

SUCROSE TREATMENT AND EXPLANT WATER CONTENT: CRITICAL FACTORS TO CONSIDER IN DEVELOPMENT OF SUCCESSFUL CRYOPRESERVATION PROTOCOLS FOR SHOOT TIP EXPLANTS OF THE TROPICAL SPECIES *Dioscorea rotundata* (YAM)

Marian D. Quain^{1*}, Patricia Berjak², Elizabeth Acheampong³ and Joseph I. Kioko²

¹ CSIR-Crops Research Institute, P.O.BOX 3785 Kumasi, Ghana

² School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban, South Africa

³ Tissue Culture Laboratory, Department of Botany, University of Ghana, Legon, Accra, Ghana

*Corresponding author : email: md.quain@cropsresearch.org, marianquain@hotmail.com

Abstract

A study was conducted to determine the optimum methods for conditioning explants to be used in the development of a simple protocol for long-term conservation of the germplasm of *Dioscorea rotundata* via cryopreservation. Shoot tips from cultures maintained *in vitro* were exposed to high concentrations of sucrose prior to silica gel-based dehydration and vitrification solution-based cryopreservation protocols. Explant water contents were determined, and ultrastructural studies were also carried out. Initially, culturing explants on medium supplemented with 0.3 M sucrose for 3-5 d considerably reduced tissue water content from about 12.2 g g⁻¹ dry mass to between 4.8 and 5.5 g g⁻¹ dry mass before cryoprotection with modified PVS2 (MPVS2) or silica gel dehydration. Ultrastructural studies indicated that cells had deposits of starch in plastids following sucrose treatments. Survival for *D. rotundata* shoot tips treated with MPVS2 vitrification solution, unloaded with 1.0 M sucrose medium and cooled to -70°C, was 16% for 15 min treatment and 44% for 40 min. After the 40 min MPVS2 treatment the TTZ test indicated 88% viability retention of explants cooled to -70°C, and 44% at -196°C. Plantlet development was obtained for -70°C-cooled shoot tips, whereas only callus development occurred from tissues exposed to liquid nitrogen. Explant regeneration was not obtained with air-dehydration techniques. It was concluded that vitrification-solution based cryopreservation presently offers the best option for conservation of this *Dioscorea* species.

Keywords: cryopreservation, desiccation, explants, *in vitro*, vitrification, yam

INTRODUCTION

Dioscorea species, colloquially known as yams, are perennial monocotyledonous climbers with underground tubers which, in some species are edible and serve as major staples in sub-Saharan Africa. Propagation is routinely vegetative, using either the tubers or vine cuttings