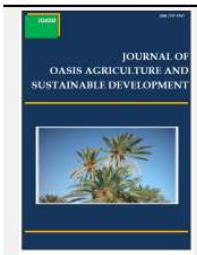

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Morphological and biochemical characterization and *in vitro* regeneration of an elite Tunisian date palm (*Phoenix dactylifera* L.) cultivar 'Boufeggous'

Zammouri Ghofrane[1], Othmani Ahmed[2,4*], Jemni Monia[3], Bayoudh Chokri[4], Sallami Amel[2] & Al-Khayri Jameel M.[5]

[1] *Faculty of Sciences, Gafsa, Tunisia.*
[2] *Laboratory for In Vitro Tissue Culture, Regional Centre for Research in Oasis Agriculture, Tozeur Km1, Degueche 2260, Tunisia.*
[3] *Laboratory for Date Palm Technology, Regional Centre for Research in Oasis Agriculture, Tozeur Km1, Degueche 2260, Tunisia.*
[4] *LR21AGR03-Production and Protection for Sustainable Horticulture (2-PHD), Regional Research Centre on Horticulture and Organic Agriculture Chott Mariem, University of Sousse, Sousse 4042, Tunisia.*
[5] *Department of Agricultural Biotechnology, College of Agriculture and Food Sciences, King Faisal University, Al-Ahsa 31982, Saudi Arabia.*

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***Correspondingauthor**
ahmedothmani630@gmail.com

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Abstract

This study was conducted to investigate morphological and biochemical characteristics and potential for in vitro regeneration of the elite and extremely rare Tunisian date palm cultivar 'Boufeggous' grown at Tozeur oasis. The results revealed that 'Boufeggous' dates are heavier (19.83 versus 11.95 g), longer (50.1 versus 42.76 mm), wider (23.3 versus 18.63 mm), and higher pulp/fruit ratio (91.96 versus 89.06%) than 'Deglet Nour'. Chemical composition study showed that 'Boufeggous' is predominantly rich in sugar and total soluble solids which could explain its unique sweetness and high energy value. The moisture content, pH level, titrable acidity and water activity were 23.16 ± 0.54, 5.3 ± 0.13, 1.7 ± 0.01 and 0.73 ± 0.01, respectively. In terms of minerals, 'Boufeggous' is rich in K and Na. In vitro regeneration on Murashige and Skoog (1962) medium comprising 1 mg/l 2,4-Dichlorophenoxyacetic acid appears to be the most effective stimulant of somatic embryogenesis and shoot organogenesis on both shoot tips and young leaf explants. Thidiazuron only acts on young leaves by stimulating embryogenic callus production with a frequency of 30%.

1. INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is widely cultivated in North African and Near Eastern countries. It constitutes the keystone species of the oasis system where it plays a considerable socio-economic and ecological role (Toutain, 1996). Presently, dates are marketed and consumed worldwide due to their dietary benefits and energizing role (Food and Agriculture Organization, 2022). Nevertheless, similar to other major producing countries, Tunisian date palm production is not only threatened by various biotic stresses (Bou Faroua disease, 'Bayoud' disease, brittle leaf disease, red palm weevil, date moth, etc.) and abiotic stresses (drought, changes in agrarian systems, intensive urbanization, climate change, soil salinization, etc.), but also by severe genetic erosion due to the establishment of mono-variety palm groves, dictated by very favorable commercial circumstances for the 'Deglet Nour' with a demanding market and a fairly high price, is now very advanced in the regions of Nefzaoua, Djerid and Gafsa. This results in the rarefaction or even total disappearance of many cultivars that do not meet current economic requirements, yet are well adapted to local conditions. Therefore, the preservation of the local date palm genetic resources is essential and has become a key priority for the various actors in this agronomic sector. To achieve these objectives, date palm growers propagate elite genotypes either by seeds or by traditional offshoots. It is well known that propagation by seeds produces heterogeneous plantations, with female plants identifiable only at a later stage, whereas propagation by offshoots remains slow,

limits genetic diversity, and can transmit diseases. The idea of creating a gene bank like that proposed by Bedjaoui et al. (2025) is also interesting, but it does not solve the problem. Taking these considerations into account, and with the aim of overcoming these difficulties, the use of in vitro culture techniques, whether via organogenesis or somatic embryogenesis, has been very successful and have enabled the rapid and large-scale multiplication of numerous selected date palm genotypes in date palm. Considering these factors, in vitro culture technique via organogenesis or somatic embryogenesis have proven highly effective, enabling the rapid and large-scale multiplication of numerous selected date palm genotypes.

