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BRIEF COMMUNICATION

Factors affecting *in vitro* multiplication of date palm

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Abstract

Rapid method of *in vitro* multiplication of date palm was developed. Shoot tips were cultured on Murashige and Skoog (MS) medium supplemented with 2 mg dm^{-3} dimethylaminopurine (2iP) + 1 mg dm^{-3} naphthalene acetic acid (NAA). Shoot buds were proliferated from white nodular cultures on hormone free medium. Shoot bud proliferation strongly enhanced when cultured on MS-medium contained 3 mg dm^{-3} 2iP + 0.5 mg dm^{-3} NAA. Culturing on full-strength MS medium showed higher multiplication rate compared with half-strength MS medium. Among four concentrations of sucrose used, 30 g dm^{-3} speeded up the bud proliferation more than 10, 20 and 40 g dm^{-3}. However, the largest shoot buds were observed with 40 g dm^{-3} sucrose. Solidification of culture media by 1.75 g dm^{-3} *Phytagel* showed the highest proliferation rate, but the largest buds were observed with 1 g dm^{-3} *Phytagel*.

Additional key words: benzylaminopurine, dimethylaminopurine, naphthalene acetic acid, *Phytagel*, shoot bud proliferation.

Date palm tree is considered the most important crop cultivated in arid regions. One of the most outstanding problems in date palm cultivation is the lack of an adequate method of vegetative propagation. Therefore, rapid *in vitro* propagation could be great advantage for quick clonal multiplication of superior date palm genotypes. Several studies have been reported on micropropagation of date palm through somatic embryogenesis and indirect organogenesis (Ammar and Benbadis 1977, Tisserat 1979, Kackar *et al.* 1989, Sudhersan *et al.* 1993). Few number of buds and subsequently plantlets were obtained by this method.

In earlier report (Saker *et al.* 2000) we described a simple method for molecular characterization of somaclonal variation in tissue culrure derived date palm. The objective of this investigation was to study different factors which affect the *in vitro* multiplication of date palm.

Date palm (*Phoenix dactylifera* L. cv. Zaghlool) offshoots detached from adult female plants were used. The outer leaves were removed and shoot apices were surface sterilized by immersion for 20 min in a 2.6 % sodium hypochlorite and rinsed three times with sterile distilled water. External leaves were removed and shoot tips 1 cm in length were excised with a small part of submeristematic tissue.

The excised tips were cultured on Murashige and Skoog (1962; MS) medium supplemented with 100 mg dm^{-3} myoinositol, 40 mg dm^{-3}adenine sulfate, 2 mg dm^{-3} 2iP and 1 mg dm^{-3} NAA. Explants were incubated in darkness and subcultured every six weeks. After three subcultures, white nodular callus was observed and then shoot buds were developed. The buds were propagated on hormone free medium to obtain stock materials. To study the effect the of different combinations of growth regulators, MS medium strength, sucrose concentrations and gelling agent on *in vitro* multiplication of date palm, uniform shoot buds (1 cm) were cultured on different media.

These experiments were designed in completely randomized design. The recorded data (number of proliferated shoot buds, shoot height and root formation) were statistically analyzed using standard error (SE) according to the method described by Snedecor and Cochran (1967). Also, callus induction and vitrification were recorded.

