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Studies on *in-vitro* organogenesis in date palm

Chander Bhan*, P.N. Sivalingam, Dhurendra Singh** and M.K. Sharma***
College of Agriculture, SK Rajasthan Agricultural University, Bikaner 334006

ABSTRACT

***In-vitro* organogenesis is the best method to overcome the problem of somaclonal variations and obtain true-to-type date palm plants. In the present investigation, activated charcoal (AC) (1.5 and 3.0 g l^{-1}), polyvinylpyrrolidone (PVP) (1.0 and 2.0 g l^{-1}) and thiourea (250.0 and 500.0 mg l^{-1}) were separately added to the half-strength Murashige and Skoog medium to test the survival, browning of tissue/ media and suitability of the growth of explants. Date palm cv. Halawy seedlings shoot tip cultured under dark condition showed that AC (1.5 g l^{-1}) was suitable for explant survival, minimized browning and supported growth for one month than other treatments. Half-strength MS medium supplemented with 1.5 g l^{-1} AC and different treatment combinations NAA (0.0, 0.1 and 1.0 mg l^{-1}) with either 2-ip or TDZ (1.5 and 3.0 mg l^{-1}) were used for axillary shoot production in dark conditions. Out of 12 treatments, 3.0 mg l^{-1} 2-iP + 0.1 mg l^{-1} NAA was superior for axillary shoots production within three months. Half-strength MS medium supplemented with different concentrations of GA_3 (0.0, 0.25, 0.5, 1.0 and 2.0 mg l^{-1}) along with 1.0 mg l^{-1} NAA was used for shoot elongation. These cultures were kept under 16 h light (2000 lux) and 8 h dark for one month. Out of five treatments tested, 0.5 mg l^{-1} GA_3 + 1.0 mg l^{-1} NAA was found optimum for shoot elongation. The results drawn from this study will be highly useful in micropropagation of date palm seedlings compared to off-shoot/ sucker by organogenesis.**

Key words: Date palm, *in-vitro,* organogenesis, somatic embryogenesis.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.), member of family Palmaceae, is a monocotyledonous, dioeceous and perennial horticultural fruit crop of arid region. Date palm is propagated traditionally by seeds and off-shoots, but the plants grown from seeds have longer juvenile period, inferior in quality, low yielder and seedlings are highly variable in nature. Due to heterozygous nature about 50% seedlings are male and they cannot be identified until tree begins to flower. Date palm plantlets produced from offshoots are better but the propagation through offshoots is slow, limited in numbers (10-15 off-shoots produced per plant in their lifespan) restricted mainly to the juvenile years of mother palm and suckers are also difficult to root. This limits the expansion of date palm cultivation on commercial scale. Hence, there is a pressing need for rapid multiplication of planting material through micropropagation. This can be achieved either through organogenesis (Khierallah and Bader, 7; Zaid and de Wet, 15) or somatic embryogenesis (Asemota *et al*., 3; Othmani *et al*., 9).

Organogenesis is based on the potentiality of meristematic tissues to form new shoots directly from the explants without callus formation and embryoid phases. This significantly reduces the total duration of *in-vitro* culture. By this method, date palm seedling production is relatively fast, easy and plantlets which are produced through this method have not shown any somaclonal variation (Asemota and Eke, 2; Zaid and de Wet, 15).

The available literatures indicate that meagre work has been reported on date palm tissue culture in India (Sharma *et al*., 11; Sharon and Shanker, 12; Bhargava *et al*., 5) with regard to multiplication of planting materials. However, multiplication of the different cultivars of date palm through organogenesis has been reported (Khierallah and Bader, 7; Othmani *et al*., 9). Keeping in view, the past prospective, the present investigation was undertaken on *in vitro* multiplication of date palm.

