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Effect Of Sodium Chloride And Fulvic Acid On The Activity Of Superoxide Dismutase And Catalase Enzymes, And Proteotype Of Date Palm Offshoot (Phoenix Dactylifera L).; A Nabaiti Variety Produced From Tissue Culture

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Abstract:

The current study was conducted at College of Agriculture and the Marshes, University of Thi-Qar, from February 2020 to February 2021, to study the effect of sodium chloride and fulvic acid and the interaction on some chemical traits of the leaves of the offshoots of date palm (Phoenix dactylifera L.), Nabaiti variety produced from tissue culture. The study was carried out by factorial experiment according to the Randomized Completely Blocks Design (RCBD), using four concentrations of sodium chloride (0, 50, 100 and 150) mM, and three concentrations of fulvic acid (0, 2.5 and 5) g L^{-1}, with three replicates for each treatment. The results showed that sodium chloride treatments had a significant effect on the studied traits, sodium chloride treatment at a concentration of (100 mM) achieved the highest mean of the activity of the enzymes, superoxide dismutase and catalase in the fresh weight trait, whereas, sodium chloride treatments affected the proteotype, as it led to the emergence of new proteins with low molecular weights, with the disappearance of other protein bands compared to the control. As for fulvic acid treatments had a significant effect on the studied traits, the treatment with a concentration (5 g L^{-1}) was the highest mean concentrations of the enzymes superoxide dismutase and catalase in the fresh weight, fulvic acid treatments affected proteotype, caused the emergence of new protein bands with small molecular weights, with the disappearance of other protein bands compared to the control treatment. Interaction between sodium chloride and fulvic acid, some of them had a significant effect on some of the studied traits, the interaction between sodium chloride at a concentration of 150 mM and fulvic acid at a concentration of (0 g L^{-1}) was recorded at the highest averages, for the concentrations of the enzymes superoxide dismutase and catalase in the fresh weight, whereas, all the interactions between sodium chloride and fulvic acid caused the emergence of new protein bands with small molecular weights, other protein bands disappeared compared to the control treatment.

Keywords: sodium chloride, fulvic acid, proteotype, superoxide dismutase, catalase, Nabaiti variety.

Introduction

The date palm (Phoenix dactlylifera L.) is a monocotyledonous plant that belongs to the family Arecaceae and the order palmae, includes more than 200 genera and nearly 4000 species of palm species. The date palm is Iraq's first tree, and it is one of the evergreen fruit trees, it grows in the tropics and subtropics between the latitudes (10-39) north and south of the world. Iraq has the widest land planted with palms in the world, it has more than 627 agricultural varieties, some varieties were distinguished by their commercial importance and abundant production, plays a major role in the national economy in addition to its great nutritional importance.The date palm Nabaiti variety is one of the good and rare varieties. Basra Governorate is considered its original home, and it is present in small numbers, it is considered one of the good varieties, and the color of the khalal is yellow and sweet in taste at this stage, devoid of tannins substance, the fruit is oval in shape and medium in size, the maturity time is also average (Al-Bakr, 1972; Matar, 1991; Ibrahim et al., 2004 and Ibrahim, 2014).

Iraq was, until recently, at the top of the first place in the global date trade for a number of date varieties, such as Al-Zuhdi, Al-Halawi, Al-Sayer, Al-Khadrawi, Al-Buraim, Al-Jibjab, and others. The data of the census in Iraq that took place in 1952 A.D., that the total number of palm trees has exceeded 32 million, the number of fruitful palm trees has reached more than 31.5 million palm trees, the total production amounted to 410 thousand tons, the number of date palms in Iraq in 1968 reached 29.9 million palm trees, production reached 380 thousand tons (Ismail, 2010).

