Micropropagation of *Capparis spinosa* L. subsp. *rupestris* Sibth. & Sm. by nodal cuttings

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In vitro propagation of *Capparis spinosa* subsp. *rupestris* was conducted using single nodal cuttings. Multiple shoots were obtained on Murashige and Skoog (MS) medium supplemented with 1 mg/L zeatin. Shoot multiplication was optimized on the same medium by subculturing shoot segments with 2-3 nodes every 6 weeks. Aptitude to proliferation was successfully maintained during nine subcultures with a mean rate exceeding 20 new shoots per explant. High rooting response of shoots (92%) was obtained after 4 h pulse treatment period in darkness with 100 mg/L IAA solution, followed by culture on solid half-strength MS basal medium. These results indicate the enormous potential of *C. spinosa* subsp. *rupestris* that could be used for large-scale multiplication.

**Keywords:** caper, *Capparis spinosa* subsp. *rupestris*, tissue culture, micropropagation

**IPC Code:** Int. Cl. A01H4/00

**Introduction**

*Capparis spinosa* L. (Caper) belonging to Capparidaceae is an indigenous perennial of most Mediterranean countries. In Lebanon, *C. spinosa* is widespread, especially in the degraded and arid soils, but has never been exploited before, probably because of the local culinary customs and the lack of home market for its products. In addition to the increasing worldwide demand for flower buds, cultivation of the caper crop could play an important role in the socioeconomic sector. Thus a whole programme has been recently established in order to select valuable Lebanese morphotypes of *C. spinosa*. For the maintenance of selected characters, clonal multiplication is essential. Difficulties in attempting conventional propagation has been reported, due to the serious rooting problems associated with its lignified and herbaceous cuttings. The use of tissue culture technique could overcome these problems and improve mass production of caper. There are only two preliminary reports on the micropropagation of *C. spinosa* using proliferating clusters, but tissues lost their proliferation capacity during successive subcultures. Micropropagation of *C. decidua* has also been reported using the culture of nodal sections. The aim of the present work is to investigate a micropropagation procedure for one Lebanese morphotype of *C. spinosa* subsp. *rupestris* Sibth. and Sm. selected for its caducous stipules.

**Materials and Methods**

Nodal segments were collected in the month of May 2000 from a selected mature shrub of *C. spinosa* subsp. *rupestris*. These explants were surface sterilized with 0.2% sodium hypochlorite for 10 min and then rinsed in sterile water six times. Cultures were initiated on Murashige & Skoog (MS) medium supplemented with 1-2 mg/L 6-benzylaminopurine (BAP) or 1-2 mg/L zeatin combined or not with 0.1 mg/L gibberellic acid (GA$_3$). For each medium, 40 nodal segments were employed (10 explants × 4 replications). Cultures initiation was conducted, at 26±2°C under fluorescent light for 16 h daily.

Shoot multiplication was conducted on the same fresh media by subculturing shoot segments with 2-3 nodes every six weeks in the same conditions as described above. For each treatment (medium × subculture), 40 shoots were employed (10 explants × 4 replications). Data on multiplication rates were collected by counting the number of shoots per explant at the end of each subculture.
For the rooting of regenerated shoots, five treatments were tested by transferring the shoots (of the 7th subculture) onto solid half-strength MS basal medium supplemented or not with 1.5 mg/L 3-indoleacetic acid (IAA) or 3-indolebutyric acid (IBA) or after a 4 h pulse treatment period in darkness with 100 mg/L IAA or IBA solution followed by a subsequent 30 d of culture on solid half-strength MS basal medium. For each treatment, 30 shoots were employed (10 cuttings × 3 repetitions). Data on the percentage of rooting were collected 45 d after initiation of the experiment. Rooted plantlets not less than 3 cm length were hardened in the greenhouse as (28-32°C, mist system) into pots containing a mixture of perlite, peat and compost (1:1:1).

Statistical Analysis
For all the experiments, differences among treatments were evaluated by performing the Duncan test (General Linear Models Procedure, SAS Institute, Cary, N.C.).

Results and Discussion
The nodal explants were sensitive to sodium hypochlorite treatment used for surface sterilization, they exhibited browning in culture and subsequently death of the explants. The percentage of responding explants did not exceed 57%. However, cultures were successfully established at intervals of 60 d on the studied media (Table 1, Fig. 1a).

