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THE SALINITY TREATMENT BY NEUTRA-SOL AND EFFECT OF ORGANIC FERTILIZER AND IRRIGATION WATER QUALITY ON PROTEIN PATTERN OF DATE PALM (*Phoenix dactylifera* L.) OFFSHOOTS CV. CHIPCHAP

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ABSTRACT

This study was conducted in Basra Governorate at the Date Palm Research Unit of the Ministry of Agriculture during the growing season 2019/2020. The 27 offshoots of date palm trees cv. Chipchap were selected at the age of 4 years. Analyzed cow manure was added as an organic fertilizer at an amount of 50 kg offshoot^{-1}, and Neutra-sol-335 industrial salinity enhancer was added to treat soil salinity at a concentration of 1.5 L per 200 L of water. The effect of irrigation water quality factor was studied in three levels, which are low salinity irrigation at 3-4 dS m^{-1}of river water and salinity irrigation water at 10 and 15 dS m^{-1}. The protein pattern of offshoots was studied at the end of the experiment. The results of the study showed that there are differences between these treatments in the number, sites and specifications of the protein bands on the polyacrylamide gel. The number of protein bands ranged between 4 - 9 bands depending on the type of treatment. There were four protein bands in the four treatments, which are each of Neutra-Sol, the salinity of irrigation water at 10 dS m^{-1}, the organic fertilizers+ 10 dS m^{-1} salinity of irrigation water and Neutra-Sol+ 15 dS m^{-1} salinity of irrigation water. Whereas, treatment of salinity of irrigation water at 15 dS m^{-1} without enhancer (Neutra-Sol) recorded the highest number of protein bands on the polyacrylamide gel, reaching 9 bands. Salt stress conditions resulted in salinity treatment of irrigation water at 15 dS m^{-1} without enhancer (Neutra-Sol) led to increasing the gene expression process and the appearance of four new protein bands compared to the control treatment to become nine protein bands, whose molecular weights ranged between 219.124 - 34.295 kDa.

Keywords: Cow manure; protein bands; salt stress; soil enhancer.

INTRODUCTION

The Date Palm is the most important species in the Arecaceae family, which includes more than 200 genera and more than 2,500 species. In addition, it is one of the most beneficial plant families after the Poaceae family, and it is of great nutritional and economic importance, which makes it a major contributor to the national income [1,2]. Date palm trees are cultivated in southern Iraq in the clay-textured areas that lie along the Shatt Al-Arab from Al-Faw in the south to Al-Qurna in the north. Al-Faw district is considered the best site for the cultivation and production of date palms [3]. The Chipchap cultivar is one of the varieties spread in the Shatt Al-Arab and southern Tigris. The most of the crop of this variety is picked in the maturation stage (Khalal), boiled with water, then dried and sold in the form of cooked fruits similar to the Brem cultivar and exported to India [4].

Iraq used to be one of the most important date palms producing countries in the world, but the productivity of date palm trees in it has become low due to the unfavorable environmental conditions, especially the problem of salinity and drought. These are among the most important problems facing agriculture on a global scale, especially in arid and semi-arid regions, which affect more than 20% of the world's irrigated lands [5]. Iraq is at the forefront of Arab and Asian countries in terms of total area affected by salinity. The salinity problem in Iraq has exacerbated in recent years, as a result of the scarcity of water and water resources, in addition to the deterioration of its quality, mismanagement and the rise of groundwater levels, which led to the salinization of the soil in the irrigated areas in central and southern Iraq [6].

Therefore, the necessity called for taking some preventive measures to treat these soils, and among these measures is the use of natural enhancers such as organic and industrial materials such as polymers and antioxidants due to their ability to improve the physical and chemical properties of the soil. In addition, the role of these enhancers are increasing the tolerance of the offshoots to unfavorable environmental stress conditions and improving most of them. The physical, biochemical and physiological characteristics of date palm offshoots [7]. Organic and chemical fertilizers are also necessary to increase and improve production. Animal manures are among the most important organic fertilizers that improve the physical and chemical properties of the soil, as it improves the ability of soil to cultivation, increases its water retention and regulates soil temperature. The organic fertilizer increases content of organic matter and improves the biological activity as well as its positive effect in killing some pathogens that are already present in the soil [8]. Salt stress tolerance involves changes in gene expression to synthesize active molecules that induce an adaptation mechanism for the growing plant under the stress conditions. These active molecules are proteins and metabolites, which are involved in regulating the biological processes that occur inside plant cells in response to the conditions of salt stress, ensuring their survival in those unfavorable conditions [9].

