BIOTIC AND ABIOTIC FACTORS INFLUENCE DURING
THE IN VITRO MULTIPLICATION PHASE OF SOME SPECIES AND CULTIVARS
OF THE ACER GENUS

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Abstract

The purpose of the research was to study the behaviour of several Acer cultivars: Acer palmatum 'Dissectum
Atropurpureum' and Acer platanoides 'Crimson King', 'Drummondii' and 'Globosum', during the first subculture of the
in vitro multiplication phase. There were used explants from the initiation phase and passed on the multiplication
nutrient media. The tested nutrient media were MS, DKW and WPM and had a different content of growth hormones.
In order to determine the multiplication rate, the influence of zeatin and thidiazuron was tested in concentration of 1.5
mg/l, on a constant level of indole-butyric acid (0.5 mg/l). In the growing chamber there was a constant temperature and
a photoperiod of 14 hours, at a luminosity of 2500 lux. The multiplication rate (microshoots/explant) for the four
genotypes was determined from the observations and registered data.

Key words: Acer palmatum 'Dissectum Atropurpureum', Acer platanoides 'Crimson King', Acer platanoides
'Drummondii', Acer platanoides 'Globosum', multiplication rate

The ornamental varieties, 'Crimson King', 'Drummondii' and 'Globosum', belong to the
species Acer platanoides, being easily to
propagate by seeds, but mostly by grafting and
cuttings. Acer palmatum 'Dissectum
Atropurpureum' - the Japanese maple – is a base
branched shrub or with short crook trunk. The
shoots are hairless and red. The flowers, situated
in small corymbs, appear once with the leaves
(Posedaru E.A., 2005). The species itself is grown
from seed. Only a few strong-growing cultivars
are commercially propagated from cuttings. Plants
of the dissected and variegated cultivars on their
own roots almost always fail to grow into good
plants (Gelderen, Oterdoom, 1994). Due to low
bud-forming capacity, propagation by grafting and
cuttings are difficult to perform, so the
micropropagation technique is widely used now
took an impressive turn, being used on large scale
at international level to obtain propagation
material.

MATERIAL AND METHOD

The viable microshoots obtained at the end
of the initiation phase (fig. 1) were transferred for
the multiplication phase on specific nutrient media
for: induction and acceleration of proliferation of
axillary budding or multiple axillary shoot (each
shoot is a potential plant) and shoot elongation
(Badea M.E., Sândulescu D., 2001). For the
ultiplcation phase, were established three
variants of nutrient media (tab. 1). Observations
regarding the multiplication rate were noted during
the first subculture, with a medium length of 35
days. For nutrient media preparation were used
stock solutions of 10x macroelements, 10x
microelements and 100x vitamins. Phytohormones were prepared as 10⁻² and 10⁻⁶
diluted solutions. Ferrous sulphate and Na₂EDTA
were added as NaFeEDTA, in concentration of 32
mg/l. Were used conic culture vessels (Ø=100mm
and h=100mm) for the first subculture, each with
25ml medium/vessel.

The work methodology respected the
standard protocol regarding aseptic conditions
transfer under laminar air flow hood. After
explants inoculation on nutrient media, the
vessels were incubated in the growth chamber.
The acclimatized premises have adjustable
photoperiodism. Illumination was done with white
light fluorescent tubes, with an intensity of 2500
luci and the photoperiod was set at 14 hours
light/24 hours. In order to study maple in vitro
multiplication capacity was organized a bifactorial
experience, having as variable factors genotype
and nutrient medium composition, and as
constant factor – photoperiod.

The experience is a 4×3 bifactorial, with a
total of 12 variants (V.1-V.12), in 3 repetitions (R1,
R2, R3), each with cu 2 culture vessels / repetition. In each culture vessel were assigned 3
microshoots from the initiation phase. The study
totalized a number of 216 inoculated explants
(tab. 2).

