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ORIGINAL ARTICLE

A combined pathway of organogenesis and somatic embryogenesis for an efficient large-scale propagation in date palm (*Phoenix dactylifera* L.) cv. Mejhoul

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Abstract

An efficient regeneration system via a combined pathway of organogenesis and somatic embryogenesis was developed for date palm (*Phoenix dactylifera* L.) cv. Mejhoul. Adventitious buds were obtained from shoot-tip explants with a frequency of 53.3% after 9 months of culture: 6 months on half-strength Murashige and Skoog (MS/2) medium containing 14.2 µM indole-3-acetic acid (IAA), 13.4 µM 1-naphthaleneacetic acid (NAA) and 0.5 µM 6-(dimethylallylamino) purine (2iP), and 3 months on MS/2 medium supplemented with 1.1 µM IAA, 1.1 µM NAA, 0.5 µM 2iP, 2.2 µM 6-benzyladenine (BA) and 0.4 µM kinetin. Adventitious bud segments were used as explants to induce somatic embryogenesis, and the effects of different concentrations (22.5, 45, 90, 225 or 450 µM) of 3,6-dichloro-o-anisic acid (dicamba) and 4-amino-3,5,6-trichloropicolinic acid (picloram) were evaluated. The optimal medium for somatic embryogenesis induction was MS medium supplemented with 45 µM picloram and 5 µM 2iP, in which the somatic embryogenesis rate was 70%. For somatic embryo maturation, the effects of sorbitol, mannitol, polyethylene glycol (PEG) and abscisic acid (ABA) were tested. The highest maturation rate (88.6 mature somatic embryos per 100 mg fresh weight callus) was observed on liquid MS medium supplemented with 20 g L^{-1} PEG. Subsequent somatic embryo germination was achieved with up to 52.0% in MS medium containing 2.5 µM NAA and 2.5 µM BA. The regenerated plantlets were transferred to the glasshouse where 76.0% of them survived.

Keywords Organogenesis · *Phoenix dactylifera* L. · Regeneration · Somatic embryogenesis

Abbreviations

2iP	6-(Dimethylallylamino) purine
ABA	Abscisic acid
BA	6-Benzyladenine
FW	Fresh weight
IAA	Indole-3-acetic acid
MS	Murashige and Skoog
NAA	1-Naphthaleneacetic acid
NOA	2-Naphthoxyacetic acid
PEG	Polyethylene glycol
PGR	Plant growth regulator

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Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most important tree species in Morocco. In the oasis areas, date palm plays important roles in creating favorable microclimates for agriculture and protecting lands from desertification (Sedra 2015). In addition, date palm cultivation contributes significantly to the incomes of oasis farmers (Sedra 2015). Unfortunately, the Moroccan date palm plantations are threatened by bayoud, a very dangerous disease caused by the fungus *Fusarium oxysporum* f. sp. *albedinis* which killed 10 million palms during the twentieth century and caused the disappearance of many cultivars (Sedra 2015). Presently, the only

way to fight bayoud is using in vitro culture techniques and planting resistant cultivars in the ravaged areas (Ferry 2011). However, the best Moroccan cultivars such as Mejhoul, Boufeggous, and Bouskri are highly susceptible to bayoud. To preserve these cultivars, the strategy used is based on the large-scale propagation through in vitro techniques, then planting regenerants in bayoud-free areas.

Large-scale propagation could be achieved through either organogenesis or somatic embryogenesis (Mazri and Meziani 2015). Propagation through organogenesis consists of initiation of meristematic buds and then their development into whole plantlets (Al-Mazroui et al. 2006). It is the main technique used for date palm propagation in Morocco. Along this line, regeneration systems have been developed for some Moroccan genotypes including Najda (Mazri 2012; Mazri and Meziani 2013), 16-bis (Mazri 2013, 2014), Boufeggous (Mazri 2015), and Mejhoul (Mazri et al. 2016; Meziani et al. 2015, 2016) among others. In many other countries, somatic embryogenesis has been used for mass propagation of date palm and was reported in many cultivars such as Barhee, Zardai, Khalasah, Muzati, Shishi, and Zart (Aslam et al. 2011), Khanizi (Eshraghi et al. 2005), and Boufeggous (Othmani et al. 2009). For both techniques, offshoots are the main source of explants.

