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Effects of growth regulators and types of culture media on somatic embryogenesis in date palm (*Phoenix dactylifera* L. cv. Degla Beida)

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ABSTRACT

In the present study, we aim to develop a complete production scheme for the propagation of Algerian date palm *Phoenix dactylifera* L. (*cv*. Degla Beïda) by somatic embryogenesis. The scheme consists of four main steps: induction of callus, multiplication of nodules on solid and liquid media, initiation of somatic embryogenesis in agitated liquid medium and maturation and germination of proembryos on solid media. Our results showed that hormonal treatments (10 mg/L 2,4-D, 1 mg/L BAP) and (50 mg/L 2,4-D, 1 mg/L BAP) combined with 300 and 1500 mg/L of activated charcoal, respectively, promoted initiation and multiplication of nodular calli on solid media. The best multiplication factor (9.75) was obtained from these calli when they were further cultivated in liquid medium containing 1 mg/L 2,4-D. Moreover, liquid medium without growth regulators promoted the development of relatively homogeneous embryogenic suspensions. The return to solid medium without growth regulators during the maturation phase improved the yield of somatic embryos and their subsequent germination.

1. Introduction

The date palm tree (*Phoenix dactylifera* L.) is a perennial monocotyledonous species adapted to the local conditions of arid and semiarid areas. Date palm cultivation in Algeria is exclusively done for the harvest of date fruit, and is considered to be a highly important socioeconomic and ecological activity. However, the Algerian phoenicicol heritage (characterized by a wide diversity of varieties) is subject to many threats, the most ominous one being Bayoud disease, caused by a telluric fungus, *Fusarium oxysporum* f.sp. *albedinis* W.L. Gordon. Appearing in Algeria in1898, this disease has already destroyed more than three million palm trees in south-western Algeria and continues to progress toward healthy palm groves of the southeast region of the country (Benkhalifa, 2006). This situation is compounded by genetic erosion caused by the extension of monovarietal cultivation imposed by market demand for high quality commercial varieties like Deglet Nour, which is unfortunately sensitive to Bayoud (Açourene et al., 2007). Current measures for controlling Bayoud disease rely exclusively on preventive measures since no effective curative control method has been developed yet. Given this fact, genetic control is the best option to slow down and to stop the spreading of the Bayoud's agent from infected areas to non-infected ones (Zaid and Arias-Jimenez, 2002; El Hadrami et al., 2005; Sedra, 2005; Benkhalifa, 2006). The use of *in vitro* culture techniques can play a crucial role in Bayoud control. Indeed, *in vitro* plant regeneration *via* somatic embryogenesis is a powerful tool for a rapid large-scale production of healthy true-to-type clonal plants thus allowing the preserving of existing natural variability but also the micropropagation of variability outcome of genetic improvement programs (crossing, somaclonal variation, mutagenesis and somatic hybridization) (Jain et al., 2011). However, the date palm remains a recalcitrant species to *in vitro* techniques because of the influence of genotype factors that affect the responsiveness of explants in culture and the frequency of maturation and germination of embryos, thus hindering the establishment of simple, reliable, and reproducible protocols (Zaid and Arias-Jimenez, 2002; Jain et al., 2011).

The present study was carried out to optimize somatic embryo production from Algerian date palm cultivar "Degla Beïda", which is sensitive to Bayoud but produces dried dates of good

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; BAP, 6-benzylaminopurine.

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nutritional and commercial value (Açourene et al., 2007; Mimouni and Siboubakeur, 2011). The approach adopted consists of making changes to the growing medium composition during the different steps of *in vitro* plant production.

2. Materials and methods

2.1. Initiation of callogenesis

The source of starting plant material was an offshoot of *P. dactylifera* L. *cv*. Degla Beïda. The offshoot, weighing 6 kg, was harvested from a palm grove of El Méniaa (north central region of Algerian Sahara). Only the apical part of the offshoot core was used to randomly collect explants of 0.5 cm^3. This part include the apical meristem, the leaf primordia, axillary buds and the base of young leaves. The explants were disinfected with a solution of $HgCl_2$ (150 mg/L for one hour), rinsed 3 times with sterile distilled water and placed on the initiation solid medium (M) (Fig. 1). The growth regulators (mg/mL) were added in combined form: 2,4-D/BAP. Seven growth regulator combinations were applied: 0/0, 1/1, 5/1, 10/1, 50/1, 100/1 and 5/5. For each mg of 2,4-D, 30 mg of activated charcoal was added to the culture medium (Table 1). Each treatment done using in 12 replicates of explants. The cultures were incubated for 2 months at 25 °C in total darkness.

