IN VITRO MULTIPLICATION OF *Acer platanoides* L.

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Abstract. Mature individuals of ''Faassens Black'' and ''Drummondii'' were used as stock plants. Hardwood nodal and apical segments, (harvested in September), softwood nodal and apical segments (harvested in May), and dormant buds (harvested in the end of April and November) were used as initial explants. Dormant buds are the most suitable explants for disinfection and culture establishment on PGRs-free WPM. The concentration of kinetin does not affect the length of the shoots within the cultivar, but in comparison with ''Drummondii'' they were statistically longer in ''Faassens Black'' on WPM with 0.5 mg l^{-1} kinetin (14.5 ± 3.5 mm). However, axillary shoots formation was not induced in both treatments and cultivars. These shoots in ''Faassens Black i'' reached maximal length (39.5 ± 11.0 mm) after the 8th subculture on WPM supplemented with 1.0 mg l^{-1} BAP and 0.1 mg l^{-1} IBA. In ''Drummondii'' only single shoots elongated but all of them gradually died and no viable shoots were found after the 8th subculture. However, the frequency of the induced axillary shoots after cultivation for 180 days was low and was noticed on single explants in ''Faassens Black'', only (0.65 ± 0.17). In juvenile explants, the multiplication rate and the length of the axillary shoots were low and were not affected by the genotype and subcultures. Maximal number (1.9 ± 0.4) and length (8.4 ± 0.6 mm) of axillary shoots was induced on the medium with 1.0 mg l^{-1} BAP and 1.0 mg l^{-1} GA_3. The combination of these PGRs provoked statistically significant additionally formation of axillary shoots but their length remained less that 5 mm in the period of cultivation. The type of cytokinin does not affect the elongation of the initial explant within the genotype. The quantity of induced axillary shoots was small and their elongation was very slow. A higher number of axillary shoots was received on WPM with 2 mg l^{-1} Kinetin (from 0.25 ± 0.16 to 2.13 ± 0.58). Probably, due to the apical dominance of the initial explant, the formation of axillary shoots, and their elongation is limited. Also, different responses to cytokinins could be attributed to the reaction of different genotypes.

Keywords: ''Drummondii'', ''Faassens Black'', Norway maple, propagation, subculture, tissue cultures.

Rezumat. Înmulţirea *in vitro* la *Acer platanoides* L. Exemplare mature de „Faassens Black” şi „Drummondii” au fost folosite ca plante stoc. Ca explante iniţiale au fost folosite segmente nodale şi apicale de lemn tare (recoltate în luna septembrie), segmente nodale şi apicale de lemn moale (recoltate în mai) şi muguri latenţi (recoltaţi la sfârşitul lunii aprilie şi noiembrie). Mugurii latenţi sunt cei mai potriviţi ca explante pentru dezinfecţie şi stabilirea culturii pe WPM fără PRG. Concentraţia de chinetină nu a afectat lungimea lăstarilor în cazul aceluiaşi soi, dar în comparaţie cu „Drummondii”, aceştia au fost statistic mai lungi în cazul „Faassens Black” pe WPM cu 0,5 mg l^{-1} chinetină (14,5 ± 3,5 mm). Cu toate acestea, formarea de muguri axilari nu a fost indusă în cazul ambelor tratamente şi soiuri. Aceşti lăstari de „Faassens Black” au atins lungimea maximă (39,5 ± 11,0 mm) după subcultura a opta pe WPM suplimentat cu 1,0 mg l^{-1} BAP şi 0,1 mg l^{-1} IBA. La „Drummondii”, numai lăstarii unici s-au alungit, dar aceştia au murit treptat şi nu au fost depistaţi muguri viabili după a opta subcultura. Cu toate acestea, frecvenţa lăstarilor induşi axial după cultivare timp de 180 de zile a fost scăzută şi a fost observată doar la explantele unice de „Faassens Black” (0,65 ± 0,17). La explantele juvenile, rata de multiplicare şi lungimea lăstarilor axilari au fost reduse şi nu au fost afectate de genotip şi subculturi. Numărul maxim (1,9 ± 0,4) şi lungimea maximă (8,4 ± 0,6 mm) a mugurilor axilari a fost indusă pe un mediu cu 1,0 mg l^{-1} BAP şi 1,0 mg l^{-1} GA_3. Combinaţia acestor PGR a determinat formarea adiţională de lăstari axiali, semnificativă din punct de vedere statistic, dar lungimea acestora nu a depăşit 5 mm în perioada de cultivare. Tipul de citokinină nu afectează alungirea explantului iniţial în cadrul aceluiaşi genotip. Cantitatea generată de lăstari axiali a fost mică şi alungirea lor a fost foarte lentă. Un număr mai mare de lăstari axilari a fost determinat în cazul WPM cu 2 mg l^{-1} chinetină (de la 0,25 ± 0.16-2.13 ± 0,58). Probabil, datorită dominaţiei apicale a explantului iniţial, formarea de lăstari axilari şi alungirea lor este limitată. De asemenea, răspunsurile diferite la citokinine ar putea fi atribuite reacţiei diferitelor genotipuri.

Cuvinte cheie: „Drummondii”, „Faassens Black”, arţar norvegian, propagare, subcultură, culturi de ţesuturi.