The essential aim of the present experiment is to study the effect of Neutra-Sol, animal manure and irrigation water salinity on the molecular and specific changes in protein pattern of date palm offshoots that have grown under salt stress.

MATERIALS AND METHODS

This study was conducted in the Al-Hartha district, north of Basra Governorate, in the Date Palm Research Unit of the Ministry of Agriculture. The 27 offshoots were selected from the date palm trees of the Chipchap cultivar, so that they were as homogeneous as possible in terms of vegetative growth strength and height and there are 4 years old. The service operations were conducted for all the offshoots. The number of leaves was equal in all the offshoots. Randomly compound soil samples were taken to represent the orchard soil at a depth of 0-30 cm to study the soil characteristics before starting the study (Table 1).

Table 1. Some of the chemical and physical characteristics of orchard soil before the beginning of the study at a depth of 0-30 cm in Al-Hartha district, Basra Governorate

Characteristics		Unit	Depth (cm)	
			0-30	30-60
Sand		g Kg^{-1}	111.44	98.21
Silt		g Kg^{-1}	229.26	335.59
Clay		g Kg^{-1}	659.30	566.20
Tissue class		—	Muddy	Muddy
True density		µg m^{-3}	2.65	2.66
Bulk density		µg m^{-3}	1.261	1.314
Total porosity		%	52.49	50.95
Organic matter		g Kg^{-1}	3.47	2.48
pH		—	7.86	7.72
E.C		dS m^{-1}	16.34	18.47
Total Carbonate		g Kg^{-1}	368.33	356.67
Total Nitrogen		g Kg^{-1}	0.088	0.023
K Uptake		mg Kg^{-1}	115.43	58.33
P Uptake		mg Kg^{-1}	11.64	1.18
Dissolved positive and negative ions	Ca^{+2}	mMol L^{-1}	14.00	15.00
	Mg^{+2}	mMol L^{-1}	6.00	7.00
	K^{+1}	mMol L^{-1}	3.31	5.22
	Na^{+1}	mMol L^{-1}	45.70	57.09
	CO_3^{-1}	mMol L^{-1}	0.00	0.00
	HCO_3^{-1}	mMol L^{-1}	3.60	4.40
	Cl^{-1}	mMol L^{-1}	61.00	64.00
	SO_4^{-2}	mMol L^{-1}	12.47	14.11
Irrigation water characteristics	pH	—	7.87	
	E.C	dS m^{-1}	3.6-4.8	

The Experiment Included the Following Factors

Soil enhancer and irrigation water factor: It included

Organic enhancer

The analyzed cow manure was used by adding 50 kg offshoot-1 to the soil by making a semicircular trench around the offshoot trunk with a depth of 50 cm and a width of 40 cm and one meter away from the trunk for all treatments. Then the trench was covered with a layer of soil and irrigating the offshoots by river water using drip irrigation.

Industrial enhancer

The industrial enhancer that used to the salinity treatment is Neutra-Sol-335 at a concentration of 1.5 L per 200 L of water, irrigating one month by adding the compound to the tank containing irrigation water and then irrigating the trees for the specified treatments in a homogeneous manner.

Irrigation water quality factor: This factor includes

A. Low salinity irrigation water (3-4 dS m^{-1}).
B. Saline irrigation water (10 dS m^{-1}).
C. Saline irrigation water (15 dS m^{-1}).

Protein Pattern of Date Palm Offshoots

Samples were dried with a freeze-dryer (Lyophilization technique) at -26°C. Protein was extracted from the samples according to the method described by Al-Najjar [10] by taking 1.0 g of the leaflet and placed in a ceramic mortar with 3 ml of Tris-HCl-buffer (0.1M, pH7.5) containing (PMSF) Phenyl methane sulfonyl fluoride (PMSF). At a temperature of (4°C), then the centrifugation process was carried out at a temperature (4°C) at a speed (18000 r /m) per minute for a period of half an hour. Then (40 µL) of the filtrate was taken to the relay device on a polyacrylamide gel. The polyacrylamide gel is using the Slab-Electrophoresis method in the presence of SDS teratogenic agents according to the method described by Al-Ani [11] and Bavei et al. [12]. The marker was used Broad Range

Protein Molecular Weight Markers from Promega and the molecular weights of the proteins were estimated and drawn through a special computer program (PhotoCapt Mw) Ver.17.