This study aims firstly to highlight the good date quality of the Tunisian 'Boufeggous' cultivar (a cultivar completely different from Moroccan 'Boufeggous') by analyzing its morphological and biochemical characteristics and comparing them with those of 'Deglet Nour' variety grown in the same oasis, the best variety in Tunisia and secondly to optimize the technique of multiplication by in vitro tissue culture. The choice of this cultivar is based on three major reasons: (i) the superior quality of its dates (Rhouma, 1994), (ii) its great rarity (Rhouma, 1994) and (iii) especially its late ripening, generally between October and November. This last feature is particularly important because it helps the cultivar adapt to climate change, which threatens the sustainability of oasis ecosystems. Indeed, in recent years, the critical period from late August to early September is now marked by very high temperatures (45–50 °C). These extreme conditions can alter the development and quality of dates of intermediate maturity cultivars, such as Deglet Nour. This alteration is of a physiological nature, it particularly affects the 'Tamar' stage and results in excessive dehydration of the fruits, thus impacting their texture, appearance and taste quality. It also affects the marketability of dates, leading to a decrease in their economic value and consequently threatening the sustainability not only of Deglet Nour, but also of all other cultivars that ripen during this hot period.

2. MATERIALS AND METHODS

2.1. Morphological and biochemical characterization

Given the influence of the geographical location (Al-Kahtani and Soliman, 2012, Zainal A'Bidin et al., 2020) and the field management practices on the morphological parameters of of date palm fruits, we compared our results with those obtained with the elite 'Deglet Nour' variety cultured under the same conditions.

Samples collection

'Boufeggous' and 'Deglet Nour' date samples (Fig. 1) were collected during the Tamar ripening stage in October 2023 from the oasis of Tozeur in South-West Tunisia. Dates were then conveyed to the workroom in cardboard boxes and kept at 4°C for the future analysis. The quality ratios were calculated using the formulas outlined by Outghouliast and Touhami (2019).

Fig. 1. Seeded fruits at Khalal (**K**), Rutab (**R**) and Tamar (**T**) stages of varieties 'Boufeggous' (right) and 'Deglet Nour' (left). Scale bar: 1 cm

Morphological characterization

The fruits and seeds of the two cultivars were morphologically characterized by measuring weight, length, width and diameter. Average weights (by means of a digital balance), length, diameter and width (using a digital calliper METRICA) were determined on 20 fruits.

Moisture content and the total soluble solids (TSS)

Moisture content was measured by loss in weight after heating a 3 g sample to constant weight at 70°C in an oven. Three repetitions were done for each measured parameter. The total soluble solids (TSS), expressed as °Brix, was determined with a refractometer and adjusted according to temperature. Three repetitions were done for each measured parameter.

Sugars concentration

Total sugars were calculated by using the method of Dubois et al. (1956) which is based on the use of the reagents phenol and concentrated sulfuric acid. It consists of first adding 200 µL of phenol (5%) to 200 µL of diluted sample extract. Second and after homogenization of the mix by vortex, we add 1mL of concentrated sulfuric acid. Third, after homogenization, the mix was incubated in a double boiler at 100°C for 5 min. Finally, the tubes were cooled in an ice bath and placed for 30 min in darkness. Afterward, the OD was determined at 492 nm. Notably, the concentration of total sugars in the extract was measured in accordance with the calibration curve obtained from a sucrose standard.

pH, titrable acidity and water activity

These measurements are made on dates without seeds which were cut into small pieces and ground into a uniform puree. The pH was measured by a digital pH-meter (Crison 501, Barcelona, Spain) according to standards NF V 05-108 (1970). Titrable acidity was determined by titrating the sample extract with 0.1 N NaOH as outlined in NF V 05-101, (1974). The water activity (aw) was measured as regards to the moisture content exploiting an aw-meter (Novasina Lab Master-aw, Swiss). The samples were ground into fine pieces and poured into a dried cup, about 2/3 of its capacity. Then, the filled cup was placed in the calculating cuvette. After removing the measuring head on the bowl, the water activity value of the sample was displayed on the device screen.