MATERIALS AND METHODS

Shoot tip of date palm cv. Halawy seedlings were used as explant for the experiment (Fig. 1). The seedlings were uprooted from containers, washed thoroughly with tap water to remove sand particles. The leaves along with leaf sheaths were removed acropetally to isolate the shoot tip. It was further trimmed to completely remove the woody tissues, keeping the succulent shoot tip intact. These isolated shoot tip explants were dipped in 0.05% bavistin (carbendazim 50 EC) for 5 min. and then were surface sterilized with 0.1% $HgCl_2$ with few drops of Tween-20 for 15 min. with continuous stirring followed by 3-4 washings with autoclaved double-distilled water.

*Corresponding author's E-mail: chander_pannu@yahoo.com
**Central Institute for Arid Horticulture, Bikaner 334006

Fig. 1. Six-month-old seedlings of date palm cv. Halawy grown in container used in experiments.

Murashige and Skoog medium was used as basal medium in all the experiments. The basal medium was dissolved in double distilled water and 30.0 g l^{-1} sucrose was added as carbon and energy source, 8.0 g l^{-1} agar-agar as solidifing agent and 1.5 g l^{-1} and activated charcoal as antioxidant. This basal medium was also supplemented with different combination of PGRs. The media had NAA as auxin either 2-iP or TDZ as cytokinin and GA_3 as gibbrelic acid. The concentration of NAA ranged from 0.0-1.0 mg l^{-1}, 2-iP/TDZ 1.5-3.0 mg l^{-1} and GA_3 0.0-2.0 mg l^{-1}. The pH of the medium was adjusted to 5.7 using 0.1 N NaOH or 0.1 N HCl solutions before adding agar. This media was autoclaved at 121.6^{0}C for 15 min. at 15 PSI. After autoclaving, test tubes and flasks containing medium were taken out and kept for solidification. Cultures were incubated in culture room and maintained at 27 ± 2°C with 35-40% RH. These cultures were provided 16/8 h, while light provided from cool white fluorescent tubes. the light intensity of 2000 lux was maintained at bench level.

The experiment was carried out with six treatment combinations (Table 1). Half-strength MS medium was supplemented with 1.5 or 3.0 g l^{-1} AC, 1.0 or 2.0 g l^{-1} PVP, 250.0 or 500.0 mg l^{-1} thiourea, 8.0 g l^{-1} agar-agar and 30.0 g l^{-1} sucrose. Intensity of shoot tip browning was measured by percent area affected by tissue browning and 1, 2, 3, 4 and 5 grades were used as follows to indicate the tissue browning. 0 = no browning, 1 = less than 10% browning, 2 = 10 to 25% browning, 3 = 25 to 50% browning, 4 = 50 to 75% browning, 5 = more than 75% browning (Asemota *et al.*, 3). Intensity of media browning was measured on the basis of colour changes in the media. Low, medium and high grades were used as follows to indicate the media browning. Low = light brown colour of medium surrounding the shoot tip. Medium = brown colour of medium surrounding the shoot tip. High = dark brown colour of the medium surrounding the shoot tip.

To determine the influence of TDZ, 2-iP and NAA, the experiment was carried out using different combinations of PGRs with half-strength MS basal medium containing 1.5 g l^{-1} AC, 8.0 g l^{-1} agar-agar and 30 g l^{-1} sucrose. The details of the experiment are given in Table 2. The axilliary shoots produced in second experiment were subjected for elongation to obtain rootable size of shoots. Different concentrations of growth regulators such as GA_3, NAA and their combination were tried. The details of the experiment are are given in Table 3. The experimental data recorded during the course of investigation were

Table 1. Effect of AC, PVP and thiourea on growth parameter of shoot tip and, tissue and media browning.

