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ORIGINAL ARTICLE

GENETIC MATCHING OF DATE PALM DEVELOPED UNDER SALINITY STRESS *IN VITRO*

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Abstract: This study was conducted during 2019-2020 on the vegetative shoots of date palm *Phoenix dactylifera* L. cultivar 'Barhee', which were grown on MS salt media containing sodium chloride (0-25-50-100-150-200 mM) supplemented with 5% sucrose. Genetic variances were assessed using molecular markers RAPD using 8 random primers. The RAPD analysis did not result a polymorphism between the amplified bundles and the absence of genetic differences. The primer (OPE-19 and OPD-10) scored the lowest similarity rate 97%, while the genetic matching was 100% in the other primers. The morphological indicators recorded a significant and gradual decrease in the growth indicators (buds number, bud length and stem length) and a significant increase in (bud diameter and branches number) in the propagation and elongation stages. Also, the chemical growth indicators recorded an increase in the chemical content (abscisic acid and proline), the effect of Sodium chloride was negative on photosynthesis, as the chlorophyll content gradually decreased with increasing sodium chloride concentration.

***Key words*:** Date palm, Salinity stress, *In vitro,* RAPD analysis.

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1. Introduction

Among the biggest challenges facing food crop productivity worldwide is the salinization of arable land and stresses from various sources of abiotic stress [El-Beltagi *et al*. (2008)]. Although, the date palm *Phoenix dactylifera*, which belongs to the family Arecaceae, shows its ability to resist live in harsh desert environments and maintain its growth in saline soils, it loses many of its distinctive properties as a fruitful tree with its resistance to various stresses as stress leads to changes harmful at the cellular level and cell structure. Among these damages is the accumulation of sodium chloride ions, which impede the absorption of water and nutrients and Na ions threaten membranes' integrity [Abul-Soad and Al-Khayri (2017), Noaema *et al*. (2020a)].

The laboratory regeneration of palm trees using different tissues, including shoot tip and multiplication of vegetative buds, has been documented by many researchers [El Hadrami *et al*. (1995), Abul-Soad and Al-khayri (2017), Walker (2016)]. This technique is from plant generation through tissue culture is usually subject to genetic differences between new plants, which may be due to the protocols followed or the length of cultivation [Matthes *et al*. (2001)]. These variations may include at the level of DNA due to a chromosomal abnormality or a rearrangement in genes. Most cases lead to a point mutation [Krishna *et al*. (2016)]. However, the variation between genotypes is the ultimate goal in plant breeding and modern technology approaches. These changes must be subjected to the improved trend and not affect the characteristics of the original variety, usually through the use of various markers, including DNA-based markers such as RFLIP

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and AFLP, SCAR amplification technique, SSR, and ISSR techniques, which depend on simple sequence repetition [Khan *et al*. (2012), Jones *et al*. (2013), Noaema *et al*. (2020b)].

RAPD technology is considered a reliable molecular technique for detecting genetic variations and genetic changes and has been used with tissue culture by many researchers [Sudha *et al*. (2019), Kumar *et al*. (2010), Al-Hasany *et al*. (2020)].

In this study, the main objective was to produce plants adapted to salt by exposing vegetative shoots to different levels of NaCl and another objective was to monitor the stability of the genetic material using RAPD parameters.

2. Materials and Methods

The study was conducted in the plant tissue culture laboratory of the Fadak Agricultural (Private Company) at Abi Al-Khasib-Basrah Governorate, for the period 2019-2021. The vegetative buds resulting from the apical buds were used in the tissue culture of the Barhi cultivar, grown on a nutrient medium [Murashige and Skoog (1962)]. Which is known as MS-media salts as a propagation medium and supplemented with sucrose 5%, acid sodium orthophosphate 200 mg, adenine sulfate 80 mg, Calcium nitrate tetrahydrate 200, Inositol 100, Thiamin10, L-Glutamine 200, Polyvinyl pyrrolidone 500. With adjusting the pH of the medium pH 00.6±0.1, Indol acetic acid (IAA), Benzyl adenine (BA) and Kinetin (Kn) 2ip were added at a concentration of 0.1 $mg.l^{-1}$, then 1 $ml.l^{-1}$ of the previously prepared vitamins was added and then added. Sodium chloride was transferred to the culture medium with concentrations (Table 1) in the phase of propagation and elongation, then morphological, chemical, and molecular measurements were taken.

