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Calcium application mitigates salt stress in Date Palm (*Phoenix dactylifera* L.) offshoots cultivars of Berhi and Sayer

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ABSTRACT

The effectiveness of exogenous application of calcium in ameliorating the adverse effects of salt stress (15.9 dS m^{-1}) on date palm offshoots (*Phoenix dactylifera* L. cultivars of Berhi and Sayer) was investigated. Ca-fertilisers Polixal and Rexene were applied either as soil amendments or foliar spray. The results showed that Polixal at 30 ml $offshoot^{-1}$ significantly increased plant height, leaf area, total chlorophyll content, RWC, proline concentration, peroxidase activity, IAA content, K^+ and K^+/Na^+ ratio in leaves of Berhi cultivar, whereas catalase activity, ABA and Cl^- content were decreased. Also Berhi cultivar responded to soil amendments more than to foliar spray. However, Ca-fertilisers mitigated salt stress in the two cultivars and Berhi cultivar was more salt stress tolerant than Sayer cultivar by maintaining the high ratio of K^+/Na^+ and regulating levels of IAA to ABA, in silty clay loam soil. These results suggest that calcium application can improve the defense system under salt stress conditions.

Key words: antioxidant enzymes, Date Palm, salt stress, IAA, ABA, calcium application, proline, RWC

IZVLEČEK

DODAJANJE KALCIJA ZMANJŠUJE SLANOSTNI STRES PRI KOKOSOVI PALMI (*Phoenix dactylifera* 'Berhi' IN 'Sayer')

Pri dveh sortah kokosove palme (*Phoenix dactylifera* 'Berhi' in 'Sayer') je bil preučevan blažilni učinek dodajanja kalcija na negativne učinke slanostnega stresa (15.9 dS m^{-1}). Ca-gnojili Polixal in Rexene sta bili dodajani ali kot talni dodatek ali kot listno pršilo. Rezultati so pokazali, da je dodatek Polixal-a v količini 30 ml na rastlino značilno povečal višino rastlin, listno površino, vsebnost celokupnega klorofila, relativno vsebnost vode (RWC), vsebnost prolina, aktivnost peroksidaze, vsebnost IAA in K^+ ter razmerje K^+/Na^+ v listih sorte Berhi, aktivnost katalaze, vsebnost ABA in Cl^- so se zmanjšali. Sorta Berhi se je bolje odzvala na dodatek gnojil v tla kot na foliarna gnojila. Calcijeva gnojila so pri obeh sortah zmanjšala slanostni stres na bogatih peščeno-ilovnatih tleh, vendar se je sorta Berhi izkazala nanj odpornejša kot sorta Sayer saj je ohranjala večje K^+/Na^+ razmerje, kar je izboljšalo tudi razmerje med IAA in ABA. Rezultati te raziskave nakazujejo, da gnojenje s Ca izboljša obrambni sistem rastlin v razmerah slanostnega stresa.

Ključne besede: antioksidacijski encimi, dateljeva palma, slanostni stres, IAA, ABA, gnojenje s Ca, prolin, RWC

1 INTRODUCTION

Inhibition of plant growth by high amounts of Na^+ and Cl^- is one of the main deleterious effects of salt stress. When present in excess amount, Na^+ and Cl^- ions enter into plant cells and can exert toxic effects on cell membranes and metabolic activities in the cytosolic part of the cell (Hasegawa *et al*. 2000; Zhu, 2001; Türkan and Demiral, 2009). The resultant effect of osmotic

