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Effect of salinity stress on physiological and biochemical traits of barley cultivars

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ABSTRACT

To assess the effect of salinity stress on physiological and biochemical traits, germination and lyisometer experiments were carried out on 15 Egyptian barley cultivars. The experiments were done during two growing seasons 2014/2015 and 2015/2016. Four different concentrations of salinity were used (0.6, 5.0, 10.0, and 15.0 dSm^{-1}). The 15 cultivars showed statistically significant responses to salinity stress. Based on germination experiment Giza 123,128, 131,136 and 2000 cultivars gave high values for germination percentage, vigorous seedling, relative water content, and salt tolerance index. The salt tolerance index can be used as an effective criterion to determine and select tolerant genotypes. From lyisometer experiment the same cultivars had high total chlorophyll content, large flag leaf area, high enzymes activity (peroxidase, POD and catalase, CAT) and high proline content. The higher efficiency of the proline content and antioxidant activities in barley can be considered as one of the factors responsible for their tolerance against salinity, which played an important role through defense system induced by salinity. The SDS–PAGE revealed that the soluble protein accumulation increased in barley shoot more than roots under the different salinity concentrations. Eighty polymorphic bands were detected in all cultivars based on their gene expression of shoot and root seedling under salinity treatments and control with molecular weight ranging from 10 to 250 KDa. The results indicated that Giza 123,128, 131,136 and 2000 cultivars could be consider as a tolerant cultivars and can be using them in salinity breeding programs

Key words: *Hordeum vulgar*, Salinity tolerance, Seed germination, Physiological parameters, SDS-PAGE protein

Introduction

Salinity stress negatively affects agricultural yield all over the world affecting production whether it is for subsistence or economic gain by more than 50 % (Ashraf *et al*., 2008). Salinity problems are increasing in many countries around the world, Egypt is one of these countries which 33% of the cultivated land which comprises only 4% of the total land area in Egypt is already salinized due to low rainfall and irrigation with saline water (Abdel-Latef, 2005). Barley is considered a major source of food and feed for a great number of animal and people especially who are living in areas affected by salinity. It is also considered more tolerant to unfavorable environmental conditions such as drought and salinity than any other cereals, which are less adapted, that are cultivated under unfavorable conditions which are for other cereals (Abu El-lail *et al.,* 2014).

Salinity breeding programs requires many steps, searching for genetic resources, searching for efficient selection criteria and tools for improvement of salt tolerant genotypes (Ashraf and Akram, 2009). Germination and seedling stages are very sensitive to salinity and widely used as screening criteria to select salt tolerant genotypes (El-Dardiry, 2007). Antioxidant enzyme activities are good criteria for selecting salt tolerance genotypes in barley of which salt tolerant cultivars have higher antioxidant enzyme activities than the salt sensitive ones (Xiaoli *et al.,* 2009).

Proline accumulation is one of the communal characteristics in many monocotyledons under saline conditions (Ashraf and Foolad, 2007), where the rate of proline accumulation is increased in barley plants under salt stress (Turkyilmaz *et al*., 2014 and Mariey *et al.* 2016).

Fingerprint markers have been widely used as express and correct analysis to identify and characterize different crop cultivars according their genetic potentialities. In barley (Mariey, 2004; El-Hamamsy and Behairy, 2015 and Hellal *et al.,* 2017) used SDS-PAGE method to screen the total

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soluble protein for salinity tolerance analysis, and found high differences parents and recommended as a good selection criteria to improvement of salt tolerance .Therefore, the objective of the current study was to evaluate the effect of using different salinity concentrations on seed germination , seedling characteristics, some physiological traits and biochemical markers in order to classify 15 Egyptian barley cultivars and to use the information in barley breeding programmers .

Materials and Methods

Fifteen Egyptian barley cultivars were kindly provided by Barley Res, Field Crops Res., Institute, Agric., Res., Center, Egypt (Table 1).

Table 1: Name, and pedigree of 15 barley cultivars used in the studied experiment

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	Baladi Bahteem/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	Deir Alla 106/Cel//As46/Aths*2"
8	Giza 130	Comp.cross"229//Bco.Mr./DZ02391/3/Deir Alla 106
9	Giza 131	CM67B/CENTENO//CAMB/3/ROW906.73/4/GLORIABAR/ 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//GLORIABAR/COPAL/3/SEN/5/AYAROS
14	Giza 136	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
15	Giza 2000	Giza117/Bahteem52// Giza118/ FAO86 / 3/Baladi16/ Gem

Germination test was carried out at growth chamber of plant breeding and biotechnology laboratory, Barley Res., Sakha Res., Station in 2015/2016. Growth conditions in incubator was (20-25 ^{o}C, relative humidity of 55-60% and 16 h light period) to study the effect of salinity stress on germination percentage and their seedling traits of 15 Egyptian barley cultivars. Under four levels of electrical conductivities ECw (C (control) tap water= 384 ppm = 0.6 dS m^{-1}, S1 = 4000 ppm = 5 dS m^{-1}, S2=8000 ppm =10 dSm^{-1} and S3= 12000 ppm =15 dS m^{-1}). Saline water was prepared by mixing tap water (0.6 dSm^{-1}) with sea water (48 dSm^{-1}) to achieve required concentration. Each cultivar was surface sterilized with 5% sodium hypochlorite solution for 10 min then rinsed with sterile distilled water three times, after that placed on filter paper into 9 cm diameter petri dishes (25 seeds per Petri dish). In each Petri dish, 5 ml of specific solution was added when needed. Germination tests were performed according to the techniques specified by the International Seed Testing Association; I.S.T.A. (2008), to evaluated germination percentage , vigorous seedling , and salt tolerance index (STI) which measured as total plant shoot and root dry weight (TDW) and calculated by the following equation: STI % = (TDW salt stress / TDW control) x 100

Lysimeter experiments were carried out at Soil, Water and Environment Research Institute at Sakha Research Station, during two successive winter growing seasons (2014/2015 and 2015 /2016)). The experimental design was conducted as split plot design arranged in a randomized complete block (RCBC). Four water salinity concentrations were used for irrigation: C (as a control) using tap water with ECw (C= 0.6, S1= 5, S2= 10 and S3= 15 dSm^{-1}) as the main plot factor and cultivars as the subplot factor. 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. The chemical properties of the saline water were analyzed according Black (1965) as shown in (Table 2). To study the effect of different salinity concentrations traits Total chlorophyll content, flag leaf area ,Relative water content (RWC) which was calculated as described by Sumithra *et al.* (2006) RWC % = 100 x [(FW – DW) / FW], Antioxidant enzymes such as Catalase (CAT) enzyme activity which was determined in the homogenates by measuring the decrease in absorption at 240 nm according to Sadasivam and Manickam (1996) and Peroxidase (POD) enzyme activity which was measured using guaiacol/H_2O_2

as substrate in absorption at 436 nm according to Lobarzewski *et al.* (1990) and Proline content according to Bates *et al.* (1973) of 15 Egyptian barley cultivars.

