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Research Article

Molecular Analysis of Anti-salinity Compounds on Date Palm offshoots (*Phoenix dactylifera* L.) cultivars using RAPD techniques

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Abstract: Date Palm, as one of the important fruit crops, has grown worldwide. The aim of the present study was to analyze the overall growth of the two cultivar growth under salinity after anti- salinity treatments. Both the cultivars analyzed for the growth contributing parameters as compared with their respective control. To study the genetic variability in both cultivars offshoots after anti-salinity treatment, DNA fingerprinting was performed using RAPD technology. The results showed that the application of anti- salinity treatments significantly increased the leaf area and the number of new leaves compared to control. Also, the anti-salinity treatments significantly increased total soluble carbohydrates and proline compared to control. Some of the anti-salinity treatments effect on DNA molecular markers, especially the treatment of Salicylic acid at 1000 ppm offshoot^{-1} gave the highest percentage of Polymorphism using OPH-6, OPH-12 and OPA-10 primers in Sayer cultivar. Whereas the treatment of Salicylic acid at 1000 ppm offshoot^{-1} gave the Polymorphism percentage with Berhi cultivar when using an OPA-10 primer only. Overall, the data suggest that anti-salinity treatment has the ability to alter the percentage of offshoots growth, and can be used to improved tolerance of date palm offshoots adaptation to salinity tolerance using RAPD techniques.

Keywords: Salinity tolerance; Proline; Adaptation; DNA Fingerprinting; Polymorphism

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a tree of life in the desert; because it tolerates high temperatures, drought and salinity more than much other fruit tree species[1]. The survival of date palm in south Iraq commonly under the effect of abiotic factors such as soil salinity, high water table, thermal stress, which lead to reduce in growth and production of date palm[2, 3]. Reduction of plant growth by high amounts of Na^+ and Cl^- the most deleterious effects of salinity stress. When present in the excess amount, Na^+, and Cl^- ions enter into the plant cells and can exert toxic effects on cell membranes and metabolic activities in the cytosolic part of the cell[4].

The genes play a key role in plant tolerance to salinity that reduces the uptake of salt from the soil and the transport of salt throughout the plant. Improved salt tolerance of plant requires new genetic sources of this tolerance. Genes that could improve salt tolerance by three main functional groups: (1) control salt uptake and transport; (2) an osmotic or protective function; and (3) make a plant grow more quickly in saline soil[5]. Assessing biodiversity for ecologically important traits using DNA sequence approaches is more indicative of variation at loci that determine the ecological and functional distribution of the species and explain the ecological-genetic pattern of a species, such as adaptation to dry or saline environment[6]. Developing new varieties adaptable to climate changes and can readily be adapted in a short period at different locations with varying agro-climatic and growing conditions[7]. Date palm cultivars commonly have similar or narrow distinguishing morphological characters that complicate cultivar identification and require genetic argument to explain phylogenetic relationships at the interspecific level. RAPD analysis is a comparatively easy, quick and less expensive procedure for generating genomic markers and evaluation of date palm varieties for salinity tolerance[8].

The role of genes encoding proteins involved in regulating other genes/proteins, signal transduction process involving hormones like ABA, Jasmonic acid (JA) and polyamines, and improve strategies to salinity stress tolerance[9]. Used Kurup *et al.*[8] RAPD technique to characterize the cultivars of date palms for salinity tolerance. The cultivars were watering with increasing concentrations of saline water, 7.8, 11.7, 15.6, 19.5, 23.4 and 27.3 dS m^{-1} at regular intervals of three months duration. The date palm cultivars fingerprinted using 14 random primers to check the DNA polymorphism. Primer OPC-02 revealed a 1400 bp fragment amplified in 'Bugal White' which known as salinity tolerance and 'Khlas' which known as drought tolerant. Primer OPD-02 distinguished 'Bugal White', which known as salinity tolerant, with a DNA fragment of about 1200 bp. The present study was designed to evaluate the Anti-salinity compounds on genetic variation of offshoots adaptation to salt stress tolerance using RAPD techniques.