Potassium and sodium determination

Potassium and sodium were determined by flame photometry on the extracts obtained by mineralization (AFNOR 05-113, 1972). Indeed, the extracts used for the determination of sodium were diluted (1/10) by nitric acid (1 mL of the extract diluted in 9 mL of 0.2 N nitric acid) although the extracts used for the determination of potassium were not diluted.

The standard solutions were prepared from the stock solutions for each element. The flame photometer was adjusted to a wavelength specific to the element sought, 768 nm for potassium and 589 nm for sodium. The emission measurements of the extracts for potassium were made on the diluted extracts, while those of sodium were made on the undiluted extracts. The emissions of the standards were also measured and the curves Emission = f (concentrations) were established.

2.2. In vitro tissue culture techniques

2.2.1. Plant materials

The apex and young leaves of 3 offshoots of the cultivar 'Boufeggous' were taken from specimen adult date palm cv. 'Boufeggous' grown at the Tozeur oasis, in Southern Tunisia. Inflorescences were collected from the same adult date plant at the end of March, after the emergence of the first 20 to 25 cm of spathe.

2.2.2. Establishment of in vitro cultures

The outer surfaces of the spathe and the cut surface were disinfected by immersion in ethanol (90%) for 20–60 s. The inflorescences and offshoot apex were then sterilized with a 0.01% mercuric chloride solution for one hour and thoroughly rinsed three times with sterile distilled water. The flower axes (spikelets) and young leaves were divided into 1.5 to 2 cm long explants, the shoot tip was divided into 4 to 6 explants and inoculated on the culture medium.

The basic medium used consists of the Murashige and Skoog (1962) solution. To this medium were added sucrose (50 g/l), adenin (25 g/l) and agar (7 g/l). As for growth regulators (hormones), we used auxins, TDZ (thidiazuron), 2,4-D (2,4-Dichlorophenoxyacetic acid), ANA (α-naphthalenacetic acid) and ANOA. The nature and concentrations are reported in the Table 1. It is important to note that all media were sterilized by autoclaving at 121°C with a

pressure of 1 bar, for 20 min. The cultures were maintained in darkness in an incubator at a temperature of 28 °C for 6–8 months. Explants showing callus and shoot induction were transfered onto multiplication medium (MM : MS medium comprising 1 mg/l IPA) in a 28 ± 2°C culture room under light conditions with 16/8 h photoperiod with 80 µmol m^{-2} s^{-1} E fluorescent light. Cultures were then transferred to an elongation medium (EM: MS medium devoid of vegetal hormones). Cultures sufficiently elongated (shoots and somatic embryos) were moved on rooting medium (RM: MS medium comprising 3 mg/l IBA).

2.3. Statistical Analysis

An ANOVA for each quality attribute was performed and values reported for the two varieties were compared to find significant differences. By the use of Info Stat (version 1), the least significant difference multiple range test at $p < 0.05$ was conducted.

3. RESULTS AND DISCUSSION

3.1. Morphological and biochemical characterization

Morphological characterization

The weight, length, diameter of 'Boufeggous' and 'Deglet Nour' dates at Tamar stage are mentioned in Table 2. The mean weight is 19.83 g, a value more than doubles of that of 'Deglet Nour' variety (8.92 g) cultured under the same conditions. Slightly superior weight values of 'Deglet Nour' dates grown in Tozeur oasis were described by Reynes et al. (1994), Jemni et al. (2014) and Taha et al. (2019), which are 10.8, 11.9 and 10.9 g, respectively. Reynes et al (1994), reported that 'Boufeggous' cultivar grown in Tozeur oasis, has mean weight close to 15.1 g. It's notable that, after studying 10 other Tunisian cultivars, Chaira et al. (2009) found fruit weights that varied from 4.34 to 11.28 g, lower than that of 'Boufeggous' fruit. As for the pulp/fruit ratio, 'Deglet Nour' variety seems to be slightly higher than that of 'Boufeggous' cultivar with 93.27% (Jemni et al, 2014) and 91.7% (Reynes et al., 1994) versus 87.36 % in our study.

