

Genetic Variation and Agro-morphological Criteria of Ten Egyptian Barley Under Salt Stress

E. A. H. Mostafa¹, H. El-Atroush², Z. M. El-Ashry¹, F. I. Mohamed¹,
S. E. El-Khodary² and S. A. Osman¹

¹Department of Genetics and Cytology, National Research Centre, Dokki, Giza, Egypt.
²Botany Department, Faculty of Science, Ain Shams University, Abassia, Cairo, Egypt.

Abstract : The present investigation was conducted to study the effect of different concentrations of NaCl (control, 9000, 12000 and 15000 PPM) on agro-morphological traits (branch number/plant, shoot length, root length, node number/plant, spike length, spike weight, grain number/spike, grain weight/spike and grain yield/plant) and protein banding patterns electrophoresis (SDS-PAGE) at three sampling time points (sampling after 24 hour, 1st week and 2nd week salt treatments). This work was carried out on seven Egyptian cultivars (Giza 123, Giza 126, Giza 127, Giza 128, Giza 129, Giza 130 and Giza 2000) and three landraces (El-Kheroba and El-Sheikh Zuwaid) from Sinai, El-Aresh and (Wadi Sedr) from Sinai, Ras Sedr. The results shown that, the more tolerance varieties to salt stress were Giza 2000 cultivar and Wadi Sedr landrace while the sensitive variety was Giza 129 depending on agro-morphological traits. The electrophoretic pattern of soluble proteins shown that a maximum polymorphism was observed after 2nd week from salt treatments and show the lowest variation, while after 24 hour and 1st week from salt treatments show a maximum variation. The protein of Mw 28 KDa and Mw 49KDa disappeared in sensitive varieties (Giza 129) and appeared in tolerant varieties after 24 hour and 1st week from salt treatments respectively.

Key words: Barley, Salt Stress, Agro-morphological Criteria, SDS-PAGE Electrophoresis.

Introduction

Barley (*Hordeum vulgare* L.) is an important cereal crop, which ranks fourth in world food production. Its value as food has been increasing due to its various health benefits¹. Barley also possesses the highest level of salt tolerance amongst cereals, thus is being used as an important species to investigate mechanisms involved in salt tolerance^{2,3}.

Drought and salinity consider two major environmental factors that reduce the productivity of plants⁴. Salinity is a serious problem affecting one third of the irrigation land nearly 950 million ha land in the world, also effect on plant growth and productivity of modern cultivars^{5,6,7}.

For overcoming salt stress, plants have evolved complex mechanisms that contribute to the adaptation to osmotic and ionic stress caused by high salinity. During the onset and development of salt stress within a plant, all the major processes such as compatible osmolytes, protein synthesis, and lipid metabolism are affected. The resistance or sensitivity to salt stress depends on the species, the genotype and the development age of the plants.

Polyacrylamide gel electrophoresis play an important role in the experimental analysis of proteins. Polyacrylamide gel electrophoresis (PAGE) is still the most widespread form of the technique, since it offers sufficient resolution for most situations coupled with ease of use and the ability to process many samples simultaneously for comparative purpose^{8,9}.

The aim of this work, study the effect of salt stress by NaCl treatment on germination percentage, germination rate and analysis of variance of agro-morphological traits and protein banding patterns, to identify which varieties more tolerance to salinity and recommend cultivating them on salty soil or exploiting primary treated sea water or salty water to irrigate it.

Material and Methods

Ten plant materials of *Hordeum vulgare* L were used in this work. seven of them; Giza 123, Giza 126, Giza 127, Giza 128, Giza 129, Giza 130 and Giza 2000 are Egyptian cultivars while the other three; El-Kheroba and El-Sheikh Zuwaid are landraces from Sinai, El-Aresh and (Wadi Sedr) from Sinai, Ras Sedr obtained through collection trip.

I- Agro-morphological criteria studies:

The ten barley plant materials were evaluated in the green house of Botany department, faculty of science, Ain Shams university, Abassia, Gairo. The experiment was conducted in a randomized complete block design with three replications: each block consist of 40 pots (30X50 cm) in which each four pots out of the 40 was planted by one variety for control, 9000 Ppm, 12000 Ppm and 15000 Ppm of NaCl concentrations. Each pot contained ten plants of each variety.

The measurment agro-morphological traits as branch number/plant, shoot length (cm), root length (cm), node number/plant, spike length (cm), spike weight (gm), no. of grains/spike, grain weight /spike (gm) and grain weight/plant (gm) are taken. The data of this agro-morphological traits measurement were analysed by CoStat_V6.303 (CoHort software, Monterey, CA) and Excel-Microsoft office 2010 programs to computerize found the analysis of variance and the means of these traits measurement.

II- Biochemical studies:-

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed, in Genetics and Cytology Department, National Research Centre, according to the method of ¹⁰, as modified by ¹¹. Water-soluble proteins (W.S.P) of studied varieties were taken from leaves of these plants. Then protein fractionations were performed exclusively on vertical slab gel (19.8 cm x 26.8 cm x 0.2 cm) using the electrophoresis apparatus manufactured by Cleaver, UK.

The images were captured by digital camera (Sony, made in japan) and transferred directly to the computer. The protein bands were analyzed by Total Lab program to find out the molecular weight of each band and that to compare the presence and absence of the band among varieties. These date were imported in MVSP (Multi-Variant Statistical Package) to find the similarity matrix and dendogram (UPGAMA, suing Jaccard,s coefficient) which reflect the relationships among the studied varieties.