INTRODUCTION

Acer platanoides L. is a valuable ornamental tree with a lot of ornamental cultivars (ARTJUSHENKO et al., 1958; KOLESNIKOV, 1958; SANTAMOUR et al., 1982; KRÜSSMAN, 1984; DIRR, 1998). It is readily propagated from seeds, but as seedlings show a wide variability in phenotypic characteristics, vegetatively propagated cultivars are preferable in urban areas. Norway maple cuttings are considered difficult to root (CHONG & DAIGNEAULT, 1986; MAYNARD & BASSUK, 1987) and depend on the age of the stock plant and etiolation of the shoots (MAYNARD & BASSUK, 1987). For this reason most of the cultivars are grafted onto seedlings origin rootstock. Furthermore, the production of large quantities of grafts is limited by the season, the period for rootstock production and the success depends on the method of grafting and climatic conditions in the field (HOWARD et al., 1974; KRÜSSMAN, 1984; DIRR & HEUSER, 1987; HOWARD, 1993; HOWARD & OAKLEY, 1997).

In vitro propagation can provide alternative of the conventional methods for cloning of valuable ornamental genotypes (BONGA, 1982; HARTMANN et al., 2002; ILIEV & BORISOWA, 2002). Studies on the *in vitro* propagation of the Norway maple have focused on wild types (ĎURKOVIČ, 1996; LINDEN & RIIKONEN, 2006) or ornamental '' Crimson Sentry'' (CHENG, 1978; LATTIER at al., 2012, 2013), ''Crimson King'' (MARKS & SIMSON, 1990, 1994), and ''Jirka'' (ŠEDIVÁ, 2009). However, some of the publications did not report the age of the stock plant and multiplication did not accomplish (CONCIOU et al., 2010; ROVINĂ et al., 2010)

This research was aimed at determining a suitable disinfection, factors affecting culture establishment, and multiplication of mature ''Faassens Black'' and ''Drummondii'' and to compare their reaction *in vitro* with juvenile *Acer platanoides* L. seedlings.

MATERIAL AND METHOD

Factors affecting the cultivation of mature genotypes

Plant material and disinfection of the initial explants

Individuals of ''Faassens Black' and ''Drummondii' were used as stock plants 8 years after their grafting. Hardwood nodal and apical segments (≈ 3 cm) were harvested in September and were disinfected consecutively by 72% ethanol for 2 min and then by 0.2% $HgCl_2$ for 15 min (variant 3). Softwood nodal and apical segments (≈ 3 cm) were harvested in May and were disinfected by 0.1% $HgCl_2$ for 3 min (variant 2). Dormant buds were harvested in the end of April and November and were disinfected consecutively by 72% ethanol for 2 min and then by 0.2% $HgCl_2$ for 8 min (variant 1). After being rinsed 3 times in sterile distilled water, the ends (≈ 2 mm) of apical and nodal segments were aseptically removed.

Culture establishment

Hardwood nodal and apical segments were cultured on plant growth regulators (PGRs)-free WPM (LLOYD & McCOWN 1980) or WPM supplemented with 1.0 mg l^{-1} 6-Furfurilaminopurine (Kinetin), and the buds were cultured on PGRs-free WPM. Softwood nodal and apical segments were cultured on PGRs-free WPM or WPM supplemented with 6-benzylaminopurine (BAP), (6-[4-Hydroxy-3-methyl-but-2-enylamino]purine (Zeatin) or Kinetin in concentration 1.0 mg l^{-1}. After two days of cultivation, the explants were subcultured on the same medium for the elimination of the separated phenols (Fig. 1).

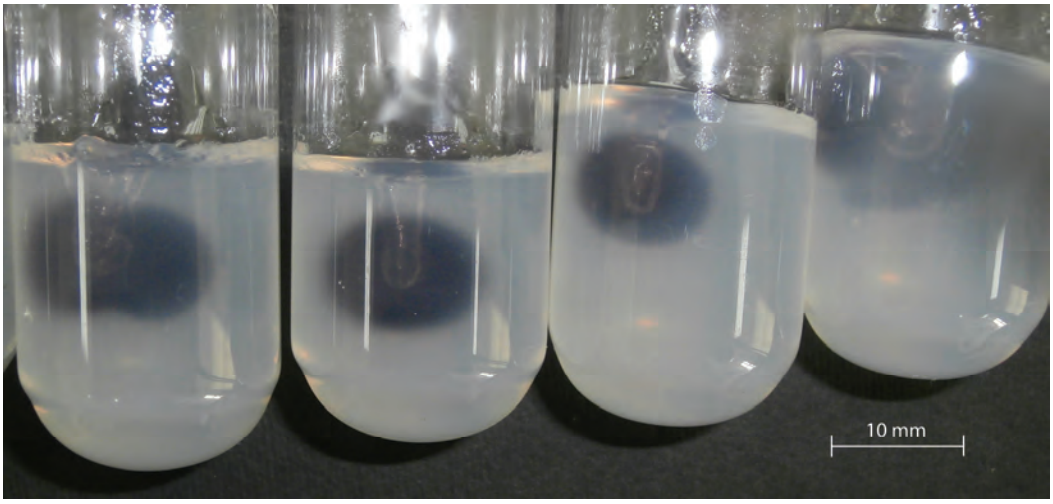

Figure 1. Released phenols on the second day after the cultivation of softwood explants (original).