Treatment	Explant survival (%)	Intensity of shoot tip (tissue) browning	Intensity of media browning	No. of days required for induction of growth in shoot tip	Length of shoot tip (cm)	No. of leaves per shoot tip
Without AC, PVP and thiourea	40	4.20	High	16.50	0.43	1.00
1.5 g l^{-1} AC	100	1.70 ^{b}	Low	4.70*	1.11^c	1.40^b
3.0 g l^{-1} AC	100	0.80^c	No browning	8.44*	1.34^d	1.80^c
1.0 g l^{-1} PVP	90	3.00^a	Medium	11.13*	0.71^b	1.22^a
2.0 g l^{-1} PVP	80	2.90^a	Medium	9.63	0.65^b	1.25^a
250 mg l^{-1} thiourea	80	3.30^a	Medium	12.14*	0.55^a	1.14^a
500 mg l^{-1} thiourea	70	3.40^a	High	8.74	0.50^a	1.34^b
CD at 5%	---	0.72	---	---	0.20	0.28

*Significant (as compared of t-calculated value is more than t- table value) from control

a, b, c and d = Significant difference between treatments and control; Low = light brown colour, Medium = brown colour, High = dark brown colour.

analysed using completely randomized design as described by Panse and Sukhatme (10).

RESULTS AND DISCUSSION

Activated charcoal (AC) @ 3.0 g l^{-1} was found effective in explant (shoot) survival (100%) and controlling tissue and media browning followed by AC @ 1.5 g l^{-1}. PVP and thiourea were not effective in support of seedling survival and control of tissue and media browning. AC @ 1.5 g l^{-1} was found effective in inducing shoot tip growth within in 4.7 days followed by AC @ 3.0 g l^{-1} (8.44 days). Average shoot tip length and number of leaves per shoot were more in the medium supplemented with AC @ 3.0 g l^{-1} followed by 1.5 g l^{-1}. PVP and thiourea were not effective in improving the growth parameters of date palm seedlings (Table 1; Fig. 2). Browning of shoot tip (explant) is one of the major problems in date palm tissue culture. The tissue browning is noticed not only in date palm but it is common to all species belonging to the family Palmaceae. This kind of browning of tissue is assumed to be due to the oxidation of polyphenols like caffeoyl shikimic acids into quinones. This quinone is more toxic than phenols (Zaid, 14), which ultimately kill the cells. To minimize the media and shoot tip browning, several additives like AC, PVP, thiourea and some antioxidants, *viz.*, citric acid and ascorbic acid have been used along with media (Bhargava *et al.*, 5).

In the above experiment, it was found that addition of AC @ 3.0 g $l^{-1}$was found best against both shoot tip and media browning, followed by 50% reduction of AC (1.5 g l^{-1}). Other additives, PVP and thiourea used in the experiment were not found effective in minimizing the browning. Similarly, the usage of PVP against browning has been very limited in date palm tissue culture (Bhargava *et al*., 5). The effectiveness of different additives against browning may depend on plant species. This finding could be correlated with

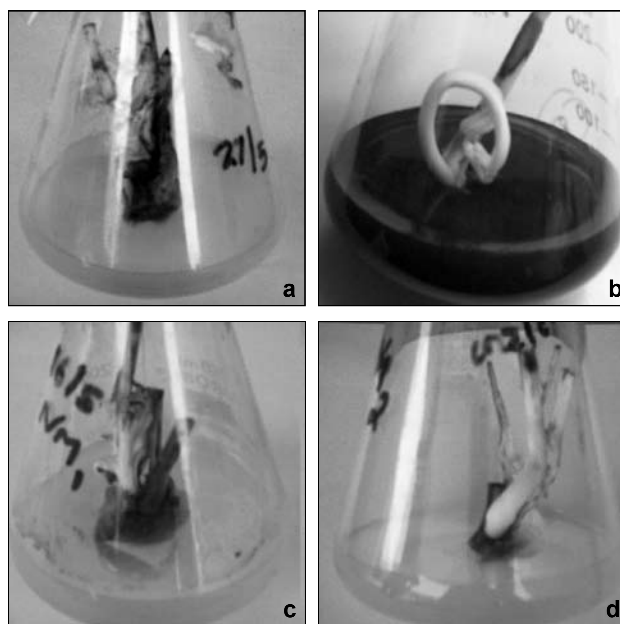

Fig. 2. Effect of activated charcoal (b), PVP (c) and thiourea (d) on tissue and media browning, with control (a).

Table 2. Effect of different combinations of cytokinins and auxin on survival and axillary shoot production.

