Australian Journal of Crop Science AJCS

AJCS 12(12):1937-1949 (2018) ISSN:1835-2707
doi: 10.21475/ajcs.18.12.12.p1267

Optimisation of *in vitro* micropropagation of different date palm cultivars

Ahmed Al-Najm, Steve Brauer, Richard Trethowan, Nabil Ahmad*

Supplementary Table 1. Callus initiation response in Barhee using three salt media supplemented with various concentrations of growth regulators.

Growth regulator	Culture salt medium		
	MS	WPM	B5
No growth regulators	-	-	-
1 mg l^{-1} TDZ	-	-	-
2 mg l^{-1} TDZ	-	-	-
3 mg l^{-1} TDZ	+	+	-
4 mg l^{-1} TDZ	+	++	-
5 mg l^{-1} TDZ	++	++++	+
6 mg l^{-1} TDZ	+	++	+
5 mg l^{-1} TDZ + the following concentrations of 2,4-D (mg l^{-1})			
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	+	++	+
7	++	+++	+
8	+	++	-
9	-	+	-
10	-	-	-
11	-	-	-
12	-	-	-
5 mg l^{-1} TDZ + the following concentrations of NAA (mg l^{-1})			
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	-
7	-	-	-
8	-	+	-
9	+	+	-
10	++	+++	++
11	+	++	-
12	+	+	-

(-) No response; (+) Indicates poor degree of callogenesis; (++) Indicates good degree of callogenesis; (+++) Indicates very good degree of callogenesis; (++++) Indicates excellent degree of callogenesis.

Supplementary Table 2. Callus induction percentage and number of somatic embryos generated from floral explants of seven cultivars cultured on WPM and supplemented with different growth regulators.

Variety	5 mg l^{-1} TDZ		5 mg l^{-1} TDZ + 7 mg l^{-1} 2,4-D		5 mg l^{-1} TDZ + 10 mg l^{-1} NAA	
	%	# GSE	%	# GSE	%	# GSE
Barhee	80.0	14	66.7	4	60.0	9
Medjool	73.3	12	53.3	3	60.0	7
Khalas	86.7	15	86.7	5	73.3	10
Jarvis	73.3	13	60.0	3	53.3	7
Nemeishi	86.7	12	73.3	5	66.7	9
Khadrawi	93.3	12	73.3	5	66.7	8
River Gem	93.3	14	80.0	5	73.3	9

L.S.D. (growth regulators) = 0.2657, L.S.D. (variety)=0.4058, L.S.D. (Interaction) =0.7029.
GSE: globular somatic embryo.

Supplementary Table 3. Effect of various growth regulators on conversion of globular somatic embryos into plantlets in several date palm cultivars.

Variety	Growth regulators										
	Zeatin (2 mg l^{-1})			2ip (3 mg l^{-1})			3 mg l^{-1} NAA + 2 mg l^{-1} Zeatin			3 mg l^{-1} NAA + 2 mg l^{-1} 2ip	
	GSE	Plantlet	%	GSE	Plantlet	%	GSE	Plantlet	%	GSE	%
Barhee	18.4	17	92.4	10	6	60	13.6	10	73.5	11.2	71.4
Medjool	21	18	85.7	11	8	72.7	17	13	76.5	13	69.2
Khalas	18.6	16	86.2	9.2	5	54.3	12.8	8	62.5	10.8	55.6
Jarvis	18.8	16	85.1	8.2	5	61.0	10.2	7	68.6	9.6	62.5
Nemeishi	11.6	10	86.2	5.8	3	51.7	8.8	6	68.2	6.6	60.6
Khadrawi	13.8	12	87.0	6.4	4	62.5	9.6	7	73.0	6.6	60.6
River Gem	10.4	10	96.2	5.6	3	53.6	7.4	6	81.1	6.8	73.5

GSE: Globular somatic embryos.For GSE: L.S.D. (growth regulators) = 0.3977, L.S.D. (variety)=0.5262, L.S.D. (growth regulators x variety) =1.0523. For plantlets: L.S.D. (growth regulators) = 0.3495, L.S.D. (variety)=0.4624, L.S.D. (growth regulators x variety) =0.9248.

Supplementary Table 4. Inflorescence proliferation and direct regeneration of plantlets from floral explants.

