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Influence of Different Media on *In Vitro* Roots and Leaves of Date Palm Somatic Embryos Cvs. Kapkap and Tharlaj

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Abstract: *In-vitro* rooting procedure for micropropagation of date palm c.v. Kapkap and Tharlaj was developed. The cv Kapkap highly responds to medium (A) which consist of full MS and 0.1 mgl^{-1}NAA without charcoal, which gave a mean of 5.5 roots per embryo, while cv Tharlaj inoculated on the same medium gave a mean of one root per embryo. The other types of medium B,C and D didn't exert any significant influence in root numbers for both cultivars, which gave a mean of one root per embryo. The type of medium apparently affected the embryos root length of both cultivars. Medium C which consist of full MS and $3gl^{-1}$ charcoal without growth regulators, gave a root length of mean 8.2cm per embryo, superior over other types of medium A,C and D which didn't apply any significant influence in root length for both cultivars.

Key words: Date palm micropropagation · *In-vitro* rooting · Cytokinens · Full MS and Charcoal

INTRODUCTION

Tissue culture is a technique mainly used for rapid propagation of several perennial fruit trees including date palm. Date palm is propagated *in vitro* by two methods; the first is by embryogenesis in which vegetative embryos are formed from embryogenic callus. The second is through organogenesis which produces date palm buds that eventually give plantlets without passing through the callus stage [1].

Date palm plantlets from callus have poor root system due to the lack of adventitious roots [2], the establishment of an effectual root system on *in vitro* is vital for subsequent success throughout acclimation to autotrophic condition, In date palm however, embryos cultured on a hormone-free medium often produce shoots only and require another step for rooting and shoot elongation, usually on a medium enriched with NAA [3]. In several plant species, rooting remains one of the most critical stages in the micropropagation technique, nevertheless, the success of *in vitro* methods in plant propagation depends not only on the number of plantlets produced but also on their survival rate upon transfer to nursery and field conditions. Several factors such as concentration of rooting media, auxin type and concentration affect *in-vitro* rooting stage.

The maturation of somatic embryos, germination, *In-vitro* rooting and plant establishment can be influenced by various *in-vitro* factors, such as sugar and auxin concentrations [3,4] the root number as well as root length in date palm differently responded to auxin treatments and their combinations with activated charcoal [5].

Root initiation is a critical stage in date palm micropropagation, as it governs the subsequent success of production of free living date palm plants. The concentration of inorganic salts plays an important role in root induction Ibrahim [6] who reported that reduction of MS salts strength to ¾ of the original concentration stimulated root formation in date palm tissue culture.

This study was carried out to determine optimum medium composition for the rooting of date palm somatic embryos to reduce the culture time, shoot development and rooting.

MATERIALS AND METHODS

Somatic embryos of date palm cv. Kapkap and Tharlaj (Fig. 5) were selected from Murashige and Skoog (MS) hormone-free medium and inoculated vertically on 4 type of MS rooting media (Table 1) dispensed in 60 ml capped culture tubes (15 ml per tube), after autoclaved for 20 min at 121°C and 1 × 105 Pa (1.1 kg cm–2). The cultures were incubated at 26 ± 2°C in 16-h photoperiods (50 mmol m^{-2} s^{-1}) and subcultured after six weeks to a fresh medium.

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Table 1: Composition of date palm rooting medium

