

Somatic embryogenesis from bud and leaf explants of date palm (*Phoenix dactylifera* L.) cv. Najda

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Abstract An efficient regeneration system through somatic embryogenesis was developed for date palm cv. Najda. Adventitious bud and proximal leaf segments cultured on Murashige and Skoog (MS) medium supplemented with various combinations of auxins and cytokinins induced embryogenesis after at least 6 months of culture. Somatic embryogenesis induction seemed correlated with the type of the explant, the induction period and the auxin used. The highest rate of somatic embryogenesis (86.0%) was obtained on bud explants cultured on MS medium supplemented with 45.0 μM 2,4-dichlorophenoxyacetic acid (2,4-D), and 4.5 μM kinetin or 4.5 μM 6-(dimethylallylamino) purine (2iP). Whereas, low levels of embryogenesis were obtained on media supplemented with 1-naphthalene acetic acid (NAA) or 2-naphthoxyacetic acid (NOA). Proximal leaf segments showed somatic embryogenesis only when cultured on media supplemented with 2,4-D or picloram. Statistical analysis revealed

significant effects of explant type and plant growth regulators (PGRs) combination on somatic embryogenesis. Somatic embryos were germinated successfully on PGR-free MS medium with or without activated charcoal (50.0–60.0 and 26.6–36.6%, respectively), and 80.0% of plantlets survived after transferring to a glasshouse for 6 months. Our results will be useful for large-scale propagation of date palm cv. Najda, characterized by high fruit quality and bayoud disease resistance.

Keywords *Phoenix dactylifera* L. · Plant growth regulator · Regeneration · Somatic embryogenesis

Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
2iP	6-(Dimethylallylamino) purine
AC	Activated charcoal
FW	Fresh weight
MS	Murashige and Skoog
NAA	1-Naphthalene acetic acid
NOA	2-Naphthoxyacetic acid
PGR	Plant growth regulator

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Introduction

Date palm (*Phoenix dactylifera* L.) is a very important crop species in the Middle East and North Africa; where it plays an essential role both in generating employment and in creating equitable oasis ecosystems (Jain 2012). In Morocco, the total area devoted to growing date palm is around 50,000 ha with 5.4 million trees (Sedra 2015). Unfortunately, bayoud disease caused by the soil fungus *Fusarium*

oxysporum f. sp. *albedinis* ravages a large part of this area. In fact, over the last century, bayoud has decimated more than 10 million trees in Morocco (Sedra 2011). To date, the only efficient way to combat bayoud and rehabilitate ravaged areas is by micropropagating bayoud-resistant cultivars (Ferry 2011). The best Moroccan commercial varieties are susceptible to bayoud (Sedra 2011) and could not be used to rejuvenate ravaged areas. Therefore, several high quality genotypes of date palm have been identified and selected (Sedra 2011), including cv. Najda (INRA-3014), which has been distributed and well accepted by farmers (Ferry 2011; Sedra 2011).

To achieve a large-scale propagation for cv. Najda, in vitro regeneration protocols via adventitious organogenesis were established (Mazri 2012; Mazri and Meziani 2013). Propagation through organogenesis was reported to be very interesting mainly because it permits the preservation of true-to-type of multiplied plants (Al-Khateeb 2006; Sedra 2005). Along this line, organogenesis has been chosen and used to produce date palm plantlets in Morocco (Ferry 2011; Meziani et al. 2015). As far as cv. Najda is concerned, more than 800,000 plants were produced and distributed to farmers (Sedra 2015). However, organogenesis has lower multiplication efficiency than somatic embryogenesis, and rehabilitating areas ravaged by bayoud would require several million trees (Ferry 2011). Thus, somatic embryogenesis seems to be the best approach to reach this purpose.

