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Propagation of two selected species of the genus *Pieris* D. Don.

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Abstract

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This work evaluates the results of propagation experiments of Mountain Pieris (*Pieris floribunda* /Pursh/ Benth. & Hook.) and Japanese Pieris (*Pieris japonica* /Thunb./ D. Don) we carried out in the Arboretum Mlyňany SAS. The material was sampled from the two exemplars of these species growing in the Arboretum. The methods used were auto-vegetative propagation by cuttings and *in vitro* micropropagation. The response of the studied woody plant species varied according to the species and the method used. In Japanese Pieris, better results were achieved by vegetative propagation by cuttings; in Mountain Pieris, much more effective propagation method was micropropagation. We also studied the effect of climatic variables on the physiological conditions of the parent plants, and the overall rooting success in primary cultures obtained by micropropagation of Mountain Pieris. The data were recorded on each sampling event in the growing seasons 2011 and 2012. The process of micropropagation in Mountain Pieris was evaluated based on the production characteristics of the regenerants after the 3rd sub-cultivation. The results confirmed statistically significant differences in the number of shoots/explants and in the concentration of chlorophyll *a* between the dates of the primary culture establishment. The maximum number of shoots/explants (10.9) was obtained in variant B (primary culture established on 07/21/2012) and the highest concentration of chlorophyll *a* 6.66 mg g^{-1} on dry matter was found in variant C (primary culture established on 08/24/2011).

Key words

climatic conditions, chlorophylls, micropropagation, propagation by cuttings

Introduction

Pieris species are evergreen shrubs or small trees belonging to the family Ericaceae. Their leaves are alternate, often gathered at the ends of branches, elongated, lanceolate, 2–8 cm long, matt or glossy. Some cultivars in the group variegata have yellow or white variegated leaves; young leaves of some improved cultivars are bronze to flame-red coloured. The pieris species are characterised with attractive flowers, some cultivars display spectacular foliage colours in spring. The flowers are 5s, with white, rosy to light-red bell-shaped crowns, 4–7 mm long, with 10 stamens. The flowers are arranged in terminal panicles long 5–18 cm. Upright inflorescences consisting of green-white to reddish buds are formed already in October. They maintain their bright colouring over the whole winter until bursting in flower in March or April. The fruits are inconspicuous five-valve spherical capsules. There are about ten pieris species growing in the North America, East Asia and the Himalayas (Horáček, 2007).

The climate conditions in Europe, however, are favourable for only four pieris species and several hundreds of cultivars have been improved in nurseries of decorative woody plants, mostly in England and in Germany. The first trial with pieris introduction in Slovakia was made in 1899, in the Arboretum Mlyňany, by the founder of the Arboretum Dr. Štefan Ambrózy-Migazzi and his gardener Jozef Mišák. In 1899, there were imported several exemplars of the Mountain Pieris (*Pieris floribunda*) from the plant nursery of Peter Smith in Germany, and several individuals of Japanese Pieris (*Pieris japonica*) were planted in the original evergreen *Semper vireo* park eight years later in 1907. Today, the living woody plant collections in the Arboretum Mlyňany SAS contain: *Pieris floribunda* /Pursh/ Benth. & Hook., *Pieris japonica* /Thunb./ D. Don, *P. japonica* cv. Debutante, *P. japonica* cv. Purity, *Pieris polita* W.

W. Sm. & Jeffey and *Pieris taiwanensis* Hayata (Hoťka and Barta, 2012).

Pieris species require soils, climate and management types similar to most of heath land plants. They need protected sites with full sun; the original species, however, thrive also in partial shade. The necessary conditions for annual flowering are sufficient air humidity and soil moisture content. The representatives of these species respond sensitively to mineral fertilizers and to pruning. In their native area, some species as *Pieris japonica* reach into high altitudes with winter temperature below –20 °C. In our climatic conditions, these species grow relatively well in protected sites, but they cannot resist black frosts in higher situated ones. The critical period is generally the end of February and the beginning of March when the buds in panicles may be damaged by spring frosts.