MATERIAL AND METHODS

Field experiment: The experiment was carried out at the General Authority of Palm station, in Hartha region – Basrah, Iraq (30o36.54'N& 30o 38.60'N to 47o44.42'E to 47o 45.18'E), 24 km from the center of Basrah, in 2014. 42 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 21 offshoots 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 the treatments at the first week of March as the following:

Treatments

1. Control (untreated)
2. Soil addition of Agrosign Humic with 100 ml/L

3. Soil addition sulphur with 200 g
4. Soil addition polixal with 30 ml/L
5. Foliar spray of Rexene ca with 2000 ppm
6. Foliar spray of Foliartal with 10 ml/L
7. Foliar spray of Salicylic acid with 1000ppm

Details of Anti-salinity compounds

Agrosign Humic: The Natural extract of leonardite rich in Humic and Fulvic acids (Humic acid 12%, Fulvic acid 3%)(Takafu Agri.co., Jorden).

Polixal: Moderating soil alkalinity (liquid) 8% calcium oxide polyhydrocarboxyl (organic acids) 20% organic material 20% total nitrogen 4.70% for alleviation salinity soil and calcium deficiency (ABONOSUALENCIA.co., Spain).

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

Foliartal: (liquid fertilizer) N 3% K 32% - potassium in the form of EDTA chelated, used in salt soil(ABONOSUALENCIA. Co., Spain).

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

Parameters of vegetative growth:

Leaf area (m^2): Leaf area (m^2) was determined according to Ahmed *et al.*[10] 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

Number of New Leaves of Offshoot: New leaves = Number of leaves when sampling - Number of leaves before treatment

Biochemical constituents

Carbohydrates content: Samples of fresh pinnae were weighed (0.2 g) and homogenized using 70 % ethanol. Then they were filtered and pigments were removed by the use benzene. An aliquot of 0.2 ml of leaf extract was added to 1.0 ml of (5 % phenol + 5 ml H_2SO_4 95%) to react in a water bath for 10 minutes at 100 ºC. Soon after, the test tube was cooled in an ice bath and then the absorbance was read at 620 nm, according to Yemm *et al.*[11]. Soluble carbohydrates were calculated by comparing sample absorbency with a standard glucose curve in a concentration range of 0 to 100 µg ml^{-1}.

Determination of proline concentration according to Irigoyen *et al.*[12].

RAPD Analysis

Extraction of Genomic DNA: The genomic DNA of date palm was isolated using the protocol of Doyle and Doyle [13] according to Bekheet[14] in the biotechnology lab. of date palm research center/ university of Basrah. Small pieces (from young leaf 0.5 g) of leaf tissue of date palm samples were Freezer-Dryer Lyophilization Technique and homogenized in 500 µl of extraction buffer (2% CTAB, 1.4 m NaCl, 20 mm EDTA pH 8.0, 100 mm Tris-HCl, pH 8.0, 0.1 m ß-Mercatoethanol). The extract was incubated at 60ºC for 20 min. To this, 500 µL phenol: chloroform: isoamyl alcohol (24:24:1)

were added and mixed by vortexing for 30 sec followed by centrifugation at 14000 rpm for 5 min at room temperature and the aqueous phase was transferred to another tube. This was once again extracted with 500 µl chloroform: isoamyl alcohol (24:1) in Eppendorf tube. To the aqueous phase, 0.6 volume of isopropanol was added, genomic DNA was precipitated and the fibrous genomic DNA was spooled. Genomic DNA was then washed three times with 70% ethanol, dissolved in TE and incubated at 37ºC for 30 min, followed by extraction with phenol: chloroform: isoamyl alcohol (24:24:1) and the aqueous phase was transferred to a fresh tube. Thereafter, the genomic DNA was precipitated by adding 0.3 ml sodium acetate, pH 5.2 (final concentration) and 2.5 vol of ethanol and collected by centrifugation at 14000 rpm for 20 min at 4ºC. The pellet was washed with 70% ethanol, vacuum dried and dissolved in TE.