2.1 Lighting and temperature after planting on the propagation medium

The plants were incubated in the incubation room at a temperature of 27±2°C, a humidity of 50 ± 10 and a light intensity of 1600 lux equipped with LED lights for 16 hours/day.

2.2 Morphological and chemical data collection

The effect of six concentrations of sodium chloride on the length, diameter and number of buds in the propagation phase and branches in the elongation phase was determined through daily observations.

Table 1: The protocol for addition NaCl.

No.	Treatment
1	MS-medium
2	MS- With Nacl25mM
3	MS-with Nacl50mM
4	MS-with Nacl100
5	MS-with Nacl 150
6	MS-With 200mM

2.3 Genomic DNA extraction

The DNA genetic material was isolated for the study samples taken from the vegetative buds of the date palm Barhee cultivar and treated with sodium chloride in addition to the standard sample and using CATB (Trimethyl ammonium bromide) as described by Doyle and Doyle (1987) the purity of the resulting DNA was estimated using a Nanodrop device.

2.4 RAPD-PCR of isolated DNA

RAPD reactions were carried out on study samples taken from genetic material isolated from vegetative shoots of date palm cultivar Barhee and treated with sodium chloride, with a volume of 50-100 ng. The materials which needed to conduct the reaction were used in the presence of the reaction master mix containing the reaction compounds to amplify the random regions by each primer, and they included dNTPs (0.4µM), Taq polymerase (0.1u), Mgcl and PCR Buffer X10 with a volume of 2.5 for each of them. Complete the volume to 25 µl by adding ddHO and the random primers shown in Table 2 and with a volume of 2.5 µl mixed within the reaction mixture, whose reaction temperature was between 35-37°C. Amplification program was 1 cycle of initial denaturation at 94°C for 1 minute followed by 45 cycles of denaturation at 95°C for 30 seconds. The double-strand then lowered the primer adhesion temperature to 35°C and 37°C, the primer binding to the DNA template. The temperature was raised to 72°C with a period of 2 minutes; the primer elongation and the last cycle, which took 5 minutes, 72°C. The size of the separated molecular bands on the agarose gel was determined using a ladder 1Kb DNA.

2.5 Statistical Analysis

The results were statistically analyzed using a software package. The morphological data were analyzed according to the CRD using least significant difference at probability level of 0.5 through GenStat

Table 2: RAPD primers used to detect genetic variation in regenerated date palm plants of cultivar'barhee'under salinity stress.

No.	Primer name	Primer Sequence	Band Score						Size range (Pb)
			cont.	25nacl	50nacl	100nacl	150nacl	200nacl	
1	OPA-03	AGTCAGCCAC	8	8	8	8	8	8	6000-500
2	OPE-08	TGGACCGGTG	7	7	7	7	7	6.5	7000-500
3	OPG-12	CAGCTCACGA	6	6	6	6	6	6	5000-700
4	OPD-10	CTGCTGGGAC	6	6	6	6	6	5	6000-200
5	MOH-9	GGACCCAACC	9	9	9	9	9	9	5000-250
6	MOH-8	GTGAGGCGTC	6	6	6	6	6	6	300-500
7	OPE-19	ACGGCGTATG	7	7	7	7	6	6	8000-350
8	OPA-05	AGGGGTCTTG	5	5	5	5	5	5	4000-250

program. The molecular weights in the RAPD indicators were determined using image 10 and the similarity coefficient was estimated based on the Index Jaccard scale.

3. Results and Discussion

3.1 Molecular analysis of RAPD

The results of the genetic study between the control and other treatment with sodium chloride, which depend on the genetic stability of the genetic material of the vegetative buds in the stage of doubling and elongation and exposed to salt stress conditions and with the concentrations mentioned in Table 2 on the basis of two replicates, to evaluate the differences resulting from exposing the shoots to saline tension compared to the non-saline treatment were used. The 8 RAPD primers which produced a total number of 320 pands, with an average number of pands of 40.06 for each primer, size ranging between (200-800pb) each primer produced a number of pands ranging from 4-9, with an average of 6.67 pands for each primer. RAPD analysis produced monomorphic patterns between the study samples and the control treatment, and no polymorphism was observed in the primers used, and as evidenced by the results of previous studies [Al-Khateeb *et al*. (2019)], there were no differences in the genotypes of buds or embryos. While, the results of the study of Lakshmanan *et al*. (2007) reported that there are differences in the genotypes resulting from tissue culture, and the change in the genotype may be attributed to the difference in the plant type and the protocols used in propagation, or from the use of growth regulators and other chemical compounds involved in preparing. In some cases, the agricultural environment causes chromosomal abnormalities or a point mutation and thus causes differences in the genotypes and somatic embryos formed [Krishna *et al*. (2016)].