Pieris are propagated mainly by seeds, some cultivars, however, are propagated in auto-vegetative way, with summer cuttings separated from semi-wood two-year-old shoots (Kamenická et al., 2004). Today, the propagation also uses *in vitro* methods (Starett et al., 1993). Plant biotechnologies are rapid and effective tools for propagation of a number of decorative and forest woody plants, and as such, they are focused appropriately in Slovakia (Kamenická and Váľka, 1997; Kamenická et al., 2005; Gajdošová et al., 2007; Ostrolucká et al., 2007; Ďurkovič, 2008).

This work compares the propagation of the Mountain Pieris and the Japanese Pieris by several propagation methods.

Material and methods

The plant material for the experiments was sampled from a 44-year-old exemplar of Mountain Pieris (*Pieris floribunda* /Pursh/Benth. & Hook.) (Fig. 1) and a 70-year-old exemplar of Japanese Pieris (*Pieris japonica* /Thunb./ D. Don) (Fig. 2), growing in the original evergreen *Semper vireo* park in the Arboretum Mlyňany SAS, both in almost identical site conditions (GPS coordinates – Mountain Pieris 48˚19’11.2″ N, 18˚22’13.9″ E; Japanese Pieris 48˚19’11.7″ N, 18˚22’13.0″ E).

Fig. 1. Flowering Mountain Pieris (*Pieris floribunda* /Pursh / Benth. & Hook.)

Fig. 2. Flowering Japanese Pieris (*Pieris japonica* /Thunb./ D. Don).

Mountain Pieris – native in moist forest hill slopes in North America. It is a high resistant, slow growing, round-shaped shrub, with an ultimate height of 1.2–2 m. The flowers appear in March to April (casually in May) clustered in terminal panicles by five. The greenish-white buds are attractive over the whole winter.

Japanese Pieris – is a native species in light open forests in mountains of the Japanese islands Shikoku, Kyushu and Honshu. It is a medium-sized shrub with attractive glossy foliage and white waxy flowers arranged in straight panicles. The flowering period is March–April. There exists a large number of cultivars of this species, such as ‘Bisbee Dwarf’ (syn.: P.j. ‘Bisbee’), ‘Blush’, ‘Brouwer’s Beauty’, ‘Chaconne’, ‘Compacta’, ‘Debutante’, ‘Dorothy Wyckhoff’, ‘Fuga’, ‘Little Heath’, ‘Nocturne’, ‘Prelude’, ‘Purity’, ‘Pygmaea’(syn.: P.j. ‘Nana Compacta’), ‘Sarabande’, ‘Snowdrift’, ‘Toccata’, ‘Valley Rose’, ‘Variegata’, ‘White Cascade’ (Horáček, 2007).

From the two parent plants, Mountain Pieris and Japanese Pieris, there was sampled material for propagation, at regular monthly intervals over the growing seasons 2011 and 2012. The material was of two types: cuttings used for auto-vegetative propagation and explants from axillary vegetative buds for *in vitro* propagation. In this work are evaluated 4 experimental variants described in Table 1. The table also contains climatic data, namely mean daily temperatures and precipitation sum from the beginning of the growing season to the first sampling date (variants A, D) and between the samplings (variants B, C). These values were measured at the Meteorological Station of the Arboretum Mlyňany SAS. In individual experimental variants, there was also sampled plant material for determining concentrations of chlorophylls (chlorophyll *a*, chlorophyll *b*, chlorophyll *a* + *b*, ratio *a/b*).

Table 1. Description of experimental variants

Variant	Sampling date	Mean daily temperature [ºC]	Precipitation sum [mm]
A	June 21, 2011	16.02	110.0
B	July 21, 2011	19.71	117.4
C	August 24, 2011	19.41	74.2
D	June 28, 2012	15.92	101.8

Auto-vegetative propagation by cuttings – in each experimental variant (A, B, C, D) were cut terminal cuttings (50 ps in each variant) from the donor plants. The cuttings were reduced to a length of 5 cm, treated with 4 different growth stimulants (R1 – Rhizopon A – 0.5% 3-indolyl acetic acid, R2 – Rhizopon A – 1% 3-indonyl acetic acid, R3 – Rhizopon AA – 0.5% 3-indonyl butyric acid, and R4 – Rhizopon AA – 1% 3-indonyl butyric acid) and rooted in substrate KLASMANN TS 5 in propagation rooms of the greenhouse. The terminal cuttings were covered with a plastic sheet to ensure sufficient moisture content. Simultaneously with these cuttings were also rooted control cuttings untreated with stimulants – control (CN).