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stress and ionic toxicity may lead to secondary effects in plants such as decreased cell expansion, production of assimilate and membrane functions, decreased cytosolic metabolism with raised production of ROS, including singlet oxygen ($^1O_2$), superoxide (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) (Lindberg *et al.,* 2012). Calcium plays a fundamental role in plant growth and development. Many extracellular signals and environmental cues including light, abiotic and biotic stress factors, elicit change in the cellular calcium levels, termed as calcium signatures (Wu *et al*., 2013). Calcium ions ameliorate the effect of salt stress by competing with sodium ions for membrane-binding sites. Salt stress reduces N, P, K, and Ca content in tissues; however, the addition of Ca restored the levels of these nutrients. In general, as external Ca^{2+} concentrations increase, Na^+ uptake and concentrations decrease while Ca^{2+} uptake and concentrations increase because Ca^{2+} interferes with non-selective cation channels and restricts Na^+ uptake. In addition, as the salt concentration in the root zone increases, the requirement for Ca^{2+} increases. However, the uptake of Ca^{2+} from the soil may be reduced as a result of ion interactions, precipitation, and increased ionic strength. These factors reduce the activity of Ca^{2+} in solution, which reduces the availability of Ca^{2+} (Grattan and Grieve 1999, Reddy, 2001 and Louchli and Grattan, 2007). Also, Na/Ca interactions can affect growth, photosynthesis, plant nutrition, water and ion transport in plants. The nature of the response will vary depending on the plant genotype (Cramer, 2002).

Zekri and Parsons (1990) found that the addition of 1, 5, or 7.5, but not 13.5, mM $CaSO_4$ to the saline solution significantly decreased the adverse effect of NaCl on shoot growth of sour orange seedlings. In salt stressed wheat (*Triticum aestivum*'Samma') Ca^{2+} improved plant height and proline content compared with treatment of 90 mM of NaCl alone (Al-Whaibi *et al.* 2011). El-Khawaga, (2013) found that the anti-salinity agents as calcium alleviated the adverse effects of salt stress on the growth of Sewy, Zaghloul and Hayany date palm cultivars. Keeping in view all these aspects, a study was planned to test the effectiveness of Ca-fertilisers in alleviating the undesirable effects of salt stress by evaluating morphological, physiological and biochemical attributes change in date palm offshoots cultivars of Berhi and Sayer.

2 MATERIALS AND METHODS

2.1 Field experiment

The experiment was carried out at the General Authority of Palm station, in Hartha region – Basrah, Iraq (30°36.54'N& 30° 38.60'N to 47°44.42'E to 47° 45.18'E), 24 km from center of Basrah, in 2014. 30 uniform, girth ± 10 cm vigorous 4-5 years-old 'Berhi' and 'Sayer' date palm offshoots were used in the experiment. The selected offshoots were planted at 5x5 m by 15 offshoot for each cultivar. Drip irrigation system was installed. Soil samples were taken from untreated offshoots; also samples of water were taken weekly. Each treatment was replicated three times, with one offshoot for each replicate. The selected offshoots were subjected to spraying foliar and addition to soil treatments to both cultivars at the first week of March as the following:

2.2 Treatments

C: Foliar spray of water as control

P1: Soil addition of POLIXAL 20-8* (15 ml offshoot^{-1})

P2: Soil addition of POLIXAL 20-8 (30 ml offshoot^{-1})

R1: Foliar spray of Rexene ca 10∗ (1000 ppm offshoot^{-1})

R2: Foliar spray of Rexene ca 10 (2000 ppm offshoot^{-1})

2.3 Ca-fertilisers compounds

***POLIXAL 20-8**: (liquid) 8 % calcium oxide polyhydrocarboxyl (organic acids) 20 % organic material 20 % total nitrogen 4.70 % for alleviation soil salinity and calcium deficiency from Company of ABONOSUALENCIA .co., Spain

∗ **Rexene ca 10**: (Solid) Chelated Calcium EDTA 9.7 % Functional Chemicals B.V. AKzoNobel, Mexio.

2.4 Average of some Environment factors at field

Average of electrical conductivity (EC) for soil in study was 15.9 dS m^{-1}, pH was 8.10. Also, average of water EC was 4.55 dS m^{-1} and pH 7.91, average of field temperature was 41.6°C.