Date palm cv. Mejhoul, also called Medjool, is the most popular, most appreciated and most sought after cultivar in the world, and one of the most preferred cultivars by farmers (Sedra 2015). Micropropagation of cv. Mejhoul has been previously established through somatic embryogenesis using offshoot-derived explants (Tisserat 1982). Unfortunately, offshoots of cv. Mejhoul are scarce and very expensive due to the spread of the bayoud disease in the Moroccan palm groves.

To induce somatic embryogenesis in date palm, the auxin 2,4-dichlorophenoxy acetic acid (2,4-D) has been widely used at the concentration of 100 mg L^{-1} (Al-Khayri 2010; Eshraghi et al. 2005). However, it was reported that high concentrations of 2,4-D may induce somaclonal variation within regenerants (Fki et al. 2011). Therefore, inducing somatic embryogenesis in date palm using other auxins at low concentrations would be of great interest. Omar and Novak (1990) reported that 3,6-dichloro-o-anisic acid (dicamba) and 4-amino-3,5,6-trichloropicolinic acid (picloram) have been used successfully in tissue culture of various species with no adverse effects on regenerated plants.

Herein, we present a new regeneration system for date palm cv. Mejhoul through a combined pathway of organogenesis and somatic embryogenesis. The purpose of this study was double: (a) inducing adventitious buds directly on the explant. These adventitious buds can be maintained in vitro through regular subcultures and used as permanently available source of explants; (b) developing an efficient regeneration system through somatic embryogenesis from the adventitious buds using auxins scarcely tested in date palm embryogenesis.

Materials and methods

Bud initiation and multiplication

Shoot tips removed from 3-year-old offshoots of date palm cv. Mejhoul were disinfected according to the protocol used in our laboratories (Mazri et al. 2016). The shoot-tip explants were cultured on half-strength Murashige and Skoog (1962) medium (MS/2) supplemented with 14.2 µM indole-3-acetic acid (IAA), 13.4 µM 1-naphthaleneacetic acid (NAA) and 0.5 µM 6-(dimethylallylamino) purine (2iP) for 6 months and then transferred to MS/2 medium supplemented with 1.1 µM IAA, 1.1 µM NAA, 0.5 µM 2iP, 2.2 µM 6-benzyladenine (BA) and 0.4 µM kinetin for 3 months under dark conditions. Adventitious buds were transferred to the multiplication medium consisting of MS/2 medium supplemented with 0.9 µM 2-naphthoxyacetic acid (NOA), 1.1 µM IAA, 1.8 µM kinetin and 1.9 µM 2iP and were maintained under 16 h photoperiod. Ten offshoots and three explants per offshoot were used in this experiment. All culture media were supplemented with 1 g L^{-1} polyvinylpyrrolidone (Duchefa Biochemie, Haarlem, The Netherlands), 30 g L^{-1} sucrose (Sigma, St. Louis, MO, USA) and solidified with 6 g L^{-1} agar (Sigma, St. Louis, MO, USA). The pH was adjusted to 5.7 before autoclaving at 121 °C for 25 min. The cultures were maintained at 25 °C and transferred to a fresh medium at 30-day intervals.

Somatic embryogenesis

Effects of auxin type and concentration on somatic embryogenesis induction

The effects of two different auxins on somatic embryogenesis were evaluated. Adventitious buds were divided into segments of 0.5 cm length and cultured on MS medium supplemented with 1 g L^{-1} activated charcoal, 30 g L^{-1} sucrose, 5 µM 2iP and various concentrations (22.5, 45, 90, 225 or 450 µM) of dicamba or picloram (induction medium). All media were gelled with 6 g L^{-1} agar (Sigma, St. Louis, MO, USA). The pH was adjusted to 5.7, and 25 ml volumes were dispensed into jars (6.5 cm in diameter and 12 cm in height), before autoclaving at 121 °C for 25 min. Five bud segments were placed per jar and ten replicate jars were used per treatment. The cultures were maintained in the dark at 25 °C for 6 months with subcultures at 1-month intervals. Afterwards, the cultures were transferred to plant growth regulator (PGR)-free MS medium for 1 month (expression medium). The rate of somatic embryogenesis, determined

as the number of calli producing globular embryos per the total number of explants, was recorded at the end of this experiment.