Somatic embryogenesis is the process by which somatic cells develop into differentiated somatic embryos. It comprises several steps: (a) formation of embryonic masses, (b) somatic embryo development, (c) maturation, (d) and germination (Mazri and Meziani 2015; von Arnold et al. 2002). However, this regeneration pathway depends greatly on many factors such as genotype, explant type and culture conditions (Gaj 2004). Somatic embryogenesis of date palm has been reviewed, and a strong influence of the genotype was reported (Fki et al. 2011; Zaid et al. 2011). Therefore, investigations are needed to perform and adapt culture media considering the plant genotype used (Abohatem et al. 2011). Regarding the cultivar Najda, somatic embryogenesis has never been reported.

Somatic embryogenesis was reported in date palm mostly from offshoot-derived explants (e.g., El Fatih and Badereldin 2010; Hassan and Taha 2012; Othmani et al. 2009a) and inflorescence tissues (e.g., Abul-Soad and Mahdi 2010; Kriaa et al. 2012). However, these approaches suffer from the limited number or even the unavailability of offshoots in some genotypes (Fki et al. 2011) and the restricted availability of spathes (Abul-Soad 2011). Moreover, bacterial contamination constitutes a limit since it could lead to a great loss. Hence, another approach

avoiding such problems could be proposed. Indeed, somatic embryogenesis could be achieved from adventitious buds and shoots developed and maintained in vitro. This innovative approach could offer great benefits. For instance, it avoids: (a) using offshoots excessively in case of limited production; (b) causing damage to the mother tree and (c) affecting fruit production by an excessive use of spathes. In addition, such technique uses a material source free of diseases and available independently of seasons.

Despite the fact that somatic embryogenesis from explants grown and maintained in vitro was successfully reported in many plant species (e.g., rhizomatous *Iris* (Laublin et al. 1991), *Camellia sinensis* O. Kunze (Kato 1996), *Rosa hybrida* and *Rosa multiflora* (Kim et al. 2004), *Pyrus communis* L. (Javadi et al. 2013) and *Olea europaea* L. (Mazri et al. 2013)), this approach has been scarcely studied in date palm (Al-Mayahi 2015). Therefore, the aim of this investigation was to develop a reproducible somatic embryogenesis pathway from buds and shoots maintained in vitro of date palm cv. Najda, adaptable for mass propagation and genetic transformation of this cultivar. Toward this end, several factors affecting the somatic embryogenesis process such as explant source, plant growth regulators (PGRs) and induction period were evaluated.

