South African Journal of Botany 114 (2018) 163–170

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

ISSR-assisted analysis of clonal fidelity supported with SEM and histology using *in vitro* propagated plants of *Moringa peregrina* (Forssk.) Fiori— An endangered desert tree

Fayas T. Purayil [a], Shyam S. Kurup [a,*], Mubarak M.S. Alkhaili [a,1], Nadia H. Tawfik [a], Salama M. Al Dhaheri [a], Abdul J. Cheruth [a], Shaikha S. Al Dhaheri [b], Sreeramanan Subramaniam [c]

[a] Department of Aridland Agriculture, College of Food and Agriculture, PO Box 15551, Al-Ain, United Arab Emirates
[b] Terrestrial and Marine Biodiversity Sector, Environment Agency, Abu Dhabi, United Arab Emirates
[c] School of Biological Sciences, Universiti Sains Malaysia (USM), Minden Heights, 11800, Georgetown, Penang, Malaysia

ARTICLE INFO

Article history:
Received 6 February 2017
Received in revised form 26 October 2017
Accepted 3 November 2017
Available online 23 November 2017

Edited by K Balkwill

Keywords:
Callus
Moringaceae
Organogenesis

ABSTRACT

Moringa peregrina is an important indigenous plant found in United Arab Emirates (UAE). It is an endangered species with important nutritive and medicinal properties. Environmental restrictions, urban expansion and unmanaged grazing endanger the plants. In this study, *in vitro* propagation of *M. peregrina* was carried out using different explants cultured in various combinations of plant growth regulators. Callus induction was successfully achieved by culturing shoot tip explants in medium containing either 2,4-D alone or in combination with thidiazuron [TDZ], 6-benzylaminopurine [BA] and kinetin [Kin]. The optimal callus induction was recorded in medium supplemented with 2,4-D (2 mg/L) and TDZ (0.1 mg/L) compared to other combinations. Callus regeneration was successfully achieved through culture in Murashige and Skoog (MS) medium containing BA and naphthalene acetic acid (NAA). Direct shoot organogenesis was achieved from nodal explants using MS medium supplemented with TDZ (0.2 mg/L). Histological and scanning electron microscopy (SEM) analyses confirmed the regeneration potential of the friable callus, which exhibited embryogenic spherical cells possessing densely stained meristematic zones amenable to differentiation into shoot primordia. The total number of roots was significantly higher in medium containing NAA when compared to medium containing indole-3-butyric acid [IBA]. The clonal fidelity of the *in vitro* plantlets developed through direct organogenesis was assessed using ISSR-DNA marker. The similarity indices between the parental plants and their progenies were above 98.2% and indicated that the progenies were highly similar to the mother plant. This technique allows mass multiplication of *M. peregrina*, hence providing a promising method of conserving the genetic resources for this plant.

1. Introduction

Moringa peregrina Fiori (*Moringaceae*) is generally known as the miracle tree in the Middle East region. There are more than twelve species in *Moringaceae*, with *M. oleifera* Lam. and *M. peregrina* among the better known species (Lalas et al., 2012). *Moringa peregrina* is found in semi-arid, arid and hyperarid regions in North-East Africa and the Middle East (Boulos, 1999). Native to the United Arab Emirates, it is locally known as *Shua* in Arabic. The height of the plant may range from 5 to 14 m. Other characteristics of the plant include grayish bark, scaly leaves and pinkish white flowers. Flowering begins annually in March, with a fruiting period of three months. The plants may produce more flowers at the expense of seed production as a result of adverse growing conditions (Hegazya et al., 2008). The fruiting body includes seed pods 20–40 cm long containing 8–15 seeds (Ghahreman, 2001). The seeds contain 42–54% oil and 23% protein. The oil is edible and has various medicinal uses (Al-kahtani, 1994). This plant is also a drought tolerant species that is able to grow in places with a deep water table (Cossalter, 1989). Xerophytic modifications in leaves and stems of Arabian *M. peregrina* were reported under drought conditions (Al-Gohary and Hajar, 1996). This species is considered as a potential crop in desert regions on account of its nutrient compositions and drought tolerance. Because of uncontrolled grazing and slow regeneration after browsing *M. peregrina* is considered to be endangered (Steinitz et al., 2009; Gomma and Pico, 2011).