PCR Amplification: RAPD analysis was carried out in Genetic Engineering Lab. of Agriculture college/ Basrah university by using three oligonucleotide primer which were OPH – 06 = 5¯– ACG CAT CGC A – 3¯, OPA – 10 = 5¯– GTG ATC GCA G – 3¯ and OPH – 12 = 5¯ – ACG CGC ATG T – 3¯ (α DNA Co.) to detect the polymorphism among the salinity and anti-salinity treatments of offshoots. The amplification was carried out in 25 µl reaction volume containing DNA master mix 12.5 µl (PCR buffer, $MgCl_2$, dNTps, Taq DNA polymerase), primer 2 µl, template DNA 2 µl and sterilized distilled water 8.5 µl. PCR amplification was performed for 40 cycles, using UNO thermal cycler of Biometra (Germany) as follows: one cycle at 92 °C for 2 min then 40 cycles at 94°C for 1 min, 32°C for 1.5 min and 72°C for 2 min (for denaturation, annealing and extension, respectively). The reaction mixture was finally incubated at 72°C for 10 min. The amplification products were analyzed by electrophoresis in 1% agarose in TBE (Tris-Borate-EDTA) buffer (pH 8.0) in the presence of DNA ladder (Promega) which was used as a marker with a molecular size of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. Then the gel was stained with ethidium bromide (0.2 mg /ml) and photographed under UV light. The banding patterns generated by RAPD-PCR marker analysis were compared to determine the variability of salinity and anti-salinity treatments offshoots. The percentage of genetic variation calculation used the following equation:

Polymorphism % = (Polymorphic bands / Total bands) x 100

Statistical analysis: Randomized completely block design of two date palm cultivars and seven treatments of Anti-salinity 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.

RESULTS

Parameters of vegetative growth: The results showed that the application of anti- salinity treatments significantly increased the leaf area and a number of new leaves compared to control (**Table 1**). Soil application of Polixal at 30 ml offshoot $^{-1}$ year^{-1} gave the highest value of leaf area and a number of new leaves compared with other treatment. Also, results of **Table 2** illustrate that the maximum value was found in Berhi cultivar, whereas Sayer cultivar recorded the lowest value in this respect.

Biochemical constituents: Table 1 shows that the anti-salinity treatments significantly increased total soluble carbohydrates and proline compared to control. The application of polixal at 30 ml offshoot-1 year-1 led to increasing of total carbohydrate and proline compared with control treatment which had the lowest value. Also, data in **Table 1** indicate that the two date palm cultivars had a clear significant difference in total carbohydrate and proline content. The maximum value was recorded in Berhi cultivar, whereas Sayer cultivar recorded the lowest value in this respect.

Table 1: Effect of Anti-salinity Treatments on Leaf area, Number of New Leaves, Total Carbohydrates Content and Proline Concentration of Berhi and Sayer Offshoots

Cultivars	Treatments	Leaf area (m^2)	Number of New Leaves	Carbohydrates (mg g^{-1})	Proline (mg g^{-1})
Berhi	Control	0.80	1.66	37.44	12.45
	Agrosign Humic 100 ml	1.01	3.33	41.21	13.40
	sulphur 200 g	1.10	3.66	46.44	14.38
	polixal 30 ml	1.20	4.00	49.45	15.40
	Rexene ca 2000 ppm	1.12	4.00	48.54	14.98
	Foliartal 10 ml	1.07	3.33	44.61	14.65
	Salicylic acid 1000 ppm	0.90	3.00	44.93	14.38
Sayer	Control	0.74	1.33	32.65	9.81
	Agrosign Humic 100 ml	0.82	2.66	40.46	11.12
	sulphur 200 g	0.89	2.66	42.83	11.14
	polixal 30 ml	1.01	3.33	45.58	11.23
	Rexene ca 2000 ppm	0.99	3.00	45.14	11.91
	Foliartal 10 ml	0.90	3.66	44.55	11.50
	Salicylic acid 1000 ppm	0.85	2.33	44.07	11.53
	R.L.S.D.	0.22	0.61	0.29	3.69