Experiment Design and Statistical Analysis

The experiment was designed using Randomized Complete Block Design (R.C.B.D.) as a two-factor (Factorial experiment). The first factor is soil and water enhancers, and the second factor is the salinity of irrigation water. The number of experimental units reached 27 trees, to which the treatments were distributed randomly. The data of results were analyzed by using analysis of variance to ensure the presence of significant differences between the treatments by the SPSS statistical program Ver.24. The means of treatments were also analyzed and the significance tested according to the revised least significant difference (R-LSD) test with a probability level of 0.05 [13].

RESULTS AND DISCUSSION

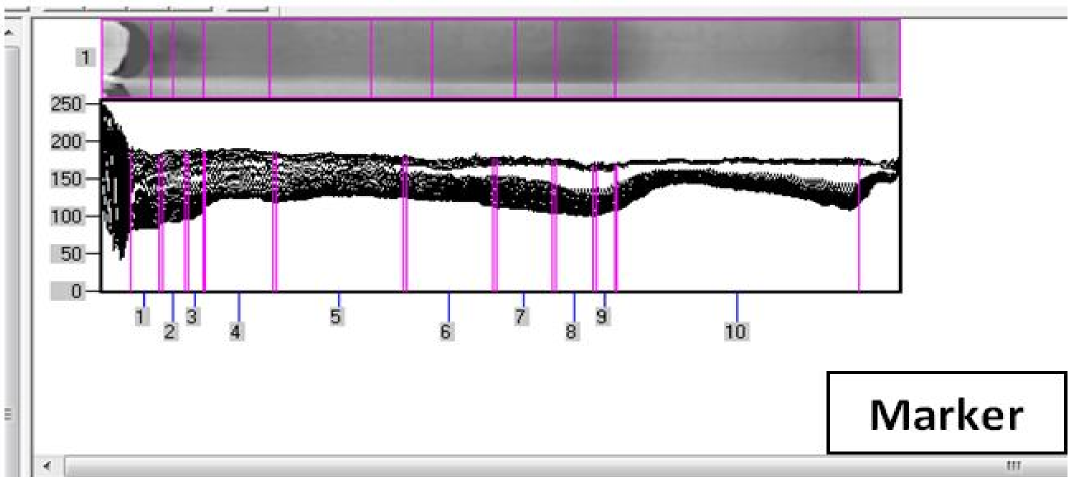
It is noticed from the results shown in Fig. 1, a, b, and c for the protein pattern of the leaves of date palm offshoots of the Chipchap cultivar, that there are significant differences between the study treatments in the protein bands on the polyacrylamide gel. The specifications of the protein bands differed in terms of size, area and height depending on the type of treatment. It is also noted from Fig. 2 that there are differences between these treatments in the number, sites and specifications of the protein bands on the polyacrylamide gel.

The number of protein bands ranged between 4 - 9 bands, depending on the type of treatment. There were four protein bands in four treatments, which are Neutra-Sol treatment+ the salinity of irrigation water at 10 dS m^{-1}, organic fertilizer treatment+ the salinity of irrigation water at 10 dS m^{-1} and Neutra-Sol treatment+ salinity of irrigation water at 15 dS m^{-1}. Whereas, the treatment of irrigation water salinity at 15 dS m^{-1} without enhancer, recorded the highest number of protein bands on the polyacrylamide gel was 9 bands. Also, five protein bands appeared on polyacrylamide gel in three treatments, which are control treatment, irrigation treatment with river water+ Neutra-Sol and irrigation treatment with river water+ organic fertilizer. This leads to the belief that these treatments did not have a gene expression if the offshoots were not subjected to saline stress, or that there might be a significant role for Neutra-Sol and organic fertilizer in improving the tolerance of the offshoots to salt stress. The stress conditions caused by the different treatments also had a clear effect on changing the position of the protein bands. As for the molecular weights of the protein bands shown in Table 2, it indicates that the molecular weights of the first protein band for all conditions converge significantly and clearly, as they ranged between 201.656 - 225.00 kDa. These results also indicate that the date palm offshoots of the Chipchap cultivar originated from

