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Original Article

Role of Sulphur in salinity tolerance of Date Palm (*Phoenix dactylifera* L.) offshoots cvs. Berhi and Sayer

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Abstract

A field experiment was conducted to investigate the role of sulphur application in salinity tolerance of Date Palm offshoots cvs Berhi and Sayer. The effect of sulphur on plant growth under saline conditions (The average of EC soil of field was (15.93 dS m^{-1}) and to EC water (4.55 dS m^{-1})), two sulphur levels (100 and 200 g offshoot^{-1} year.$^{-1}$ were addition to Soil), The results showed that the sulphur at level of 200g caused a significant increase the offshoot height, leaf area, number of leaves and girth of plant with cv. Berhi compared with control. Also, sulphur application significantly influenced biochemical characteristics such as (Total Chlorophyll, Dry weight, RWC, Carbohydrates, proline concentration soluble protein, peroxidase enzyme activities and endogenous indol acetic acid (IAA) content of two cultivars compared with control. As well as, treatment of sulphur at 200g offshoot^{-1} year.$^{-1}$ to Berhi cultivar gave the highest values in these respects. While the results significantly showed decrease in respect of Catalase enzyme activities and content of ABA. Howover, using sulphur at 200 g offshoot^{-1} year.$^{-1}$ to Berhi cultivar significantly decreased Na^+ and Cl^- concentration of leaves compared with control and improved salinity tolerance of offshoots by maintaining high ratio of K/Na.

Key words: Sulphur, Date Palm, salinity tolerance, proteins, Antioxidant enzymes, IAA, ABA.

Introduction

The salinity in nature imposes two primary harmful effects on plants: one is osmotic stress and the other, ionic toxicity. Due to the presence of high salt, salinity stress increases the osmotic pressure in the soil solution over the osmotic pressure in plant cells. As a result, plant loses its ability for uptake of water and minerals, especially the uptake of K + and Ca^{2+} [1], [2], [3].

Plant growth responds to salinity in two phases: a rapid, osmotic phase that inhibits growth of young leaves, and a slower, ionic phase that accelerates senescence of mature leaves. Plant adaptations to salinity are of three distinct types: osmotic stress tolerance, Na^+ or $Cl^−$ exclusion, and the tolerance of tissue to accumulated Na^+ or $Cl^−$ [4]. Date palms *Phoenix dactylifera* L. establish most of their growth in hot weather when salinity has the most adverse influence on plants. Furthermore, some varieties can tolerate salinity levels up to 22000 ppm (EC 34 dS m^{-1}). However, their growth and yield productivity are affected [5]. Sulphur is a macronutrient with essential roles in plant development compounds are synthesized from Sulphur metabolism useful in reversing the adverse effects of abiotic stress because of their free radicals scavenging property. Sulfur-containing metabolites, amino acids (cysteine and methionine), vitamins (biotin and thiamine), thioredoxin system, glutathione lipoic acid and glucosinolats have potential to promote or modify physiological and molecular processes under salinity stress in plants. Thus, modulation of sulphur metabolites production could alter physiological and molecular mechanisms to provide tolerance against salinity [6].

Sulphur has a role to play in increasing chlorophyll formation and aiding photosynthesis. S also plays a role in the activation of enzymes, nucleic acids [7]. Sulphur is strongly involved in improving nutrient assimilation and in stimulating the anti-oxidative defense system of plants through its metabolite glutathione[8], [9]. Also, the acidity produced during elemental sulphur oxidation increases the availability of nutrients such as P, Mn, Ca, and SO4 in soils which may enhance growth performance of plants[10]. [11] found that Leaf length of Mishrig Wad Laggai date palm cultivars was increased as a result of Sulphur application, 400 g elemental Sulphur per tree to the soil were recommended to date palm growers in calcareous soil or alkaline soil reaction with soil pH 8.3-8.8. The study conducted by [12] to assessed the effect of salinity and sulphur element on alleviating the adverse effects of salinity on growth of Sewy, Zaghloul and Hayany Date Palm Cultivars, showed that Sulphur element at 100 g date palm.-1 significantly increased the leaf area compared to untreated trees.