2.5 Parameters of study

Were taken on October 15; all the physiological measurements were performed as following:

2. 5.1 Parameters of vegetative growth

2. 5. 1.1 Increase in offshoot height (cm)
Measured by a measuring tape to third fully expanded leaf.
The increase in plant height = plant height when sampling - plant height before treatment

2. 5.1. 2 Leaf area (m^2)
Leaf area (m^2) was determined according to Ahmed and Morsy, (1999) in four pinnae taken from the middle parts of each leaf, following the equation:
Leaf area (m^2) = (0.37 (length × width) + 10.29 × No. of pinnae) / 1000

2.5.2 Biochemical constituents

2.5.2.1 Total chlorophyll content
The extraction of total chlorophyll was carried out according to Lichtenthaler and Wellburn, (1983). The fresh tissue of leaves was collected and froze 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.)
Vol = the final volume (ml)
Wt. = sample weight (g)

2.5.2.2 Relative water content (RWC)
Leaf samples were weighed (fresh biomass) immediately after harvesting, soaked in distilled water at 25°C for 24 hr to determine the turgid mass then ,the samples were dried in an oven at 80°C for 48 hr and their dry biomass was determined. RWC was calculated by the following equation:

RWC = (fresh mass- dry mass) / (turgid mass- dry mass) x 100.

2.5.2.3 Determination of proline concentration according to Irigoyen *et al.*, (1992).

2.5.3 Antioxidant enzyme activity assays

2.5.3.1 Enzyme extraction after Luhova *et al.* (2003)

2.5.3.2 Enzyme activity was determined spectrophotometricaly.

2.5.3.3 Peroxidase activity was measured by using a guaiacol assay Angelini *et al.* (1990).

2.5.3.4 Catalase activity was measured by hydrogen peroxide assay based on formation of its stable complex with ammonium molibdate (Goth, 1991). 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 molibdate and the concentration of the yellow complex of molibdate and hydrogen peroxide was measured at 405 nm. Activity was expressed on a fresh mass basis (Units mg $protein^{-1}$FW).

2.5.4 Extraction and purification of IAA and ABA
Extraction, purification and quantitative determination of free and bound IAA and ABA were done, with minor modifications, according to the methods of Rastegar *et al.* (2011). Spectrophotometric techniques were used to determine the amounts of IAA and ABA. One gram fresh weight of each sample was taken and extracted with 60 ml of methanol: chloroform: 2N ammonium hydroxide mixture (12:5:3 v/v/v). Each extract (60 ml) was kept in a bottle in deep freeze for further analysis. Extract was then treated with 25 ml of distilled water and 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 11 with 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 of 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 Rastegar *et al.,* (2011). TLC separated IAA and ABA were isolated from the glass plaques according to the standard synthetic IAA and ABA Rf values. IAA and ABA were dissolved in 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.

2.5.5 Determination of potassium and sodium concentration was according to Creser and Parsons, (1979). This solution became transparent and used for determinations of K and Na concentrations by emission flame photometer (model 129, Shanghai Lingguang int. trade co., ltd.)

2.5.6 Determination of chloride concentration Chloride (Cl^-) in plant tissue extracts was determined by potentiometric titration. With use 0.2 g of dried ground leaf tissue and addition of 50 ml 2 % acetic acid with shacking through 30 min and filtered by Whatman No.1, tritrated against 0.01 N silver nitrate using potassium chromate as an indicator to a bricked end point (Kalra, 1998).

2.6 Statistical analysis

Randomized completely block design of two date palm cultivars and five treatments of calcium 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). Revised Least Significant Differences (R.L.S.D.) among treatments was considered at the $P \leq 0.05$ levels.