The rooting success (%) in the individual experimental variants was evaluated after 60 days of cultivation.

Propagation by the method *in vitro* – plant explants sampled from axillary vegetative buds of the donor plants, Mountain Pieris and Japanese Pieris**,** by 20 ps in each variant, were washed in water, cut into shorter segments and sterilised by 5 min immersion in a 0.3% light agar solution supplemented with 25 ml l^{-1} PPM (PPM™, Plant Cell Technology, Inc., Washington, DC USA) and then by immersion for 1–2 min in a 0.1% solution of mercury chloride – to prevent exogenous contamination. After a thorough rinsing (3 times with redistilled sterile water), the shoots were shortened to 1–2 cm long explants and transported in sterile conditions onto a modified WPM cultivation medium (Lloyd and McCown, 1980; Starett et al., 1993) enriched with cytokinin N^6-[2-Isopentenyl]adenine (2iP) at a concentration of 8 mg l^{-1}, pH values of cultivation media were adjusted to 5.2 either with 1M KOH or with 1M HCl, and supplemented with 20 g l^{-1} sucrose, 8 g l^{-1} agar, poured in cultivation dishes and sterilised in an autoclave for 20 min at a temperature of 121 °C and a pressure of 120 kPa.

The explants were cultivated in controlled conditions, for a 16-hour cultivation period, at temperatures of 24 °C ± 1 °C during day and 20 °C ± 1 °C at night, and illumination intensity 40–50 µmol s^{-1} m^{-2}. After 10 weeks of cultivation, there was evaluated the number of vital primary explants and the differentiated shoots were used repeatedly for sub-cultivation. The micropropagation process in the experimental variants A, B, C was evaluated after the 3rd sub-cultivation, on the background of production characteristics of the regenerating material (number and length of shoots, biomass production). At the same time, chlorophyll concentrations were determined in the regenerating segments (chlorophyll *a*, chlorophyll *b*, chlorophyll *a* + *b*, ratio *a*/*b*).

Determining of chlorophyll concentrations

The chlorophyll concentrations were determined by spectrophotometry, as proposed by Lichtenthaler (1987). Chlorophylls in plants occur in form of chlorophyll-protein complexes, consequently, their extraction from plant material requires using non-polar solvents (ethanol, acetone, benzene). The extraction from plant material discussed in this work was carried out with 80% solution of acetone. The pigment extracts were prepared from material taken from aboveground parts of the tissue cultures. From each culture, there were taken 10 discs, each with a diameter of 5 mm. The discs were homogenised in a grinding mortar with a small amount of quartz sand, waterless magnesium carbonate and 3 ml of 80% acetone. The obtained homogeneous substance was filtered through a glass porous filter (S_3). From the filtered substance, there were sampled 20 ml amounts (Dykyjová et al., 1989) whose absorbance was measured in a spectrophotometer V-600 (Jasco, Japan), at wave lengths λ_a = 663.2 nm and λ_b = 646.8 nm corresponding to the absorption maxima of chlorophyll *a* and chlorophyll *b*. The measured absorbance values were substituted in the equations proposed by Lichtentahler (1987) for calculating concentrations of photosynthetic pigments of chlorophylls *a*, *b*, total chlorophylls *a* + *b* and chlorophyll ratio *a/b*. Finally the results were converted to dry-mass corresponding values.

The biomass production was assessed based on dry biomass, gravimetrically, after drying out the specimens to the constant weight at 105 °C.

