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Effect of medium type, TDZ, PG, and their interactions on *In Vitro* Regeneration (*Phoenix dactylifera* L.) cv. Barhee

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Abstract

Date palm micropropagation still faces many problems, such as reduced growth and development of callus and organogenesis has lower multiplication efficiency. The effect of Phloroglucinol (PG) (HA; 0, 25, 50 and 100 mg l^{-1}), and Thidiazuron (TDZ) (0.0 , 0.5 and 1.0 mg l^{-1}) were studied on *in vitro* regeneration of *Phoenix dactylifera* L., cv. Barhee. Liquid media supplemented with 50 mg l^{-1} PG in combination with 0.5 mg l^{-1} TDZ gave the highest callus weight (6.94 gm). This treatment was also more effective in obtaining the highest number of buds and average shoots formation (23.0, and 41.0) for each of them, respectively. The liquid medium supplemented with 50 mg l^{-1} PG in combination with 1.0 mg l^{-1} TDZ gave the highest shoots content of nitrogen (0.867%), while the highest shoots content of phosphor and Potassium (0.4567 % and 0.9033 %) were found in liquid media supplemented with 50 mg l^{-1} PG and 0.5 mg l^{-1} TDZ combination. Furthermore, we found that the combined application between 50 mg l^{-1} PG and 0.5 mg l^{-1} TDZ in the liquid medium. resulted in the highest shoots content of endogenous IAA levels (3.644 µg.kg^{-1}), compared with other treatments. The data revealed that the maximum chlorophyll content of shoots was observed in a medium supplemented with 50 mg l^{-1} PG and 0.5 mg l^{-1} TDZ with the liquid medium.

Keywords: Callus induction, Multiple shoots, macronutrient content, Endogenous IAA.

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Introduction

Micropropagation is a promising way for obtaining large amounts of uniform plants that are disease-free and without pests during plant material exchange, genetically identical, and high-quality planting material. The increasing demand for rare and excellent quality date palm cultivars makes us use micropropagation as an unavoidable propagation method (Jasim et al., 2009; Al Mayahi, 2019; Al Mayahi et al.,2010, 2020). Plant tissue culture success as a plant propagation method is greatly influenced by the composition of the growth medium (Ibrahim et al., 2013; Shareef et al., 2016; Al-Mayahi et al., 2018). The researchers were interested in using chemical compounds to stimulate the generation of plant cells, among those used compounds (Thidiazuron TDZ) and (Phloroglucinol PG 1,3,5 tryhydroxybenzene). The TDZ gained great attention because it is one of the most efficient and effective synthetic cytokinins in tissue culture for woody plants (Huettemam and Prcecc,1993). TDZ has been widely used to stimulate and propagate the shoots in many plant species, including the date palm (Al-Mayahi, 2014). As for PG, adding it to the medium caused an increase in the rate of induction of shoots (Kumar et al., 2005), As well as the inclusion of the medium with the compound PG encouraged induction and production of shoots and their multiplication (Buthuc-keul and Deliu, 2001).

The aim of this study was to find the effects of TDZ and PG supplementation on the growth and multiplication of in vitro date palm buds and analysis of some physicochemical parameters.

Materials and Methods

The present study was achieved in The Tissue Culture Laboratory of Date Palm Research Centre, Basra University, Basrah, Iraq. During 2017 and 2018. Induced callus from the apical buds was separated, and for its propagation, it was cultured on MS medium (Murashige and Skoog, 1962), with additional 100 mg l-1 glutamine, 5 mg l-1 thiamine HCl, 1 mg mg l-1 biotin, 30 g l-1 sucrose, and solidified with agar at 7.0 g l-1 and 0.5 gl-1 activated charcoal, with the addition of growth regulators Naphthalene Acetic Acid (NAA) at 6 mg l-1 and 6-(dimethylallyl amino) purine (2iP) at 2 mg l-1. To study the effects of Phloroglucin (PG) and Thiaduzuron (TDZ) on callus growth, supplementation of these compounds at different concentrations in the growth medium was assessed. MS medium was modified at four concentrations of HA (0, 25, 50, and 100) mg l-1 or TDZ (0.0 , 0.5 and 1.0) mg l-1. The pH of the medium was adjusted to 5.7-5.8 before the addition of agar. Media dispensed into culture containers. All culture containers with media were autoclaved at 121°C and 1.04 kg cm-2 for 20 min. The cultures were kept in the culture room at 27 ± 2°C with 16 h light and eight-hour dark provided by white by florescent light. The weight of the callus was recorded after six weeks from the callus culture application.

For differentiation and multiplication, callus bloomed on growth media were divided and subculture on differentiation and multiplication media supplemented as mentioned above, except for the plant growth regulators 1 mg l-1 (NAA), 0.5 mg l-1 (BA) and 0.5 mg l-1 kinetin (K), (Al-Mayahi, 2021). It was also supplemented with the same concentrations of PG and TDZ to study their effects on the multiplication of buds and some changes in phytochemicals properties. Cultures were incubated at 27 ± 2 °C and irradiated for 16 h with a diffuse light provided by daylight fluorescent lamps. The results of the experiments regarding the percentage of bud

regeneration and bud number per jar were recorded after 12 weeks after the inoculation of callus on the media.

Macronutrients content in in vitro grown shoots

Content of total nitrogen, phosphorus and potassium in shoots was analyzed according to the method described by Cresser and Parsons (1979).0.2 g of shoot samples (as dry weight) were taken into a caldal flask with a capacity of 100 cm3 and digested with a mixture of sulfuric acid (69 %) and perchloric acid (62 %) under heating for one hour, subsequently, the digested solution was transferred into volumetric flask 50 cm3, and volumes were completed in size with distilled water. Chemical analyses were performed using the following methods: total N was measured— by the distillation method according to Kjeldahl (Page et al. 1982), Phosphorus was measured by spectrophotometer at 880 nm, according to Murphy and Riley (1962). K, Ca, Mg, and Na were determined by atomic absorption spectrometry, according to the method described by Black (1968).