3 RESULTS AND DISCUSSION

3.1 Effect of calcium on plant height, leaf area, total chlorophyll and RWC under salt stress

Results presented in Table 1 revealed that calcium treatments significantly ($P \leq 0.05$) increased the height and leaf area of offshoots compared with control. Using polixal at 30 ml offshoot^{-1} to Berhi cultivar gave the highest values of height and leaf area (34.3 cm, 1.20 m^2), respectively, whereas control with Sayer cultivar recorded the lowest value in this respect (11.3 cm, 0.7 m^2), respectively. However, the increase of growth may be attributed to the expansion of cells and activation of photosynthesis by increased total chlorophyll and RWC, proline concentration and peroxidase activity. Protective role common to concentration, of Ca^{+2} might be attributed to its role in the maintenance of the structural integrity of the plasma membrane and thus controlling the uptake of Na^+ and Cl. Larkindale and Knight, (2002) suggested that calcium role is in protecting against oxidative damage by the protection of calcium channel blockers and calmodulin inhibitors under heat stress. During our experiments field temperature was up to 40 °C. The resultant transient Ca^{2+} increase caused potential stress signal transduction and led to salt adaptation (Gul and Ajmal, 2006). Effect of calcium on height of the plant is in agreement with that obtained by Al-Whaibi *et al.,* (2011) on wheat plant and effect of calcium on leaf area is in concordance with findings of El-Khawaga, (2013) on date palm. The increased offshoot height of Berhi cultivar may be attributed to its ability to restrict Cl^- movement into the shoot more effectively than the Sayer cultivar. Thus, the concentrations of potentially harmful Cl^- ions would be lower in the photosynthetically active tissues, or different in the genotype of vigorous the growth rate in term of Berhi cultivar, and the largest in Sayer cultivar. Table 1 reveals that the total chlorophyll content and RWC of leaves was increased by polixal at 30 ml offshoot^{-1}. Analysis of Berhi cultivar gave the highest values of total chlorophyll and RWC (1.8 mg.g^{-1}, 6.5 g, 83.7 %), respectively, whereas control with Sayer cultivar gave the lowest value in this respect (0.8 mg.g^{-1},

65.3 %). Ca^{2+} retarded the loss of chlorophyll, protein and intercellular space, suggesting that the ion plays a regulatory role in maintaining and controlling membrane structure and function (Hepler, 2005). From those studies and our results suggesting that the Ca^{2+}plays a regulatory role in maintaining of chlorophyll, it acts as an antioxidant system regulator by increase proline in chloroplasts for scavenging of ROS. Effect of Ca^{2+} is in agreement with that obtained by Jafari *et al.,* (2009) on sorghum plant by calcium. The role of Ca^{2+} in increased relative water content might be attributed to its ability to regulate the compatible solutes and osmotic adjustment, subsequently increasing turgor. Jafari *et al*. (2009) suggested that the protective effect of Ca^{2+} in salinized plants is probably due to its role in maintaining membrane integrity, because one of the primary effects of salt stress is a disruption of membrane integrity caused by displacement of Ca^{2+} from the cell surface by Na^{+}.

Table 1: Averages of plant height (cm), leaf area **(**m^2**)**, total chlorophyll (mg g^{-1}) and RWC (%) of Berhi and Sayer