Each treatment contained three repetitions, and 15 explants were used per replication. The explants with developed buds and elongated shoots as well as dead explants were evaluated after 2 weeks.

Factors affecting the elongation and multiplication of the shoots

After 2 weeks on PGRs-free WPM, the buds with removed scales harvest in April from ''Faassens Black'' and ''Drummondii'' were cultured on WPM supplemented with 0.5 and 1.0 mg l^{-1} kinetin for elongation and multiplication. Three replications, each containing 6-7 were used for each variant of the medium. After 14 days, the explants were subcultured on the same variant of the medium and the results were evaluated after 28 days from the beginning of the experiment.

The elongated shoot tips (~ 2 cm) were cultured on WPM supplemented with 1.0 mg l^{-1} BAP and 0.1 mg l^{-1} indole-3-butyric acid (IBA). The effect of 10 subcultures (each with a duration of 14 days) was studied on the reaction of initial explants and axillary shoots formation. The results were evaluated after each 2nd subculture and the formed axillary shoots were isolated and cultured on the same medium. Finally, the isolated shoots were subcultured after 20 days, and the results were evaluated 20 days later. For each cultivar at least 14 initial explants were used at the beginning of the experiment.

Factors affecting the cultivation of juvenile genotypes

Effect of the genotype on the multiplication rate

To study the effect of genotype, the formed axillary shoots from 8 seedlings were cultured on WPM supplemented with 1.0 mg l^{-1} kinetin and 1.0 mg l^{-1} Gibberellic acid (GA_3). Due to the limiting initial material, 3 to 11 axillary shoots were use per genotype. The explants were subcultured after 14 days and the results were evaluated 14 days later.

Effect of subculture and genotype on the axillary shoots formation

To study the effect of genotype and subculture axillary shoots of three clones (2, 5, and 8) were cultured on WPM enriched with 1.0 mg l^{-1} BAP and 0.1 mg l^{-1} indole-3-butyric acid (IBA). The formed axillary shoots were subcultured 5 times and each subculture was with duration of 14 days. The number of formed axillary shoots was evaluated after each subculture.

Effect of PGRs on the axillary shoots formation

Axillary shoots (5-8 mm) from genotype 2 with small part of the callus (1-2 mm) on the base (Fig. 2) were cultured on WPM supplemented with 3.0 mg l^{-1} BAP and 0.1 mg l^{-1} IBA, 1.0 mg l^{-1} BAP and 0.1 mg l^{-1} IBA, 1.0 mg l^{-1} BAP and 1.0 mg l^{-1} GA_3, and 1.0 mg l^{-1} N^6-(2-Isopentenyl)adenine (2iP). Each treatment contained 3 repetitions and each of them - 6 or 7 explants. The explants were subcultured after 20 days on the same variants of the medium and the results were evaluated on the 40th day from the beginning of the experiment.

Figure 2. Axillary shoots with little callus on the base, used as initial explants (original).

Axillary shoots (10-15 mm), were used as initial explants (Fig. 3). They were cultivated on WPM medium supplemented with 1 mg l^{-1} BAP, mg l^{-1} 2iP, and 2.0 mg l^{-1} Kinetin. Each treatment contained 8 explants. The shoots were transferred on the 20th day on the same variants of medium, after removing only the necrotic callus or leaves. Number and length of initial elongated and new formed axillary shoots were evaluated after 40 days. No mean length was calculated when the new formed shoots were less than 5 mm.

Figure 3. Axillary shoots, used as initial explants (original).

Conditions of the cultivation

Each variant of the media contained 7 g l^{-1} agar (Sigma) and pH was adjusted to 5.6 before autoclaving (under pressure of 118 kPa and 120ºC for 20 min). The cultures were grown in a cultivation chamber at 23 ± 1ºC with 16 hrs of cool white fluorescent light at a photosynthetic photon flux density of 40 µmol m^{-2} s^{-1}, daily.

Statistical analysis

The results were analysed by One-Way ANOVA followed by a post hoc LSD test at $p < 0.05$, using SPSS 20.0 for Windows. Percentage values were transformed using arcsine square root (√P) (COMPTON, 1994) to normalize error distribution prior variance analysis.

RESULTS AND DISCUSSION

Factors affecting the cultivation of mature genotypes

Disinfection of the explants and culture establishment

The results from disinfection experiments demonstrated that the highest statistically significant level of exogenous contamination was observed on softwood explants from both cultivars harvested in May. It could be due to the lower concentration of $HgCl_2$ and shorter duration of disinfection necessary for this type of explants. The level of exogenous contamination in other types of explants was low in the stage of their dormancy and significant differences between them and the time of their collection was not observed in both investigated cultivars. The highest statistically significant level of endogenous contamination in both cultivars was found in hardwood segments, collected in September. The level of endogenous contamination in other type of explants was low and significant differences between them and time of their collection was not observed in both investigated cultivars. The highest level of aseptic explants in both cultivars was obtained when buds were collected in April and November in the stage of their dormancy (Table 1). All our explants were resistant to the action of $HgCl_2$ i.e. they were not damaged from the treatment and were viable. Softwood explants and buds demonstrated development (Fig. 4). The elongation of the shoot was induced only from the dormant buds with removed scales, harvested in the beginning of the spring and cultivated on PGRs-free WPM. However, the percentage of the buds with elongated shoots was low in both cultivars (24.4 ± 8.0 and 11.1 ± 4.4, resp.) and the length of the induced shoots reached not more than 5 mm in the 14 days of cultivation (Table 2, Fig. 5).

