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Development of zygotic and somatic embryos of *Phoenix dactylifera* L. cv. Deglet Nour: Comparative study

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Abstract

In order to improve somatic embryogenesis production in date palm *Phoenix dactilyfera* L. cv. Deglet Nour (DN), a comparative study between somatic (SE) and zygotic (ZE) embryos developments was carried out. The data showed that ZE maturation occurred from 10 to 19 weeks after pollination (WAP). During this period, the fresh weight (FW) and the dry weight (DW) of ZE increased progressively to reach a maximum level at 19 WAP. SE development occurred in three distinct stages. The DW remained constant during the two first stages, and declined slightly during the third and final stage. Embryo protein analysis revealed significant differences between ZE and SE. The ZE total protein level was initially low and increased to the maximum at mature stage. However, no significant change in total protein was detected during SE development. SDS-PAGE analysis showed a poor protein profile for SE, compared to that of ZE. In the latter, a 22 kDa protein was identified by N-terminal sequencing as a glutelin. This protein was accumulated rapidly during early development and remained at a relatively constant level during ZE development, and then declined progressively 12 days after embryo germination (DG). This protein seems to be absent in SE.

Keywords: Zygotic embryo; Somatic embryo; *Phoenix dactylifera* L. cv. DN; Total protein; Glutelin

1. Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most economically important fruit crop and is cultivated across North Africa and the Middle-East. Its nutritive value and widespread cultivation underscore the need for improving propagation methodologies, especially in vitro techniques (Al-Khayri and Al-Bahrany, 2004). Clonal propagation via somatic embryogenesis – the production of embryos without recourse to sexual reproduction – is the most promising approach to improve propagation in date palm. This technique has been widely and successfully applied to many cultivated plant species (Shah et al., 2000). In vitro protocols are already available for date palm, which permit the production of many synchronous somatic embryos in liquid culture (Fki et al., 2003). Date palms derived from somatic embryos are less susceptible to somatic variation compared to those derived from organogenesis (Ammirato, 1987; Merkle et al., 1990; Osuga et al., 1999). Although somatic embryo production is a well-established process for date palm (Al-Khayri, 2005), there are still possibilities for improvement. For example, a number of biochemical approaches have been proposed to distinguish embryogenic calli (Al-Khayri, 2005). The somatic embryo (SE) differs from the zygotic embryo (ZE), as it lacks a dormancy phase, and it also lacks a seed integument and an endosperm, both of which are required for seed survival and germination (Brownfield et al., 2007). This may explain why SE seedlings are less vigorous than those raised from true seed. More knowledge is required concerning SE physiology, and especially of the differences in protein content between SE and ZE, which may improve the quality of SE and SE-derived seedlings. The poor vigour of SE-derived seedlings seems to be related to their incomplete maturation under standard in vitro conditions (Roberts et al., 1990). Major changes take place in the latter stages of ZE maturation, including a switch from cell specification over to the accumulation of carbohydrates, thus preparing the embryo for full seed development (Yadegari and Goldberg, 1997).

Abbreviations: DG, days of germination; DN, Deglet Nour; DW, dry weight; ELISA, enzyme-linked immunosorbent assay; FW, fresh weight; MW, molecular weight; OPD, orthophenylene diamine; PBS, phosphate buffered saline; PBS-T, phosphate buffered saline tween; SE, somatic embryo; WAP, weeks after pollination; ZE, zygotic embryo.

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Therefore maturation is considered as a critical step in embryo development.

Histocytological observations indicated that storage protein accumulation in SE is poor compared to ZE (Aberlenc-Bertossi et al., 1999; Sané et al., 2006; Zouine et al., 2005). In *Eucalyptus nitens*, the cells of SE and ZE contain similar lipid-rich globular bodies, except in the meristematic regions. SE have slightly lower levels of storage proteins in their cotyledonary cells compared to ZE (Saumitra and John, 2000). These carbohydrate reserves play a vital role in seedling survival and development, as they sustain the seedling until photosynthesis (Galau et al., 1986). The storage proteins of dicots are predominantly albumins and globulins, whereas prolamin and glutelin are the major ones in monocots (Derbyshire et al., 1976). In the case of rice seeds, glutelin is the predominant storage protein, reaching 80% of total endosperm proteins (Takaiwa et al., 1999).

In the present report, a comparative study between protein content of date palm ZE and SE is given. The evolution of the glutelin as an important known storage protein during embryo's development was also investigated. This latter protein may be a useful biochemical marker.

