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THE EFFECT OF COMBINATIONS OF GROWTH REGULATORS ON THE ELONGATION OF THE TISSUE STEMS OF THE MEDJOOL DATE PALM AND THE USE OF RHABDITE TECHNOLOGY TO DETECT GENETIC CHANGES IN THE TISSUE DEFORMED LEAVES

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Abstract:
The effect of combinations of growth regulators on the elongation of the tissue branches of the Medjool date palm, where the T5 treatment (1 mg L^{-1} of each of Kin, 2iP, BA, IAA) outperformed most of the experimental characteristics by giving the highest values in terms of the average number of new leaves (2.66 leaves per plant part^{-1}).) and the percentage of shoots rooting (80%) and the average of; number of roots (6 roots per plant part - 1), shoot length (2.66 cm), and increase in stem length (4.33 cm). Genetic variations (one of the major production defects in date palm tissue farms) resulting from the tissues of the unknown variety of date palms produced by the indirect organogenesis method were studied to show the ratio of genetic differences between the distorted and normal leaves in the tissue to indicate their exclusion from the micropropagation protocol using the rhabd technology to detect mutations. Using seven random primers after amplification of the total DNA, three primers revealed polymorphisms and gave results in detecting heterogeneity, as the study showed a genetic difference of 10% between the two distorted tissues and the normal plant.

Keywords: Date palm, elongation, tissue culture, leaf deformities, RAPD-PCR, genetic variations.

INTRODUCTION

Propagating date palms using plant tissue culture technology is the best way to produce large quantities of plants with high quality and efficiency (Abdalla et al., 2022). The resulting plants are free of diseases and pests and uniform in size (Fki et al., 2011), and the elongation stage is one of the important stages in palm tissue culture (Al-Sumaidai, 2017), as tissue branches are grown on nutrient media provided with growth regulators to enhance growth and increase branch length (Mazri and Meziani , 2013). Gene expression in date palms takes several years, so DNA markers are used to study genetic stability and ensure that genetic mutations do not occur (Jahan et al., 2020). Somaclonal Variations or genetic mutations may appear, which depends on the method of direct or indirect tissue propagation, the type and concentrations of growth regulators (cytokinins and auxins have a role in the occurrence of genetic variations in date palms) (Imran et al., 2022; Park et al., 2023) and the number of times Reimplantation and genetic recombination These differences may be permanent (genetic resulting from changes in DNA) or temporary (epigenetic resulting from changes in the tissue environment in tissue culture) (Faissal et al., 2013), and these differences result in somatic cells, which are considered a defect. In commercial production. Phenotypic differences increase with the age of the tissue culture (Bairu et al., 2006), and the use of 2,4-D auxin in inducing callus when establishing tissue cultures works to increase genetic variation in tissue cultures (Saker et al., 2006). Several cases have been recorded. Of the deformities of date palm seedlings resulting from tissue culture, including deformities of the fronds due to their abnormal growth (Mirani et al., 2019). To demonstrate the best combination of growth regulators to enhance the growth of the tissue stems of date palms. Due to the lack of sufficient studies on deformities of plant parts in the laboratory and the appearance of deformities in the leaves of the Medjool variety produced by organogenesis indirectly, the current study aimed to detect these genetic changes in a manner using the rhabd method.

These deformities result from duplication or translocation of genes, or point mutations through deletions, insertions, or methylation, or they may be at the chromosome level (Dennis, 2004). Laboratory growth conditions also play a role in causing genetic mutations, and the number of mutations accumulates in commercial tissue cultures over time (Duncan , 1997). Most of the phenotypic abnormalities that occur in date palms resulting from tissue culture are of the type of epigenetic variations, and these variations are characterized by being unstable and not inherited, and are most likely physiological changes that disappear after a period of time, usually after the influencer disappears (Al-Khatib, 2005).

