PRELIMINARY RESULTS REGARDING *IN VITRO* BEHAVIOUR OF TWO *ACER* VARIETIES DURING THE INITIATION PHASE

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Abstract
Taking into account the economic importance of Acer species and their frequent use in landscape architecture, both nationally and internationally, it is necessary to develop an in vitro biotechnology research to propagate some of the most popular and requested Acer varieties. The study presents data on the partial results of in vitro initiation for two varieties of Acer platanoides: 'Globosum' and 'Crimson King'. During the initiation phase, Acer platanoides 'Globosum' showed the best initiation percentage, using the next nutrient composition: MS macroelements, MS microelements, MS vitamins, auxins/cytokines 0,2:1 mg/l, while Acer platanoides 'Crimson King' variety recorded the best results on the nutrient medium with a different composition: MS macroelements, MS microelements, LF vitamins and auxins/ cytokine0,4:1,2 mg/l.

Keywords: Acer platanoides , 'Globosum', Acer platanoides , 'Crimson King', in vitro culture, explants

1. INTRODUCTION

'Globosum' and 'Crimson King' varieties belong to *Acer platanoides* species and are easy to propagate by seeds, buds and mostly by cuttings and grafting. Due to their reduce bud regeneration capacity, grafting and propagation by cuttings are hard to perform and as a result, is used the *in vitro* propagation technique.

2. MATERIAL AND METHOD

For the initial phase of *in vitro* culture, explants were taken from cuttings harvested in a single phonological moment, respectively the dormant phase (November), from the stock-plants.
Harvesting of shoots was carried out following the protocol requirements of the *in vitro* initiation phase. The two nutrient media V1 and V2, tested for the initiation phase had a different composition, in terms of vitamins and phytohormones (Table 1).
Before sharing the media in culture vessels, the pH of nutrient media was adjusted at 5.6-5.8.
Culture media distributed in vessels were sterilized by autoclaving, at 1,1 atmospheres, 120^0C, for 25 de min.
For the initiation phase, the experience has a total of 4 variants, with 3 repetitions each.
Variable factors were:
A - genotype with two graduations, A1 - variety 'Globosum'
A2 - variety 'Crimson King'
B - the nutrient medium, with two graduations, B1 and B2 (Table 2).

Table 1. ***Nutritive media composition tested during the initiation phase***

Composition	V1	V2
Macroelements	MS	MS
Microelements	MS	MS
Vitamins	MS	LF
Agar (g/l)	7	7
Dextrose (g/l)	40	40
NaFeEDTA (mg/l)	32	32
Benzil aminopurine (BAP) (mg/l)	1,0	1,2
Indolil acetic acid (IAA) (mg/l)	0,2	-
Naftil acetic acid (NAA) (mg/l)	-	0,4

Legend: MS = Murashige-Skoog (1962), LF = Lee-Fossard (1977)
(Lee E.M.C., De Fossard R.A. 1977 and Murashige T., Skoog F. 1962**)**

Table 2. ***Experimental variants for in vitro initiation of cultures***

Variants	Variable factors	
	Genotype	Nutritive medium
V1	A1	B1
V2	A1	B2
V3	A2	B1
V4	A2	B2

The ornamental varieties used were:

- '**Globosum**' - an ornamental tree by 5-6 m height, with globular canopy, round, flatted, with dense and compact branching. Its leaves are initially round, 5 lobed, dull lobes in turn lobate, all peaks acuminate. Leaf color is dark green, becoming golden in autumn (Posedaru, E.A., 2005) (Figure1);

- **'Crimson King'** - an ornamental variety with oval canopy, reaches by 15 m in height, with dark purple leaves during summer, very decorative (Figure1).

Biological material was processed as followed (Figure 2):

- bending;
- microcutting at 2 - 2.5 cm;
- disinfection by washing with water and 2-3 drops of chlorine based disinfectant, followed by sterilization in 96% ethanol for 10 minutes and 6% calcium hypochlorite for 15 minutes;
- rinse in distilled water sterilized by autoclaving.

Explants, consisting in meristematic tissue with 2-3 leaflets were taken under the binocular in aseptic conditions in laminar air flow hood. The explants were maintained on nutrient media during the initiation phase of *in vitro* culture under laboratory conditions: 24° C ± 2ºC temperature, 14 hours photoperiodism and 3500 lux light intensity in the growing chamber.

3. RESULTS AND DISCUSSIONS

Results were recorded 30 days after *in vitro* culture initiation (Figure 3).

In the initiation phase of *in vitro* culture for the two ornamental maple varieties growth was influenced by two variable factors: nutrient medium composition and genotype.

