The effect of leaf and shoot tip removal and explant orientation on axillary shoot proliferation of *Codiaeum variegatum* Blume var. *pictum* Muell. Arg. cv. Excellent

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Abstract

The removal of all developed leaves from 1 to 1.5 cm shoots of *Codiaeum variegatum* Blume var. *pictum* Muell. Arg. ‘Excellent’, cultured on the Murashige–Skoog medium containing 4.4 μM (1 mg l⁻¹) 6-benzylamino-purine (BAP) and 5.8 μM (2 mg l⁻¹) gibberellic acid (GA₃), doubled the number of axillary shoots in comparison to non-defoliated controls. An additional increase in shoot numbers was achieved when the shoots were placed on the medium horizontally or vertically in an inverted position with the shoot tip down. Shoot tip removal slightly improved axillary branching, and additional defoliation strengthened this effect. Exogenous indole-3-acetic acid (IAA) decreased shoot numbers, and 2,3,5-triiodobenzoic acid (TIBA) did not replace defoliation nor diminish the size of callus formed at the base of the shoots. Almost all the shoots obtained were able to root on the medium with 4.9 μM (1 mg l⁻¹) indole-3-butyric acid (IBA), and acclimatize in the greenhouse.

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1. Introduction

*Codiaeum variegatum* Blume var. *pictum* Muell. Arg. is a shrub plant originated from Malaysia. Dwarf forms of this species are cultivated as ornamental pot plants. *Codiaeum variegatum* is propagated through leaf and...
stem cuttings, a practice that requires large stock plantations. An alternative method of propagation is the multiplication of axillary shoots in vitro. Although reports regarding micropropagation of *Codiaeum* through axillary shoots are limited, this method of propagation is used on a small scale in commercial laboratories (Tymoszuk, 1989). There are several obstacles limiting the introduction of micropropagation on a larger scale: a low propagation coefficient (1–2 new axillary shoots per explant in six weeks), a long subculture period (6–8 weeks), and a tendency to produce adventitious shoots from callus growing out of shoot bases. Regeneration of multiple shoots from callus grown on leaf cut edges of *Codiaeum* (Orlikowska et al., 1995) can be used for the generation of new genotypes, but it is disadvantageous for true-to-type propagation. These juvenile adventitious shoots were very convenient explants for further effective axillary shoot production giving rise to 4–5 new axillary shoots in six week-subculture, but after 3–4 subcultures shoot branching decreased to 1–1.5 shoots (Orlikowska, unpublished). Shibata et al. (1996) reported very efficient axillary shoot production in cultures of related species *Croton sublyratus*, which were initiated from seedling buds. However, the authors did not mention if efficient shoot proliferation was maintained over several subcultures.

Cultures initiated in our laboratory from shoot tips and nodal buds of Excellent cultivar grew slowly, developed large leaves, and only few axillary shoots on media with BAP within 6–8 weeks. Preliminary experiments have shown that an increase in BAP concentration above 1 mg l$^{-1}$ or its replacement by TDZ did not improve axillary shoot production, but instead stimulated growth of hard organogenic callus at the shoot bases. Exogenous cytokinin is not the only means of regulating axillary shoot growth in vitro. Increase in number of rose microcuttings was stimulated by tipping of initial shoots or by application of TIBA (1 mg l$^{-1}$) on the shoot apices (Bressan et al., 1982). Horizontal orientation of shoots without apices stimulated in vitro branching of *Quercus robur* (Vieitez et al., 1994). A significant increase in axillary shoot number of five woody species due to horizontal orientation of tipped and defoliated shoots was reported by McClelland and Smith (1990). Tipping of shoots and/or leaf removal from mother plants is often used in traditional propagation by cuttings. Wilkins (1988) reported that defoliation in addition to apex removal of *Euphorbia pulcherrima* increased axillary shoot numbers. In order to increase the efficiency of *Codiaeum variegatum* axillary shoot production in vitro we have checked different approaches: shoot defoliation, shoot tip removal and change of shoot orientation. The results of these experiments are presented in this paper.

