTGCGTACGAATTGAC	15	12	80.0	0.89
10	me6+em5	F: TGA GTC CAA ACC GGA CA R: GACTGCGTACGAATTTGC	11	8	72.7	0.73
Average			9.8	6.4	61.4	0.63
Total			98	64		

F, Forward primer sequences; R, Reverse primer sequences; NTF, Number of Total fragment; NPF, number of polymorphic fragments; PPF, Percentage of polymorphic fragments; PIC, polymorphic information content.

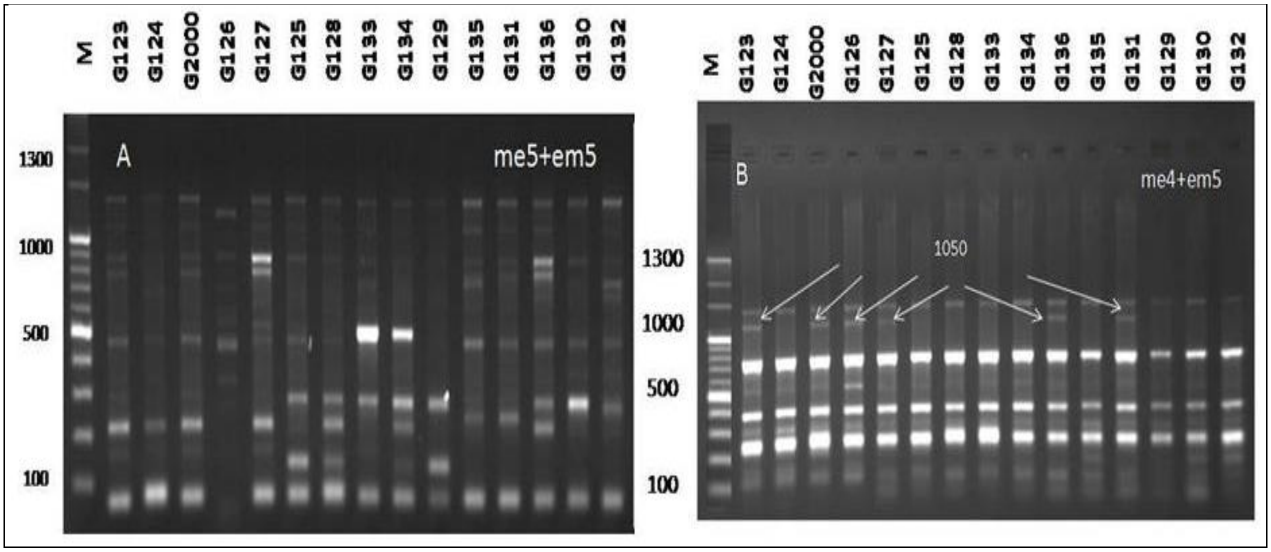

Fig. 2. Amplification results of the primers combination of SRAP primers (A) me5+em5, (B) me 4+ em5 in 15 Egyptian barley cultivars.

Second PCA2 clarified that 14.37 % of the total variability influenced by number grains spike^{-1} and 1000 grain weight with positively correlated with MP, STI and GMP and negatively correlated with SSI and TOL. Biplot diagram and cluster analysis based on PCA1, PCA2 and stress tolerance indices, were graphically shown in a (Fig. 1a and b).

Both biplot and cluster analysis had divided all cultivars into four groups named A, B, C and D groups. Group A include tolerant cultivar (T) which had high yield under control and under stress conditions such as Giza 123, Giza 131, Giza 136 and Giza 2000. Group B containing cultivars Giza 126, Giza 127, Giza 133, Giza 130 and Giza 135, showed moderate tolerance (MT) against saline water stress,. D group (S) include the saline water stress sensitive cultivars had lowest yield under control and stress conditions such as Giza 129, Giza 124 and Giza 132). C group include the moderate sensitive saline water stress cultivars (MS) include Giza 134, Giza 128 and Giza 125 cultivars. These results are similar with the earlier reports (Fernandez, 1992; Sharafi et al., 2013; Taherian et al., 2017; Yan and Kang , 2003) whom used multivariate analysis to classification barley genotypes for salinity.