3.2 Genetic stability of vegetative shoots of date palm cultivar Barhee

In Table 3, it is also noted that the primers (MOH-9, MOH-8) recorded the highest genetic stability rate of 100% and there was no monomorphic polymorphism in these markers, while the primer (OPE-19) gave the lowest genetic stability rate of 95 % compared to other primers. It is noted that the primer (OPD-10) was amplified by three significant association sites and gene duplication, which indicates the accuracy of this primer in determining the resulting differences. While the other primers produced monomorphic sites and no polymorphism was observed between the NaCl and control treatments, which indicates the stability of the genetic material in the concentrations used for salt and from the results of amplification of the primers (OPE-8, OPD-10, OPE-19) and there were no differences between concentrations (25-50-100 mM) and the control treatment. The stability of the genetic material and the absence of significant variations in the genetic material may result from this protocol used in the propagation of vegetative shoots and the study treatments successfully maintaining the stability of the genetic material.

In this study, the concentrations of sodium chloride that can be used to produce plants tolerwnce to salt stress conditions and are not genetically differentiated were determined. The slight variation that was diagnosed in concentration 200 mM may be due to basic changes in certain genes or variation at the chromosomal level (Table 3).

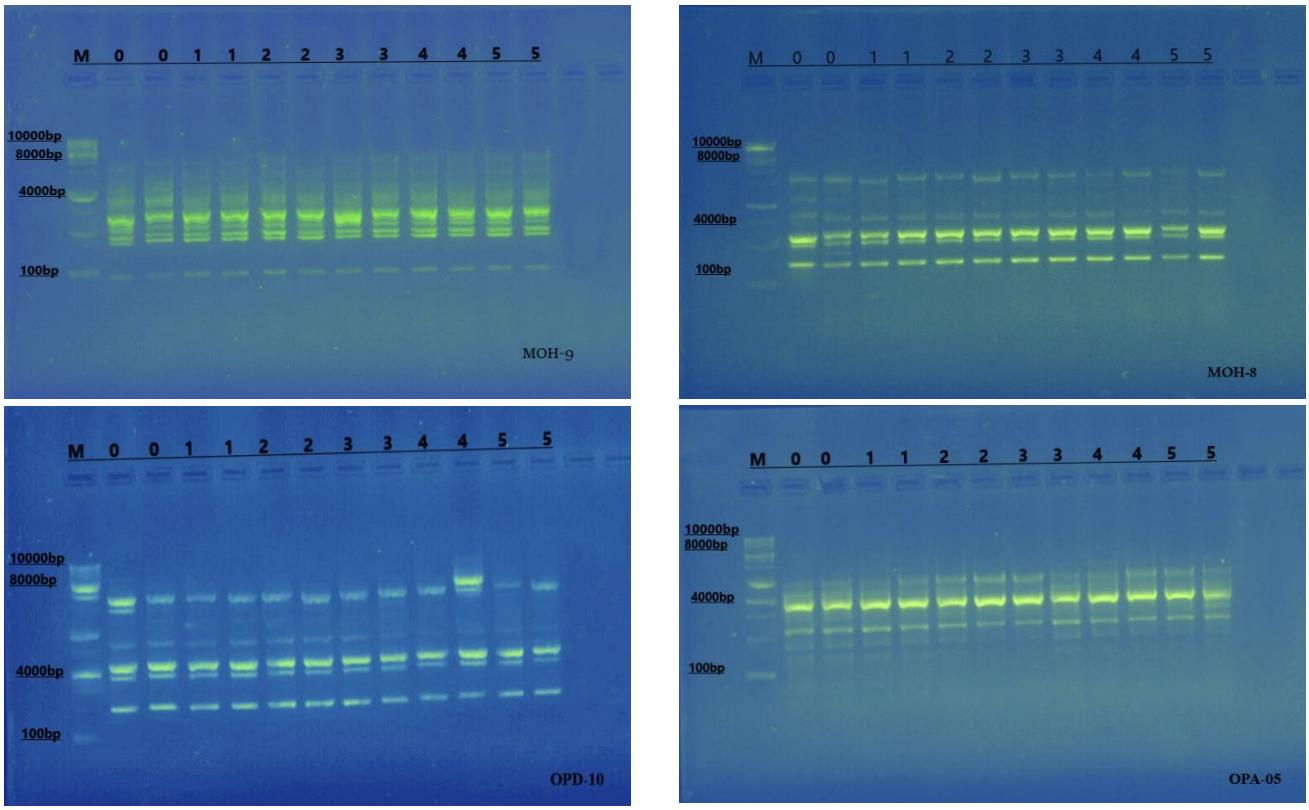

Fig. 1 : Gel Electrophoresis of RAPD fragments in *Phoenix dactylifera* .cv. barhee

Table 3: Genetic similarity between regenerated plants from the Barhee cultivars and comparison treatment.