Effects of abscisic acid (ABA) and osmotic solutes on somatic embryo maturation

In this experiment, 100 mg fresh weight (FW) embryogenic callus was transferred to jars containing 20 ml of liquid MS medium supplemented with 0.5 g L^{-1} activated charcoal, 30 g L^{-1} sucrose, 10–50 µM ABA, and/or 30 g L^{-1} sorbitol, mannitol or polyethylene glycol (PEG). Based on the results of this experiment, the effect of different concentrations (5–50 g L^{-1}) of PEG on somatic embryo maturation was evaluated. The pH of all media was adjusted to 5.8 before autoclaving. All the cultures were incubated in the dark at 25° C over a shaker (60 rpm) for a period of 9 weeks with transfers to fresh medium at 3-week intervals. Five to ten replicates were used per treatment and the number of mature somatic embryos was recorded after 9 weeks of culture.

Somatic embryo germination and conversion into plantlets

Mature somatic embryos were transferred to jars containing 25 ml of MS medium supplemented with 0.5 g L^{-1} activated charcoal, 30 g L^{-1} sucrose, 6 g L^{-1} agar and various combinations of NAA and BA. The pH of all media was adjusted to 5.8 before autoclaving. Embryos were cultured under a 16-h-photoperiod at 25 °C with transfers to fresh medium at 1-month intervals. Five jars, each containing five embryos, were used per treatment and the percentage of germinated embryos was recorded after 4 months of culture.

Plantlet acclimatization

Regenerated plantlets with three leaves and well-developed root system were removed from the jars. The root system was washed with tap water and then soaked in a fungicide solution for 15 min (1 g L^{-1} Pelt 44 PM; Bayer CropScience, Bayer Maghreb SA, Casablanca, Morocco). The plantlets were then potted in a mixture of peat and gravel (1:1) for acclimatization. After 2 weeks in a tunnel covered with a polyethylene bag (98% relative humidity, 27 °C), the bag was gradually removed to allow plantlets hardening under glasshouse conditions (70% relative humidity, 27 °C).

Statistical analyses

Experiments were carried out in a completely randomized design. Data were analyzed by ANOVA using SPSS v. 16.0 (IBM, Chicago, IL, USA) and the means were compared using Student–Newman–Keuls test ($P \leq 0.05$). Percentage data were arcsine-transformed prior to analysis.

Results

Bud initiation and multiplication

The media used for bud initiation showed interesting results: after 6 months of culture on MS/2 medium supplemented with IAA, NAA, and 2iP, 70% shoot-tip explants became swollen with yellow-white color (Fig. 1a), while the rest became brown or did not show any response to the culture medium. Transferring explants to the second medium resulted in bud formation (Fig. 1b). After 3 months of culture, 53.3% of the total explants showed shoot buds. The organogenic cultures were then transferred to the multiplication medium, where an average of 12.7 shoot buds per explant was observed after 3 months of culture (Fig. 1c). These organogenic cultures can be maintained in the multiplication medium with regular subcultures and used as a source of explants to induce somatic embryogenesis (Fig. 1d).

Somatic embryogenesis induction

After 2 months of culture on induction media, white friable calli were visible at the cut-end of the bud segments in contact with medium before invading the complete explant surface. After 6 months on the induction medium and 1 month on the expression medium, embryogenic calli were observed. They were white or yellow and friable with globular embryos (Fig. 2a). Somatic embryogenesis varied significantly depending on the culture medium used. The highest rate of somatic embryogenesis (78.0%) was observed on MS medium supplemented with 225 µM picloram. However, there was no significant difference with media containing 45, 90 and 450 µM picloram, which showed somatic embryogenesis rates of 70.0, 72.0 and 74.0%, respectively (Table 1). On the other hand, the use of dicamba showed very low rates of somatic embryogenesis (4.0–20.0%).