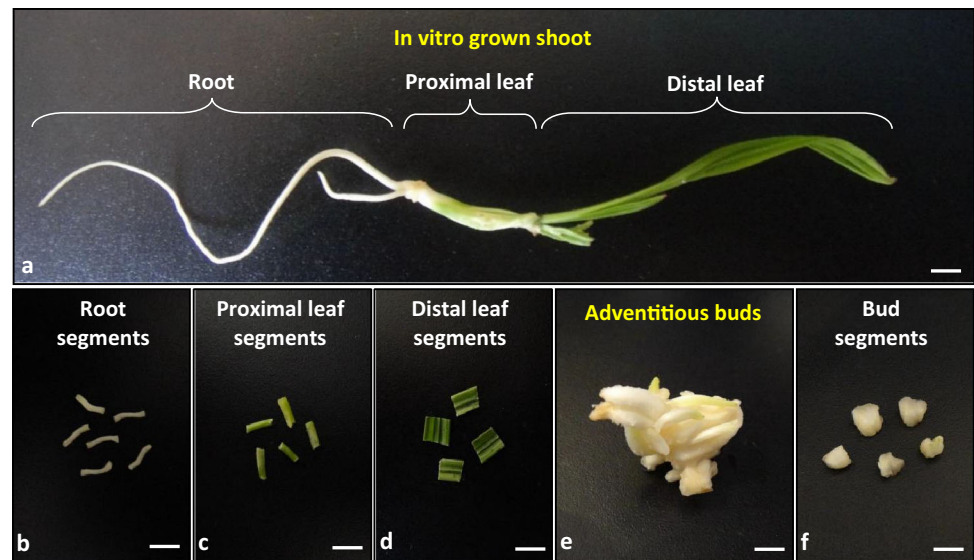
Materials and methods

Plant material and culture conditions

Adventitious buds of cv. Najda, obtained as described by Beauchesne et al. (1986) and different parts (distal leaf, proximal leaf, and root) of in vitro grown shoots obtained as described by Mazri and Meziani (2013) were used as explants. The adventitious buds were obtained after 12 months of culture (9-month initiation and 3-month multiplication) while the shoots were obtained after 15 months of culture (9-month initiation, 3-month multiplication and 3-month elongation-rooting). The explants were cut into 0.5 cm length segments (Fig. 1). Leaf segments were placed with the abaxial surface on the culture medium.

The mineral medium used was MS medium (Murashige and Skoog, 1962) supplemented with 30 g L⁻¹ sucrose (Sigma, Steinheim, Germany) and gelled with 6 g L⁻¹ agar (Sigma, St. Louis, MO, USA). The pH was adjusted to 5.7 with NaOH or HCl (1 N) then the medium was distributed in 30 mL aliquots in 300 mL jars (6.5 cm in diameter and 12 cm in height). The culture medium was autoclaved for 25 min at 121 °C. The cultures were transferred onto fresh medium every month.

Fig. 1 Origin of the explants used to induce somatic embryogenesis. **a** Shoot obtained through in vitro organogenesis used as source of explant. **b** Root segments. **c** Proximal leaf segments. **d** Distal leaf segments. **e** Adventitious buds obtained through in vitro organogenesis used as source of explant. **f** Bud segments. Bars correspond to 0.5 cm



Callus induction

Eight media sequences (M1–M8) were tested to induce somatic embryogenesis (Table 1). The explants were cultured on MS medium supplemented with 1 g L⁻¹ activated charcoal (AC) and various combinations of 45 μM auxin (2,4-dichlorophenoxyacetic acid (2,4-D); 1-naphthalene acetic acid (NAA); 2-naphthoxyacetic acid (NOA) or picloram) and 4.5 μM cytokinin (6-(Dimethylallylamino) purine (2iP) or kinetin). All PGRs were purchased from Sigma, Steinheim, Germany. The cultures were incubated for 4–6 months in darkness at 25 °C.

Somatic embryogenesis expression

Induced calli were transferred to the expression medium which consisted of MS medium basal formulation supplemented with 1 g L⁻¹ AC and 10% PGRs concentration of the induction medium (Table 1). The cultures were maintained for one month under dark conditions at 25 °C.

Somatic embryo maturation and germination

Embryogenic cultures were divided into 100 mg fresh weight (FW) calli then transferred to maturation medium, which consisted of PGR-free MS basal medium supplemented with 1 g L⁻¹ AC, under dark conditions at 25 °C for 2 months. Tubular embryos were then transferred to PGR-free MS medium, with or without 1 g L⁻¹ AC, at 25 °C under a 16 h light photoperiod for germination. Shoots from the germinated embryos were cultured for 3 months (25 °C, 16/8 photoperiod) on PGR-free MS medium supplemented with 1 g L⁻¹ AC.

Plantlets transplantation

Plantlets with 3–4 leaves and elongated roots were removed from the culture medium; the root system was washed in running tap water then soaked for 15 min in a solution of 1 g L⁻¹ Pelt 44 PM systemic fungicide (Bayer CropScience, Bayer Maghreb SA, Casablanca, Morocco).

Table 1 Composition of media used for embryogenic calli induction and embryo regeneration. All media were supplemented with 30 g L⁻¹ sucrose, 1 g L⁻¹ activated charcoal and 6 g L⁻¹ agar