Total protein from shoot and root of 15 barley cultivars from four saline water treatments were analyzed using SDS–polyacrylamide gel electrophoresis according to the method of Laemmli, (1970) as modified by Studier, (1973).

Table 2: Chemical properties of tap water and different irrigation water used in the experiment

Chemical properties	(C= 0.6 dsm^{-1})	(S1=5 dsm^{-1})	(S2=10 dsm^{-1})	(S3= 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 ^{1} g 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 $meq100^{-1}$ g soil				
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

Statistical Analysis:

The data of two seasons were homogeneous and statistically analyzed as the randomized complete block design (RCBD) .All statistical analyses were performed using the computer software MSTAT-C Computer Program according to (Snedecor and Cochran, 1969). Cluster analysis was performed to produce a dendrogram using a computer software program Minitab v.12 to get the cluster and genetics similarity based on the morphological data according Kovach (1995). However the PAST program (PAleontological Statistics Version 1.94b) was used to produce a dendrogram based on protein banding pattern which were scored as a binary data as present (1) or absent (0) adapted by Hammer *et al*. (2001).

Results and Discussion

The two-way analysis of variance for germination percent (GP%) , vigorous seedling traits such as shoot and root length, shoot and root fresh weight and shoot and root dry weight and physiological parameters such as leaf area index, total chlorophyll content, relative water content, Catalases (CAT), Peroxidases (POD) and Proline content for 15 Egyptian barley cultivars are shown in (Table 3). The data showed that the cultivars, salinity and interaction between cultivar × salinity expressed significant differences in all traits. These results were in agree with (Hagh *et al*. 2017) who suggested that the effect of salinity, cultivars and their interaction was significant at one percent for germination percentage and seedling traits, (Askari *et al*. 2017) for salt tolerance index, and with El-Hamamsy and Behairy, (2015); Behrouzi *et al.* (2015) and Mariey *et al.* (2016) for physiological parameters.

The effect of different salinity concentrations on seed germination and vigorous seedling are shown in (Table 4). For Germination percentages were decreased in all barley cultivars under the four concentration of saline water. The results revealed that Giza 131,123, 125, 128, 2000, 136 and Giza 135 scored high values of germination percentage of the four concentration of saline water scoring 100% GP under control conditions. Also the same cultivars scored height values under high salinity concentration with values of 78, 74, 70, 70, 68, 67 and 63 % GP, respectively. The lower values were observed by Giza 124 (54%), Giza129 (45%) and Giza 132 (20%) under high salinity treatment.

Concerning Root and Shoot length and their ratio, data in (Table 4 & Fig 1) showed that roots and shoots lengths of the 15 cultivars were decreased as different salinity concentrations increased. The maximum lengths for both root and shoot value was expressed by Giza 131,123, 125, 128, 2000,136 and Giza 135 under all salinity treatments. Whereas, the minimum lengths for both root and shoot values were found in Giza 124, and 132 were under high salinity treatment 15 dS m^{-1}. Root / shoot ratio was affected by different salinity concentrates, the higher ratio under control treatment was

(52.8%) recorded by Giza 128. Whereas under high salinity treatment 15 dS m^{-1} Giza 2000 gave high root /shoot ratio (45.3%).

Regarding the effect of the four concentrations of saline water on shoot and root fresh and dry weight shown in (Table 4). Cultivars Giza 136, 123, 131 and 2000 scored the highest values under all salinity levels for dry and fresh weight, while Giza 124 and132 recorded the lowest values.

The cultivars differed in their sensitivity and tolerance to salinity under different salinity treatments and it was clear that Giza 123,131, 125,128,135,136 and 2000 had high germination percentage, high seedling traits and had more advantages than the other cultivars in their response to salinity stress. Moreover, the increase of salt concentration had a negative effect on seed germination and seedling traits. Similar results have also been reported by in barley (El Goumi *et al.* 2014; El-Hamamsy and Behairy, 2015; Askari *et al.* 2017; Hellal *et al*. 2017 and Hagh *et al*. 2017) they reported that the seed germination and seedling traits were decreased with increasing salinity levels. Such decrease might be caused by the high osmotic pressure of the solutions slowing down the intake of necessary water for germination and by the toxic effect of high salt concentration on embryo growth.

Table 3: Analysis of variance of studied traits for 15 barley cultivars under four salinity concentrations

Studied Traits	Cultivars	Salinity	Salinity x Cultivar	CV%	L.S.D.0.05
Germination percentage (GP %)	**	**	**	**2.31**	**1.06**
vigorous seedling					
Shoot length cm (SL)	**	**	*	**1.32**	**10.5**
Root length cm (RL)	**	**	**	**0.82**	**0.39**
Shoot/root ratio %	**	**	**	**0.42**	**0.14**
Shoot fresh weight mg/plant (SFW)	**	**	**	**1.25**	**7.40**
Root fresh weight mg/plant (RFW)	**	**	**	**3.58**	**0.05**
Shoot dry weight mg/plant (SDW)	**	*	*	**3.92**	**0.005**
Root dry weight mg /plant (RDW)	**	**	**	**13.61**	**0.327**
Slat tolerance (ST)	**	**	**	**10.9**	**7.52**
Physiological traits					
Flag Leaf area cm $^{-2}$ (LA)	**	**	**	**1.04**	**0.46**
Total chlorophyll content SPAD	**	**	**	**0.12**	**0.95**
Relative water content %	**	**	**	**6.98**	**3.02**
Catalaes activity µmol g^{-1}/sec (CAT)	**	**	**	**5.64**	**2.56**
Porxidases activity µmol g^{-1}/sec (POD)	**	**	*	**6.55**	**1.33**
Proline activity µ/mg (PRO)	**	**	**	**1.79**	**1.56**