Table 11. Genetic diversity among 15 barley cultivars using 10 SRAP primer combinations

Cultivars	Total Polymorphic Band	PPF	Shannon's Information Index
G123	80	81.63	4.38
G124	67	68.37	4.21
G125	79	80.61	4.37
G126	71	72.45	4.26
G127	76	77.55	4.33
G128	76	77.55	4.33
G2000	77	78.57	4.34
G136	72	73.47	4.28
G133	72	73.47	4.28
G134	77	78.57	4.34
G135	73	74.49	4.29
G136	81	82.65	4.39
G129	70	71.43	4.25
G130	75	76.53	4.32
G131	90	91.84	4.50
Average	75.7	77.3	4.3

PPF, Percentage of polymorphic fragments

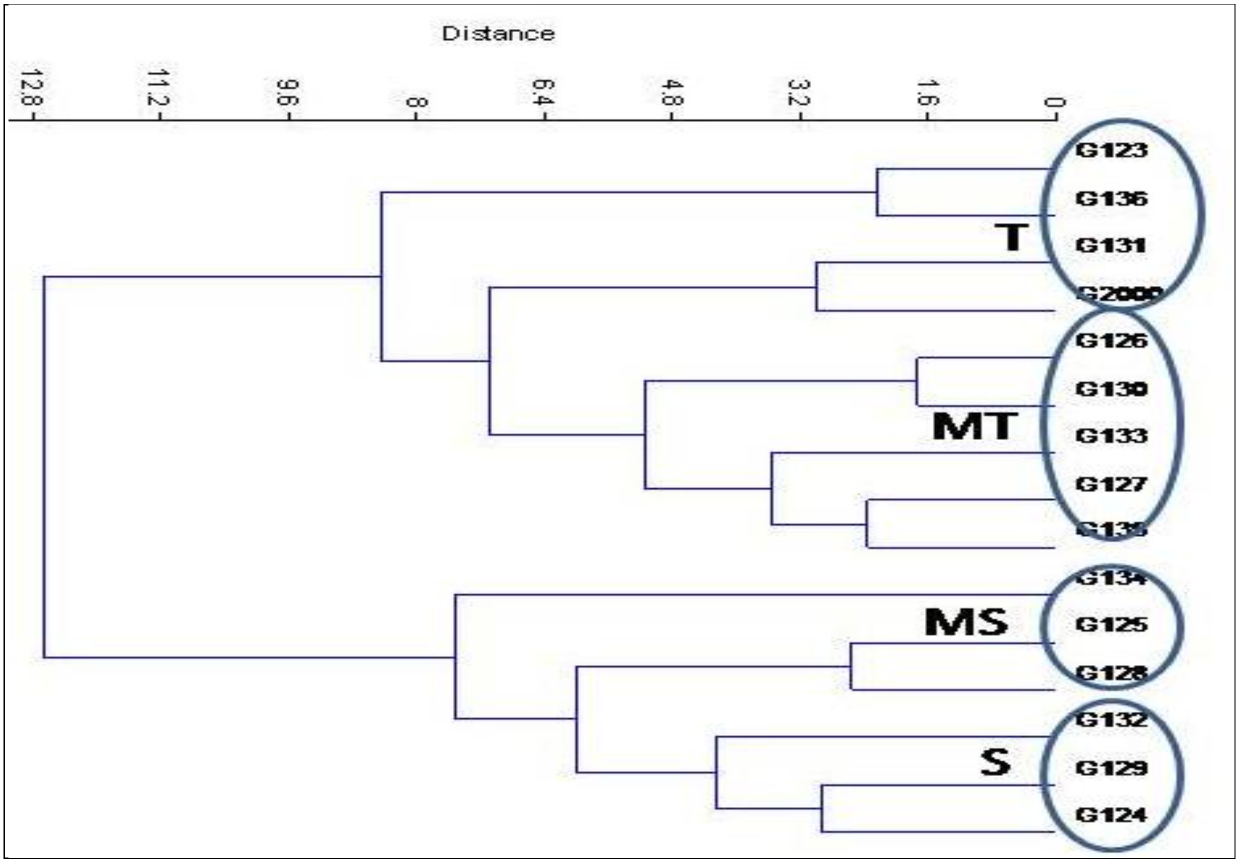

Fig. 3. Cluster analysis of 10 primers combination of SRAP for 15 Egyptian barley cultivars.