Results and discussion

The auto-vegetative propagation of Japanese Pieris by cuttings treated with various growth stimulants exhibited the best mean establishment rate (53.5%) in variant B. The lowest percent of rooted shoots (14.5%) was obtained in variant A (sampling date 21 June 2011). Over the study period, there were not recorded any strong fluctuations in the mean daily temperature, with the lowest values at the beginning of the growing season. As for the precipitation sum, the most distinct drop was found in variant C (Table 1). In variant B (21st of July 2011 sampling date), when the rooting of cuttings was the best, both temperature and precipitation reached the highest values what suggests good physiological stage of the parent plant from which the cuttings have been taken for rooting. This finding is in agreement with the results of Walter (2011) which recommends for the propagation of genus *Pieris* D. Don mature terminal shoots cut from July to September.

Among the growth stimulants, 1% 3-indolyl acetic acid (R2) was the most effective – with 40.4% average rooting rate. With 1% 3-indolyl butyric acid (R4), the average rooting success was by 5% lower. The rooting

success values obtained with using growth stimulants in lower concentrations (R1, R3) were changed only a little (R1 = 29.7%, R3 = 28.9%) compared to R2 and R4 (Fig. 3). Application of auxins affected considerably not only the root quality but also the speed of root system development. In case of shoots without growth stimulants, the first roots appeared with a two-week delay and in lower abundance.

According to Spethmann (1990) the success in propagation by cuttings is determined decisively by the age and fitness of the plant and, consequently, by the physiological viability of the cuttings. Some woody plants better propagate with green – non-lignified (summer, soft cuttings), the other exhibit more success with winter (hard) cuttings. Physiological fitness of parent plants is also considerably affected by the stand microclimate. From this point of view, the sampling date is important factor affecting root development in shoots. Therefore, we also investigated the influence of selected microclimatic variables (air temperature, precipitation) on assimilatory pigments concentrations in parent woody plants. We found the concentration of chlorophyll *a* in the parent plant of Japanese Pieris in the individual experimental variants in the range 3.26–5.82 mg g^{-1} (Table 2). The highest concentrations occurred in variant B during intensive biomass creation, the lowest in variant C – probably due to the precipitation deficit in this period. Similar trends were found for the rate of chlorophyll *a* to chlorophyll *b*, *a/b*, with the values ranging from 2.24 to 2.94. Analysis of variance (Table 3) and consecutive Duncan test confirmed statistically significant differences in assimilatory pigments concentrations in all experimental variants (Table 2). Strong influence of precipitation sum on chlorophyll *a* concentration was also confirmed by linear regression analysis (Fig. 4) with a high correlation coefficient (r = 0.9148). For temperature, no similar influence was detected. The effects of environmental factors on assimilatory pigments contents were studied by Demming-Adams et al. (1996), Seifermann-Harms (1994) and Kirchgessner et al. (2003). The last author suggests the following rank of climatic factors affecting pigments: air temperature, solar radiation, global radiation, atmospheric precipitation. Exploration of the dependence of rooting success of Japanese Pieris cuttings on climatic conditions and on chlorophyll content in parent woody plant (Table 4) by correlation analysis resulted in a conclusive relation for the precipitation sum under using stimulant R2 (r = 0.5204) and for control (r = 0.6455). The correlation between the shoot rooting success and mean daily temperature during sampling was only weak. Significant to highly significant was dependence of rooting success on concentration of chlorophyll *a*, total concentration of chlorophylls *a* and *b* and concentration ratio *a/b* with using growth stimulants R1, R2, R3, R4. The closest dependence was found in the control variant (r values: chlorophyll *a* 0.8987; chlorophyll *a* + *b* 0.8660; chlorophyll *a/b* 0.9965) (Table 4). Considerable differences in rooting success of cuttings of Japanese Pieris among individual variants of experiments were also due to insufficiently stable temperature and moisture conditions in the propagation compartment in the greenhouse in the Arboretum.