*and **; indicate significance at 0.05 and 0.01levels, respectively

About Salt Tolerance Index (STI), significant differences among all cultivars were obvious with the four different salinity levels. Data in (Table 4) showed that salt tolerance index was negatively affected by salt stress in all cultivars and varied between 20 % recorded by Giza 132 on high salinity S3 to 97.8% recorded by Giza 2000 in low salinity S15dSm^{-1}. This result is agree with El Goumi *et al.* (2014) and Askari *et al*. (2017) which they reported that the salt tolerance index, is a function of both seed germination and total seedling dry weight, is considered to be a reliable criterion for salt tolerance.

Fig. 1: Effect of four concentration of saline water on shoot and root length of fifteen barley cultivars

Table 4: Effect of different salinity concentrations on morphological traits of 15 barley cultivars from germination test

Cultivars	Salinity Treatments dSm^{-1}	Germanton Percentage %	Length		Root /shoot ratio	Fresh weight		Dry weight		Salt tolerance %
			Shoot cm	Root cm		Shoot mg/plant	Root mg/plant	Shoot mg/plant	Root mg/plant	
Giza 123	C= 0.6	100	15.67	5.67	36.17	1.555	0.955	0.71	0.45	100.0
	S1= 5	93	14.67	5.50	37.50	1.031	0.531	0.64	0.41	96.6
	S2=10	83	13.67	5.00	36.57	1.010	0.410	0.58	0.30	94.2
	S3=15	74	12.67	3.00	23.67	0.739	0.339	0.27	0.15	85.2
Giza 124	C= 0.6	90	13.67	5.33	39.02	0.752	0.406	0.27	0.21	100.0
	S1= 5	82	12.33	4.00	32.43	0.637	0.277	0.26	0.19	85.9
	S2=10	73	7.67	2.00	26.07	0.269	0.230	0.15	0.06	72.9
	S3=15	50	1.50	0.50	33.00	0.200	0.015	0.10	0.02	65.3
Giza 125	C= 0.6	100	15.00	6.33	42.22	1.077	0.537	0.88	0.49	100.0
	S1= 5	95	13.67	5.33	39.02	0.983	0.383	0.81	0.41	92.9
	S2=10	80	10.33	4.00	38.72	0.825	0.225	0.65	0.31	76.1
	S3=15	70	6.00	2.00	33.33	0.355	0.140	0.25	0.15	61.3
Giza 126	C= 0.6	93	12.67	5.33	42.11	1.006	0.477	0.78	0.33	100.0
	S1= 5	90	11.67	4.00	34.29	0.777	0.333	0.75	0.23	90.0
	S2=10	82	9.67	2.67	27.59	0.591	0.250	0.54	0.15	78.8
	S3=15	58	6.00	1.33	22.22	0.451	0.120	0.35	0.10	70.5
Giza 127	C= 0.6	95	14.00	5.67	40.50	1.222	0.622	0.67	0.26	100.0
	S1= 5	93	12.67	4.50	35.51	1.058	0.558	0.60	0.24	91.5
	S2=10	83	8.67	3.00	34.60	0.637	0.237	0.51	0.22	86.9
	S3=15	65	7.00	2.00	28.57	0.569	0.169	0.45	0.16	80.9
Giza 128	C= 0.6	100	12.00	6.33	52.78	0.938	0.486	0.77	0.25	100.0
	S1= 5	98	10.33	4.67	45.16	0.774	0.348	0.67	0.21	97.7
	S2=10	93	8.00	3.67	45.83	0.618	0.274	0.37	0.18	81.3
	S3=15	70	6.00	2.67	44.44	0.348	0.133	0.19	0.12	70.1
Giza 129	C= 0.6	93	12.33	6.67	54.09	0.730	0.355	0.73	0.27	100.0
	S1= 5	90	11.00	5.50	50.00	0.616	0.253	0.62	0.25	92.7
	S2=10	82	7.67	3.00	39.11	0.464	0.116	0.45	0.21	73.0
	S3=15	45	2.97	1.0	33.67	0.340	0.090	0.35	0.17	63.8
Giza 130	C= 0.6	95	13.67	6.50	47.45	0.860	0.618	0.86	0.27	100.0
	S1= 5	90	12.00	5.50	45.83	0.750	0.260	0.75	0.25	88.4
	S2=10	82	8.67	3.67	45.83	0.555	0.250	0.56	0.21	85.8
	S3=15	64	4.33	2.00	46.15	0.253	0.143	0.46	0.17	72.7
Giza 131	C= 0.6	100	15.33	5.67	36.96	1.553	0.953	0.91	0.28	100.0
	S1= 5	98	11.67	5.00	42.86	1.029	0.529	0.84	0.25	95.7
	S2=10	88	10.00	3.67	36.67	0.985	0.385	0.79	0.15	93.9
	S3=15	78	6.67	2.33	35.00	0.633	0.233	0.65	0.13	88.1
Giza 132	C= 0.6	93	15.33	5.33	34.78	0.655	0.317	0.74	0.24	100.0
	S1= 5	93	13.67	3.67	26.83	0.536	0.213	0.66	0.23	86.5
	S2=10	78	10.67	1.67	15.63	0.464	0.140	0.45	0.20	82.3
	S3=15	20	0.30	0.10	20.00	0.050	0.010	0.01	0.00	20.0
Giza 133	C= 0.6	98	15.00	6.50	43.33	0.914	0.464	0.57	0.25	100.0
	S1= 5	94	14.67	5.33	36.33	0.842	0.342	0.53	0.23	85.2
	S2=10	79	5.50	2.00	36.03	0.792	0.227	0.43	0.16	75.6
	S3=15	62	3.67	1.33	36.36	0.227	0.192	0.23	0.11	49.6
Giza 134	C= 0.6	97	13.67	5.33	39.02	0.949	0.349	0.78	0.25	100.0
	S1= 5	88	13.00	5.00	38.46	0.784	0.284	0.65	0.23	87.1
	S2=10	80	12.50	3.33	26.64	0.485	0.166	0.49	0.14	82.9
	S3=15	59	4.67	1.00	21.43	0.390	0.077	0.39	0.09	69.1
Giza 135	C= 0.6	100	14.67	5.67	38.64	0.977	0.377	0.58	0.35	100.0
	S1= 5	93	13.33	4.67	35.03	0.822	0.322	0.55	0.34	92.2
	S2=10	85	6.67	2.33	30.43	0.742	0.142	0.42	0.23	72.2
	S3=15	63	4.00	1.50	30.00	0.566	0.083	0.21	0.12	70.3
Giza 136	C= 0.6	100	15.67	7.00	44.68	1.676	0.921	0.86	0.43	100.0
	S1= 5	98	13.50	6.00	44.44	1.024	0.524	0.87	0.41	97.6
	S2=10	88	9.50	4.00	42.01	0.856	0.256	0.47	0.30	89.7
	S3=15	67	7.67	2.50	32.59	0.471	0.160	0.34	0.24	87.3
Giza2000	C= 0.6	100	15.67	7.67	48.94	0.921	0.553	0.73	0.44	100.0
	S1= 5	98	13.00	6.00	46.15	0.740	0.321	0.63	0.36	97.8
	S2=10	86	11.00	5.00	45.45	0.579	0.240	0.45	0.28	93.0
	S3=15	68	7.67	3.50	45.25	0.274	0.127	0.23	0.14	78.6
Average		83.72	10.01	4.15	43.19	0.73	0.32	0.54	0.23	85.53
C.V %		2.31	1.32	0.82	0.40	1.25	3.58	3.92	13.61	10.9
LSD 0.05	Salinity(S)	3.11	0.83	0.41	0.45	0.09	0.07	0.06	0.03	4.68
LSD 0,05	Cultivars (C	12.2	3.23	1.34	8.26	0.22	0.14	0.15	0.07	12.63
LSD 0.05	SXC	1.06	1.05	0.39	0.14	7.40	0.05	0.005	0.227	7.52