The process of cultivation, production and processing of dates has become completely paralyzed and in continuous decline, especially in recent decades, not investing this national wealth in an optimal way to develop the fields of agriculture, production, manufacturing and marketing, this also included the decline in the number of palm trees, and the damage it suffered as a result of wars and neglect, smuggling of good items out of the country, the migration of large numbers of producers to this profession and salinity of water and soil and other reasons. In 2020, the number of date palm trees in Iraq reached 17,348,741 palm trees, the actual product of them is 11,225,016 palm trees. There are 1,046.754 palm trees in Thi-Qar Governorate, the product is actually 423.702 palm trees. The total production of dates in Iraq for the same year amounted to 735,353 thousand tons, 49,597 tons are in Thi-Qar Governorate (Dates production report, 2020).

The size of the significant decline in the number of palm trees and the quantities of dates produced in Iraq is clearly shown, water scarcity and high salinity (salinity of water and soil) have contributed, especially in the orchards of the southern region, in addition to the previously mentioned reasons, a significant role in this decline, because the problem of soil salinity and irrigation water, it was one of the most important problems facing agriculture worldwide, especially arid and semi-arid regions (Munns and Tester, 2008; Alturki, 2018).

The use of saline water for irrigation, it affects approximately one third of the irrigated lands in the world in humid areas as well as arid and semi-arid areas (Yaish and Kumar, 2015). The percentage of salt accumulation increased from 1983 to 2005 to about 40% in arable lands (Guo et al., 2017). Therefore, Iraq is at the forefront of the Arab and Asian countries in terms of the total area affected by salinity (Batanoay, 1996).

The harmful effects of salinity are due to plant growth, to ion poisoning, especially ions of sulfur, chloride and sodium, in addition to osmotic tension, lack of necessary elements and oxidative stress, also ionic imbalance (Munns and Tester, 2008).

It has become necessary to use some possible alternative techniques at the present time in order to improve the salt tolerance of plants (Jasim et al., 2010). Many recent studies have shown, there are a lot of chemical compounds, nutrients and growth stimulants, which can be used to reduce the effects of salinity on the growth and production of plants, one of these compounds is fulvic acid, it is a plant organic acid that is naturally produced from humic matter, resulting from the decomposition of organic matter, adding them to the soil or plant causes an increase in the absorption of nutrients, especially when exposed to salt stress (Cimrin et al., 2010).

Fulvic acid also improves the physical, chemical and biological properties of the soil, reduces the problems and damages of excessive salinity and alkalinity, thus, it increases the strength of the root group and its ability to absorb (Shaaban et al., 2009).

Through the foregoing, this study came, in which date palm cuttings of the Nabaiti variety were used, produced from tissue culture in order to improve its salt tolerance, using fulvic acid, to determine the effects of sodium chloride and fulvic acid on the plant.

Materials and Methods:

This study was conducted at the University of Thi-Qar, College of Agriculture and the Marshes, for the period from March 2019 to March 2020 Using 36 offshoots of date palm cultivar Nabaiti, the output from tissue culture and identical in size and at the age of three years. The offshoots were watered with low saline concentrations, gradually increasing by 25 mM in each watering, until the required study concentrations are reached, and stability on it for the purpose of avoiding the exposure of plants to shock (shock) by the effect of salt stress, then the plants were watered with the same concentration for 10 waterings between one watering and another 10 days. The plants were irrigated with aqueous solution of fulvic acid and for 10 waterings between one watering and the other for 10 days. The parameters of the soil and irrigation water used for the study were as shown in the following table:

Table (1): Soil and water analysis.

No.	Parameters	EC	pH
1	Soil	2.73	6.93
2	Water	11.17	7.10

Experimental measurements:

Determination of the activity of superoxide dismutase enzyme:

The activity of the enzyme superoxide dismutase was measured according to the method of Marklund and Marklund (1974). Based on the enzyme's ability to prevent the oxidation of Pyrocalol at pH 8.20.

Estimation of the activity of the enzyme catalase:
The activity of the enzyme catalase was estimated according to the method (Hadwan and Kadhum, 2018).

Electrophoresis for proteins:
The protein was extracted according to (Wu et al., 2014). Migration was carried out by taking 5 micro-liter of sample solution according to (Sambrook and Russell, 2001; Hashim et al., 2011). Silver staining was performed and the image analyzed by CS analyzer (Blum et al., 1987).