Hormones Analysis

Extraction and measurement of auxins: Auxins were extracted and quantified according to the method of Nagar and Sood, (2006). Five grams of buds tissue after various treatments with Phloroglucin (PG) and Thiaduzuron (TDZ) and their combinations were homogenized using 80% methanol. The extract was filtered through the Whatman filter paper (no.1) and evaporated under vacuum under dark conditions at 4 oC. The supernatant has been dried in vacuum, removed by a buffer of 0.1 M potassium phosphate (pH 8.1). Eleuate was obtained by using 1 N Hydrochloric Acid (HCI) and by using a partitioning (4 x) with diethyl ether, in dryness, in water with a pH set to 2.5. The injection in reversed HPLC, C18 column, in the isocratic elution mode by the concentrate determined phytohormones using a portable acetone step (26:74) with 30 mM of phosphoric acids. A UV detector (2996 PDA detector) with 280 nm was passed through the column eluants and auxins were characterized and quantified. Standard auxins were used as reference (IAA).

Measurement of chlorophyll content

The concentration of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl (a + b), was estimated according to the method described by (Lichtenthaler, 1987). The readings recorded with a spectrophotometer at wavelengths of 663 and 644 nm for Chl a, and Chl b, respectively. Then we applied the equations shown below:

Chl a = (13.36A663) - (5.19A644).

Chl b = 27.49A644 - 8.12A663.

total chlorophyll= Chl a+Chl b.

Statistical analysis

The experiments were carried out as a factorial experiment by three-factor for each experiment using a completely randomized design(CRD). Statistical variance analysis was performed using Analysis of Variance (ANOVA) table. Using the GenStat software. Differences were compared using the least significant difference (L.S.D) at the 1% probability level.

Results

Effect of medium type, TDZ and PG on physical traits

Callus weight.

According to the results obtained in Table (1), the callus tissues grown at liquid medium showed significantly superior in callus weight (5.61) g. they were compared with the callus grown on a solid medium, which was (5.16) gm. Data (Table 1) showed there was a significant effect of TDZ on the mean callus weight. The highest mean callus weight was obtained on the media supplemented with TDZ at a concentration of 0.5 mg l^{-1}, where it reached (6.24) gm., which has significantly superior on the two concentrations (1 and 0) mg l^{-1}, while the lowest weight of callus was at the control treatment (4.74) gm.; additionally, TDZ treatment at 1 mg l^{-1} showed significant superiority compared with control treatment. Table (1) show that the optimal treatment was observed in the medium containing 50 mg l^{-1} of PG, which showed significant superiority compared with other concentrations (0 and 25) mg l^{-1} in the mean weight of callus after 8 weeks of culture, where the highest value of callus weight was recorded (6.07) gm. The lowest weight of callus was at the control treatment (4.80) gm. Data in Table (1) indicate that the treatment between TDZ and medium type had a significant effect on callus's wet weight after 8 weeks from culture. TDZ treatment at concentration 0.5 mg l^{-1} with the solid medium significantly superiority on the other treatments except for treatment 0.5 mg l^{-1} of TDZ with the liquid medium, which recorded the highest value reached (6.26) gm., while the control treatment recorded the lowest average weight of callus reached (4.53) gm. The results are given in Table (1) indicate that the PG treatment at 50 mg l^{-1} with the liquid medium significantly superior on other treatments except for the treatment 50 mg l^{-1} PG with the solid medium, which recorded the highest mean weight of callus (6.31) gm. In contrast, for the control treatment with the solid media recorded the lowest average weight of callus where it reached (4.75) gm. According to the results obtained, a combination of 0.5 mg l^{-1} of TDZ with 50 mg l^{-1} of PG application had the highest mean weight of callus (6.91) gm. Compared with other treatments after 8 weeks of culture, the control treatment recorded the lowest value reached (3.32) gm. As for the triple interaction, Table (1) indicates the superiority of the treatment 50 mg l^{-1} of PG and 0.5 mg l^{-1} of TDZ in the liquid medium, which recorded the highest value reached (6.94) gm, compared the treatments others. In contrast, the control treatment with the solid medium recorded the lowest value reached (3.11)gm., Fig (1) .

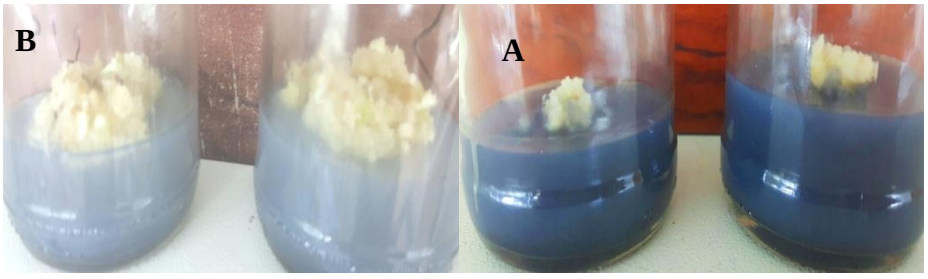

Fig (1) Callus proliferation on MS medium with (A) control treatment. (B) 50 mg l^{-1} PG with 0.5 mg l^{-1} TDZ,

Table (1) Effect of medium type TDZ, PG, and their interactions on mean wet weight of callus(g) after 8 weeks of culture

