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Original Article

Proliferation of female inflorescences explants of date palm

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KEYWORDS

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Abstract This study was conducted to determine the effect of Abscisic Acid (ABA) and Ancymidol on proliferation of female inflorescences explants of date palm. In the first experiment two lengths of spath at (5–7 cm) or at (7–10 cm) were cultured on nutrient media which consists of half macro and full micro-salts of MS medium supplemented with gradual decreasing in concentration of Abscisic Acid (ABA) and Ancymidol from 4.5, 3.0, 1.5 to 0.5 mg^{-1}. In the second experiment two phases of nutrient medium (solid and liquid) and two source of carbon were investigated. Gradual decreasing of ABA concentrations from 4.5 mg^{-1} to 1.5 mg^{-1} in culture medium, stimulated the production of direct somatic embryos and accelerated callus initiation, but at last decrement (0.5 mg^{-1}) of Ancymidol concentration few embryos were produced. Callus initiation from inflorescences explants gave high production and well development of somatic embryos when cultured on liquid medium supplemented with 40 g^{-1} sucrose. All direct or indirect somatic embryos obtained in these experiments were converted successfully to healthy normal plantlets which could be transferred to acclimatization stage.

Introduction

Since tissue culture was developed as means of large scale propagation of the date palm (*Phoenix dactylifera* L.), the offshoots growing at the base of the mother tree constituted the only source of explants used for the initiation on stage. Because of the low success level at this starting, a large number of plant material is not always available, and there are even case where no offshoots are produced at all. Immature inflorescences represent an important explants source for micropropagation of elite palm trees (Tisserat, 1981; Zaid and Tisserate, 1983). Inflorescences tissue can be collected with limited damage to the upper parts of the donor plant. Immature inflorescences are protected by sheaths which prevent infestation by fungi and bacteria and avoid chemical damage from sterilization solution. To date there are no specific ways to determine the somatic embryogenesis and plant regeneration from young inflorescences explants of date palm. In this study we try to determine a protocol for micropropagation somatic embryogenesis and plant regeneration from female inflorescences explants of date palm.

Materials and methods

This work was carried out in central laboratory of developing of date palm research at Giza, Egypt during period from 2009

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to 2010. Two length female of spathes 5–7 and 7–10 cm were collected from the adult mother tree on February. Spathes were carefully removed with a clean knife and after cleaning with 70% ethanol the, spathes were wrapped in aluminum foil to start the sterilization process in the laboratory.

Sterilization technique

Two distinct steps were followed:

- The first step had been done outside the laminar flow hood and before spathes were opened then sprayed with 70% ethanol and burned for a few seconds to burn the external hairs.
- The second step took place under aseptic condition into laminar air flow hood. The spathes were gently opened with sterilized scalpel then the spikelets were soaked in mercuric chloride ($HgCl_2$) at 0.1% for 15 min. The explants of inflorescences were rinsed three times with sterilized distilled water.

Each spikelet of the two lengths (5–7 cm and 7–10 cm) were cut into 1–2 cm long pieces which each piece carries many florets (The initials of female flowers) as shown in (Fig. 1a and b).

Experiment (1): Effect of gradual decreasing of Abscisic Acid and Ancymidol concentration culture medium:
Components of nutrient medium: Each pieces of spikelets explants of the two lengths (5–7 cm and 7–10 cm) were cultured on half macro and full micro-elements of Murashige and Skoog (1962) combined with 40 mg^{-1} adenine – sulfate, 5 mg^{-1} thiamin-HCl, 100 mg^{-1} myoinositol, 200 mg^{-1} glutamine, 5 mg^{-1} 2,4-D, 1 mg^{-1} 2ip, 0.5 g^{-1} activated charcoal, 50 g^{-1} sucrose and 5.0 g^{-1} agar. Spikelet explants of the two lengths (5–7 cm and 7–10 cm)) were cultured periodically for 6 weeks intervals on medium supplemented with gradually decreasing in Abscisic Acid or Ancymidol concentration starting with the highest concentration (4.5 mg^{-1}) and ending with the lowest concentration (0.5 mg^{-1}).

pH of each medium was adjusted to 5.7 ± 0.1 prior to addition of agar, the medium were distributed into culture test tubes 15 ml × 150 ml. Each one continued 30 ml, the culture test tubes were immediately capped with poly propylene closure and then medium were sterilized by autoclaving at 121 °C and 15 Ibs/in^2 for 20 min culture test tubes of all treatments were incubated under complete darkness at 27 ± 2 °C.