The main objectives of this study were to investigate the effect of sulphur element on alleviation of salt stress of date palm offshoots and how to improve salt stress tolerance and the growth these characteristics of offshoots under saline conditions.

Materials and Methods

Plant materials and experimental procedure: The study was carried out at a General Authority of Palm station, in Hartha region – Basrah, Iraq (30o36.54'N& 30o 38.60'N to47o44.42'E to 47o 45.18'E), 24 Km from center of Basrah, during 2014 season on 30 Trees uniform in vigour and girth (±10 cm), 4- 5 years- old Berhi and Sayer Date Palm cultivars offshoots effected by salt strass (on Basins Agriculture of 9 offshoot per each cultivar) Texture of Basins is Silty clay loam . The selected offshoots are planted at 5x5m. Drip irrigation system was followed. Soil samples take from untreated offshoots Basins, as well as samples of water were carried out weekly. Addition of sulphur element to soil were carried out doing the first week of March in the morning. Each treatment was replicated three times, with one offshoot for each replicate. The selected offshoots were subjected to treatments as following:

C: control (untreated)

S1 : sulphur at (100 g offshoot^{-1} year.$^{-1}$) addition to Soil

S2 : sulphur (200 g offshoot^{-1} year.$^{-1}$) addition to Soil

Replacted same treatments to both two cultivars

*Average of EC soil to season of study was (15.93 dS m-1), pH was (8.10). Also, Average of EC water (4.55 dS m-1) and pH (7.91), Average of Temperature of field was (41.63)°C and Average of Relative humidity was (47.33).

Parameters of study took them On October 15, after all the physiological measurements were performed. ***Parameters of vegetative growth: Height of offshoot (cm):*** Measured by a measuring tape to third leaf

The increase in plant height = plant height when sampling - plant height before treatment

***Leaf area (m2)*:** Leaf area (m2) was determined according to [13]. In four pinnae taken from the middle parts of each third leaf, following the equation:

Leaf area (m2) = (0.37 (length × width) + 10.29 × No. of pinnae) /1000

Total chlorophyll content: The extraction of Total chlorophyll was carried out according to [14]. The fresh tissue of leaves collected and freeze in freezen then, the leaves (0.25 g) were homogenized with 80% acetone. The optical density (O.D.) of the extracted chlorophyll was measured at 645 and 663 nm by using spectrophotometer PD-303. Total chlorophyll content was calculated by the following formulae:

Total chlorophyll (mg/g) = 20.2 (OD 645) + 8.02 (OD 663) x Vol./ Wt.)

Analysis of soluble carbohydrates: Samples of fresh leaves were weighed (0.2 g) and homogenised using 70 % ethanol according to [15].

Relative water content (RWC) and Dry weight: Leaf samples were weighed (fresh weight) immediately after harvesting, soaked in distilled water at 25°C for 24 h to determine the turgid weight then , the samples were dried in an oven at 80° C for 48 h and their dry weights were determined. RWC was calculated by the following equation: RWC = (fresh Weight- dry weight)/(turgid weight- dry weight) x 100.

Determination of proline concentration: according to [16].

Determination of protein content: The proteins were determined by Bradford's method[17].

***Antioxidant enzyme activity assays: Enzyme extraction*:** according to[18].

Enzyme activity: were assayed by spectrophotometric methods.

Peroxidase activity: was measured an assay guaiacol [19].

Catalase activity: was measured an assay hydrogen peroxide based on formation of its stable complex with ammonium molybdate [20] 0.2 ml of plant extract was incubated in 1 ml reaction mixture containing 65 mM hydrogen peroxide in 60 mM potassium phosphate buffer,pH 7.4 at 25 °C for 4 min. The enzymatic reaction was stopped with 1 ml of 32.4 mM ammonium molybdate and the concentration of the yellow complex of molybdate and hydrogen peroxide was measured at 405 nm. Activity was expressed on a fresh weight basis (Units.mg protein $^{-1}$ FW).