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Abbreviation : BAP - benzylaminopurine; 2iP - dimethylaminopurine; MS - Murashige and Skoog; NAA - naphthaleneacetic acid.
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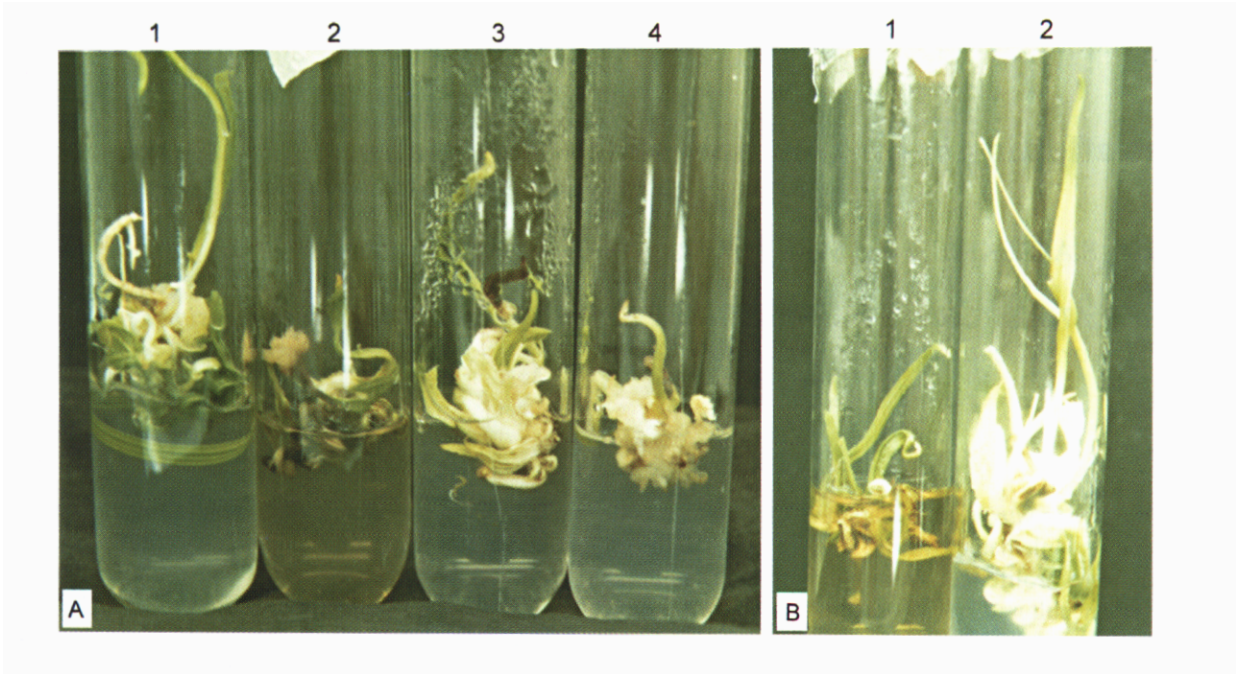

Fig. 1*A*. Proliferated shoot buds of date palm after six weeks of culturing on MS-hormone free medium (*1*), MS + 3 mg dm^{-3} BA (*2*), MS + 3 mg dm^{-3} 2iP + 0.5 mg dm^{-3} NAA (*3*), and MS + 2 mg dm^{-3} 2iP (*4*).
Fig. 1*B*. Proliferated buds of date palm after six weeks of culturing on half (*1*) and full strength (*2*) of MS-medium.