Results and Discussion

I-Agro-morphological Criteria

The analysis of variance of (branch number/plant, shoot length, root length, node number/plant, spike length, spike weight, grain number/spike, grain weight/spike and grain yield/plant) shown highly significant differences among ten studied Egyptian *Hordeum vulgare* L. varieties. The salinity with different sodium chloride concentrations (control, 9000, 12000 and 15000 Ppm) shown highly significant differences between the treated varieties and untreated varieties (control). No significant differences were found between replicates and in the interaction between varieties and treatment as shown in **table (1)**. Salt stress of NaCl treatments effect on all studied agro-morphological traits on ten barley varieties. In **table (2)** the heights mean values were recorded after control treatment whereas the lowest ones was recorded after treatment with 15000 Ppm. The

differences between means of such traits after control and 15000 PPM treatment were significant whereas between control and 12000 were significant in respect to all studied traits except branch number, spike weight and grain weight. Only shoot length, spike length, grain number and grain yield showed significant differences between control and 9000 PPM.

The mean performance of all studied agro-morphological traits of studied barley varieties under salt stress of NaCl treatments represented that Giza 128 gave the highest number of branch, while Giza 123 and Giza 130 gave the lowest number of branch under salt stress of NaCl, while Giza 2000 and wadi Sedr gave the maximum value in all the other agro-morphological criteria while Giza 129 gave the minimum value (**fig. 1 and Table 3**). The salinity had a great effect on plant growth, activity of major cytosolic enzymes by disturbing intracellular potassium homeostasis, causing oxidative stress and programmed cell death. Also reduced nutrient uptake, metabolic toxicity, inhibition of photosynthesis, reduce CO₂ assimilation and reduced root respiration^{12, 13, 14, 15}. The present results agreement with the results of¹⁶, who studied the effect of salt stress on mean performances on some agro-morphological traits of six barely genotypes (Giza 123, Rihaen 03, Saiko, Becheer, Line 1 and Line 2), and concluded that, the two genotypes (Giza 123 and Rehan-03) had the highest mean performance values and the mean squares for all genotypes were significant and high significant in all traits under salt stress, control and their combined data but the mean squares were non-significant for plant height and grain yield in Sakha only. **Mariey and Oraby**^{17, 18} found that the salinity reduced number of tillers, plant height, days to heading, number of spike/plant and grain yield, their results agreement with our results except in plant height and grain. Our results agreement with^{19, 20, 17, 21, 22}, who observed a different types of significant of agronomic traits in barley under salinity.

Table (1): Mean square (MS) of analysis variance for studied traits on 10 barley varieties under salt stress of NaCl.

Source	df	Mean Square (MS)								
		Branch Number	Shoot Length	Root Length	Node Number	Spike Length	Spike Weight	Grain Number	Grain Weight	Grain Yield
Replicates	2	0.08 ^{ns}	39.00 ^{ns}	14.85 ^{ns}	1.38 ^{ns}	9.52 ^{ns}	0.12 ^{ns}	236.96 ^{ns}	0.09 ^{ns}	0.05 ^{ns}
Treatments	3	0.39*	775.01***	25.08***	0.93***	1.90***	0.02**	119.22***	0.02**	0.05***
Varieties	9	1.46***	196.37***	12.46***	2.55***	3.73***	0.03***	53.42***	0.02***	0.03***
Treatments x varieties	27	0.10 ^{ns}	3.05 ^{ns}	0.33 ^{ns}	0.01 ^{ns}	0.10 ^{ns}	0.00 ^{ns}	2.43 ^{ns}	0.00 ^{ns}	0.00 ^{ns}
Error	78	0.105	3.477	2.757	0.108	0.219	0.006	8.879	0.004	0.006
Total	119									

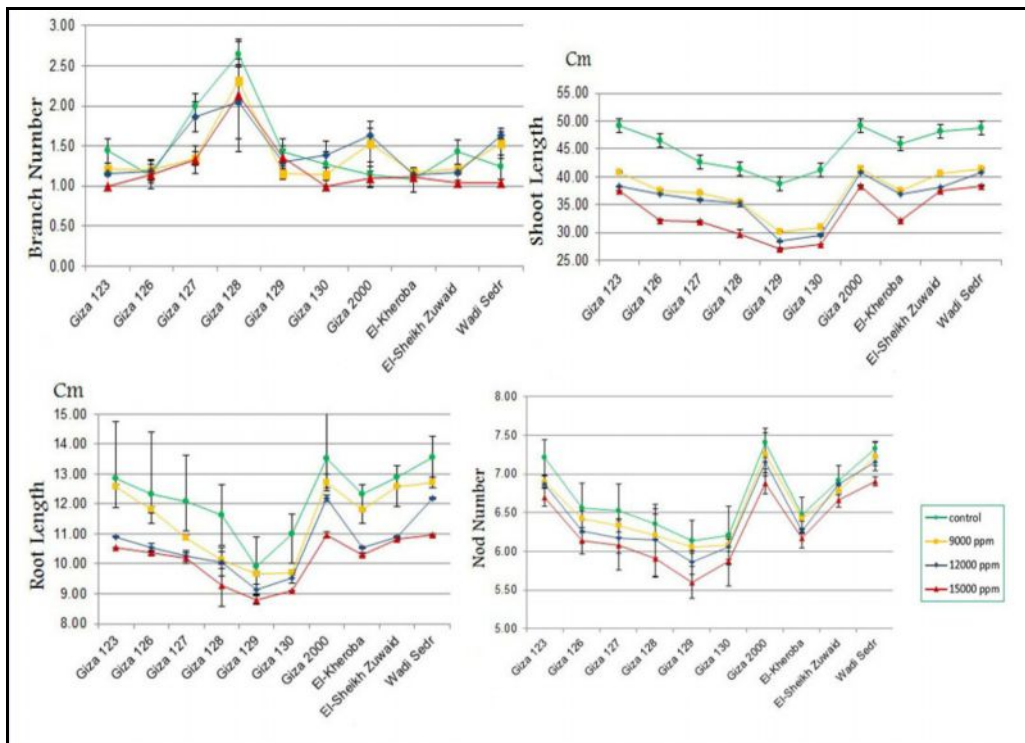
*, **, ***: Significant at 0.05 level of probability. ^{ns}: no significant.