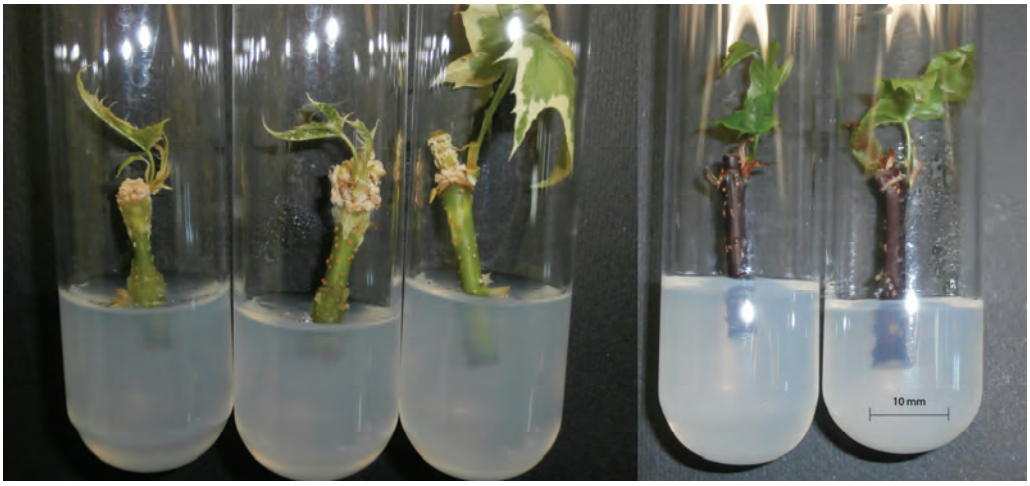

Figure 4. Softwood explants from *Acer platanoides* ‘‘Drummondii’’ and ‘‘Faassens Black’’, after 14 days of cultivation. No shoot elongation noticed (original).

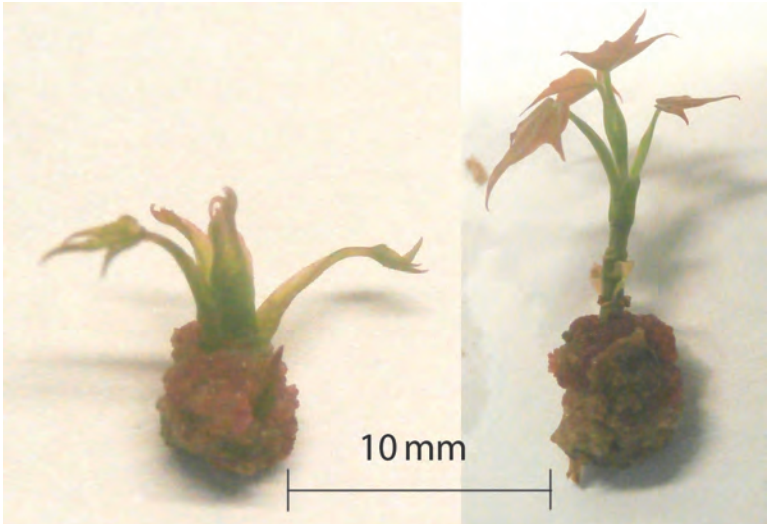

Figure 5. Non elongation and elongation of buds from *Acer platanoides* ‘‘Faassens Black’’.

The initial explants have been treated with 70% ethyl alcohol for 1 min before applying the disinfection agent (LINDEN & RIIKONEN, 2006). NaClO (5.0-6.15%) has been used for 15-20 min for disinfection of actively growing shoots (PREECE et al., 1991; LATTIER et al., 2013) or dormant segments (CONCIOU et al., 2010). Dormant apical and axillary buds were disinfected in 0.5% NaClO for 20 min (LINDEN & RIIKONEN, 2006). However, in some reports, the physiological condition of the initial explant is not indicated (ŠEDIVA, 2009; ROVINĂ et al., 2010). It is well known that $HgCl_2$ is the most effective disinfectant and for softwood explants it is used in 0.1% for 1-3 min and for hardwood explants in 0.2% for 8-10 min (ILIEV et al., 2010). Actively growing explants were disinfected by 0.5% $HgCl_2$ for 5 min followed by 5% NaClO for 15 min (MARKS & SIMPSON, 1990). However, in all these publications the percentage of aseptic explants is not reported. ROHR & HANUS (1987) reported that the receiving of aseptic softwood segments in *Acer pseudoplatanus* is very difficult after disinfection with 7.5% calcium hypochlorite for 15-20 min. ĎURKOVIČ (2003) reported that dormant buds with removed scales have been successfully disinfected with 0.1% $HgCl_2$ for 2-5 min and the contamination rate of juvenile buds was 39.6% and of mature twice less (ĎURKOVIČ 2003). Our results are in agreement with these findings and showed that dormant buds, isolated in April are the most suitable explants for disinfection (Table 1) and culture establishment (Table 2).