Somatic embryo maturation

The highest number of mature somatic embryos (88.0) was observed on MS medium supplemented with 30 g L^{-1} PEG (Fig. 2b; Table 2). Embryogenic calli cultured on ABA-free media produced significantly more mature embryos than those cultured on media containing ABA. In fact, the culture media supplemented with ABA showed few mature embryos (9.2–16.2) after 9 weeks of culture, while the majority of somatic embryos were still at the globular stage. MS medium with neither ABA nor osmotic solutes

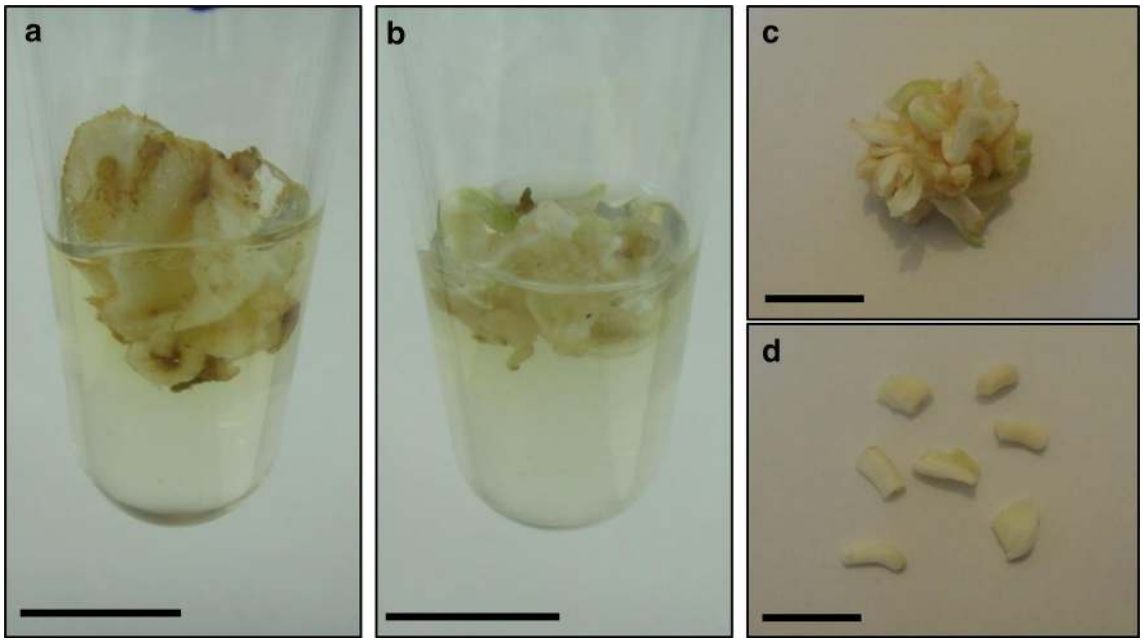

Fig. 1 Adventitious bud formation in date palm cv. Mejhoul. **a** Explant swelling after 6 months of culture on MS/2 medium supplemented with 14.2 µM IAA, 13.4 µM NAA and 0.5 µM 2iP. **b** Adventitious bud formation after 3 months of culture on MS/2 medium supplemented with 1.1 µM IAA, 1.1 µM NAA, 0.5 µM 2iP, 2.2 µM BA and 0.4 µM kinetin. **c** Bud multiplication after 3 months of culture on MS/2 medium supplemented with 0.9 µM NOA, 1.1 µM IAA, 1.8 µM kinetin and 1.9 µM 2iP. **d** Bud explants used for somatic embryogenesis induction. Bars correspond to 1 cm