As regards flag leaf area (cm^2), data in Table 5, clearly showed that the large flag leaf area were produced by, Giza 131 under control and the different salinity treatments with value of 12.9, 12.4, 11.2 and 8.9 cm respectively, followed by Giza 128 at control and the three different salinity levels with value of 12.9, 12.2, 11.1 and 8.8 cm^2, respectively. The small leaf areas were produced by Giza

129, 132 and Giza 124 cultivars. Flag leaf area were decrees in all cultivars with increasing salinity treatments compared with control. These results were in a harmony with Abo-El-lail *et al.* (2014) and Mariey *et al.* (2016 &2017) found that flag leaf area in barley cultivar was decreased with increasing salinity concentrations.

For total chlorophyll content, data in (Table 5) showed that the high total chlorophyll content was recorded by Giza 131 with values of 51.1, 49.2, 47.2 and 42.6 under the four salinity treatments respectively. While, the lower total chlorophyll content was found by Giza 132 with values of 41.5, 40.8, 37.3 and 33.8 under all salinity concentrations respectively. At higher salinity levels, the chlorophyll content was decreased this might be possibly due to changes in the lipid protein ratio of pigment protein complexes or increased chlorophyll activity. These results were agree with Yildiz and Terzi, (2013), El-Hamamsy and Behairy (2015) and Mariey *et al.* (2016) they found that the total chlorophyll content in barley cultivar was decreased with increasing salinity concentrations

The Relative water content (RWC) significantly reduced by the increase of salinity concentrations for all cultivars as shown in (Table 5). High means values of RWC were recorded under condition control and found in Giza 136 with 78.6%. Low values of RWC were recorded under high salinity levels which found in for Giza 132 (20.0%). Parallel results were reported by El Goumi *et al.* (2014) and Kamboj *et al.* (2015).

Concerning the activities of antioxidant enzyme peroxidase (POD) and catalase (CAT) of 15 Egyptian barley cultivars under different salinity concentrations are shown in (Table 5). POD activity was increased with increasing salinity concentrations. The results showed that high activity of POD enzymes was observed in Giza 123, Giza 125, Giza 127, Giza 131, Giza 136 and Giza 2000 cultivars under the four salinity treatments. Moreover, CAT activity was significant under all salt concentration and was increased with increasing salinity levels. High values of CAT activity were found in Giza 123, Giza 125, Giza 128, Giza 131, Giza 136 and Giza 2000 under control and the three salinity treatments.

Antioxidant enzymes play important role through the defense system induced by salinity stress. From these results, CAT enzymes showed the high rate of activity changes under different salinity levels. However, the POD activity was relatively low when compared with CAT activity. This indicated the major role of CAT enzyme in the antioxidant defense of barley plants under salt stress conditions. Yildiz and Terzi, (2013), Turkyilmaz *et al.* (2014), Behrouz *et al*. (2015) and Mariey *et al.* (2016) stated that catalase enzyme was more important than POD enzyme in barley defense under salt stress since CAT enzyme goes through H_2O_2 in cells, so the reduction in CAT activity will purportedly due to inhibition of enzyme synthesis or protein degradation

The effect of different salinity concentrations on proline content are shown in (Table 5). High values of proline content was found in Giza 136 (0.87, 1.39, 2.87 and 2.54 mg/g) at control, 5, 10 and 15dSm^{-1} of saline water concentrations respectively. Followed by Giza 123 with values of 0.82, 1.23, 2.11 and 2.50 mg/g under the four salinity different treatments respectively. 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 increasing salinity concentrations. These results were in agreement Yildiz and Terzi, (2013), El-Hamamsy and Behairy (2015), Behrouz *et al.* (2015) and Mariey *et al.* (2016) they confirmed that the accumulation of free proline during barley exposure to salinity stress showed high degree of tolerance to salinity.