Amongst two cytokinins tested, zeatin proved to be more effective than BAP for initiating shoots and a higher yield of shoots per explant (Table 1). Zeatin at 1 mg/L seemed to be the best concentration, since it facilitated a high rate of proliferation and shoot development without altering their elongation. Besides, no callus formation occurred at this concentration. Although at a higher concentration, zeatin (2 mg/L) stimulated a similar yield of shoots, but significantly reduced their elongation and also associated with callus induction.

Conversely, BAP (1-2 mg/L) induced limited proliferation but stimulated shoot elongation. Besides, no callus formation was observed at the base of the explants.

During the proliferation stage, the medium containing 2 mg/L zeatin was discarded in order to avoid poor elongation of shoots and also a possible formation of adventitious shoots from callus. Regenerated shoot segments were successfully subcultured on the three remaining media. All of them

<table>
<thead>
<tr>
<th>Cytokinin (mg/L)</th>
<th>Explants responding (%)</th>
<th>Mean shoot number per explant ± SD</th>
<th>Mean shoot length (cm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>20.4±1.8 c</td>
<td>1.0±0.2 c</td>
<td>1.1±0.3 c</td>
</tr>
<tr>
<td>BAP (1)</td>
<td>30.6±4.6 bc</td>
<td>3.1±0.9 b</td>
<td>3.0±0.5 a</td>
</tr>
<tr>
<td>BAP (2)</td>
<td>57.4±3.1 b</td>
<td>4.7±1.7 b</td>
<td>2.9±0.6 a</td>
</tr>
<tr>
<td>Zeatin (1)</td>
<td>75.5±4.8 a</td>
<td>7.6±2.4 a</td>
<td>2.8±0.4 a</td>
</tr>
<tr>
<td>Zeatin (2)</td>
<td>78.8±2.6 a</td>
<td>8.4±2.5 a</td>
<td>0.7±0.2 b</td>
</tr>
</tbody>
</table>

Values followed by the same letter are not significantly different (P=0.05)

Table 1—The effect of cytokinin on shoot induction and elongation of C. spinosa subsp. rupestris after 60 d of culture initiation of single nodal sections on MS medium

Fig. 1(a-d)—Stages of micropropagation of C. spinosa subsp. rupestris: a, Shoots induced from nodal segments on modified MS medium supplemented with 1 mg/L BAP; b, Multiplication of shoots on modified MS medium supplemented with 1 mg/L zeatin; c, Four-week old rooted shoots on solid half-strength MS basal medium after a 4 h pulse treatment period in darkness with IAA solution (100 mg/L); d, 9 to 10-week-old hardened plantlet in pot under the greenhouse conditions.

proved to be favourable to induce the development of axillary buds (Table 2, Fig. 1b). The proliferation capacity increased over the successive subcultures onto fresh media with a mean rate exceeding 20 new shoots per explant at the end of the 5th subculture. However, significant difference was noticed in the responses according to cytokinin type used. Compared to BAP (1-2 mg/L), zeatin at 1 mg/L significantly enhanced proliferation with a higher
After the fifth subculture, the proliferation rate decreased significantly with BAP (1-2 mg/L) due to the occurrence of vitrification that progressively caused the decline of proliferating clusters. Conversely, 1 mg/L zeatin succeeded to maintain proliferation capacity up to 9 subcultures and was not associated with any visible problem related to shoot development (Table 2).

Isolated single shoots were capable of rooting with different treatments, even on half strength MS medium devoid of growth regulators (Table 3). However, the rate of rooting and the sprouting speed of the roots was found to be significantly different in various treatments. Incorporation of IAA or IBA in solid half strength MS medium caused callus formation (data not shown) that delayed the development of roots. Shoots of these two treatments rooted within 30-45 d with the rates of 19.5 and 33.9%, respectively. The best rooting response was obtained after a 4 h pulse treatment period in darkness in concentrated IAA solution, followed by a subsequent culture on solid half-strength MS basal medium (Fig. 1c). Shoots of this treatment started to root within 15 d and a rate of 87% of rooted shoots was obtained after 45 d. The varying number of roots and their length was not found to be statistically significant for the different treatments. In general, standard deviation was generally high reflecting the heterogeneity of shoots response to rooting treatments (Table 3).

