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IN-VITRO PROPAGATION OF DATE PALM
(Phoenix dactylifera L.)

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Plantlets have been obtained from offshoot apex callus of "Khalas" date palm. Callus was produced from culture explant on RM medium having NAA and cytokinin. The combinations of NAA 1.5 mg/l and 0.1-1.0 mg/l of cytokinin proved to be the best hormonal treatments for callus subculture, NAA and 2,4-D were applied with cytokinin. 2,4-D did not form any root in the medium whereas NAA did form root and shoot.

INTRODUCTION

The major commercial use of tissue culture is the rapid propagation of selected plants with desirable characteristics. Date palm is vegetatively propagated by the offshoots which arise at the base of the petiole, and are adventitious in origin. This production is relatively low to a certain period in the palm life span. The development of faster propagation method is particulary important for the date palm growers. Propagation of plants by tissue culture has been successfully employed in many plant species especially where monoclone populations are desired (Murashige, 1974).

Tissue culture technique was tried as a possible method of propagation because of the universal interest in rapid propagation of palm species. Several investigators have employed the technique of tissue culture to generate the plantlets from date plam tissues (Reuveni,1979; Khalil, *et al.*, 1982 and Tisserat, 1979a, 1979b and 1981).

This investigation is concerned with the application of tissue culture technique to the vegetative propagation of date palms.

MATERIALS AND METHODS

Well known data palm variety named "Khalas" grown in Al-Hassa was selected for the propagation of tissue culture technique. The clonal material.

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was obtained from offshoots of palm trees grown at an Al-Hassa private farm near King Faisal University, Saudi Arabia. Offshoots were dissected and viable lateral buds were excised from the leaf axils. Buds (8 x 10 mm) with preformed leaves were disinfected in 10% sodium hypochlorite solution for 10 minutes and rinsed three times with sterile water. Primordial leaves were removed very carefully before culturing in the nutrient medium.

Nutrient medium consisted of macro and micro elements plus3% sucrose, 0.8% agar, 100 mg/l myoinositol and 0.4 mg/l thiamine hydrochloride (Reinert and Mohr, 1967). Hormones added to the basal medium included, Naphthalene acetic acid (NAA),2,4-dichlorophenoxy acetic acid (2,4-D) and kinetin (6-furfuryl aminopurine). 0.3% neutralized activated charcoal was used for the oxidation and to avoid the browning effect of callus. The pH of the medium was adjusted to 5.7 prior to the addition of agar. The medium was filled in tubes (25x150mm) and flask capped with non-absorbing cotton and autoclaved for 15 minutes at 120°C. To secure aseptical conditions, explant tissues were cultured in a Laminar Flow Cabinet. Cultures were incubated at 28°C in a controlled cabinet. Callus was initiated from explants after 2 months in culture. Callus was sub-cultured in the fresh medium devoid of hormones.

RESULTS

Callus Formation : Callus was initiated from bud explant apical tissue on a medium containing NAA and cytokinin. Moderate levels of NAA (1.0-5.0 mg/l) with low levels of cytokinin (0.1-1.0 mg/l) were found to be the good combinations for the callus formation (Table 1). This callus was characterized as friable, yellow white in colour and was found to contain numerous modular bodies. There were pro-embryonic bodies which upon subculture in the medium devoid of hormones, formed the roots.

Root and Shoot Initiation : Rooting and shooting efficiency of date palm was improved with combinations of NAA and kinetin. 2,4-D did not give any root with any of the combinations. However, there were some shoots initiated from callus. NAA at 1 or 5 mg/l with 0.5 and 1 mg/l of kinetin yielded 60-70% rooted plantlets (Table 2). With 2,4-D, 1.0 mg/l treatment plus 0.1 mg/l kinetin a small number of roots in a few tubes were formed.

Table 1. *Callus produced from each combination of 10 tubes*

		NAA			Means
	mg/l	1.0 mg/l	3.0 mg/l	5.0 mg/l	
Cytokinin	0.1	7	6	4	5.66
	0.5	7	5	3	5.00
	1.0	6	5	2	4.33
Means		6.66	5.33	3.00	

Table 2. *The effect of NAA and 2,4-D with cytokinin on rooting of date palm (rooting percentage out of 10 tubes).*

Kinetin (6-furfuryl aminopurine)	Naphthalene acetic acid (NAA)				2,4-dichlorophenoxy acetic acid (2,4-D)			
mg/l→	1.0	5.0	10.0	20.0	1.0	5.0	10.0	20.0
0.1	70	60	60	30	20.0	—	—	—
0.5	60	60	40	30	10.0	10.0	—	—
1.0	40	50	30	—	10.0	—	—	—
1.5	20	20	30	—	20.0	—	—	—

DISCUSSION

Highly viable callus was found in date palm tissue. Several plantlets have been initiated from the clonal explants of "Khalas" variety. Tisserat (1981) reported that callus was initiated when 2,4-D was used whereas in this study, NAA proved to be the best auxin for the callus formation in date palm. Khalil *et al.* (1982) found the best results of callus formation when they used NAA with different levels of cytokinin. They advised to avoid the use of growth regulators as far as possible. Beauchesne (1982) also used the lowest concentration of 2,4-D in his experiments.

It was observed that callus formation induced due to the combination of NAA and cytokinin was superior in growth rate. This callus continued proliferating and initiated roots when subcultured in other nutrient media. However, the root differentiation was very poor in 2,4-D when used in combinations with cytokinin. Some plantlets had shoots but no roots. Rooted plantlets were transplanted to a mixture containing equal parts of peat and sand but failed to

survive in the green house. Tisserat (1981) reported that plantlets survived when they were carefully removed from nutrient medium and kept under high humidity conditions until acclimated to free living conditions.

Further studies on root and shoot development, nutritional requirements and potting soil for transplants of date palm are going on in Tissue Culture Laboratory at King Faisal University, Al-Hassa, Saudi Arabia.

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