

***In vitro* Morphogenesis of Arabian Date Palm (*Phoenix dactylifera* L.)**

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Abstract

After inoculation of young leaves of date palm offshoot required about six months to complete the morphogenesis process. Fourteen weeks were required for embryogenic callus formation under continuous dark condition and nine weeks for shoot initiation (under 16/8 h light/dark). The highest number of explants (80%) produced callus in modified MS containing 5 mg/l 2,4-D + 2 mg/l 2ip. Sixty per cent of explants produced callus in the modified medium containing 5 mg/l 2,4-D + 5 mg/l NAA. While only 50 per cent of the explants formed callus in the same medium when supplemented with only 5 mg/l 2,4-D. The induced calli were transferred to modified MS for shoot proliferation. A combination of two cytokines showed better performance than single ones in shoot induction. The highest percentage (70) of shoot developed in modified MS containing 2 mg/l BAP + 1 mg/l Kn. Forty per cent shoot induction was found in the same medium supplemented with 2 mg/l of BAP. Thirty per cent shoot formed in MS containing 1 mg/l of Kn. The shoots were subcultured at three-four week intervals throughout culture duration.

Introduction

The Arabian date palm (*Phoenix dactylifera* L.) a diploid ($2n = 36$) is an outbreeding perennial monocotyledon of the family Arecaceae cultivated in North Africa and the Middle East. The date palm is a major fruit crop in most Arab countries (Loutfy and El-Juhany 2010) and the top ten producing countries were Egypt, Saudi Arabia, Iran, United Arab Emirates, Pakistan, Algeria, Sudan, Oman, Libya, and Tunisia (Kader and Hussein 2009). Due to its outbreeding and heterozygous nature, date palm progenies consist of approximately 50 : 50 male and female trees (Carpenter and Ream 1976). Conventional propagation is by

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offshoots making them slow to establish new plantations. In addition, seed propagated palms do not bear true to type due to heterozygosity and require up to seven years fruiting. The need for rapid and efficient vegetative propagation systems for elite genotypes has therefore become urgent.

Since 1970 intensive efforts have been undertaken for large scale micropropagation of date palm using somatic embryogenesis and organogenesis (Drira 1983, Drira and Benbadis 1985, Tisserat 1979, El Hadrami et al. 1998). Somatic embryogenesis proved to be a quick and efficient method for large scale propagation and highly useful for breeding programs (El Hadrami et al. 1998), because the production of selected healthy female plants which are disease and pest-free, genetically uniform and true to type that are produced within three - four years, to avoid seasonal effect plants are multiplied under controlled (laboratory and greenhouse) conditions round the year, when large scale production is required (Zaid and Wet 2002). Date palm fruits contain a variety of vitamins and minerals which have different physiological functions (El-Sohaimy and Hahez 2010).

In vitro improvement of date palm through somatic embryogenesis and their subsequent physiological and morphological responses have been the focus of recent study (Omar and Novak 1990, Sudharsan et al. 1993, El- Hadrami and Baaziz 1995, Zaid and Hughes 1995, Veramendi and Navarro 1996, 1997, Al-Khayri 2001, 2002, 2003, Al-Khayri and Al-Bahrany 2001, Fki et al. 2003). Progress has been made in distinguishing embryogenic and non-embryogenic date palm calli based on peroxidase activity (Baaziz et al. 1994). Calli were usually initiated from the cut ends of the explants.

The objective of this study was to investigate the role of different concentrations and combinations of 2,4-D, NAA, 2ip, BAP and Kn to induce somatic embryogenesis and shoot initiation from juvenile leaf explants of date palm.

Materials and Methods

An efficient morphogenesis protocol has been developed through *in vitro* culture technique from juvenile leaves of offshoot of date palm were used in various combinations and concentrations of PGR in modified MS. This is the first report of *in vitro* date palm morphogenesis in Bangladesh. Offshoots were obtained from adult Arabian date palm cv. Ajwah growing in Bangladesh Sugarcane Research Institute (BSRI) date palm garden.

About 6 - 8 cm long leaf base was cut from the unexpanded leaf. These collected parts were first washed thoroughly in running tap water for 10 - 15 min, then they were cleaned with 5% Savlon and 70% ethyl-alcohol and rinsed

with sterile double distilled water. Thereafter ~~then~~ they were cleaned with liquid detergent Tween 20 (1% v/v) for 5 - 10 min and rinsed with sterile double distilled water. Finally they were surface sterilized with 0.1% HgCl₂ (w/v) solution for 20 min and again washed well in sterile distilled water three - four times to remove all traces of HgCl₂ (Badawy et al. 2005).