Following the genotype influence on each nutrient medium we can note that explants from 'Globosum' variety grew faster than 'Crimson King' explants. The best growing percent of explants was obtained on the B1 nutrient medium by 'Globosum' variety with 93.33%, followed by 'Crimson King' with only 53.33% (Figure 4).

Following the culture media influence on explants' growth, we can observe that the B2 nutrient medium is not indicated for A1 genotype, explants showing a 66.66% growing percentage, while 'Crimson King' variety on the same nutrient medium presents the greatest value of 86.66% (Figure 5).

Figure 1. Acer platanoides 'Globosum' and 'Crimson King' stock plants

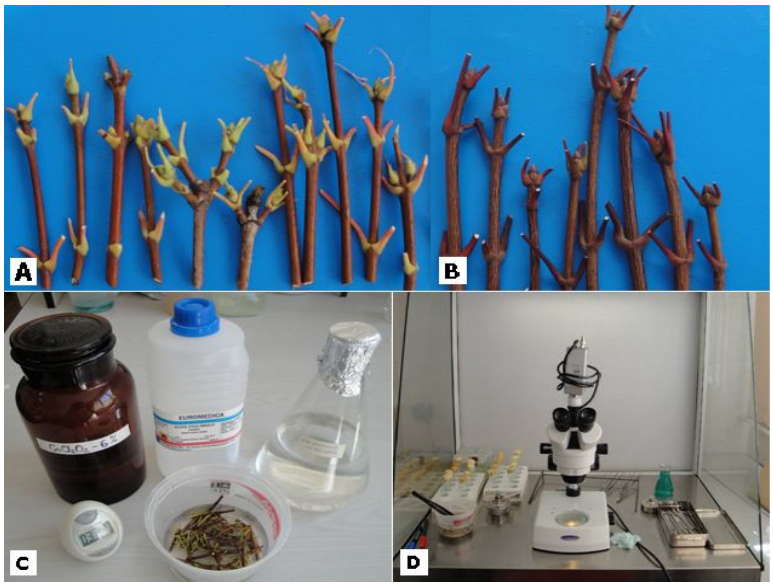

Figure 2. Initiation protocol: A. 'Globosum' cuttings; B. 'Crimson King' cuttings; C. Disinfection; D. Explants' sampling

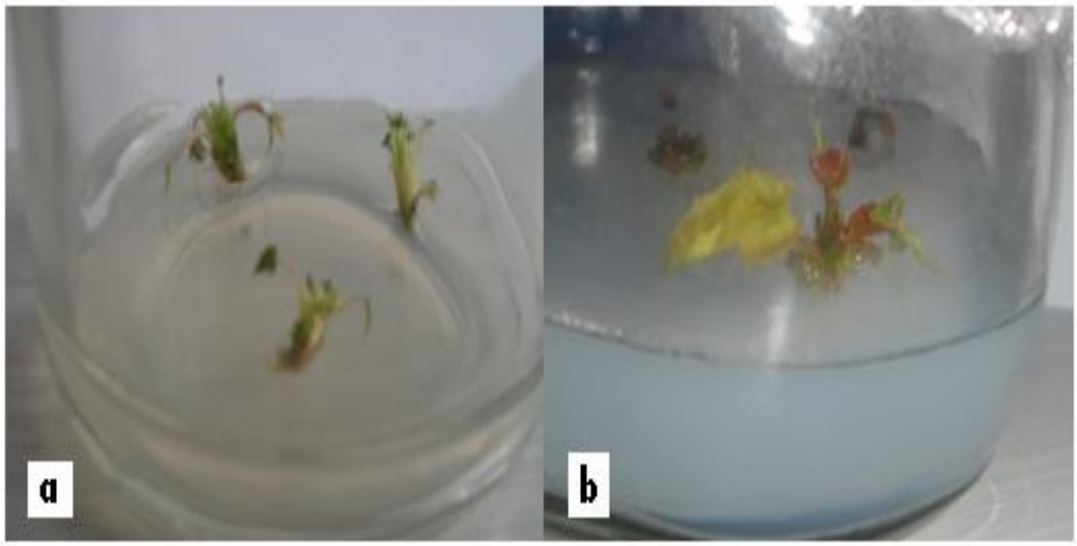

Figure 3. Explants' growing a) 'Globosum' (b) 'Crimson King'