No.	Primer name	Total band number	Number of monomorphic bands	Similarity%
1	OPA-03	48	48	100
2	OPE-08	41	40	97.59
3	OPG-12	36	36	100
4	OPD-10	35	34	97.14
5	MOH-9	54	54	100
6	MOH-8	36	36	100
7	OPE-19	40	38	95
8	OPA-05	30	30	100
	Total	320.5	316.5	98.75

3.3 Morphological and chemical analysis

The results of the morphological data in Table 4 indicate a significant decrease in the mean of the studied morphological characteristics of the Barhee cultivar treated with sodium chloride in both the number of buds formed and the length of the bud. The treatment 200 mM recorded the least significant difference of 18.16 and 6.53 cm, respectively. In contrast, the control treatment recorded the highest rate for the number of buds and their length, which amounted to 33.66-11.8 cm and the results of the table indicate the moral effect of adding sodium chloride on the bud diameter, as the two treatments (150-200 mM) recorded the highest average of bud diameter, which amounted to (3.43-3.92) compared to the control treatment. It is noted from the results of the table that in the vegetative elongation stage, the addition of sodium chloride significantly affected the measured characteristics (stem diameter, branches number), as the 100 mM treatment was significantly superior to the rest of the treatments and the comparison treatment. In contrast, a significant gradual decrease in stem length was observed with an increase in the concentration of sodium chloride. The reason for the increase in the bud diameter in the propagation phase and the stem diameter in the elongation phase may be attributed to the increase in the concentration of NaCl salt to the mechanism used by plants in preventing or maintaining a minimum growth