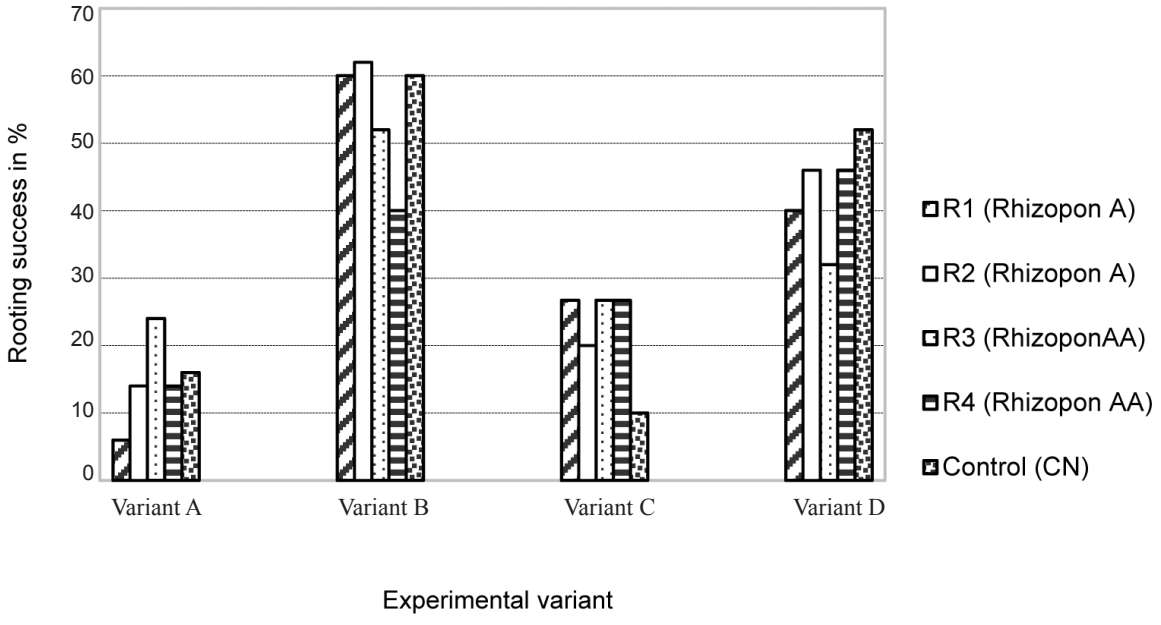


Fig. 3. Rooting success in cuttings of Japanese Pieris in individual experimental variants.

Table 2. Chlorophylls concentrations in parent plants of Japanese Pieris and Mountain Pieris in individual experimental variants

Donor plants	Experimental variant	Chlorophyll *a* [mg g^{-1}] ± SE[1]	Chlorophyll *b* [mg g^{-1}] ± SE[1]	Chlorophyll *a* + *b* [mg g^{-1}] ± SE[1]	Chlorophyll *a* / *b* [mg g^{-1}] ± SE[1]
Pieris japonica	A	4.56 ± 0.372 b	1.94 ± 0.367 a	6.50 ± 0.722 b	2.37 ± 0.256 ab
	B	5.82 ± 0.317 c	2.03 ± 0.287 a	7.85 ± 0.221 c	2.94 ± 0.579 c
	C	3.26 ± 0.287 a	1.52 ± 0.399 a	4.78 ± 0.675 a	2.24 ± 0.433 a
	D	5.09 ± 0.845 c	1.84 ± 0.393 a	6.93 ± 1.227 bc	2.79 ± 0.191 bc
Pieris floribunda	A	4.06 ± 0.829 b	1.76 ± 0.619 b	5.82 ± 1.404 b	2.41 ± 0.431 b
	B	5.27 ± 0.707 c	1.60 ± 0.328 ab	6.86 ± 1.023 bc	3.34 ± 0.292 c
	C	1.71 ± 0.315 a	1.05 ± 0.389 a	2.75 ± 0.642 a	1.77 ± 0.486 a
	D	5.47 ± 0.320 c	2.13 ± 0.184 b	7.60 ± 0.466 c	2.58 ± 0.159 b

SE[1], standard error of arithmetic mean.
Differences among values labelled with the same symbols (a)–(d) are not statistically significant at 95% significance level (Duncan test).