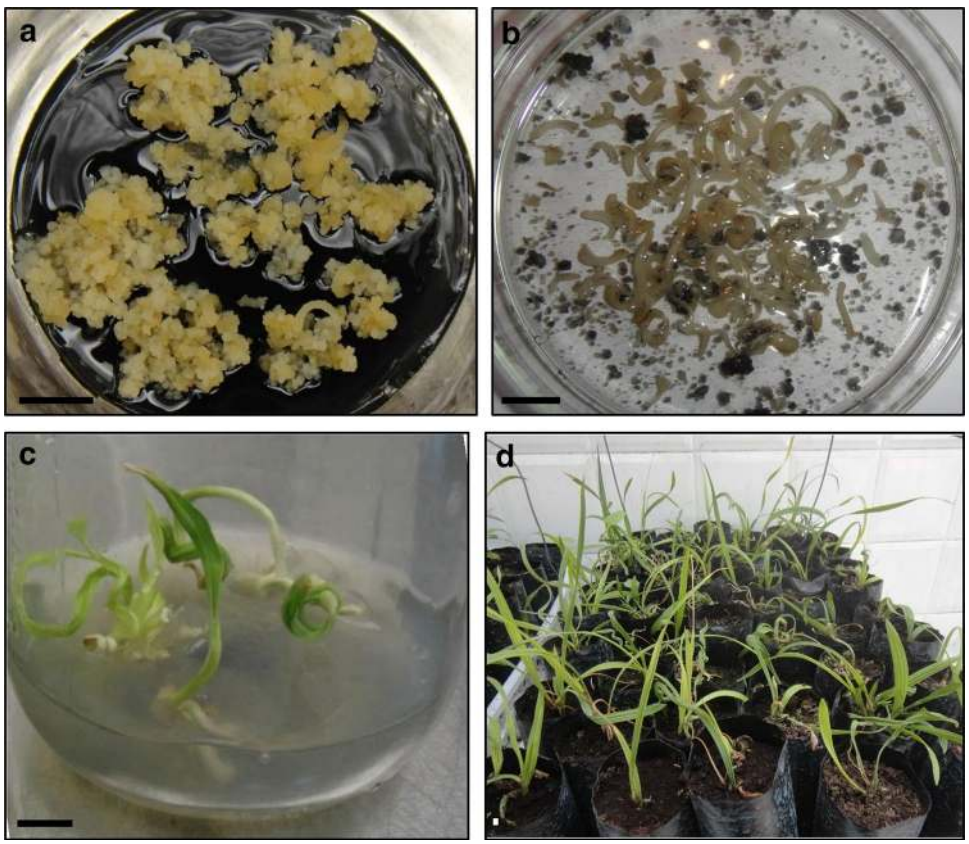

Fig. 2 Somatic embryogenesis and plantlet regeneration in date palm cv. Mejhoul. **a** Embryogenic callus induction after 6 months of culture on MS medium containing 45 µM picloram and 5 µM 2iP and 1 month on PGR-free MS medium. **b** Mature somatic embryos after 9 weeks of culture on liquid MS medium containing 30 g L^{-1} PEG. **c** Embryo germination after 2 months of culture on MS medium containing 2.5 µM NAA and 2.5 µM BA. **d** Plantlet survival after 3 months in the glasshouse. Bars correspond to 1 cm

Table 1 Somatic embryogenesis after 6 months of culture on MS medium containing various concentrations of picloram and dicamba and 1 month on PGR-free MS medium

PGRs in the induction medium			Somatic embryogenesis (%)
Picloram (µM)	Dicamba (µM)	2iP (µM)	
22.5	–	5	44.0 ± 15.7 b
45	–	5	70.0 ± 16.9 c
90	–	5	72.0 ± 13.9 c
225	–	5	78.0 ± 17.5 c
450	–	5	74.0 ± 23.1 c
–	22.5	5	4.0 ± 8.4 a
–	45	5	10.0 ± 10.5 a
–	90	5	12.0 ± 10.3 a
–	225	5	14.0 ± 9.6 a
–	450	5	20.0 ± 16.3 a