During the hardening stage, in vitro rooted plantlets longer than 3 cm were successfully acclimatized with a survival rate of 92% (Fig. 1d). After 2 months, the length of hardened plants ranged between 19 and 27 cm.

The whole micropagation system, and proliferation stage in particular, optimized for the mass propagation of C. spinosa subsp. rupestris is a much effective method than the ones reported earlier. According to earlier reports, the aptitude for proliferation of these species was limited to only five or six subcultures due to basal disorganization and vitrification of the axillary shoots in the presence of BAP13,14. In comparison, although similar results were obtained with BAP, the protocol described here with 1 mg/L zeatin involved nine subcultures resulting in higher yield of axillary shoots. Moreover, the rooting rate reported here is higher than the one previously obtained (70%)14. In hardening process, according to the present protocol using plantlets of 3 cm length, survival rate (92%) is much greater than the one reported before13.

These results indicate the enormous potential of this method for the mass clonal propagation C. spinosa subsp. rupestris. The protocol developed here will be useful for cloning of selected Lebanese genotype(s) of caper with caducous stipules and should allow their cultivation as a new promising marketing crop.

**References**


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**Table 2**—The effect of cytokinin on shoot multiplication rate (average number of shoots per explant ± standard deviation) of *C. spinosa* subsp. *rupestris* over nine successive subcultures on MS medium

<table>
<thead>
<tr>
<th>Cytokinin (mg/L)</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
<th>7th</th>
<th>8th</th>
<th>9th</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP (1)</td>
<td>3.4 ± 0.5</td>
<td>5.3 ± 1.2</td>
<td>7.3 ± 1.5</td>
<td>10.2 ± 2.1</td>
<td>20.8 ± 4.5</td>
<td>15.3 ± 5.1</td>
<td>12.6 ± 4.5</td>
<td>10.7 ± 2.1</td>
<td>9.8 ± 2.5</td>
</tr>
<tr>
<td>BAP (2)</td>
<td>4.5 ± 1.3</td>
<td>5.9 ± 1.5</td>
<td>10.6 ± 2.3</td>
<td>14.6 ± 3.5</td>
<td>23.7 ± 5.2</td>
<td>17.7 ± 3.1</td>
<td>11.7 ± 4.2</td>
<td>11.0 ± 3.3</td>
<td>11.7 ± 2.1</td>
</tr>
<tr>
<td>Zeatin (1)</td>
<td>6.5 ± 1.9</td>
<td>7.9 ± 0.9</td>
<td>12.8 ± 2.9</td>
<td>18.5 ± 2.7</td>
<td>33.4 ± 4.8</td>
<td>32.6 ± 4.5</td>
<td>33.7 ± 5.1</td>
<td>33.2 ± 4.4</td>
<td>30.3 ± 5.2</td>
</tr>
</tbody>
</table>

**Table 3**—Effect of five treatments on rooting of shoots of *C. spinosa* subsp. *rupestris* after 45 d. Data represent the mean ± SD of three 16-shoots replicates per treatment

<table>
<thead>
<tr>
<th>% Rooting</th>
<th>MS/2 growth regulators free</th>
<th>MS/2 + 1.5 mg.I⁻¹ IAA</th>
<th>MS/2 + 1.5 mg.I⁻¹ IBA</th>
<th>4 h in darkness / 100 mg.I⁻¹ IAA</th>
<th>4 h in darkness / 100 mg.I⁻¹ IBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root number</td>
<td>56.9 ± 36.8 b</td>
<td>19.5 ± 26.4 d</td>
<td>37.3 ± 43.9 c</td>
<td>92.5 ± 35.4 a</td>
<td>53.8 ± 26.3 c</td>
</tr>
<tr>
<td>2.5 ± 1.0 a</td>
<td>2.1 ± 0.8 a</td>
<td>2.5 ± 1.2 a</td>
<td>1.7 ± 0.5 a</td>
<td>3.0 ± 0.4 a</td>
<td></td>
</tr>
<tr>
<td>Root length (mm)</td>
<td>6.4 ± 8.3 a</td>
<td>6.5 ± 5.0 a</td>
<td>8.5 ± 8.4 a</td>
<td>4.7 ± 6.5 a</td>
<td>1.2 ± 0.5 a</td>
</tr>
</tbody>
</table>

Values followed by the same letter are not significantly different (P=0.05)