Cultivars	Treatments	Plant height (cm)	Leaf area **(**m^2**)**	Total chlorophyll (mg g^{-1})	RWC (%)
Berhi	C	15.0 ± 5.0^{d}	0.8 ± 0.02^{ef}	0.9 ± 0.02^{fg}	67.3 ± 1.2^{d}
	P1	26.6 ± 1.5^{b}	0.9 ± 0.06^{cde}	1.4 ± 0.05^{b}	74.3 ± 0.7^{c}
	P2	34.3 ± 2.0^{a}	1.2 ± 0.16^{a}	1.8 ± 0.03^{a}	83.7 ± 0.8^{a}
	R1	25.0 ± 2.0^{bc}	0.9 ± 0.04^{cde}	1.0 ± 0.05^{de}	78.9 ± 0.5^{b}
	R2	31.6 ± 2.5^{ab}	1.1 ± 0.23^{ab}	1.1 ± 0.0^{c}	83.3 ± 0.5^{a}
Sayer	C	11.3 ± 1.5^{d}	0.7 ± 0.0^{f}	0.8 ± 0.07^{g}	65.3 ± 0.9^{e}
	P1	21.6 ± 2.5^{c}	0.9 ± 0.01^{cde}	1.0 ± 0.00^{d}	75.0 ± 1.2^{c}
	P2	30.3 ± 0.57^{ab}	1.0 ± 0.02^{bc}	1.1 ± 0.02^{c}	79.5 ± 0.6^{b}
	R1	25.0 ± 2.0^{bc}	0.8 ± 0.01^{def}	0.9 ± 0.02^{ef}	74.6 ± 0.9^{c}
	R2	28.6 ± 2.0^{b}	0.9 ± 0.02^{bcd}	0.9 ± 0.02^{f}	78.1 ± 0.6^{b}
R.L.S.D. ($P \leq 0.05$)		4.1	0.16	0.06	1.4

cultivars response to Ca-fertilisers under salt stress

Value represents mean ± standard error of three replicates.

C: Foliar spray of Water as control, P1: Soil addition of POLIXAL 20-8* (15ml offshoot^{-1}), P2: Soil addition of POLIXAL 20-8 (30 ml offshoot^{-1}), R1: Foliar spray of Rexene ca 10∗ (1000 ppm offshoot^{-1}), R2: Foliar spray of Rexene ca 10 (2000 ppm offshoot^{-1})

3.2 Effect of calcium on proline concentration, POD and CAT activities, IAA and ABA under salt stress

Table 2 reveals that calcium treatments resulted in significantly ($P \leq 0.05$) higher proline concentration and peroxidase activity of treated leaves compared to control. The application of polixal 30 ml offshoot^{-1} to Berhi cultivar led to increase proline concentration and peroxidase activity (15.4 mg g^{-1}, 7.2 unit mg^{-1} FW), respectively, compared with control to Sayer cultivar which had the lowest values in this respect (9.1 mg g^{-1} ,4.2 unit mg^{-1} FW), when using polixal at 30 ml offshoot^{-1}, Experiment with Berhi cultivar gave the lowest value of catalase activity (0.7 units mg protein^{-1} FW) compared to control to Sayer cultivar, which gave the highest value of catalase activity (2.3 units mg protein^{-1} FW). Effect of calcium in the alleviation of salt stress and the increase of proline reflects the ability of salt-tolerant offshoot to prevent damage of ROS by maintaining better enzymatic (POD and CAT) and non-enzymatic (proline) defense systems. Proline plays a major role in osmoadaptation through an increase in osmotic stress that shifts the dominant osmolyte from glutamate to proline (Tripathi *et al*., 1998). From the results in this work, it seems that proline might confer salt stress tolerance to

