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EFFECT OF MEDIUM TYPE AND PLANT GROWTH REGULATORS ON THE *in vitro* SHOOT PROLIFERATION OF *Cotinus coggygria* Scop. 'ROYAL PURPLE'

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Abstract. The smoke bush (*Cotinus coggygria* Scop**.)** is a popular and highly demanded ornamental shrub whose propagation can be problematic. The aim of the study was to test the effect of the mineral salt composition in the medium, concentration of plant growth regulators and the medium pH on the *in vitro* multiplication and shoot growth of *C. coggygria* Scop. 'Royal Purple'. Shoot tips were cultured on the following media: Anderson (AN), Murashige and Skoog (MS) (full- , half- and quarter strength), Nitsch and Nitsch (NN), Quoirin and Lepoivre (QL) or Lloyd and McCown (WPM). Media were supplemented with 6-benzyladenine (BA) in combinations with 1-naphthaleneacetic acid (NAA). The medium type and a concentration of the cytokinin significantly affected the regeneration rate of explants, the number, length and weight of axillary shoots as well as callus growth. The MS medium containing full strength of mineral salts with pH 5.8 was the best. High shoot proliferation (100%) and the highest number of shoots per explant (4.6) were obtained due to the use of 1.0 mg·dm^{-3} BA in combination with 0.1 mg·dm^{-3} NAA .

Key words: AN, micropropagation, NN, QL, smoke bush, WPM

INTRODUCTION

Cotinus coggygria Scop. 'Royal Purple' is a member of the cashew family (Anacardiaceae). It is commonly known as a smoke tree or smoke bush. The genus contains two species, one in North America and the other in Europe. They are small shrubs or trees with yellowish wood and rich maroon-red foliage and purplish red inflorescenses. The smokebush is an important part of outdoor and public spaces in modern landscape archi-

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tecture [Dirr 1990]. Vegetative propagation of *Cotinus coggygria* by conventional cutting, although used, can be slow, difficult, and cultivar-dependent. Micropropagation can provide an opportunity to obtain large number of homogenous plants [Pospíšilová et al. 1999]. The efficiency of micropropagation is related to the *in vitro* cultural conditions as a type of medium, growth regulators or medium pH. One of the most important factors is the composition of the cultural medium. Each plant species has its own characteristic elementary compostion which can be used to adapt the medium formulation [Nas and Read 2004]. Besides the cultural media, the present techniques involved in the tissue culture, the hormonal balance is crucial for organogenesis. The influence of hormones is expressed both individually and also by changing the ratio between the stimulators and inhibitors. Nevertheless, there is a lack of scientific literature on this topic. Metiever et. al. [2006] reports about the *in vitro* rooting of microshoots of *Cotinus coggygria* Scop. 'Royal Purple'. However, there is a lack of information regarding the mineral salt composition of the medium, medium pH or growth regulator requirements for stimulating shoot proliferation. The present investigation on *C. coggygria* Mill. 'Royal Purple' was undertaken to establish the optimal conditions for fast multiplication of smoke bush by the *in vitro* method.

MATERIALS AND METHODS

Four experiments were conducted to optimize proliferation conditions. Microcuttings of *C. coggygria* 'Royal Purple' consisting of few nodes were multiplied and maintained on medium supplemented with MS micronutrients, vitamins, 30 $g \cdot dm^{-3}$ sucrose and 8 $g \cdot dm^{-3}$ agar in clear jars. In the first three experiments media were adjusted to 5.8 with 0.1 M NaOH and 0.1 M HCl.

In the first experiment, shoot tips explants (1 cm) were cultured on following media: Anderson (AN) [1980], Murashige and Skoog (MS) [1962], Nitsch and Nitsch (NN) [1969], Quoirin and Lepoivre (QL) [1977] and Lloyd and McCown (WPM) [1980].

In the second shoot culture experiment, the effect of the full-, half- and quarter strength Murashige and Skoog medium (MS) [1962] was studied.

In the third experiment the cytokinin 6-benzylaminopurine (BA) in combination with auxin 1-naphthaleneacetic acid (NAA) was submitted to MS medium to promote the axillary and adventitious shoot regeneration as well as their elongation. Concentration ranges of plant regulators used in this experiment are presented in Table 3.

