*Asian J. of Bio Sci. (2006) Vol. **1** No. 2 : 112-113*

Cryopreservation of *Citrus reticulata* embryonic axes by vitrification technique

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(Accepted : June, 2006)

The importance of loading and treatment with a vitrification solution on the survival of *Citrus reticulata* embryonic axes cryopreserved using vitrification technique (Derudde *et al*., 1990) were investigated in this paper. Loading treatment was carried out for 20 min. at 25 ^{0}C before chemical dehydration with 100 per cent PVS2 for 20 min. After treatment with PVS2 rapid immersion in liquid nitrogen, rapid rewarming, unloading in a 1.2 M sucrose solution for 20 min. Then the culture on solid medium with 0.3 M sucrose for 1 day and growth recovery for 4 weeks on standard medium, survival of *C. reticulata* embryonic axes reached 60 per cent following the process.

Key words : Citrus, Vitrification, Cryopreservation.

INTRODUCTION

CITRUS reticulata is member of Rutaceae family, which is most important fruit crop in India. Citrus are not only delicious to eat, but also provide vitamin, minerals and many other nutrients.

In-situ conservation of plant genetic resources may not be adequate and feasible for each and every plant species therefore, the *ex situ* conservation provides the opportunity for safe and reliable conservation of plant genetic resources.

Various cryopreservation techniques are presently available for long-term conservation of plant genetic resources, among them the vitrification technique (Dereudde *et al.,* 1990). However, in recent years, vitrification has also been used for freezing zygotic embryos or embryonic axes of *C sinensis* (Cho *et al.,* 2002). Seeds of *C. reticulata* display sensitivity to desiccation and freezing in such cases, embryonic axes are employed for cryopreservation. This study investigates the effect of the loading and vitrification treatment on the survival of excised *C.reticulata* axes cryopreserved using a vitrification protocol.

MATERIALS AND METHODS

The investigation was carried out at National Bureau of Plant Genetic Resources, New Delhi in 2003, to study seed storage behavior and cryopreservation of *Citrus reticulata* embryonic axes by vitrification technique.

Seed storage behavior of *C. reticulata* was carried out by studying polyembryony, morphological characters such as seed size, texture, mucilaginous deposition and physiological features especially desiccation and freezing sensitivity. In vitrification technique first embryonic axes were excised from the *C.reticulata* seed and kept in laminar airflow for desiccation. Then of freshly excised embryonic axes were pre-cultures on MS medium supplemented with 0.1 M sucrose, followed by a day culture on medium supplemented with 0.3 M sucrose + 0.5 glycerol. Then loading treatment was carried out for 20 minutes at 25 ^{0}C before chemical dehydration with PVS2 (Sakai *et al.,* 1991). To avoid detrimental recrystalization rapid thawing of tissues at round 35-40 ^{0}C was essential after removing from liquid nitrogen.

After rewarming, the highly concentrated PVS2 solution should be washed or replaced with an unloading solution. Usually liquid medium supplemented with 1-2 M sucrose so after thawing. Pipette out the leftover PVS2 and add 1 ml of unloading solution. After gentle swirling, replace the unloading solution and allow the treatments for 20 min at 25^0 C.

Then the axes are placed on agar solidified regrowth medium in petriplate lined with sterile filter and incubate over night in culture room (Murashige and Skoog, 1962).

RESULTS AND DISCUSSION

Seed characteristics of Citrus reticulata :

Seeds of *C. reticulata* were glossy, beaded having average seed of 7.3 x 4 mm. The results obtained in the present investigation are in accordance with the results of Mumford and Grout (1979) and Radhamani *et al.* (1991). They reported that role of seed coat have found to be inhibitory for germination in many species of citrus**.** The detailed data is given in Table 1.

Moisture content and Viability of seeds of C. reticulata:

The results revealed that, in *C. reticulata*, the moisture content decreased due to desiccation from 40.27 (fresh) to 9.2 (desiccated) per cent which resulted lowering 100 to 80 per cent germination. Similar results were obtained by Hong and Ellis (1995), who categorized many species of citrus under intermediate and recalcitrant category orthodox on the basis of moisture content and size of seed. The data on moisture content and viability of fresh and desiccated seeds are given in Table 2.

Polyembryony of *C. reticulata*:

Polyembryony is the common feature of *Citrus* and allied genera. It was found that all the accessions invariably showed polyembryonic nature but the percent varied with accessions. The detailed data is given in Table 3.

In *C. reticulata*, the lowest polyembryony observed in MD-35 (10.0 per cent) followed by MD-2 and MD-57 (25 per cent) with an average of 2-3 seedlings. This is in confirmation with the results obtained by Prasad and Ravishankar (1983), who reported that citrus sp. vary considerably with regard to poly-embryony, number of nucellar, and embryos per seed.

Effect of vitrification on survival of embryonic axes of *C. reticulata*:

The effect vitrifications on embryonic axes are presented in Table.4.

The results in Table –4 and Figure-1 showed that 75 to 100 per cent and 50 to 80 per cent viability before and after cry preservation, respectively.

The above findings in good conformity with the results of Cho *et al.* (2002) who studied the seven different loading solutions of vitrification, among the solution containing 2M glycerol + 0.4M sucrose was the most efficient. Of the six vitrification solutions tested, PVS2 vitrification solution, applied for 20 min. at 25^{0}C ensured the highest survival of embryonic of *Citrus madurensis.*

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