We have used the following abbreviations in this paper: AC — activated charcoal, BAP — 6-benzylamino-purine, GA$_3$ — gibberellic acid, IAA — indole-3-acetic acid, IBA — indole-3-butyric acid, TIBA — triiodo-benzoic acid.
2. Material and methods

Shoot tips and nodal buds collected in the early summer from pot plants of *Codiaeum variegatum* ‘Excellent’ were used for culture initiation. The plants were obtained from a commercial source, were about 30–40 cm tall, and had been grown in a heated greenhouse under conditions typical of this area and time of year. Shoots without leaves were washed carefully in a kitchen detergent solution, rinsed under running tap water, and cut into sections with 1–2 nodal buds. They were surface disinfected by immersion in 75% ethanol for 15 s, followed by shaking in 4% of “Chloramine T” with a few drops of kitchen detergent for 20 min, and finally rinsed three times in sterile distilled water. Explants were placed on MS medium (Murashige and Skoog, 1962) without growth regulators for six days. Shoots without visible contamination were tested on KING B bacterial medium, and then incubated on MS medium supplemented with 4.4 μM BAP (1 mg l\(^{-1}\)) and 5.7 μM IAA (1 mg l\(^{-1}\)) for initial multiplication. The basic multiplication medium selected after preliminary experiments consisted of MS mineral salts, WPM vitamins (Lloyd and McCown, 1981), 30 g l\(^{-1}\) sucrose, 7 g l\(^{-1}\) Plant Agar (Duchefa), BAP 4.4 μM (1 mg l\(^{-1}\)) and GA\(_3\) 5.8 μM (2 mg l\(^{-1}\)) and pH 5.8.

Experiments were carried out on 1–1.5 cm shoots derived from the basic multiplication medium, within 10 months, beginning from June 1998. In the first experiment the effect of defoliation on axillary shoot production was tested. All the leaf blades, with the exception of the youngest ones wrapping the top buds, were removed. This experiment was repeated three times in three subsequent subcultures, with 60 × 3 shoots used in each treatment. In the first subculture experimental shoots were taken from non-defoliated shoots, in the second and third subculture from multishoots produced by defoliated ones. In the second experiment, the effect of the orientation of defoliated shoots (vertical with shoot tip up or down and horizontal), and shoot tip removal of defoliated and non-defoliated shoots on axillary shoot proliferation was studied. In the third experiment, the effects of IAA or TIBA at 0.5 and 1 μM on defoliated and non-defoliated shoots were tested. IAA and TIBA were filtered and added to cooled medium. The second and third experiments were repeated twice with 28 × 2 shoots used per treatment.

In all the experiments the cultures were evaluated after a six-week period. Axillary shoots were divided into two length classes (＞1 cm and ＜1 cm, but not shorter than 3–4 mm) and presented in Tables as total shoot numbers and number of shoots ＞1 cm. The size of calluses formed on the shoot bases was visually estimated.

The shoots obtained from these experiments were grown and/or rooted on media with 4.9 μM (1 mg l\(^{-1}\)) IBA or with 3 g l\(^{-1}\) activated charcoal (AC). The rooted shoots were acclimatized in the greenhouse in a peat-perlite mixture.
The stock mother and experimental cultures were maintained at 22°C, under a 16 h/8 h photoperiod of 70 µmol s⁻¹ m⁻² provided by cool-white fluorescent lamps. Initially, a dark period of five days was applied to shoots cultured on the medium with IBA.

The experiments were conducted in 330 ml jam jars containing four shoots each, with ventilated polypropylene twist lids. The data obtained from each jar averaged on a per explant were subjected to analysis of variance. The significance of differences was determined by Duncan’s multiple range test at 0.05 level.

3. Results

Defoliated shoots produced significantly more axillary shoots in comparison to non-defoliated ones. The difference was very well visible already after three weeks (Fig. 1). After six weeks defoliated shoots produced at least twice as many axillary shoots as non-defoliated ones (Tables 1 and 2, Figs. 2 and 3). The positive effect of defoliation on induction of axillary shoots was especially clear in the class of shoots <1 cm. In subsequent subcultures the number of shoots <1 cm, both defoliated and non-defoliated decreased, but in proportion to each other.