Table (2): Mean performance of salt stress of NaCl treatment on all studied traits of 10 barley varieties.

	Branch Number	Shoot Length	Root Length	Node Number	Spike Length	Spike Weight	Grain Number	Grain Weight	Grain Yield
Control	1.48 ^a	45.17 ^a	12.21 ^a	6.71 ^a	4.12 ^a	0.22 ^a	16.12 ^a	0.17 ^a	0.21 ^a
9000 PPM	1.38 ^{ab}	37.30 ^b	11.45 ^{ab}	6.57 ^{ab}	3.74 ^b	0.19 ^a	12.98 ^b	0.15 ^a	0.16 ^b
12000 PPM	1.45 ^a	36.09 ^c	10.63 ^{bc}	6.48 ^b	3.72 ^b	0.19 ^{ab}	12.89 ^b	0.14 ^a	0.16 ^b
15000 PPM	1.22 ^b	33.31 ^d	10.14 ^c	6.29 ^c	3.51 ^b	0.15 ^b	11.38 ^b	0.11 ^b	0.11 ^c

Table (3): Mean performance of 10 barley varieties under salt stress of NaCl treatment on all studied traits.

	Branch Number	Shoot Length	Root Length	Node number	Spike Length	Spike Weight	Grain Number	Grain Weight	Grain Yield
Giza 123	1.20 ^c	41.45 ^a	11.72 ^{ab}	6.92 ^{ab}	4.09 ^b	0.22 ^{abc}	15.07 ^a	0.17 ^{abc}	0.19 ^{abc}
Giza 126	1.16 ^c	38.33 ^b	11.26 ^{abc}	6.34 ^c	3.76 ^{bc}	0.19 ^{abcd}	13.63 ^{ab}	0.15 ^{abcd}	0.16 ^{abcd}
Giza 127	1.64 ^b	36.91 ^{bc}	10.86 ^{abc}	6.28 ^c	3.58 ^{cd}	0.17 ^{bcd}	13.05 ^{abc}	0.13 ^{cd}	0.14 ^{cd}
Giza 128	2.29 ^a	35.46 ^c	10.27 ^{bcd}	6.15 ^{cd}	3.37 ^{cd}	0.15 ^{cde}	11.24 ^{bcd}	0.12 ^{cde}	0.13 ^{cde}
Giza 129	1.31 ^c	31.12 ^d	9.39 ^d	5.91 ^d	2.78 ^e	0.11 ^e	9.89 ^d	0.07 ^e	0.08 ^e
Giza 130	1.20 ^c	32.35 ^d	9.84 ^{cd}	6.05 ^{cd}	3.24 ^d	0.13 ^{de}	10.63 ^{cd}	0.10 ^{de}	0.11 ^{de}
Giza 2000	1.35 ^c	42.47 ^a	12.35 ^a	7.17 ^a	4.52 ^a	0.25 ^a	15.62 ^a	0.20 ^{ab}	0.22 ^{ab}
El-Kheroba	1.13 ^c	38.13 ^b	11.24 ^{abc}	6.33 ^c	3.74 ^{bc}	0.19 ^{abcd}	13.66 ^{ab}	0.14 ^{bcd}	0.16 ^{bcd}
El-Sheikh Zuwaid	1.21 ^c	41.08 ^a	11.80 ^a	6.80 ^b	4.09 ^b	0.22 ^{ab}	15.09 ^a	0.16 ^{abc}	0.19 ^{abc}
Wadi Sedr	1.36 ^c	42.35 ^a	12.36 ^a	7.15 ^a	4.53 ^a	0.24 ^{ab}	15.55 ^a	0.20 ^a	0.24 ^a