Growth regulator	Culture salt medium		
	MS	WPM	B5
No growth regulators	-	-	-
1 mg l^{-1} 2ip	-	-	-
2 mg l^{-1} 2ip	-	-	-
3 mg l^{-1} 2ip	+	+	-
4 mg l^{-1} 2ip	+	+	-
5 mg l^{-1} 2ip	++	++	-
6 mg l^{-1} 2ip	+	+	-
5 mg l^{-1} 2ip + the following concentrations of 2,4-D			
0	-	-	-
0.5	-	-	-
1	-	+	-
1.5	++	+++	+
2	+	+	-
5 mg l^{-1} 2ip + the following concentrations of NAA			
0	-	-	-
0.5	+	++	+
1	++	+++	+
1.5	+	+	-
2	-	-	-
5 mg l^{-1} 2ip + the following concentrations of 2,4,5-T			
0	-	-	-
0.5	+	++	-
1	++	+++	+
1.5	-	+	-
2	-	-	-

(-) No response; (+) Indicates poor degree of proliferation; (++) Indicates good degree of proliferation; (+++) Indicates excellent degree of proliferation

Supplementary Table 5. Effect of various cytokinins combined with 0.3 mg l^{-1} NAA on adventitious bud multiplication in seven date palm cultivars.

Variety	Zeatin (3 mg l^{-1})			2ip (4 mg l^{-1})			BA (2 mg l^{-1})		
	Shoot	Plantlet	% Plantlet	Shoot	Plantlet	% Plantlet	Shoot	Plantlet	% Plantlet
Barhee	12	11	91.66	9	7	77.77	8	6	75
Medjool	11	10	90.90	10	7	70	10	7	70
Khalas	14	12	85.71	10	8	80	11	8	72.72
Khadrawi	12	10	83.33	11	8	72.72	10	7	70
Nemeishi	12	11	91.66	10	7	70	11	8	72.72
River Gem	10	8	80	7	5	71.42	7	5	71.42
Jarvis (male)	10	9	90	7	5	71.42	8	7	78.5
Average	11.57	10.14	87.60	9.14	6.71	73.33	9.28	6.8	74.05

L.S.D. (growth regulators) = 0.505, L.S.D. (variety)=0.772, L.S.D. (growth regulators x variety) =1.336. For Number of shoots: L.S.D. (growth regulators) = 0.3754, L.S.D. (variety)=0.5734, L.S.D. (growth regulators x variety) =0.9931.

Supplementary Table 6. Effect of various GA_3 concentrations on shoot elongation (shoot length in cm) in seven date palm cultivars after 8 weeks of culture in the presence of 3.0 mg l^{-1} zeatin.

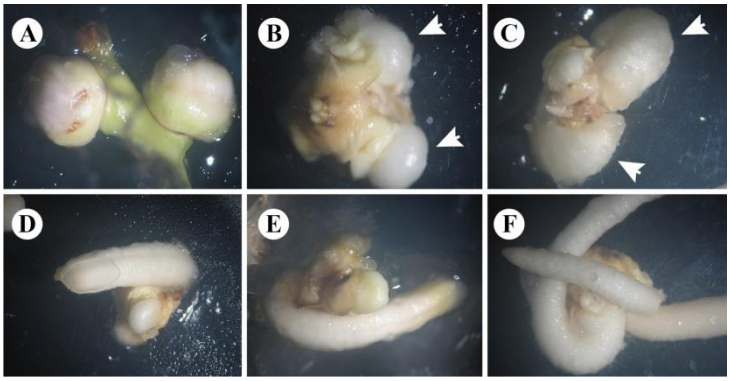
Cultivar	GA_3 concentration (mg l^{-1})			
	0	0.2	0.5	1.0
Barhee	3.30	5.90	6.95	6.85
Medjool	3.45	5.98	7.26	7.18
Khalas	3.97	6.25	7.79	7.92
Khadrawi	3.27	5.80	7.76	8.23
Nemeishi	2.70	5.14	8.12	8.71
River Gem	2.69	5.40	6.92	7.27
Jarvis (male)	3.10	6.0	8.69	9.49

L.S.D. (GA_3) =0.1350, L.S.D. (variety) =0.1786, L.S.D. (GA_3 x variety) =0.3572.