3.4. SRAP Marker Analysis

SRAP marker systems using selected ten different SRAP primer combinations was applied to assess the level of genetic diversity in fifteen Egyptian barley cultivars for saline water tolerant. Data in Table 10 showed that the total fragments were 98, in which 64 fragments were polymorphic, however 34 were monomorphic.

The band number for each pair of primers was ranged from six bands in (me2+em2) to fifteen bands in (me5+ em6) with an average of 6.4% per primer combination. The percentage of polymorphism for each primer combination varied from 33.3% (me3+em4) to 100% (me5+em5) with average 61.4% (Fig. 2a). The amplified fragments ranged from 50bp to 1300 bp as shown in (Fig. 2a and b).The primer me4+em5 had specific bands found in tolerant cultivars with size of 1050 bp (Fig. 2 b).Polymorphic information content (PIC) values were evaluated to assess the genetic diversity for ten selected primers, ranging from lowest PIC value (0.35 %) related to primer combination me3+em4 to highest PIC value (0.96%), which was related to primer combination me5+em5. The primer combination me5+em5 was highly informative and could be useful primer set to confirm the genetic differences among barley cultivars for saline water tolerant.

The percentage of polymorphic loci of fifteen cultivars ranged from 71.43% (Giza 129) to 91.64% (Giza 131). The obtained Shannon's information index ranged from 4.21 (Giza 124) to 4.50 (Giza 131) (Table 11). In this study SRAP marker gave 98 alleles which were amplified by ten primer combinations in 15 cultivars. This alleles number was higher than any other DNA marker used for the genetic diversity for salinity tolerant on the barley such as RPAD (Mariey, 2004), SSR (Mariey et al., 2013; Mariey et al., 2016) and ISSR (Khatab and Mariey, 2013). The high polymorphic rate (100%) and PIC value (0.96), together with the moderated genetic similarity (0.62) observed among 15 cultivars suggested a high level of heterogeneity. The high polymorphism rate coincides with those reported earlier (Mariey et al., 2105; Yang et al., 2008; Yang et al., 2010). They used SRAP marker to evaluate the genetic diversity in barley.

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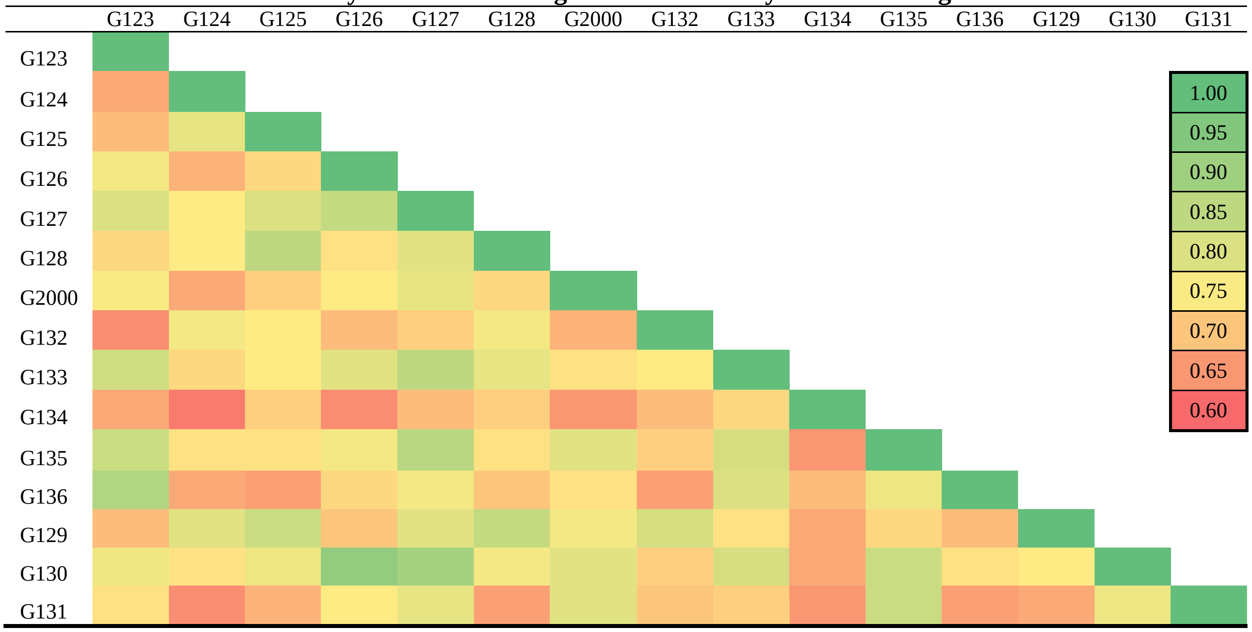
Table 12. Genetic similarity coefficient among the studied barley cultivars using SRAP