Table 3. Analysis of variance (ANOVA) of chlorophylls concentrations in parent plants of Japanese Pieris and Mountain Pieris in individual experimental variants

Source of variance	Degrees of freedom	F-test							
		Pieris japonica				*Pieris floribunda*			
		Chlor. *a*	Chlor. *b*	Chlor. *a* + *b*	Chlor. *a* / *b*	Chlor. *a*	Chlor. *b*	Chlor. *a* + *b*	Chlor. *a* / *b*
Among treatments	3	22.55*	1.87	13.09*	3.55*	39.33*	5.21*	22.47*	14.95*
Residual (within treatments)	16								
Total	19								

*statistically significant differences at 95% level ($P < 0.05$).

Table 4. Correlation between rooting success in cuttings of Japanese Pieris, vitality of primary explants in Mountain Pieris, climatic conditions and chlorophylls concentrations in parent plants

Woody plant	Symbol	Precipitation sum	Mean temperature	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a* + *b*	Chlorophyll *a* / *b*
Pieris japonica	R1	0.2811	0.3147	0.6236*	0.2945	0.5744*	0.8371*
	R2	0.5204*	0.3155	0.8119*	0.5234*	0.7719*	0.9581**
	R3	0.2250	0.2779	0.7631*	0.5629*	0.7378*	0.8416*
	R4	–0.0238	–0.0150	0.5315*	0.1486	0.4718*	0.7995*
	CN	0.6455*	0.0592	0.8987**	0.6457*	0.8660**	0.9965**
Pieris floribunda	In vitro	0.5955*	–0.9106**	0.7261*	0.9535*	0.7910*	0.3083

$|r| < 0.40$ – poor correlation (very weak relation); $0.40 < |r| < 0.85$ – good correlation (significant)*; $0.85 < |r| < 1.0$ – strong correlation (highly significant)**.

The successful micropropagation of Mountain Pieris (Fig. 6), contrarily to the Japanese Pieris, was consistent with the former knowledge according to which the morhopgenetic response of explants can exhibit considerable differences, even intraspecific or among cultivars of the same species (Salaj and Blehová, 2006). Evaluation of establishment success in primary cultures revealed (Fig. 5) that the highest percentage of vital primary explants (80% and 85%) was obtained in case of juvenile shoots (variants A, D). Similarly as in Japanese Pieris, assimilatory pigments were also investigated in Mountain Pieris. These pigments showed statistically significant differences among the variants (Table 2). The highest concentration of chlorophyll

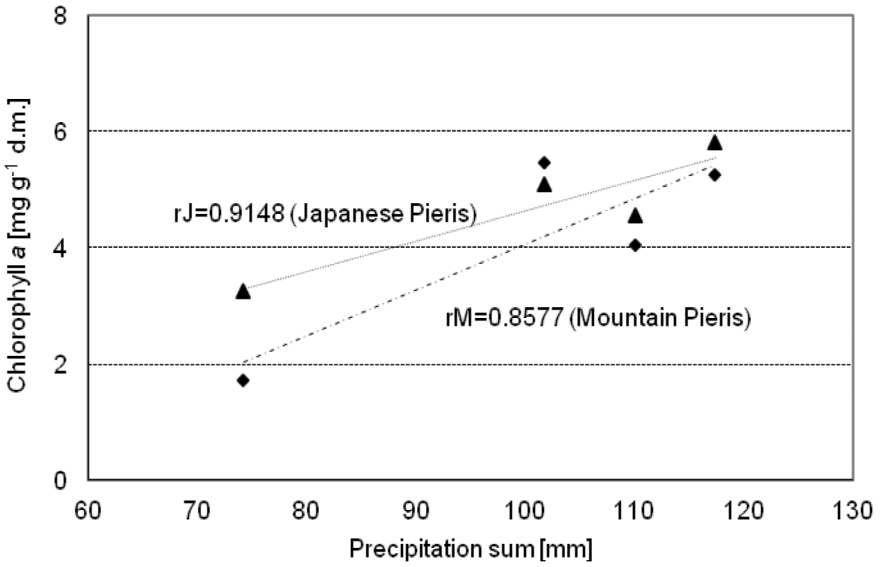


Fig. 4. Linear regression analysis of precipitation sum's effect on chlorophyll *a* concentrations.