Effect of Anti-salinity Compounds on genetic variation of DNA Finger Printing

Using OPH – 06 Primer: Figure 1 and Table 2 show the use of OPH – 06 primer to reveal the effect of anti-salinity treatments on Polymorphism of Berhi cultivar under salt stress. It was clear that (8) bands in the control treatment. Using Humic acid at 100 ml, sulphur at 200 g, polixal at 30 ml, Rexene Ca at 2000 ppm and salicylic acid at 1000 ppm offshoot^{-1} gave (8) bands, all of which were identical to the control treatment. Thus, the percentage of Polymorphism (0 %), but the treatments of Foliartal at 10 ml offshoot^{-1} gave (7) bands. all of which were identical to the control treatment. Thus, the percentage of Polymorphism was (0%). However, using OPH – 06 primer on Berhi cultivar showed that the total bands were (55), without Polymorphic bands and so the percentage of Polymorphism was (0%). Also, Figure 1 and Table 2 show that using OPH – 06 primer to reveal the effect of anti-salinity treatments on Polymorphism of Sayer cultivar under salt stress. It is clear that there were (7) bands in the control treatment. Using Humic acid at 100 ml gave (7) bands too, all of which were identical to the control treatment. Thus, the percentage of Polymorphism was (0 %), but both the treatments of sulphur at 200 g and polixal at 30 ml offshoot^{-1} gave (8) bands. One of them was Polymorphic compared with control treatment and reached the percentage of Polymorphism (12.5 %). Also, the treatment of spraying Rexene Ca at 2000 ppm gave (7) bands. One of them was Polymorphic compared with control treatment and reached the percentage of Polymorphism (14.20 %) whereas the treatment of Foliartal at 10 ml offshoot^{-1} gave (9) bands. One of them was Polymorphic compared with control treatment and reached the percentage of Polymorphism (11.11%). Besides, the treatment of salicylic acid at 1000 ppm gave (8) bands, two of them are Polymorphic compared with control treatment and reached the percentage of Polymorphism (25 %).

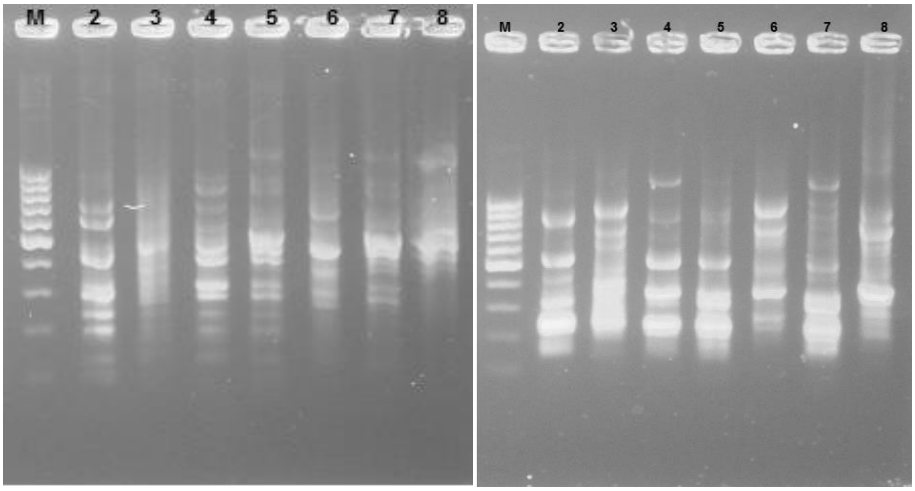

Fig. 1: RAPD-PCR gel image showing DNA polymorphism during the effect of anti-salinity compounds on Berhi and sayer cultivars using OPH – 06 primer under salt stress

