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Cryopreservation of *In Vitro* - Cultured Pre-Embryo of *Sapindus mukorossi* Gaertn.

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Objectives

The world is plant resources is declining at un precedented rates and the loss of biological diversity is a major environmental concern. Genetic conservation of plant species will be needed to maintain broad genetic polymorphism. A combination of in situ and ex situ techniques might be incorporated for this purpose. To overcome so many obstacles in methodologies, the cryopreservation could play an important role in gene conservation. It is reported th crypreservation procedure of *S. mukorossi* by vitrification #1, vitrification #2, encapsulation-dehydration and encapsulation-vitrification method.

Materials and Methods

1. Materials: *in vitro* culture pre-embryos of *S. mukorossi*
2. Methods: four cryopreservation methods
   1) Vitrification #1
   Pre-embryos were loaded for 20 min 25°C with a cryoprotectant in cryotube and replaced with the PVS2 vitrification solution on ice. Pre-embryos were preserved in -196°C for a day by plunging directly into LN(liquid nitrogen). Pre-embryos were rapidly warmed in a water bath at 40 °C And PVS2 was removed and plunging on liquid B5 medium containing 1.2 M sucrose for 15 min. After, pre-embryos was planted on regeneration medium.
   2) Vitrification #2
   Pre-embryos were loaded for 20 min 25°C with a cryoprotectant in cryotubes and replaced with the PVS2 vitrification solution at room temperature. Next process made use of vitrification #1 method.
   3) Encapsulation-dehydration
   Making alginate gel beads within pre-embryo precultured for 18 hr in MS medium supplemented with 0.75M sucrose. Place beads in sterile silica gel and dry in the air flow for 0 ~ 360 min. Place beads in cryotube and submerge for a day in LN. Thaw at room temperature for 15 min in liquid MS medium to rehydrate and place on regeneration medium.
   4) Encapsulation-vitrification
   Preculture is used of encapsulation-dehydration with same method. Place beads in PVS2 vitrification solution on ice and submerge for a day in LN. Thaw at room temperature for 15 min in MS medium with 2 M glycerol and 0.4 M sucrose to rehydrate and place on regeneration medium.

Results and Discussion

As survival rate, it was seen the better vitrification methods than encapsulation methods. The case of encapsulation, we showed survival rate that it almost near to 100 % both all. The case of vitrification, the frist method was showed survival rate about 80 % and the most good result found out that vitrification time was from 40 min to 80 min on ice. The second method was showed survival rate about 90 % and the most good result found out that vitrification time was from 100 min to 120 min at the room temperature. As a whole, we had got a good survival rate, because the vital power of the material, pre-embryo stage, is very good.

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