Treatment (mg l^{-1})	Explant survival (%)	No. of shoots per culture	No. of leaves per culture	Leaves length (cm)
Control	70	1.00	1.29	1.06
1.5 2-ip + 0.0 NAA	80	1.00	1.38[a]	1.33[a]
1.5 2-ip + 0.1 NAA	90	1.00	1.44[a]	1.39[b]
1.5 2-ip + 1.0 NAA	90	1.78[b]	2.44[d]	1.86[c]
3.0 2-ip + 0.0 NAA	90	1.56[a]	1.56[b]	1.73[c]
3.0 2-ip + 0.1 NAA	100	3.10[d]	3.70[e]	2.48[e]
3.0 2-ip + 1.0 NAA	80	1.38[a]	1.50[b]	1.61[c]
1.5 TDZ + 0.0 NAA	100	2.50[a]	2.90[d]	2.06[c]
1.5 TDZ + 0.1NAA	100	2.90[c]	3.50[e]	2.18[d]
1.5 TDZ + 1.0 NAA	90	1.67[b]	2.22[c]	1.79[c]
3.0 TDZ + 0.0 NAA	80	1.38[a]	1.75[b]	1.66[b]
3.0 TDZ + 0.1 NAA	90	1.67[b]	2.00[c]	1.72[c]
3.0 TDZ + 1.0 NAA	80	1.00	1.38[a]	1.20[a]
CD at 5%	---	0.28	0.16	0.32

a, b, c, d and e = Significant difference between treatments and control.

the findings of other researchers (Sharma *et al*., 11; Othmani *et al*., 9).

The experimental results revealed that the combination of 3.0 mg l^{-1} 2-iP and 0.1 mg l^{-1} NAA was found very effective in axillary shoot production and survival of seedling shoot tip (Fig. 3). Maximum number of axillary shoots (3.10) per culture, number of leaves and length of leaves were also observed in the same medium followed by 1.5 mg l^{-1} TDZ + 0.1 mg l^{-1} NAA. At concentrations lesser than 3.0 mg l^{-1} 2-iP and more than 1.5 mg l^{-1} TDZ added there was a reduction of axillary shoot production and they were not supported survival of shoot tip explants.

The experimental data on effect of different combination of cytokinin and auxin on axillary shoot production revealed that the half-strength MS medium containing 3.0 mg l^{-1} 2-iP + 0.1 mg l^{-1} NAA and 1.5 mg l^{-1} TDZ + 0.1 mg l^{-1} NAA was more suitable for inducing more number of axillary shoots and leaves. The combination of the cytokinin and auxin also promoted the survival of seedling shoot tip at the initial stage of culture (Table 2; Fig. 1.3). The average length of leaves was increased by 2-iP than TDZ and for more axillary shoot production and number of leaves 0.1 mg l^{-1} NAA is required with both 2-iP and TDZ. While comparing the different combinations of cytokinin requirement, it was found that 3.0 mg l^{-1} of 2-iP was found more suitable for all growth parameters tested in the experiment. The average number of shoots induced by 3.0 mg l^{-1} 2-iP + 0.1 mg l^{-1} NAA and 1.5 mg l^{-1} TDZ + 0.1 mg l^{-1} NAA were comparable with the results obtained by Taha *et al.* (13). However, it was found to be low while comparing with earlier reports (Asemota and Eke, 2; Khierallah and Bader, 7). The reasons for less number of axillary shoots induced by the combination of cytokinins and auxin used in the present experiment could be attributed to the usage of half-strength MS medium, single cytokinin with auxin, cultivar and genotype (Balal and El-deep, 4), type of explants and other additives (Taha *et al*., 13; Khirallah and Bader, 7). More number of shoots per culture may be possible, while using full-strength MS medium (Taha *et al*., 13) with two or more cytokinins along with suitable auxin according to the genotypes or cultivars with longer duration (seven months) (Khierallah and Bader, 7).