2.2. Calli maintenance and multiplication

Two media were tested for calli multiplication:

Solid medium "M": This medium is identical to the callogenesis initiation medium (Table 1). The explants of the previous step with or without callogenesis, were subcultured on fresh media every two months. The cultures were submitted to the same growing and environmental conditions of the callogenesis initiation phase. Observations were carried out over a period of 20 months (10 subculture periods) (Fig. 1).

Liquid medium "MM": After ten months of culture on the solid medium, liquid medium (MM) was used to multiply the mass of nodular calli (Table 1). During this step, only growth regulator combinations that resulted in callus on solid medium (M) (2,4-D/BAP: 5/1, 10/1, 50/1, 100/1 (mg/L)) and intense nodular callogenesis were selected. In addition, a new growth regulator combination (1/0) was introduced (Table 1). Nodular callus weighing 0.5 g were slightly fragmented and placed into 650 mL rectangular flasks Corning® Costar® each containing 150 mL of liquid medium MM. Four flasks/treatment were used. The medium was renewed every month. The liquid medium cultures were incubated on a rotary shaker (100 rpm) at 25 ± 2 °C under a 16 h, fluorescent light, 2000 Lx/8 h dark. For a more intense multiplication, callus produced on the medium MM were re-transferred again to the solid culture medium (M) at the end of the second month of culture in MM (Fig. 1).

2.3. Induction of embryogenesis, maturation and germination of somatic embryos

For the induction of embryogenesis and proembryo maturation, somatic embryogenesis was induced in liquid medium (ME) devoid of growth regulators and with a half mineral fraction (Table 1). The suspension preparation step consists of slight fragmentation and crushing with a scalpel 0.4 g of nodular callus in the presence of 150 mL of liquid medium followed by filtration through a sieve of 500 μm mesh size. Cell suspensions thus obtained into 500 mL Erlenmeyer flasks (3 suspensions) were placed on a rotary shaker (100 rpm). After decanting the cell suspension, the liquid culture medium was renewed every month. At this stage, the formation of somatic proembryos was accomplished through one of two ways: directly in the liquid medium, or after plating out the fraction of cells from suspension cultures that did not yield structured somatic embryos on a new solid (MG_1) or semi-solid (MG_2) medium (150 mL per 500 mL Erlenmeyer flask) to allow maturation (Table 1).

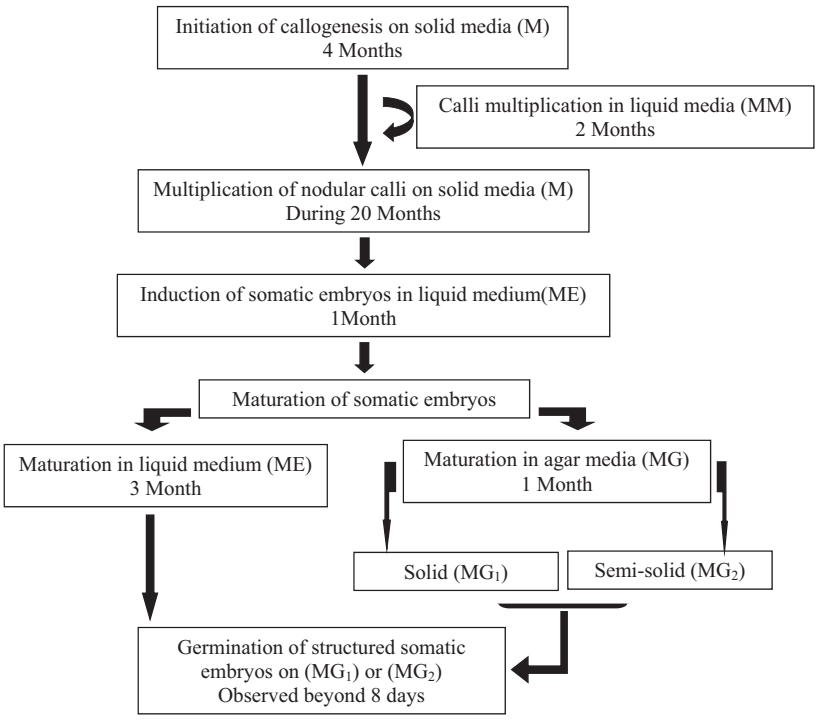

Fig. 1. Schematic representation of the experimental protocol.