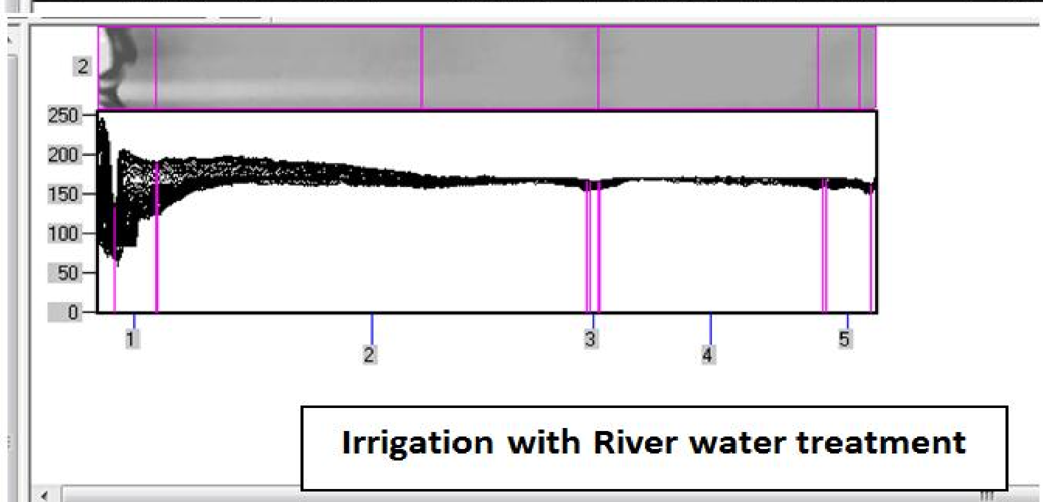
Table 2. Protein bands and their molecular weights (kDa) in date palm offshoots of the Chipchap cultivar

protein packs	1	2	3	4	5	6	7	8	9	10
Marker	219.12	182.03	147.79	130.47	102.8	85.66	65.27	57.37	48.46	34.79
Comparison	201.65	85.19	48.46	35.335	33.38	0	0	0	0	0
River water Irrigation with Nutrosol	204.53	130.02	78.43	48.25	38.34	0	0	0	0	0
River water Irrigation with organic fertilizer	207.43	130.47	78.86	48.46	34.29	0	0	0	0	0
Irrigation water 10 dS/m^{-1}	210.34	136.72	122.36	96.12	48.67	34.39	0	0	0	0
Irrigation water 10 dS/m^{-1} with Nutrosol	213.26	146.90	82.89	37.15	0	0	0	0	0	0
Irrigation water 10 dS/m^{-1} with organic fertilizer	216.19	132.26	71.72	48.25	0	0	0	0	0	0
Irrigation water 15 dS/m^{-1}	219.12	166.35	124.62	100.48	82.89	54.51	48.05	36.56	34.29	0
Irrigation water 15 dS/m^{-1} with Nutrosol	222.06	149.55	81.53	38.81	0	0	0	0	0	0
Irrigation water 15 dS/m^{-1} with organic fertilizer	225.00	150.00	100.00	75.00	50.00	35.00	0	0	0	0

a single origin (vegetative propagation) due to their sharing in the same band. The second, third, fourth, and fifth bands converged in the site and the molecular weight of the two treatments (irrigation with river water+ Neutra-Sol) and (irrigation treatment with river water+ organic fertilizer). It is also noted that a sixth protein band with a molecular weight of 34.396 kDa was observed in the treatment of irrigation water at 10 dS m^{-1} without enhancer.

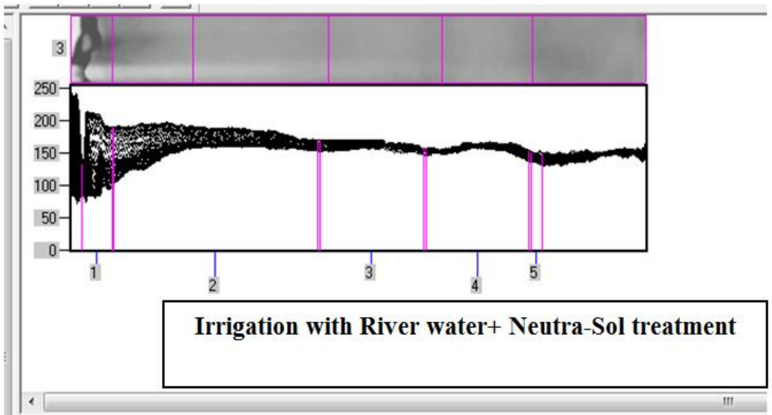


Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	112178	192	954		219.124	6	379376	184	2756		85.661
2	94086	188	742		182.032	7	238776	180	1802		65.272
3	69852	190	530		147.791	8	149888	176	1219		57.379
4	306050	190	2173		130.474	9	77320	172	636		48.466
5	571216	186	4028		102.869	10	1087954	178	7632		34.799