Table 2: Effect of Anti-salinity Compounds on Genetic Variation of Berhi and Sayer Offshoots using OPH- 06 primer under Salt Stress

cultivars	Symbol	Treatment	Total bands	Polymorphic bands	Polymorphism %	bands size (bp)
Berhi	2	Control	6	-	-	0.878 - 0.200
	3	Agrosign Humic 100 ml	6	0	0	0.878 - 0.200
	4	sulphur 200 g	6	0	0	0.878 - 0.200
	5	polixal 30 ml	6	0	0	0.878 - 0.200
	6	Rexene ca 2000 ppm	6	0	0	0.878 - 0.200
	7	Foliartal 10 ml	2	0	0	0.337 - 0.200
	8	Salicylic acid 1000 ppm	5	2	40	0.700 - 0.200
Sayer	2	Control	6	-	-	0.792 - 0.007
	3	Agrosign Humic 100 ml	5	0	0	0.792 - 0.007
	4	sulphur 200 g	3	0	0	0.381 -0.007
	5	polixal 30 ml	3	1	33.33	0.339 - 0.007
	6	Rexene ca 2000 ppm	5	2	40	0.761 - 0.007
	7	Foliartal 10 ml	5	3	60	0.761 - 0.007
	8	Salicylic acid 1000 ppm	6	2	33.33	0.745 - 0.007

Using OPH – 12 Primer: Figure 2 and Table 3 show using an OPH-12 primer to reveal the effect of anti-salinity treatments on Polymorphism of Berhi cultivar under salt stress. It is clear that there are (8) bands in the control treatment. Using Humic acid at 100 ml, sulphur at 200 g, polixal at 30 ml, Rexene Ca at 2000 ppm and salicylic acid at 1000 ppm offshoot^{-1} gave (8) bands, all of which were identical to the control treatment. Thus, the percentage of Polymorphism was (0 %), but the treatments of Foliartal at 10 ml offshoot^{-1} gave (7) bands. all of which were identical to the control treatment. Thus, the percentage of Polymorphism was (0%). However, using OPH – 06 primer on Berhi cultivar showed that total bands reached (55) bands, without Polymorphic bands and so the percentage of Polymorphism was (0%).

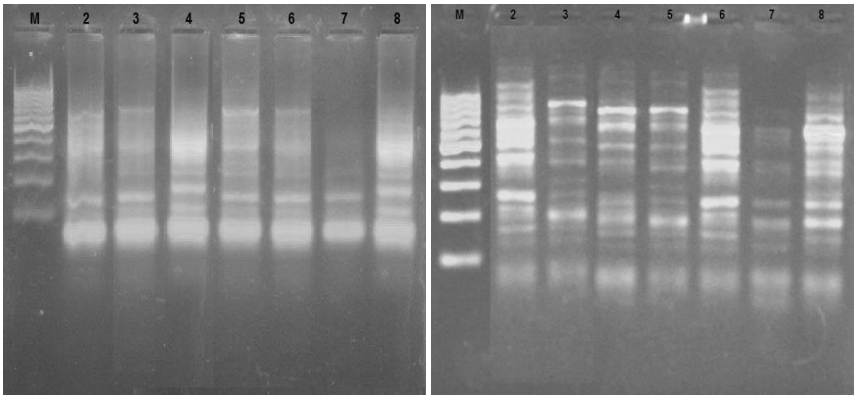

Fig. 2: RAPD-PCR gel image showing DNA polymorphism during the effect of anti-salinity compounds on Berhi and Sayer cultivars using OPH-12 primer under salt stress