MATERIALS AND METHODS

He used the tissue branches of the date palm Phoenix dactylifera L., the Medjool variety (grown by indirect adventitious shoots in Iraq, Jannah Al-Nakheel Company in 2021) as a source of plant parts, and the study was conducted at Jannah Al-Nakhil Company - Baghdad in April 2022. The study included the elongation of tissue branches using MS medium with full salt strength provided with combinations of regulators (all regulators used at the same concentration of 1 mg L^{-1} according to Meziani et al. (2019) and Almusawi et al. (2017)) and shown in Table 1. The tissue stems resulting from the multiplication stage, with a length of 3-5 cm, were used at a rate of ten replicates, with each branch being counted as a duplicate, and at the rate of three branches per jar, in order to determine the best combination of growth regulators for the development of the tissue stems of the Medjool date palm. The data were collected after 8 weeks, as a primary experiment with six levels using a completely randomized design (C.R.D.). SPSS was used to analyse the data, and the averages were compared according to Duncan's multinomial test at a probability level of 5%. The experiment studied were: the average number of new leaves (leaf per vegetative part^{-1}), the percentage of rooting (%), the average number of roots (root per vegetative part^{-1}), the average length of roots (cm), and the average increase in stem length (cm). Deformities were diagnosed in the leaves of date palm tissues growing in agricultural containers (Figure 1) during the growth of the branches and their elongation in the branches, represented by the leaves curling and curling. A sample of the deformities and a sample of healthy leaves were taken to demonstrate the effect of the deformity, physiological or genetic, using RAPD-PCR technology with seven primers (Table 2). in the laboratories of Wahaj Al-Ahli DNA Company - Baghdad, I used the Geneid DNA extraction kit, and I used the primers in Table 1, and the PCR reaction program in Table 3.

Table 1. Combinations of growth regulators used in the elongation of tissue branches of date palm variety Medjool.

Treatment						
T6	**T5**	**T4**	**T3**	**T2**	**T1**	
0	1	1	0	1	1	Benzyl adenine (**BA**) mg L^{-1}
1	1	1	1	1	1	Kintein (**KIN**) mg L^{-1}
1	1	0	1	1	1	6-(γ-γ-Dimethylallylamino) Purine (**2iP**) mg L^{-1}
1	0	1	1	0	0	*Meta*-**Topolin** mg L^{-1}
0	0	0	1	0	1	1-Naphthaleneacetic Acid (**NAA**) mg L^{-1}
0	0	1	0	1	0	2-Naphthoxyacetic acid (**NOA**) mg L^{-1}
1	1	0	0	0	0	Indole-3-acetic acid (**IAA**) mg L^{-1}

Figure 1. Deformed leaves of the Medjool variety for three samples: A, wrinkled, B, curled, C, normal leaf.

Table 2. Sequences and nucleotide sequences of primers used in the RAPD-PCR technique

Primer name	5→3 Primer sequences
OP-V19	GGGTGTGCAG
OP-V02	AGTCACTCCC
OP-V14	AGATCCCGCC
OP-V09	TGTACCCGTC
OP-M06	CTGGGCAACT
OP-M05	GGGAACGTGT
OP-P04	GTGTCTCAGG

Table 3. PCR reaction program used to detect genetic variations in date palm leaf tissues

Stages	Temperature (°C)	Time (min)	Number of cycles
Primary denaturation	95	3	40
Denaturation	95	1	
Correlation	37	1	
Elongation	72	1	
Final elongation	72	10	

The RAPD results that appeared in the gel were analysed after converting the descriptive results into digital data by placing the number 1 in the presence of the band and 0 in its absence on the agarose gel and analysing it statistically using SPSS. The genetic distance ratio was used based on the results of the two primers used to find the genetic distance ratio between the samples, which is based on the presence of common bands between a pair of those samples, and was estimated based on the 72 s'Nie factor (Nie and Li, 1979) according to the following equation in the SPSS program:

$$\text{Genetic Distance} = 1 - ((2*N\_xy) / (N\_y+N\_x))$$

Whereas: G.D. represents the genetic dimension, Nxy represents the number of shared packages between the two samples x and y, which represent two samples, Nx represents the number of total packages in sample x and Ny represents the number of total packages in sample y. As for the cluster analysis scheme, it was carried out according to the UPGMA method, to obtain the genetic dimension tree (Sneath and Sokal, 1973) using the NTSYS-pc (Numerical Taxonomy System) program, thus obtaining a cluster analysis scheme that shows the close and distant genetic groups for all tested samples.

RESULTS AND DISCUSSION

The effect of combinations of growth regulators on the elongation of date palm branches

Average number of new leaves

The results (Table 4 and Figure 8) showed the superiority of T3 (1 mg L^{-1} for Kin, 2iP, MT, NAA) and T5 (1 mg L^{-1} for Kin, 2iP, BA, IAA) treatments to the rest of the study treatments, except for the T6 treatment (1 mg L^{-1} for each of Kin, 2iP, MT, IAA), which gave the highest average number of new leaves, which amounted to 2.66 leaves for branch^{-1} for both treatments, while the T2 and T5 treatments gave the lowest average number of new leaves, which amounted to 1.33 leaves for the branch. $^{-1}$ for both.