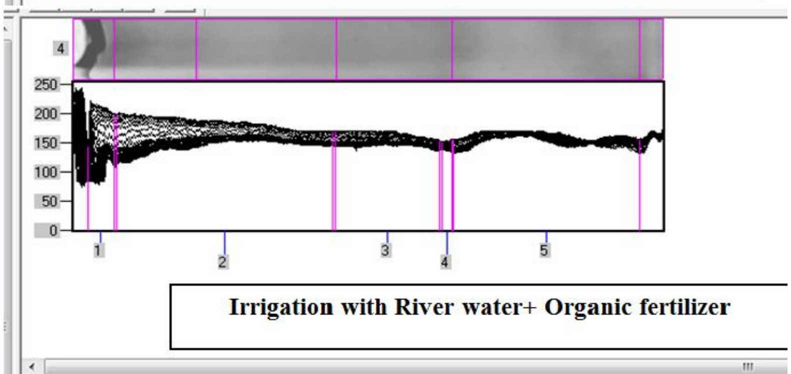


Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	182958	206	1378		201.656	4	1216160	172	7208		35.335
2	2330982	198	13833		85.197	5	240890	170	1484		33.389
3	50996	166	318		48.466						

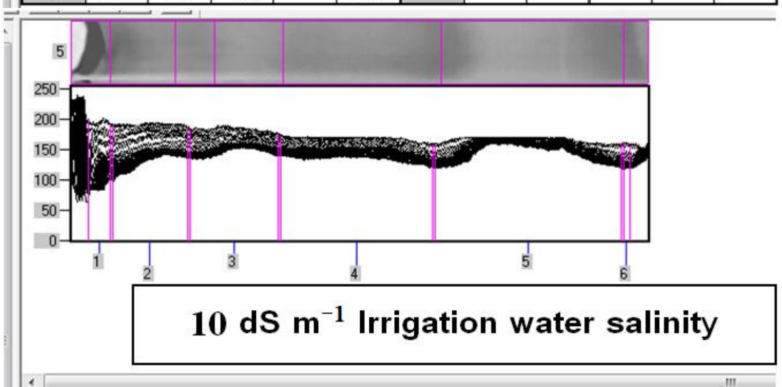
Fig. 1a. Some specifications of protein bands on polyacrylamide gel for date palm offshoots of the Chipchap cultivar (Photocapt program)



Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	173290	214	1378		204.539	4	699464	168	4505		48.257
2	1439698	198	8904		130.026	5	75034	154	530		38.344
3	729532	170	4558		78.430						

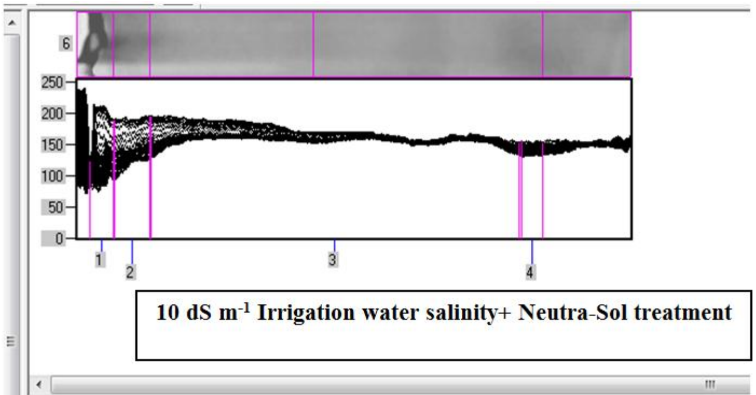


Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	143314	222	1166		207.436	4	67864	156	477		48.466
2	1421038	202	9169		130.474	5	1230032	170	7897		34.295
3	679734	170	4505		78.868						