Table 1
The influence of leaf removal on axillary shoot production of *Codiaeum variegatum* ‘Excellent’ in three subsequent passages (means followed by the same letter do not differ at P = 0.05)

<table>
<thead>
<tr>
<th>Passage</th>
<th>Total number of shoots</th>
<th>Number of shoots &gt;1 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-defoliated</td>
<td>Defoliated</td>
</tr>
<tr>
<td>I</td>
<td>2.5a</td>
<td>4.4b</td>
</tr>
<tr>
<td>II</td>
<td>2.0a</td>
<td>3.7b</td>
</tr>
<tr>
<td>III</td>
<td>1.6a</td>
<td>3.3b</td>
</tr>
<tr>
<td>Mean</td>
<td>2.0</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Fig. 1. Axillary shoots produced within three weeks on non-defoliated (left) and defoliated explants. Bar represents 1 cm.
Table 2
The influence of defoliated shoot orientation and apex removal of defoliated and non-defoliated shoots on axillary shoot production of Codiaeum variegatum ‘Excellent’ (means are given from two parallel repetitions of the experiment)

<table>
<thead>
<tr>
<th>Characteristic of explants</th>
<th>Total number of shoots</th>
<th>Number of shoots &gt;1 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-defoliated, vertically, shoot tip up</td>
<td>1.5a</td>
<td>0.5a</td>
</tr>
<tr>
<td>Non-defoliated, apex removed</td>
<td>2.4b</td>
<td>1.3b</td>
</tr>
<tr>
<td>Defoliated, vertically, shoot tip up</td>
<td>3.9c</td>
<td>1.9c</td>
</tr>
<tr>
<td>Defoliated, apex removed</td>
<td>3.5c</td>
<td>1.0b</td>
</tr>
<tr>
<td>Defoliated, horizontally</td>
<td>5.1d</td>
<td>0.5a</td>
</tr>
<tr>
<td>Defoliated, vertically, shoot tip down</td>
<td>4.7d</td>
<td>0.4a</td>
</tr>
</tbody>
</table>

Fig. 2. Axillary shoots produced in six-week culture period. From left to right: on non-defoliated shoot, on defoliated shoot, on defoliated and horizontally orientated shoot. Bar represents 1 cm.

Fig. 3. Axillary shoots produced in six-week culture period: top row — from non-defoliated shoot, middle row — from defoliated shoot, bottom row — from defoliated and horizontally orientated shoot. Bar represents 1 cm.
This effect was caused by the weakening of shoots (marked by small diameter) due to repetitive defoliation. Our observations indicated that the number of axillary shoots produced on defoliated explants was correlated with shoot diameter and length (data not shown). It is important, therefore, to grow a number of shoots without defoliation in order to produce initial explants for effective propagation in the next culture period and to use shoots longer than 1 cm. Shoots produced on defoliated explants did not demonstrate higher branching potential in further multiplication when leaves were not removed. Axillary shoots produced on defoliated explants had a light green colour in contrast to axillary shoots from non-defoliated ones which had a stronger green colour. Leaf colour variegation became visible later, after 2–4 weeks when grown on the media supplemented with IBA or AC.

The second experiment demonstrated that orientation of defoliated shoots significantly influenced axillary shoot production. The highest number of shoots was produced by shoots orientated horizontally or vertically with the shoot tip down (Table 2, Figs. 2 and 3), although in these treatments the number of shoots longer than 1 cm was lower. Apex removal from non-defoliated shoots slightly improved axillary branching. Defoliation, in addition to apex removal strengthened this effect but was less efficient than only defoliation.

The addition of 0.5 and 1.0 μM of IAA to proliferation medium decreased the total number of axillary shoots produced, but only on defoliated explants. This effect was achieved by the decreased production of shoots <1 cm, while production of shoots >1 cm was not affected (Table 3). The addition of TIBA slightly increased the number of shoots >1 cm grown on non-defoliated and decreased the total number of shoots grown on defoliated shoots. The total number of shoots produced on non-defoliated explants was not affected but the total number of shoots produced on defoliated shoots was lower in comparison to the control although this effect was weaker than the effect of IAA.