	Date palm rooting medium			
Ingredients mg/l	A	B	C	D
NH_4NO_3	1650	825	1650	825
KNO_3	1900	950	1900	950
$CaCl_2 \cdot 2H_2O$	440	220	440	220
$MgSO_4 \cdot 7H_2O$	370	185	370	185
KH_2PO_4	170	85	170	85
$Na_2EDTA \cdot 2H_2O$	37.3	18.65	37.3	18.65
$FeSO_4 \cdot 7H_2O$	27.8	13.9	27.8	13.9
H_3BO_3	6.2	3.1	6.2	3.1
$MnSO_4 \cdot 4H_2O$ KI	22.3	11.15	22.3	11.15
$ZnSO_4 \cdot 7H_2O$	8.6	4.3	8.6	4.3
kI	0.83	0.415	0.83	0.415
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.125	0.25	0.125
$CuSO_4 \cdot 5H_2O$	0.025	0.0125	0.025	0.0125
$CoCl_2 \cdot 6H_2O$	0.025	0.0125	0.025	0.0125
I-Inositol	100	50	100	50
Nicotinic acid	0.5	0.25	0.5	0.25
Pyridoxine HCl	0.5	0.25	0.5	0.25
Thiamine HCl	0.1	0.05	0.1	0.05
Glycine	2	1	2	1
IAA	-	0.1	-	0.1
NAA	0.1	0.5	-	0.5
2ip	-	0.25	-	0.25
Kinetin	-	0.25	-	0.25
Charcoal	-	-	3000	0.25
Sucrose	30000	30000	30000	30000
Agar	7000	7000	7000	7000

The experiment media consisted of 1-full strength MS-medium with 0.1 mgl^{-1} NAA (A) or with 3 gl^{-1} charcoal without hormone (C), 2- half strength MS-medium with growth regulators(B) or with growth regulator and little amount of charcoal (d) and two cultivars (Kapkap and Tharlaj) in a complete randomize design.

Ten replicates (culture tube) were assigned per treatment with one embryo per tube. Data were analyzed using the Statistical Analysis System, general linear model (GLM procedure, SAS Institute Inc., 2004) and means were evaluated by LSD (least significant difference).

The response was assessed 12 weeks later, on the number, length of embryonical roots in addition to number and length of leaves.

RESULTS AND DISCUSSIONS

There is a significant interaction between the cultivars and medium type on the number of roots as shown in (Fig. 1). The cv Kapkap highly responds to

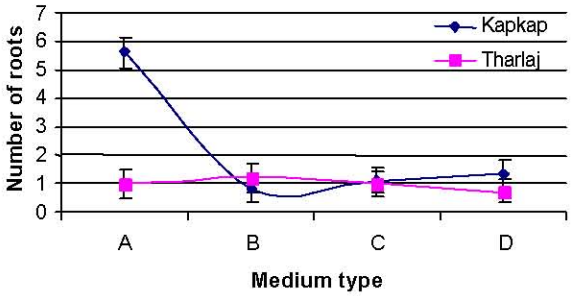

Fig. 1: Effect of medium on roots number of two date palm cultivars

medium (A) which consist of full MS and 0.1 mgl^{-1}NAA without charcoal, which gave a mean of 5.5 roots per embryo (Fig 6 A), while cv Tharlaj inoculated on the same medium gave a mean of one root per embryo (Fig. 6 B), it is genotype dependent and may due to the interaction between exogenously added plant growth regulators over the concentration of endogenous hormones, together with the involvement of sensitivity of the tissues to particular hormone groups, might help clarifying the occurrence of divergent patterns in somatic embryogenesis and in tissue culture in general [7].

The other types of medium B,C and D didn't exert any significant influence in root numbers for both cultivars, which gave a mean of one root per embryo (Fig. 6 C). Faisal *et al.* [8] on Tyophora indica reported that, the *in vitro*-regenerated shoot induced roots when transferred to full- and half strength MS medium. Half-strength growth regulator-free medium was found superior to full-strength MS medium for root development. On the other hand, Zong *et al.* [9] showed that ½ or full strength MS medium inhibited root elongation of Incarvillea sinensis and shoot turned brown, but shoot browning was greatly decreased on ¼ strength MS medium.

The type of medium apparently affected the embryos root length of both cultivars (Fig. 2), Medium C which consist of full MS and 3gl^{-1} charcoal without growth regulators, gave a root length mean of 8.2cm per embryo, superior over the other types of medium A,C and D which didn't apply any significant influence in root length for both cultivars, it may be due to the effect of growth regulators mainly NAA in root initiation but once the roots commence, hormone free medium was preferred. Also medium C consist of 3gl^{-1} charcoal which helped the root to grow up and increase in length while the other medium consist of no charcoal or little amount as in medium D. Activated charcoal is commonly used in tissue culture media. Its addition to culture medium may

