ASPECTS OF “IN VITRO” CULTIVATION OF CHELIDONIUM MAJUS L.

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Abstract: The initiation of “in vitro” cultures of Chelidonium majus L aimed not only to assess the dedifferentiation capacity depending on explant origin and growth regulators, but also to develop a multiplication protocol based on direct regeneration from shoots explants, followed by roots development induction. The proliferative capacity was tested on leaf and shoots explants, cultivated on Murashige-Skoog basal medium, testing two auxins: 2.4 dichlorophenoxiacetic acid (2,4D) and indolylacetic acid (IAA) in combination with a cytokinin: kinetine (K). The biomass accumulation was measured by regular weighing on analytical balance. Regenerative capacity was evaluated on shoots explants, cultivated on different inductive variants of MS medium. Benzylaminopurine (1 mg/l) and in combination with 2.4 dichlorophenoxiacetic acid (0.5 mg/l) stimulated growth and multiplication of shoots. Root system development was achieved on MS medium without growth regulators.

Keywords: Chelidonium majus, callus, micropropagation

Introduction

Chelidonium majus L. (Family Papaveraceae) is an herbaceous perennial plant, native to Europe and western Asia and introduced widely in North America. It has an erect habit, and may reach 30 to 120 cm high. The leaves are lobed and crenate, 30 cm long. The sap is yellow to orange.

Crude extracts of C. majus as well as purified compounds [1], [2], [3], [4], derived from it exhibit a broad spectrum of biological activities (antiinflammatory, antimicrobial, antitumoral, analgesic, hepatoprotective).

“In vitro” cultivation of Chelidonium majus L was initiated in order to evaluate the cell dedifferentiation and redifferentiation [5], as an unconventional alternative for plant biomass multiplication, the main source of bioactive compounds with pharmaceutical value (chelidonine, sanguinarine, chelerythrine with antibacterial activity [6], anti-tumour activity.

Materials and methods

The cultures of Chelidonium majus L were based on explants taken from mature individuals, harvested from spontaneous flora, in flower stage of development.

Callus induction was performed on leaf and shoot explants, cultivated on different variants of MS medium (Tab. I).

Table I. Variants of MS medium for callus induction

<table>
<thead>
<tr>
<th>MS</th>
<th>2,4 D</th>
<th>mg/l IAA</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>III</td>
<td>0,5</td>
<td>-</td>
<td>0,5</td>
</tr>
<tr>
<td>IV</td>
<td>-</td>
<td>0,5</td>
<td>0,5</td>
</tr>
</tbody>
</table>

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The preparation of explants for inoculation was the chemical sterilization, using a solution of 3% Na hypochlorite. Treatment duration was 12 minutes, followed by washing repeatedly with sterile distilled water.

The diversification of MS induction media was based on two types of auxins: 2.4 diclorphenoxyacetic acid (2,4D) and indolylacetic acid (IAA) and a cytokinine: kinetine (K). The biomass accumulation was measured by regular weighing on analytical balance.

Direct micropropagation consisted in shoots explants, cultivated on different variants of MS medium, supplemented with benzalaminopurine alone and in combination with 2.4 diclorphenoxyacetic acid (Tab. II).

All the cultures were maintained at 25°C under 16 hr photoperiods. The cultures growing on various levels of growth regulators were scored for the number of shoots per culture and rooting after every 2 weeks.

### Table II. Variants of MS medium for shoot induction

<table>
<thead>
<tr>
<th>MS</th>
<th>BAP mg/l</th>
<th>2,4D mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>0,5</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>0,1</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>0,5</td>
</tr>
<tr>
<td>V</td>
<td>0,5</td>
<td>0,25</td>
</tr>
<tr>
<td>VI</td>
<td>0,1</td>
<td>0,05</td>
</tr>
</tbody>
</table>

### Results and discussions

*Chelidonium majus* L. has gained considerable attention because of its increasing use as a medicinal plant. The objectives of this work were not only to develop an efficient *in vitro* multiplication method for *Chelidonium majus* L. shoot explants, but also to study the dedifferentiation process on different explants, as a potential source of biomass production.

“In vitro” multiplication of this important drug-yielding plant is an efficient unconventional alternative. For callus induction it were tested leaf and shoot explants, collected from mature individuals of *Chelidonium majus* L.

The explants were surface sterilized and cultured on Murashige- Skoog media containing different combination of two types of auxins: 2.4 diclorphenoxyacetic acid (2,4D) and indolylacetic acid (IAA) and a cytokinine: kinetine (K), in concentrations of 0,5 and 1 mg/l.

The beginning of callus proliferation took place differently, depending especially on type of explants, concentration and combination of growth regulators.

After 7 days of inoculation, callusing of the cultured explants was observed at the cut ends. The callus covered the entire explant for 3 weeks.

Primary callus culture was obtained from shoot explants, cultivated on MS, supplemented with 0,5 mg/l IAA and 0,5 mg/l K, after about 4 weeks, after cultures initiation (Photo 1 and 2).

The highest frequency of callus proliferation was recorded on the same variant, after several stages of subculturing.

Leaf explants did not respond similarly to this combination of growth regulators. The most common reaction was tissue necrosis without cell proliferation.
Regenerative capacity was tested directly on shoot fragments, grown on MS medium, based on a diversification of hormonal balance.

This media was supplemented with different concentrations of 6-benzylaminopurine (BAP) 1 mg/l, 0,5 mg/l, 0,1 mg/l alone or in combination with 2,4 diclorphenoxiacetic acid (2,4 D), in concentration of 0,5; 0,25; 0,05 mg/l.

Benzylaminopurine (1mg/l) and in combination with 2,4 diclorfenoxiacetic acid (0,5 mg/l) stimulated growth and multiplication of shoots (Photo 3, 4, 5).

Proliferating shoot cultures were achieved on explants associated with a high number of shoots per explant (5 shoots/explant).

For rooting induction, elongated shoots were transferred to basal medium without growth regulators (Photo 6).

Conclusions

Primary callus culture was obtained from shoots explants, cultivated on variants IV of MS medium (0,5 mg/l IAA and 0,5 mg/l K).

Benzylaminopurine (1mg/l) and in combination with 2,4 diclorfenoxiacetic acid (0,5 mg/l) stimulated growth and multiplication of shoots

Root system development was achieved on MS medium without growth regulators.

REFERENCES

Photo 1. Shoot explant

Photo 2. Primary callus cultures

Photo 3. Early stage of caulogenesis

Photo 4. Shoots multiplication

Photo 5. Regenerated plant

Photo 6. Roots development