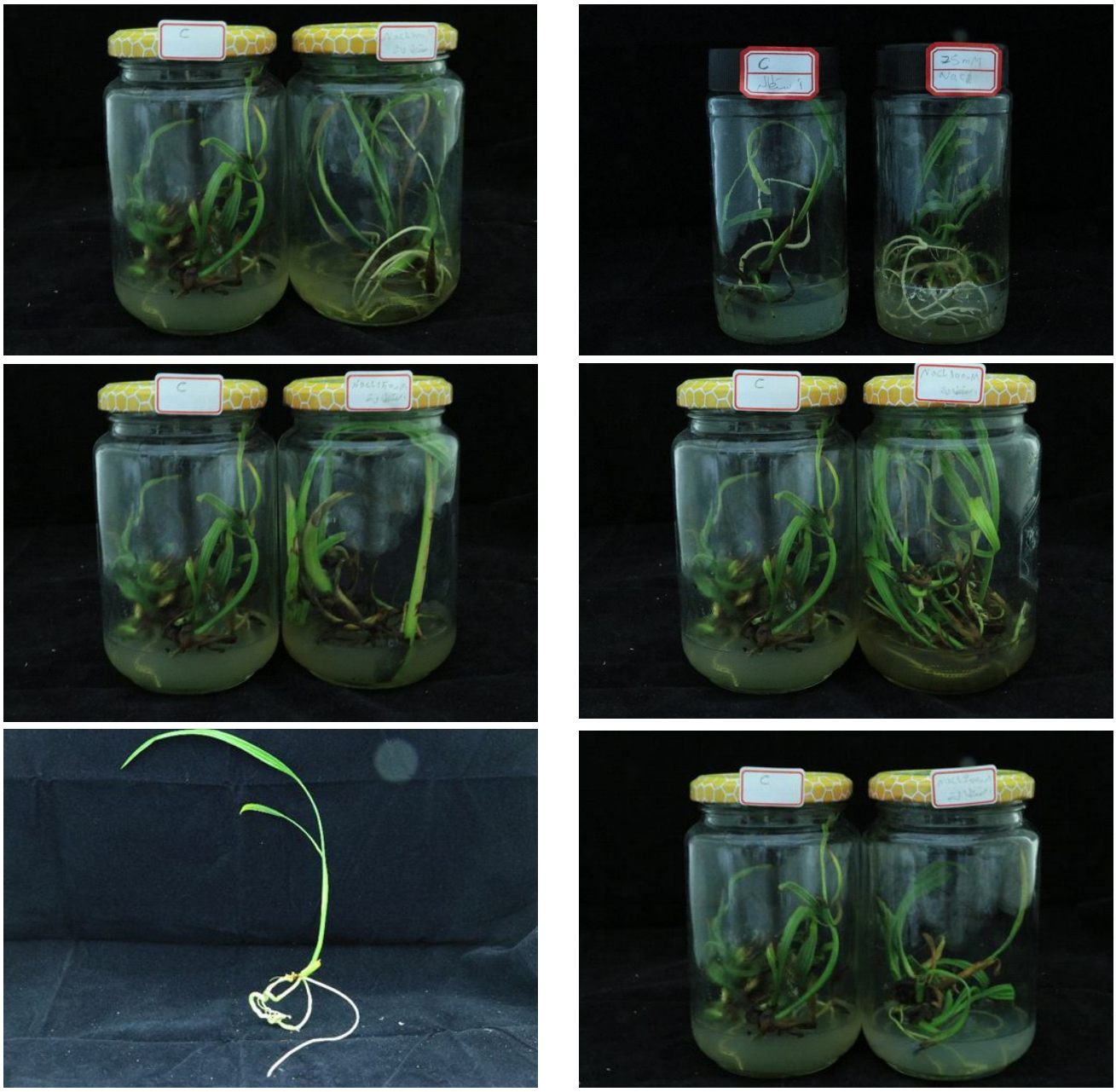

Fig. 2 : Buds growing on the elongation medium containing sodium chloride and at concentrations (0, 25, 50, 100, 150, 200) mmol of sodium chloride

Table 4: *In vitro* effect of salt stress on growth characters of the date palm cultivar Barhee.

Treatments	Embryo germination			Elongation stages		
	Number buds	Length buds (cm)	Diameter buds (mm)	Shoot length (cm)	Shoot diameter (mm)	Shoot number
Control	33.66±0.96^{a}	11.80±1.44^{a}	1.46±0.05^{d}	13.73±0.48^{a}	1.73±0.08^{b}	13.33±0.58^{d}
25mM	32.05±0.31^{a}	11.53±2.13^{a}	1.60±0.20^{d}	13.77±0.86^{a}	1.80±0.26^{b}	15.07±0.90^{bc}
50mM	31.77±0.98^{a}	10.63±0.66^{ab}	1.80±0.10^{d}	13.81±0.09^{a}	2.00±0.50^{b}	16.00±0.56^{ac}
100mM	32.40±1.96^{a}	9.30±0.55^{bc}	2.76±0.15^{bc}	11.07±0.01^{b}	4.16±0.52^{a}	18.50±0.87^{a}
150mM	26.00±2.19^{b}	7.06±1.10^{d}	3.43±0.35^{ac}	8.63±0.32^{c}	3.66±0.17^{a}	8.93±3.99^{e}
200mM	18.16±1.27^{c}	6.53±0.70^{d}	3.92±0.82^{a}	8.52±0.02^{c}	3.60±0.53^{a}	6.70±2.34^{e}

level under the influence of salt stress by reducing the loss of water from the cell by closing the stomata and maintaining vital growth systems are obsolete by accumulating a number of compatible solutes that include proteins, carbohydrates, amino acids and quaternary ammonium compounds, as this accumulation leads to the activation of free radical scavenging antioxidants (ROS) [Taïbi *et al*. (2016), Yaish *et al*. (2017)].

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