Statistical Analysis
The experiment was carried out as a two-factor experiment, the first factor is sodium chloride with four concentrations (0, 50, 100 and 150 mM), the second factor is fulvic acid in three concentrations, which are (0, 2.5 and 5) g L^{-1}, with three replicates for each treatment, by 36 experimental units, according to the Randomized Completely Block design (RCBD). The data were statistically analyzed according to the ANOVA Table, using the statistical analysis program Genstat 31 dec (2012). The averages were compared using the Revised Least Significant Difference test (R.L.S.D.) at the level of significance of 5% (Al-Rawi and Khalaf Allah, 1980).

Results and Discussion

Superoxide dismutase activity:
Table (2) shows that there was a significant increase in the concentration of superoxide dismutase enzyme with an increase in the concentration of sodium chloride. The treatment with concentration 100 mM gave the highest mean of 10.86 enzymatic absorption units g^{-1} fresh weight, which did not differ significantly from the treatment with a concentration of 150 mM, it gave an average of 10.54 enzymatic absorption units g^{-1} fresh weight, compared with the control treatment, which recorded the lowest mean of 9.08 enzymatic uptake units g^{-1} fresh weight.

As for the effect of fulvic acid, it was significant in increasing the concentration of superoxide dismutase with an increase in the concentration of fulvic acid, the treatment with a concentration of 5 g L^{-1} gave the highest mean of 10.81 enzymatic absorption units g^{-1} fresh weight, compared with the control treatment, which recorded the lowest mean of 8.96 enzymatic absorption units g^{-1} fresh weight.

The interaction between sodium chloride and fulvic acid, some of them had a significant effect on the concentration of superoxide dismutase enzyme, for the leaves of the date palm, a tissue culture-produced cultivar, the interaction treatment between sodium chloride (150 mM) and fulvic acid at a concentration of 0 g L^{-1} was significantly superior, it gave the highest mean of 12.38 enzymatic absorption units g^{-1} fresh weight, compared to the control treatment, which recorded the lowest mean of (5.17 enzymatic absorption units g^{-1} fresh weight).

Table (2): Effect of sodium chloride and fulvic acid and the interaction between them on the concentration of superoxide dismutase (enzymatic absorption unit g^{-1} fresh weight).

NaCl concentration (mM)	Fulvic acid concentration g L^{-1}			NaCl Mean
	0	2.5	5	
0	5.17	10.73	11.33	9.08
50	7.74	9.40	10.80	9.31
100	10.53	10.61	11.43	10.86
150	12.38	9.55	9.68	10.54
Fulvic acid Mean	8.96	10.08	10.81	
R.L.S.D.$_{0.05}$	**Fulvic acid**	**NaCl**	**Interaction**	
	0.52	0.45	0.90	

Catalase enzyme activity:

Table (3) showed that sodium chloride had a significant effect by increasing the activity of catalase enzyme by increasing the concentration of sodium chloride in the growth medium, the concentration treatment (100 mM) outperformed and gave the highest mean of 2.74 enzymatic absorption units g^{-1} fresh weight, which did not differ significantly with the concentration treatment (150 mM), which recorded an average of 2.71 enzymatic absorption units g^{-1} fresh weight, compared with the control treatment, which gave the lowest rate (1.52 enzymatic absorption units g^{-1} fresh weight).

As for folic acid, it had a significant effect on the activity of the enzyme catalase, and the concentration treatment was outperformed (5 g L^{-1}), it gave the highest mean of 2.67 enzymatic absorption units g-1 fresh weight, compared with the control treatment, which recorded the lowest mean of (2.26 units of enzymatic absorption unit g^{-1} fresh weight, which did not differ significantly from the treatment with concentration (2.5 g L^{-1}), which recorded an average of 2.04 enzymatic absorption units g^{-1} fresh weight.