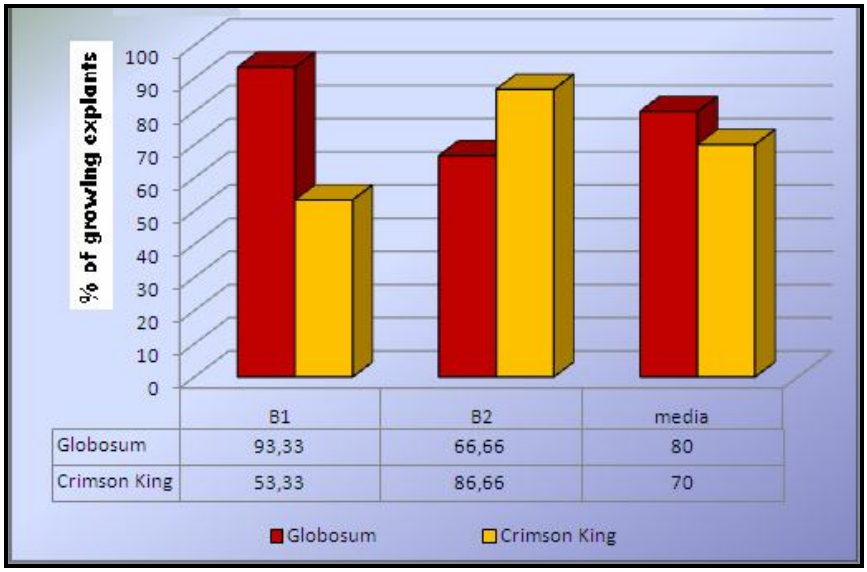

Figure 4. Explants' growing depending on genotype influence

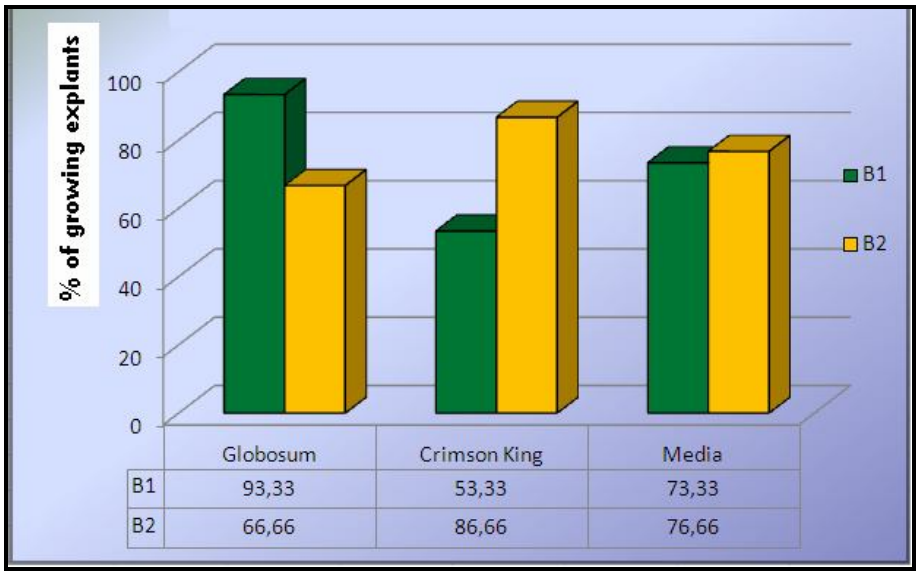

Figure 5. Explants' growing depending on nutritive medium

4. CONCLUSIONS

We can conclude that the *Acer platanoides* varieties studied, 'Globosum' and 'King Crimson King' had a good behavior during the initiation of *in vitro* culture.

The observations and recorded data led to the following conclusions:

- 'Globosum' variety registered the best growing percentage on the nutrient medium with the next composition: MS macroelements, MS microelements, MS vitamins, cytokines/auxins 1:0,2 mg/l;
- 'Crimson King' variety recorded the best results on the nutrient medium with a different composition, such as: MS macroelements, MS microelements, LF vitamins and cytokines/auxins 1,2:0,4 mg/l;

This study presents only data obtained during the initiation phase. Because our goal is to establish the *in vitro* propagation biotechnology for these ornamental varieties, we will continue to perform research regarding the vitroplants behavior during the multiplication, rooting and acclimatization phases.

5. REFERENCES

George E.F., Puttock D.J.M. and George, H.J. (1987) Plant Culture Media, Vol. I., Formulations and Uses, Exercise Limited, Edington, Westbury, p. 12-13.

Lee E.M.C., De Fossard R.A. (1977) Some factors affecting multiple bud formation of strawberry (x *Fragaria ananassa* Duchesne). Acta Hort. 78: 187-195.

Murashige T., Skoog F. (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Posedaru E.A. (2005) Plante ornamentale recomandate pentru amenajarea spațiilor verzi din România, Arboricultură ornamentală, Editura Universității din Pitești, p. 81-86.