Medium type	PG (mg .l-1)	TDZ (mg .l-1)			PG and medium type
		0	0.5	1	
Liquid (temporary immersion)	0	3.53	5.34	5.69	4.85
	25	5.05	6.40	5.55	5.67
	50	6.23	6.94	5.75	6.31
solid medium	0	3.11	5.64	5.49	4.75
	25	4.46	6.27	4.01	4.91
	50	6.03	6.87	4.58	5.83
					Mean of medium
TDZ and medium	Liquid	4.94	6.23	5.66	5.61
	Solid	4.53	6.26	4.96	5.16
					Mean of PG
TDZ and PG	0	3.32	5.49	5.59	4.80
	25	4.75	6.34	4.78	5.29
	50	6.13	6.91	5.16	6.07
Mean of TDZ		4.74	6.24	5.18	
LSD 0.01					

Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.34	0.42	0.42	0.59	0.59	0.72	1.03

Number of buds

Table (2) shows the liquid medium superiority to the solid medium in the number of buds. The number of buds in the liquid medium reached (18.00) buds after 12 weeks of culture, while the number in the solid medium (16.15) buds. The same table shows there was a significant effect of TDZ on the average number of buds. The highest mean of buds number was obtained on the media supplemented with TDZ at a concentration of 0.5 mg l^{-1} where it reached (18.83) buds., which has significantly superior on the two concentrations (1 and 0) mg l^{-1}, while the lowest number of buds was at the control treatment (15.44) buds. The table also shows the superiority of treatment PG at a concentration 50 mg l^{-1}, which recorded (19.94) buds, while the lowest value was at the control treatment reached (15.33) buds after 12 weeks of culture. Table (2) show that the optimal treatment was observed in the medium containing 0.5 mg l^{-1} of TDZ with 50 mg l^{-1} of PG, which showed significant superiority compared with other treatments in the average number of buds after 12 weeks of culture, which recorded the highest value reached (22.17) buds. In contrast, the control treatment recorded the lowest value reached (13.50) buds.

Data in Table (2) indicate that the treatment interaction between TDZ and medium type had a significant effect on the average number of buds after 12 weeks of culture. TDZ treatment at concentration 0.5 mg l^{-1} with the liquid medium showed significant superiority on the other treatments except for treatment interaction between 1.0 mg l^{-1} of TDZ with the liquid medium, which recorded the highest value reached (19.78) buds. The control treatment in the solid medium recorded the lowest average (15.11) buds.

The same table also indicates the PG treatment in the liquid medium at 50 mg l^{-1} has significantly affected compared with other treatments in the average number of buds except for the 50 mg l^{-1} PG in the solid medium, where its highest value was recorded (20.44) buds, while the lowest value was at the control treatment in the solid medium, which reached (14.33) buds.
As for the triple interaction, Table (2) indicates the significant superiority of the treatment 50 mg l^{-1} of PG and 0.5 mg l^{-1} of TDZ in the liquid medium in the mean number of buds compared the other treatments except for 50 mg l^{-1} of PG and 0.5 mg l^{-1} TDZ in the solid medium, which recorded the highest value reached (23.00) buds after 12 weeks of culture, while the control treatment with the solid medium recorded the lowest number reached (12.66) buds. Fig (1).

Table (2) effect of medium type, TDZ , PG and their interactions on the average number of buds.

Medium type	PG (mg .l^{-1})	TDZ (mg .l^{-1})			PG and medium type
		0	0.5	1	
Liquid (temporary immersion)	0	14.33	18.00	16.67	16.33
	25	14.33	18.33	19.00	17.22
	50	18.67	23.00	19.67	20.44
solid medium	0	12.67	16.67	13.67	14.33
	25	14.67	15.67	13.67	14.67
	50	18.00	21.33	19.00	19.44
					Mean of medium
TDZ and medium	Liquid	15.78	19.78	18.44	18.00
	solid	15.11	17.89	15.44	16.15
					Mean of PG
TDZ and PG	0	13.50	17.33	15.17	15.33
	25	14.50	17.00	16.33	15.94
	50	18.33	22.17	19.33	19.94
Mean of TDZ		15.44	18.83	16.94	

LSD 0.01						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
1.03	1.27	1.27	1.80	1.80	2.20	3.11

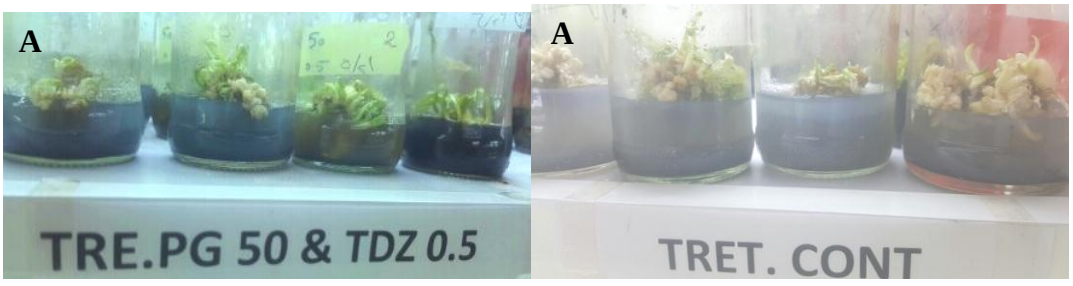

Fig (2) Bud regeneration on MS medium with (A)control treatment (B) 50 mg.l^{-1} PG with 0.5 mg.l^{-1} TDZ,