Biochemical fingerprinting was developed to identify proteins involved in salt stress response in 15 Egyptians barley cultivars. The SDS–PAGE profile revealed that the soluble protein accumulation increased in barley shoot more than root. Banding pattern of total protein was shown in (Table 6) and (Fig.2, 3 &4). Eighty polymorphic bands were detected in all cultivars based on their gene expression in barley seedling shoot and root under salinity treatments and control with molecular weight ranging from 10 to 250 KDa.

Table 5: Effect of different salinity concentrations on physiological traits of 15 barley cultivars

Cultivars	Salinity Treatments dSm^{-1}	Total chlorophyll content (SPAD)	Flag leaf Area (cm)	Relative water content %	Peroxidase µmol g^{-1}/sec	Catalase µmol g^{-1}/sec	Proline mg/g
Giza 123	C= 0.6	48.73	10.31	59.7	1.09	7.36	0.82
	S1= 5	48.63	9.76	55.3	3.85	8.76	1.23
	S2=10	47.43	8.62	43.6	4.85	10.75	2.11
	S3=15	41.93	8.05	38.1	4.00	9.00	2.50
Giza 124	C= 0.6	45.47	8.93	52.2	0.32	1.50	0.37
	S1= 5	43.13	7.32	48.5	0.43	3.56	0.58
	S2=10	39.43	6.85	39.7	0.53	5.08	0.80
	S3=15	33.27	5.40	32.0	0.50	5.00	0.75
Giza 125	C= 0.6	50.17	10.00	62.5	2.15	2.10	0.46
	S1= 5	49.70	9.70	53.7	2.35	4.50	0.96
	S2=10	47.20	8.30	45.6	3.35	6.04	1.15
	S3=15	41.67	7.61	32.0	3.00	6.00	0.93
Giza 126	C= 0.6	48.00	10.21	67.4	0.72	2.98	0.49
	S1= 5	47.07	9.44	60.6	1.74	4.59	1.02
	S2=10	42.93	7.33	53.1	2.74	6.01	1.48
	S3=15	34.47	5.99	47.5	2.00	5.30	1.30
Giza 127	C= 0.6	48.60	10.40	61.3	0.67	1.54	0.33
	S1= 5	47.80	7.30	59.9	2.74	3.22	0.78
	S2=10	46.17	7.22	55.0	3.74	3.75	0.82
	S3=15	37.20	6.97	53.6	3.00	3.00	0.76
Giza 128	C= 0.6	48.83	12.97	56.8	0.78	7.34	0.63
	S1= 5	47.13	12.18	54.3	1.64	8.50	1.23
	S2=10	44.93	11.05	53.3	2.64	9.11	2.13
	S3=15	41.23	10.83	51.7	2.00	9.12	2.20
Giza 129	C= 0.6	42.53	7.10	61.0	0.75	1.12	0.45
	S1= 5	40.10	5.95	58.9	0.96	3.20	0.51
	S2=10	35.20	4.55	57.4	1.96	3.75	0.82
	S3=15	33.63	4.05	36.0	1.00	3.00	0.78
Giza 130	C= 0.6	48.20	10.13	66.2	0.35	2.78	0.41
	S1= 5	44.13	7.02	58.6	1.45	3.63	0.56
	S2=10	43.93	6.29	56.8	2.45	4.77	0.91
	S3=15	40.87	5.43	48.2	2.00	4.30	0.87
Giza 131	C= 0.6	50.23	12.98	71.5	2.21	9.98	1.43
	S1= 5	48.53	12.41	69.9	3.21	11.34	3.09
	S2=10	47.30	11.23	58.2	4.21	12.99	2.43
	S3=15	42.93	8.88	50.1	4.00	11.40	2.10
Giza 132	C= 0.6	41.50	7.25	60.7	0.47	1.64	0.34
	S1= 5	40.80	6.81	52.5	1.65	2.30	0.54
	S2=10	37.33	5.75	49.9	2.65	2.92	0.80
	S3=15	33.67	5.19	20.3	2.00	2.00	0.65
Giza 133	C= 0.6	48.57	9.63	68.4	0.26	1.78	0.73
	S1= 5	46.53	9.88	58.3	0.36	2.83	0.85
	S2=10	45.43	9.67	51.7	0.86	3.70	0.94
	S3=15	38.83	8.84	33.9	0.54	3.00	0.75
Giza 134	C= 0.6	49.43	10.33	57.8	1.11	4.45	0.48
	S1= 5	48.03	11.01	52.9	1.35	5.00	1.00
	S2=10	45.53	9.62	50.3	2.35	6.89	1.23
	S3=15	36.13	8.37	46.8	2.00	6.34	1.10
Giza 135	C= 0.6	50.07	9.54	50.6	2.08	3.55	0.75
	S1= 5	47.07	8.62	49.4	2.24	5.73	0.85
	S2=10	45.93	7.11	47.0	3.24	6.82	0.96
	S3=15	41.93	6.61	39.7	3.00	4.64	0.90
Giza 136	C= 0.6	51.13	11.04	78.6	2.08	4.26	0.87
	S1= 5	49.17	9.98	68.5	4.021	7.89	1.39
	S2=10	47.87	8.56	65.2	6.021	10.52	2.87
	S3=15	42.57	6.35	54.4	5.30	9.76	2.34
Giza 2000	C= 0.6	50.67	10.56	73.8	1.12	6.42	0.59
	S1= 5	48.13	9.94	68.0	2.18	8.76	1.20
	S2=10	47.23	8.73	53.2	3.18	10.07	1.99
	S3=15	41.27	6.41	51.8	3.00	9.20	1.50
Average		44.39	8.58	53.90	2.14	5.61	1.10
C.V%		1.04	0.12	6.98	5.64	6.55	1.79
LSD 0.05	Salinity	1.38	0.73	3.21	0.47	1.17	0.24
LSD 0,05	Cultivars	3.13	1.08	7.39	0.69	1.02	0.35
LSD 0.05	S XC	0.46	0.95	3.02	1.13	2.56	1.56

The band (20 KDa) appeared in all cultivars shoots under different salinity concentrations and control (Fig. 2 & 3), but did not appear in all cultivars roots except for Giza 129 and Giza 124 (Fig. 4) where was found in roots under control and the different salinity concentrations.