Data correspond to means ± standard deviations. Means with the same letter are not significantly different at 0.05 probability level

Table 2 Effect of sorbitol, mannitol, PEG and different concentrations of ABA on somatic embryo maturation after 9 weeks of culture

Maturation medium				Number of mature somatic embryos
ABA (µM)	Sorbitol (g L^{-1})	Mannitol (g L^{-1})	PEG (g L^{-1})	
–	–	–	–	43.8 ± 9.4 b
10	–	–	–	12.8 ± 1.9 a
30	–	–	–	11.4 ± 3.0 a
50	–	–	–	9.2 ± 3.0 a
–	30	–	–	56.2 ± 12.0 c
–	–	30	–	61.4 ± 4.6 c
–	–	–	30	88.0 ± 4.0 d
10	30	–	–	15.6 ± 4.0 a
30	30	–	–	12.2 ± 2.8 a
50	30	–	–	10.2 ± 3.1 a
10	–	30	–	12.6 ± 6.8 a
30	–	30	–	12.0 ± 4.9 a
50	–	30	–	9.8 ± 1.3 a
10	–	–	30	16.2 ± 3.5 a
30	–	–	30	13.4 ± 3.0 a
50	–	–	30	12.6 ± 1.8 a

Data correspond to means ± standard deviations. Means with the same letter are not significantly different at 0.05 probability level

produced 43.8 mature embryos while ABA-free media supplemented with sorbitol and mannitol showed 56.2 and 61.4 mature embryos, respectively. Mannitol showed a high level of embryo browning, which subsequently led to their death.

The concentration of PEG had a significant effect on somatic embryo maturation. The optimal concentration of PEG was 20 g L^{-1} which yielded 88.6 mature embryos. Increasing PEG concentration to 40 and 50 g L^{-1} did not promote somatic embryo maturation (Table 3).

Table 3 Effect of different concentrations of PEG on somatic embryo maturation after 9 weeks of culture

Maturation medium PEG (g L^{-1})	Number of mature somatic embryos
5	53.6 ± 12.1 a
10	73.9 ± 10.0 b
20	88.6 ± 5.9 d
30	88.0 ± 4.0 d
40	83.6 ± 4.4 cd
50	76.8 ± 7.8 bc

Data correspond to means ± standard deviations. Means with the same letter are not significantly different at 0.05 probability level

Somatic embryo germination and plantlet acclimatization

The highest rate (52.0%) of somatic embryo germination (Fig. 2c) was observed on MS medium supplemented with 2.5 µM NAA and 2.5 µM BA, with no significant difference with media containing 5.0 µM NAA–2.5 µM BA; 2.5 µM NAA–5.0 µM BA and 5.0 µM NAA–5.0 µM BA (Table 4). The addition of either NAA or BA alone to the culture medium showed lower germination frequencies. Some of the embryos developed shoots without roots or formed callus instead of roots, while 28.0–84.0% of somatic embryos turned brown and died (Table 4). Successfully germinated embryos were transferred to the glasshouse where 76.0% of them survived after 3 months (Fig. 2d).

Discussion

In the present study, we report a successful regeneration system for date palm cv. Mejhoul using a combination of organogenesis and somatic embryogenesis. Adventitious buds were developed from shoot-tip explants with a rate of 53.3%. Studies on adventitious bud initiation in date palm are scarce, and different PGR combinations were suggested depending on the genotype. Al-Mayahi (2014) suggested MS medium supplemented with 1 mg L^{-1} BA and 0.5 mg L^{-1} TDZ to induce organogenesis in date palm cv. Hillawi. Bekheet (2013) used MS medium supplemented with 1 mg L^{-1} NAA and 2 mg L^{-1} 2iP to induce organogenesis in cv. Zaghlool. Hussain et al. (2001) induced adventitious buds from the explants of cvs. Asil, Hussaini and Zaidi using MS medium supplemented with 4 mg L^{-1} IBA and 1 mg L^{-1} BA, while Khierallah and Bader (2007) suggested

