Journal of Genetic Engineering and Biotechnology (2016) 14, 363–370

Academy of Scientific Research & Technology and
National Research Center, Egypt

Journal of Genetic Engineering and Biotechnology

www.elsevier.com/locate/jgeb

ORIGINAL ARTICLE

In vitro conservation of embryogenic cultures of date palm using osmotic mediated growth agents

M.K. El-Bahr [a], A. Abd EL-Hamid [b], M.A. Matter [a,*], A. Shaltout [b], S.A. Bekheet [a], A.A. El-Ashry [a]

[a] *Plant Biotechnology Department, Genetic Engineering and Biotechnology Division, National Research Centre, 33 El Bohouth St. (Former El-Tahrir St.), Dokki, Giza P.O. 12622, Egypt*
[b] *Department of Horticulture, Ain-Shams Univ., Cairo, Egypt*

Received 9 June 2016; revised 30 July 2016; accepted 21 August 2016
Available online 3 September 2016

KEYWORDS

Date palm;
In vitro preservation;
Mannitol;
Sorbitol;
Sucrose

Abstract This study was carried out to investigate the effect of mannitol, sorbitol and sucrose as osmotic agents on *in vitro* conservation of embryogenic cultures of date palm (*Phoenix dactylifera*, L.) Bartamoda and Sakkoty cultivars. Embryogenic cultures was obtained using MS medium supplemented with 10 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D) and 3 mg/l isopentenyl adenine (2iP). Among the three types of osmotic substances used for slow growth conservation, sucrose at all concentrations gave the highest percentage of survival with Sakkoty cultivar. However, addition of 40 g/l or 60 g/l mannitol and 20 g/l sorbitol showed the highest percentage of survival percentage with Bartamoda cultivar. The different sucrose concentrations caused higher numbers of germinated embryos of the two cultivars compared with mannitol or sorbitol. Also, the number of germinated embryos was increased with increasing the storage periods till the ninth month. Genetic stability was determined using random amplified polymorphic DNA (RAPD) analysis. There were no clear genetic differences between the two osmotic agents used for preservation. The preserved cultures of Sakkoty cultivar gave the high percent of similarity while Bartamoda cultivar gave low percent of similarity. From the obtained results we can recommend using 40 g/l mannitol or 20 g/l sorbitol for *in vitro* preservation of Bartamoda cultivar of date palm and 20 g/l of sucrose for Sakkoty cultivar.

© 2016 Production and hosting by Elsevier B.V. on behalf of Academy of Scientific Research & Technology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The date palm (*Phoenix dactylifera*, L.), is a standout among the most monetarily imperative natural product crop developed in Middle East and North Africa [1]. The fruit is highly nutritious and rich as a source of sugar, minerals, and vitamins and it is considered the most important economical product of date palm tree. Furthermore, all the plant parts of the date palm tree have integrated in traditional or industrial applications [2]. Date palms are propagated sexually via seeds and vegetatively via offshoots. Vegetative propagation method is limited by both the numbers of offshoots produced from a superior selected plant and the development of useful offshoots

* Corresponding author.
Peer review under responsibility of National Research Center, Egypt.

http://dx.doi.org/10.1016/j.jgeb.2016.08.004
1687-157X © 2016 Production and hosting by Elsevier B.V. on behalf of Academy of Scientific Research & Technology.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

from a single plant, which occurs only during the juvenile phase of the palm's life [3].

At the present, the most widely recognized technique used to conserve date palm germplasm is as whole plant in field [4]. While *in situ* conservation is essential to maintain the evolution of the species and allow new diversity to be created through natural selection processes, it presents many disadvantages for conservation. The collections are exposed to natural disasters and attacks by pests and pathogens. In addition, distribution and exchange from field genebank are difficult because of the vegetative nature of the material and the greater risk of disease transfer, also, it needs great areas and cost labor. The most limiting factor is that the trees lose their abilities to give new off shoots after about 15–20 years of culturing which may lead to genetic erosion. *In vitro* methods have benefits and allow the successful preservation of date palm germplasm. The plant material is not exposed to different destructive factors and can be illimitably multiplied all year round.

Plant tissue culture and molecular biology techniques are of great interest for collecting, characterization, multiplication and storage of date palm germplasm. *In vitro* conservation involves the maintenance of explants in a sterile, pathogen-free environment. Miniaturization of explants allows reduction in space requirements and consequently labor cost for the maintenance of germplasm collections [5]. There are two main methods of *in vitro* conservation of plant germplasm. First is by reducing growth which was achieved by modifying the culture medium or low temperature incubation [6]. Second is cryopreservation which is understood as storage between −79 and −196 °C, the low extreme being the temperature of liquid nitrogen. *In vitro* storage under slow growth conditions delays the necessity for subculturing and consequently allows efficient utilization of labor year round [7]. Therefore, tissue culture considered an alternative method for date palm conservation that can eliminate the obstacles of field conservation.