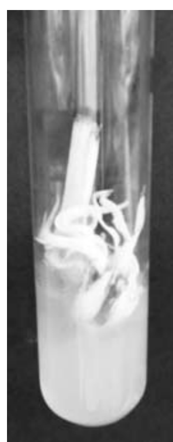

Fig. 3. Multiple shoots induction due to 3.0 mg l^{-1} 2-ip + 0.1 mg l^{-1} NAA supplementation.

The result revealed that shoot elongation was enhanced by 0.5 mg l^{-1} GA_3 along with 1.0 mg l^{-1} NAA (1.81 cm) followed by 0.25 mg l^{-1} GA_3 with 1.0 mg l^{-1} NAA. Increase or decrease in the concentration of GA_3 from 0.5 mg l^{-1} reduced the shoot length. The present experimental data on shoot elongation clearly indicated that while increasing the concentration of GA_3 upto 0.5 mg l^{-1} along with 1.0 mg l^{-1} NAA have a positive influence on shoot length, thickness leaf length and number of leaves per shoot. Further, increase in the GA_3 concentration (more than 0.5 mg l^{-1} along with 1.0 mg l^{-1} NAA) had a negative influence on shoot length, thickness and other growth parameters measured in the present experiment. It was also found that 1.0 mg l^{-1} NAA alone had some positive effect on shoot elongation as it was significantly higher than control (Table 3; Fig. 4).

The effect of GA_3 along with NAA on shoot elongation obtained in this experiment is contrary to the observations of Khierallah and Bader (7) as they reported that with increasing GA_3 concentration in the medium there was corresponding increase in the shoot length. However, the shoots grown on medium with more than 0.5 mg l^{-1} GA_3 concentration showed some malformation like slenderness of shoots, difficult in rooting and they were not suited for transplanting. Thus, it reflects the differential response of GA_3 on the type of explants used. Results of earlier studies and the present experiment clearly showed that 1.0 mg l^{-1} NAA along with 0.5 mg l^{-1} GA_3 is required to obtain optimum elongation and development of both sucker and seedling shoot tips (Loutfi and Chlyah, 8; Abdul-Soad *et al.*, 1; Khierallah and Bader, 7).

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Table 3. Effect of different combinations of GA_3 and NAA on shoot elongation in date palm.

NAA + GA_3 (mg l^{-1})	Shoot length (cm)	Shoot thickness (mm)	Leaf length (cm)	No. of leaves per shoot
Control	0.68	1.30	0.41	1.20
1.0 + 0.00	1.15[b]	1.70[a]	0.77[a]	1.30[a]
1.0 + 0.25	1.50[d]	2.40[c]	0.93[b]	1.90[b]
1.0 + 0.50	1.81[e]	2.70[c]	1.12[c]	2.50[c]
1.0 + 1.00	1.29[c]	2.10[b]	0.86[b]	1.60[a]
1.0 + 2.00	0.96[a]	1.60[a]	0.72[a]	1.50[a]
CD at 5%	0.15	0.49	0.14	0.49

a, b, c, d and e = Significant difference between treatments and control.

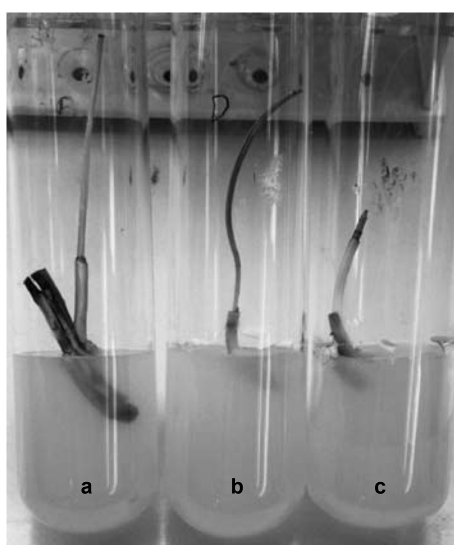

Fig. 4. Effect of NAA + GA_3 on shoot elongation, (a) 1.0 mg l^{-1} NAA + 0.5 mg $l^{-1}GA_3$, (b) 1.0 mg l^{-1} NAA + 0.25 mg l^{-1} GA_3, and (c) without hormone.

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