a (5.47 mg g^{-1}) was recorded in variant D, the lowest one (1.71 mg g^{-1}) in variant C. The Mountain Pieris exhibited more pronounced differences in the values of *a/b* ratio, ranging from 1.77 to 3.34. Linear regression analysis (Fig. 4) confirmed a strong correlation between the precipitation sum and chlorophyll *a* concentration in parent plants (r = 0.8577). The correlation analysis also confirmed that the percentage of vital shoots of Mountain Pieris was significantly negatively correlated (r = –0.9106) with the mean daily air temperature in the individual experimental variants (Table 4). Evidential correlation was also found between the primary culture initiation in Mountain Pieris and chlorophyll *a* concentration (r = 0.7261) and between this culture initiation and chlorophyll *a/b* ratio (r = 0.7910). In case of chlorophyll *b*, the correlation was highly significant – r = 0.9535 (Table 4).

The same results were obtained by Šedivá (1998) working with propagation of evergreen plants in the family Ericaceae who succeeded in establishing primary cultures for 6 from 14 taxons. The most serious problems seemed to be connected with Japanese Pieris and its cultivars. For the Mountain Pieris, Šedivá obtained the best results on WPM cultivation medium supplemented with 5 mg l^{-1} 2iP.

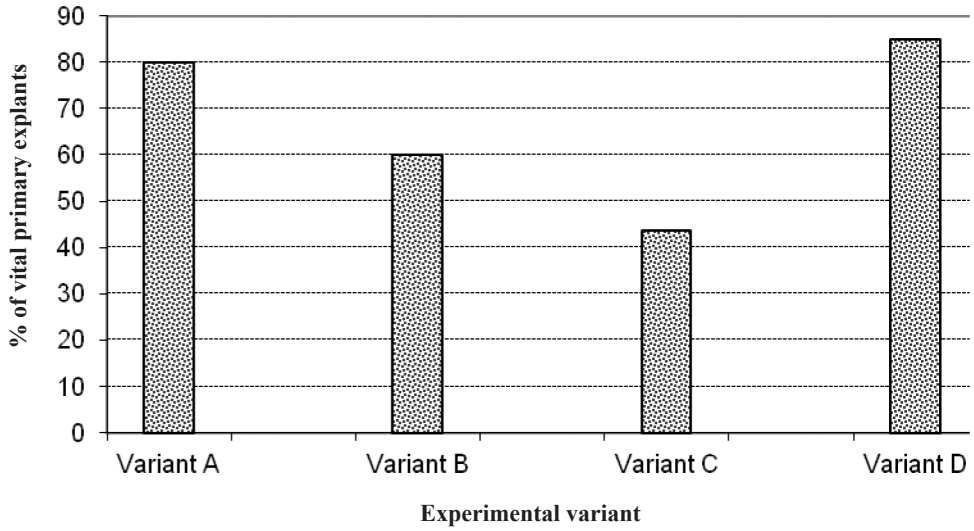


Fig. 5. Vitality of primary explants of Mountain Pieris in individual experimental variants.

The micropropagation process of Mountain Pieris was evaluated based on the production characteristics in the regenerants (variants A, B, C) after the 3rd sub-cultivation. The results of variance analysis showed statistically significant differences in the number of vital shoots, dry biomass amount (Table 5) and concentrations of chlorophylls *a*, *a* + *b*, *a*/*b* (Table 7) dependent on the date of establishment of the primary culture (Fig. 6a). The maximum number of 10.9 shoots/explants with ultimate length of 19.88 mm was obtained in variant B (primary culture established on July 21, 2011) (Fig. 6b). In this variant was also obtained the highest value of dry biomass 0.0632 g (Table 6). The highest concentration of chlorophyll *a* making 6.66 mg g^{-1} in dry mass (Table 8) was found in variant C (primary culture established on August 24, 2011), which was also reflected in vitality and leaf area of shoots (Fig. 6c). Synthesis of assimilatory pigments in tissue cultures is also controlled by amounts of cytokinines in the cultivation media (Kaul and Sabharwal, 1971; Salaj and Blehová, 2006).

Differences among values labelled with the same symbols (a)–(d) are not statistically significant at 95 % significance level (Duncan test).