Media sequence	Induction medium	Expression medium	Maturation medium
M1	MS + 45 μM 2,4-D + 4.5 μM 2iP	MS + 4.5 μM 2,4-D + 0.45 μM 2iP	MS
M2	MS + 45 μM 2,4-D + 4.5 μM kinetin	MS + 4.5 μM 2,4-D + 0.45 μM kinetin	MS
M3	MS + 45 μM NAA + 4.5 μM 2iP	MS + 4.5 μM NAA + 0.45 μM 2iP	MS
M4	MS + 45 μM NAA + 4.5 μM kinetin	MS + 4.5 μM NAA + 0.45 μM kinetin	MS
M5	MS + 45 μM NOA + 4.5 μM 2iP	MS + 4.5 μM NOA + 0.45 μM 2iP	MS
M6	MS + 45 μM NOA + 4.5 μM kinetin	MS + 4.5 μM NOA + 0.45 μM kinetin	MS
M7	MS + 45 μM picloram + 4.5 μM 2iP	MS + 4.5 μM picloram + 0.45 μM 2iP	MS
M8	MS + 45 μM picloram + 4.5 μM kinetin	MS + 4.5 μM picloram + 0.45 μM kinetin	MS

The plantlets were planted in a mixture of peat-gravel substrate (1:1, w/w) in plastic bags, then sprayed with 0.5 g L^{-1} Pelt 44 PM solution. Thirty days later, plantlets were transferred to open benches in the glasshouse with $27 \text{ }^\circ\text{C}$ and 70% relative humidity.

Data collection and statistical analysis

Observations on the following variables were performed: percentage of explants with callus after 4–6 months on the induction medium; percentage of embryogenic cultures after 1 month on the expression medium; mean number of tubular somatic embryos per 100 mg FW embryogenic culture after 2 months on the maturation medium; percentage of somatic embryos germinated after 2 months on the germination medium and percentage of plantlet survival after 6 months in the glasshouse.

For callus induction and somatic embryogenesis expression, we used 5 explants per jar. Each jar was considered a replicate and each treatment was replicated 10 times. The mean number of tubular somatic embryos was averaged from 10 calli (100 mg weight each) per experiment. For somatic embryo germination, 6 isolated tubular embryos were cultured per jar, considered as one replicate, for each treatment, we used 5 replicates. Data were examined using analysis of variance (ANOVA). The experimental design used in this research was completely randomized design. Statistical analysis was performed using SPSS v. 16.0 (SPSS Inc., IBM, Chicago, IL, USA) and the means were separated by Student–Newman–Keuls test. Percentage data were subjected to arcsine transformation prior to statistical analysis.

Results

Callus induction

Calli started to appear during the fourth week of culture on induction media. After 4 months of culture, the callus formation rate remained stable while callus weight kept increasing. Bud explants showed significantly ($P \leq 0.05$) higher formation of callus than shoot-derived explants. The highest percentage (90.0%) of bud explants producing callus was observed on MS medium supplemented with $45 \text{ } \mu\text{M}$ 2,4-D and $4.5 \text{ } \mu\text{M}$ 2iP. Proximal leaf, distal leaf and root explants showed callus formation rates of 36.0–60.0, 12.0–28.0 and 8.0–20.0%, respectively. However, not all of the calli produced were embryogenic. Callus induced on root and distal leaf was limited to explant borders. All the explants showed white or pale-yellow, friable and granular calli.

Effect of induction period on somatic embryogenesis induction

Explants incubated for 4–5 months in the induction medium produced non-morphogenic calli. Indeed, when transferred onto the expression medium, callus growth decreased and no somatic embryo was observed. However, explants incubated for 6 months in the induction medium then transferred to the expression medium formed globular embryos on their surface (Fig. 2a). Occasionally, root formation could be observed. This shows that for date palm cv. Najda, the switch of cells to the embryonic state occurs after at least 6 months on the induction medium.

Effect of explant type on somatic embryogenesis

After one month of culture in the expression medium, somatic embryogenesis was greatly affected by the explant source. The somatic embryogenesis rate was higher when calli were obtained from bud explants, with a range of 60.0–86.0%. Proximal leaf segments showed embryogenic cultures only when 2,4-D or picloram were used; with a range of 12.0–16.0% (Table 2). Distal leaf and root explants did not show any embryogenic response in culture.