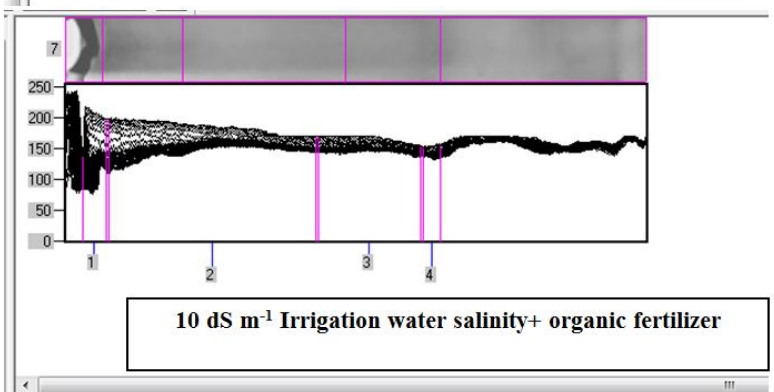


Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	110640	202	1007		210.345	4	961176	174	6625		96.126
2	467484	194	3286		136.720	5	1206476	170	8109		48.678
3	595654	190	3869		122.360	6	40010	162	318		34.396

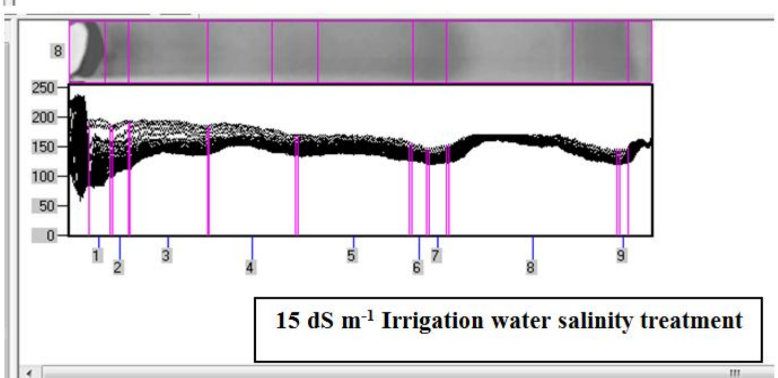
Fig. 1b. Some specifications of protein bands on the polyacrylamide gel of date palm offshoots of the Chipchap cultivar (Photocapt program)



Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	134844	214	1113		213.264	3	2665962	196	16642		82.899
2	234352	196	1643		146.908	4	142086	156	1007		37.152

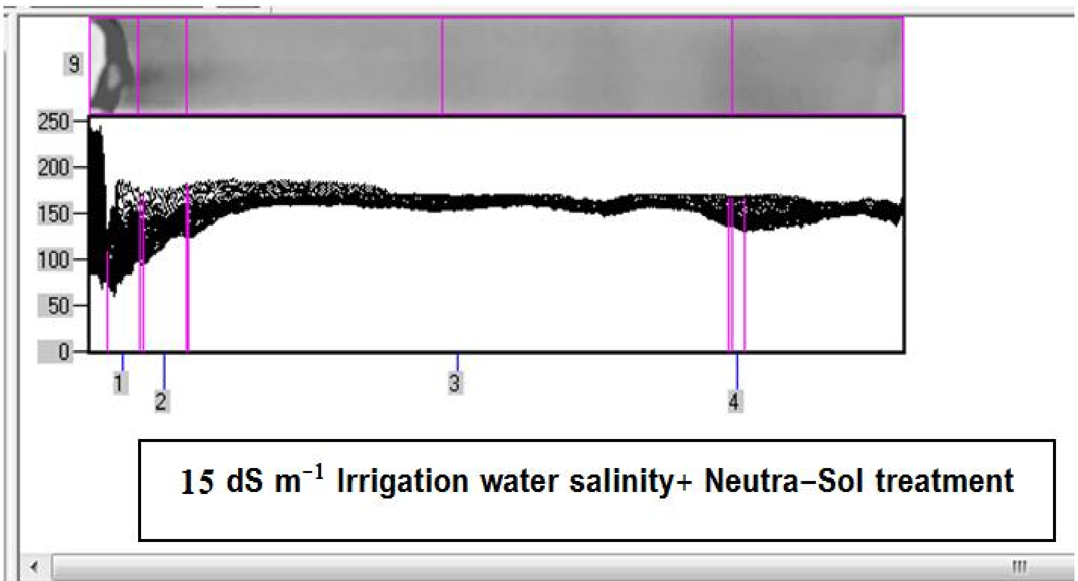


Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	125312	218	1060		216.191	3	668412	170	4452		71.721
2	1373948	200	8957		132.263	4	112898	158	795		48.257