Extraction and purification IAA and ABA content: Extraction, purification and quantitative determination of free and bound IAA and ABA were done, with minor modifications, according to the methods of [21]. Spectrophotometric techniques were used to determine the amounts of IAA and ABA. One gram fresh weight of each sample was taken and combined with 60 ml of methanol: chloroform: 2N ammonium hydroxide (12:5:3 v/v/v). Each combined extract (60 ml) was kept in a bottle at in deep freeze for further analysis. Combined extract was treated with 25 ml of distilled water. The chloroform phase was discarded. The water-methanol phase was evaporated. The water phase was adjusted to the extract pH value of 2.5 or 7 or 11with (1N HCl) or (1N NaOH) respectively and 15 ml ethyl acetate was added at each of three steps. This procedure provided the isolation of free-form IAA and ABA from the extraction solvent. After an incubation period of 1 hour at 70 °C, the same procedure was used for the isolation of bound-form IAA and ABA from the extraction solvent. Evaporation of ethyl acetate was performed at 45°C using a rote-evaporator system (B.chi Instruments). Thin-layer chromatography (TLC) was done using silica gel GF254 (Merck Chemicals, Germany) according to the method of [21].TLC separated IAA and ABA were isolated from the glass plaques according to the standard synthetic IAA and ABA Rf values. IAAand ABA were dissolved with 2 ml of methanol for filtration and separation from silica using cotton-glass filled transferring pipettes. Spectrophotometeric assay was done at 280 nm for IAA and 263 nm for ABA and for all standard synthetic IAA and ABA and isolation samples.

Table 1: Effect of Sulphur application on plant height (cm), leaf area (m^2), leaves Number (leaf $plant^{-1}$), girth (cm) under salt stress

Cultivars	Treatments	Height plant (cm)	Leaf area (m^2)	leaves Number Leaf $plant^{-1}$	Girth of offshoot (cm)
Berhi	C	14.33	0 .80	1.66	17.00
	S1	26.33	1.00	3.33	19.66
Sayer	S2	30.00	1.10	3.66	22.00
	C	11.66	0.74	1.33	15.66
	S1	19.00	0.91	2.33	17.33
	S2	23.33	0.89	2.66	17.33
	R.L.S.D.	2.44	0.20	1.45	2.44

Table 2: Effect of Sulphur application in Total Chlorophyll content (mg g^{-1}), Dry weight (g), RWC (%), Carbohydrates (mg g^{-1}), proline (mg g^{-1}) under salt stress

Cultivars	Treatments	Total Chlorophyll ($mg.g^{-1}$)	Dry weight (g)	RWC (%)	carbohydrates ($mg.g^{-1}$)	Proline ($mg.g^{-1}$)
Berhi	C	0 .94	5.23	67.43	37.44	12.45
	S1	1.36	6.01	77.33	41.43	12.95
Sayer	S2	1.59	6.34	82.50	46.44	14.38
	C	0.93	4.31	65.13	32.65	9.81
	S1	1.04	4.25	77.69	40.35	11.12
	S2	1.10	5.13	77.91	42.83	11.14
	R.L.S.D.	0.07	0.58	1.63	4.87	0.17

Determination of Potassium and sodium concentration: according to [22].This solution became transparent and used for determinations of K and Na concentrations emission flame photometer.

Determination of Chloride concentration: according to[23].

Statistical analysis: A completely randomized block design of two date palm cultivars and three treatments of sulphur replicated three times were used to conduct the experiment . Experimental data on all variables were subjected to analysis of variance (ANOVA) procedures using a statistical package, SPSS version 16.0 (SPSS, Chicago, IL). Significant differences among treatments were considered at the $P \leq 0.05$ levels.