Effect of PGRs on somatic embryogenesis

Somatic embryogenesis was strongly influenced by PGRs. When bud explants were used, somatic embryogenesis occurred on all PGRs combinations (Table 2). There were no significant differences ($P > 0.05$) between 2,4-D (86.0%) and picloram (80.0–82.0%) with regard to embryogenesis expression. However, these auxins induced significantly higher somatic embryogenesis responses than NAA (62.0–68.0%) and NOA (60.0–62.0%). When shoot-derived explants were used, somatic embryos were produced only from proximal leaf calli on media supplemented with 2,4-D (16.0%) or picloram (12.0–14.0%). Within the same explant type, 2iP and kinetin incorporated into the culture medium with the same auxin did not differ significantly in embryogenic culture formation (Table 2).

Somatic embryo maturation, germination and plantlet acclimatization

Somatic embryos in globular stage started to develop into tubular form after transferring them to maturation medium (Fig. 2b). Quantitatively, bud explants produced significantly ($P \leq 0.05$) higher number of tubular somatic embryos per 100 mg FW callus than proximal leaf segments (Table 3). The highest average number of tubular

Fig. 2 Somatic embryogenesis and plantlet regeneration from bud segments of date palm cv. Najda. **a** Embryogenic culture after 6 months on MS medium supplemented with 45 μ M picloram and 4.5 μ M 2iP then 1 month on MS medium supplemented with 4.5 μ M picloram and 0.45 μ M 2iP. **b** Tubular somatic embryos isolated after 2 months on the maturation medium. **c** Somatic embryo germination after 2 months on the germination medium. **d** Shoot elongation and rooting after 3 months of culture on the elongation-rooting medium. Bars correspond to 1 cm

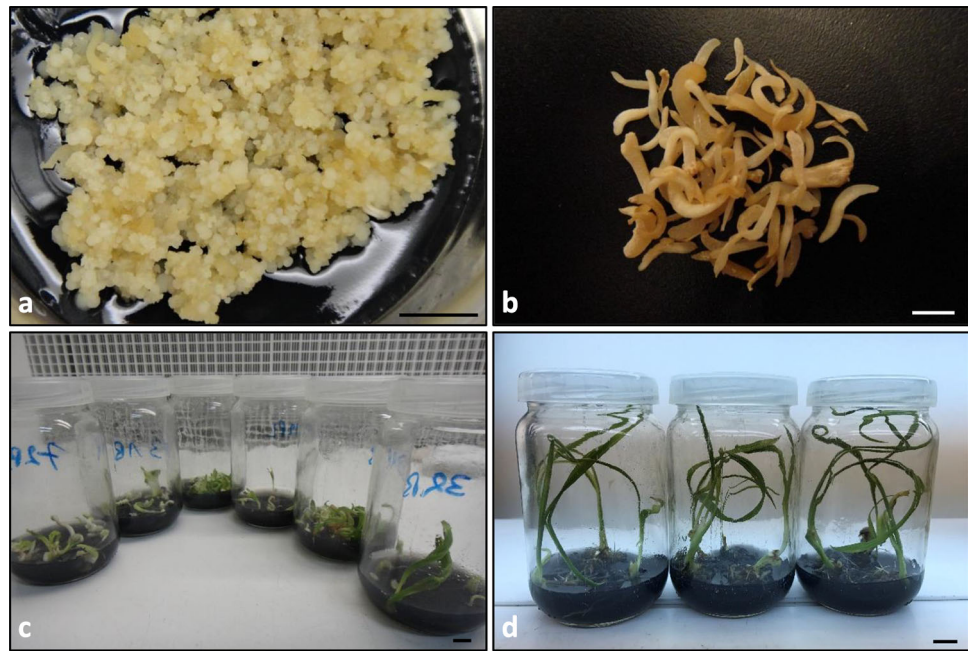


Table 2 Percentage of embryogenic culture ($\% \pm$ SD) after 7 months of culture (6 months on induction medium followed by 1 month on expression medium) as influenced by explant type and media sequence