In the fourth experiment the MS medium pH was tested. The media were adjusted to pH 4.8, 5.8 or 6.8 with 0.1 M NaOH and 0.1 M HCl before autoclaving.

All media were autoclaved at 121°C at 110 kPa for 20 min. The cultures were maintained in the light at a photon fluence rate of 80–100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under a 16-h photoperiod with F40T12 Gro-WS lamps (Sylvania Gro-Lux^{TM}) in a culture room at 24°C. Relative humidity was kept at 55–65%. There were 3 replicates per treatment, each including 10 microshoots. In all experiments the following parameters were measured: mean shoot number, mean shoot length and proliferation rate. In the first and second experiment dry mass was determined: 1 g samples were dried at 105°C to constant weight [Strzelecka et al. 1982]. For all multiplication treatments, nondestructive obser-

vations were performed after 8 week cultures. The results were analyzed statistically using a standard statistical procedure with one factorial ANOVA and the means were compared by the Duncan's test at $\alpha = 0.05$.

RESULTS

Results of the *in vitro* studies on differentation and multiplication of *C. coggygria* 'Royal Purple' presented in Table 1 show a varied smokebush response to the types of media tested. The best regeneration rate (96%) occurred on MS and QL media.

The medium type affected a number of shoots formed from an explant. The largest number of shoots was obtained on MS medium, whereas the lowest number on QL and WPM (fig. 1). Shoots growing on the MS medium formed the significantly longest axillary shoots while the significantly shorter shoots were obtained on the NN and WPM media. A medium type affected as well the dry mass of shoots and callus shoot dry mass obtained on AN and WPM medium was larger whereas the low dry weight was noted on QL. However, the highest callus dry mass was observed on NN and WPM medium.

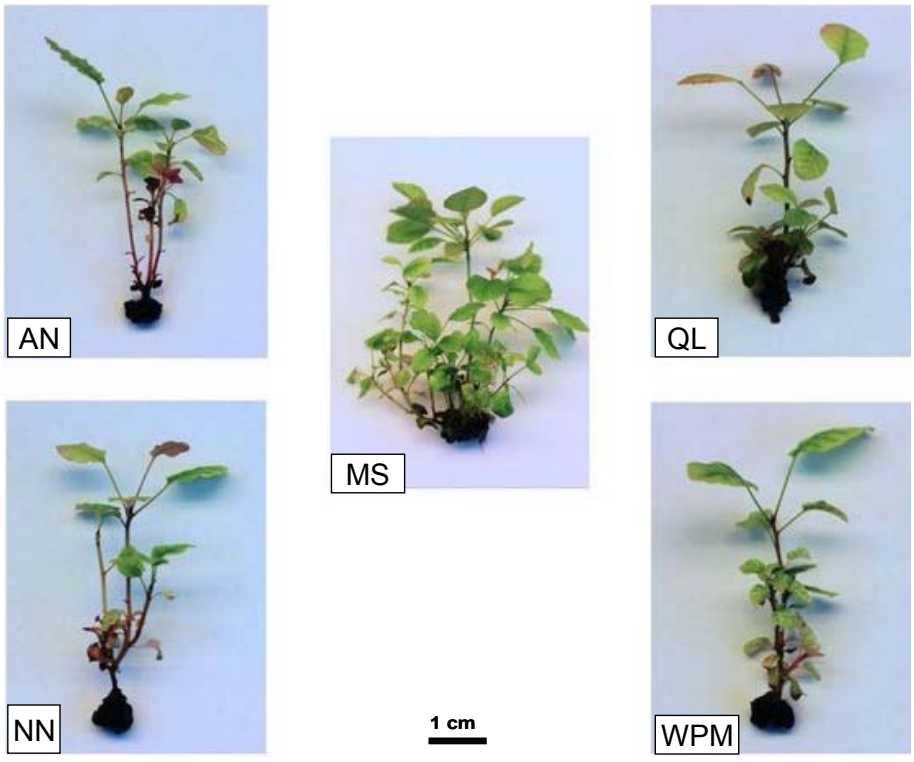

Fig. 1. Effect of the medium type on shoot differentiation in *C. coggygria* 'Royal Purple'
Rys. 1. Wpływ rodzaju pożywki na regenerację eksplantatów *C. coggygria* 'Royal Purple'

