Effect of 2,4-D and NAA on callus induction in date palm cultivars Halawy and Medjool

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ABSTRACT

In the present investigations two popular cultivars (Halawy and Medjool) of date palm were selected for in vitro callus formation. The effect of different levels of 2,4-D (25, 50 and 100 mg/l) and NAA (25, 50 and 100 mg/l) in combination with BA (3 mg/l) was studied. The maximum responsive culture was observed with 2,4-D (100 mg/l) + BA (3 mg/l). The addition of BA 3 gm/l activated charcoal in MS basal medium when taken from shoot tip explant. This treatment was found to induce earliest callus formation (146 days) as compared to other treatments. However higher concentration of auxin was found to aggravate the growth of the callus and maximum weight of callus was recorded with low concentration of auxin. The quality of callus was also affected differently with all combinations of treatments. The embryogenic calli were developed after 6 months of callus which was sub-cultured on fresh MS basal medium without hormone.

Keywords: Callus induction; date palm; Halawy; Medjool; 2,4-D; NAA

INTRODUCTION

Date palm (*Phoenix dactylifera* L) a monocotyledonous and dioecious plant species belonging to the Arecaceae family is widely cultivated in arid regions of India. It is cultivated mainly in Kuchchh region of Gujarat and arid regions of Rajasthan. Naturally date palm can be propagated through seeds. The seed-propagated plants are not all female due to dioecious nature of plant and male plants do not bear fruits. Rooted offshoots can also be preferred for conventional propagation because they produce true to type plants with better fruit quality (Al-Khayri et al 2001, Omar et al 1992). However there are some problems associated with this system eg the availability of offshoots is limited because the number produced by each palm is low. The offshoot must remain attached to its parent tree for a long time until the development of adequate root system. In addition to this method of excision is complicated, time consuming and difficult to root. Some reports are available on micropropagation of date palm through somatic embryogenesis (Tisserat 1979, Sharma et al 1984). However the refinement of protocols developed for in vitro callus induction and somatic embryogenesis is controlled by the type and concentration of plant growth regulators added to basal medium.

MATERIAL and METHODS

The explants for callus inititian of date palm were taken from the plants of date palm (*Phoenix dactylifera* L) growing in the mother block of Hi-Tech Nursery, Central Institute for Arid Horticulture, Bikaner, Rajasthan and Date Palm Research Centre, Rajasthan Agricultural University, Bikaner, Rajasthan. The offshoots were detached from mother plants during the month of December 2008.

After careful elimination of all leaves and adjourning tissues the shoot tips were excised from the offshoots. These explants were dipped in 70 per cent ethanol for 1 minute and surface-sterilized with 0.1 per cent $HgCl_2$ containing few drops of Tween-20 with continuous stirring for 10-12 minutes followed by washing with autoclaved distilled water 6-8 times.

Shoot tips were cut into (1.5 cm) long explants. The explants were inoculated on MS (Murashige and Skoog 1962) basal media incorprating different concentrations of 2,4-D (25, 50 and 100 mg/l) and NAA (25, 50 and 100 mg/l) with BA (3 mg/l). MS medium without plant growth regulators was also tested for the induction of callus. The embryogenic calli were matured, germinated and shoot proliferation was done on basal MS media without plant growth regulators and activated charcoal.

All media contained sucrose (30 g/l) and 3.0 g/l activated charcoal and were solidified with 0.7 per cent agar. The pH of all media was adjusted to 5.7 proir to autoclaving. Cultures were sub-cultured every four weeks. The cultured medium was poured in conical flasks and test tubes for sterilization. Autoclaving was done for 15 minutes at 121°C and 15 psi (1.1 kg/cm^2) pressure. After autoclaving the media were stored in dark conditions for 48 hours at 25 ± 2°C. The callus cultures were incubated in culture room at 26 ± 1°C and 16/8 hours photoperiod was provided with the help of cool white fluorescent tubes in complete darkness. The light intensity was maintained at 2000 lux at bench level. The somatic embryogenesis maturation, germination and shoot proliferated cultures were incubated under constant light (1000 lux) at 27 ± 2ºC temperature.

RESULTS and DISCUSSION

Effect of 2,4-D, NAA and BA on the time taken for callus induction

The data presented in Table 1 show the average period (days) taken for callus induction in cvs Halawy and Medjool. Without plant growth regulators treatment the callus was not induced. In both cultivars (Halawy and Medjool) the time taken for callus induction was maximum in treatment NAA (25 mg/l) followed by 2,4-D (50 mg/l) and minimum in 2,4-D (100 mg/l). At higher concentration (100 mg/l) the callus was induced earlier than at lower concentration. The pronounced effect of different combinations of 2,4-D, NAA and BA was observed on callus induction process in date palm cultivars. The mechanism of auxin and cytokinin-mediated morphognesis has been observed as the subject of extensive studies on differentiation of tissues and organs in various plant species.