Media sequence	Percentage of explants forming embryogenic culture (%)			
	B	PL	DL	R
M1	86.0 \pm 13.5 a	16.0 \pm 15.7 c	–	–
M2	86.0 \pm 9.6 a	16.0 \pm 18.3 c	–	–
M3	68.0 \pm 16.8 b	–	–	–
M4	62.0 \pm 22.0 b	–	–	–
M5	60.0 \pm 23.0 b	–	–	–
M6	62.0 \pm 22.0 b	–	–	–
M7	82.0 \pm 19.8 a	14.0 \pm 9.6 c	–	–
M8	80.0 \pm 9.4 a	12.0 \pm 10.3 c	–	–

Mean values with the same letters are not significantly different ($P > 0.05$)

B bud segment, PL proximal leaf segment, DL distal leaf segment, R root segment

somatic embryos formed on bud explant callus (16.4) was observed on media sequence M8, with no significant difference with media sequences M1, M2 and M7 that displayed 15.0, 15.8 and 15.8 tubular somatic embryos per 100 mg FW callus, respectively. Our results showed that PGRs combinations used for somatic embryogenesis induction and expression affected somatic embryo maturation (Table 3). Indeed, there was a significant difference ($P \leq 0.05$) in the mean number of tubular somatic embryos

Table 3 Mean number (\pm SD) of tubular somatic embryos per 100 mg FW callus after 9 months of culture (6 months on induction medium followed by 1 month on expression medium then 2 months on maturation medium) as influenced by explant type and media sequence

Media sequence	Mean number of somatic embryos	
	B	PL
M1	15.0 \pm 1.1 a	4.2 \pm 1.8 c
M2	15.8 \pm 1.5 a	5.4 \pm 1.4 c
M3	9.0 \pm 0.4 b	–
M4	10.2 \pm 1.0 b	–
M5	9.4 \pm 1.7 b	–
M6	10.4 \pm 0.9 b	–
M7	15.8 \pm 1.8 a	4.8 \pm 1.2 c
M8	16.4 \pm 1.7 a	5.2 \pm 0.4 c

Mean values with the same letters are not significantly different ($P > 0.05$)

B bud segment, PL proximal leaf segment

between media sequences M1, M2, M7 and M8, and M3, M4, M5 and M6. As regard to proximal leaf explants, the embryogenic cultures showed an average of 4.2, 5.4, 4.8 and 5.2 tubular somatic embryos per 100 mg FW callus in media sequences M1, M2, M7 and M8, respectively.

The germination stage (Fig. 2c) was achieved with tubular somatic embryos cultivated in PGR-free MS medium with or without AC. Nevertheless, AC improved the germination frequency. The highest germination percentage (60.0%) was obtained when somatic embryos were derived from bud segments in media sequence M8 and germinated on MS medium supplemented with AC. On the

Table 4 Somatic embryo germination (% \pm SD) as influenced by explant type, media sequence and activated charcoal (AC)

Media sequence	Germination frequency on MS medium supplemented with AC		Germination frequency on AC-free MS medium	
	B	PL	B	PL
M1	56.6 \pm 9.1 a	53.3 \pm 13.9 a	36.6 \pm 7.4 b	33.3 \pm 23.5 b
M2	56.6 \pm 9.1 a	50.0 \pm 11.7 a	33.3 \pm 16.6 b	36.6 \pm 21.7 b
M3	53.3 \pm 13.9 a	–	30.0 \pm 24.7 b	–
M4	50.0 \pm 16.6 a	–	30.0 \pm 21.7 b	–
M5	53.3 \pm 13.9 a	–	26.6 \pm 9.1 b	–
M6	56.6 \pm 14.9 a	–	30.0 \pm 13.9 b	–
M7	56.6 \pm 22.3 a	56.6 \pm 22.3 a	33.3 \pm 20.4 b	30.0 \pm 13.9 b
M8	60.0 \pm 9.1 a	53.3 \pm 13.9 a	33.3 \pm 0.0 b	33.3 \pm 16.6 b