offshoots by increasing the antioxidant system and osmotic adjustment mediator. Thus proline facilitates water uptake. Effect of calcium is in agreement with that obtained by Al-Whaibi *et al.*, (2011) on wheat plant by used calcium. Also, it has been commonly reported that salt stress is one of the major causes of oxidative damage to plant tissues. Though, plants can reduce the damaging effects of reactive oxygen species by developing a physiologically powerful defense system together with antioxidant enzymes like POD and CAT (Rao *et al.* 2013). Salinity intensity leads to reduce water accessibility and/or absorption and therefore lowered leaf turgor and, at last, leads to stomata closure (Azizpour *et al.* 2010). CO_2 attainment influenced by stomata closure is a basis for fluctuations and imbalances in ongoing light reactions and CO_2 fixation. The final result of these nonstandard conditions would be reduced $NADP^+$/NADPH, H^+ ratio and increased ROS production (Esfandiari *et al.* 2007). Our results showed that peroxidase to activity increased more in salt-stressed plants supplied with calcium than salt stress alone, whereas catalase activity decreased in calcium treatments. The mechanism of CAT and POD activities regulated by external calcium is still vague. Whereas peroxidase has ability to stimulate growth and improve tolerance to salt stress, its primary function is to oxidize molecules at the expense of hydrogen peroxide. They play a major role in four physiological processes; auxin metabolism, lignin fortification, defense mechanisms against pathogens and some respiratory processes (Baaziz, 1989), it is also a part of antioxidant defense systems which work in concert to control cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging of ROS. Also, data in Table 2 indicate that two date palm cultivars had significant differences in IAA content. The maximum value was presented in using polixal at 30 ml offshoot^{-1} to Sayer cultivar (84.6 μg g^{-1}), whereas control of Berhi cultivar recorded the minimum value in this respect (43.3 μg g^{-1}). While the results showed a reverse effect on the content of ABA that the treatment of polixal at 30 ml offshoot^{-1}to Berhi cultivar gave the lowest values of ABA content (54.2 μg g^{-1}), and control of Sayer cultivar recorded the highest value in this respect (85.8 μg g^{-1}). The positive action of calcium compounds on increased endogenous IAA and decreased endogenous ABA content might be attributed to mitigated the adverse effects of salt stress on offshoots by osmoregulation which is possibly mediated by increased production of carbohydrates (data not shown) as well as increased proline concentration by regulating the membrane stability, photosynthetic pigments and modify the balance between these hormones and metabolites. Further, protection under salt stress was achieved through enhanced activities of antioxidant enzymes, to POD and CAT. Thus enhanced recover and stimulate growth. These phytohormones can positively or adversely affect preceding plant growth, while interacting with each other (Fahad *et al.* 2014). One of the fast and sensitive auxin-induced reactions is an increase of Ca^{2+} cytosolic concentration, which is suggested to be dependent on the activation of Ca^{2+} influx through the plasma membrane and auxin increases plasma membrane permeability to Ca^{2+} (Kirpichnikova *et al.* 2014). The role of calcium in increase IAA and decrease ABA content might be to IAA acts as a signal for increases plasma membrane permeability to Ca^{2+}, thus maintains and modifies the balance between these hormones for the purpose of reducing the damage by salinity and stimulate growth by alleviation of ABA content.

Table 2: Averages of proline (mg g^{-1}), POD (unit mg^{-1} FW), CAT (unit mg $protein^{-1}$ FW), IAA (μg g^{-1}) and ABA (μg g^{-1}) of Berhi and Sayer cultivars response to Ca-fertilisers under salt stress

Cultivars	Treatments	Proline (mg g^{-1})	POD (Unit mg^{-1}FW)	CAT (Unit mg $protein^{-1}$FW)	IAA (μg g^{-1})	ABA (μg g^{-1})
Berhi	C	12.4±0.04 ^{d}	5.1±0.11 ^{c}	1.3± 0.10^{c}	43.3±3.9^{e}	80.6±5.8 ^{ab}
	P1	13.3±0.13^{c}	6.6±0.21^{b}	0.9±0.02 ^{ab}	70.7±4.5^{d}	67.9±3.0 ^{cd}
	P2	15.4±0.35 ^{a}	7.2±0.23 ^{a}	0.7±0.01 ^{a}	81.0±2.0 ^{ab}	54.2±4.3^{e}
	R1	13.4±0.25 ^{c}	6.5±0.08^{b}	1.3±0.05 ^{c}	73.8±2.8 ^{cd}	77.1±4.5 ^{b}
	R2	14.9±0.34 ^{b}	7.1±0.06 ^{a}	1.2±0.10 ^{bc}	78.3±1.9 ^{bc}	63.2±3.3 ^{d}
Sayer	C	9.1 ±0.07 ^{h}	4.2±0.18 ^{e}	2.3±0.09 ^{e}	46.1±1.6 ^{e}	85.8±3.4 ^{a}
	P1	11.1±0.01 ^{g}	4.6±0.03 ^{d}	1.6±0.07 ^{d}	73.6± 2.0^{d}	71.5±4.1 ^{bc}
	P2	11.2 ±0.06^{fg}	5.0±0.07 ^{c}	1.3±0.11 ^{c}	84.6±2.0 ^{a}	66.9±2.9 ^{cd}
	R1	11.5±0.12 f	4.6 ±0.02^{d}	1.8±0.57 ^{de}	69.6±2.9 ^{d}	69.9±2.8^{c}
	R2	11.9±0.12 ^{e}	5.0 ±0.11^{c}	2.1± 0.26^{e}	81.8±1.5^{ab}	68.4±1.8 ^{cd}
R.L.S.D. ($P \leq 0.05$)		0.3	0.2	0.3	4.6	6.4