Number of shoots

The results in Table (3) indicate the liquid medium superiority over the solid medium in the average number of shoots. The number of shoots in the liquid medium reached (36.61) shoots. In contrast, the solid medium gave the lowest rate in the number of shoots (33.56) shoots after 12 weeks of culture. The same table also shows a significant effect of TDZ on the average number of shoots. The highest mean of shoots number was obtained on the media supplemented with TDZ at a concentration of 0.5 mg l^{-1} where it reached (37.39) shoots., which has significantly superior to control treatment, while the lowest number of shoots was at the control treatment (32.58) shoots. The cultures grown at PG (50 mg l^{-1}) showed better results in a number of shoots (36.00) shoots compared with the cultures grown at PG (0 "control treatment" and 1 mg l^{-1}), which were 34.42, 34.83, respectively. However, the differences between 50 mg l–1 PG and the other two concentrations (1 and 0) mg l^{-1} are not significant. Table (3) show that the optimal treatment was observed in the medium containing 0.5 mg l^{-1} of TDZ with 50 mg l^{-1} of PG, which showed significant superiority compared to the interaction treatments (0 mg l^{-1} of PG with 0, 0.5 mg l^{-1} of TDZ, and 25 mg l^{-1} PG with 0 mg l^{-1} TDZ) in the average number of shoots after 12 weeks of culture, which recorded the highest value reached (38.83) shoots. In contrast, the control treatment recorded the lowest value reached (29.92) shoots. Table (3) shows the significant superiority of the treatment of 0.5 mg l^{-1} TDZ with the liquid medium in the mean number of shoots compared to the interaction treatments (0 mg l^{-1} TDZ with the solid and liquid media, 1 mg l^{-1} TDZ with the solid medium) reached (38.78) shoots, after 12 weeks of culture, while the lowest average number of shoots was recorded at the control treatment in the solid medium reached (30.33) shoots. Also, the table shows the PG treatment in the liquid medium at 50 mg l^{-1} was the highest value for the number of shoots (37.66) shoots. In contrast, the lowest average number of shoots was recorded at the control treatment in the solid medium and reached (32.67) shoots, Fig (3) . As for the triple interaction, the treatment of 50 mg l^{-1} PG and 0.5 mg.l-^{1} TDZ in the liquid medium was superior significantly on the by giving it the highest number of vegetative shoots reached (41.00) shoots after 12 weeks of cultivation, while the lowest number of shoots was recorded at the control treatment with the solid medium of (26.33) Fig (1) **.**

Fig (3) Shoots regeneration on MS medium with (A)control treatment (B) 50 mg.l^{-1} PG with 0.5 mg.l^{-1} TDZ

Table (3) The effect of medium type, TDZ, PG, and their interactions on the average number of shoots.

Medium type	PG (mg.l^{-1})	TDZ (mg .l^{-1})			PG and medium type	
		0	0.5	1		
Liquid (temporary immersion)	0	33.50	39.00	36.00	36.17	
	25	35.33	36.33	36.33	36.00	
	50	35.67	41.00	36.33	37.67	
solid medium	0	26.33	37.00	34.67	32.67	
	25	32.00	34.33	34.67	33.67	
	50	32.67	36.67	33.67	34.33	
					Mean of medium	
TDZ and medium	Liquid	34.83	38.78	36.22	36.61	
	Solid	30.33	36.00	34.33	33.56	
					Mean of PG	
TDZ and PG	0	29.92	33.00	35.33	34.42	
	25	33.67	35.33	35.50	34.83	
	50	34.17	38.83	35.00	36.00	
Mean of TDZ		32.58	37.39	35.28		
LSD 0.01						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
2.12	2.60	2.60	3.68	3.68	4.51	6.37

Fig (4) Multiple shoot regeneration of date palm cv. Barhee in the container of liquid (temporary immersion) at 1 mgl^{-1} TDZ.

Nitrogen content in *in vitro* grown vegetative growths.

It is noticed from the results in Table (5) that the liquid medium was significantly superior to the solid medium in the content of the vegetative growth of the nitrogen component, as the percentage of nitrogen in the liquid medium reached (0.75)%, while in the solid medium is

reached (0.69)%. It was also found that there was a significant effect of TDZ, as it was observed that the treatment was significantly superior to the control treatment by 0.5 mg l^{-1} TDZ by registering the highest percentage of nitrogen amounting to (0.76)%, while the lowest percentage was in the control treatment, which amounted to (0.66)%. Also, PG had a significant effect, as Table (5) showed that the treatment was superior to 50 mg l^{-1} PG significantly on the two concentrations (0 and 25) mg l^{-1} PG by giving it the highest percentage, which was (0.78)%. In contrast, the lowest percentage was recorded when the standard treatment reached (0.66)%. As for the interactions between the medium and PG, the table above explained that there is a significant effect on the content of vegetative growths of nitrogen, as the treatment surpassed 50 mg l^{-1} PG with the liquid medium, with a significant difference over the rest of the treatments by giving it the highest ratio of (0.80)%, while it was The lowest percentage recorded is when the standard treatment with the solid medium was (0.63)%. Simultaneously, the superiority was not significant for the treatment 50 mg l^{-1} PG with the liquid medium on 25 mg l^{-1} PG with the liquid medium; and the 50 mg l^{-1} PG in solid medium. As for the bilateral interaction between the medium and the TDZ, the treatment of 0.5 mg l^{-1} TDZ with the liquid medium superiority all treatments, recording the highest rate of nitrogen reaching (0.78)%, while the lowest percentage of nitrogen was recorded at the control treatment with the solid medium was (0.63)%.

As for the interactions between the two compounds TDZ and PG, the same table showed that the treatment 0.5 mg l^{-1} TDZ with 50 mg l^{-1} PG was superior to other treatments, by giving it the highest percentage of nitrogen as it reached (0.84)%, while the lowest percentage was at the treatment was (0) PG with 1 TDZ) mg l^{-1}, which recorded (0.64)%. While Table (5) showed the effect of triple interactions, where the two interaction treatments (1 mg l^{-1} TDZ with 50 mg l^{-1} PG with the liquid medium) and the treatment (0.5 mg l^{-1} TDZ with 50 mg l^{-1} PG with the solid medium) showed a significant superiority compared to the other interactions, they recorded the highest percentage of nitrogen, which was (0.86)%. In comparison, the lowest percentage was recorded for nitrogen at the two interaction treatments (0 mg l^{-1} TDZ with 25 mg l^{-1} PG) in the solid medium and the treatment (0 mg l^{-1} TDZ with). 50 mg l^{-1} PG) in the solid medium, which reached (0.62)%.