These results are in close conformity with the findings of Yadav et al (2001) and Kacker et al (1989) who obtained callus in Medjool and Halawy cultivars on MS medium containing 2,4-D 100 mg/l and other additives. In vitro callus induction, growth and differentiation are controlled by the type and concentration of plant growth regulators added to the basal medium as well as the interaction between auxin and cytokinin in culture media. Tisserat (1979), Mater (1990), Madhuri et al (1998) and Saker et al (1998) proved that addition of auxins to media was necessary for induction of callus.

Effect of 2,4-D, NAA and BA on periodical response of culture in Halawy and Medjool

The information on periodical response of callus growth and differentiation after each month in cvs

Table 1. Effect of 2,4-D, NAA and BA on the time taken for callus induction in two cultivars

Treatment	Concentration of 2,4-D/NAA (mg/l) (+ BA 3 g/l)	Average number of days taken in cv	
		Halawy	Medjool
Control	0 + 0	-	-
NAA	25	225	247
NAA	50	190	210
NAA	100	160	157
2,4-D	25	205	217
2,4-D	50	180	187
2,4-D	100	159	146

Table 2. Effect of 2,4-D, NAA and BA on periodical response of culture in two date palm cultivars Halavy and Medijool

Treatment	Concentration of 2,4-D/NAA (mg/l) (+ BA 3 g/l)	Periodical response after each month							
		1	2	3	4	5	6	7	8
Halawy									
Control	0 + 0	-	-	Tissue browning	Tissue browning	Dead	-	-	-
NAA	25	No response	No response	No response	No response	Cell enlargement	Cell enlargement	Cell differentiation	Callus initiation
NAA	50	No response	No response	No response	Cell differentiation	Callus initiation	Callus formation	Callus formed	-
NAA	100	No response	No response	No response	Cell differentiation	Callus initiation	Callus formed	-	-
2,4-D	25	No response	No response	No response	Cell enlargement	Cell enlargement	Cell differentiation	Cell differentiation	Callus formed
2,4-D	50	No response	No response	No response	Cell enlargement	Cell enlargement	Cell differentiation	Cell differentiation	Callus initiation
2,4-D	100	No response	No response	No response	Cell differentiation	Callus initiation	Callus formed	-	-
Medjool									
Control	0 + 0	-	Tissue browning	Tissue browning	Tissue browning	Dead	-	-	-
NAA	25	No response	No response	No response	No response	Cell enlargement	Cell enlargement	-	Callus formation
NAA	50	No response	No response	No response	Cell enlargement	Cell enlargement	Cell enlargement	Callus formation	-
NAA	100	No response	No response	Cell enlargement	Cell enlargement	Callus formation	-	-	-
2,4-D	25	No response	No response	No response	Cell enlargement	Cell enlargement	Callus formation	-	-
2,4-D	50	No response	No response	No response	No response	Cell enlargement	Cell enlargement	Callus formation	-
2,4-D	100	No response	No response	Cell enlargement	Cell enlargement	Callus formation	-	-	-

Plate 1. Friable creamish callus at lower concentration of auxin

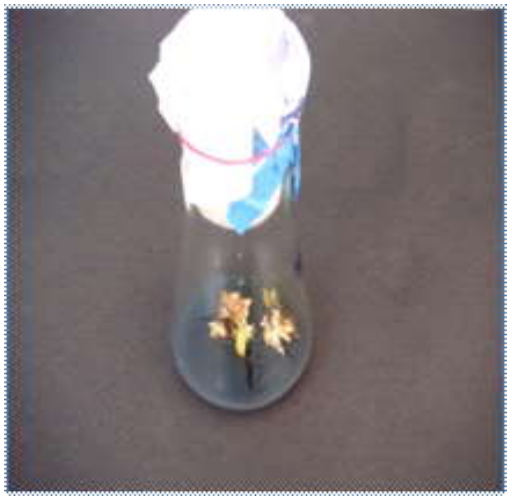

Plate 2. Compact nodular callus at higher concentration of auxin

Plate 3. Somatic embryo germination

Plate 4. Shoot proliferation

Halawy and Medjool is given in Table 2. The data show that in every sub-culture several changes like tissue browning, cell enlargement, cell differentiation and callus initiation were noticed under different treatments. The culture morphogenetic responses as observed in both the cultivars were gradual changes in terms of both negative and positive in every month.

