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EFFECT OF EXPLANT SOURCE ON *IN VITRO* REGENERATION OF PLANTS THROUGH TISSUE PROLIFERATION IN *PHOENIX DACTYLIFERA* L. CV. *FUSLI*

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Abstract

The effect of explant source on *in vitro* regeneration of plants through tissue proliferation in *Phoenix dactylifera* L. cv Fusli is reported.

Introduction

Commercial cultivation of superior cultivars of date palm has been restricted due to slow asexual propagation through offshoots. Propagation of date palm by seeds is not satisfactory since date palm is heterozygous and about half of the progeny will be male or female and hence not true to type. There is therefore, need for rapid clonal multiplication of date palm. Several attempts to grow date palm in tissue culture have been carried out (Ammar, 1977; Mohammad, 1978; Eeuwen, 1978; Tisserat, 1981). Propagation of palms through tissue culture has been primarily by the production of plants from embryogenic callus. Asexual embryogenic callus has been obtained from a variety of somatic and zygotic embryos (Omar, 1988; Kacker *et al.*, 1989; Falcone & Marcheshi, 1988).

Reports on clonal propagation of date palm through tissue proliferation rather than callus formation are however few (Tisserat, 1979; Zaid & Tisserat, 1983; Matter, 1986; Omar, 1988 Reuveni, 1979). Axillary bud proliferation may be the most effective methodology for the *in vitro* propagation because there is no callus cycle and genetic stability can be maintained. Plant regeneration via somatic embryogenesis is preferred because genetic stability can be maintained and a great number of embryos with complete plantlets can be obtained. Organogenesis from callus can cause somaclonal variation and it is not an ideal system for *in vitro* propagation except when other methods are not possible. The present report describes the *in vitro* multiplication in *Phoenix dactylifera* L. cv. Fusli.

Materials and Methods

Apical, lateral buds and segments of leaves from young suckers of *P. dactylifera* L. cv. Fusli were excised as explant source. The ex-plants were dipped for 24h in anti-oxidant solution containing 100 mg/l ascorbic and 150 mg/l citric acid used to control browning. After surface sterilization in 2.6% Sodium hypochlorite solution containing few drops of Tween 20 followed by three successive washings of 15 minutes each in

Table 1. Effect of explant source on *in vitro* regeneration and somatic embryogenesis in *Phoenix dactylifera* cv. Fusli.

Treatments		Explants used		
		Apical Buds	Axillary buds	Other tissues
M-R1	NAA 0.01 + 2-4D 5.0 mg/l	++++	-	-
M-R2	NAA 0.1 + 2i-P 5.0 mg/l	++	++	+++
M-R3	IBA 1.0 mg/l	++	+	-
M-R4	2-4D 5.0 mg/l	+	++	+
M-R5	NAA 3.0 + BAP 3.0 mg/l	***	++	-
M-R6	2i-P 0.1 + IBA 3.0 mg/l	++	+++	-

*** = Somatic Embryogenesis, ++++ = Excellent, +++ = Very good, ++ = Good, + = Poor, - = Nil

autoclaved distilled water, explants were placed on basal medium (Murashige & Skoog, 1962) containing micro and macro nutrients with 100 mg/l Myoinositol, 0.4 mg/l Thiamine HCl, 20 mg/l Adenine sulphate, 3 g/l activated charcoal, 30 g/l sucrose and 6 g/l agar. Other essential supplements were cytokinins and auxins viz., Benzyl amino purine (BAP), 2,4-dichlorophenoxy acetic acid (2.4-D), Naphthalene acetic acid (NAA), 2i-P and Indole Butyric acid ranging from 0.0 to 5.0 mg/l (Table 1). Shoot tips consisting of the apical dome with 2 to 4 leaf primordia, axillary, lateral buds and segments of newly growing leaves were also cultured on basal nutrient medium consisting of various combinations of growth regulators. The cultures were incubated either in complete darkness or under 16h photoperiod with 2000 lux light intensity at $25 \pm 2^{\circ}C$.

Results and Discussion

Apical Buds: Where apical buds were used the cultures maintained in complete darkness showed tissue proliferation after 3 weeks on media containing 0.01 mg/l NAA and 5.0 mg/l 2,4-D and when transferred to regenerating medium, somatic embryogenesis was observed after 25 days on medium containing 0.1 mg/l 2,iP and 3.0 mg/l IBA (Fig.1). Embryogenic tissues were retained by subsequent reculturing on fresh media containing the same ingredients as used for tissue proliferation. Dark green shoots were observed after 55 days of culturing without root formation. Rooting was observed on medium containing 0.01 mg/l NAA. The rooted plants were transferred to the green house for establishment. Sharma *et al.*, (1984) established callus only from axillary buds and shoot tips on MS medium containing $NaH_2PO_4.2H_2O$(170 mg/l), $KH_2PO_4.2H_2O$ (200 mg/l), 2,4-D (100 mg/l), BAP(5 mg/l) and Thiamine HCl (0.4 mg/l) and obtained somatic embryos viable plants from medium devoid of growth regulators in cv Khadravi. We have used low levels of 2,4-D i.e., 5.0 mg/l to minimize the chances of aberrant progeny in the cultures in the cultivar Fusli.

Axillary Buds: Where axillary buds were used good tissue proliferation was achieved on media containing 0.1 mg/l 2iP + 3.0 mg/l IBA (Fig.2). Proliferated tissue were light

Fig.1. Somatic embryogenesis and plant regeneration from apical bud.

yellow in colour and granular in texture. The cultures were maintained by regular subculturing after every 6th week and were later transferred to regeneration medium. Direct shoot regeneration was induced in case of axillary buds. The shoots were separated aseptically and were cultured on rooting medium. Best root initiation was achieved on Murashige & Skoog (1962) medium with 0.1 mg/l NAA. Bouguedoura *et al.*, (1990) tested axillary buds as explant source on different regimes of auxins and cytokinins and obtained vegetative buds from cultures under 16-h photoperiod on MS medium supplemented with 30 g/l sucrose and 1.0 mg/l IBA.

Fig.2. Tissue proliferation from axillary bud.

Where sections of new emerging leaves were used, tissue proliferation occurred only on media containing 0.1 mg/l NAA and 0.5 mg/l 2iP. On further sub-culturing, however, the cultures did not survive. Omar (1988) obtained shoots from cotyledonary sheath segments at low concentrations of NAA (1.0 mg/l), with root proliferation under high NAA levels (10-30 mg/l), with two types of regeneration from leaf segments viz., direct organogenesis on media containing 100 mg/1 NAA and 3 mg/l kinetin and callus induction on media containing 3 mg/l 2-iP and 100 mg/l NAA. It would suggest that apical buds could be used as a source for *in vitro* regeneration of *P. dactylifera* plants through somatic embryogenesis as compared to axillary buds.

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