Table 3: Effect of Anti-salinity Compounds on Genetic Variation of Berhi and Sayer Offshoots using OPH – 12 Primer

cultivars	Symbol	Treatment	Total Bands	Polymorphic bands	Polymorphism %	bands size (bp)
Berhi	2	Control	8	-	-	0.684 - 0.094
	3	Agrosign Humic 100 ml	8	0	0	0.684 - 0.094
	4	sulphur 200 g	8	0	0	0.894 - 0.094
	5	polixal 30 ml	8	0	0	0.841 - 0.094
	6	Rexene ca 2000 ppm	8	0	0	0.841 - 0.138
	7	Foliartal 10 ml	7	0	0	0.841 - 0.138
	8	Salicylic acid 1000 ppm	8	0	0	1.036 - 0.138
Sayer	2	Control	7	-	-	0.789 - 0.031
	3	Agrosign Humic 100 ml	7	0	0	0.789 - 0.031
	4	sulphur 200 g	8	1	12.5	0.992 - 0.031
	5	polixal 30 ml	8	1	12.5	0.868 - 0.031
	6	Rexene ca 2000 ppm	7	1	14.20	0.868 - 0.127
	7	Foliartal 10 ml	9	1	11.11	0.992 - 0.031
	8	Salicylic acid 1000 ppm	8	2	25	1.200 - 0.127

Also, Figure 2 and Table 3 show using an OPH-12 primer to reveal the effect of anti-salinity treatments on Polymorphism on Sayer cultivar under salt stress. It is clear that there were (10) bands in the treatment of control whereas the treatments of Humic acid at 100 ml, sulphur at 200 g offshoot^{-1} gave (6) bands, all of which were identical to the control treatment. Thus, the percentage of Polymorphism was (0 %).

Also, the treatment of polixal at 30 ml offshoot^{-1} gave (3) band. One of them was Polymorphic compared with control treatment and reached the percentage of Polymorphism (33.33%) whereas the treatment of spraying Rexene Ca at 2000 ppm offshoot^{-1} gave (8) bands. This was identical to the

control treatment. Thus, the percentage of Polymorphism was (0%). Besides, the treatment of Foliartal at 10 ml offshoot^{-1} gave (2) bands, all of which were identical to the control treatment. Thus, the percentage of Polymorphism was (0 %). The treatment of spraying salicylic acid at 1000 ppm offshoot^{-1} gave (7) bands, one of them was Polymorphic compared with control treatment and reached the percentage of Polymorphism (14.28 %).

Using OPA -10 primer: Figure 3 and Table 4 show using an OPA-10 primer to reveal the effect of anti-salinity treatments on Polymorphism of Berhi cultivar under salt stress. It is clear that there were (6) bands found in control treatment. Also, the treatments of Humic acid at 100 ml, sulphur at 200 g, polixal at 30 ml and Rexene Ca at 2000 ppm offshoot-1 gave (6) bands, all of which were identical to the control treatment. Thus, the percentage of Polymorphism was (0%), but the treatment of Foliartal at 10 ml gave (2) bands, all of which were identical to the control treatment. Thus, the percentage of Polymorphism was (0%) whereas the treatment of Salicylic acid at 1000 ppm gave (5) bands, two of which only were Polymorphic and so the percentage of Polymorphism was (40%).

Also, **Figure 3** and **Table 4** show using an OPA-10 primer to reveal the effect of anti-salinity treatments on Polymorphism of Sayer cultivar under salt stress. It is clear that (6) bands were found in the control treatment whereas the treatment of humic acid at 100 ml gave (5) bands, all of which were identical to the control treatment. Thus, the percentage of Polymorphism was (0%), sulphur at 200 g gave (3) bands, all of which were identical to the control treatment. Thus, the percentage of Polymorphism was (0%), but polixal at 30 ml gave (3) bands, one of which only was Polymorphic and so the percentage of Polymorphism was (33.33%). Also, spraying Rexene Ca at 2000 ppm gave (5) bands, two of which only were Polymorphic and so the percentage of Polymorphism (40%), however, Foliartal at 10 ml offshoot-1 gave (5) bands, three of which only were Polymorphic and so the percentage of Polymorphism was (60%), and Salicylic acid at 1000 ppm offshoot-1 gave (6) bands, tow bands of which only were Polymorphic and so the percentage of Polymorphism was (33.33%).