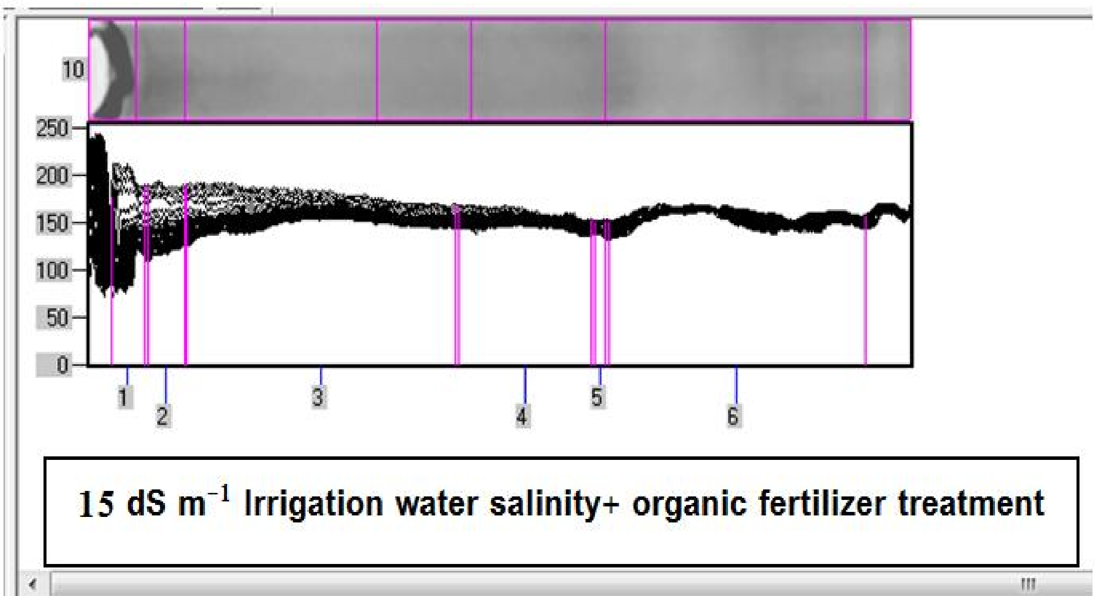


Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	104284	198	954		219.124	6	91462	156	689		54.516
2	94198	194	742		166.354	7	102282	152	795		48.050
3	493264	196	3392		124.625	8	1088788	170	7261		36.565
4	578204	190	3763		100.480	9	53770	150	424		34.295
5	702922	170	4823		82.899						

Fig. 1c. Some specifications of protein bands on the polyacrylamide gel for date palm offshoots of the Chipchap cultivar (Photocapt program)

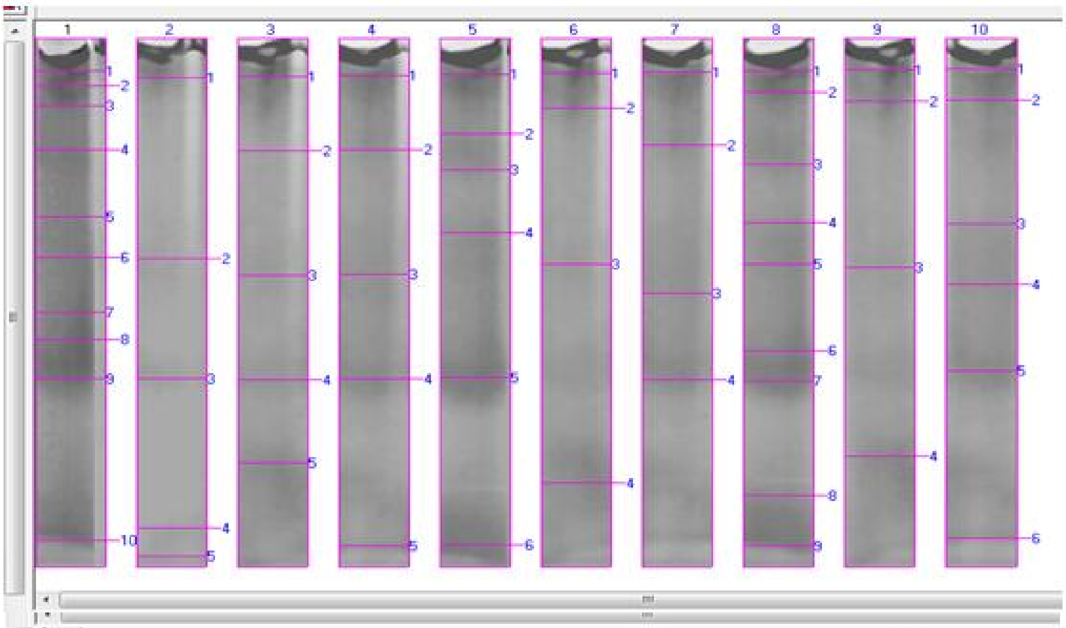


Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	118398	186	1060		222.061	3	2653236	188	16642		81.539
2	187680	182	1378		149.558	4	61228	168	424		38.819