The phosphorous content in *in vitro* grown vegetative growths(%).

It is noted from the results in Table (6) the superiority of the liquid medium over the solid medium in the content of the vegetative growth of phosphorus, as the percentage of phosphorus in the liquid medium was (0.43)%. In comparison, in the solid medium, the percentage of phosphorus was (0.41)%. The same table also showed the effect of TDZ on vegetative growth content of phosphorus where it noted that the treatment 0.5 TDZ was superior on the two concentrations (0 and 1) mg l^{-1} TDZ by giving it the highest percentage of phosphorus (0.43)%, while the lowest percentage was at the control treatment reached (0.40). The table also shows the significant superiority of treatment PG at a concentration of 50 mg l^{-1}, on the control treatment, which recorded the highest percentage of phosphorous(0.44)%, while the lowest percentage was at the control treatment reached (0.40)%. As for the interaction between the medium and PG, the same table showed that the treatment 50 mg l^{-1} PG with the liquid medium was superior to other treatments, by giving it the highest percentage of phosphorous (0.44)%, while the lowest

percentage of phosphorous was at the control treatment in the solid medium (0.39)%. Also, the interaction between the medium and TDZ had an insignificant effect on the content of phosphorus's vegetative growth, as the treatment recorded 0.5 mg l^{-1} TDZ with the liquid medium. The highest percentage of phosphorus was (0.43)%. The lowest percentage was recorded at the control treatment in the solid medium was (0.39)%. Table (6) show that the optimal treatment was observed in the medium containing 0.5 mg l^{-1} of TDZ with 50 mg l^{-1} of PG, which showed significant superiority on the control treatment in the phosphorus content, which recorded the highest value reached (0.45)%. In contrast, the control treatment recorded the lowest value reached (0.38)%.

Table (5) The effect of medium type, TDZ, PG and their interactions on the vegetative growths content of nitrogen (%)

Medium type	PG (mg .l^{-1})	TDZ (mg .l^{-1})			PG and medium type
		0	0.5	1	
Liquid (temporary immersion)	0	0.690	0.707	0.687	0.694
	25	0.660	0.820	0.767	0.749
	50	0.733	0.823	0.867	0.808
solid medium	0	0.663	0.630	0.607	0.633
	25	0.627	0.770	0.663	0.687
	50	0.623	0.863	0.773	0.75
					Mean of medium
Effect TDZ and medium	Liquid	0.694	0.783	0.773	0.750
	solid	0.638	0.754	0.681	0.691
					Mean of PG
TDZ and PG	0	0.677	0.668	0.647	0.664
	25	0.643	0.795	0.715	0.718
	50	0.678	0.843	0.820	0.781
Mean of TDZ		0.666	0.769	0.727	

LSD 0.01						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.0524	0.0642	0.0642	0.0907	0.0907	0.1111	0.1572

Table (6) indicates the effect of triple interaction on the vegetative growth content of phosphorus. The treatment of 0.5 mg l^{-1} TDZ with 50 mg l^{-1} PG with the liquid medium was superior with a

non-significant difference from all treatments, including the control treatment in both liquid and solid mediums. It reached (0.45)%, while the lowest percentage was recorded at the control treatment with the solid medium, which was (0.37)%.

Table (6) The effect of medium type, TDZ, PG and their interactions on the vegetative growths content of phosphor (%)

Medium type	PG (mg .l^{-1})	TDZ (mg .l^{-1})			PG and medium type
		0	0.5	1	
Liquid (temporary immersion)	0	0.3867	0.4267	0.4133	0.4089
	25	0.4167	0.4333	0.4533	0.4344
	50	0.4500	0.4567	0.4333	0.4467
solid medium	0	0.3733	0.4067	0.4167	0.3989
	25	0.3867	0.4400	0.4367	0.4211
	50	0.4300	0.4467	0.4367	0.4378
					Mean of medium
TDZ and medium	Liquid	0.4178	0.4389	0.4333	0.4300
	solid	0.3967	0.4311	0.4300	0.4193
					Mean of PG
TDZ and PG	0	0.3800	0.4167	0.4150	0.4039
	25	0.4017	0.4367	0.4450	0.4278
	50	0.4400	0.4517	0.4350	0.4422
Mean of TDZ		0.4072	0.4350	0.4317	

LSD 0.01						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.02871	0.0351	0.0351	0.0497	0.0497	0.0609	0.0861

Potassium content in *in vitro* grown vegetative growths.