Data were calculated after each culture as follows:

1. Percentage of direct somatic embryos.
2. Callus initiation degree/explant.

(This data Scored visually according to Pottino (1981) as follow):

Negative results (−) 1
Below average results (+) 2
Average results (+ +) 3
Good results (+ + +) 4
Very good result (+ + + + +) 5

Experiment (2): Effect of the culture medium phase (solid or liquid) and carbon source (sucrose or mannitol) on proliferation of inflorescences explants:

Smoothly friable callus which obtined from previous experiments were subcultured on liquid or solid nutrient medium. Two types of carbon source (sucrose or mannitol) at different concentrations (30, 40, 60 g^{-1}) were added to culture medium which consists of half salt strength of Murashige and Skoog nutrient medium combined with 0.4 mg^{-1} thiamin-HCl, 120.0 mg^{-1} potassium dehydrogenate orthophosphate (KH_2PO_4), 100 mg^{-1} glutamine, 100 mg^{-1} myoinositol, 0.5 mg^{-1} pyridoxine, 0.5 mg^{-1} nicotinic acid, 0.05 mg^{-1} benzyl adenine (BA) 0.1 mg^{-1} naphthalene acetic acid (NAA), 0.25 mg^{-1} Abscisic Acid (ABA), 0.3 mg^{-1} activated charcoal, 20 g/l sucrose were added in all mannitol treatments and 5 g^{-1} agar were added for solid medium. The pH of liquid medium was adjusted to 5.2 ± 0.1. Medium were distributed into small jars (50 ml) where each one contained 20 ml.

Culture jars were immediately capped with polypropylene closure and then medium were sterilized by autoclaving at 121 °C and 15 Ibs/in2 for 20 min. The inoculated patch of callus explants was about 0.5 g in each treatment. Incubation conditions were at 27° temperatures under low light with 2000 lux. The treatments of liquid culture medium were kept on the shaker at 120 rpm. Data were taken on the number of differentiated mature somatic embryos per explant after two subcultures (8 weeks).

Results and discussions

Culturing the explants periodically every 6 weeks on medium gradual decreasing in the concentrations of either ABA or Ancymidol showed variable morphogenesis characters as production of direct somatic embryogenesis or callus initiation.

Direct somatic embryos production

Table 1 showed that, the average values of direct somatic embryos percentage were significantly higher with ABA concentration than with Ancymidol concentration as found by Baochun and Wolyn (1996) who reported that ABA induced the

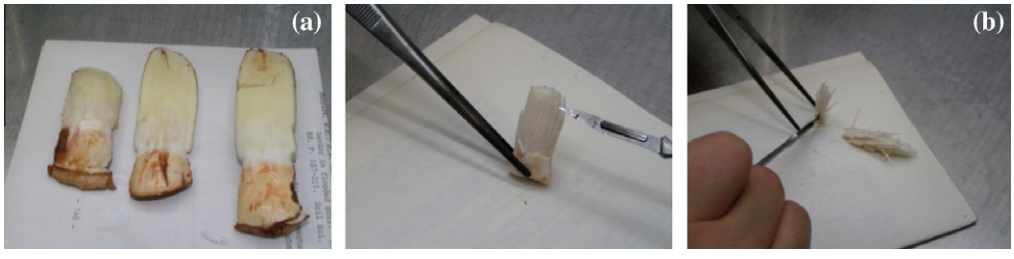

Fig. 1 (a) Different age spathes and (b) cut of the spikelets to 2–3 segments.