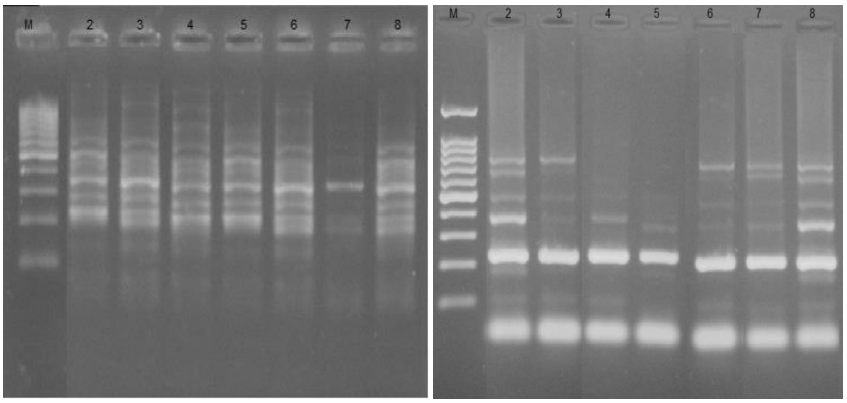

Fig. 3: RAPD-PCR gel image showing DNA polymorphism during the effect of anti-salinity compounds on Berhi and sayer cultivars using OPA – 10 primer under salt stress

Table 4: Effect of Anti-salinity Compounds in Genetic Variation of Berhi and Sayer Offshoots using OPA – 10 Primer

cultivars	Symbol	Treatment	Total Bands	Polymorphic bands	Polymorphism %	Bands size (bp)
Berhi	2	Control	8	-	-	0.684 - 0.094
	3	Agrosign Humic 100 ml	8	0	0	0.684 - 0.094
	4	sulphur 200 g	8	0	0	0.894 - 0.094
	5	polixal 30 ml	8	0	0	0.841 - 0.094
	6	Rexene ca 2000 ppm	8	0	0	0.841 - 0.138
	7	Foliartal 10 ml	7	0	0	0.841 - 0.138
	8	Salicylic acid 1000 ppm	8	0	0	1.036 - 0.138
Sayer	2	Control	7	-	-	0.789 - 0.031
	3	Agrosign Humic 100 ml	7	0	0	0.789 - 0.031
	4	sulphur 200 g	8	1	12.5	0.992 - 0.031
	5	polixal 30 ml	8	1	12.5	0.868 - 0.031
	6	Rexene ca 2000 ppm	7	1	14.20	0.868 - 0.127
	7	Foliartal 10 ml	9	1	11.11	0.992 - 0.031
	8	Salicylic acid 1000 ppm	8	2	25	1.200 - 0.127

DISCUSSION

The positive effect of anti-salinity treatments on alleviating the damage effects of salinity on growth might be attributed to increased mesophyll thickness, activate photosynthesis by increasing total chlorophyll, RWC, carbohydrate, proline and the balance between endogenous hormones IAA and ABA[15]. It is also possible that the protection of the endogenous antioxidant systems often correlated with increased tolerance to oxidative stress or controlling the level of free radicals in plant tissues[16]. The positive role of calcium in alleviating the damage of salinity on growth may be attributed to decreased Na^+ uptake and increased K^+ uptake as well as increased mesophyll thickness and activate photosynthesis by increasing total chlorophyll, RWC, carbohydrate, proline[15]. Sayer cultivar recorded the lowest value in this respect.