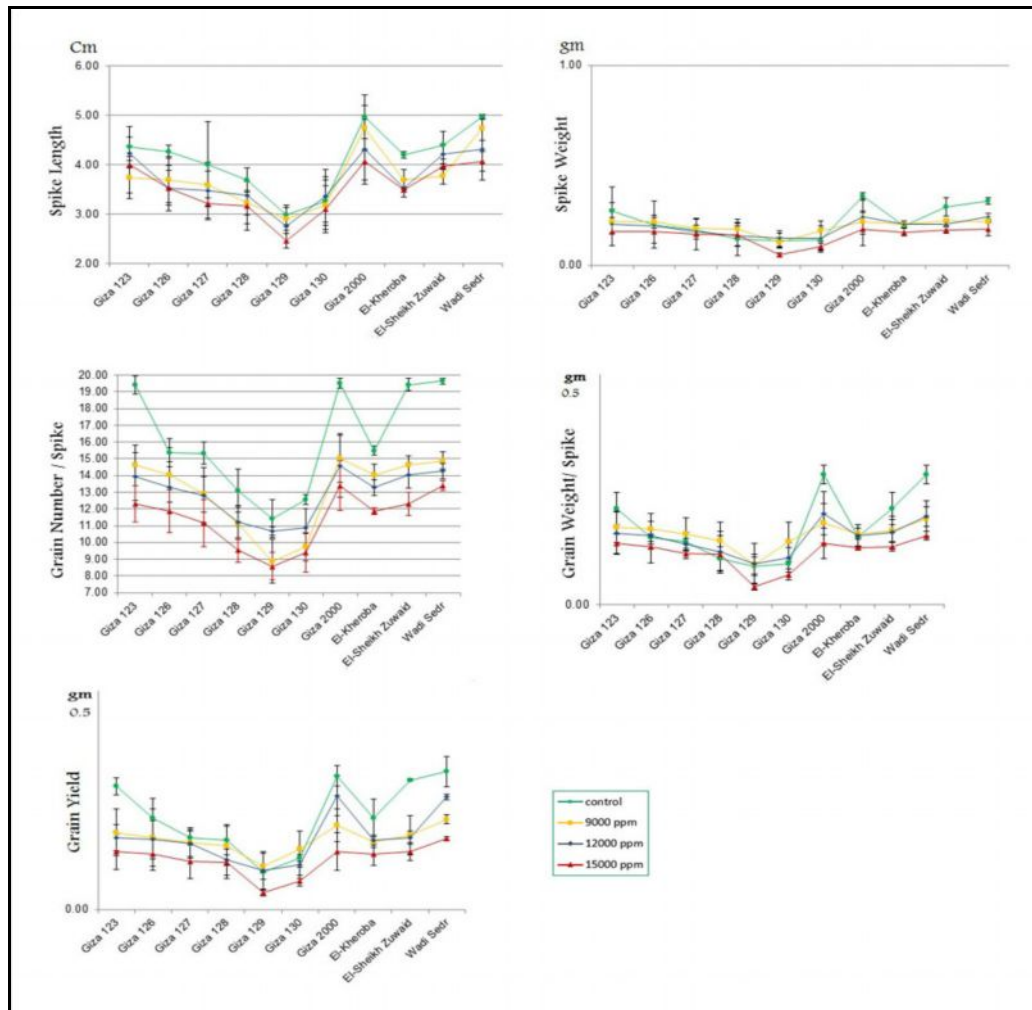


Figure (1): Effect of different concentration of NaCl on some agro-morphological criteria

II-Biochemical studies:

The salinity had a great effect on genes expression in plants so, the protein content affected by salinity and caused a great variance in the electrophoretic patterns of protein. Also protein banding pattern different with time of treatment.

After 24 hour from first treatments, the total number of bands were 30 bands with molecular weight range from 12 to 300 KDa, eight of them were polymorphic bands at Mw 250, 155, 125, 110, 60, 49, 45 and 28 KDa. There are some genes unexpressed into protein in control. While it's expression enhancement under salinity stress with 9000, 12000, 15000 PPM of NaCl such as, protein of Mw 250 KDa in Giza 130 and El-Kheroba; protein of Mw 155 KDa in Giza 123, Giza 130, El-Kheroba, El-Sheikh Zuwaid, Wadi Sedr; protein of Mw 110 KDa in Giza 130; protein of Mw 28 KDa in three studied landraces only. There are some genes expressed into proteins only under 12000 and 15000 PPM of NaCl such as protein of Mw 250 and 125 KDa in Giza 126 and Wadi Sedr respectively. While protein of Mw 49 KDa in El-Sheikh Zuwaid and Wadi Sedr were presented only under 15000 PPM of NaCl treatment, there were some genes expressed to proteins under 9000 and 12000 PPM only such as protein of MW 49 KDa in El-Kheroba (**fig. 2 and tables 4&5**).

After 1st week from first treatments, The total number of bands were 34 bands with molecular weight range from 7 to 300 KDa, seven of them were polymorphic bands at Mw 160, 155, 125, 110, 83, 78 and 49 KDa. There are some genes unexpressed into protein in control. While it's expression enhancement under salinity stress with 9000, 12000, 15000 PPM of NaCl such as, protein of Mw 155 KDa in Giza 123, Giza 130 and Wadi Sedr; protein of Mw 110 KDa in El-Kheroba and protein of Mw 83 KDa in El-Kheroba. There are some genes expressed into proteins only under 12000 and 15000 PPM of NaCl such as protein of Mw 125 KDa in Wadi Sedr; protein of Mw 83 KDa in El-Sheikh Zuwaid and protein of Mw 78 KDa in Giza 127 and Giza

128. In contrast there are some proteins present in control while absent under all salinity treatments such as proteins of molecular weight 155 in Giza 129; proteins of molecular weight 110 in Wadi Sedr; proteins of molecular weight 49 in Giza 129 (**fig. 3 and tables 6&7**).

After 2nd week from first treatments, The total number of bands were 25 bands with molecular weight range from 7 to 300 KDa, seven of them were polymorphic at Mw 250, 155, 125, 110, 40, 33 and 30 KDa. There are some genes unexpressed into protein in control. While it's expression enhancement under salinity stress with 9000, 12000, 15000 Ppm of NaCl such as, protein of Mw 155 KDa in Giza 123; protein of Mw 33 KDa in Giza 130 and Giza 2000 and protein of Mw 30 KDa in Giza 123. There are some genes expressed into proteins only under 12000 and 15000 Ppm of NaCl such as protein of Mw 33 KDa in El-Sheikh Zuwaid and protein of Mw 30 KDa in Giza 2000. In contrast there are some proteins present in control while absent under all salinity treatment such as proteins of molecular weight 110 KDa in Giza 130; proteins of molecular weight 40 KDa in Giza 128 and proteins of molecular weight 33KDa in Giza 123 (**fig. 4 and tables 8&9**).