Chemical characterization

Table 3 below summarizes the main chemical characteristics of 'Boufeggous' and 'Deglet Nour' dates.

Moisture content

The moisture content of date palm fruit is one of the essential parameters for the determination and careful guidance of harvesting and conservation operations (Elbar et al., 2024).

Table 1. Growth regulator composition of media for inducing morphogenetic responses.

Initiation medium	Growth regulator (mg.l^{-1})	Activated charcoal (g.l^{-1})
M_1	ANA-ANOA (1-1)	0
M_2	2,4-D = 1	0.3
M_3	TDZ = 0.1	0

Table 2. Morphological and physical characteristics of fresh 'Boufeggous' and 'Deglet Nour' date fruit.

variety	Weight (g)			Length (mm)		Diameter (mm)		Pulp/fruit ratio (%)
	Fruit	Seed	Pulp	Fruit	Seed	Fruit	Seed	
'Boufeggous'	19.83±0 .99a	1.59 ±0.27a	18.24 ± 1,02a	45.4 ±1.44a	27.2 ±1.23a	23.30 ±1.16a	9.4 ±0.84a	91.96 ±1.43a
'Deglet-Nour'	8.92 ±1.37b	0.89 ±0.106b	7.96 ±1.38b	40.41 ±1.83a	25.71 ±3.13b	16.91 ±1.07b	7.1 ±0.9b	89.06 ±2.16b

Data are means (n = 20) ± SD. Means in the column followed by different letters are significantly different (p ≤ 0.05) according to LSD test.

Table 3. Biochemical parameters of dates of 'Boufeggous' and 'Deglet Nour' date fruit.

Parameter	Moisture content (%)	TSS (Brix°)	ST (g/100g)	pH	ACT (g eqAC/100 g MF)	AW	K (mg/g MS)	Na (mg/g MS)
'Boufeggous'	23.16 ±0.54a	65.43 ±0.64a	67.87 ±5,20a	5.30 ±0.13a	0.18 ± 0.01b	0.73 ±0.01a	0.36 ±0,00b	0,38 ±0,00a
'Deglet Nour'	16.44 ±0.26b	61.71 ±1.68b	70.57 ±1.95a	5.32 ±0.085a	0.38 ±0.035a	0.63 ±0.03b	0.96 ±0.05a	0.38 ±0. 05a

Data are means (n = 3) ± SD. Means in the column followed by different letters are significantly different (p ≤ 0.05) according to LSD test.

Kenfhar (2004) reported that dates with moisture content between 10 and 24% have a good character. In the present study, moisture content was about 23.16 ± 0.54%, therefore 'Boufeggous' cultivar has good quality and seems to be suitable for consumers like that of 'Deglet Nour' with 16.445±0.265% moisture content. Borchani et al. (2010) reported lower moisture content in the same cultivar with 11.3 %. Taha et al. (2019) and Elbar et al. (2024) reported slightly higher values with 'Deglet Nour' variety. According to FAO/WHO (1985), 26% is the necessary moisture content of common date varieties for marketing. This moisture content permits to store dates for a long period at room temperature without risking deterioration (Benyahia-Krid et al., 2021).

