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Organogenesis of *Phoenix dactylifera* L. cv. Mejhoul: Influences of natural and synthetic compounds on tissue browning, and analysis of protein concentrations and peroxidase activity in explants

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ABSTRACT

The effect of some chemical and natural antioxidants on date palm organogenesis was evaluated. Shoot tip explants of *Phoenix dactylifera* L. cv. Mejhoul were cultured on half-strength Murashige and Skoog (1/2MS) medium supplemented with various concentrations (1.5, 3 or 4.5 g l^{-1}) of activated charcoal (AC), polyvinylpyrrolidone (PVP) or date stone-based activated carbon (DSAC); or with the methanolic extracts of *Rosmarinus officinalis* and *Thymus satureioides*, either alone (0.16-0.48 g l^{-1}) or in combination (0.08-0.24 g l^{-1}). The use of PVP at 1.5 g l^{-1} provided the optimal conditions during the initiation stage with an organogenesis frequency of 52.5% and a tissue browning frequency of 30.0%. During the multiplication stage, 1.5 g l^{-1} DSAC showed significantly better results in terms of shoot bud proliferation (13.9 shoot buds per explant) and tissue browning (7.5%). The total protein concentration and peroxidase activity varied significantly depending on the anti-browning additive, and positive correlations were revealed between protein concentration and bud proliferation and morphology, and between peroxidase activity and tissue browning. Shoot elongation and rooting were achieved in plant growth regulator-free 1/2MS medium and up to 97.5% of plantlets survived after acclimatization. It has been revealed that DSAC is an efficient natural anti-browning compound for date palm organogenesis.

1. Introduction

Date palm (*Phoenix dactylifera* L.) is an agronomically and economically important crop species in the MENA region, where it is involved in reducing desertification risks and creating equable microclimate for agriculture within oasis ecosystems (Jain, 2011). In Morocco, date palm covers an area of 50,000 ha with 5.4 million palms (Sedra, 2015), and it is the most economically important food crop in the oasis areas (Sedra and Lazrek, 2011). However, date palm is threatened by bayoud, a dangerous disease caused by the vascular pathogen *Fusarium oxysporum* f. sp. *albedinis* and causes severe reduction in date palm cultivation and expansion in Morocco and Algeria (Jaiti et al., 2007). Indeed, bayoud has decimated more than 12 million palms (Saker, 2011). Unfortunately, the best Moroccan and Algerian date palm varieties such as Mejhoul, Boufeggous, Jihel, Bouskri, Deglet Noor and Ghras are sensitive to bayoud (Sedra, 2011). Moreover, chemical treatments are not effective to control this disease (Jaiti et al., 2007). Among the elite date palm cultivars threatened by bayoud, Mejhoul is the most famous and most sought after cultivar worldwide (Sedra 2015). It is also the most popular and most desired cultivar by the Moroccan farmers and consumers. To date, large-scale propagation of sensitive varieties then planting them in bayoud-free areas is by far the only practicable way to preserve them from extinction.

Abbreviations: AC, activated charcoal; DSAC, date stone-based activated carbon; FW, fresh weight; MS, Murashige and Skoog; PGR, plant growth regulator; PVP, polyvinylpyrrolidone.

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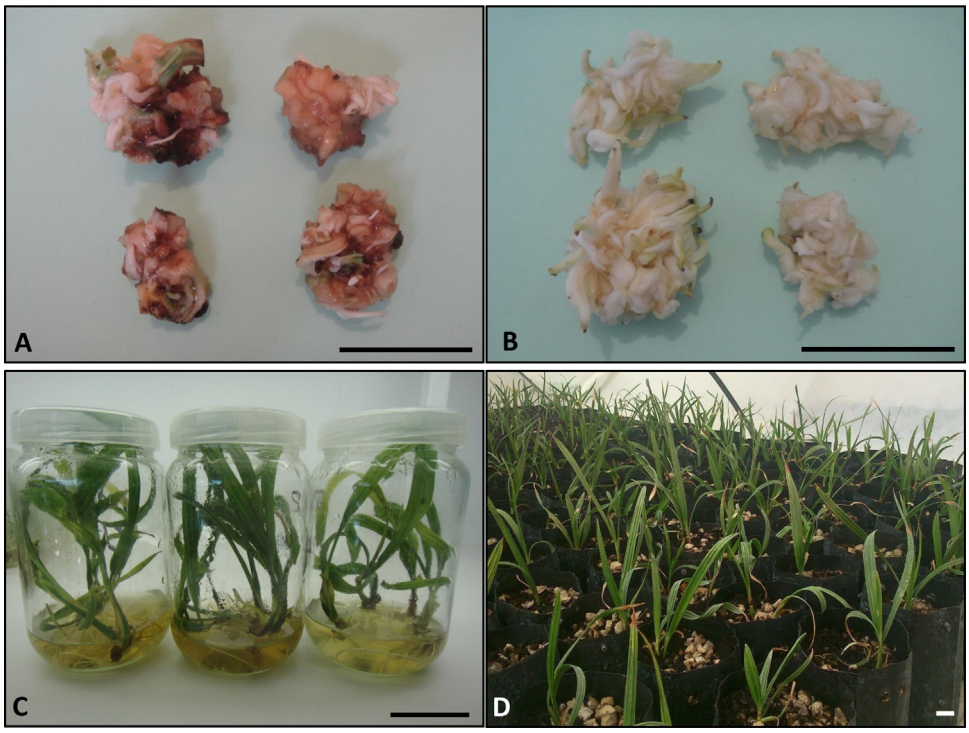