SRAP can be used to perform genetic diversity analysis in a wide range of living organisms (Li et al., 2013). It has shown a high degree of reproducibility and discriminatory power and mainly targets exons which are expected to be evenly distributed along all chromosomes with GC-rich regions and introns with AT-rich regions (Li and Quiros, 2001).

3.5. Cluster Analysis Based on SRAP Marker

Cluster analysis formed a dendrogram among the 15 Egyptian barley cultivars based on ten SRAP primer combinations using Jaccard's genetic similarity coefficient was outlined by the Un weighted Pair-Group Method (Fig. 3). The dendrogram of SRAP markers had clustered all the Egyptian cultivars in to four groups. Each group include the most closed cultivars together according their response to saline water stress and the genetic similarity coefficient among barley cultivars ranged from low similarity (62%) between Giza 134 and Giza 124 which proposed that these cultivars were the least-related cultivars to highest similarity (92%) between Giza 126 and followed by (89%) similarity between Giza 127, Giza 130 and GCS was 87% between Giza 136 and Giza 123 indicating that was a very close relationship among these cultivars as shown in Table 12. Cluster analysis demonstrated clear genetic relationships among 15 barley cultivars. Similarly, Li et al., (2013) and Mariey et al., (2015) used SRAP marker system is simple and efficient system and was applicable for the molecular characterization and the investigation of phylogenic relationships among cultivar or genotypes and has potential for marker-aided selection, linkage mapping, evolutionary studies and breeding purposes.

4. Conclusion

The results of this study suggested that barley genotypes Giza 123, Giza 131, Giza 136 and Giza 2000 could be selected to grow under saline water irrigation (up to 15 dSm^{-1}). Overall, it can be concluded that substantial variation in salt tolerance among barley cultivars at the harvest stage was found in this study. Most importantly, STI, MP, GMP parameters could be considered as useful selection criteria for screening the salt tolerance in terms of grain yield among studied barley cultivars. SRAP marker found to be efficient to evaluate the genetic diversity of salinity tolerance of Egyptian barley cultivars.

List of Abbreviations: ECw, Electrical Conductivities for water; GMP, Geometric mean productivity; NPF, number of polymorphic fragments; NTF, Number of Total fragment; PIC, Polymorphism information content; PPF, Percentage of polymorphic fragments; RCBD, Randomized complete block design; SRAP, Sequence related amplified polymorphism; SSI, Stress susceptibility index; STI, Stress tolerance index; TOL, Tolerance index; UPGMA, Un weighted pair-group method with arithmetical average.

Authors Contribution: All authors contributed to the design and implementation of the research, to the

analysis of the results and to the writing of the manuscript.

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Conflict of Interest: The authors declare that they have no competing interests.

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