Number	Volume	Height	Area	------	M.W.	Number	Volume	Height	Area	------	M.W.
1	128030	212	1080		225.000	4	621676	168	4158		75.000
2	163350	194	1188		150.000	5	53666	154	378		50.000
3	1298626	192	8370		100.000	6	1234908	170	7992		35.000

Fig. 1d. Some specifications of protein bands on the polyacrylamide gel of date palm offshoots of the Chipchap cultivar (Photocapt program)



	M.W.									
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
1	219.124	201.656	204.539	207.436	210.345	213.264	216.191	219.124	222.061	225.000
2	182.032	85.197	130.026	130.474	136.720	146.908	132.263	166.354	149.558	150.000
3	147.791	48.466	78.430	78.868	122.360	82.899	71.721	124.625	81.539	100.000
4	130.474	35.335	48.257	48.466	96.126	37.152	48.257	100.480	38.819	75.000
5	102.869	33.389	38.344	34.295	48.678			82.899		50.000
6	85.661				34.396			54.516		35.000
7	65.272							48.050		
8	57.379							36.565		
9	48.466							34.295		
10	34.799									

Fig. 2. Number and sites of protein bands and their molecular weights for date palm offshoots of the Chipchap cultivar (Photocapt program)

1- Marker; 2- control; 3- Irrigation with river water+ Neutra-Sol; 4- Irrigation with river water+ organic fertilizer; 5- 10 dS m^{-1}Irrigation water; 6- 10 dS m^{-1} Irrigation water+ Neutra-Sol; 7- 10 dS m^{-1}irrigation water + organic fertilizer; 8- 15 dS m^{-1}Irrigation water; 9- 15 dS m^{-1}Irrigation water+ Neutra-Sol; 10- 15 dS m^{-1}Irrigation water+ organic fertilizer

This may be due to gene expression resulting from salt stress. Salt stress conditions caused salinity treatment of irrigation water at 15 dS m^{-1} without enhancer to increase the gene expression process and the appearance of four new protein bands compared to the control treatment, to become nine protein bands whose molecular weights ranged between 219.124 - 34.295 kDa. These results indicate that the exposure of plants to stress conditions may lead to a deficiency in the synthesis of natural proteins, as well as a change in the transcription and translation processes, which leads to the production of new proteins by the gene expression process according to the plant's need and in response to the type of stress that it is subjected to, ensuring the tolerance of plants for those conditions [14]. Salt stress is one of the most important types of stress affecting plant growth and development. As this type of stress leads to the plant's exposure to the second type of stress, which is water stress, where salinity reduces the plant's ability to absorb water. In general, most of the stresses that plants are exposed to cause the apparent lack of protein transformations and the lack of synthesis of nucleic acids in plant tissues [15]. Stress tolerance includes active molecules that induce an adaptation mechanism for plants growing under stress. These active molecules are Proteins and Metabolites produced by the plant by the process of gene expression, which interferes with the regulation of biological processes inside the plant as a result of its response to stress conditions, in order to ensure the plant's tolerance to these conditions [9]. Through these results, it is possible to rely on the process of gel electrophoresis of proteins on the polyacrylamide gel to find the genetic variances between date palm trees growing in different environmental conditions as it is an accurate and simple chemical method. These results of the study were similar to results of Abdul Wahid and Aati [16] to a number of date palm cultivars using the electrophoresis technique, where the results of their study showed differences in the number and sites of protein bands and their molecular weights ranged between 32.58- 67.939 kDa. The results of this study were also consistent with what Al-Najjar [17] found when he studied on a relationship of environmental stress to the protein pattern in date palms; the study concluded the efficiency of the protein pattern analysis method in determining the nature of the stress effect of different environmental factors on different varieties of date palms.

CONCLUSION

The results of the study showed that there are differences between these treatments in the number, sites and specifications of the protein bands. The number of protein bands ranged between 4 - 9 bands depending on the type of treatment. The salt stress conditions when used treatment of irrigation water at 15 dS m^{-1} without enhancer (Neutra-Sol) led to increasing the gene expression process and the appearance of four new protein bands compared to the control treatment which were nine protein bands.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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