Value represents mean ± standard error of three replicates.
C: Foliar spray of Water as control, P1: Soil addition of POLIXAL 20-8* (15 ml $offshoot^{-1}$), P2: Soil addition of POLIXAL 20-8 (30 ml $offshoot^{-1}$), R1: Foliar spray of Rexene ca 10∗ (1000 ppm $offshoot^{-1}$), R2: Foliar spray of Rexene ca 10 (2000 ppm $offshoot^{-1}$)

3.3 Effect of calcium on Na^+, K^+, Cl^- and Na/K ratio under salt stress

Results presented in Table 3 revealed that calcium treatments significantly ($P \leq 0.05$) decreased Na^+ and Cl^- content of leaves compared with control. The using of polixal at 30 ml $offshoot^{-1}$to Berhi cultivar gave the lowest values of Na^+ and Cl^- content (4.0 mg g^{-1}, 4.0 mg g^{-1}), respectively, whereas, control treatment of Sayer cultivar recorded the highest value in this respect (11.6 mg g^{-1}, 15.1 mg g^{-1}), respectively. Also, data in Table 3 showed that the using polixal at 30 ml $offshoot^{-1}$ to Sayer cultivar gave the highest values of K^+ content (18.0 mg g^{-1}) compared with control treatment to Berhi cultivar (10.6 mg g^{-1}). Calcium treatments significantly increased K^+/Na^+ ratio of leaves compared with control. Using polixal at 30 ml $offshoot^{-1}$ to Berhi cultivar gave the highest values of K^+/Na^+ ratio (4.0) compared with control treatment of Sayer cultivar (1.2). Increasing K^+ concentrations under saline conditions may help to decrease sodium uptake required for maintaining the osmotic balance (Tuteja and Mahajan, 2007). Grattan and Grieve, (1999) reported that addition of Ca restored the levels of N, P, K in tissues. Our results cleared that increasing K^+ related with increase Na^+ in both cultivars and the cultivar is more salt tolerant when accumulates less Na^+, Cl^- and K^+ ions. The role of calcium in decreased Na^+ content and promoted uptake of potassium, thus increased K^+/Na^+ ratio might be attributed to that Ca^{2+} promoted the uptake of K^+ in the presence of sodium. Thus, Ca^{2+} by some mechanisms imparts selectivity to the ion transport process (Hepler, 2005). Ca^{2+} decreased roots Na^+ accumulation, increased shoots K^+ accumulation, and enhanced the selective absorption and transport capacity for K^+ over Na^+ in thr plant (Wu and Wang, 2012). The obtained results go in line with the findings of Zekri and Parsons, (1990) on sour orange seedlings. K^+/Na^+ and Ca/Na ratios are useful indicators of the degree of plant resistance to salinity that a greater degree of salinity tolerance in plants are associated with a more efficient system for selective uptake of K^+ and/or Ca^{2+} over Na^+ (Wu and Wang, 2012; Wu *et al.* 2013). It is suggested that K^+ and Ca^{2+} play key roles in several physiological processes, such as stabilization of membranes and control of enzyme