Effect of 2,4-D, NAA and BA on the quality parameters of callus: At lower concentrations (25 and 50 mg/l) of auxin the callus was white creamish and friable (Plate 1) whereas at higher concentration (100 mg/l) the callus was nodular compact in cv Halawy (Plate 2) but in Medjool it was white creamish and friable (Table 3). The growth and weight varied in different concentrations. The growth of callus was good depending on the type of cultivar and its weight was more with low concentrations of auxin. Similar findings were also reported by Tisserat (1979), Eke et al (2004), Asemota et al (2005) and Kacker et al (1989) with respect to quality of callus. Yadav et al (2001) also obtained creamish white and friable callus with 100 mg/l 2,4-D. Later the callus was transferred on the MS basal media without hormone for somatic embryo maturation, germination and shoot proliferation (Plates 3, 4).

REFRENCES

Al-Khayri JM 2001. Optimization of biotin and thiamine requirements for somatic embryogenesis of date palm (*Phoenix dactylifera* L). In Vitro Cellular and Developmental Biology- Plant **37(4):** 453-456.

Asemota O, Eke CR and Odewale JO 2005. Date palm (*Phoenix dactylifera* L) in vitro morphogenesis in response to growth regulators, sucrose and nitrogen. African Journal of Biotechnology **6(20):** 2353-2357.

Eke CR, Akomeah P and Asemota O 2004. Somatic embryogenesis in date palm (*Phoenix dactylifera* L) from apical meristem tissues from Zebia and Loko landraces. African Journal of Biotechnology **4(3):** 224-246.

Table 3. Effect of 2,4-D, NAA and BA on the quality parameters of callus in two cultivars

Treatment	Concentration of 2,4-D/NAA (mg/l) (+ BA 3 g/l)	Quality parameters of callus			
		Nature	Colour	Weight (g)	Growth
Halawy					
Control	0 + 0	Friable	Creamish white	0.60	Little
NAA	25	Friable	Creamish white	0.30	Fair
NAA	50	Nodular compact	Brown	0.28	Little
NAA	100	Friable	Creamish white	0.40	Little
2,4-D	25	Friable	Creamish white	0.50	Little
2,4-D	50	Nodular compact	Brown	0.20	Little
Medjool					
Control	0 + 0	Friable	Creamish white	0.80	Little
NAA	25	Friable	Creamish white	0.48	Fair
NAA	50	Friable	Creamish white	0.45	Good
NAA	100	Friable	Creamish white	0.50	Fair
2,4-D	25	Friable	Creamish white	0.60	Little
2,4-D	50	Friable	Creamish White	0.40	Good

Kacker NC, Solanki KR and Joshi SP 1989. Micropropagation of date palm (*Phoenix dactylifera* L) cv Khadrawy using tissue culture technique. Annals of Arid Zone **28:** 137-141.

Madhuri S, Shankar P and Sharon N 1998. Somatic embryogenesis and plant regeneration from primordial leaf of *Phoenix dactylifera* L cv Yakubi. Indian Journal of Experiment Biology **36(5):** 526-529.

Mater AA 1990. Effect of auxin-cytokinin on micropropagation of date palm. King Faisal University Saudi Arabia Journal of Agricultural Sciences **2:** 211-223.

Murashige T and Skoog F 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum **15:** 473-497.

Omar MS, Hameed MK and Al-Rawi MS 1992. Micropropagation of date palm (*Phoenix dactylifera* L). In: Biotechnology in agriculture and forestry 18, High-tech and micropropagarion II (YPS Bajaj ed), Springer-Verlag, Heidelberg, Berlin, pp 471-492.

Saker MM, Moursy HA and Bekheet SA 1998. In vitro propogation of Egyptian date palm: I. Morphogenic responses of immature embryos. Bulletin of Faculty of Agricultural University, Cario. **49(2):** 203-214.

Sharma DR, Sunita D and Chowdhury JR 1984. Somatic embryogenesis and plant regeneration in date palm (*Phoenix dactylifera* L) cv 437 Khadravi through tissue culture. Indian Journal of Experimental Biology **22:** 596-598.

Tisserat B 1979. Propagation of date palm (*Phoenix dactylifera* L) in vitro. Journal of Experimental Botany **30(6):** 1275-1283.

Yadav N, Yadav RC, Chowdhury VK and Chowdhury JB 2001. Explant and cultivar response to in vitro clonal propagation of female date palm (*Phoenix dactylifera*). Paper presented, 2nd International Conference on Date Palms, Al-Ain, UAE, 25-27 March 2001.