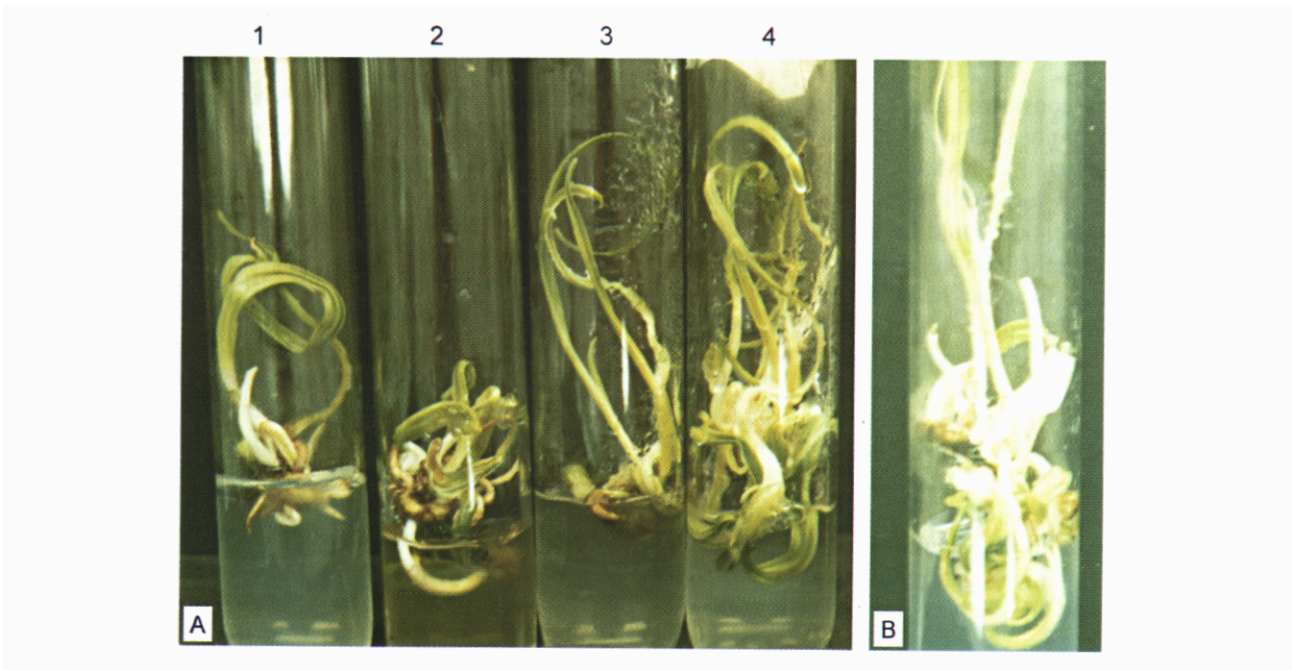

Fig. 2*A*. Proliferated shoot buds of date palm after six weeks of culturing on MS-medium containing 0 (*1*), 10 (*2*), 20 (*3*), and 30 (*4*) g dm^{-3} of sucrose.
Fig. 2*B*. Proliferated buds of date palm after six weeks of culturing on MS-medium containing 1.75 g dm^{-3} *Phytagel*.

Shoot bud proliferation strongly was enhanced by culturing on medium contained 3 mg dm^{-3} 2iP + 0.5 mg dm^{-3} NAA in comparison with hormone free medium (Table 1, Fig. 1*A*). However, the highest shoot length was noticed after six weeks of culturing on hormone free medium. This results are in agreement with those obtained by Al-Marri and Al-Ghamdi (1995) and Bekheet and Saker (1998). They used media contained 2iP and BA for *in vitro* multiplication of date palm. However, Tisserat (1984) used medium contained NAA and BA for shoot bud proliferation of date palm *in vitro*. Root formation was observed only on hormone free medium

(15 %). On the other hand the highest callus induction was observed in MS + 2 mg dm^{-3} 2iP.

The influence of half and full strength of MS-medium on *in vitro* shoot bud proliferation of date palm was also studied. Number of proliferated shoot buds was 8.80 ± 0.30 on full-strength MS medium and only 3.60 ± 0.50 on half-strength MS medium. Similarly, high of cultures was higher on full-strength MS medium than a half-strength MS medium (Fig. 1*B*). It is clear from the results that, there is negative influence of nutrients starvation on *in vitro* shoot bud proliferation of date palm.

Table 1. Effect of various combinations of phytohormones added to MS medium on *in vitro* multiplication of date palm. Means ± SE, $n = 20$.

Phytohormones	[mg dm^{-3}]	Number of shoot buds	Height of culture [cm]
control	0	2.20 ± 0.25	6.00 ± 0.25
BA	3	1.20 ± 0.30	1.40 ± 0.23
2iP + NAA	3 + 0.5.	8.10 ± 0.20	2.80 ± 0.33
2iP	2	4.80 ± 0.11	1.80 ± 0.20

Sucrose is considered as a good source of carbon in plant tissue culture media. In date palm tissue culture, sucrose is essential for embryo development and its concentration had an strong influence on embryogenesis (Veramendi and Navarro 1996). In this experiment, the number of shoot buds was gradually increased as sucrose concentration increased in culture medium till 30 g dm^{-3} then decreased. Also, no root and callus formation or vitrification were observed with this concentration. The highest shoot were observed with 40 g dm^{-3} sucrose, and some evidences of vitrification were noticed with this concentration. 3 % sucrose was also used for *in vitro* shoot proliferation of date palm by Tisserat (1984). However, Veramendi and Navarro (1996) mentioned that, sucrose starvation of nodular callus of date palm had promoting effect on somatic embryogenesis.

Table 2. Effect of different concentrations of sucrose on *in vitro* multiplication of date palm. Means ± SE, $n = 20$.

Sucrose conc. [g dm^{-3}]	Number of shoot buds	Height of culture [cm]	Root formation [%]
10	2.20 ± 0.15	3.60 ± 0.22	5
20	3.00 ± 0.25	3.20 ± 0.60	20
30	8.50 ± 0.50	5.80 ± 0.20	-
40	4.60 ± 0.33	6.40 ± 0.23	-

The effect of gelling agent (*Phytagel*) concentrations (1.0, 1.75 and 2.0 g dm^{-3}) on shoot bud proliferation of date palm was also studied. The best results of shoot bud proliferation was registered with 1.75 g dm^{-3} *Phytagel*. This concentration also show no root and callus formation or vitrification. However, the highest shoots were observed with 1g dm^{-3} phytagel. Some vitrificated cultures were noticed when the low concentration (1 g dm^{-3}) of phytagel was used.

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