TSS (Brix°)

Most of the TSS in dates consists of different types of soluble sugars; sucrose, glucose and fructose as well as acids and it constitutes a good means used for total sugar content estimation (Farag and Al-Masri, 1999). The total soluble solid (TSS) content is one of the important quality attributes employed for the assessment of the commercial value of dates (Manickavasagan et al., 2014). Compared with 'Deglet Nour' dates with 61.71%, those of 'Boufeggous' have a higher sugar content 65,43%. Djafri et al. (2021), reported lower TSS in Algerian varieties namely, 'Tinissine' 55.16%, 'Ghars' 50%, 'Tantanboucht' 50 % and 'Deglet Nour' 25%.

Sugar concentration

Like all date fruits, the studied dates were rich in sugar with 67.87±5,20 g/100g FM, which is lower than that of 'Deglet Nour' 70.576±1.959 g/100g FM. This result was also confirmed by Chaira et al. (2007), Elleuch et al. (2008), Besbes et al. (2009), and Jemni et al. (2014) when studying sugar concentration of 'Deglet Nour' variety with 72.82, 79.1, 87.55 and 78.25 g/100g, respectiveley. Meanwhile, for the same 'Deglet Nour' variety, El Arem et al. (2011) have revealed a lower sugar concentration with 63.16±1.59%. According to Al-Farsi et al. (2005), several factors can intervene and influence the quantity of sugars in the date for example, growing conditions, the geographical origin, sun exposure, soil type, season, storage conditions, fertilization, maturity, time and harvest period. Reynes et al. (1994) reported that the predominant sugars in cultivar 'Boufeggous' are reducing sugars (fructose and glucose), as opposed to 'Deglet Nour' variety which is rich of sucrose (Jemni et al., 2014; Cherif et al., 2024).

pH level

This parameter reflects the degree of acidity of dates and constitutes among the main factors determining the quality of dates. According to Barreveld (1993), a good quality date generally has a pH of about 6; however, a poor quality date has a pH less than 5. Hence, with a pH of 5,3 ± 0,13, dates of 'Boufeggous' cultivar like those of 'Deglet Nour' are of acceptable taste quality. According to Borchani et al. (2010) the pH of 'Deglet Nour' and 'Boufeggous' are 5.45±0,02 and 5.58±0.01, respectively. Such pH values largely reflected the stability of the 'Deglet Nour' variety microbes. Besides, Audigié et al. (1984) reported that when the pH of dates is less than 5.5, these dates are considered acid and thus their quality taste is reduced.

Titrable acidity

Expressed as citric acid equivalent, reflected fruit quality and indicated the sourness. 'Boufeggous' cultivar had 1.7±0.01, a value highly superior to that of 'Deglet Nour' variety wihch is 0.385±0.035. This aligne with results described by Jemni et al. (2014) in 'Deglet Nour' variety with 0.10±0.02 g citric acid 100 g^{-1} fw. Alahyane et al. (2021) reported that titrable acidity of seventeen Moroccan dates varieties and clones was ranged from 0.29 to 1.40%. However, our results remain largely inferior to those reported by Al-Farsi et al. (2007) who found contents between 1.9 and 2.7%, i.e. 19 to 27 g/kg. Al-Shahib and Marshall (2003) have mentioned that dates acidity was susceptible to change depending on the content of organic acids (malic, citric and oxalic acids) and residues of polyphenols.

Water activity (AW)

The water activity of the studied dates is 0.73±0.01 which is higher than that of 'Deglet Nour' variety with 0.635±0.033. Jemni et al. (2014) found a similar result with 'Deglet Nour' variety ; AW between 0.625 and 0.645. According to Borchani et al., (2010), the AW of 'Boufeggous' is about 0,64±0,01 which is lower than our results. Besbes et al., (2009) postulated that the low AW may protects dates against both many bacterial species by inhibiting their ability to produce toxins and the development of certain osmophilic yeasts, a phenomenon that requires an AW around 0.6 (Pitt, 1975).