Table 1. Factors affecting the disinfection of the initial explants.

Period of harvesting	Variant of disinfection	''Faassens Black''			''Drummondii''		
		Exogenous contamination (%)	Endogenous contamination (%)	Aseptic explants (%)	Exogenous contamination (%)	Endogenous contamination (%)	Aseptic explants (%)
Hardwood segments							
September	3	13.3 ± 6.7 a	77.8 ± 8.0 c	8.9 ± 8.9 a	8.9 ± 2.2 a	73.3 ± 3.8 c	17.8 ± 4.5 ab
Softwood segments							
May	2	51.7 ± 5.8 b	3.9 ± 4.0 ab	34.4 ± 5.1 b	45.6 ± 6.8 b	23.9 ± 4.5 b	30.5 ± 3.7 b
Buds							
April	1	4.5 ± 2.2 a	3.3 ± 3.8 ab	82.2 ± 2.2 c	6.7 ± 6.7 a	8.9 ± 4.4 ab	84.5 ± 9.7 c
November	1	4.5 ± 2.2 a	3.3 ± 3.8 ab	82.2 ± 2.2 c	11.1 ± 4.4 a	4.4 ± 4.4 a	84.5 ± 4.4 c

The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$. The equally named columns of the two cultivars are estimated together.

Table 2. Factors affecting the culture establishment.

		''Faassens Black''			''Drummondii''		
Month of harvesting	PGRs (mg l^{-1})	Explants with developed buds (%)	Explants with elongated shoots (%)	Dead explants (%)	Explants with developed buds (%)	Explants with elongated shoots (%)	Dead explants (%)
Hardwood explants							
September	1.0 Kinetin + 0.1 IBA	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Softwood explants							
May	PGRs-free	17.8 ± 4.5 bcd	0.0 ± 0.0 a	0.0 ± 0.0 a	31.1 ± 9.7 d	0.0 ± 0.0 a	0.0 ± 0.0 a
	1.0 BAP	13.3 ± 3.8 abc	0.0 ± 0.0 a	0.0 ± 0.0 a	28.9 ± 5.9 cd	0.0 ± 0.0 a	0.0 ± 0.0 a
	1.0 Zeatin	26.6 ± 3.8 cd	0.0 ± 0.0 a	0.0 ± 0.0 a	17.8 ± 8.0 bcd	0.0 ± 0.0 a	0.0 ± 0.0 a
	1.0 Kinetin	2.2 ± 2.2 ab	0.0 ± 0.0 a	0.0 ± 0.0 a	4.4 ± 4.4 ab	0.0 ± 0.0 a	0.0 ± 0.0 a
Buds with removed scales (1) or with scales (2)							
April (1)	PGRs-free	82.2 ± 2.2 f	24.4 ± 8.0 b	0.0 ± 0.0 a	80.0 ± 4.9 f	11.1 ± 4.4 ab	4.5 ± 2.2 ab
November (2)	PGRs-free	55.5 ± 2.2 e	0.0 ± 0.0 a	26.7 ± 3.8 c	71.1 ± 8.0 ef	0.0 ± 0.0 a	13.3 ± 3.8 b

The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$. The equally named columns of the two cultivars are estimated together.

Elongation and multiplication of the shoots

On kinetin enriched WPM all explants formed callus. In some of them it was light-green but in a lot of them it was brown and necrotic outside (Fig. 6, 7). On these variants of the medium from 5.6 ± 5.6% to 22.2 ± 14.7% of explants died and the explants with elongated shoots reached from 44.4% to 50.0% (Fig. 8). It was found that 0.5 mg l^{-1} Kinetin induced maximal percentage of shoot-forming explants (ĎURKOVIČ, 1996) but statistical difference between both our treatments and cultivars was not found. The concentration of kinetin did not affect the length of the shoots within the cultivar but in comparison with ''Drummondii'' they were statistically longer in ''Faassens Black'' on medium with 0.5 mg l^{-1} kinetin (14.5 ± 3.5 mm). However, axillary shoots formation was not induced in both treatments and cultivars studied (Table 3).

Figure 6. Axillary shoot formation and callus necrosis on explant from *Acer platanoides* ‘‘Faassens Black’’.