From the present results we concluded that, protein patterns of the ten varieties under different salt stress condition were inspected visually and compared with each other. Maximum polymorphism was observed after 2nd week from salt treatments and show the lowest variation, while after 24 hour and 1st week from salt treatments show a maximum variation. This may be due to the plant reclaimed with the salinity and produce the needed protein which help in defense themselves from salt stress. **Konarev**²³ stated that, proteins are primary products in the realization of heredity information and reflect the genetic structure of the organisms, SDS-PAGE, which is the most employed techniques for separation and identification of proteins according to their molecular weight^{24,25,26}.

The protein of Mw 28 KDa disappeared in sensitive varieties (Giza 129) while appeared in tolerant varieties after 24 hour salt treatment, under salinity stress with 9000, 12000, 15000 Ppm of NaCl, this agreement with^{27, 28, 29, 30}. While after 1st week salt treatment, the protein of Mw 49 disappeared in sensitive varieties (Giza 129) while appeared in tolerant varieties under salinity stress 9000, 12000 and 15000 Ppm of NaCl, this agreement with³¹. The accumulation of this kind of proteins under salt stress may enable to elucidate the mechanism of salt tolerance of plant cells, at least, in relation to the mechanism of regulation of gene expression³².

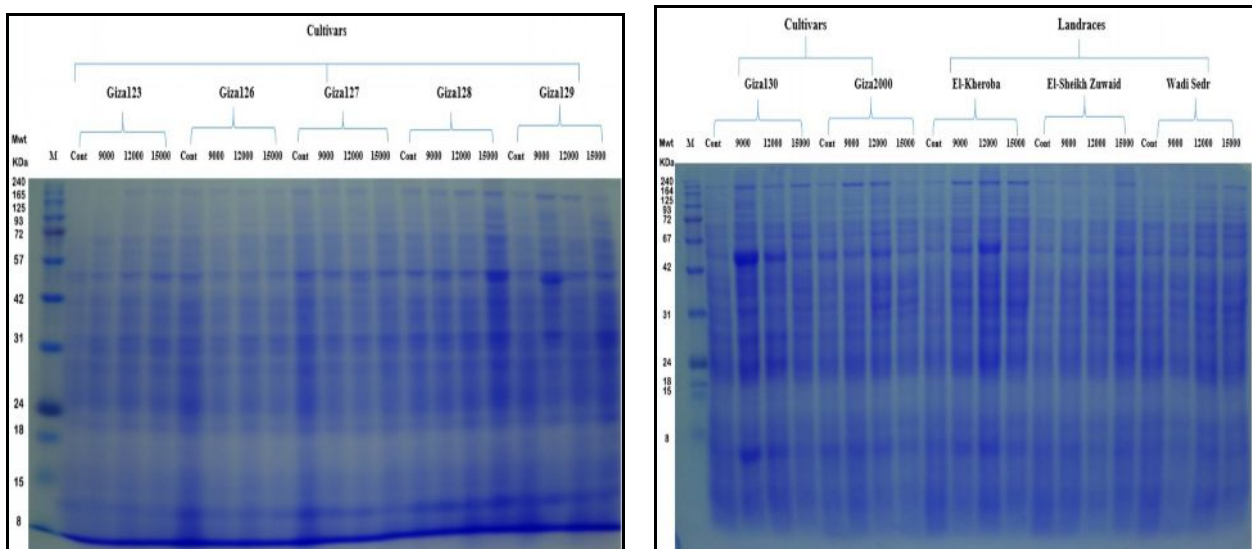


Figure (2): Effect of salinity on protein electrophoresis patterns of ten *Hordeum Vulgare* L. after 24 hour from salt treatments