Table 4 Effect of different concentrations of NAA and BA on somatic embryo germination

PGRs in the germination medium		Germination response		
NAA (μM)	BA (μM)	Somatic embryos converted into plantlets (%)	Somatic embryos forming shoot only (%)	Somatic embryos turned brown and died (%)
0	0	8.0 ± 10.9 a	8.0 ± 10.9 a	84.0 ± 16.7 b
2.5	0	28.0 ± 17.8 ab	12.0 ± 17.8 a	60.0 ± 14.1 ab
5.0	0	24.0 ± 8.9 ab	12.0 ± 10.9 a	64.0 ± 26.0 ab
0	2.5	12.0 ± 17.8 a	32.0 ± 30.3 a	56.0 ± 16.7 ab
2.5	2.5	52.0 ± 22.8 b	12.0 ± 10.9 a	36.0 ± 26.0 a
5.0	2.5	48.0 ± 10.9 b	20.0 ± 20.0 a	32.0 ± 22.8 a
0	5.0	16.0 ± 16.7 ab	48.0 ± 30.3 a	36.0 ± 38.4 a
2.5	5.0	52.0 ± 26.8 b	20.0 ± 14.1 a	28.0 ± 17.8 a
5.0	5.0	52.0 ± 26.8 b	20.0 ± 14.1 a	28.0 ± 22.8 a

Data correspond to means ± standard deviations. Means with the same letter in the same column are not significantly different at 0.05 probability level

MS medium supplemented with 1 mg L^{-1} NAA, 1 mg L^{-1} NOA, 2 mg L^{-1} 2iP and 1 mg L^{-1} BA for cv. Maktoom. In the present study, shoot-tip explants were cultured on MS/2 medium supplemented with 14.2 μM IAA, 13.4 μM NAA, and 0.5 μM 2iP for 6 months then transferred to MS/2 medium supplemented with 1.1 μM IAA, 1.1 μM NAA, 0.5 μM 2iP, 2.2 μM BA, and 0.4 μM kinetin for 3 months. The use of different PGR combinations may be explained by different requirements among date palm cultivars. Along this line, Jain (2012) reported that organogenesis in date palm is highly genotypic dependent.

Somatic embryogenesis was induced from adventitious buds developed and maintained in vitro. Interestingly, high somatic embryogenesis rates were obtained. To the best of our knowledge, this is the first time that somatic embryogenesis is reported in date palm cv. Mejhoul from adventitious bud segments. Such explants were successfully used to induce somatic embryogenesis in date palm cv. Najda (Mazri et al. 2017). Our results showed that the rate of somatic embryogenesis varies depending on the auxin type and concentration. In fact, picloram at 45–450 μM resulted in the highest somatic embryogenesis rates. Picloram has been scarcely used to induce somatic embryogenesis in date palm. Othmani et al. (2009) used picloram at different concentrations to induce somatic embryogenesis from juvenile leaves of date palm cv. Boufeggous and reported that this auxin failed to produce embryogenic callus. This is not consistent with our findings. Khierallah et al. (2015) succeeded to produce embryogenic calli in date palm cv. Bream using 50 mg L^{-1} picloram. More recently, we succeeded to induce somatic embryogenesis in cv. Najda using 45 μM picloram (Mazri et al. 2017). These conflicting results may be related to the genotype. Indeed, it is well known that in date palm, somatic embryogenesis is influenced by the genotype. On the other hand, dicamba showed significantly lower somatic embryogenesis rates than picloram. Dicamba has been scarcely used in date palm somatic embryogenesis. Omar and Novak (1990) used dicamba to sustain and produce embryos on calli already established in medium containing 2,4-D. However, these calli failed to produce somatic embryos. The different responses observed with the different auxins used are due to the different degree of activity of each auxin (Davies 2004). In fact, the added auxins interfere with explant endogenous hormones and affect their levels (Gaspar et al. 1996). This creates stress conditions that promote embryogenic transition in somatic cells (Fehér et al. 2003). The observed differences among auxins could be the result of different signal transduction involved (Pacurar et al. 2014).