Percentage of root emergence

The results of Table 4 showed that T3 (1 mg L^{-1} for each of Kin, 2iP, MT, NAA) and T5 (1 mg L^{-1} for each of Kin, 2iP, BA, IAA) were significantly superior to the rest of the study's treatments by giving it the highest percentage of root growth, which amounted to 80%, while the T1 treatment (1 mg L^{-1} of each of Kin, 2iP, BA, NAA) gave the lowest percentage of root growth, which amounted to 30%.

Average number of roots

The results (Table 4) indicated that the treatment of T5 (1 mg L^{-1} for each of Kin, 2iP, BA, IAA) and T6 (1 mg L^{-1} for each of Kin, 2iP, MT, IAA) was significantly superior to the rest of the study treatments except for the two T4 treatments (1 mg L^{-1} for each of Kin, 2iP, MT, NAA), the superior treatments gave the highest average number of roots, reaching 6 and 5.33 roots for branch^{-1}, respectively, while the T1 treatment gave (1 mg L^{-1} for each of Kin, 2iP, BA, NAA) The lowest average number of roots was 1.33 roots for branch^{-1}.

Table 4. The effect of different combinations of growth regulators on the elongation of tissue shoots of date palm variety Medjool after 8 weeks of planting.

Treatment	Studied characteristics				
	Number of new leaves	Rooting percentage	root number	Root length	Increase in stem length
T1	1.50 b	30 d	1.33 d	1.50 b	2.66 a
T2	1.33 b	50 c	1.66 cd	1.66 b	4.00 a
T3	2.66 a	80 a	3.33 bc	2.33 ab	4.00 a
T4	1.33 b	70 b	5.00 ab	1.63 b	4.33 a
T5	2.66 a	80 a	6.00 a	2.66 ab	4.33 a
T6	1.66 ab	70 b	5.33 a	3.00 a	4.33 a

**Means with similar letters are not significantly different from each other at the 5% probability level according to Duncan's multinomial test.

T1=1 Kin,2iP, BA, NAA, T2=1 Kin,2iP, BA, NOA T3=1 Kin, 2iP, MT, NAA, T4=1 Kin, BA, MT, NOA, T5=1 KIN, 2iP, BA, IAA, T6=1 KIN, MT, 2iP, IAA.

Average root length

The results of Table 4 showed that the T6 treatments (1 mg L^{-1} for each of Kin, 2iP, MT, IAA) were significantly superior to the rest of the study treatments except for the T3 treatments (1 mg L^{-1} for each of Kin, 2iP, MT, NAA) and T5 (1 mg L^{-1} for Kin, 2iP, BA, IAA) by giving it the highest average number of roots, which amounted to 3 cm, while the T1 treatment (1 mg L^{-1} of each of Kin, 2iP, BA, NAA) gave the lowest average number of roots, which amounted to 1.5 cm.

Average increase in stem length

The results of Table 4 showed that there were no statistically significant differences between the study parameters.

The results of the current study support the general framework that plant growth regulators enhance the elongation and rooting of the tissue branches of the date palm Meziani et al. (2019). It is known that auxins control cell division and elongation and promote the formation of adventitious roots (Machakova et al., 2008), as well as cytokinins, which are essential for cell division (Van Stadon et al., 2008). Abahmane (2011) showed that the most commonly used growth regulators at this stage are BA, 2iP, Kin, IBA, and NAA, and that combinations of auxins and cytokinins in high concentrations benefit the branches grown in the elongation stage. This effect may be attributed to the role of auxins and cytokinins in cell division and elongation, and that studies were limited to studying one of them without the other, and that the studied treatment of the two groups by adding them as a combination improved the metabolic processes (Al-Sumaidaie, 2017). These results are consistent with Meziani et al. (2019) using combinations of auxins and cytokinins, as they improved the length of tissue branches and root formation in them.

DNA extraction and determination of quantity and purity

The results of DNA extraction (Table 5) for the three samples showed that an appropriate amount of DNA was obtained, which showed its effectiveness in the electrophoresis results and ranged between 8.8-12.9 nanograms $microliter^{-1}$, with a purity percentage of the extracted DNA between 1.81-1.87, which indicates the efficiency of the extraction method.

Table 5. Purity and concentrations of DNA extracted from the studied samples.