Results

Height, leaf area, number of leaves and girth plant: Results presented in Table 1 reveal that sulphur treatments were significantly increased the offshoot height, leaf area, number of leaves and girth of plant of both two cultivars compared with control. Using sulphur at 200 g $offshoot^{-1}$ $year.^{-1}$ to Berhi cultivar gave the highest values with respect to of offshoot height, leaf area, number of leaves and girth of plant (30.00 cm, 1.10 m^2 , 3.66 leaf $plant^{-1}$, 22.00 cm), respectively. whereas control of Sayer cultivar recorded the lowest values in these respects (11.66 cm, 0.74 m2, 1.33 leaf $plant^{-1}$, 15.66 cm), respectively.

Biochemical characteristics: Sulphur application significantly influenced biochemical characteristics (Table 2) The addition of sulphur to soil at 200 g $offshoot^{-1}$ $year.^{-1}$ to Berhi cultivar recorded the highest values of Total Chlorophyll, Dry weight, RWC, Carbohydrate and proline concentration (1.59 mg g^{-1} , 6.34 g, 82.50 %, 46.44 mg g^{-1}, 14.38 mg g^{-1}), respectively. Whereas control of Sayer cultivar recorded the lowest values in these respects (0.93 mg g^{-1}, 4.31 g, 65.13 %, 32.65 mg g^{-1}, 9.81mg g^{-1}), respectively.

protein, peroxidase enzyme activities and IAA content: Results shown in Table 3 illustrate the use of Sulphur as anti-salinity chemical. This compound significantly increased soluble protein, peroxidase enzyme activities, endogenous indol acetic acid (IAA) content of both cultivars compared with control. As well as, treatment with sulphur at 200 g $offshoot^{-1}$ $year.^{-1}$ to Berhi cultivar gave the highest values with respect to (5.77 mg g^{-1}, 6.59 Unit.mg.-1FW, 77.78 µg g^{-1}), respectively.

Catalase enzyme activities and ABA content: The results significantly showed decrease of Catalase enzyme activities and content of ABA that the treatment of sulphur at 211 g offshoot-1 year.-1 to Berhi cultivar gave the lowest values with respect to (0.85 Units.mg protein.-1FW, 65.82 $\mu g.g^{-1}$). Respectively. However, untreated treatment of Sayer cultivar recorded the highest value in this respect (2.30 Units.mg protein.-1FW, 81.06$\mu g.g^{-1}$). Respectively.

Na, Cl, K concentration and K/Na Ratio: Results presented in Table 4 revealed that sulphur treatments significantly decreased Na+, Cl- concentration of leaves compared with control. The use of sulphur at 200 g $offshoot^{-1}$ $year.^{-1}$ to Berhi cultivar gave the lowest values of Na and Cl concentration (4.28 mg g^{-1} , 4.83 mg g^{-1}), respectively. Whereas, control treatment of Sayer Cultivar recorded the highest value of those ions (11.66 mg g^{-1}, 15.33 mg g^{-1}), respectively. Also, data in Table 4 showed that the using sulphur at 200 g $offshoot^{-1}$ $year.^{-1}$ to Sayer cultivar gave the hightest values of K+ concentration (15.80 mg g^{-1}) compared with control treatment of Berhi cultivar (10.69 mg g^{-1}). While sulphur treatments significantly increased K/Na Ratio of leaves compared with control. The use of sulphur at 200 g $offshoot^{-1}$ $year.^{-1}$ to to Berhi Cultivar gave the hightest values of K/Na Ratio (3.14). compared with control treatment of Sayer cultivar

Table 3: Effect of sulphur application in protein (mg g^{-1}), POD (Unit mg^{-1}FW), CAT (Unit mg $protein^{-1}$FW), IAA (µg g^{-1}), and ABA (µg g^{-1}) under salt stress

Cultivars	Treatments	Protein (mg.g^{-1})	POD (Unit.mg^{-1}FW)	CAT (Units.mg $protein^{-1}$FW)	IAA (µg.g^{-1})	ABA (µg.g^{-1})
Berhi	C	4.00	5.02	1.34	42.17	81.06
	S1	5.43	5.20	0.87	63.62	66.39
	S2	5.77	6.59	0.85	77.78	65.82
Sayer	C	2.21	4.19	2.30	47.71	85.51
	S1	3.16	4.84	1.39	76.00	71.00
	S2	4.26	4.89	1.35	78.58	69.20
	R.L.S.D.	0.28	0.44	0.15	4.070	5.48