In contrary, the band (50 KDa) were found in all cultivars seedling shoots and roots under the three different salinity treatments and control except for Giza 126 roots (Fig.3) the protein diaper in all salinity concentrations and control.

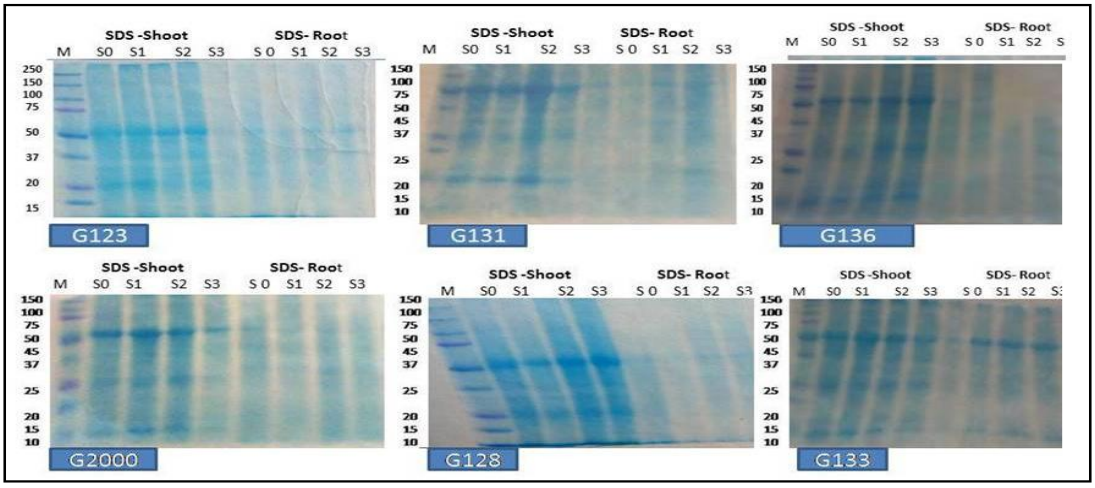

Fig 2: Gel Electrophoresis of SDS-PAGE of total soluble proteins of three salinity concentration in shoot and root of Giza 123,131,136,2000.128 and Giza 133 subjected to S0= 0.6 , S1=5, S2=10, S3=15 dSm^{-1} in shoot and root respectively. M = molecular weight marker

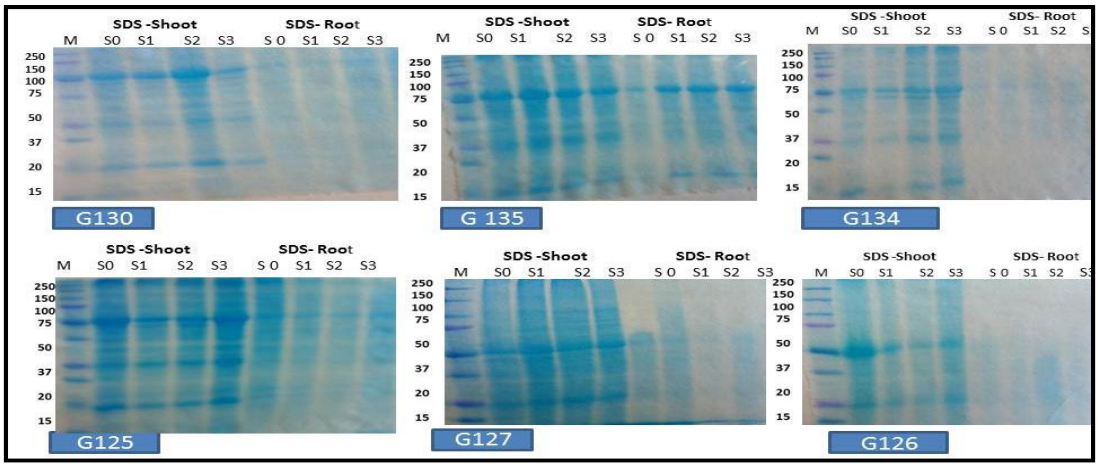

Fig. 3: Gel Electrophoresis of SDS-PAGE of total soluble proteins of three salinity concentration in shoot and root of Giza 130, 135, 134, 125, 127 and Giza 126 subjected to S0= 0.6 , S1=5, S2=10, S3=15 dSm^{-1} in shoot and root respectively. M = molecular weight marker.

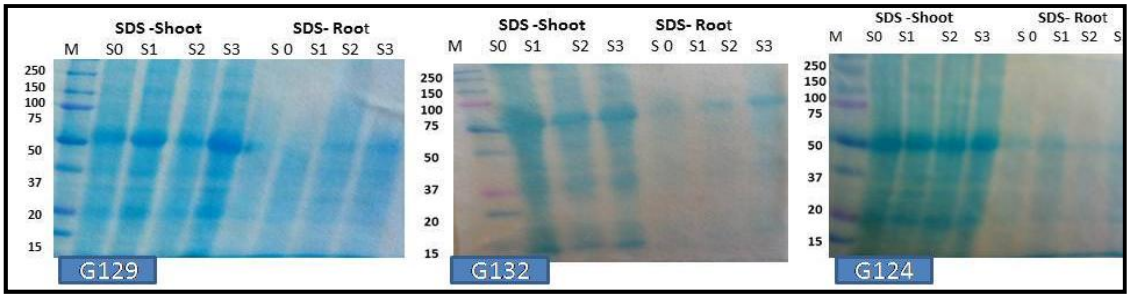

Fig. 4: Gel Electrophoresis of SDS-PAGE of total soluble proteins of three salinity concentration in shoot and root of Giza 129,Giza 132 and Giza 124 subjected to S0= 0.6 , S1=5, S2=10, S3=15 dSm^{-1} in shoot and root respectively. M = molecular weight marker.

