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Cryopreservation of Seeds of Lily [Lilium Iedebourii (Baker) Bioss.]: Use of Sucrose and Dehydration

B. Kaviani Livani

***Abstract*—Cryopreservation of germplasm at liquid nitrogen (-196°C) is a perfect method for the long-term conservation of plant genetic resources. A cryopreservation process using dehydration was performed for seeds of lily [Lilium Iedebourii (Baker) Bioss.]. Seeds were subjected to a rapid freezing protocol in liquid nitrogen following dehydration and treatment with 0.75 M sucrose for 1 h. Survival after freezing was nil for control seeds and, 75% for seeds treated with sucrose and dehydration.**

***Key words*— conservation, dehydration, germplasm, lily, sucrose.**

I. INTRODUCTION

Lilium Iedebourii (Chelcheragh lily) is distributed in the Damash of Ammarloo and Kalchooleh of Dorfak areas of Guilan province in the north of Iran. It is a perennial plant that has good ornamental value, especially as a pot plant [1]. This plant attracts lots of tourists from all over the world. Lilium Iedebourii (Baker) Bioss., a threatened and rarest lily, is an endiginous species to Iran. Seeds produced in June, are thin and papery. In vitro conservation of the plant germplasm is essential for plant breeding programs, also, provides a source of compounds to the pharmaceutical, food and crop protection industries. In the last decade, some reliable cryogenic procedures, have been developed and the number of cryopreserved species has enormously been increased [2], [3]. For the long-term conservation of plant germplasm, cryopreservation, at ultra-low temperature (liquid nitrogen, -196°C), is actually the valuable technique.

Cryopreservation of biological tissues can be successful only if intra-cellular ice crystal formation is avoided. Crystal formation, can be prevented through vitrification [4]. Two requirements must be met for a cell to vitrify; rapid freezing and a concentrated cellular solution [5]. Sugars play a very important role in the acquisition of resistance to desiccation and to freezing in LN [6], [7]. Generally, tissues which have low water content, such as meristematic tissues, embryonic axes and seeds are more resistant to the stress of these techniques [8], [9].

In current study, dehydration and sucrose, a non-penetrating cryoprotective substance, used as the pretreatments.

B. Kaviani Livani is with the Department of Horticultural Science, Faculty of Agriculture, Islamic Azad Universiy, Rasht Branch, Rasht, Iran
Phone: +989111777482, +981314240149; Fax: +981313462255

II. Materials and methods

Seeds of Lilium Iedebourii (Baker) Bioss. were collected from Damash area of Guilan province in the north of Iran. Seeds were disinfected in ethanol 70% (v/v) for 1 min followed by sodium hypochlorite 0.5% (v/v) for 10 min. For osmoprotection, seeds were suspended in MS [10] liquid medium containing 0.75 M sucrose for 1 h with agitation. Seeds were transferred to empty open petri dishes and desiccated in the air current of a laminar flow chamber for 1 h. To determine the moisture content, 20 seeds were maintained in MS liquid medium with 0.75 M sucrose for 1 h with agitation. After that they were dessicated 1 h under laminar flow. The dehydrated seeds were weighted and dried in oven at 110°C for 20 h. Moisture content was expressed as a percentage of their initial fresh weight. Moisture content in control seeds is very low (about 10-15%) but after disinfection the moisture content is high. Seeds become dehydration for decreasing of moisture content. For cryopreservation, control and osmoprotected seeds were placed in a 1.8 ml cryotube and directly plunged into LN and held for 24 h. Cryotubes were thawed in a water-bath at 37-38°C for 3 min. Following cryopreservation, samples were cultured on solid MS medium (Agar-agr 0.8%) with 3% sucrose. Cultures were incubated at 25°C under a 16-h photoperiod. After growth the percentage of seeds surviving were recorded.

In every experiment approximately 12 seeds were treated for each of three replicates. Data were subjected to ANOVA (analysis of variance) and significant differences between treatments were determined by Duncnn's Multiple Range Test (DNMRT) using the MSTATC software package.

III. RESULTS AND DISCUSSION

Non-pretreated seeds, control, did not survive after exposure to LN. The same results were reported in many plants [11], [12]. Contrary to our results, embryonic axes of Camellia sinensis withstood after freezing in LN without any pretreatment [8]. The percentage of germination of seeds pretreated with sucrose and dehydration was 75%. In contrary, embryonic axes of Melia azedarach L. even those pretreated with sucrose and dehydration for 1 h, did not survive after exposure to LN. Reference [13] shows that the pretreatment of embryonic tissues of Ipomoea batatas with high levels of sucrose alone resulted in up to 28.6% survival. After dehydration the maximum survival demonstrated was 9.1%. The study of references [6], [7] on Gentiana scabra

germplasm have revealed that preculturing with sucrose and desiccation induce high dehydration tolerance, the method has been found to be effective for cryopreservation. Preculture with a high concentration of sucrose greatly increases the intracellular concentration which will act as the principal agent of tolerance to desiccation [7].

Statistical analysis showed that the difference between the survival rates of control and pretreated seeds was significant. Similar results were obtained with a few plants such as Melia azedarach L. [11], Cynodon spp. [12] and Camellia japonica L. [8].

Current study demonstrated that the moisture content of Lilium Iedebourii (Baker) Bioss. seeds before exposure to LN was 15-20%. The study on cryopreservation of Melia azedarach L. seeds was revealed that the optimum moisture content was 16-18% [11]. It is appears the optimum moisture content for germplasms of the more plants before exposure to LN is normally about 20% [12]-[14]. Reduction of water content to a critical level seems to be a necessary step for successful cryopreservation.

In conclusion, cryopreservation is now a viable long-term storage technique for plants germplasm.

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