To conserve this plant species from extinction, micropropagation is considered a feasible technique for clonal production and regeneration of superior planting materials under controlled conditions. This method supports the conservation of genetic characteristics of the plants which are frequently affected by environmental limitations (Kant et al., 2010). Compared to conventional propagation, micropropagation has many advantages, such as the multiplication of genetically and physiologically

* Corresponding author.
E-mail address: skurup@uaeu.ac.ae (S.S. Kurup).
[1] Part of the Ph.D. dissertation of the author

https://doi.org/10.1016/j.sajb.2017.11.006
0254-6299/

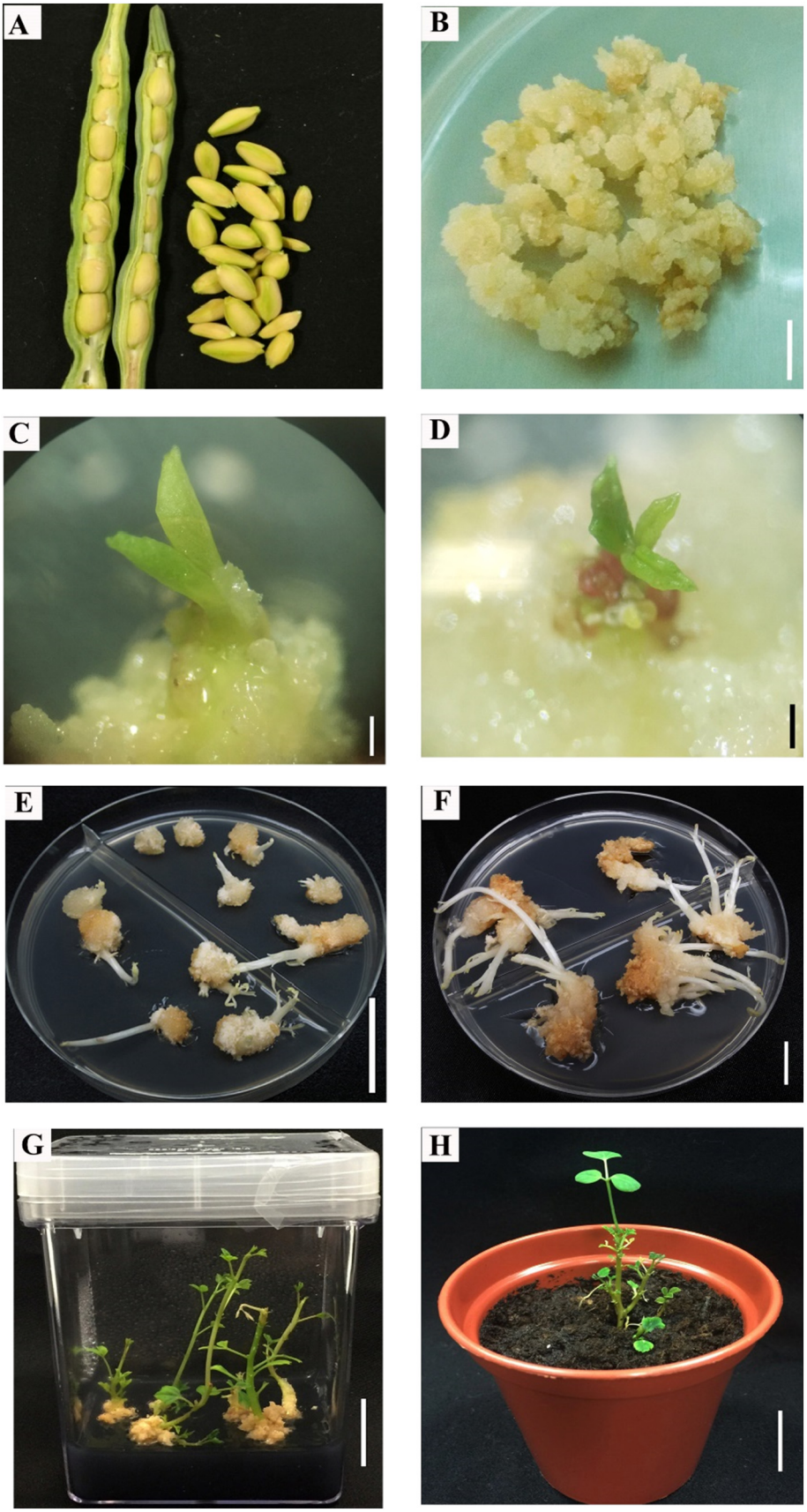

Fig. 1. *In vitro* multiplication of *Moringa peregrina*. **A:** *Moringa peregrina* fruits and seeds, **B**: *In vitro* callus from shoot cutting after 3 weeks of culture initiation, **C&D:** Formation of leaf primordia observed in callus, **E:** *In vitro* germination of nodal explants after 2 weeks of inoculation, **F:** Multiple shoot induction after 5 weeks of inoculation in media supplemented with 0.2 mg/L TDZ, **G**: Regenerated plantlets after 12 weeks of culture initiation, **H**: Healthy plantlets transferred to pot filled with soil and peat moss (1:1). Scale bar in B, C & D = 0.5 cm, E & F = 2 cm, G & H = 3 cm.