	Samples code	DNA concentration ($ng.\mu l^{-1}$)	280 \ 260 Purity
Normal leaves	**1**	**12.9**	**1.86**
Wrinkled leaves	**2**	**8.8**	**1.87**
Curled Leaves	**3**	**9.1**	**1.81**

The seven primers gave positive results by successfully amplifying DNA bands for all the studied samples by 63 bands, the highest for primer OP-V14 (15 bands) and the lowest for primer OP-V19 (3 bands). The bands with the highest molecular size were 1450 base pairs from primer OP-M05. While the band with the lowest molecular size was 100 base pairs for primer OP-V19, only three primers successfully differentiated between samples (OP-V19, OP-V14 and OP-V09) and showed PolymorPhic bands, the highest of which was for primer OP-V19. V14 (15 bands), with a PolymorPhic band percentage of 100%. The results of Table 6, based on the results of RAPD-PCR, showed the extent of the distance between the samples that were tested, as the highest genetic distance was 0.1068 between sample 1 and sample 2, while the lowest genetic distance was found between sample 1 and 3, which amounted to 1028. The genetic distance between samples depends on the number of polymorphism bands. The greater the number of shared packages, the lower the genetic distance. Therefore, the polymorphism bands between samples indicate similarity in the genetic material in that region of the genome.

Table 6. Genetic dimension among the studied samples.

	Treatment	1	2	3
Normal leaves	1	0		
Wrinkled leaves	2	0.1068	0	
Curled Leaves	3	0.1028	0.10526	0

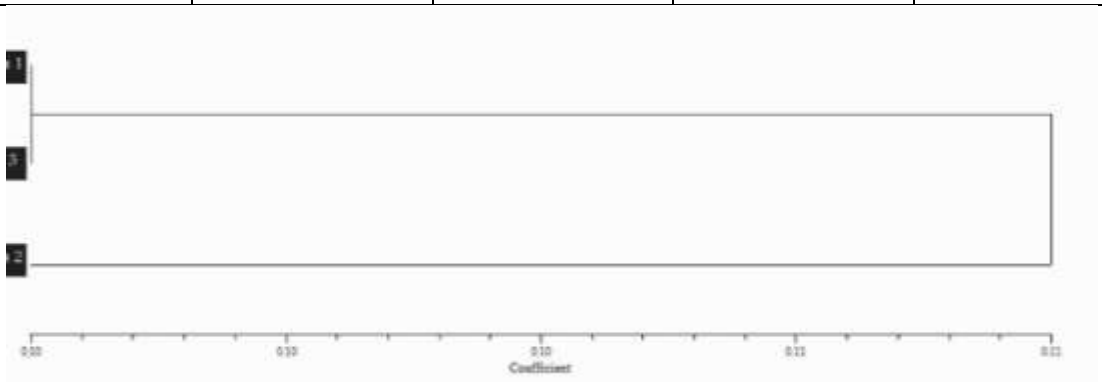

Figure 2. Cluster analysis chart (relationship tree) for the samples under study based on RAPD-PCR indicators, where sample **1** represents normal leaves, sample **2** represents wrinkled leaves, and sample **3** represents curled date palm leaves.

The arrangement of the studied samples within the cluster analysis diagram (Figure 11) depends on the genetic affinity with which the main groups are related. The diagram shows that the samples fall into two main groups, the first of which included samples 1 and 3 and the second representing sample 2 only.
The results from Table 6 and Figure 2 showed that there is a genetic distance between samples 2 and 3 (deformed leaves) and sample 1 (the mother plant) of the leaves of the Medjool date palm, as a genetic distance of approximately 10% from the mother plant was recorded for the two samples (Table 40), which proves the existence of genetic divergence. Between the mutant samples and the mother plant.
The loss of bands in some samples may be due to the absence of sites complementary to the primers on the DNA of the studied samples (Bastianel et al., 2006). The size of the duplicated fragment is based on the binding sites of the primers on the two DNA strands, that is, it is equal to the point between the two binding sites. Because these sites spread randomly on the two DNA strands, any change in the nucleotide sequence causes a change in the binding sites and thus a change in the size of the duplicated fragments (He et al., 1994).
The change in the nucleotide sequence may be due to the presence of mutations resulting from the induction of callus at the beginning of the establishment of the Medjool date palm plantation.
Genetic variations in the DNA of plant cells, whether resulting from the effect of growth regulators, the number of times replanting, the length of the plantings' survival, or the use of auxins, is an appropriate mechanism for creating a genetic makeup that is more adapted to the new environmental conditions, but it is not suitable for the commercial production of date palms (Parfitt and Arulsekar, 1987).
The current study confirmed that the RAPD-PCR technique is effective in detecting genetic variations and helps as tools in facilitating the detection of mutations and studying the genetic stability of date palm tissues. These results are consistent with what was found by El-Bahr et al. (2019), Al-mayahi (2021), and Eshrahi et al. (2005).

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