activity, Na^+ does not function as a macro-nutrient, and thus the substitution of K^+ by Na^+ and the decrease in Ca^{2+} concentration may cause ion imbalances (Tuna *et al.* 2007). Therefore, control of Na^+ accumulation, and high K^+/Na^+ and Ca^{2+}/Na^+ ratios may enhance salinity tolerance in plants (Wu *et al.* 2013). However, Berhi cultivar had higher 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 a better capacity to maintain intracellular K^+ and Na^+ homeostasis, and thus is subjected to less damage under salt stress. The relatively stronger tolerance of this cultivar to salinity may be related to the ability of plants to accumulate high levels of proline, RWC and maintain the high ratio of K^+/Na^+ and regulate levels of IAA to ABA.

Table 3: Averages of Na^+, K^+, Cl^- (mg g^{-1}) and K^+/Na^+ ratio of Berhi and Sayer cultivars response to Ca-fertilisers under salt stress

Cultivars	Treatments	Na (mg g^{-1})	K (mg g^{-1})	Cl (mg g^{-1})	K/Na Ratio
Berhi	C	8.3±1.7 ^{b}	10.6±2.2 ^{b}	9.6±0.5 ^{de}	1.3±0.4 ^{c}
	P1	5.5±0.7 ^{a}	11.0±0.6 ^{d}	6.1±0.7 ^{b}	2.0± 0.1^{c}
	P2	4.0±1.1 ^{a}	15.6±0.5 ^{bc}	4.0±0.5 ^{a}	4.0±0.9 a
	R1	4.9±1.0 ^{a}	12.3±1.5 ^{d}	8.0±0.5^{c}	2.5±0.8 ^{bc}
	R2	4.8±1.0 ^{a}	14.8±0.8 ^{bc}	6.0±0.5^{b}	3.1±0.4 ^{ab}
Sayer	C	11.6±2.0 ^{c}	14.7±0.6 ^{c}	15.1±1.0 ^{g}	1.2±0.2 ^{c}
	P1	6.8±0.5 ^{b}	15.0±2.0 ^{bc}	10.0±0.5^{e}	2.2±0.4 ^{bc}
	P2	6.0±1.0 ^{ab}	18.0±1.0 ^{a}	8.9±0.6 ^{cd}	3.0± 0.4^{b}
	R1	8.0±1.0 ^{b}	15.5±1.7 ^{bc}	11.5±0.5 ^{f}	1.9±0.4 ^{c}
	R2	6.6±0.5 ^{b}	17.1±1.0 ^{ab}	10.5± 0.5^{ef}	2.6±0.4 ^{bc}
R.L.S.D. ($P \leq 0.05$)		2.0	2.3	1.0	0.9

Value represents mean ± standard error of three replicates.
C: Foliar spray of Water as control, P1: Soil addition of POLIXAL 20-8* (15ml $offshoot^{-1}$), P2: Soil addition of POLIXAL 20-8 (30 ml $offshoot^{-1}$), R1: Foliar spray of Rexene ca 10* (1000 ppm $offshoot^{-1}$), R2: Foliar spray of Rexene ca 10 (2000 ppm $offshoot^{-1}$)

4 CONCLUSIONS

The study involving calcium with different concentrations and type of fertilisers indicated that the addition of POLIXAL 20-8 (30 ml $offshoot^{-1}$) to the soil is more effective than foliar spray of Rexene ca 10 (2000 ppm $offshoot^{-1}$) in all the parameters of study. We can deduce from the results that calcium effect was due to the protection against oxidative damage by protecting calcium channel blockers and calmodulin inhibitors under salt and heat stress. Also, maybe Berhi cultivar is more salt stress tolerant than Sayer cultivar by maintaining a high ratio of K^+/Na^+ and regulating levels of IAA to ABA. We suggest also that there is a common link between the peroxidase enzyme and calcium ion in stimulating growth and improving salt stress tolerance.

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