Table 1. Effect of the medium type on shoot differentiation in *C. coggygria* 'Royal Purple'
Tabela 1. Wpływ rodzaju pożywki na regenerację eksplantatów *C. coggygria* 'Royal Purple'

Type of the medium Rodzaj pożywki	% regeneration * % eksplantatów regenerujących *	No. of shoots per explant Liczba pędów/eksplantatów	Shoot length (mm) Długość pędów	Dry mass Sucha masa (mg)	
				shoots pędy	callus kalus
AN	92 b**	2.2 ab	12.1 b	0.46 b	0.15 a
MS	96 c	3.7 c	19.0 c	0.36 a	0.17 a
NN	82 a	2.6 b	9.0 a	0.35 a	0.27 b
QL	96 c	2.1 a	11.9 b	0.33 a	0.17 a
WPM	89 b	1.9 a	10.2 a	0.46 b	0.20 b
Mean Średnia	91	2.5	12.4	0.39	0.19

*100% was 30 explants – 100% stanowiło 30 eksplantatów
**Means followed by the same letter are not significantly different at α = 0.05 – średnie oznaczone tą samą literą nie różnią się istotnie przy α = 0,05

Table 2. Effect of the macronutrients concentration in medium on shoot differentiation in *C. coggygria* 'Royal Purple'
Tabela 2. Wpływ stężenia soli mineralnych na regenerację eksplantatów *C. coggygria* 'Royal Purple'

Type of the medium Rodzaj pożywki	% regeneration * % eksplantatów regenerujących *	No. of shoots per explant Liczba pędów/eksplantatów	Shoot length (mm) Długość pędów	Dry mass Sucha masa (mg)	
				shoots pędy	callus kalus
MS	96 c**	3.7 b	1.9 b	0.36 a	0.17 a
½ MS	95 b	3.1 a	0.7 a	0.36 a	0.32 b
¼ MS	87 a	2.7 a	0.6 a	0.34 a	0.29 b
Mean Średnia	92.67	3.2	1.1	0.35	0.26

*100% was 30 explants – 100% stanowiło 30 eksplantatów
**Means followed by the same letter are not significantly different at α = 0.05 – średnie oznaczone tą samą literą nie różnią się istotnie przy α = 0,05

The effect of the MS macronutrient concentration on shoot differentiation is shown in Table 2. The highest shoot regeneration rate was obtained on the full strength MS medium (96%), whereas the lowest regeneration on ¼ MS (87%). Explants cultured on the full strength MS medium produced the highest number of axillary shoots which were also the longest (19 mm) (tab. 2). There was no significant difference between the dry mass of shoots obtained on MS with different macronutrient concentrations, whereas the lowest callus dry mass was recorded on the full strength MS.

To induce shoot proliferation, five media formulations were used (tab. 3), with different BA concentrations. The ones containing 0–1.0 mg·dm^{-3} BA with 0.1 mg·dm^{-3} NAA were found optimal for the shoot regeneration (100%). Presence of the cytokinin in a higher concentration decreased the regeneration by 15%.

The treatment with 1.0 mg·dm^{-3} BA plus 0.1 mg·dm^{-3} NAA was significantly more efficient for the number of shoots than all other BA concentrations tested, producing 4.6 shoots per explant. Presence of the cytokinin in higher concentrations diminished proliferation.

Table 3. Effect of the concentration of plant growth regulators on the regeneration of *C. coggygria* 'Royal Purple'
Tabela 3. Wpływ stężenia regulatorów wzrostu na regenerację eksplantatów *C. coggygria* 'Royal Purple'

Concentration of plant growth regulators (mg·dm^{-3}) Stężenie regulatorów wzrostu		% regeneration * % eksplantatów regenerujących *	No. of shoots per explant Liczba pędów/eksplantatów	Shoot length (mm) Długość pędów
BA	NAA			
0	0.1	100 b	1.0 a	32.6 c
0.5	0.1	100 b	2.8 b	30.7 c
1.0	0.1	100 b	4.6 c	18.1 a
2.0	0.1	85 a	3.0 b	28.2 bc
3.0	0.1	85 a	1.4 a	24.2 b
Mean – Średnia		94	2.6	26.8

*100% was 30 explants – 100% stanowiło 30 eksplantatów
**Means followed by the same letter are not significantly different at α = 0.05 – średnie oznaczone tą samą literą nie różnią się istotnie przy α = 0,05

