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

Manuela Elena CONCIOIU[1], Mihaela Ileana OPREA[1]

e-mail: manuela.concioiu@hotmail.com

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 lucs. 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 axilary budding or multiple axilary shoot (each shoot is a potential plant) and shoot elongation (Badea M.E., Săndulescu D., 2001). For the ultiplication 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 Na_2EDTA 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 lucsi 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:

[1]U.S.A.M.V. Bucharest

A. Genotype:
A.1 – *Acer palmatum* 'Dissectum Atropurpureum';
A.2 – *Acer platanoides* 'Crimson King';
A.3 – *Acer platanoides* 'Drummondii';
A.4 – *Acer platanoides* 'Globosum'.
B. Culture medium:
B.1 – MS medium;
B.2 – DKW medium;
B.3 – WPM medium.

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 axilary shoot are influenced by the composition of the nutritend 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 respectivly, and genotype had a great influence.

Table 1

Composition of nutrient media tested for in vitro multiplication phase

Composition (mg/l)	Variants of nutrient media		
	B1	B2	B3
Macroelements	MS	DKW	WPM
Microelements	MS	DKW	WPM
Vitamins	MS	DKW	MS
Indole-butyric acid (mg/l)	0.5	0.5	0.5
Zeatin (mg/l)	-	1.5	1.5
Thidiazuron (mg/l)	1.5	-	-
NaFeEDTA (mg/l)	32	32	32
Dextrose (g/l)	40	40	40
Agar (g/l)	7.5	7.5	7.5

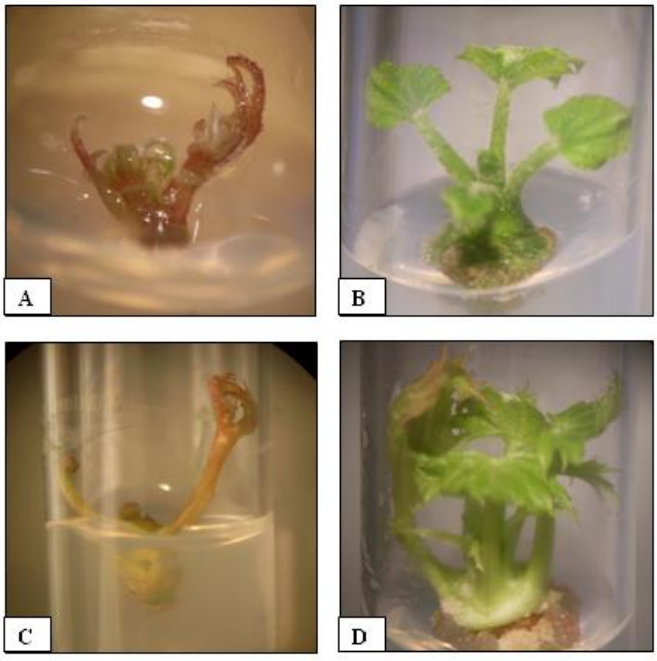

Figure 1 **Acer explants at the end of in vitro initiation phase: Acer plamatum 'Dissectum Atropurpureum' (A), Acer platanoides 'Globosum' (B), Acer platanoides 'Crimson King' (C), Acer platanoides 'Drummondii' (D) (original)**

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, respectivly *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.

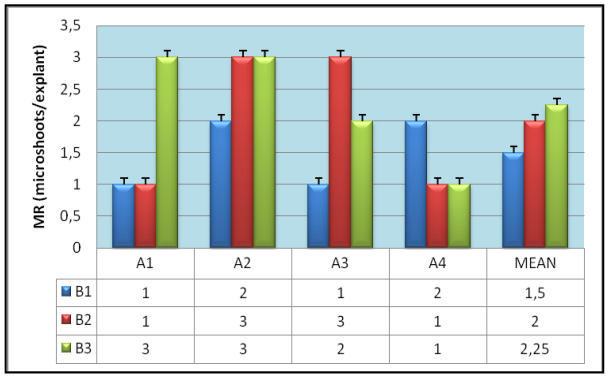

Figure 2 **Multiplication rate for subculture 1, depending on culture medium for different genotypes**

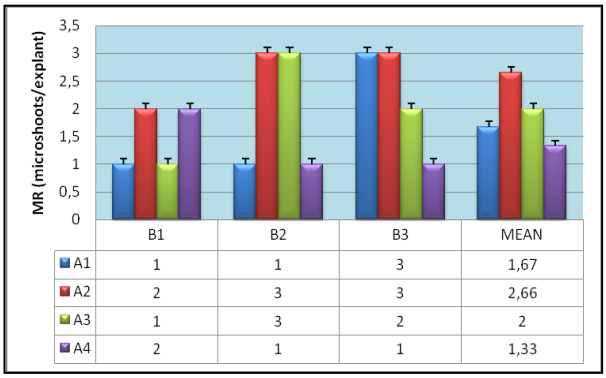

Figure 3 **Multiplication rate for subculture 1, depending on genotype for different culture media**

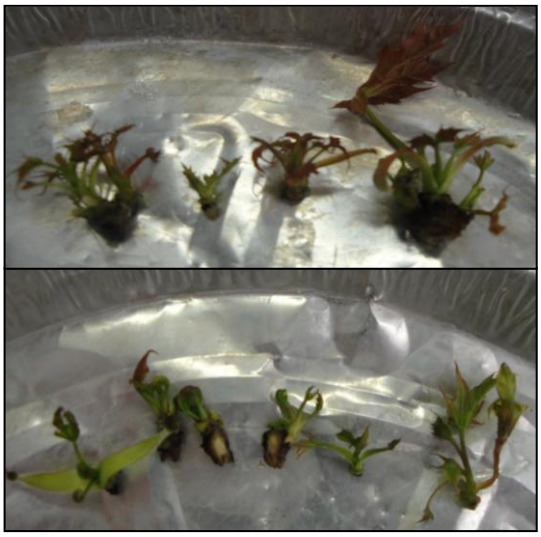

Figure 4 ***Acer platanoides* 'Crimson King', micromultiplication aspects: microshoots at the end of initiation phase (up);microshoots individualisation and shortning (down) (original)**

Table 2

Experimental variants for multiplication phase

Constant factor: photoperiod	Variant	Variable factors	
		A: Nutrient medium	B: Photoperiod
	V.1	A.1	B.1
	V.2	A.1	B.2
	V.3	A.1	B.3
	V.4	A.2	B.1
	V.5	A.2	B.2
	V.6	A.2	B.3
	V.7	A.3	B.1
	V.8	A.3	B.2
	V.9	A.3	B.3
	V.10	A.4	B.1
	V.11	A.4	B.2
	V.12	A.4	B.3

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.

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