Table 6: Molecular weight (MW) KDa of SDS- PAGE total proteins for shoot and root of fifteen Egyptian Barley cultivars their name as listed from 1 to 15 in (table 1) under different salinity levels 0.6, 5, 10 and 15 dSm^{-1}, for each of them. (+) means presence and (-) means absence of band

MW		Salinity dSm^{-1}	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
250		C= 0.6	+	+	+	+	+	+	-	-	-	+	-	+	+	-	+
250	Shoot	S1=5	+	+-	+	+	+	+	+	-	-	+	-	+	+	-	+
250		S2=10	+	+	+	+	+	+	-	-	-	+	-	+	+	-	+
250		S3=15	-	+	+	+	+	+	-	-	-	+	-	+	+	-	+
250		C= 0.6	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-
250	Root	S1=5	-	-	+	--	-	-	-	-	-	-	-	-	+	-	-
250		S2=10	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-
250		S3=15	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-
150		C= 0.6	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
150	Shoot	S1=5	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
150		S2=10	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
150		S3=15	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
150		C= 0.6	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
150	Root	S1=5	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
150		S2=10	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
150		S3=15	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
100		C= 0.6	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
100	Shoot	S1=5	-	+	-	-	+	-	+	-	-	_	-	-	-	-	-
100		S2=10	-	+	-	-	+	-	+	-	-	_	-	+	-	-	-
100		S3=15	-	-	-	-	-	-	+	-	-	_	-	+	-	-	-
100		C= 0.6	-	-	-	-	-	-	-	-	-	_	-	-	-	-	-
100	Root	S1=5	-	-	-	-	-	-	-	-	-	_	-	-	-	-	-
100		S2=10	-	-	-	-	-	-	-	-	-	_	-	-	-	-	-
100		S3=15	-	-	-	-	-	-	-	-	-	_	-	-	-	-	-
75		C= 0.6	+	-	-	-	-	-	-	_	-	-	-	-	-	-	-
75	Shoot	S1=5	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-
75		S2=10	+	+	_	-	+	-	-	-	-	-	-	+	-	-	-
75		S3=15	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
75		C= 0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75	Root	S1=5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75		S2=10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75		S3=15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50		C= 0.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
50	Shoot	S1=5	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
50		S2=10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
50		S3=15	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
50		C= 0.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
50	Root	S1=5	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
50		S2=10	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
50		S3=15	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
45		C= 0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	Shoot	S1=5	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
45		S2=10	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
45		S3=15	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
45		C= 0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	Root	S1=5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45		S2=10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45		S3=15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37		C= 0.6	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-
37	Shoot	S1=5	+	-	-	-	-	+	-	+	-	+	-	-	-	+	-
37		S2=10	+	-	-	-	-	+	-	+	-	+	-	-	-	+	-
37		S3=15	+	-	-	-	-	+	-	+	-	-	-	-	-	+	-
37		C= 0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
37	Root	S1=5	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
37		S2=10	+	-	-	-	-	+	-	+	-	-	-	-	-	-	+
37		S3=15	+	-	-	-	-	+	-	+	-	-	-	-	-	-	+
20		C= 0.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	Shoot	S1=5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20		S2=10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20		S3=15	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+
20		C= 0.6	-	+	-	+	+	-	-	-	-	+	-	-	-	-	-
20	Root	S1=5	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-
20		S2=10	-	+	+	+	-	-	-	-	-	+	-	-	-	-	-
20		S3=15	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
15		C= 0.6	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+
15	Shoot	S1=5	-	-	-	-	+	-	-	-	-	-	-	+	+	-	+
15		S2=10	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+
15		S3=15	-	-	-	-	+	-	-	-	-	-	-	+	+	-	+
15		C= 0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Root	S1=5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15		S2=10	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
15		S3=15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10		C= 0.6	+	-	+	-	_	-	-	-	+	-	+	-	+	+	-
10	Shoot	S1=5	+	-	-	-	_	-	-	-	+	-	+	-	+	+	-
10		S2=10	+	-	+	-	_	-	-	-	+	-	+	-	+	+	-
10		S3=15	+	-	-	-	_	-	-	-	+	-	+	-	+	+	-
10		C= 0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
10	Root	S1=5	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+
10		S2=10	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+
10		S3=15	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+

Furthermore, the results indicated that the salt stress led to increase in the number of some new polypeptides in barley seedling compared with control, such as the protein with molecular weight of

45 KDa was found only in barley seedling shoots under all different salinity treatments ,while it was not found in Giza 125 and Giza 134 under control condition.

Moreover, there was another protein with molecular weight of 37 KDa was found in shoot and root of both Giza 129 and Giza 131 under two different salinity concentrations 10 and 15 dS m^{-1}. The results also indicated that there were two protein induce only in the roots of Giza 2000 in all salinity treatments and not expressed in their shoots, which give a specific bands with 37 and 10 KDa molecular weights.

Cluster Analysis and Genetic Similarity (GS%)

The results of cluster analysis based on seed germination, seedling traits and physiological parameters using Euclidean distance, average linkage were displayed in Table 7 and graphically illustrated in dendrogram Fig5. Low similarity level (23%) was recorded between Giza 123 and Giza 132. Followed by was (44.6%) obtained between Giza 123 and Giza 129. From dendrogram, it is obvious that pairs of cultivars (Giza 126 and Giza 135), (Giza 136 and Giza 2000) and (Giza 126 and Giza 134) were closely related to each other where the similarity levels among them were more than 80 recording 83.2, 82.8 and 80.4, respectively. On the other hand, the remaining similarity levels among the pairs of cultivars ranged between 70 and 80%. From the previous results, it could be concluded, based on similarity levels, that cultivars Giza 124, 125, 129 and 132 have low similarity levels (dissimilarity) with Giza 123 and may produce good results if they are crossed with. These results are in harmony Ravari *et al.* (2016), and Mariey *et al.* (2016& 2017) who reported that cluster analysis based on morphological parameters considered a valuable tool for subdividing any number of cultivars in groups including similarity and dissimilarity cultivars or genotypes, which would help the breeder to plan an effective breeding program for stress conditions.