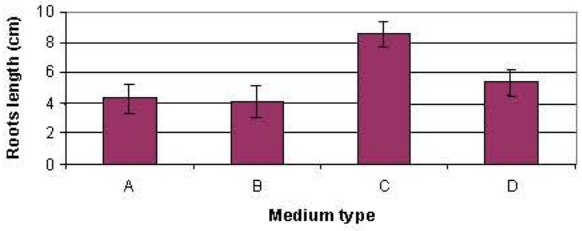

Fig. 2: Effect of medium on the roots length of over all date palm cultivars tested

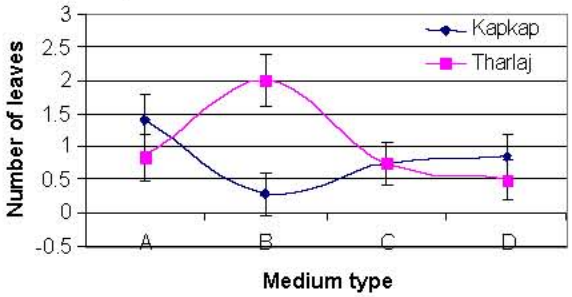

Fig. 3: Effect of medium on leaves number of two date palm cultivars

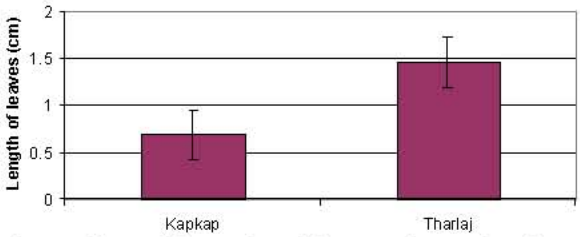

Fig. 4: Effect of date palm cultivars on leaves length

promote or inhibit *in-vitro* growth, depending on species and tissues used. The effects of activated charcoal may be attributed to establishing a darkened environment; adsorption of undesirable/inhibitory substances; adsorption of growth regulators and other organic compounds, or the release of growth promoting substances present in or adsorbed by activated charcoal [10].

As shown in Fig. 3 the leaves number of date palm embryo was significantly effected by cultivars and medium type, the cv Tharlaj is highly responds to medium

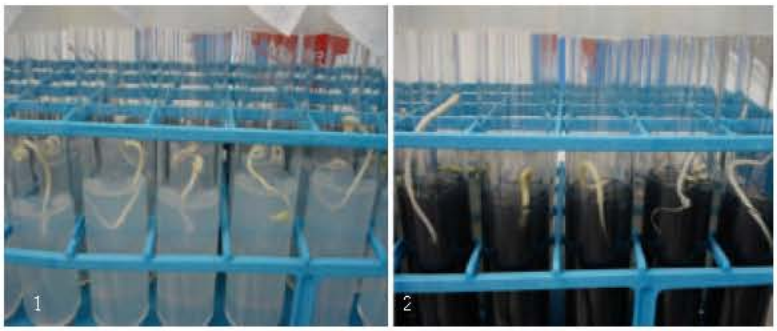

Fig. 5: Somatic embryos of date palm cv. Kapkap and Tharlaj inoculated vertically on (1) Medium A and (2) medium C

Fig. 6: (A) Cv Kapkap in medium A which is consist of full MS and 0.1 mgl^{-1}NAA without charcoal.
(B) Cv Tharlaj inoculated on the same medium.
(C) Cv Kapkap and Tharlaj in medium C which consist of full MS and 3000 mgl^{-1}charcoal without growth regulators.

B which is half MS that consist of cytokinens without charcoal and gave a mean of 2 leaves per embryo while the cv Kapkap in the same medium gave a mean of less than 0.5 leaf per embryo.

The other types of medium A,C and D didn't exert any significant influence in leaves numbers for both cultivars, it may due to the lack of cytokinens in medium A and C and due to availability of charcoal in medium D.

The leaves length was effected by date palm cultivars genotype, Tharlaj have more leaf length reach up to 1.5cm, while Kapkap have a mean of leaf length of 0.5 cm (Fig. 4). In many plant species has been shown that the response of shoot cultures to different carbohydrate treatments appears to be genotype dependent to some extent [11].

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