Potassium and sodium concentrations

In our analysis, potassium shows relatively low level (0.36%) in cultivar 'Boufeggous' compared with first other varieities including 'Deglet Nour' with 0.96 (in our study) and 0.639% as reported by Reynes et al. (1994) and second the same variety but cultured in other conditions with 0.724% (Reynes et al., 1994). Even with this concentration, potassium concentration in 'Boufeggous' cultivar seems to be higher than that of 13 date palm varieties of the United Arab Emirates (UAE) which were studied by Dghaim et al. (2021). Concerning the sodium, it shows the same concentration (0.38) in the two studied varieties (0.38). This value is higher than that of 'Deglet Nour' variety reported by Reynes et al. (1994) and 15 Tunisian variety studied by Ben salah and Rachid (2006). Mainly, dates are naturally low in sodium (0.1 to 0.2%) (Dghaim et al. 2021). According to Booij et al. (1992), the composition of mineral elements in general contributes to the characterization of a particular geographical origin. Regarding their poverty of sodium and richness in potassium, dates can be considered a good option for people suffering from high blood pressure (Al-Farsi and Lee 2008). Reynes et al. (2014) have shown that the 'Boufeggous' date is also rich in calcium and phosphorus.

3.2. Regeneration of the Boufeggous cultivar by *in vitro* tissue culture

3.2.1. Effect of plant growth regulators on the evolution of completely matured flower explants

Results presented in Table 4 indicated that regardless of the tested culture medium, the majority of explants swell and remain in this state without the slightest sign of differentiation of calli or shoots. Nevertheless, on MS medium supplemented with NAA and ANOA, protected floral pieces (petals and carpels), grow significantly, harden and remain in this state for several months before finally becoming completely necrotic.

Our results do not align with those of Kriaa et al. (2012), who succeeded to produce vitroplants from completely matured flowers of the 'Deglet Nour' variety despite the use of the same hormone (2,4-D) and with the same dose (1 mg/l). Likewise, Othmani et al. (2024), arrived to

Table 4. Frequency of shoots and embryogenic callus induction (%) from completely mature female flowers, shoot tips and young leaf explants of variety 'Boufeggous' on MS medium comprising NAA, ANOA, 2,4D and TDZ.

Growth regulators (mg/l)				Completely mature female flowers		Shoot tips		Young leaves	
NAA	ANOA	2,4D	TDZ	shoots	callus	shoots	callus	shoots	callus
0.00	0.00	0.00	0.00	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c
1.0	1.0	0.00	0.00	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c
0.00	0.00	1.0	0.00	0.00c	0.00c	50±25a	33.33±14.43a	26.66±2.88b	23.33±5.77 b
0.00	0.00	0.00	0.10	0.00c	0.00c	0.00c	0.00c	0.00c	33.33±5.77a

Means of shoots followed by different letters and means of callus followed by different letters are significantly different ($p \leq 0.05$) according to LSD test.

a **b** **c** **d**

Fig. 2. The different stages passed by shoot tip explants of the Boufeggous cultivar to regenerate somatic embryos after 12 months of culture on M2 culture medium. **a**: apparition of nodules, **b**: beginning of somatic embryo differentiation, **c**: differentiated somatic embryos and **d**: conversion of somatic embryos to plantlets. Scale bar: 3 mm.

regenerate tetraploid vitroplants from completely matured tetraploid flowers by using 2,4-D (1 mg/l). In the same context, Zayed (2017) was succeeding in in vitro regeneration of date palm plantlets by using the technique of floral reversion. He was cultured mature female inflorescences on MS induction medium comprising the plant growth regulators; 2,4-dichlorophenoxyacetic acid, 2-isopentenyladenine, paclobutrazol or abscisic acid with 10, 3, and 2 or 2 mg/L, respectively. In our study, the failure of in vitro production of vitroplants from mature female flowers could be due to the relatively limited number of inflorescences used in our case or to the stage of their development which is different from that of the 'Deglet Nour' variety. This recalcitrant character of older inflorescences of the 'Boufeggous' variety could then require the use of a higher dose of 2,4-D or other vegetative hormones separately or in combination.