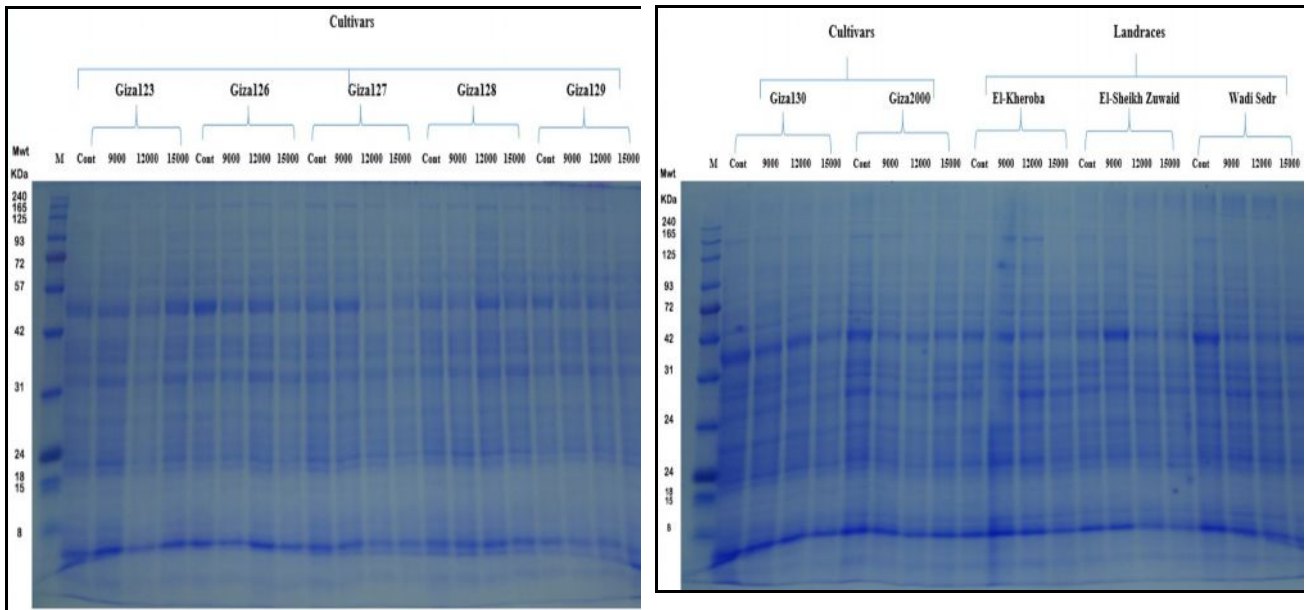


Figure (3): Effect of salinity on protein electrophoresis patterns of ten *Hordeum Vulgare L.* after 1st week from salt treatments.

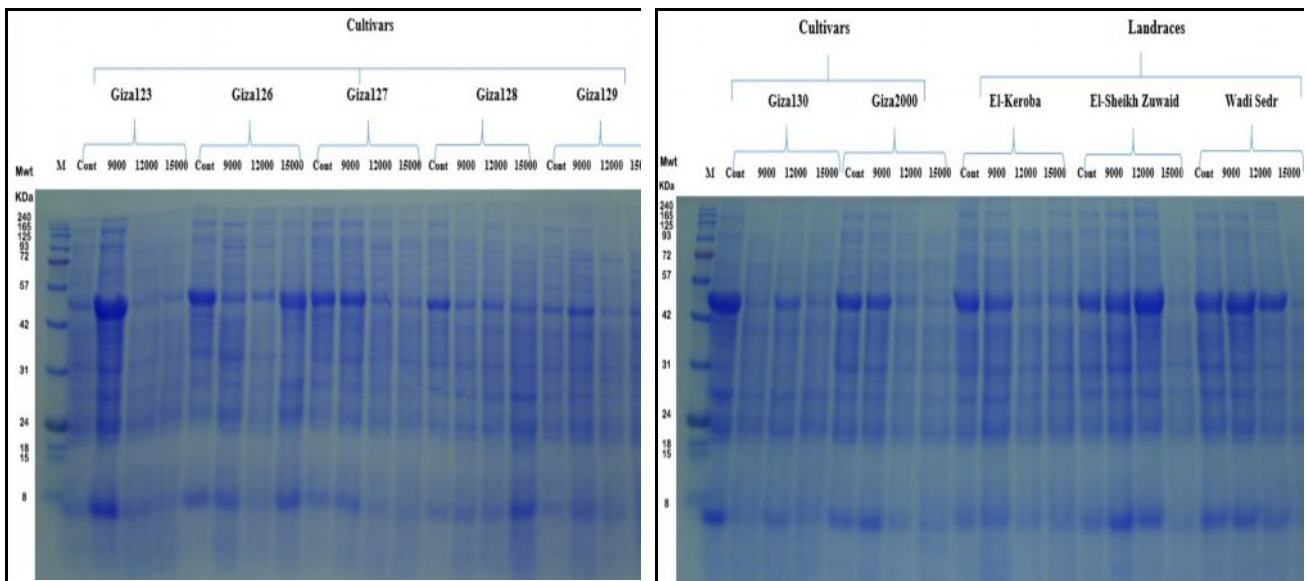


Figure (4): Effect of salinity on protein electrophoresis patterns of ten *Hordeum Vulgare L.* after 2nd week from salt treatments.

Table (4): Effect of salinity on protein electrophoresis patterns of ten *Hordeum Vulgare* L. after 24 hour from salt treatment

No	MW	Cultivars																			
		Gizal23				Gizal26				Gizal27				Gizal28				Gizal29			
		Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000
1	300	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	250	+	-	-	+	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+
3	160	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
4	155	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
5	125	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	110	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	95	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	85	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	74	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	69	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	68	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
12	65	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	60	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
14	58	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
15	55	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	52	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
17	49	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	45	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-
19	40	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
20	35	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
21	33	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
22	31	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
23	29	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
24	28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	25	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
26	22	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
27	18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29	15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	12	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Total		27	25	25	26	28	27	29	27	30	30	30	28	30	30	29	29	30	29	26	27

(-) absent band (+, ++ and +++) gradual increase in the band intensity.

Table (5): Effect of salinity on protein electrophoresis patterns of ten *Hordeum Vulgare* L. after 24 hour from salt treatment.

No	MW	Cultivars																			
		Giza130				Giza2000				El-Kheroba				Landraces El-Sheikh Zuwaid				Wadi Sedr			
		Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000
1	300	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	250	-	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
3	160	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
4	155	-	+	+	+	+	-	+	+	-	+	+	+	-	+	+	+	-	+	+	+
5	125	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	110	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	95	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	85	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	74	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	69	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	68	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
12	65	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	60	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	58	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
15	55	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	52	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
17	49	+	+	+	-	-	-	-	-	+	+	+	-	-	-	+	-	-	-	-	+
18	45	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	40	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
20	35	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
21	33	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
22	31	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
23	29	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
24	28	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+
25	25	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
26	22	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
27	18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29	15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	12	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Total		27	30	30	29	29	28	29	29	26	30	30	29	27	26	27	30	25	26	28	29

(-) absent band (+, ++ and +++) gradual increase in the band intensity.

