IN VITRO GERMPLASM CONSERVATION OF *Magnolia sirindhorniae* Noot. & Chalermglin IN MINIMAL GROWTH CONDITION AND BY CRYOPRESERVATION

Sirikool Kesa [*1,2], Patchra Limpanavech[2], Sumitra Kongchuensin[2], Piyarat Chareonsap[1] and Pornchai Juthamas[1]

[1] *Plant Genetics Conservation Project as The Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn, Bangkok 10303, Thailand*
[2]*Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10400 Thailand*

Magnolia sirindhorniae Noot. & Chalermglin is a new and the only *Magnolia* species existing in fresh water swamp forest and endemic to Thailand. *In vitro* conservation of *Magnolia sirindhorniae* Noot. & Chalermglin shoot tips was conducted to study the effects of 1) the different concentrations of macronutrients: MS, 3/4MS and 1/2MS 2) the concentrations of two types of sugar: sucrose 20, 30 g/l in combination with mannitol 0, 10, and 20 g/l and 3) the concentrations of growth retardant, paclobutrazol 0, 10 and 20 mg/l. It was found that the shoot tips in 3/4MS with 20 g/l sucrose and the addition of 10 mg/l paclobutrazol was found to extend the storage period to eight months with the survival rate of 46.6±9.2% from all of which the recovery plantlets on regeneration medium were achieved. The cryopreservation of shoot tips by encapsulation-vitrification method was also experimented to study the effects of cold hardening during preculturing, the immersion period of shoot tips in osmoprotective solution and PVS_2 at 0°c for long term storage. The most suitable method obtained was done by preculturing the shoot tips on MS medium containing 0.3 M sucrose at 25 °c for two days. The shoot tips were then encapsulated and left in the osmoprotective solution for 60 min. then dehydrated with PVS_2 at 0°c for 60 min. before plunging into liquid nitrogen. After thawing and reculturing on MS medium, the survival rate of the shoot tips observed was 33.3±8.7% while recovery growth on regeneration medium was 26.6±8.2%. Cold hardening at 15°c during preculturing, using osmoprotective solution and PVS_2 for over 60 min. were found to decrease the survival rate of the shoot tips.

Keywords: *Magnolia sirindhorniae*, minimal growth storage, Cryopreservation , encapsulation-vitrification

*corresponding author: Sirikool Kesa (E-mail: piyarat@rspg.org, sirikool.k@rspg.org)

Introduction : *Magnolia sirindhorniae* Noot. & Chalermglin was the new species of the Family Magnoliaceae, endemic to Thailand, found in fresh water swamp forest in Lopburi province (Nooteboom and Chalermglin, 2000). Due to its narrow adaptation and limited quantity in natural habitat, this species is concerned risky of loss. *In vitro* conservation offers a suitable alternative to field collections. Storage under minimal growth by manipulation of the culture media, altering the nutrient condition and use of growth retardants has been widely applied for short and medium term conservation. Cryopreservation using liquid nitrogen (-196 ^{0}C) is believed to be the safe and cost-effective option for long-term storage of plant germplasm; however, the protection of plant cells from damages caused by the crystallization of intracellular water into ice is necessary (Engelmann, 2000). The encapsulation-vitrification technique is the currently promising method to accomplish proved in some species (Sakai *et al*., 1991, Matsumoto *et al.,* 1995). This paper reports the successful method both for short to medium term and long term conservation of *Magnolia sirindhorniae*.

Methods : *In vitro* grown shoot tips of *Magnolia sirindhorniae* Noot. & Chalermglin were used in this study. At least 10 shoot tips were used in each experiment with 3 replications. For minimal growth storage experiment, three sets of consequential experiments were carried out. In the first set, 1 cm. long shoot tips were aseptically transferred to modified Murashige and Skoog,

1962 medium which varied macronutrient concentration to full strength (MS), 3/4 strength (3/4MS) and half strength (1/2MS). The cultures were maintained under 25±2°c 40 μmol m^{-2} s^{-1} light intensity, 16 h photoperiod till more than half of the shoot tips in each experiment showed chorotic base up to the first node. The treatment which gave the longest storage period of time, was selected to use for the second set of experiment to examine the kind and concentration of sugar: sucrose 20 and 30 g/l in combination with mannitol 0, 10 and 20 g/l. The treatment with best result was again selected to use in the third set of experiment to study the effect of growth retardant, Paclobutrazol: 0, 10 and 20 mg/l. The cultures were maintained under the same mentioned condition without subculturing and survival rate and the vigor of shoots by score ranking were recorded and calculated for standard error then the data from the best selected experiment were statistically analyzed and compared monthly by ANOVA and DMRT. All of the shoot tips were transferred to the medium, MS+2 mg/lBA, for 2 month to observe the plantlet regeneration.