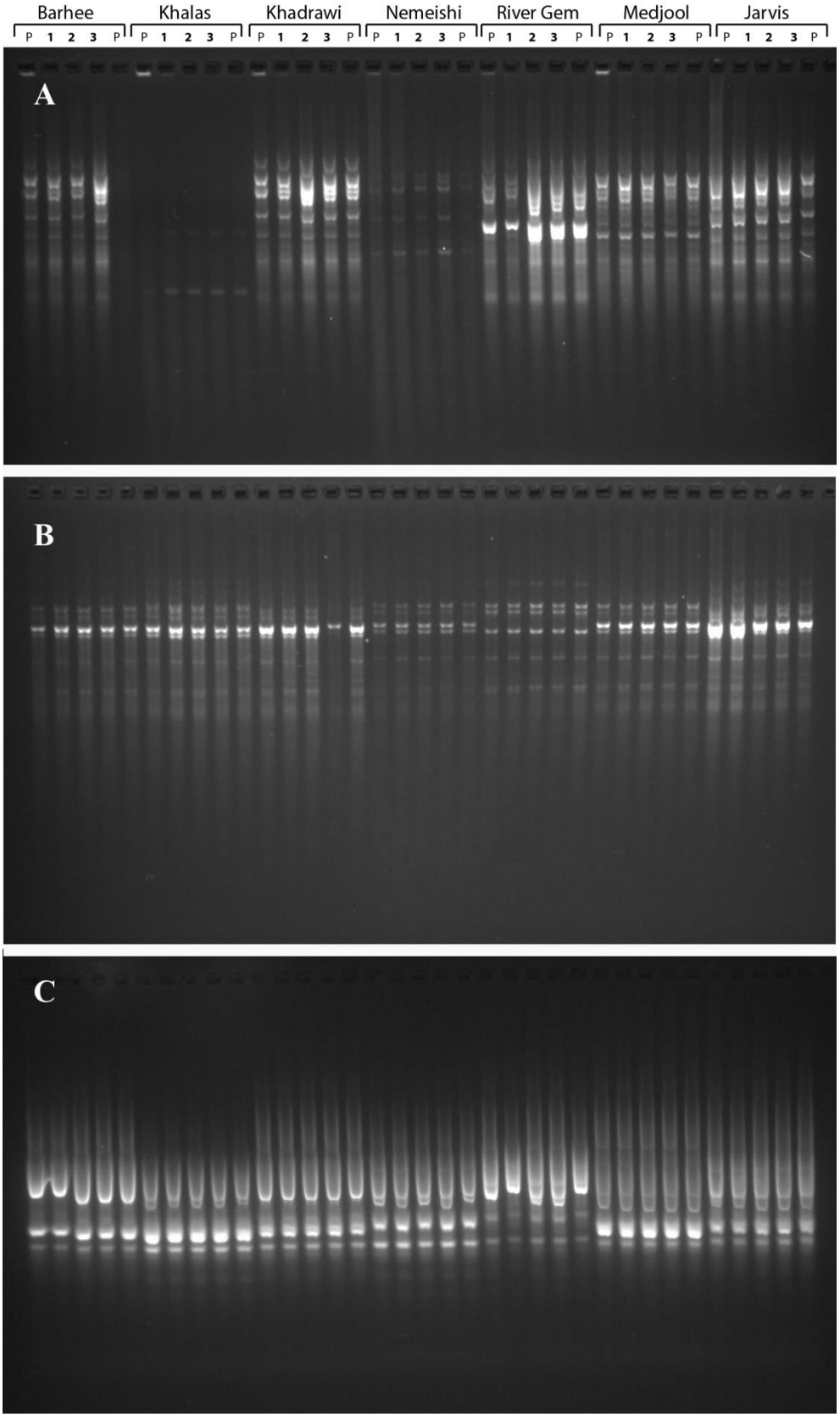
Supplementary Table 7. Effects of different NAA concentrations on the rooting percentage, number of roots, and root length of seven date palm cultivars after 12 weeks.

Variety	Root Length (cm) NAA Concentration				Root number NAA Concentration				Rooting % NAA Concentration			
	0	0.2	0.5	1	0	0.2	0.5	1	0	0.2	0.5	1
Barhee	2.9	5.90	4.07	4.0	0.6	3.9	2.1	2.1	50	80	70	60
Medjool	3.0	5.11	4.0	3.79	0.7	4.6	3.0	2.2	50	70	70	60
Khalas	2.27	6.52	5.19	3.97	1.1	3.6	2.0	1.2	60	90	80	60
Jarvis	3.15	5.12	4.62	3.87	0.4	4.0	3.0	1.9	40	70	60	70
Nemeishi	3.08	7.20	5.17	4.0	0.5	5.0	3.1	2.0	30	90	60	50
Khadrawi	1.97	6.27	4.90	3.37	0.6	4.7	2.1	1.9	40	90	80	60
River Gem	2.02	5.80	4.23	3.80	1.2	4.80	3.7	2.9	60	80	80	70