Mean values with the same letters are not significantly different ($P > 0.05$)

B bud segment, PL proximal leaf segment



Fig. 3 Plantlets of date palm cv. Najda after 6 months in the glasshouse. Bars correspond to 5 cm

other hand, only 33.3% of somatic embryos obtained from M8 germinated on AC-free MS medium (Table 4). Indeed, the majority of somatic embryos turned brown and died. Embryos obtained from proximal leaf explants showed germination frequencies of 50.0–56.6% on media supplemented with AC and 30.0–36.6% on AC-free media. Shoots from the germinated embryos showed normal growth and rooted well. After 3 months of culture, the average shoot length was 11.3 cm, the average number of roots per shoot was 3.5, and their average length was 2.6 cm (Fig. 2d). The plantlets were then transferred to the glasshouse, where 80.0% survived after 6 months (Fig. 3).

Discussion

For date palm, somatic embryogenesis seems to be a powerful tool for large-scale propagation. However, this regeneration pathway is highly genotype-dependent in this species (Jain 2012). Therefore, it is important to define and

optimize culture conditions for elite date palm cultivars. In Morocco, Najda is an important cultivar characterized by bayoud disease resistance and high fruit quality (Sedra 2011). Developing a regeneration protocol through somatic embryogenesis for this cultivar could help in rehabilitating areas ravaged by the bayoud disease. Up to now, there is no report concerning cv. Najda somatic embryogenesis.

Date palm belongs to recalcitrant species to tissue culture, and establishing *in vitro* regeneration protocols is affected by many factors (Abohatem et al. 2011). In the present study, it has been shown that the induction period affects somatic embryogenesis. In fact, after 4–5 months in the induction medium, obtained calli were non-embryogenic. Embryogenesis was observed on callus which remained in induction medium for at least 6 months. Generally, the induction period varies considerably among genotypes. In fact, Othmani et al. (2009a) reported 6–7 months induction to observe embryogenesis in date palm cv. Boufeggous. Hassan and Taha (2012) reported 9 months induction for cvs. Zaghlol, Amry and Malakaby while Eshraghi et al. (2005) observed embryogenesis in cultivars Khanizi and Mordarsing after a 12-month induction period.

Somatic embryogenesis in date palm has been reported mostly from shoot-tip explants (e.g., El Hadrami et al. 1995; Eke et al. 2005) and inflorescences (e.g., Bhaskaran and Smith 1992; Abul-Soad and Mahdi 2010). Recently, a somatic embryogenesis pathway was described from leaf segments of *in vitro* shoots of date palm cv. Quntar (Al-Mayahi 2015); such explants would be of great benefit for large-scale propagation due to their somatic origin, seasonal independency and availability. In the present study, bud and shoot-derived explants, obtained and maintained *in vitro*, were used; and a significant effect of explant source on somatic embryogenesis has been revealed. The observed differences might be due to various levels of

endogenous growth hormones. Indeed, the level of endogenous hormones is considered as one of the crucial factors influencing embryogenic potential of explants (Gaj 2004), and it varies considerably among explants (Jiménez 2001). Our results showed that bud explants have the highest embryogenesis potential.

We also observed somatic embryogenesis on proximal leaf segments taken from shoots developed *in vitro*. Such response is not common regarding date palm somatic embryogenesis. Gueye et al. (2009a, b) have succeeded to establish callus from seedling leaves of cv. Ahmar. However, embryo formation was not reported. More recently, Al-Mayahi (2015) reported embryo regeneration from leaf explants of cv. Quntar. This novel pathway could certainly open new opportunities for improving *in vitro* propagation of date palm cultivars. Nevertheless, further studies are needed to increase somatic embryogenesis frequency from leaf explants.