The interaction between sodium chloride and fulvic acid had a significant effect in increasing the activity of catalase enzyme, the interaction treatment between (sodium chloride at a concentration of 150 mM and fulvic acid at a concentration of 0 g L^{-1} gave the highest mean of 3.28 enzymatic absorption units g^{-1}- fresh weight, which did not differ significantly with the interaction treatment between NaCl 100 mM and fulvic acid at a concentration of 5 g L^{-1}, which recorded a rate of (3.15 enzymatic absorption units g^{-1} fresh weight, compared with the control treatment, which recorded the lowest rate (0.89 units of enzymatic absorption unit g^{-1} fresh weight)

Table (3): Effect of sodium chloride and fulvic acid and the interaction between them on the concentration of catalase enzyme (enzymatic absorption unit g^{-1} fresh weight).

NaCl concentration (mM)	Fulvic acid concentration g L^{-1}			NaCl Mean
	0	2.5	5	
0	0.89	1.26	2.41	1.52
50	2.07	2.20	2.72	2.33

100	2.81	2.26	3.15	2.74
150	3.28	2.45	2.41	2.71
Fulvic acid Mean	2.26	2.04	2.67	
R.L.S.D.$_{0.05}$	**Fulvic acid**	**NaCl**	**Interaction**	
	0.32	0.27	0.55	

The results in tables (2 and 3) showed a significant increase in the concentrations of the enzymes superoxide dismutase and catalase by the effect of sodium chloride and fulvic acid. The reason for this increase may be due to the effect of sodium chloride, that salt stress can induce a state of oxidative stress, as it stimulates and activates the plant's defense systems to remove or reduce its harmful effects as it produces Reactive Oxygen Species (ROS), such as O_2^- (Super Oxide), Hydroxyl radicals (OH^-) and Hydrogen Peroxide (H_2O_2), that cause severe damage when the plant remains under stress conditions (Ashraf and Harris, 2004; Jehan et al., 2012). The most important of which is the damage to the metabolic processes in the plant due to the oxidative stress that it causes to the membrane lipids, proteins and nucleic acids (Chookhampaeng, 2011; Elsahookie, 2013), which prompts the plant to stimulate its defense systems, the most important of which is the enzymatic defense system. The most important of these antioxidant enzymes are superoxide dismutase and catalase (Ashraf and Foold, 2007; Ashraf et al., 2008; Ashraf, 2009).

As the superoxide dismutase enzyme is considered the first line of defense in the plant, where it was found by (Geebelen et al., 2002; Alscher et al., 2003). The enzyme superoxide dismutase (SOD) looks for the superoxide radical onions and converts it to hydrogen peroxide, while the enzyme catalase is the second line of defense in the plant or cell, which works to convert the very harmful hydrogen peroxide into water and an oxygen molecule, because the concentration of hydrogen peroxide increases in the cell as a result of the oxidative stress of salinity, in addition to the quantities resulting from the action of the enzyme superoxide dismutase (Alscher et al., 2003; Al-Desuquy, et al., 2012).

The levels of (ROS) inside the plant cell remain at their lowest level, by multiple protection mechanisms, however, an increase in ROS occurs during certain periods of development, it was also a response to certain types of stress, the most important of which is salt stress. Plant cells possess both enzymatic and non-enzymatic defense mechanisms, which can overcome oxygen toxicity, delaying the harmful effects of free radicals (Larson, 1988; Ames et al., 1993). Enzymatic protection system, includes Superoxide Dismutase (SOD), Catalase (CAT) and Acrobat Peroxidase (APX), they are key mechanisms in the removal of ROS (Asada et al., 1973; Bowler et al., 1992; Wellekens et al., 1997). These enzymes are able to remove or neutralize ROS, the balance between SOD and APX or the activity of catalase in cells, it has a major role in removing ROS (Bowler et al., 1992), without these defenses, plants cannot convert light energy into mechanical energy efficiently, where the SOD acts as the main line of defense, to catalyze the conversion of superoxide radicals to molecular oxygen and hydrogen peroxide, then the enzymes CAT and APX remove the hydrogen peroxide, SOD and CAT are the most important in cells as antioxidant enzymes, its combined activity converts (O_2^-) and hydrogen peroxide into water and oxygen, thus

avoiding cellular damage, mitochondria and chloroplasts contain mechanisms of ROS removal (Mittler et al., 2004).

