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RESEARCH ARTICLE

Vitrification-Based Cryopreservation of In Vitro-Grown Apical Meristems of *Chlorophytum borivilianum* Sant et Fernand: A Critically Endangered Species

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Abstract This article reports the cryopreservation of apical meristems of *Chlorophytum borivilianum*, a tropical and IUCN critically endangered species. Initially, in vitro cultured shoots were pre-adapted on 12% (w/v) sucrose for 2-months and were found appropriate stock material for further experimentations. Furthermore, the preculture of meristems excised from pre-adapted in vitro shoots on 12% (w/v) sucrose containing MS medium with 50 mg/l abscisic acid for 48 h, followed by treatment with loading solution (LS), and plant vitrification solution (PVS2) was found crucial for recovery following cryostorage. Thereafter, durations of exposure to the LS and PVS2 were optimized to enhance the regeneration efficiency of apical meristems. Treatment with LS for 20 min followed by 30 min exposure to PVS2 was standardized for the vitrification of the apical meristems before plunging them into liquid nitrogen. Moreover, after cryoexposure thawing was performed for 1 min at 38 °C ± 2 in a water-bath followed by the treatment with unloading solution for 10 min resulted in enhanced recovery up to 33% on 2 mg/l 6-benzyladenine (BA) and 0.2 mg/l α-naphthalene acetic acid containing MS medium.

Keywords Abscisic acid · Liquid nitrogen · PVS2 · Sucrose · Vitrification

Significance Statement: For the cryopreservation of *Chlorophytum borivilianum* abscisic acid was found crucial that helps in freeze tolerance. Moreover, the exposure times to cryoprotectants were optimized which minimizes the toxicity and helps in regeneration of meristems.

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Introduction

Chlorophytum borivilianum Sant et Fernand is a tropical and vegetatively propagated species. Its tuberous root contains steroidal saponins, which vary considerably among the genotypes and ranges between 2 and 17% of its dry weight [1]. A number of reports are there revealing the impact of this herb on diabetes, arthritis, rheumatism and its aphrodisiac potential [2, 3]. Huge commercial and pharmaceutical importance is one of the major causes of overexploitation of this species from its natural habitats [3]. Moreover, seed germination rate is very poor i.e., 8–16% only [4]. Therefore, it has been documented as a critically endangered herb in the Red List of the IUCN [5] and as a rare plant species by the Botanical Survey of India [6]. A number of literatures are available for in vitro propagation of this herb; however, conservation through cryopreservation was not attempted till now.

The conventional method of germplasm conservation includes maintenance of whole plants in the field [7]. Field maintenance of plant materials not only carries the risks of infections of viral, fungal, bacterial diseases and insect-pests, but also includes losses due to environmental disasters, which has led to the erosion of valuable germplasm resources [8]. The most appropriate method suggested for long-term ex situ conservation of any species is storage of their seeds. However, in the case of vegetatively propagated species or of species with low germination rate,

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