2. Materials and methods

2.1. Plant material and embryo collection

ZE were collected from date palm kernels (cv. Deglet Nour), beginning 10 weeks after pollination (WAP) when the embryo endosperm had hardened and finishing after 19 weeks. Fifty ZE were collected at each harvest. Mature seeds were imbibed in water to induce germination and raised at 25 ± 1 °C in the dark for 25 days. Every 3 days 20 seeds were collected.

Somatic embryos were obtained from embryogenic suspension cultures derived from the DN variety as previously described (Fki et al., 2003). Before being structured, samples of 0.5 g of embryos were removed from their maintenance media (M3) and transferred on a fresh medium. Embryos were subcultured weekly until maturation (structured embryos). Three development stages of SE were identified (Fig. 1B).

2.2. Protein extraction

The embryos were frozen in liquid nitrogen and ground into a fine powder and mixed with 50 mM Maleate Tris Buffer (pH 8.3) containing 2% SDS, 0.5 mM EDTA, 2 mM PMSF, 1 mM DTT and 2 mM β-mercaptoethanol. After centrifugation, the quantity of soluble proteins in the supernatant was estimated using Bradford's Method (Bradford, 1976). Proteins were then separated by SDS-PAGE according to Laemmli (1979) and stained with Coomassie Brilliant Blue-R250. The molecular weights (MW) were obtained by comparison to standard protein markers (SDS-PAGE Standards, 161-0304, Bio-Rad). The protein N-terminal sequencing was performed using an Applied Biosystems Procise 492 equipped with 140 C HPLC systems.

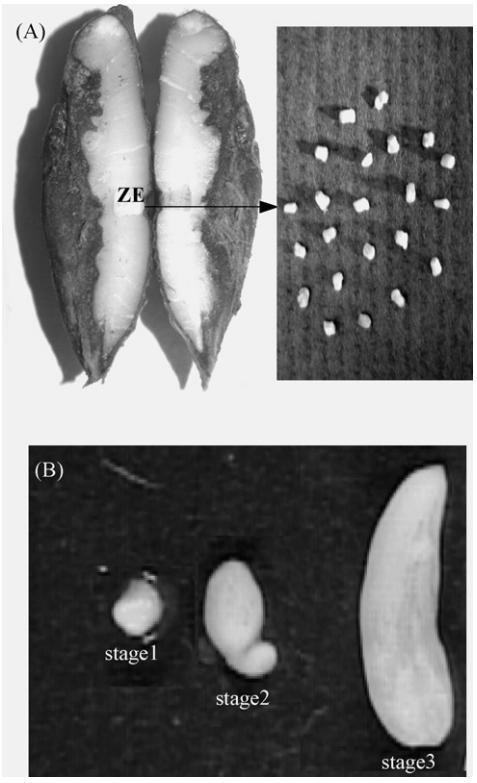

Fig. 1. Date palm seed cv. DN incised in the middle showing the position of the ZE (A). The three stages of date palm SE (B): stage 1: ovoid embryo; stage 2: longed embryo and stage 3: structured embryo.

2.3. Immunological analysis

After SDS-PAGE electrophoresis, the glutelin protein band was cut from the gel and used as antigen for rabbit immunization. The gel slice was dissolved in 0.5 ml of PBS and the mixture was injected into a rabbit in presence of complete Freund's Adjuvant (F-5881, SIGMA). Four subsequent injections were performed every 10 days in presence of incomplete Freund's Adjuvant (F-5506, SIGMA). The rabbit immunological reaction was tested by ELISA using an anti-rabbit serum (1/1000) conjugated to the peroxidase. The optical density was measured at 492 nm. The obtained rabbit antiserum was used for protein gel blot analysis as follows: after electrophoresis in SDS-PAGE, the proteins were electroblotted on a nitrocellulose membrane using 20 mM Tris Buffer containing 150 mM glycine (pH 8.5) at 70 V for 1 h. The fresh membrane was blocked for 2 h with PBS buffer 3% milk containing rabbit polyclonal antiserum (1/1000) of date palm glutelin. The membrane was then incubated with an anti-rabbit IgG conjugated with peroxidase for 2 h. The reaction was revealed using the ECL Plus Western Blotting Detection Reagents from Amersham Biosciences, as described by the Supplier.

Glutelin protein was quantified in crude protein extract by indirect ELISA. Proteins (20 ng) extracted from embryos were diluted in PBS buffer. Immunoplate wells were coated with 100 μl of protein solution for 24 h at 4 °C. The plate wells were then blocked with 3% milk in PBS for 2 h at 37 °C. Polyclonal