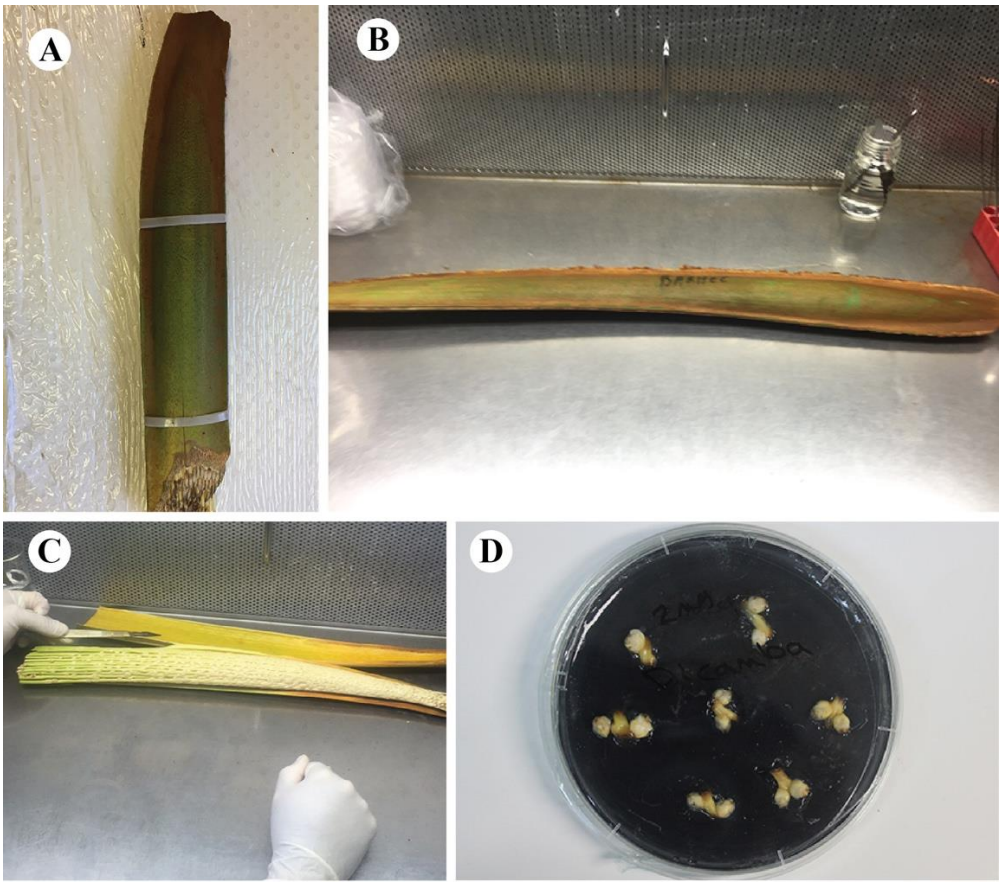
For root length: L.S.D. (NAA) = 0.02647, L.S.D. (variety)=0.03501, L.S.D. (NAA x variety) =0.07003.
For root number: L.S.D. (NAA) = 0.2067, L.S.D. (variety)=0.2734, L.S.D. (NAA x variety) =0.5468.



Supplementary Fig 1. Direct organogenesis and floral reversion of mature flowers into the vegetative state. (A) Inflorescence explant on semi-solid medium. (B-C) Adventitious bud initiation shown as a swelling of carpels and indicated by arrow heads. (D-F) Buds growth and elongation of shoots directly from the floral organs.



Supplementary Fig 2. iPBS profile of 3 in vitro regenerated plants and the parent plant (P) of each of 7 date palm cultivars using primer 2074 in A, primer 2381 in B and primer 2374 in C resolved on 2.0% agarose gel. The bands obtained shows the true to type genetic material present.



Supplementary Fig 3. Explant preparation of the cultivar Barhee for inoculation onto nutrient culture medium of known composition. (A) Spathe excised from the mother tree at maturity stage and tied to prevent splitting and exposing the mature inflorescence during transportation. (B) Spathe inside the laminar flow cabinet during the surface sterilization process. (C) Opening the spathe under aseptic conditions. (D) culturing 1-2 cm inflorescence explants onto semisolid medium in 55 mm sterile Petri dishes.