Journal of Applied Research on Medicinal and Aromatic Plants xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

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

En-masse production of elite clones of *Dendrobium crepidatum*: A threatened, medicinal orchid used in Traditional Chinese Medicine (TCM)

Paromik Bhattacharyya[a,b], Suman Kumaria[a,*], Nikhil Job[a], Pramod Tandon[a]

[a] *Plant Biotechnology Laboratory, Centre for Advanced Studies, Department of Botany, North-Eastern Hill University, Shillong 793022, Meghalaya, India*
[b] *Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa*

ARTICLE INFO

Article history:
Received 10 January 2016
Received in revised form 12 March 2016
Accepted 11 April 2016
Available online xxx

Keywords:
Medicinal orchid
Traditional Chinese Medicine
Secondary metabolites
Natural antioxidants
Gene targeted markers

ABSTRACT

Orchids are one of the promising medicinal plant families of which *Dendrobium crepidatum*. Lindl & Paxton figures out prominently because of its multi directional medicinal attributes. In the present report, *in vitro* regeneration protocol has been developed from the nodal segments of *D. crepidatum*, to cater sustainable commercial exploitation and conservation needs. Thidiazuron (TDZ) at 3 mg/l singly resulted in the response frequency of 55% which could be increased to 97% by incorporating NAA at 0.5 mg/l and TDZ at 2 mg/l in combination in the medium. Shoot induction rate was further enhanced with the use of polyamines and at 0.8 mM putrescine along with 2 mg/l TDZ and 0.5 mg/l NAA in the medium 11.8 shoots/explant could be obtained. Highest rooting frequency of the shoots was achieved in medium containing 2 mg/l IBA. Genetic stability of the acclimatized plants was assessed using Start Codon Targeted (SCoT) polymorphism and inters simple sequence repeats (ISSR). SCoT revealed a total variability of 10% within the micropropagated plants whereas the cumulative ISSR and SCoT data revealed 6.25% clonal variability indicating high genetic fidelity amongst the regenerates. A comprehensive higher yield of the secondary metabolites along with significant higher antioxidant potentials as compared to the mother plant was revealed using DPPH and FRAP assays. An increased regeneration frequency with a comparative higher yield of secondary metabolite and genetic stability reported in the present communication ensure the ingenuity of this clonal propagation protocol developed for *D. crepidatum* which can be further utilized in the sustainable commercial utilization and conservation of other medicinally important orchid species.

1. Introduction

The genus *Dendrobium*, is one of the most highly evolved and diversified group represented by more than 1100 species worldwide with its major distribution areas ranging from Southeast Asia to New Guinea and Australia (Puchooa, 2004). India has rich orchid diversity and Northeast India, being part of the Indo-Burma mega biodiversity hotspot harbors one of the richest reserves of orchids comprising around 750–800 orchid species (Chowdhery, 2001). The use of orchids as the source of medicines for the treatment of diseases dates back many centuries and was first reported by the Chinese (Bulpitt, 2005). Orchids are a rich source of various important secondary metabolites of biological importance like alkaloids, sesquiterpenes, polyphenols etc (Okamoto et al., 1966; Lawler and Slaytor, 1969; Elander et al., 1973). The dried stems obtained from 30 medicinal dendrobes including *Dendrobium crepidatum* Lindl. & Paxton, are used in traditional Chinese pharmacopeias as a "Yin tonic" to nourish the stomach, promote the body fluid secretion, prevent cataract development, relieve throat inflammation, reduce peripheral vascular obstruction and enhance the levels of body immunity (Bao et al., 2001). The medicinal importance of *D. crepidatum* is primarily due to the presence of various secondary metabolites predominantly alkaloids in its tissue parts in significant amounts. It has been reported that the alkaloid, crepidatin, from *D. crepidatum* has potent cancer cell killing properties and the plant extract also upregulates the activities of nerve growth factor (NGF) mediated neurite outgrowth in PC12 cell lines (Majumder and Chatterjee, 1989; Hossain, 2009; Gutiérrez, 2010; Li et al., 2013). As the principal importance of medicinal plants is being highlighted for being the source of natural antioxidant and functional foods, the role of clonal propagation attracts much attention (Vaidya and Devasagayam, 2007). Several epidemiological studies revealed that a high intake of herbal materials rich in natural antioxidants reduces the risk of chronic diseases (Hossain and Rahman, 2011).

* Corresponding author.
E-mail address: sumankhatrikumaria@gmail.com (S. Kumaria).

http://dx.doi.org/10.1016/j.jarmap.2016.04.001
2214-7861/

Please cite this article in press as: Bhattacharyya, P., et al., *En-masse* production of elite clones of *Dendrobium crepidatum*: A threatened, medicinal orchid used in Traditional Chinese Medicine (TCM). J. Appl. Res. Med. Aromat. Plants (2016), http://dx.doi.org/10.1016/j.jarmap.2016.04.001

Fig. 1. Micropropagation of *D. crepidatum* from nodal segments: (A) mature plant in the green house (B) initiation of shoot buds after 3 weeks of culture in MS + 3 mg/l TDZ (bar = 5 mm) (C) initiation of primary PLBs in MS + 3mgl TDZ after 3 weeks of culture (bar = 5 mm) (D) proliferation of multiple shoots in MS + 2 mg/l TDZ and 0.5 mg/l NAA (bar = 10 mm) (E) multiplication of shoots in MS + 2 mg/l TDZ + 0.5 mg/l NAA + 0.8 mM putrescine (bar = 10 mm) (F) rooting in half-strength MS + 2 mg/l TDZ + 0.5 mg/l NAA + 8 mM putrescine + 2 mg/l IBA (bar = 1 cm) (G) complete plantlets with roots (bar = 1 cm) (H) greenhouse acclimatized plantlets.

Various chemical compounds are present within the medicinal plants amongst which polyphenol has received much importance mainly because of its multifaceted role in the treatment of various degenerative and aging related disorders. Researches revealed that polyphenols exhibit potent antioxidant activity in various *in vitro* cellular assays (Brewer, 2011; Halliwell, 2008; Procházková et al., 2011).

Conventionally, dendrobes are propagated through cuttings, separation of offshoots and keikis produced from the old stems resulting in a few propagules after 2–3 years. Furthermore, the orchid diversity in the Northeast India and the country as a whole is

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