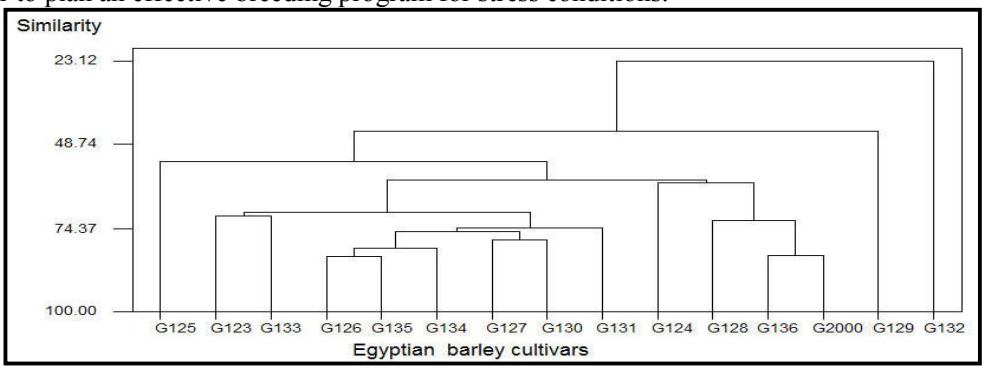

Fig. 5: Cluster analysis of fifteen Egyptian barley cultivars based on germinated and physiological traits

Table 7: Similarity levels for 15 Egyptian barley cultivars based on seed germination and physiological trait under four different concentration of sea water across two seasons

Step	Number of Clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of obs. in new cluster
1	14	83.19	23.398	G126	G135	4	2
2	13	82.81	23.929	G136	G2000	14	2
3	12	80.42	27.253	G126	G134	4	3
4	11	78.00	30.622	G127	G130	5	2
5	10	75.38	34.261	G126	G127	4	5
6	9	74.30	35.762	G126	G131	4	6
7	8	72.13	38.779	G128	G136	6	3
8	7	70.73	40.737	G123	G133	1	2
9	6	69.66	42.221	G123	G126	1	8
10	5	60.58	54.857	G124	G128	2	4
11	4	59.61	56.208	G123	G124	1	12
12	3	53.86	64.212	G123	G125	1	13
13	2	44.61	77.076	G123	G129	1	14
14	1	23.12	106.992	G123	G132	1	15

Regarding the results of cluster analysis based on data scored banding protein pattern from the polyacrilamide gel electrophoresis using Ward's method, Jaccard distance was illustrated in dendrogram Fig 6. and the genetic similarity was displayed in Table 8. The dendrogram were divided all the 15cultivars in to four clusters according to their gene expression of protein accumulation under different salinities concentration in both shoot and root of the fifteen barley cultivars. Each cluster included the most closed cultivars together according to their gene expression for salinity response to tolerance or sensitivity or moderated tolerance or moderated sensitive. For the genetics similarity, the results showed that the high GS was found between Giza 123 and Giza 136 was 73%. The low GS was found between Giza 126 and Giza 131 was 18%. Moreover, the GS between Giza 124 and Giza 129 was 69% which both of these cultivars were found in one cluster. The gene expression of total protein was couriers about the molecular basis of salinity response in all cultivars and express about their tolerance, sensitivity, moderated tolerance and sensitive. So we could consider that Giza 124 and Giza 129 as sensitive cultivars under all salinity treatments. Moreover, we could consider that Giza 123,136 and 2000 as salinity tolerant cultivars. These resulted were in a good harmony with (Mariey, 2004, EL- Mouhamady *et al*.2012, El-Hamamsy and Behairy, 2015 and Hellal *et al*.2017) who reported that biochemical fingerprint method using SDS-PAGE could be used as efficient tools to identify and distinguish among barley genotypes and landraces under salinity stress.

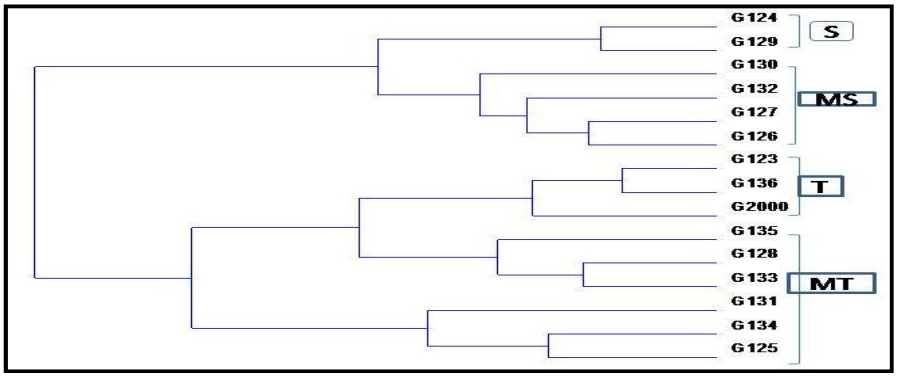

Fig. 6: Similarity levels for 15 barley cultivars calculated by cluster analysis based on gene expression of total protein using SDS-PAGE.

Table 8: Similarity coefficient values among 15 barley cultivars based on protein banning pattern generated by SDS- PAGE

	G123	G124	G132	G129	G125	G126	G127	G128	G130	G131	G135	G134	G133	G136
G123	1.00													
G124	0.45	1.00												
G132	0.40	0.38	1.00											
G129	0.37	0.69	0.30	1.00										
G125	0.39	0.44	0.25	0.33	1.00									
G126	0.48	0.30	0.32	0.27	0.29	1.00								
G127	0.50	0.54	0.43	0.43	0.37	0.45	1.00							
G128	0.53	0.34	0.48	0.28	0.32	0.29	0.41	1.00						
G130	0.32	0.31	0.33	0.37	0.24	0.35	0.45	0.30	1.00					
G131	0.33	0.33	0.27	0.30	0.33	0.18	0.42	0.32	0.29	1.00				
G135	0.38	0.26	0.29	0.24	0.59	0.33	0.33	0.50	0.21	0.27	1.00			
G134	0.53	0.35	0.33	0.26	0.57	0.44	0.52	0.38	0.35	0.39	0.43	1.00		
G133	0.53	0.26	0.32	0.22	0.56	0.33	0.33	0.67	0.28	0.25	0.64	0.54	1.00	
G136	0.73	0.44	0.53	0.35	0.26	0.40	0.57	0.67	0.41	0.39	0.30	0.48	0.44	1.00
G2000	0.60	0.32	0.34	0.30	0.31	0.41	0.39	0.52	0.28	0.33	0.44	0.39	0.51	0.45

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