This may be due to the increased activity of the enzymes superoxide dismutase (SOD) and catalase (CAT), increasing the concentration of fulvic acid, role fulvic acid play, in improving the tolerance of plants to salt stress conditions, it acts as a protective substance by stimulating the protective effects of the plant, the most important of which is to increase the effectiveness of antioxidant enzymes, including the enzymes superoxide dismutase and catalase, they were mentioned (Zaki, 2017; Fahim, 2019).

Fulvic acid has a natural chelating ability, it helps to chelate and facilitate nutrients in the soil, which helps to facilitate the microelements fixed in the soil, elements such as iron, zinc, manganese and copper, as a result of facilitating these microelements, because SOD is a mineral enzyme, it was found in plants of three types called isozyme, depending on the metal used as a catalyst, were MnSOD, FeSOD and Cu/ZnSOD, there was a great similarity in the functions of these types, which is (detoxification of superoxide ions), but their presence in different places inside the cell (Van Comp et al., 1994; Morgan et al., 2008).

Cu/ZnSOD found in the cytosol, MnSOD found in mitochondria, the FeSOD found in chloroplasts. Nevertheless, MnSOD has also been observed in chloroplasts (Coratao et al., 2006). As a result, it is highly likely that fulvic acid plays a major role in increasing the activity of the enzyme superoxide dismutase, as a result of facilitating and increasing the absorption of the mineral elements that are associated with it, therefore, there is an increase in the activity of this enzyme, as shown by the results of the current study in Table (2). The SOD enzyme acts as the main line of defense, to catalyze the conversion of superoxide radicals into molecular oxygen and hydrogen peroxide then comes the role of the enzyme catalase (CAT) by removing hydrogen peroxide, therefore, it is necessary to increase the activity of the enzyme catalase with the increase of the activity of the enzyme superoxide dismutase, because it depends on increasing its activity on the amounts of hydrogen peroxide, resulted from the oxidative stress of salinity and the activity of the enzyme superoxide dismutase (Mittler et al., 2004).

Gel electrophoresis (proteotype):

The results showed in Figures (1 and 2) and Table (4) that the increase in the concentrations of sodium chloride and fulvic acid and their interactions, it led to changes in the protein phenotype of the leaves of histologically produced date palm offshoots, as the control treatment gave nine protein bands with molecular weights of 10, 12, 13, 18, 28, 30, 33, 48 and 52 kDa, while the treatment of sodium chloride at a concentration of 50 mM gave eleven protein bands whose molecular weights reached 10, 12, 13, 15, 18, 27, 28, 30, 33, 36 and 50 kDa. Four new bands (15, 27, 36 and 50) kDa compared to the control treatment, while two protein bands (48 and 52) kDa disappeared, it appeared in the control treatment and disappeared in the sodium chloride treatment (50 mM), as for the rest of the bands, which were 10, 12, 13, 18, 28, 30 and 33 kDa, they were quite similar to bands featured in a control treatment.

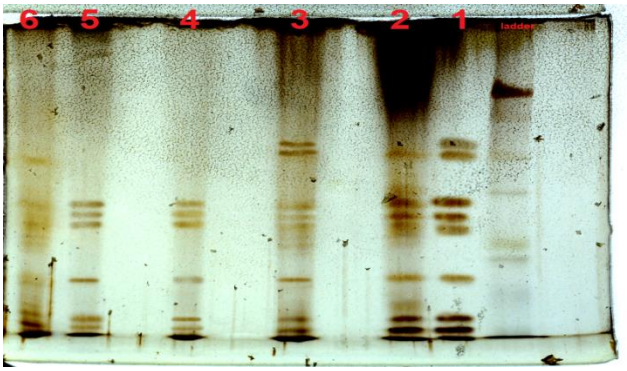
Treatment of sodium chloride at a concentration of 100 mM, it gave eleven protein bands whose molecular weights reached 10, 12, 13, 18, 24, 27, 28, 30, 32, 50 and 52 kDa, of them, four new bands

were 24, 27, 32 and 50 kDa, compared to the control treatment. Two protein bands (33 and 48) kDa, which appeared in the control treatment, disappeared, vanished in NaCl treatment (100 mM), as for the rest of the bands (10, 12, 13, 18, 28, 30 and 52) kDa, they were completely similar to the bands that appeared in the control treatment.