It is noticed from the results in Table (7) that the liquid medium was significantly superior to the solid medium in the vegetable growth content of the potassium, as the percentage of potassium in the liquid medium reached (0.77)%, while in the solid medium is reached (0.71)%. The same table also showed the effect of TDZ on the content of vegetative growths of potassium, were observed that the treatment TDZ at 0.5 mg l^{-1} was significantly superior to the two concentrations (0 and 1) mg l^{-1} TDZ, with the highest percentage of potassium (0.78)%, while the lowest percentage was at the control treatment, which reached (0.71)%. As for the effect of PG, the same table showed that the treatment 50 mg l^{-1} PG was significantly superior to the two concentrations (0 and 25) mg l^{-1} PG by giving it the highest percentage of potassium (0.79)%. Simultaneously, the lowest was recorded percentage at the control treatment, reaching (0.71)%. Table (7) showed the interaction between the medium and PG, as the treatment 50 mg l^{-1} PG with the liquid medium significantly superior to other treatments, as it gave the highest percentage of (0.82)%,

while the lowest percentage of potassium was when the control treatment in the solid medium was reached (0.68)%. The bilateral interaction between the medium and TDZ showed a significant effect on the content of potassium vegetative growths, as the treatment at 0.5 mg l^{-1} TDZ with the liquid medium significantly outperformed all treatments, which amounted to (0.82)%. The lowest percentage was recorded when the control treatment in the solid medium was (as for the bilateral interaction between TDZ and PG, it is evident from the same table that the treatment 0.5 mg l^{-1} TDZ with 50 mg l^{-1} PG was superior to all treatments, where the highest percentage of potassium was recorded at (0.86)%, while the lowest percentage was at the control treatment, which amounted to (0.68)%. At the same time, Table (7) showed the effect of triple interactions on the content of potassium in vegetative growths, where the treatment superior 0.5 mg l^{-1} TDZ with 50 mg l^{-1} PG with liquid medium significantly over the control treatment, by giving it the highest percentage of (0.90)%, while the lowest percentage recorded when the control treatment was in the solid medium, which was (0.65)%.

Table (7) The effect of medium type, TDZ and PG and their interactions on the vegetative growths content of Potassium (%)

Medium type	PG (mg .l^{-1})	TDZ (mg .l^{-1})			PG and medium type	
		0	0.5	1		
Liquid (temporary immersion)	0	0.7133	0.7833	0.7367	0.7444	
	25	0.7500	0.7800	0.7300	0.7533	
	50	0.7667	0.9033	0.7967	0.8222	
solid medium	0	0.6500	0.7233	0.6800	0.6844	
	25	0.6733	0.7200	0.6933	0.6956	
	50	0.7133	0.8267	0.7600	0.7667	
					Mean of medium	
TDZ and medium	Liquid	0.7433	0.8222	0.7544	0.7733	
	solid	0.6789	0.7567	0.7111	0.7156	
					Mean of PG	
TDZ and PG	0	0.6817	0.7533	0.7083	0.7144	
	25	0.7117	0.7500	0.7117	0.7244	
	50	0.7400	0.8650	0.7783	0.7944	
Mean of TDZ		0.7111	0.7894	0.7328		
LSD 0.01						
Medium	PG	TDZ	TDZ and media	PG and media	TDZ and PG	TDZ, PG and media
0.03591	0.0439	0.0439	0.0621	0.0621	0.0761	0.1077

Indole acetic acid (IAA) content in *in vitro* grown vegetative growths It is observed from the results in Table (8) the significant superiority of the solid medium on the liquid medium in the content of the vegetative growth of auxin; it was reached (28.98) µg. Kg^{-1}, while in the liquid

medium, it reached (28.87) µg Kg^{-1} . The same table shows that the optimal treatment was observed in the medium containing 0.5 mg l^{-1} of TDZ (30.33) µg. Kg-1, which showed significant superiority in increasing the content of IAA vegetative growths compared with the two concentrations (0 and 1) mg l^{-1}, while the lowest value was at the control treatment, which reached (27.28) µg. Kg^{-1}, PG also significantly affected the vegetative growth content of auxin, where table (8) shows that the optimal treatment was observed in the medium containing 50 mg l^{-1} of PG, which showed significant superiority compared with other concentrations (0 and 25) mg l^{-1} after 8 weeks of culture, which recorded the highest value (29.96) µg. Kg-1, while the lowest value at control treatment, reached (27.90) µg. Kg-1.[1] As for the interaction between the type of medium and PG, Table (8) indicates that the PG treatment at 50 mg l^{-1} with the liquid and solid medium significantly superior to other treatments except for the treatment 25 mg l^{-1} PG with the solid medium, where they recorded the highest value (29.96) µg. Kg-1 for each of them, while the lowest value was recorded at the control treatment with both medium (27.90) µg. Kg^{-1}. The interaction between the type of medium and TDZ had a significant effect, the treatment at 0.5 mg l^{-1} TDZ with the solid medium showed a significantly superior treatment except for the treatment at 0.5 mg l^{-1} TDZ with the liquid medium, which recorded the highest value reached (30.41) µg. Kg^{-1}. While the lowest value was recorded at the control treatment with the solid medium (27.82) µg. Kg^{-1}. As for the interactions between the TDZ and PG, table 8 shows the significant superiority of the treatment TDZ at 0.5 mg l^{-1} with the 50 mg l^{-1} PG in auxins content compared to other interaction treatments recorded the highest value reached (32.34) µg. Kg^{-1} after 12 weeks of culture, while the lowest value recorded at the control treatment (26.51) µg. Kg^{-1}. As for the triple interaction, Table (8) indicates the significant superiority of the two treatments(0.5 mg l^{-1} TDZ with 50 mg l^{-1} PG with the liquid and solid media), recording the highest value (32.34) µg. Kg^{-1} compared to other interaction treatments; the lowest auxin content was recorded at the control treatment in both media (26.51) µg. Kg^{-1}.

Chlorophyll content of vegetative growths (mg. 100^{-1} fresh weight)

The results in Table (9) indicate the superiority of the liquid medium over the solid medium in the content of chlorophyll , it reached in the liquid medium (157.3), respectively, while it reached in the solid medium (140.6). Superiority was significant in total chlorophyll. The same table also shows the TDZ compounds effect on the chlorophyll content, where the treatment superiority of 0.5 mg l^{-1} significantly in the chlorophyll content which reached (156.2), while the lowest value recorded for chlorophyll at the control treatment was (141.3). The PG at 50 mg l^{-1} was a significant effect on the two concentrations (0 and 25) mg l^{-1} in the chlorophyll content, which recorded (160.9), while the lowest value was at the control treatment (143. 8).

