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EXPLORING THE USE OF DMSO AND ASCORBIC ACID TO PROMOTE SHOOT DEVELOPMENT BY EXCISED EMBRYONIC AXES OF RECALCITRANT SEEDS

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Abstract

Seeds of *Trichilia dregeana, T. emetica* and *Protorhus longifolia* are recalcitrant (desiccation-sensitive), hence cryopreservation is the only *ex situ* means feasible for long-term conservation of these germplasm. For cryopreservation of these species, the excised embryonic axis is the explants of choice due to their small size and higher tolerance to desiccation. However, for many species with seeds having fleshy cotyledons, shoot development fails to occur after excision, which has been attributed to a reactive oxygen species (ROS) burst during excision wounding. This is a critical limiting step in developing cryopreservation protocols for such species. In embryos of *T. dregeana, T. emetica* and *P. longifolia*, the cotyledonary insertions are in close proximity to the shoot apical meristem and oxidative stress upon excision of the axis from cotyledons has been consistently associated with shoot tip necrosis, which precludes shoot development. This study tested the effects of dimethyl sulphoxide (DMSO) pre-culture prior to complete removal of the cotyledons, and post-excision soaking in DMSO or in the antioxidant, ascorbic acid, on shoot development by axes of *T. dregeana* and *P. longifolia*. These treatments had a significant ($p<0.05$) positive effect on shoot production with a 6 h DMSO pre-culture combined with a DMSO post-excision soak being optimal for promoting shoot production in 70% of the axes of *T. dregeana* and 60% of those of *P. longifolia*. Embryonic axes of *T. emetica* responded best to a 6 h DMSO pre-culture alone, with 55% of axes producing shoots. It was further shown that two different post-harvest developmental stages of *T. dregeana* axes differed significantly initially ($p<0.05$) in their response to DMSO and ascorbic acid treatments.

Keywords: antioxidants, ascorbic acid, cryopreservation, desiccation sensitive, DMSO, excision injury, Me_2SO, oxidative stress, *Protorhus*, recalcitrant seeds, ROS, *Trichilia*

INTRODUCTION

Recalcitrant (desiccation-sensitive) seeds are characterised by high water content and an ongoing developmental or germinative metabolic state at shedding; by their desiccation- and, in some cases, chilling-sensitivity; and by a short post-shedding life span (reviewed in 6). Seeds of *Trichilia dregeana* Sond., *Trichilia emetica* Vahl. (Meliaceae), and *Protorhus longifolia* (Berrh.) Engl. (Anacardiaceae) have been categorised as recalcitrant (18,20) and