Treatment of sodium chloride at a concentration of 150mM, it gave seven protein bands with molecular weights of 11, 12, 13, 18, 28, 31 and 33 kDa, of them, two new bands were 11 and 31 kDa, while four bands (10, 30, 48 and 52) kDa disappeared in the sodium chloride (150mM) treatment, which were visible in the control treatment, as for the rest of the bands (12, 13, 18, 28 and 33) kDa, they were completely similar to the bands that appeared in the control treatment.

Fulvic acid treatment at a concentration of 2.5 g L^{-1} gave seven protein bands with molecular weights of 11, 12, 13, 18, 28, 30 and 32 kDa, among them were two new bands, 11 and 32 kDa, while four bands (10, 33, 48 and 52) kDa disappeared, compared to the control treatment, as for the rest of the bands (12, 13, 18, 28 and 30) kDa, they were completely similar to the bands that appeared in the control treatment.

The treatment of fulvic acid at a concentration of 5 g L^{-1} gave eleven protein bands whose molecular weights reached 12, 14, 15, 18, 19, 25, 26, 28, 30, 32 and 47 kDa, seven new bands appeared (14, 15, 19, 25, 26, 32 and 47) kDa, compared to the control equation as for the rest of the bands, which were 12, 18, 28 and 30 kDa,, they were completely similar to the bands that appeared in the control treatment, while five bands (10, 13, 33, 48 and 52) kDa had appeared in the control treatment and disappeared in the fulvic acid treatment at a concentration 5 g L^{-1}.



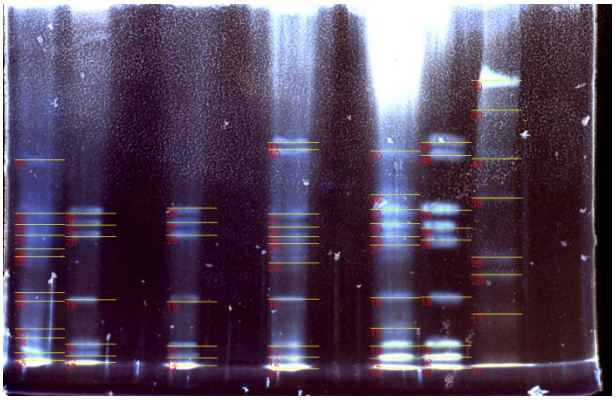


Figure (1): Gel electrophoresis of the treatments (1-6).

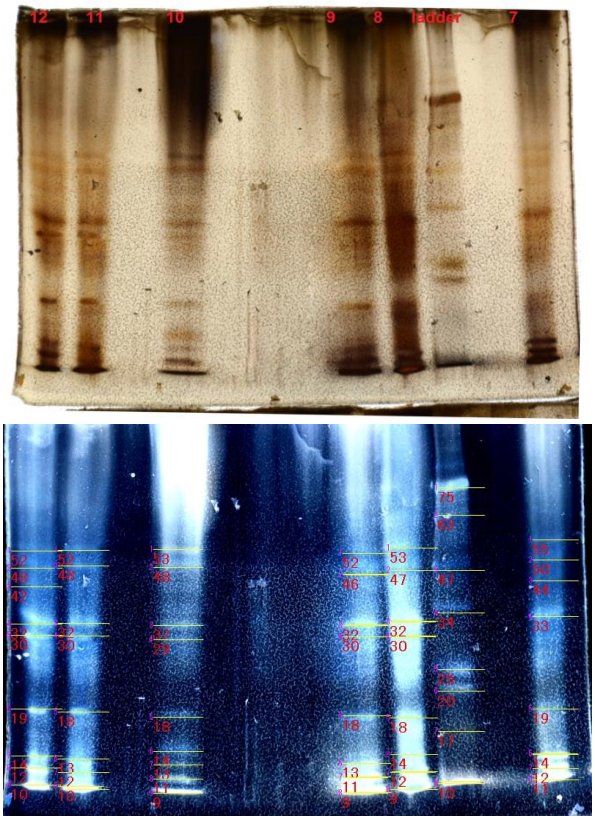


Figure (2): Gel electrophoresis for treatments (7-12).