In previous studies on date palm somatic embryogenesis, the maturation-promoting effect of liquid media has been reported. Fki et al. (2003) indicated that 200 mature somatic embryos were produced per 100 mg FW callus on liquid medium while only 10 mature somatic embryos were observed on solid medium. Thus, many other authors have used liquid media to enhance somatic embryo maturation, e.g., Zouine and El Hadrami (2007) for date palm cvs. Jihel and Bousthami Noir, Abohatem et al. (2011) for cvs. Boufeggouss and Bouskri, Al-Khayri and Al-Bahrany (2012) for cv. Nabout Saif, and Boufis et al. (2014) for cv. Degla Beida. George et al. (2008) reported that somatic embryos undergo faster growth in liquid medium than on agar-solidified medium. Our results revealed that osmotic solutes promote somatic embryo maturation, and that PEG is more suitable for embryo maturation than sorbitol and mannitol. This is in good agreement with Yaseen et al. (2013), who mentioned that PEG permits quick maturation of somatic embryos. In date palm cv. Nabout Saif, Al-Khayri and Al-Bahrany (2012) reported that increasing PEG concentration in the culture medium to 10 and 15% increased

the percentage of large size (6–8 mm) and extra large size (> 8 mm) somatic embryos, respectively. On the other hand, our data showed that ABA does not promote somatic embryo maturation. This is in good agreement with the findings of Al-Khayri and Al-Bahrany (2012), according to which increasing the concentration of ABA increased the percentage of small-size embryos (< 3 mm) of date palm cv. Nabout Saif and hampered embryo growth. However, Zouine et al. (2005) reported that ABA at the concentration of 10^{-5} M increased sugar and total protein accumulation in somatic embryos of date palm cvs. Bousthami Noir and Jihel.

In the present study, the highest rate (52.0%) of somatic embryo germination was observed on MS medium supplemented with 2.5 µM NAA and 2.5 µM BA. In previous studies, many factors have been associated with somatic embryo germination. Zouine et al. (2005) found that activated charcoal promotes somatic embryo germination in date palm cvs. Bousthami Noir and Jihel. Ibrahim et al. (2012) reported that genotype and desiccation influence embryo germination in cvs. Malkaby and Barhee. The size of somatic embryos (Al-Khayri and Al-Bahrany 2012) and the texture of the culture medium (Boufis et al. 2014) were also reported to influence somatic embryo germination in date palm. As regards to PGRs, Othmani et al. (2009) suggested 1 mg L^{-1} NAA for date palm cv. Boufeggous while Zouine and El Hadrami (2007) used a combination of NAA, IBA and BAP to enhance somatic embryo germination in date palm cvs. Bousthami Noir and Jihel.

Plantlet acclimatization was successfully achieved with a high survival rate of 76.0%. Different survival rates were reported in the literature, depending on the cultivar. For example, in date palm cv. Boufeggous, a survival rate of 60% was reported by Othmani et al. (2009) while Al-Khayri (2010) obtained a survival rate of 72–84% in cvs. Khasab and Nabout Saif. In cv. Najda, a survival rate of 80% was reported (Mazri et al. 2017). A high rate of acclimatization is a good indicator of a successful micropropagation system.

Conclusions

We developed an efficient regeneration system for date palm cv. Mejhoul via a combination of organogenesis and somatic embryogenesis. Adventitious buds maintained in vitro proved to be very interesting explants for somatic embryogenesis. The formation of embryogenic calli was affected by auxin type and concentration, and the use of picloram resulted in high rates of embryogenesis. The use of PEG promoted somatic embryo maturation while a combination of NAA and BA showed an acceptable rate of embryo germination. This regeneration system will be valuable for large-scale propagation of date palm cv. Mejhoul. We are currently assessing the genetic stability of the regenerated plants using molecular markers.

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Compliance with ethical standards

Conflict of interest We declare that we have no conflict of interest.

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