Table 4: Effect of sulphur application in Na, K , Cl concentration (mg g^{-1}) and K/Na Ratio under salt stress

Cultivars	Treatments	Na (mg g^{-1})	K (mg g^{-1})	Cl (mg g^{-1})	K/Na
Berhi	C	8.67	10.69	10.16	1.24
	S1	5.16	12.95	6.50	2.53
	S2	4.28	13.44	4.83	3.14
Sayer	C	11.66	14.14	15.33	1.10
	S1	8.43	14.29	10.50	1.69
	S2	7.66	15.80	9.83	2.13
	R.L.S.D.	2.04	2.34	1.42	0.48

(1.10). **Discussion**

Sulphur application increased the tolerance of Date Palm offshoots to salinity as indicated an increase in height, leaf area, number of leaves and girth of plants. Sulphur is strongly involved in improving nutrient assimilation and in stimulating the anti-oxidative defense system of plants through its metabolite glutathione [8], [9]. Also, the acidity produced during elemental sulphur oxidation increases the availability of nutrients such as P, Mn, Ca, and SO4 in soils which may enhance growth performance of plants [10]. The addition of sulphur increased many metabolites that are known as “compatible (organic) solutes” (charbohydrate, proline and proteins) in the cytoplasm to increase their hyperosmotic tolerance against salt stress-induced water loss from the cells. Also, sulphur improved chlorophyll content might be attributed to increased the antioxidant system as well as an osmotic adjustment mediator by increasing the charbohydrate, protein, proline and Peroxidase activiti . In term of the chloroplast is the major source of ROS production in cells under stressful conditions [24]. There was a significant positive action of sulphur as anti-salinity compound by increase dry weight and RWC might be attributed to increased the solutes as protein, proline, and carbohydrate which increase turgor subsequently cause high RWC. Improved proteins and Peroxidase activitie content might be attributed to stimulate growth and improve tolerance salt stress by increased uptake K+ which required the formation of new proteins by stimulating the process of gene expression. Such proteins act as protective proteins or a free radical scavenger. Also, by recovery chlorophyll content which is the major source of ROS production in cells under stressful conditions [24]. And increased IAA content. Whereas reduce of Catalase activity was in result of reduce of oxidation stress by decreased Na+ in leaves. Plants adapt to stress conditions by activation of cascade(s) of molecular mechanisms, which result in alterations in gene expression and synthesis of protective proteins/compounds [25]. The positive action of sulphur application on increased endogenous IAA and decreased endogenous ABA content might be attributed to mitigated the adverse effects of salinity on offshoots by osmoregulation which is possibly mediated by increased production of carbohydrate as well as proline By regulating the membrane stability, photosynthetic pigments and modify the balance between these hormones. Further protection under salt stress was achieved through enhanced activities of antioxidant enzymes, POD and CAT .thus enhanced recover and stimulate growth.

These phytohormones can positively or adversely affect preceding plant growth, while interacting with each other [26]. Moreover, sulphur application led to decrease Na concentration and promoted the uptake of potassium, Thus increased K/Na ratio might be attributed to enhanced the selective absorption and transport capacity for K+ over Na+ in roots of offshoot An increased K/Na ratio in leaves is an indication of increased potassium and reduced sodium ion uptake. However, Berhi cultivar had the highest K/Na ratio than sayer cultivar. This was due to the result of both a higher K+ and a lower Na+ concentration in Berhi cultivar, indicating that Berhi cultivar has better capacity to maintain intracellular K+ and Na+ homeostasis, and thus are subjected to less damage under salinity stress. The relatively stronger tolerance of this cultivar to salinity may be related to the ability of plants to accumulate high levels of proline, proteins, RWC, dry weight and soluble sugars and maintain high ratio of K/Na.

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