Table (6): Effect of salinity on protein electrophoresis patterns of ten *Hordeum Vulgare* L. after 1st week from salt treatment

No	MW	Cultivars																				
		Gizal23			Gizal26			Gizal27			Gizal28			Gizal29								
		Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	
1	300	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	250	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	160	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	-
4	155	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
5	125	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
6	110	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
7	95	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	85	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	78	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+	-
11	74	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	69	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	68	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
14	58	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
15	55	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	52	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
17	49	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-
18	45	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	40	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
20	38	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
21	35	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
22	31	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	29	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	28	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
25	25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
27	22	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
28	18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
31	12	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
32	10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
33	8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
34	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Total		32	33	33	33	33	33	33	32	32	32	32	31	32	31	33	31	33	31	31	31	27

(-) absent band (+, ++ and +++) gradual increase in the band intensity.

Table (7): Effect of salinity on protein electrophoresis patterns of ten *Hordeum Vulgare* L. after 1st week from salt treatment

No	MW	Cultivars												Landraces								
		Giza130				Giza2000				El-Kheroba				El-Sheikh Zuwaid				Wadi Sedr				
		Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	
1	300	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	250	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	160	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
4	155	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	125	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	110	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
7	95	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	85	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	83	-	-	-	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+
10	78	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	74	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	69	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	68	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
14	58	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
15	55	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	52	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
17	49	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	45	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	40	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
20	38	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
21	35	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
22	31	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	29	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	25	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
26	24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
27	22	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
28	18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
31	12	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
32	10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
33	8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
34	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Total		32	33	33	32	34	33	34	34	32	34	34	34	33	32	32	33	32	32	33	33	33

(-) absent band (+, ++ and +++) gradual increase in the band intensity.

Table (8): Effect of salinity on protein electrophoresis patterns of ten *Hordeum Vulgare* L. after 2nd week from salt treatment

No	MW	Cultivars																			
		Gizal23				Gizal26				Gizal27				Gizal28				Gizal29			
		Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000
1	300	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	250	+	+	+	+	-	+	+	-	+	+	-	+	+	-	-	-	-	-	-	-
3	160	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
4	155	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
5	125	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	110	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-	-
7	95	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	85	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	74	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	65	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
11	58	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
12	55	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	52	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
14	40	++	+	-	-	+	-	-	+	+	+	-	-	+	-	-	-	-	+	+	-
15	38	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
16	35	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
17	33	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
18	31	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
19	30	-	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
20	29	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
21	28	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
22	25	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
23	22	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
24	12	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
25	7	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Total		23	24	23	23	22	22	22	23	24	24	22	22	24	23	22	22	22	23	21	20

(-) absent band (+, ++ and +++) gradual increase in the band intensity.

Table (9): Effect of salinity on protein electrophoresis patterns of ten *Hordeum Vulgare* L. after 2nd week from salt treatment

No	MW	Cultivars																			
		Gizal30				Giza2000				El-Kheroba				Landracen				Wadi Sedr			
		Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000	Cont	9000	12000	15000
1	300	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	250	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	160	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
4	155	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	125	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	110	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	95	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	85	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	74	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	65	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
11	58	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
12	55	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	52	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
14	40	++	-	+	-	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+
15	38	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
16	35	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
17	33	-	+	+	+	-	+	+	+	-	-	-	+	-	-	+	+	+	+	+	+
18	31	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
19	30	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	29	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
21	28	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
22	25	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
23	22	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
24	12	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
25	7	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Total		23	20	23	20	23	24	24	25	24	24	24	25	23	23	25	25	25	25	25	25

(-) absent band (+, ++ and +++) gradual increase in the band intensity.