Axillary shoots produced in these experiments were not vitrified and callus from shoot bases did not form adventitious shoots. Large differences in the size of

### Table 3

<table>
<thead>
<tr>
<th>Medium</th>
<th>Total number of shoots</th>
<th>Number of shoots &gt;1 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-defoliated</td>
<td>Defoliated</td>
</tr>
<tr>
<td>–</td>
<td>2.1abc</td>
<td>5.0e</td>
</tr>
<tr>
<td>IAA 1 μM</td>
<td>1.8a</td>
<td>2.3bc</td>
</tr>
<tr>
<td>IAA 0.5 μM</td>
<td>1.8a</td>
<td>2.5c</td>
</tr>
<tr>
<td>TIBA 1 μM</td>
<td>2.0ab</td>
<td>3.4d</td>
</tr>
<tr>
<td>TIBA 0.5 μM</td>
<td>1.8a</td>
<td>3.3d</td>
</tr>
</tbody>
</table>
calluses between explants were observed but means between treatments and repetitions did not differ significantly (data not shown).

All shoots, independently on their length, produced roots on the medium with 1 mg l\(^{-1}\) IBA. They formed roots within three weeks but an additional three weeks of growth were necessary to enable them to reach a condition which allowed them to acclimatise in the greenhouse (Fig. 4). Shoots on the medium with AC elongated first and by the end of the sixth week 70% of them were rooted usually with one long, branched root (Fig. 4). The shortest shoots <0.5 cm remained unrooted on medium with AC although they elongated to 1–1.5 cm. Almost all the rooted shoots acclimatized well in the greenhouse if they were treated twice with GA\(_3\) solution (200 mg l\(^{-1}\)), i.e., immediately and one week after planting out in the greenhouse.

4. Discussion

Endogenous plant growth substances play a key role in the regulation of the growth of axillary shoots. It is generally accepted that auxin which plays a role in the regulatory process of apical dominance is predominantly produced in shoot apices and the youngest leaves. In traditional propagation, the removal of apices with the youngest leaves stimulates axillary branching. The removal of the apices and whole leaves is used practically in propagation of *Euphorbia pulcherrima* (belonging to the same family as *Codiaeum*) via shoot cuttings (Grueber and Wilkins, 1985, cited in Wilkins, 1988).

Our attempts to increase the number of axillary shoots of *Codiaeum variegatum* cv. Excellent through leaf removal, apex removal and shoot orientation
(horizontal or tip down) were successful. In all experiments the number of useful shoots for further multiplication or rooting was at least two times higher on defoliated than control shoots. Produced shoots, even if most of them were shorter than 1 cm, were able to root on medium with IBA and acclimatize in the greenhouse. The preparation of such explants is not difficult and time consuming because it can be done by one scalpel movement. It is not necessary to remove the petioles or maintain their particular length. A further increase in axillary shoot numbers was achieved by changing the shoot position to horizontal or vertical with tip down, although the latter position was unstable when new leaves started to grow. The increase in branching through horizontal and opposite shoot orientation could be explained as an effect of release from apical dominance caused by a disturbance in the auxin basipetal transport. Against the exclusive role of endogenous auxin in retarding growth of axillary shoots is a fact that the effect of shoot removal, which eliminates the main source of endogenous auxin, was weaker than that of defoliation. Consequently, it is possible that horizontal and opposite shoot orientation facilitated better absorption of medium ingredients.

However, the results of the third experiment support the role of auxin in the arrest of axillary shoots because exogenous IAA decreased the total number of shoots from defoliated explants. On the other hand TIBA, which is known as the inhibitor of auxin transport, did not replace defoliation at least in the concentrations tested. Voyiatzi and Voyiatzis (1988) reported that TIBA at 1, 3 and 10 mg l\(^{-1}\) (2, 6 and 20 μM) increased shoot numbers and reduced shoot callus in rose cultures at a level similar to (10 mg l\(^{-1}\)) or greater than (1 and 3 mg l\(^{-1}\)) apex removal. A similar effect of TIBA at 5, 10 and 15 μM on the increase in axillary shoot growth of *Acer saccharinum* was reported by Marks and Simpson (1994). In our experiment TIBA did not reduce the size of calluses, but did increase the number of shoots longer than 1 cm grown on non-defoliated explants and reduced the total number of shoots grown on defoliated explants.

In our experiments fully developed leaves prevented the growth of axillary shoots more than young leaves and apices, although we can expect more auxin to be produced in the latter. This effect could be caused by putative additional factor(s) inhibiting the axillary shoot growth, present in the matured leaves.

The increase in number of high quality axillary shoots of *Codiaeum variegatum* by simple treatments — defoliation and horizontal orientation of shoots on the medium has practical value for the large scale micropropagation of this species.

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References


