Researches concerning the behavior of *Cymbidium sp.* protocorms cultured in “in vitro” cell culture

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**Abstract** Protocorms of *Cymbidium sp.* grow and present balanced, organogeneses, when cultured on MS basal medium, supplemented with 30 g/l sucrose, 7 g/l agar and 0.1 mg/l BA. They produce normal plantlets, which can be transferred “ex vitro” and aclimatized to the conditions of the non-aseptic life.

**Key words** Cymbidium sp., protocorms in vitro culture, organogenesis

Orchids are highly valued ornamentals, due to their beautiful flowers. As orchid cultivars are heterozygous, vegetative multiplication is the only way to pass the features of one individual, obtained by selection, over to its descendents. Unfortunately, traditional propagation methods allow a very low multiplication rate. The culture of orchids extended quickly, only after the method to eliminate viral infections—which reduce plant quality and efficiency—had been found [4]. This method used stem meristems cultured on aseptic media. Soon afterwards, quick and efficient ways to multiply orchids “in vitro” even in large industrial amounts, have been worked out [2]. The technique for the micropropagation of *Cymbidium* cultivars is well know, while no methods with satisfactory commercial efficiency could be established [6,7]. Therefore, there are still problems as concerns the “in vitro” culture of certain orchid genera, species and cultivars, mostly when their efficient micropropagation is the aim. The regenerative and organogenetic potential of explants is, generally, influenced not only by the culture conditions, but by the nature of explants and the genotype to be multiplied [2]. The meristems of many orchids produce structures called protocorms or “protocorm-like buds” [6].

Protocorm glomerules isolated and cultured on fresh, liquid or solid media, undergo protocorm multiplication or organogenesis; in the latter case, normal plantlets, that can be aclimatized to live under natural conditions, are generated [1].

As we already have “in vitro” cultures of protocorms of *Cymbidium sp.* we studied protocorm behaviour, when cultured on a classical, Murashige-Skoog (MS) medium.

Data cited in the literature recommend the Knudson medium to be used for the “in vitro” culture of *Cymbidium* protocorms aimed at both protocorm multiplication and organogenesis [1]; on the contrary, other orchid genera require other media, such as Vacin-Went medium [8], modified by Sagawa & Kunisaki [6] who added natural extracts to the basal medium. In spite of the fact that protocorms multiply and grow well even on hormone-free media, growth regulators promote organogenesis [1,2].

As we intended to set up the best culture media for the “in vitro” micropropagation of *Cymbidium sp.*, we studied the behaviour of protocorms cultured on MS basal medium, supplemented with various growth regulators.

**Materials and Method**

The protocorms, cultured initially on Knudson medium, have been transferred to MS basal medium, supplemented with 30 g/l sucrose and 7 g/l agar; medium pH was 5.2. Different types and concentrations of growth regulators have been used. First of all, we studied the behaviour of protocorms cultured on MS medium, containing the following growth regulators (in mg/l):

<table>
<thead>
<tr>
<th>Experimental variants</th>
<th>BA</th>
<th>NAA</th>
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<tbody>
<tr>
<td>MS0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MS - 1</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>MS - 2</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>MS - 3</td>
<td>0.3</td>
<td>-</td>
</tr>
<tr>
<td>MS - 4</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>MS – 5</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>MS – 6</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

The protocorms have been incubated in the growth chamber, under the following conditions: 24°C ± 1°C and 16 hrs light/8 hrs darkness; we used fluorescent light with an intensity of 2500 lux.

All cultures have been compared with the control, i.e. with the protocorms grown on hormone-free basal medium. Comparative data have been
Results and Discussions

Generally, protocorms responded very well when cultured on MS medium, even without growth regulators. The Cymbidium sp. cultured on hormone-free media for 60 days, presented a temperate caulogenesis; 50% of the cultures produced 2.5 cm long shoots, and 6 cm long roots. The Cymbidium sp. protocorms cultured on MS - 1 medium, containing 0.1 mg/l BA (fig. 1 - A), present on a good caulogenesis, as 80% of the protocorms generated 8 cm long shoots; rhizogenesis was similar or a little weaker with respect to the control (fig. 1 – B,C). Protocorm multiplication and organogenesis were not satisfactory in the other experimental variants. Thus, neither 0.2 or 0.3 mg/l BA added to the medium, led to conspicuous differences in organogenesis with respect to the occurring on a medium containing 0.1 mg/l BA (MS - 1); little was gained by enhancing BA level to 10 mg/l (MS - 4), as organogenesis was not improved and only root covers formed.

We have also noticed that the presence of NAA in the culture medium, together with BA or alone, induced senescence (basal leaves turned yellow), with time; 1 mg/l NAA (MS - 6) caused the necrosis of protocorms and plantlets, in about 2-3 weeks since inoculation. Senescence occurred much slower on media containing both NAA and BA, in equal concentrations (MS - 5).

“In vitro” generated plantlets have been transferred “ex vitro” after a culture period of 120-130 days, when they have gained a normal conformation, via organogenesis. Plantlets transferred “ex vitro” have been gradually acclimatized to greenhouse conditions (fig. 1- D,E). The culture substrate that has been used in this phase, consisted of a mixture of leaf soil, sphagnum and beech bark in small pieces (1:2:1). This mixture has been sterilized by autoclaving, at least two weeks before using it. Plantlets have been protected from dryness by covering them with flasks, for 2-3 weeks; they have been watered with water at greenhouse temperature. The percentage of surviving plantlets has been about 70%.

Conclusions

The protocorms of Cymbidium sp. grow and collected 60,90 and 120 days, since the beginning of the experiments.
present balanced, organogeneses, when cultured on MS basal medium, supplemented with 30 g/l sucrose, 7 g/l agar and 0.1 mg/l BA. They produce normal plantlets, which can be transferred “ex vitro” and aclimatized to the conditions of the non-aseptic life.

References