Variable factors:
RESULTS AND DISCUSSIONS

The recorded data are expressed in ratio of multiplication (microshoots/explant) for the first subculture in the multiplication phase. During the multiplication phase, growth and axillary shoot are influenced by the composition of the nutritent medium and genotype. The notes and recorded data emphasized that on a constant level of the photoperiod at 14 hours and the same indole-butyric acid concentration as growth regulator, nutrient media, MS, DKW and WPM respectively, and genotype had a great influence.

Table 1

<table>
<thead>
<tr>
<th>Composition (mg/l)</th>
<th>Variants of nutrient media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B1</td>
</tr>
<tr>
<td>Macroelements</td>
<td>MS</td>
</tr>
<tr>
<td>Microelements</td>
<td>MS</td>
</tr>
<tr>
<td>Vitamins</td>
<td>MS</td>
</tr>
<tr>
<td>Indole-butyric acid (mg/l)</td>
<td>0.5</td>
</tr>
<tr>
<td>Zeatin (mg/l)</td>
<td>-</td>
</tr>
<tr>
<td>Thidiazuron (mg/l)</td>
<td>1.5</td>
</tr>
<tr>
<td>NaFeEDTA (mg/l)</td>
<td>32</td>
</tr>
<tr>
<td>Dextrose (g/l)</td>
<td>40</td>
</tr>
<tr>
<td>Agar (g/l)</td>
<td>7.5</td>
</tr>
</tbody>
</table>

From the interaction of nutrient medium with genotype (AxB) it shows that in the first subculture of the multiplication phase, the genotypes A.1, A.2 and A.3, respectively Acer palmatum 'Dissectum Atropurpureum', Acer platanoides 'Crimson King' (fig. 4) and Acer platanoides 'Drummondii', had the highest multiplication rate (fig. 2).
Looking at the culture media, the B.3 graduation (V3, V6, B9 and V12 variants) had the highest multiplication rate (fig. 3), with 3 microshoots/explant, thus WPM culture medium was recommended for multiplication phase. The lowest performance had MS culture medium, with 1 microshoot/explant, for A.1 and A.2 genotypes.

**Table 2**

<table>
<thead>
<tr>
<th>Variant</th>
<th>A: Nutrient medium</th>
<th>B: Photoperiod</th>
</tr>
</thead>
<tbody>
<tr>
<td>V.1</td>
<td>A.1</td>
<td>B.1</td>
</tr>
<tr>
<td>V.2</td>
<td>A.1</td>
<td>B.2</td>
</tr>
<tr>
<td>V.3</td>
<td>A.1</td>
<td>B.3</td>
</tr>
<tr>
<td>V.4</td>
<td>A.2</td>
<td>B.1</td>
</tr>
<tr>
<td>V.5</td>
<td>A.2</td>
<td>B.2</td>
</tr>
<tr>
<td>V.6</td>
<td>A.2</td>
<td>B.3</td>
</tr>
<tr>
<td>V.7</td>
<td>A.3</td>
<td>B.1</td>
</tr>
<tr>
<td>V.8</td>
<td>A.3</td>
<td>B.2</td>
</tr>
<tr>
<td>V.9</td>
<td>A.3</td>
<td>B.3</td>
</tr>
<tr>
<td>V.10</td>
<td>A.4</td>
<td>B.1</td>
</tr>
<tr>
<td>V.11</td>
<td>A.4</td>
<td>B.2</td>
</tr>
<tr>
<td>V.12</td>
<td>A.4</td>
<td>B.3</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

The results regarding the multiplication phase of *Acer palmatum ‘Dissectum Atropurpureum’* and *Acer platanoides ‘Crimson King’, Acer platanoides ‘Drummondii’* and *Acer platanoides ‘Globosum’, Kanzan’* varieties led to the following conclusions:

- for the first subculture of the multiplication phase, the genotypes *Acer palmatum ‘Dissectum Atropurpureum’, Acer platanoides ‘Crimson King’ and ‘Drummondii’* had the best in vitro behavior;
- regarding the nutrient medium, the highest rates of multiplication (3 microshoots/explant) were achieved using DKW and WPM media.

**REFERENCES**