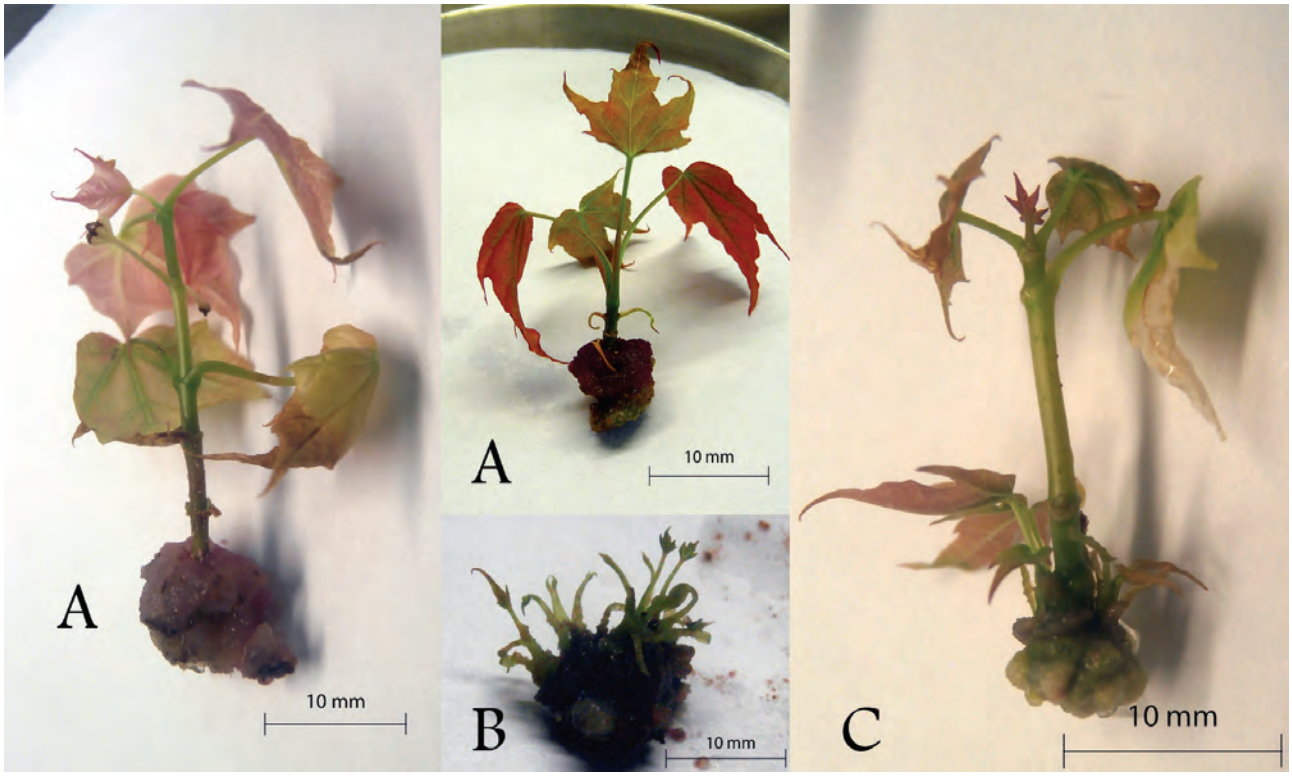

Figure 7. Different types of callus formed. A) Pink or red soft callus, easy to be removed, B) Dark brown callus on the surface, C) Rear occasion of light green callus formation.

Figure 8. Shoot elongation from cultivated buds, from both cultivars (‘Faassens Black’’, left and ‘‘Drummondii’’, right) on WPM supplemented with Kinetin.

Table 3. Elongation of the shoots from buds with removed scales after two subcultures.

	‘‘Faassens Black’’			‘‘Drummondii’’		
Kinetin (mg l^{-1})	**Explants with elongated shoots (%)**	**Length of the shoots (mm)**	**Dead explants (%)**	**Explants with elongated shoots (%)**	**Length of the shoots (mm)**	**Dead explants (%)**
0.5	50.0 ± 9.6 a	14.5 ± 3.5 b	11.1 ± 5.6 a	50.0 ± 9.6 a	6.2 ± 0.9 a	11.1 ± 5.6 a
1.0	50.0 ± 19.2 a	9.9 ± 1.9 ab	5.6 ± 5.6 a	44.4 ± 5.6 a	7.6 ± 2.2 a	22.2 ± 14.7 a

The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$. The equally named columns of the two cultivars are estimated together.

Effect of the subculture on multiplication rate

In the end of each subculture, reddish and soft callus was formed on the base of explants. However, its inside part was fresh and green (Fig. 7). The duration of subcultivation in our experiment did not provoke statistical significant necrotizing of the explants in ‘‘Faassens Black’’ and significant rate of the elongated initial explant was found after the 10th subculture. These shoots reached the significantly maximal length after the 8th subculture. It was indicated that rejuvenation in mature trees could sometimes be obtained by repeated subcultures (BALESTER et al., 1990; BONGA & VON ADERKAS, 1992; GIOVANNELLI & GIANNINI, 2000) and the proliferation rate could be increased (BALESTER et al., 1990; SANCHEZ et al., 1991). However, the frequency of the induced axillary shoots was low and was noticed on single explants. Also, their length was small and was not affected by the subculture (Fig. 9).

Opposite tendency was found in ‘‘Drummondii’’. Not only single elongated shoots but all of them gradually died and no viable shoots were found after the 8th subculture (Table 4).