Somatic embryogenesis was highly affected by PGRs. In fact, 2,4-D and picloram were found to be more effective than other auxin treatments. Along this line, it was reported that the auxin effect depends on both auxin nature and concentration (Teale et al. 2006). Picloram has been scarcely used to induce somatic embryogenesis in date palm. In a recent study on cv. Bream, Khierallah et al. (2015) succeeded to produce embryogenic calli using 50 mg L⁻¹ picloram. On the other hand, Othmani et al. (2009a) reported that explants of cv. Boufeggous failed to produce embryogenic callus in the presence of picloram. Such difference may be due to the genotype and/or the origin of the explant.

Generally, somatic embryogenesis in date palm was observed when auxins were used at high concentrations; e.g. Al-Khayri (2011), Eke et al. (2005) and Eshraghi et al. (2005) used 100 mg L⁻¹ 2,4-D to induce somatic embryogenesis. Interestingly, El Hadrami et al. (1995) showed that somatic embryos could be observed at a concentration of 5 mg L⁻¹ 2,4-D in cvs. Iklane and Jihel. Othmani et al. (2009a) reported that somatic embryogenesis occurred at 5–10 mg L⁻¹ 2,4-D in cv. Boufeggous. Low concentrations of auxins would be greatly better for genetic stability and cost saving. Our results showed that 45 µM 2,4-D (9.94 mg L⁻¹), NAA (8.37 mg L⁻¹), NOA (9.09 mg L⁻¹) or picloram (10.86 mg L⁻¹) induced embryogenic calli from bud explants of cv. Najda. Lower concentrations did not induce somatic embryogenesis (data not shown).

Our results showed a somatic embryo germination frequency of 26.6–60.0%. In other date palm cultivars, somatic embryo germination was reported with different rates. For example, Al-Khayri and Al-Bahrany (2012) reported germination frequencies ranging from 17.5 to 72.5% in cv. Naboul Saif. Somatic embryos obtained from

cvs. Khasab, Berny and Barhee showed germination frequencies ranging from 60 to 75% (Al-Khayri 2011). In date palm cv. Boufeggous, a germination frequency of 83% was observed (Othmani et al. 2009a). Our results showed that the use of AC increased significantly the germination frequency of somatic embryos. This is in good agreement with Zouine et al. (2005).

Achieving a high survival rate of plantlets under *ex vitro* conditions is primordial for commercial scale propagation (Hazarika 2006). For many species, a considerable number of micropropagated plants do not survive transfer from *in vitro* to *ex vitro* conditions (Kumar and Rao 2012). In date palm micropropagated plants, the survival rate under *ex vitro* conditions depends upon several factors; e.g., genotype, *in vitro* culture conditions and the micropropagation technique. Othmani et al. (2009a, b) reported a survival rate of 60% in date palm cv. Boufeggous and 80% in cv. Deglet Nour. Al-Khayri (2010) observed a survival range of 72–84% in cvs. Khasab and Naboul Saif. In previous works on cv. Najda organogenesis, we demonstrated that the survival rate depends upon the elongation-rooting medium; and a high survival rate of 100% was obtained in plantlets that have been cultured on PGR-free solid medium before acclimatization (Mazri 2012; Mazri and Meziani 2013). High survival rates (88–92.5%) were also observed in other Moroccan cultivars propagated through organogenesis (Mazri 2014, 2015; Mazri et al. 2016; Meziani et al. 2015). In the present study, plantlets transferred to *ex vitro* conditions have exhibited a survival rate of 80%. This is in good agreement with McCubbin and Zaid (2007) who indicated that date palm plantlets regenerated through organogenesis show higher survival rates than those obtained through somatic embryogenesis.

Conclusions

We developed a new and efficient approach for large-scale propagation of date palm cv. Najda through somatic embryogenesis. Bud-derived explants displayed a high embryogenic potential when cultured on media supplemented with 2,4-D or picloram and a high survival rate was obtained after acclimatization. Our protocol will be useful to overcome obstacles encountered with the use of shoot-tip explants or inflorescences and to reconstitute groves destroyed by bayoud disease. Finally, molecular analyses and field evaluation of agro-morphological characteristics are desirable to assess the genetic conformity of regenerants.

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Compliance with ethical standards

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

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