Table 9 also show an effect of the TDZ compound with the liquid medium on the chlorophyll content, where the treatment is 0.5 mg l^{-1} exceeded on the liquid medium significantly in the chlorophyll content (a and total), which reached (106.1 and 164.0), respectively, while not noted a significant superiority in the chlorophyll b (57.9) mg $100g^{-1}$. The lowest value was recorded for chlorophyll (a, b and total) at the control treatment with solid medium (133.3). The PG at 50 mg l^{-1} with the liquid medium has significantly over the other treatment in the chlorophyll (a, b and total), the highest value was recorded (108.1, 60.9 and 169.0) respectively, while the lowest value

was recorded with the control treatment with the solid medium reaching (88.3, 46.9 and 135.3) Sequentially. The same table showed the superiority of the treatment of 0.5 mg l^{-1} TDZ with 50 mg l^{-1} PG significantly in the content of chlorophyll (a and total) over the rest of the treatments except for the treatment (1 mg l^{-1} TDZ with 50 mg l^{-1} PG), which recorded the highest value (108.4 and 171.4) respectively, while the lowest value at the treatment 0 mg l^{-1} TDZ with 25 mg l^{-1} PG, which was (80.8 and 132.4), respectively, the lowest value for chlorophyll (B) was reached at the control treatment (46.6) mg.100g^{-1}. The triple interaction we are noted in table (9) which shows that the treatment 50 mg. L-1 PG and 0.5 mg l^{-1} TDZ with the liquid medium was significantly higher over the control treatment in the solid medium by giving it the highest value for the plant tissue content of chlorophyll (183.1), while the lowest chlorophyll content was recorded when treatment 0 mg l^{-1} TDZ with 25 mg l^{-1} PG in the solid medium reaching (121.8).

Table (8) Effect of medium type, TDZ, PG and their interactions on vegetative growths content of auxin (µg. Kg^{-1})

Medium type	PG (mg .l^{-1})	TDZ (mg .l^{-1})			PG and medium type
		0	0.5	1	
Liquid (temporary immersion)	0	26.51	28.97	28.21	27.90
	25	28.63	29.42	28.19	28.74
	50	28.31	32.34	29.24	29.96
solid medium	0	26.51	28.97	28.21	27.90
	25	28.63	29.93	28.64	29.07
	50	28.31	32.34	29.24	29.96
					Mean of medium
TDZ and medium	Liquid	27.82	30.24	28.55	28.87
	solid	27.82	30.41	28.70	28.98
					Mean of PG
TDZ and PG	0	26.51	28.97	28.21	27.90
	25	28.63	29.67	28.42	28.91
	50	28.31	32.34	29.24	29.96
Mean of TDZ		27.82	30.33	28.62	

LSD 0.01						
Medium	PG	TDZ	TDZ and medium	PG and medium	TDZ and PG	TDZ,PG and medium
0.595	0.728	0.728	1.030	1.030	1.262	1.784

Table (9) The effect of medium type, TDZ, PG and their interactions on buds content of total chlorophyll a (mg.100g^{-1})

Medium type	PG (mg .l^{-1})	TDZ (mg .l^{-1})			PG and medium type
		0	0.5	1	
Liquid (temporary immersion)	0	151.5	153.6	151.6	152.2
	25	142.9	155.3	154.0	150.8
	50	153.3	183.1	170.5	169.0
solid medium	0	129.5	142.0	134.4	135.3
	25	121.8	143.3	135.7	133.6
	50	148.6	159.7	150.3	152.8
					Mean of medium
TDZ and medium	Liquid	149.2	164.0	158.7	157.3
	solid	133.3	148.3	140.1	140.6
					Mean of PG
TDZ and PG	0	140.5	147.8	143.0	143.8
	25	132.4	149.3	144.8	142.2
	50	150.9	171.4	160.4	160.9
Mean of TDZ		141.3	156.2	149.4	

LSD 0.01						
Medium	PG	TDZ	TDZ and media	PG and media	TDZ and PG	TDZ, PG and media
6.20	7.60	7.60	10.74	10.74	13.16	18.61

Discussion

It is clear from Tables (1, 2, 3, and 4) that TDZ treatment has a significant effect on the studied physical characteristics, including the weight of callus and buds, the shoots' weight, the number of buds and number of shoots formed. The reason may be that TDZ is one of the phenylurea Substituted compounds that It is known at present with the activity of cytokinins, which are characterized by their high efficacy, superior to most other types of cytokines, including Zeitin, The natural product in the plants (Huetteman and Preece, 1993). The use of TDZ led to the formation of new callus cells and achieved the highest rate of cell proliferation compared to other growth regulators, The stimulation of callus induction at low levels is an indication of endogenous vital activity, as Murthy et al. (1998) mentioned. The stimulation of the development of callus from the axillary buds is due to the effect of TDZ at a very high rate. The use of TDZ has stimulated at low concentrations (0.1-10) Mmol, lateral shooting, and formation of transverse buds and direct or indirect shoots grown *in vitro* (Guo et al., 2011). The positive effect of TDZ on date palm growth was in a good agreement with studies which revealed that the TDZ at the appropriate concentrations enhanced the plant growth and development of many plants (Zambre et al., 2001; Casanova et al., 2004; Nhut et al., 2006; Jheng et al., 2006; Jones et