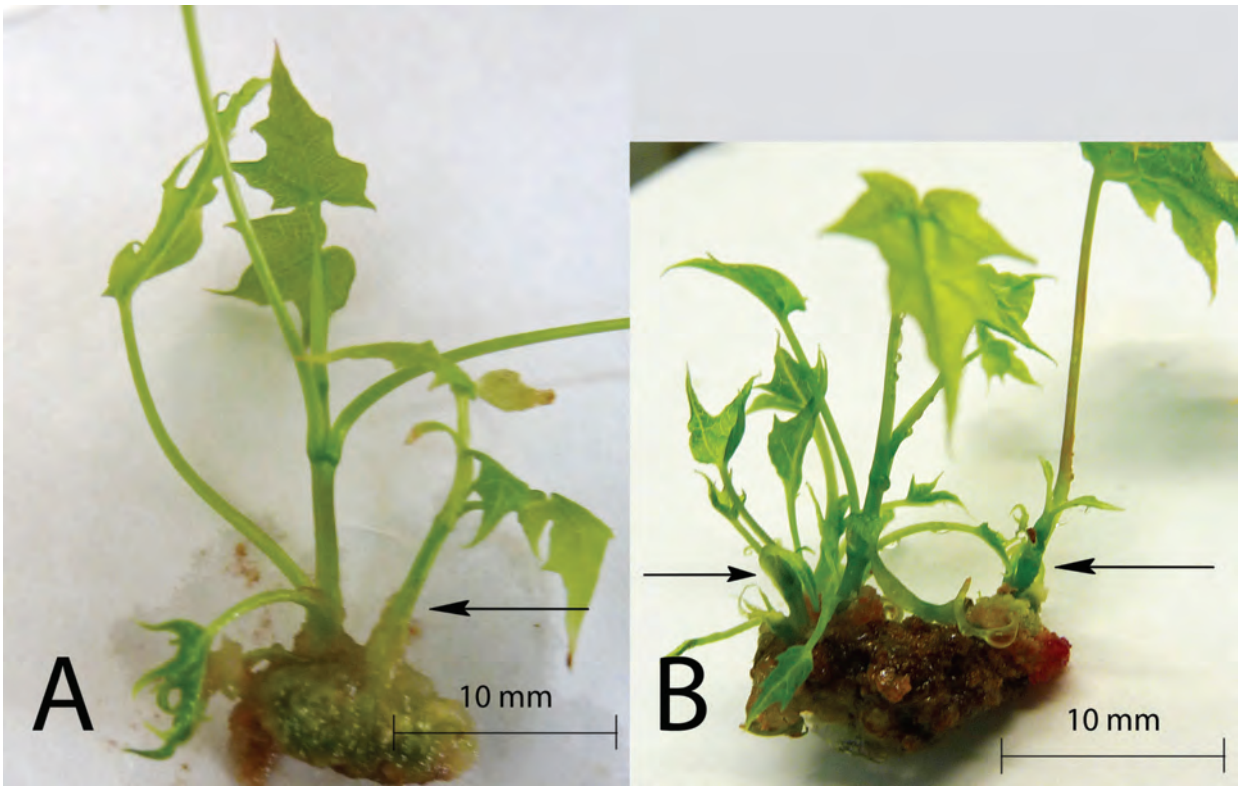

Figure 9. Axillary shoot formation A) longer than 5 mm and B) shorter than 5 mm.

Table 4. Effect of the subculture on multiplication rate.

Subculture (day)	''Faassens Black''					''Drummondii''				
	Dead explants (%)	Reaction of the initial explant		Axillary shoots formed		Dead explants (%)	Reaction of the initial explant		Axillary shoots formed	
		Elongated explants (%)	Length of the explant (mm)	Number	Length (mm)		Elongated explants (%)	Length of the explant (mm)	Number	Length (mm)
28	4.2 ± 4.2 a	8.3 ± 4.2 ab	5.0 ± 0.0 a	0.04 ± 0.04 a	5.0 ± 0.0 abc	6.7 ± 6.7 a	0.0 ± 0.0 a	-	-	-
56	4.2 ± 4.2 a	16.7 ± 8.3 bc	7.2 ± 1.6 a	0.25 ± 0.11 ab	8.0 ± 1.6 bc	8.3 ± 8.3 a	8.3 ± 8.3 ab	44.0 ± 0.0 c	-	-
84	0.0 ± 0.0 a	9.3 ± 0.3 ab	22.7 ± 2.8 b	0.19 ± 0.08 ab	7.3 ± 1.2 abc	16.7 ± 8.3 a	0.0 ± 0.0 a	-	-	-
112	0.0 ± 0.0 a	11.1 ± 2.8 ab	39.5 ± 11.0 c	0.23 ± 0.13 ab	8.0 ± 2.2 c	22.0 ± 11.1 a	0.0 ± 0.0 a	-	-	-
140	12.8 ± 2.6 a	25.7 ± 6.8 c	11.6 ± 2.5 ab	0.15 ± 0.10 ab	5.0 ± 0.0 ab	100.0 ± 0.0 b	-	-	-	-
180	14.3 ± 0.0 a	11.9 ± 6.3 ab	9.6 ± 3.0 ab	0.65 ± 0.17 c	6.1 ± 0.4 abc	-	-	-	-	-

The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$. The equally named columns of the two cultivars are estimated together.

Factors affecting the cultivation of juvenile genotypes

Effect of the genotype and subculture on the axillary shoot formation

Reddish and soft callus was formed on the base of explants (Fig. 7). The multiplication rate and the length of the axillary shoots were low, were not affected by the genotype, and all explants from genotypes 1 died (Table 5). Also, the subcultures did not affect significantly the number of the induced shoots and they remained smaller than 5 mm (Fig. 9). Single explants formed axillary shoots in genotype 8 and all explants died after the 3rd subculture (Table 6).

Table 5. Effect of the genotype on the multiplication rate and elongation of the axillary shoots.