Cryopreservation by encapsulation-vitrification: To find out the suitable procedures for encapsulation-vitrification before cryopreservation, the excised 2-3 mm. shoot tips were transferred to MS+0.3 M sucrose for preculturing at 25°c compared to cold-hardening at 15 °c for 2 day. Then the shoot tips were encapsulated into 3% Na-alginate gel beads and osmoprotected with the solution containing 2.0 M glycerol+0.4 M sucrose for 0, 30 and 90 min on a rotary shaker and then dehydrated with PVS_2 solution for 0, 60 and 120 min at 0 °c. The tested shoot tips were cultured on MS medium for 1 month and survival rate was recorded. Then the suitable procedures that gave more than 50% survival shoot tips were selected to prepare the shoot tips again then plunged into liquid nitrogen. After rapid warming in a water bath at 38 °c, the shoot tips were then replaced PVS_2 with 1.2 M sucrose for 20 min. The encapsulated shoot tips were transferred onto MS+0.3 M sucrose for 1 day then reculture on MS medium for 1 month to observe the survival rate. The shoot tips were transferred to MS+ 2.0mg/l BA for recovery. The rate of shoot formation was expressed as a percentage of the total number of normal shoot recovery after 1 month culturing.

Results and Discussion : The modified MS medium containing 3/4 MS macronutrients, can be used to store shoot tips of *Magnolia sirindhorniae* for 3 months. If sucrose was reduced to the level of 20 g/l, the conservation period could be extended to 6 months and the addition of 10 mg/l Paclobutrazol was found to increase the storage life to 7 months with the same survival rate of 73.3±8.2%. The regenerated plantlets were found 100% recovered and vigorous. From 18 treatments of encapsulation-vitrification method, the suitable procedure for cryopreservation of shoot tips of *Magnolia sirindhorniae* was preculturing the shoot tips on MS+0.3 M sucrose at 25°c for 2 days, then encapsulating the shoot tips and adding the osmprotective solution for 60 min then dehydrating with PVS_2 at 0°c for 60 min. before plunging into LN. Survival rate of shoot tips observed after thawing and reculturing on MS medium was 33.3±8.7% while shoot tip recovery on MS+2.0mg/l BA was 26.6±8.2%. Cold hardening at 15°c during preculturing, using osmoprotective solution and PVS_2 for more than 60 min. decreased the survival rate of the shoot tips

Acknowledgment : This study was supported by Novartis scholarship to commemorate the Queen's 72 years Anniversary and Plant Genetics Conservation Project as The Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn, Bangkok Thailand.

References :

(1) Engelmann, F. 2000. Importance of cryopreservation for the conservation of plant genetic resources. In F. Englemann and H. Takagi (eds.), Cryopreservation of tropical plant germplasm current research progress and application, pp. 1-7. Rome : IPGRI.

(2) Matsumoto, T., Sakai, A., Takahashi, C. and Yamada, K. 1995. Cryopreservation of *in vitro* grown apical meristems of wasabi (*Wasabi japonica*) by encapsulation-vitrification method. CryoLetters. 16: 189-196.

(3) Murashige, T. and Skoog, F. 1962. A revised meium for rapid growth and bioassays with tobacco tissue culture. Physiologia Plantarum. 15: 473-497.

(4) Nooteboom, H.P. and Chalermglin, P.2000. A new species of *Magnolia* (Magnoliaceae) from Thailand. Blumea. 45:245-247.

(5) Sakai, A., Kobayashi, S. and Oiyama, I. 1991. Survival by vitrification of nucellar cell of navel orange (*Citrus sinensis* var 'brasiliensis'Tanaka) cooled to -196°C. Plant physiology. 137: 465-470.