Surface sterilized juvenile leaves of 0.5 - 1 cm were aseptically inoculated on modified MS with different concentrations of 2,4-D (3, 5, 10, 20 and 40 mg/l), in combination with 2,4-D + NAA (3 + 3, 5 + 5, 10 + 10 and 20 + 10 mg/l) and 2,4-D + 2ip (3 + 1, 5 + 2, 10 + 2 and 20 + 3 mg/l) for callusing (Othmani et al. 2009b, El-Shiaty et al. 2004, Jain and Gupta 2005 and Taha et al. 2002). The primary callus obtained from the explants was subcultured in the same medium. MS without PGRs was also tested for the induction of embryogenic cells. Cultures were kept in darkness at 28 ± 2°C and subcultured ~~after~~ every three - four weeks for 14 weeks under the same culture conditions until maturation. To promote proliferation, the entire expanding explants with resultant callus were transferred to modified MS supplemented with 0.1 mg/l 2,4-D and devoid of AC. Once embryogenic culture mass increased they were maintained and proliferated by subculturing at three - four weeks intervals and incubated under the same conditions as for initiation (Othmanit et al. 2009a).

For shoot multiplication, embryogenic callus was transferred to modified MS supplemented with different concentrations of BAP and Kn separately (1, 2, 3, 4, and 5) and different combined concentrations of BAP + Kn (1 + 1, 1 + 2, 1 + 3, 2 + 1, 2 + 2, 2 + 3 mg/l) and obtaining morphogenetic responses from calli, cultures were exposed to maintain at 28 ± 2°C under 16 h light and 8 h dark cycle for nine weeks. Lighting was provided using white cool fluorescent tubes of 40 µmol/m²/s light intensity. Roots were also produced above the condition for long time maintained (Junaid and Saeed 2009).

For all the above studies, modified MS contained 3% (w/v) sucrose, 0.5 g/l activated charcoal (AC), 10% coconut milk, NaH₂PO₄ (170 mg/l), citrate (100 mg/l), biotin (2 mg/l) and were solidified with 0.7% agar. The pH of all media was adjusted to 5.7 prior to autoclaved at 121°C at 15 psi for 20 min. Experiments were repeated at least three times and at least ten cultures were employed per treatment (Jain and Gupta 2005).

Results and Discussion

It was found that the highest percentage (50) of explant produced callus in modified MS supplemented with 5 mg/l 2,4-D in dark condition (Fig.1a). It was revealed that callus initiation started at 3 mg/l 2,4-D and reached the peak (50%) at 5 mg/l. After that it was gradually decreased up to 20 mg/l, Moreover, no

callus was initiated in the modified MS fortified with the concentration of 2,4-D (control and 40 mg/l) (Table 1). Othmani et al. (2009b) and Aslam et al. (2009) found similar results in their research. After eight weeks, callus was maintained at low concentration of 1 mg/l 2,4-D and kept in dark condition for proliferation of embryogenic callus. Roots also formed from the embryogenic callus within six weeks. (Othmani et al. 2009a, Zaid and Wet 2002 and Gueye et al. 2009).

The maximum embryogenic callus (60%) was revealed in modified MS containing 5 mg/l 2,4-D + 5 mg/l NAA within 14 weeks in dark condition (Fig. 1b). It was found that callus initiation started at 3 mg/l 2,4-D + 3 mg/l NAA and reached the peak (60%) at 5 mg/l 2,4-D + 5 mg/l NAA. After that it was gradually decreased up to 10 mg/l 2,4-D + 10 mg/l NAA, no callus was induced in the MS fortified with the concentration of 2,4-D + NAA (control and 20 + 10 mg/l) (Table 1). Zaid and Wet (2002) were used high concentration of 2, 4-D (100 mg/l) for embryogenic callus. After eight weeks at low concentration of 1 mg/l 2,4-D + 1 mg/l NAA were added in the modified MS in dark condition for proliferation of embryogenic callus and induction of roots (Othmani et al. 2009a and Al-Khalifah 2006).

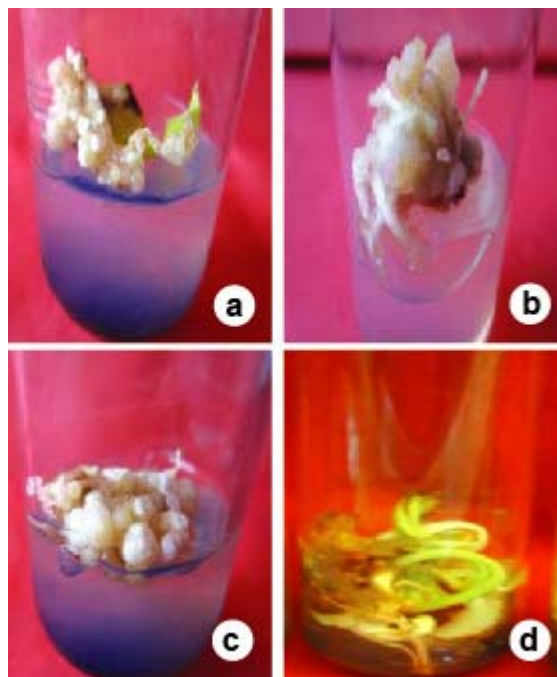


Fig. 1a-d. Morphogenesis in *in vitro* grown explants of a juvenile leaf offshoot a-c. Embryogenic Callus. d. Shoot induced from embryogenic callus.