Treatment of the interaction between sodium chloride at a concentration of 50 mM and fulvic acid at a concentration of 2.5 g L^{-1}, it gave eight protein bands with molecular weights of 11, 12, 14, 19, 34, 43, 50 and 55 kDa, of these, seven new bands were 11, 14, 19, 34, 34, 50 and 55 kDa, one band is completely similar to what appeared from the control treatment, which was 12 kDa, while the eight protein bands that appeared in the control treatment disappeared, they did not appear in the interaction treatment, which were 10, 13, 18, 28, 30, 33, 48 and 52 kDa.

The interaction treatment between sodium chloride (50 mM) and fulvic acid (5 g L^{-1}) gave eight protein bands with molecular weights of 10, 12, 14, 18, 30, 33, 47 and 53 kDa, of these, three new bands were 14, 47 and 53 kDa, as for the rest of the bands, which were five bands (10, 12, 18, 30 and 33) kDa, they are quite similar to the bands featured in a control transaction whereas, four bands (13, 28, 48 and 52) kDa disappeared from this treatment and appeared in the control treatment.
The interaction treatment between sodium chloride at a concentration 100 mM and fulvic acid at a concentration 2.5 g L^{-1} gave eight protein bands with molecular weights of 9, 12, 13, 18, 30, 32, 46 and 52 kDa, of which three new bands were 9, 32 and 46 kDa compared to the control treatment, as for the rest of the bands, which were 12, 13, 18, 30 and 52 kDa, they are completely similar to what appeared in the control treatment, while four bands were 10, 28, 33 and 48 kDa disappeared from this treatment, compared to the control treatment.

The interaction treatment between sodium chloride at a concentration 100 mM and fulvic acid at a concentration 5 g L^{-1} showed nine protein bands with molecular weights of 9, 11, 13, 14, 18, 30, 32, 48, and 53 kDa, of which five new bands were 9, 11, and 14, 32 and 53 kDa compared to the control treatment, while the rest of the bands (13, 18, 30 and 48) kDa were completely similar to the bands that appeared in the control treatment, while five bands (10, 12, 28, 33 and 52 kDa) disappeared from this treatment compared to the control treatment.

The interaction treatment between sodium chloride at a concentration 150 mM and fulvic acid at a concentration 2.5 g^{-1} gave eight protein bands whose molecular weights reached 10, 12, 14, 18, 30, 32, 48 and 52 kDa, of which two new bands were 14 and 32 kDa, and the rest of the six bands were 10, 12, 18, 30, 48 and 52, they were completely similar to the bands that appeared in the control treatment, while three bands were 13, 28 and 33 kDa disappeared from this treatment compared to the control treatment.

The interaction treatment between sodium chloride at a concentration 150 mM and fulvic acid at a concentration of 5 g L^{-1} gave nine protein bands with molecular weights of 10, 13, 14, 19, 30, 32, 42, 48 and 52 kDa, of which four were new bands, were 14, 19, 32 and 42 kDa, as for the rest of the bands, which are five bands (10, 13, 30, 48 and 52) kDa, they are completely similar to the bands that appeared in the control treatment, while four bands (12, 18, 28 and 33) disappeared from the This treatment compared to the control treatment.

Table (5): The effect of sodium chloride and fulvic acid and the interaction between them on the protein pattern of leaves of date palm cuttings, a Nabaiti variety.