Genotype	Dead explants (%)	Number of shoots	Length of shoots (mm)
1	33	0.0 ± 0.0 a	-
2	0	1.0 ± 0.3 b	21.9 ± 4.0 a
3	0	0.3 ± 0.3 ab	7.0
4	0	0.5 ± 0. 9 ab	12.0 ± 2.0 a
5	0	1.2 ± 0.4 b	19.5 ± 4.4 a
6	33	0.5 ± 0.9 ab	11.5 ± 2.5 a
8	0	1.1 ± 0.2 b	15.1 ± 2.6 a

The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

Table 6. Effect of the genotype and subculture on multiplication rate of axillary shoots.

Subculture (days)	Genotype					
	2		5		8	
	Dead explants (%)	Number of shoots	Dead explants (%)	Number of shoots	Dead explants (%)	Number of shoots
14	8.7	0.6 ± 0.2 abc	7.7	0.8 ± 0.4 abc	0	-
28	10.0	0.7 ± 0.2 abc	5.0	0.6 ± 0.2 abc	25	0.5 ± 0.5 ac
42	15.6	0.6 ± 0.2 abc	12.9	0.5 ± 0.2 ab	66	-
56	15.0	1.0 ± 0.3 c	11.7	0.2 ± 0.1 a	100	-
70	12.7	0.4 ± 0.1 a	8.8	0.9 ± 0.3 bc	-	-

The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$. The equally named columns of the two cultivars are estimated together.

Effect of PGRs on the axillary shoots formation

The combination of BAP and IBA led to the mortality of some explants and it increased in the highest concentration of BAP. Maximal number and length of axillary shoots was induced on the medium with BAP and GA_3. The combination of these PGRs provoked statistically significant additional formation of axillary shoots but their length remained less that 5 mm in the period of cultivation (Table 7).

Table 7. Effect of PGRs on the number and length of the formed axillary shoots after 40 days of cultivation.

PGRs (mg l^{-1})	No of the shoots (> 5 mm)	Length of the shoots (> 5 mm)	No of the shoots (< 5 mm)	Dead explants (%)
3 BAP + 0.1 IBA	1.0 ± 0.4 a	7.2 ± 0.7 ab	1.6 ± 0.5 a	45 ± 12 b
1 BAP + 0.1 IBA	1.2 ± 0.2 ab	6.2 ± 0.4 a	3.1 ± 0.6 b	10 ± 7 a
1 BAP + 1 GA_3	1.9 ± 0.4 b	8.4 ± 0.6 b	4.7 ± 0.6 c	0 ± 0 a
1 2iP	1.7 ± 0.2 ab	7.1 ± 0.5 ab	2.8 ± 0.4 ab	0 ± 0 a

The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

LINDÉN & RIIKONEN (2006) demonstrated axillary buds formation in Norway maple with 0.01 to 0.1 µM of TDZ. Kinetin in 0.5 mg l^{-1} was also effective for the induction of axillary shoots (ĎURKOVIČ, 1996). It was reported that 2 µM BAP promoted higher rate of shoots regeneration and longer shoots in 'Crimson Sentry' in comparison with other cytokinins (LATTIER et al., 2013). The type of cytokinin does not affect the elongation of the initial explant within the genotype. When BAP was used, elongation of the shoots in genotype 2 was not observed and statistical difference in the length of shoots in genotype 5 and ''Faassens Black'' was not found. Higher number of axillary shoots in genotype 5 was received after the treatment with Kinetin, but different cytokinins do not affect significantly their number in genotype 2 and ''Faassens Black''. Generally, the quantity of induced axillary shoots was small. The elongation of the shoots was very slow and few of them reached a bit more than 5 mm (Table 8, Fig. 9).

Possibly due to the apical dominance of the initial explant, the formation of axillary shoots and their elongation is limited. Also, different responses to cytokinins could be attributed to the reaction of different genotypes. For this reason the *in vitro* proliferation and elongation of the axillary shoots in *Acer platanoides* and its cultivars remained difficult.

Table 8. Effect of genotype and cytokinins on the reaction of initial explant, number, and length of the formed axillary shoots.

Genotype	PGRs (mg l^{-1})	Reaction of the initial explant		Axillary shoots formed	
		Number of elongated explants	Length of the explant (mm)	Number	Length (mm)
	1 BAP	0	-	1.1 ± 0.3 bc	-
Genotype 2	1 2iP	2	12.5 ± 1.6 a	1.1 ± 0.5 bc	-
	2 Kinetin	2	14.6 ± 3.8 a	1.6 ± 0.5 cd	6.0 ± 0.4 a
	1 BAP	4	19.4 ± 5.1 ab	1.1 ± 0.4 bc	8.8 ± 1.3 a
Genotype 5	1 2iP	7	20.5 ± 3.6 ab	0.1 ± 0.1 a	-
	2 Kinetin	2	11.8 ± 1.1 a	2.1 ± 0.6 d	-
	1 BAP	6	18.5 ± 3.6 ab	0.0 ± 0.0 a	-
'Faassens Black'	1 2iP	7	27.4 ± 3.2 b	0.0 ± 0.0 a	-
	2 Kinetin	5	19.4 ± 3.2 ab	0.3 ± 0.2 ab	-

The means (M) ± standard error (SE) within a column followed by the same letter are not significantly different estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

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