References

- Priebe, M. G.; H. Wang; D. Weening; M. Schepers; T. Preston and R. J. Vonk (2010). Factors related to colonic fermentation of nondigestible carbohydrates of a previous evening meal increase tissue glucose uptake and moderate glucose-associated inflammation. *Am. J. Clin. Nutr.* 91: 90-97.
- Munns, R. and M. Tester (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59: 651-681.
- Kaur, R.; K. Singh; and J. Singh (2013). A root-specific wall associated kinase gene, HvWAK1, regulates root growth and is highly divergent in barley and other cereals. *Funct. Integr. Genomics*, 13: 167—177.
- Serrano, R.; F.C. Macia and V. Moreno (1999). Genetic engineering of salt and drought tolerance with yeast regulatory genes. *Sci. Hortic.*, 78: 261-269.
- Maas, E.V. and G.J. Hoffman (1977). Crop salt tolerance current assessment. *ASCEJ. Irrig. Drainage Div.*, 103: 115-135.
- Babu, S.; A. Sheeba; P. Yogameenakshi; J. Anbumalarnathi and P. Rangasamy (2007). Effect of salt stress in the selection of salt tolerance hybrids in rice (*Oryza Sativa* L.) under in vitro and in vivo condition. *Asian. J. Plant Sci.*, 6(1): 137-142.
- Munns, R. (2002). Comparative physiology of salt and water stress. *Plant Cell Environ.*, 25:239-250.
- Hames, B.D. (1990). One-dimensional polyacrylamide gel electrophoresis .In: Gel electrophoresis of protein. Hames, B.D. and Rickwood, D. first edd. Oxford university press.
- El-Atroush. H. M.; A. E. El-Shabasy; M. A. Tantawy and H. M. S. Barakat (2015). Pollen morphology and protein patternof *Nitraria retusa* and some selected taxa of *Zygophyllaceae* in Egypt. *Egypt. J. of Genet. And Cytology*, (under press).
- Laemmli, U.K. (1970). Cleavage of structural prteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.
- Studier, F.W. (1973). Analysis of bacteriophage T1 early RNAs and proteins of slab gels. *J. Mol. Biol.*, 79: 237-248.
- Sairam, R. K. and G. C. Srivastava (2002). Changes in antioxidant activity in sub-cellular fraction of tolerant and susceptible wheat genotypes in response to long term salt stress. *Plant Sci.*, 162(6): 897-904.
- Cuin, T. A. and S. Shabala (2007). Compatible solutes reduce ROS-induced potassium efflux in *Arabidopsis* roots. *Plant Cell Environ.*, 30(7): 875-885.
- Demirkiran, A.; S. Marakli; A. Temel and N. Gozukirmizi (2013). Genetic and epigenetic effects of salinity on in vitro growth of barley. *Genet Mol Biol.*, 36(4): 566-570.
- Liu, J.; H. Gao; X. Wang; Q. Zheng; C. Wang; X. Wang and Q. Wang (2014). Effects of 24-epibrassinolide on plant growth, osmotic regulation and ion homeostasis of salt stressed canola. *Plant Biol.*, 16(2): 440-450.
- Khatab, I. A. and M. A. Samah (2013). Development of Agronomical and Molecular Genetic Markers Associated with Salt Stress Tolerance in Some Barley Genotypes. *Research Journal of Biological Sciences*, 5(5): 198-204.
- Mariey, A.S. (2004). Genetical and molecular studies on barley salt tolerance. M.Sc. Thesis, Tanta Univ., Egypt.
- Oraby, H.F.; C.B. Ransom; A.N. Kravchenko and M.B. Sticklen (2005). Barley HVA1 gene confers salt tolerance in R3 transgenic oat. *Crop Sci.*, 45: 2218-2227.
- Naseer, Sh.; A. Nisar and M. Ashraf (2001). Effect of salt stress on germination and seedling growth of barley (*Hordeum vulgare* L.). *Pak. J. Biol. Sci.*, 4(3): 359-360.
- Ahmad, A.N.; U.H.J. Intshar; A. shamshad and A. Muhammad (2003). Effects of Na, SO and NaCl salinity levels on different yield parameters of barley genotypes. *Intl. J. Agric. Biol.*, 5(2): 157-159.
- Eleuch, L.; A. Jilal; S. Grandi; S. Ceccarelli; M.K. Schmising; H. Tsujimoto; A. Hajer; A. Daaloul and M. Baum (2008). Genetic diversity and association analysis for salinity tolerance, heading date and plant height of barley germplasm using simple sequence repeat markers. *J. Integr. Plant Biol.*, 50: 1004-1014.
- Taghipour, F. and M. Salehi (2008). The study of salt tolerance in Iranian barley (*Hordeum vulgare* L.) genotypes in seedling growth stage. *Am. Eu. J. Agric. Environ. Sci.*, 4: 525-529.
- Konarev, V. G. (1983). Belki rastenii kak geneticheskie marker (Plant Proteins As Genetic Markers), Moscow: Nauka, 14.

24. Haidar, A. S.; M. F. Walid; A. S. Mohamed and A. B. Mohamed (2013). Variability of orphological characters, protein patterns and random amplified polymorphic DNA (RAPD) markers in some Pisum genotypes. *Afr. J. Agric. Res.*, 8(17):1608-1616.
25. Moradpour, K.; A. Najaphy; S. Mansoorifar and A. Mostafaie (2014). Evaluation of leaf protein pattern in wheat genotypes under drought stress. *Int. J. Adv. Biol. Biom. Res.*, 2(3): 840-846.
26. Savithiry, S. N. (2014). Analysis of Soyabean Seed Proteins Using Proteomics. *J. Data Mining Genomics Pproteomics*, 5(1): 2.
27. Ericson, M. C. and S. H. Alfinito (1984). Proteins produced during salt stress in tobacco cell culture. *Plant physiol.*, 74: 506-509.
28. Hurkman, W.J. and C.K. Tanaka (1987). The effects of salt on the pattern of protein synthesis in barley roots. *Plant Physiol.*, 83: 517-524.
29. Takebayashi, N.; M. Hachisuka and S. Mori (1987). Peptide related to NaCl stress in barley roots. *Jpn. J. Soil Sci. Plant Nutr.*, 58: 696-701.
30. Ben-Heyyim, G.; Z. Faltin; S. Gepestein; A. L. Camoin; D. Strosberg; Y. Eshdat (1993). Isolation and characterization of salt associated protein in citrus. *Plant Sci.*, 88: 129-140.
31. Bavei, V.; B. Shiran; M. Khodambashi and A. Ranjbar (2010). Protein electrophoretic profiles and physiochemical indicators of salinity tolerance in sorghum (*Sorghum bicolor* L.). *African Journal of Biotechnology*, 10(14): 2683-2697.
32. Shirata, H. and H. Takgishi (1990). Salt induced accumulation of 26 and 27 KDa proteins in cultured cells of rice plant. *Soil Sci. plant Nutr.*, 36(1): 153-157.