Treatments	Bands No.	Divergent bands	Molecular weights (kDa)
Ladder	8		11, 17, 20, 25, 34, 47, 63 and 75
0	9		10, 12, 13, 18, 28, 30, 33, 48 and 52
NaCl (50 mM)	11	4	10, 12, 13, 15, 18, 27, 28, 30, 33, 36 and 50

NaCl (100 mM)	11	4	10, 12, 13, 18, 24, 27, 28, 30, 32, 50 and 52
NaCl (150 mM)	7	2	11, 12, 13, 18, 28, 31 and 33
Fulvic acid (2.5 g L^{-1})	7	2	11, 12, 13, 18, 28, 30 and 32
Fulvic acid (5 g L^{-1})	11	7	12, 14, 15, 18, 19, 25, 26, 28, 30, 32 and 47
NaCl (50 mM)+ Fulvic acid (2.5 g L^{-1})	8	7	11, 12, 14, 19, 34, 43, 50 and 55
NaCl (50 mM)+ Fulvic acid (5 g L^{-1})	8	3	10, 12, 14, 18, 30, 33, 47 and 53
NaCl (100 mM)+ Fulvic acid (2.5 g L^{-1})	8	3	9, 12, 13, 18, 30, 32, 46 and 52
NaCl (100 mM)+ Fulvic acid (5 g L^{-1})	9	5	9, 11, 13, 14, 18, 30, 32, 48 and 53
NaCl (150 mM)+ Fulvic acid (2.5 g L^{-1})	8	2	10, 12, 14, 18, 30, 32, 48 and 52
NaCl (150 mM)+ Fulvic acid (5 g L^{-1})	8	4	10, 13, 14, 19, 30, 32, 42, 48 and 52

It can be seen from the results in Figures (1 and 2) and Table (4), the treatments of sodium chloride and fulvic acid and their interactions, it has caused the disappearance of proteins and the emergence of new proteins, the reason may be that salt stress conditions cause significant changes in the process of gene expression, leads to changes in the pattern of accumulated proteins, cause the disappearance of some proteins or the emergence of new proteins (Munns, 2005; Yamaguehi and Blymwad, 2005). In addition, the exposure of plant cells to salt stress, leads to the production of the growth inhibitor abscisic acid ABA, this hormone is closely related to plants' tolerance to salt stress conditions, by transmitting the signal leading to genetic induction, the formation of proteins that have an osmotic protection function (Munns, 2005).

The results showed that sodium chloride caused the accumulation of very close proteins with small molecular weights ranging from 52-10 kDa, the reason for the formation of these proteins may be due to the lengthening of the adaptation period to salinity, cause an increase in the production of these proteins and can be considered as osmotin proteins, confirmed by a number of researchers that there are proteins that accumulate repeatedly in saline media, Osmo Protectants Proteins (Hare et al., 1998; Chen and Murata, 2002; Rodes et al., 2002).

In addition, the construction of these proteins may be in response to sodium chloride levels in the growth medium, which may be beneficial to the plant, it was mentioned Boner (2000) that the proteins that are made in the plant with small molecular weights are formed in response to the stress conditions of salt, it increases the osmotic pressure of the cells and thus improves the efficiency of water absorption by the plant.

The effect of fulvic acid on the disappearance of proteins and the appearance of other proteins, especially with small molecular weights in its treatments or in its interactions with sodium chloride the reason may be due to the role of fulvic acid in treating soil salinity by chelating the calcium element

present in the soil, calcium becomes free, active and facilitating. It was easily absorbed and accumulated in the plant. The calcium ion is a second messenger in many physiological processes, including inducing gene expression in the construction of proteins that help the plant adapt to salt stress conditions (Fahim, 2019). In addition to the role of fulvic acid in breaking the ionic bond of sodium chloride in the soil, thus, the sodium element can be expelled through the washing process of the soil. The sodium element is expelled with the puncture water, which reduces its effect on the plant and increases its adaptation to salt stress conditions (Pardo et al., 1998; Kawano and Muto, 2000; Kim et al., 2007).

Conclusions

The results of this study confirmed that fulvic acid led to a significant increase in the concentrations of the enzyme superoxide dismutase and catalase, this is one of the important indications for increasing the plant's tolerance to the salt stress condition, may have stimulated the gene expression process of the plant, leads to the disappearance of proteins and the emergence of new proteins, especially those with small molecular weights.

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