3.2.2. Effect of plant growth regulators on vegetative explants (shoot tips and young leaves)

Shoot tips

Our results revealed that when shoot tips were cultured on MS medium comprising 2,4-D (1 mg/l), they showed the higher ability to regenerate vitroplants through at once somatic embryogenesis (Fig. 2) and shoot organogenesis which can be direct (Fig. 3a and b) or indirect via the callus phase (Fig. 3c and d). Similarily, *in vitro* regeneration of date palms by organogenesis from the terminal bud was reported by Sidky (2017) using MS medium supplemented with the hormonal combination of NAA-ANOA-BAP-IPA (1-1-2.5-2.5 mg/l). Furthermore, Aslam et al. (2009) were able to produce embryogenic calli that differentiated into somatic embryos from explants taken from the terminal bud of the date palm ('Khalas' variety) and cultured on MS medium comprising 45.24 µM 2,4-D and 54.21 µM 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Abahmane (2017) reported that the organogenesis induction directly from shoot tips without passing by callus phase ensures the genetic fidelity of the produced date palm plantlets. Further, Al-Khayri and Naik (2017) found that the use of 2,4-D associated with other growth regulators in MS medium effectively promotes embryogenic callus and stimulates bud multiplication from shoot tips of various date palm varieties. To obtain vigorous plantlets, the

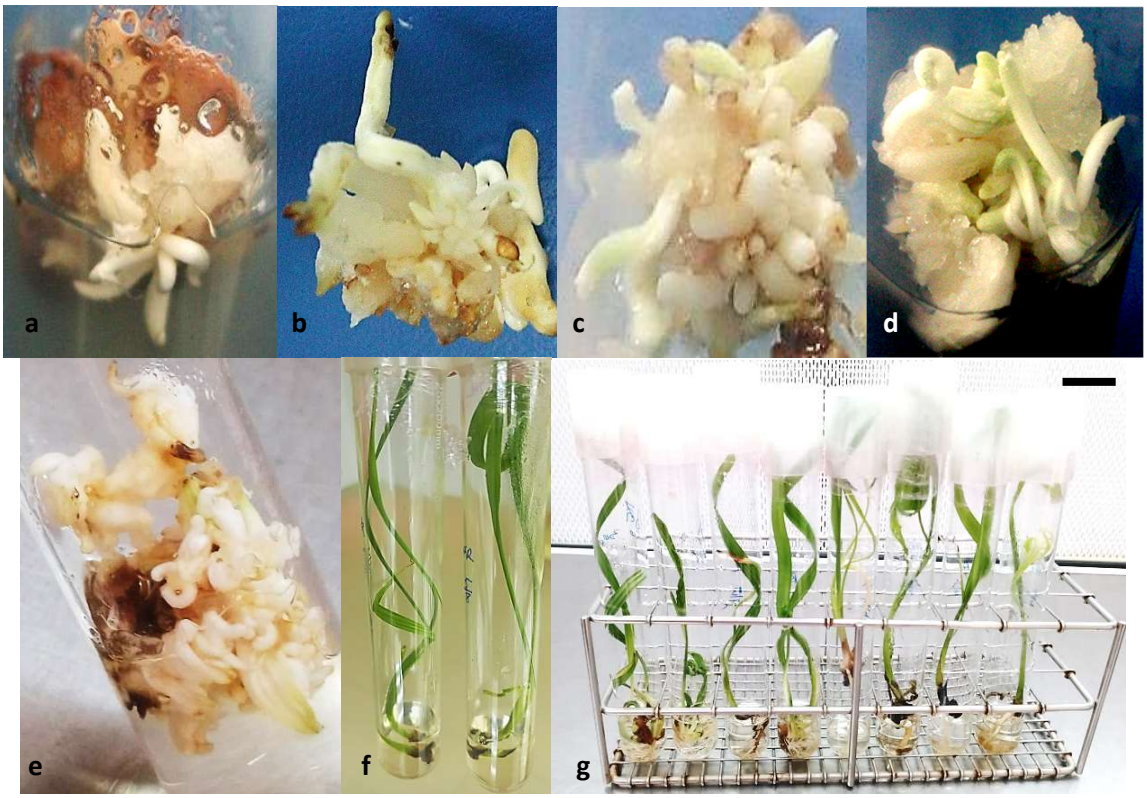

Fig. 3. The different stages passed by shoot tip explants of the 'Boufeggous' variety to regenerate shoots directly (**a** and **b**) and indirectly (via callus phase: **c** and **d**) after 8-9 months of culture on M2 culture medium, e: shoot multiplication, f: shoot elongation and g: rooting of shoots. Scale bar: **a**, **b** and **c** (7 mm), **e** (10 mm) and **f** (3 cm).

new vegetative shoots pass through three stages: active multiplication (Fig. 3e), elongation and rooting (Fig. 3f).