The addition of osmotica such as mannitol, sucrose and sorbitol to the culture media has been proved to be efficient in reducing growth and increasing the storage life of many *in vitro* grown tissues of different plant species [8]. Healthy shoot bud cultures of date palm were obtained after 6 months of storage on a medium containing sorbitol. This period was extended for 9 months in the case of callus cultures [9]. Bekheet [10] mentioned that the presence of mannitol or sorbitol in culture medium had a retardant effect on the growth and development of globe artichoke cultures. In this respect, preservation of wild shih microshoots on a medium supplemented with sucrose, mannitol or sorbitol under light at 25 °C was able to inhibit the growth rate and maintain explant quality up to 12 weeks [11]. This study was planned to develop an applicable method for *in vitro* conservation of two Egyptian date palm cultivars i.e., Sakkoty and Bartamoda by adding mannitol, sorbitol and sucrose into the culture medium.

2. Materials and methods

2.1. Plant materials and sterilization

Offshoots about 10–15 kg of date palm cvs. Bartamoda and Sakkoty were detached from the adult females grown in Aswan governorate and used as plant materials. The outer leaves were removed with the hard bottom and fibrous sheaths belled off. Then the internal leaves were gradually removed and shoot apices in length (5 cm) were taken and kept in an antioxidant solution (100 mg/l ascorbic acid + 150 mg/l citric acid) to avoid explants browning. For disinfection, shoot tips were immersed in 70% ethanol for 1 min followed by 50% of sodium hypochlorite solution for 30 min and then explants were rinsed three times by sterile distilled water.

2.2. Explanting and culture media

Shoot tips were trimmed to about 1 cm in length and they were excised with part of sub-meristematic tissues then aseptically cultured on MS medium [12] supplemented with 2 mg/l dimethyl amino-purine (2iP), 1 mg/l naphthalene acetic acid (NAA). Culture medium was solidified by 7 g agar, the pH was adjusted to 5.8 using 0.1 N of either KOH or HCl then autoclaved at 121 °C and at a pressure of 1.2 kg cm^2 for 20 min. Cultures were incubated in a growth chamber at 25 ± 2 °C under darkness conditions and recultured on the same fresh medium for three times (six weeks intervals). Calli were transferred to MS medium supplemented with 10 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D) and 3 mg/l 2iP for six weeks and incubated at normal growth conditions (25 ± 2 °C under 16 h light (2000 Lux) and 8 h dark) for induction of embryogenic cultures (Fig. 1).

2.3. In vitro conservation using osmotic active agents

For evaluating the role of mannitol, sorbitol and sucrose as osmotic agents on conservation of embryogenic cultures of the two date palm cvs. Bartamoda and Sakkoty, equal pieces (250 mg) of embryogenic cultures of each cultivar were transferred to maintenance medium (MS + 2.5 mg/l kin + 0.5 mg/l 2,4-D) supplemented with 20, 40 or 60 g/l of mannitol, sorbitol or sucrose and cultures were incubated at 24 ± 2 °C at dark conditions. Survival percentage was recorded after 3, 6, 9 and 12 months of storage. The number of germinated embryos was also recorded. Each treatment consisted of three replicates (each replicate was represented by three culture jars).

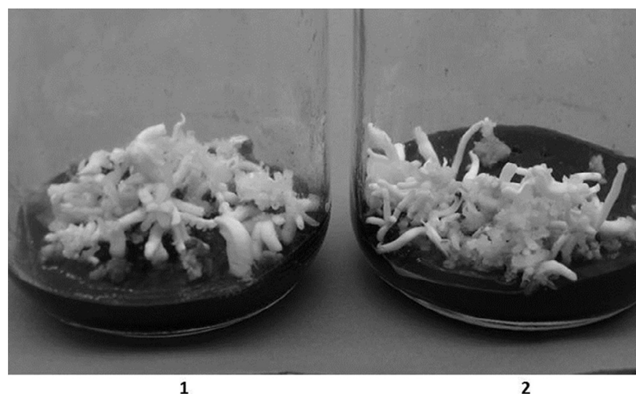

Figure 1 Embryogenic cultures of Bartamoda (1) and Sakkoty (2) cultivars proliferated on MS medium supplemented with 10 mg/l 2,4-D and 3 mg/l 2iP.

Download English Version:

https://daneshyari.com/en/article/8416580

Download Persian Version:

https://daneshyari.com/article/8416580

Daneshyari.com