al., 2007) As indicated by Naif (2019), the medium provided with cytokinin was superior to the control treatment in the weight of callus. The number of buds, as well as between cultures with Bioreactor system, which superior on solid medium, the reason may be attributed to the benefit of the nutrient medium being limited in the solid system, as the part in contact with the medium is the one that benefits from the components of the nutrient medium without the other parts of the tissue. The immersion of a part tissue in a solid medium leads to a decrease in gas exchange, which affects the growth and development of the cultivated plant tissue. The superiority in the bioreactor may be due to the abundance of nutrients and their increased readiness, which causes ease of absorption in the moving liquid medium compared to the solid medium, and the movement of the parts implanted in the liquid medium works to exchange gases and overcome the phenomenon of deficiency of some elements that may appear on the tissue parts as a result of their assembly. (Ducos et al., 2003). As for cultivation in solid media, it impeded the absorption of nutrients by the implanted tissue, which negatively affected the growth and multiplication of crops compared to the liquid medium (Al Khateeb and Alturki, 2014). The emergence of adventitious buds from tissues and organs cultivated in-vitro has been observed in many plants; that the emergence of adventitious buds is from cells exposed to the nutrient medium that is in contact with it, as these cells lose their differentiation and return to the meristematic state and then re-differentiate them by the effect of the composition of the medium and conditions surrounding environment into meristematic regions that grow and develop into shoots with the same phenotypic composition as the buds found in the axils of leaves (Torrey, 1967). These results are consistent with what was found by Huetteman and Preece (1993), and Husain et al. (2007). PG reduces the peroxidase activity within the cultivated plant part and thus protects endogenous auxin from oxidative stress (Kumar et al., 2005; Rustaei et al., 2009; Wang et al., 2011; Salekjalali, 2012; Jani et al., 2015, Londe, et al., 2017), Fig 4 . It was also noted from the above tables that the liquid medium was superior in the temporary immersion technique in the content of the plant tissue of auxins. The vegetable part of auxin was higher in the bioreactor compared to the solid medium. There is much evidence proving that cytokinins activate the synthesis of proteins and plant pigments and differentiate membranes in chloroplasts (Kulaeva; etal. 2002). Studies also indicate that treatment with cytokinins contributed to prolonging the life of plastids (Reski et al., 1986), the cytokinins react with a light then stimulating gene expression and increased formation of plastid proteins (Reski et al., 1991). The treatment with cytokinin increases the DNA of chloroplasts, which increases protoplast construction, maintains the level of pigments and changes the permeability of the membranes, as well as stimulates the replication of plastids and the formation of grana membrane (Van Staden et al., 1988). High chlorophyll retention efficacy, especially in high concentrations (Sabovljevi et al., 2010). Table (8) illustrate the effect of TDZ on the content of developing tissues from plant auxin (IAA), as it increased the content of plant tissue from auxin, due to the presence of direct effects of TDZ on endogenous auxin. Its direct effect on apical meristems during the initial developments in tissue culture, as the addition of 1.8 µmol of TDZ had a direct effect on auxins compared to plants grown with TDZ-free medium (Ferreira et al., 2006). Nayef (2019) also showed that adding cytokinins to the

nutritional environment increased the vegetable tissue content of auxins, cytokinins, Gibberellins, and decreased abscisic acid content.

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تأثير نوع الوسط و TDZ و PG وتداخلاتهم على التوالد المختبري لنخيل التمر (*Phoenix dactylifera* L.) صنف البرحي.

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الخلاصة

الإكثار الدقيق لنخيل التمر لا يزال يواجه العديد من المشاكل ، مثل: بطئ نمو الكالس وانخفاض قدرته على تكوين الأعضاء، لذا تمت دراسة تأثير Phloroglucinol (PG) بالتراكيز (0 ،25 ، 50 و 100) ملغم لتر$^{-1}$ و Thidiazuron (TDZ) بالتراكيز (0 ، 0.5، 1) ملغم لتر$^{-1}$ في تحفيز الإكثار الدقيق وتضاعف الأفرع لنخيل التمر *Phoenix dactylifera* L. ، صنف البرحي. وقد اظهرت نتائج الدراسة ان الوسط السائل المضاف اليه 50 ملغم لتر$^{-1}$ PG مع 0.5 ملغم لتر$^{-1}$ TDZأعطى أعلى معدل لوزن للكالس بلغ (6.94 غم)، كما تفوقت هذه المعاملة معنويا على باقي المعاملات من حيث كفاءتها في الحصول على أعلى معدل لعدد البراعم والافرع الخضرية (23.0 و 41.0) لكل منها على الترتيب. كما بينت الدراسة تفوق الوسط السائل المضاف إليه 1.0 ملغم لتر $^{-1}$ TDZ مع 50 ملجم لتر $^{-1}$ PG في الحصول على أعلى محتوى للأفرع من عنصر النيتروجين (0.867٪) ، بينما سجلت الافرع الخضرية المزروعة في وسط سائل مكمل بـ 0.5 ملغم لتر $^{-1}$ TDZ و 50 ملغم لتر $^{-1}$ PG أعلى محتوى من عنصري الفوسفور والبوتاسيوم بلغ (0.4567٪ و 0.9033٪) لكل منهما على الترتيب. علاوة على ذلك ، وجد أن الاستخدام المشترك بين 50 ملغم.لتر$^{-1}$ PG و 0.5 ملغم لتر$^{-1}$ TDZ في الوسط السائل اعطى أعلى محتوى للأفرع من مستوى الاوكسين IAA (3.644 ميكروغرام .كغم$^{-1}$) مقارنة بالمعاملات الأخرى. كما أظهرت البيانات أن الحد الاعلى لمحتوى الأفرع من الكلوروفيل سجل في الوسط السائل المزود ب 50 ملغم لتر$^{-1}$ PG و 0.5 ملغم لتر$^{-1}$ TDZ .

الكلمات المفتاحية: استحثاث الكالس ، تضاعف الأفرع ، العناصر الكبرى، الاندول استك اسد IAA.