Young leaves

The results recorded in Table 4 suggest that the hormonal factor largely influences the capacity of leaf explants to regenerate vitroplants in the studied cultivar. Indeed, if the hormonal combination NAA-ANOA (1-1 mg/l) does not appear to induce none somatic embryogenesis or shoot organogenesis, the addition of 1 mg/l 2,4-D to the culture medium was shown to be effective to stimulate vitroplants production via both the neoformation of buds and somatic embryos. The stimulating effect of 2,4-D on the regeneration capacity of the date palm from young leaves has also been described by Othmani et al. (2009) and Fki et al. (2010). It's to note that, young leaves appear the only tissue able to regenerate somatic embryos when cultured on MS medium comprising the cytokine TDZ at 0.1 mg/l. This cytokinin has been successfully used by Taha et al. (2021) to regenerate vitroplants from young inflorescences of the 'Medjool', 'Barhee' and 'Selmi' varieties.

3. CONCLUSION

The aim of this study was to characterize morphologically and biochemically the fruits of the rare Tunisian 'Boufeggous' cultivar, while comparing them with those of the 'Deglet Nour' variety grown under the same conditions. Our results showed that, in terms of morphology, 'Boufeggous' dates collected from the Tozeur oasis can compete with the most commercially available 'Deglet Nour' dates in Tunisia, with a fruit weight, length, and width of 19.83 ± 0.99 g, 45.4 ± 1.44 mm, and 23.3 ± 1.16 mm, respectively. Using the same parameters, the 'Boufeggous' cultivar can even compete with the 'Medjool' cultivar, the most marketable cultivar worldwide. Indeed, studies conducted on the 'Medjool' cultivar in Morocco reported a maximum fruit weight of 17.37 ± 2.22 g, a maximum length of 42.01 ± 3.89 mm, and a maximum width of 21.55 ± 1.79 mm (Goubi et al., 2025). However, in terms of chemical composition, 'Boufeggous' dates do not surpass either 'Deglet Nour' or 'Medjool'. Indeed, 'Boufeggous' are characterized by a softer texture and a sweeter taste, but they are more perishable. In contrast, 'Deglet Nour' is drier, better balanced in sugars and acids, and richer in minerals (notably potassium), which gives it greater stability and nutritional value. Despite these factors, the combination of its high fruit weight and late ripening makes the large-scale propagation of this cultivar likely to significantly influence the date palm industry in Tunisia. To achieve this objective, we used in vitro tissue culture with vegetative and reproductive explants. Results showed that optimal multiplication through organogenesis was obtained when the apical bud was grown on MS medium with 2,4-D (1 mg/L). We were also able to produce somatic embryos indirectly from young leaves cultured on MS medium with thidiazuron (0.1 mg/L) or 2,4-D (1 mg/L). However, this regeneration pathway should be used carefully because of its potential risk of somaclonal variation (Duta-Cornescu et al., 2023; Das et al., 2025). The latter suggested that auxins may induce somaclonal variation in tissue culture, which can occur either at the chromosomal level (alterations in chromosome number or structure, such as aneuploidy, polyploidy, deletions, duplications, insertions, or translocations) or at the DNA sequence level, mainly as point mutations.

Abbreviations

ANOA : β-Naphthoxy Acetic Acid
BAP: 6-Benzylaminopurine
EM: Elongation medium
IBA: Indole Butyric Acid
IPA: Indole-3-Propionic Acid
MS : Murashige and Skoog (1962) medium
MM: Multiplication medium
NAA: 1-Naphthaleneacetic Acid
RM: Rooting medium
TDZ: Thidiazuron

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