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Short Communication

Cryopreservation of seeds of lily [*Lilium ledebourii* (Baker) Bioss]: Use of sucrose and dehydration

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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 ledebourii* (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: Dehydration, germplasm conservation, lily and sucrose.

INTRODUCTION

Lilium ledebourii (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 (Ghahreman, 1997). This plant attracts lots of tourists from all over the world. *L. ledebourii* (Baker) Bioss, a threatened and rarest lily, is an endogenous 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 (Grout, 1995; Sakai, 1997). For the long-term conservation of plant germplasm and 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 verification (Sakai, 2000). Two requirements must be met for a cell to vitrify; rapid freezing and a concentrated cellular solution (Panis and Lambardi, 2005). Sugars play a very important role in the acquisition of resistance to desiccation and to freezing in liquid nitrogen (Suzuki et al., 2005, 2006).

Generally, tissues which have low water content, such as meristematic tissues, embryonic axes and seeds are more resistant to the stress of these techniques (Janeiro et al., 1996; Radhamani and Chandel, 1992). In current study, dehydration and sucrose, a non-penetrating cryoprotective substance, used as the pretreatments.

MATERIALS AND METHODS

Seeds of *L. ledebourii* (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 (Murashige and Skoog, 1962) 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 (Murashige and Skoog, 1962) liquid medium with 0.75 M sucrose for 1 h with agitation. After that they were desiccated 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 liquid nitrogen

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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 Duncan's Multiple Range Test (DNMRT*)* using the MSTATC software package.

RESULTS AND DISCUSSION

Non-pretreated seeds control, did not survive after exposure to liquid nitrogen. The same results were reported in many plants (Bernard et al., 2002; Reed et al., 2006). Contrary to our results, embryonic axes of *Camellia sinensis* withstood after freezing in liquid nitrogen without any pretreatment (Janeiro et al., 1996).

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 liquid nitrogen. Blakesley et al. (1995) showed 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 Suzuki et al. (2005 and 2006) 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 (Suzuki et al., 2006).

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 *M. azedarach* L. (Bernard et al., 2002), *Cynodon* spp. (Reed et al., 2006) and *Camellia japonica* L. (Janeiro et al., 1996).

Current study demonstrated that, the moisture content of *L. Iedebourii* (Baker) Bioss. seeds before exposure to liquid nitrogen were 15-20%. The study on cryopreservation of *C. japonica* L seeds was revealed that the optimum moisture content was 16-18% (Bernard et al., 2002). It is appears that, optimum moisture content for germplasms of the more plants before exposure to liquid nitrogen is normally about 20% (Reed et al., 2006; Blakesley et al., 1995; Dumet et al., 2000). 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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