Fig. 1. Shoot bud multiplication, elongation and plantlet acclimatization of *Phoenix dactylifera* L. cv. Mejhoul. (A) Shoot buds after 3 months of multiplication on the anti-browning-free 1/2MS medium (CM1' medium). (B) Shoot buds after 3 months of multiplication on 1/2MS medium supplemented with 1.5 g l^{-1} date stone-based activated carbon (CM5 medium). (C) Shoot elongation and rooting after 3 months on PGR-free CM5 medium. (D) Plantlet acclimatization after 3 months. Bars correspond to 3 cm.

Large-scale propagation of date palm might be achieved through either somatic embryogenesis or organogenesis (Mazri and Meziani, 2015). Hegazy and Aboshama (2010) reported that somatic embryogenesis allows higher multiplication efficiency than organogenesis. However, some authors have associated regeneration through somatic embryogenesis with somaclonal variation (Saker et al., 2000; Saker et al., 2006). On the other hand, it was reported that organogenesis preserve genetic fidelity within regenerants (Sedra, 2005). Regeneration through organogenesis in date palm is genotype dependent (Jain, 2012), and successful protocols were reported for some cultivars such as cv. Sukry (Al-Khateeb, 2006), cv. Maktoom (Khierallah and Bader, 2007), cv. Dhakki (Khan and Bi Bi, 2012), cv. Najda (Mazri and Meziani, 2013) and cv. Boufeggous (Mazri, 2015). Nevertheless, certain physiological disorders that decrease the proliferation capacity of shoot buds, namely hyperhydricity, precocious rooting and tissue browning were associated with regeneration through organogenesis (Abahmane, 2011; Mazri, 2014, 2015).

Browning of date palm explants is caused by the high levels of caffeoylshikimic acids contained in date palm tissues (Loutfi and El Hadrami, 2005). This phenomenon, observed during either somatic embryogenesis (Abohatem et al., 2011) or organogenesis (Mazri, 2014, 2015), hampers the multiplication efficiency of explants and leads to subsequent necrosis. Abohatem et al. (2011) demonstrated that reducing the time between subcultures decreased the intensity of browning in somatic embryos while Mazri (2014, 2015) indicated that plant growth regulators (PGRs) and carbon sources might affect the intensity of tissue browning during organogenesis. Generally, compounds such as activated charcoal (AC) and polyvinylpyrrolidone (PVP) have been widely used to limit date palm tissue browning (Loutfi and El Hadrami, 2005). The use of some plant extracts or derivative compounds might have a protective effect against date palm tissue browning. In fact, it was reported that the extracts of *Rosmarinus officinalis* (Erkan et al., 2008) and *Thymus satureioides* (Ramchoun et al., 2015) as well as date palm seeds (Ardekani et al., 2010; Mohamed and Al-Okbi, 2005) have an antioxidant activity. Nevertheless, their protective effect against date palm tissue browning has never been evaluated.

The purpose of this investigation was to evaluate the effects of different concentrations of AC, PVP, date stone-based activated carbon (DSAC) as well as *R. officinalis* and *T. satureioides* methanolic extracts on adventitious bud initiation, multiplication and browning during in vitro organogenesis of *P. dactylifera* L. cv. Mejhoul, and to determine the relationship between total protein concentrations, peroxidase activity and explant reactivity.

2. Materials and methods

2.1. Plant material and disinfection

Offshoots of *P. dactylifera* L. cv. Mejhoul (3-year-old) were collected from Erfoud, Morocco. The outer leaves were removed and each shoot tip (7–8 cm in length, 3–3.5 cm in diameter) was treated with 0.03% solution of potassium permanganate (Sigma, Steinheim, Germany) in commercial liquid chlorine bleach (5% v/v NaClO; ACE, Industries Marocaines Modernes, Casablanca, Morocco) for 20 min, then rinsed three times in sterile distilled water (10 min each time). Afterwards, each shoot tip was cut longitudinally and 10 segments (7–9 mm in length) consisting of the apical meristem region were excised and transferred to the culture medium.

2.2. Basal medium composition

The composition of the basal medium (1/2MS) used in all experiments was as follows: ½ MS macro-elements (Murashige and Skoog, 1962) supplemented with MS microelements, MS vitamins, 3% sucrose (Sigma, St. Louis, MO, USA) and gelled with 0.6% agar (Sigma, St. Louis, MO, USA). This culture medium was selected based on preliminary experiments (data not shown). The medium pH was adjusted to 5.8 prior to autoclaving at 121 °C for 25 min.