Table 5. Analysis of variance (ANOVA) of growth characteristics of tissue cultures of Mountain Pieris after the 3rd sub-cultivation, in three experimental variants

Variance	Degrees of freedom	F-test		
		Number of shoots	Shoot length	Dry mass
Among treatments	2	8.72*	1.03	2.24*
Residual (within-treatments)	22			
Total	24			

*statistically significant differences at 95% significance level (P < 0.05).

Table 6. Average values of growth characteristics of tissue cultures of Mountain Pieris after the 3rd sub-cultivation

Experimental variant	Number of shoots / explants ± SE[1]	Shoot length [mm] ± SE[1]	Dry mass / explants [g] ± SE[1]
A	7.60 ± 3.253 a	17.53 ± 4.391 a	0.0322 ± 0.0013 a
B	10.90 ± 3.253 b	19.88 ± 2.229 a	0.0632 ± 0.0039 ab
C	5.35 ± 4.600 a	16.81 ± 7.572 a	0.0399 ± 0.0049 c

SE[1], standard error of arithmetic mean.

Table 7. Analysis of variance (ANOVA) of chlorophylls concentrations in regenerants of Mountain Pieris after the 3rd subcultivation, in individual experimental variants

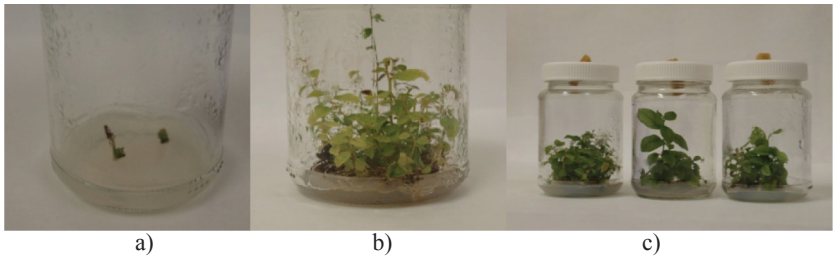
Variance	Degrees of freedom	F-test			
		Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a* + *b*	Chlorophyll *a*/*b*
Among treatments	2	5.73*	1.04	39.28*	2.93*
Residual (within treatments)	22				
Total	24				

*statistically significant differences at 95% significance level (P < 0.05).

Table 8. Chlorophylls concentrations in regenerants of Mountain Pieris after the 3rd sub-cultivation, in individual experimental variants

Experimental variant	Chlorophyll *a* [mg g^{-1}] ± SE[1]	Chlorophyll *b* [mg g^{-1}] ± SE[1]	Chlorophyll *a* + *b* [mg g^{-1}] ± SE[1]	Chlorophyll *a*/*b* [mg g^{-1}] ± SE[1]
A	3.86 ± 1.691 a	1.76 ± 0.815 a	5.82 ± 2.497 a	2.23 ± 0.278 a
B	4.94 ± 1.609 a	2.16 ± 1.001 a	7.10 ± 2.524 ab	2.47 ± 0.623 ab
C	6.66 ± 0.582 b	2.36 ± 0.267 a	9.02 ± 0.508 b	2.87 ± 0.471 b

SE[1], standard error of arithmetic mean.
Differences among values labelled with the same symbols (a)–(d) are not statistically significant at 95% significance level (Duncan test).



a) b) c)

Fig. 6. Propagation of Mountain Pieris by the method *in vitro* – a) primary culture; b) multi-shoot tissue culture after the 3rd sub-cultivation – experimental variant B; c) multi-shoot tissue culture after the 3rd sub-cultivation – experimental variant C.

Propagation of plants in the family Ericaceae *in vitro* was also studied by Norton, M. E. and Norton, C. R. (1985) who compared the effects of cytokinines: N^6-benzyladenine (BA) and 2iP. These authors as well as most of the others obtained better results with using 2iP, as BA was toxic for many species and the shoots became necrotic. Malá and Šíma (2000) report that the success in micropropagation depended primarily on the appropriate cultivation environment (appropriate cultivation medium, temperature, moisture, light). However, important are also so called indirect factors – sampling date of primary explants, age and physiological fitness of donor plants, surface sterilisation and preparation of explants.

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