Seed disinfestation methods for in vitro cultivation of epiphyte orchids from Southern Brazil

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ABSTRACT

Disinfestation of orchid seeds is necessary when the germination is performed in vitro. In such case, cultures are supplemented by a source of sugar and salts in order to obtain energy and other requirements. However, the presence of sugar and salts easily allows the development of unwanted microorganisms. To avoid such phenomenon, an efficient procedure must be done as a pretreatment of the seeds with liquid or gaseous substances. In this study with several Brazilian orchids, the seeds were disinfested with sodium hypochlorite solutions containing 0.4% or 0.8% active chlorine for five minutes and sodium hypochlorite or formaldehyde fumes up to two hours, this procedure being efficient for such purpose and making it possible to be used routinely.

Keywords: orchids, in vitro culture, germination, sodium hypochlorite, formaldehyde.

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Epiphyte orchids have been multiplied routinely from seeds, using a non-symbiotic culture as a propagation method (Knudson, 1922). The inoculation in vitro is the main technique for micro-propagation without symbiotic mycorrhizae association. In order to properly develop this technique, sugar and salts are required in a sterile culture medium, where seeds are free from microorganisms.

Orchid embryos can be cultivated in vitro, using immature seeds (Kerbauy & Handro, 1981). In such case, the seeds are taken from the closed capsule to prevent contamination when no other inhibition of germination is found (Van der Kinderen, 1987; Rasmussen et al., 1990). This procedure is not suitable for a germplasm bank, because seeds must reach maturity and have low water content to be stored (Pritchard & Seaton, 1993). For germination, the disinfestation is mandatory before inoculation.

The disinfestant liquid should remove or kill all microorganisms from the seeds' surface without damaging the embryos. For the purpose of disinfesting orchid seeds, calcium hypochlorite is the most used one (Wilson, 1915; Thompson, 1980; Arditti et al., 1982), besides hydrogen peroxide (Snow, 1985) and sodium hypochlorite (Pierrick, 1990). Calcium hypochlorite needs to be filtered before utilization, and cannot be stored over 12 hours (Arditti et al., 1982). Sodium hypochlorite also needs to be fresh, since both are alkaline and lose chloride, the active oxidizing ion, which captures oxygen, killing the aerobic microorganisms and fungi spores, which in turn, are mainly responsible for most of the contamination. These chemicals can improve somehow the germination in a few species by light scarification of the seeds (Haus-von Schmude et al., 1986; Van Wae & Debergh, 1986).

Sodium and calcium hypochlorite are prescribed to be used at different concentrations and times of exposure to disinfest seeds (Singh, 1988; Butcher & Marlow, 1989; Seaton & Hailes, 1989; Oddie et al., 1994; Chu & Mudge, 1994). In spite of it, some contamination may appear due to small cracks on the surface of seeds that may keep a few spores. Applying sodium hypochlorite fumes for 5 to 30 minutes can solve this problem, allowing a complete disinfestation (Arditti & Ernst, 1992).

In the attempt to attain an efficient method for several Brazilian epiphyte
and 99% formaldehyde fumes).

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chloride), and two with gaseous
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MATERIAL AND METHODS

Bifrenaria inodora Lindl.,
Brassavola tuberculata Hook.,
Cattleya bicolor Lindl.,
C. intermedia R.Grah.
C. intermedia var. pallida Lindl.,
Enccycia pygmeae (Hook.)
Dressler, Epidendrum fulgens A.Brong.
Laelia purpurata Lindl. &
Paxt, Miltonia regnellii Reichb.
Oncidium pumilum Lindl.
Pleurothallis glumacea Lindl.

Moisten bath: Twenty milligrams
of seeds from each of the eleven species
were packed in 10 centimeters of plastic
net (60 µm mesh) closed by plastic clips.

RESULTS AND DISCUSSION

The eleven species examined
showed over 75% of initial viability
(Table 1). The disinfestation, with
increased concentration of sodium
hypochlorite solution, decreased the
germination, and is related to the
exposure time (Table 1). The species
B. tuberculata, C. intermedia and
C. intermedia pallida were the most
tolerant to the effects of the disinfestant.

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chemicals are required, followed by several rinsing with sterile water. These procedures are time consuming, mainly when a large quantity of seeds should be processed.

The utilization of gaseous disinfestation devised in this paper showed good results. The disinfestation period was not as restricted as that of sodium hypochlorite, and besides less manipulation was required. Thirty minutes to four hours can be used without the loss of viability (Figure 2A). The tetrazolium test used as a fast viability test could show a decrease of viability after 3 hours in the presence of formaldehyde fumes, probably by acidification of seed surface (Figure 2B). The use of buffered tetrazolium solution should overcome this trouble. After one hour of treatment, no further contamination was observed. This method is recommended because of its efficacy and ease. Formaldehyde fumes can be applied also when a vial with several explants shows a localized contamination. For rare orchid materials, the recovery of in vitro contaminated explants or plantlets can be very suitable.

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