The effect of anti-salinity treatments on alleviating the harmful effect of salinity and increased organ osmolytes might be attributed to increasing sugars that are compatible solutes which accumulate in plant tissues that are exposed to abiotic stress, such as water deficit, extreme temperatures, and salt stress. The accumulation of sugars may play an important role in the plant defensive mechanisms of osmoregulation and energy preservation[17], and molecule as sucrose protects biological macromolecules (DNA and RNA) against the damaging effects of salinity [9]. Also, increased metabolic by the accumulation of proline which is often regarded as a basic strategy for the protection and survival of plants under abiotic stress[18]. The proline accumulation is possible due to increasing in the enzymes of its synthesis or decrease in the enzymes of its oxidation[19].Therefore, it can be inferred that exogenous application of anti-salinity treatments improved the osmoprotective system of the offshoots. Moreover, proteomic, genomic and metabolic reviews have uncovered that the capacity of proline is not as immediate as at first accepted. Inquire about reviews on plants, particularly those on

proline synthesis and catabolic genes, have shown that the proline created under distressing conditions can go about as a good solute in osmotic regulate, a free radical scavenger, a metal chelator, an activator of detoxification pathways, a cell redox balance, a cytosolic pH buffer, a source of energy, nitrogen and carbon source, a stabilizer for subcellular structures and membranes including photosystem II, or go about as a signaling molecule[20]. The effect of calcium in the alleviation of salt stress and the increase of proline reflects the ability of salt-tolerate offshoots to prevent the formation of ROS and K^+ loss by maintaining enzymes as (POD and Cat) and non-enzymatic (proline, soluble carbohydrates, and proteins) defense systems[21]. RAPD technology used in the current study showed Polymorphisms between some of the treatments of anti-salinity compounds. Those Polymorphisms in DNA fragments may result from differences in the links of the primers with DNA or for the case of deletion or addition to the base or the number of nitrogen bases that composed of the bar the DNA or obtained one of substitution, Inversion and Rearrangement processes. These processes are working on changes in bands and Molecular weights that appear after separating bands process depending on the molecular weights in the gel agarose using electrophoresis. This would serve to activate specific genes (gene on) or silence other genes (gene off) [22, 23].

Plants undertake evolved four strategies to subsist forth environmental stresses which look on release match lodgings of sundry genes flip the introduction of epigenetic modifications, such as DNA methylation. DNA methylation plays a primary traffic in gene enunciation by attractive RNA-directed DNA methylation (RdDM) of genes and by faith some histone modifications DNA methylation may be significantly affected by the environment and cannot be experimentally manipulated or maintained. Answer for, co-conspirator meticulousness forced to be taken for granted at the drop of a hat pre-eminent strategies fitted on opus plants with regard to novel traits based on variations in DNA methylation. Reducing the equilibrium of DNA methylation eternally has an adverse perform on the plant's ability to tolerate environmental stresses. Epigenetic modifications produce to an adaptive evolutionary mechanism in plants[24]. DNA methylation at a medicine is genetic through meiosis [24,25]. As well as DNA methylation shows gene utterance distinction gets the tight-fisted of the matching plant species when grown under diverse environmental conditions[24]. However, it is noted that there is an effect on the spraying treatments more Polymorphism than the treatments of the addition to soil. Further, the treatment of salicylic acid was more Polymorphism than others. Also, Sayer cultivar was more Polymorphism than Berhi cultivar that considers high salt tolerant. Besides, it is noted that there is flowering in the second season to most of the offshoots exposed to treatments.

CONCLUSIONS

Anti-salinity treatments on date palm offshoots showed improved Parameters of vegetative growth such as leaf area and a number of new leaves, and Biochemical constituents such as total soluble carbohydrates and proline compared to control. Further, Berhi cultivar was more tolerance to salinity than Sayer cultivar. It is assumed that used Anti-salinity treatments improve the overall Parameters of growth with the capability to alter at the genetic level.

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