The best profuse callus (80%) was found (Figs 1b and c) in modified MS containing 5 mg/l 2,4-D + 2 mg/l 2ip within eight weeks in dark condition. It was also observed that callus initiation started at 3 mg/l 2,4-D + 1 mg/l 2ip and reached 80% at concentration 5 mg/l 2,4-D + 2 mg/l 2ip. After that it gradually decreased up to 10 mg/l 2,4-D + 2 mg/l 2ip, no callus was formed in the concentration of 2,4-D + 2ip (control and 20 + 3 mg/l) (Table 1) (Bekheet et al. 2008, El-Shiaty et al. 2004 and Badawy et al. 2005). After eight weeks at low concentration of 0.5 mg/l 2,4-D + 0.1 mg/l 2ip were added in modified MS and kept in dark condition for proliferation of embryogenic callus. Roots were formed from embryogenic callus within six weeks. Therefore, It was revealed that 5 mg/l 2,4-D + 2 mg/l 2ip is the best performance for callus induction in above concentrations.

Table 1. Effect of different concentrations of 2,4-D, 2,4-D + NAA and 2,4-D + 2iP in modified MS on callus initiation.

PGR	PGR conc. (mg/l)	Number of explant inoculated	Weeks to callus initiation	Degree of callusing	Callus initiated (%)
2,4-D	Control	1	14	-	-
	3	20	"	++	20
	5	20	"	+++	50
	10	20	"	++	30
	20	20	"	-	10
	40	20	"	-	-
2,4-D + NAA	Control	1	"	-	-
	3 + 3	20	"	++	30
	5 + 5	20	"	+++	60
	10 + 10	20	"	++	35
	20 + 10	20	"	-	-
	Control	1	"	-	-
2,4-D + 2ip	3 + 1	20	"	++	35
	5 + 2	20	"	+++	80
	10 + 2	20	"	++	40
	20 + 3	20	"	-	-

Degree of callusing: '-' means no response, '+' means slight callusing, '++' means medium callusing, '+++ means profuse callusing.

The embryogenic calli derived from young leaves of offshoot were transferred to shoot induction medium (modified MS) supplemented with different concentrations of BAP and Kn alone and or in combination. The embryogenic calli were kept in 16 hrs light conditions. The highest percentage of shoots (70) were regenerated from embryogenic calli when cultured in modified MS containing 2 mg/l BAP + 1 mg/l Kn (Fig. 1d). Forty per cent shoots were

formed in modified MS containing only BAP @ 2 mg/l while only 30 per cent shoots were formed in the same medium containing 1 mg/l Kn (Table 2). It was revealed that shoot induction percentage increased up to 2 mg/l BAP and or up to 1 mg/l Kn, and declined the percentage of shoot formation beyond the above concentration. Therefore, it could be mentioned that the combined effect of different cytokinins showed better performance than single one for shoot induction from embryogenic calli (Aslam et al. 2009). Two cytokinin tested BAP was more active than Kn during multiple shoot formation. The superiority of BAP than Kn for multiple shoot formation was also demonstrated in other plants like *Jatropha integerrima* (Sujatha and Dhingra 1993), *Sapium sebiferum* (Siril and Dhar 1997), and *Bombaxceiba* (Chand and Singh 1999, 2004). Shoot multiplication rate decreased with increasing BAP concentrations up to 3 mg/l. Similar effect was also found in many other plant tissue cultures (Jouira et al. 1998, Biroscikova et al. 2004, Junaid et al. 2007).

Table 2. Effect of different concentrations of BAP and Kn alone and in combination with modified MS on shoot induction from embryogenic callus.

PGR	PGR conc. (mg/l)	Shoot formation (%)
BAP	Control	-
	1	10
	2	40
	3	10
	4	-
	5	-
Kn	Control	-
	1	30
	2	10
	3	-
	4	-
	5	-
BAP + Kn	Control	-
	1 + 1	20
	1 + 2	-
	1 + 3	-
	2 + 1	70
	2 + 2	30
	2 + 3	-

'-' means no response.

Present study elucidated an efficient protocol for plant morphogenesis *via* embryogenesis of date palm and this will be helpful in conducting micropropagation.

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