Table 4. Effect of the medium pH on the differentation of *C. coggygria* 'Royal Purple'
Tabela 4. Wpływ pH pożywki na regenerację eksplantatów *C. coggygria* 'Royal Purple'

pH of the medium pH pożywki	% regeneration * % eksplantatów regenerujących *	No. of shoots per explant Liczba pędów/eksplantatów	Shoot length (mm) Długość pędów
4.8	58 a	3.1 a	17.7 a
5.8	60 a	3.7 b	16.5 a
6.8	66 a	2.7 a	15.0 a
Mean – Średnia	61	3.2	16.4

*100% was 30 explants – 100% stanowiło 30 eksplantatów
**Means followed by the same letter are not significantly different at α = 0.05 – średnie oznaczone tą samą literą nie różnią się istotnie przy α = 0,05

In the treatment with lack of BA in the medium and that with its lowest concentration (0–0.5 mg·dm^{-3} BA + 0.1 mg·dm^{-3} NAA) the shoots were the longest (tab. 3). However, also the length of those from plantlets cultured in presence of 2 mg·dm^{-3} BA did not differ significantly from the latter treatments.

The statistical analysis showed no significant influence of the medium pH on regeneration rate (tab. 4): 58–66% explants regenerated in the pH range 4.8–6.8. The best for proliferation was pH 5.8 giving significantly more shoots per explant (3.7). There was no effect of pH on shoot length.

DISCUSSION

In vitro propagation of woody plants has always been difficult due to problems with establishment of aseptic cultures, microbial contamination and varied nutritional medium requirements [Purohit and Kukda 1994, Agrawal et al. 2002]. Nutritional requirements for the optimal *in vitro* growth depend on a species involved. Similarly, tissues from different plant organs may have different requirements for respective growth [Murashige and Skoog 1962]. For this reason, one single medium cannot be suitable for each type of plant tissue and organ. Therefore, when starting with a new species/cultivar, it is crucial to develop a medium that can fulfill the specific requirements of the tissue [Bhojwani and Razdan 1996]. In *C. coggygria* 'Royal Purple' the optimum response was found on MS medium (96% regeneration). This may be attributed to a greater demand of smoke bush for nitrogen and potassium which stimulate production of new proteins [Guru et al. 1999]. These components are higher in MS medium as compared to AN, NN, QL and WPM. It is worth mentioning that, althought the average number of shoots was significantly increased on MS medium, both MS and QL medium were equally effective for shoot multiplication. However, again the average shoot length was the highest on MS. The similar results were obtained by Rovină et. al. [2009] who examined a possibility of a rapid propagation of two smoke bush cultivars: 'Royal Purple' and 'Simfonia verii' and found that their response to three media: MS, QL and Lee Fossard (LF) [1972].

The effect of growth regulators is crucial in many plant processes, also in clonal propagation [Blakesley et al. 1991]. Difference in the hormone levels has been hypothesised to be related to ability to regeneration in *Cotinus coggygria*. As far as we know, the adenine exerts its morphogenetic effect on growth and organ formation of plant tissues by affecting nucleic acid metabolism [Miller and Skoog 1953]. Frequently, shoot proliferation can be significanlty enhanced by the addition to the proliferation medium an auxin and a cytokinin in low concentrations and in a proper ratio. Combinations of cytokinin and auxin can result in a better shoot proliferation than when both growth regulators are used singly. Our preliminary trials showed that presence of 0.1 mg·dm^{-3} NAA in the medium is necessary for smoke bush proliferation. In this work benzyladenine (BA) has been shown to stimulate shoot organogenesis in *C. coggygria* 'Royal Purple'. This study demonstrated that high shoot multiplication rate (100%) occurs due to the treatment with 0–1.0 mg·dm^{-3} BA in combination with 0.1 mg·dm^{-3} NAA. Higher amount of 6-benzyladenine may result in the loss the regeneration potential of the

shoots as observed in this experiment and by other authors. For example, Ďurkovič [2008] reported the highest shoot multiplication rate (90%) of *Cornus mas* 'Macrocarpa' with 0.7 mg·dm^{-3} BA with 0.05 mg·dm^{-3} NAA. That treatment increased as well a number of shoots. On the other hand, with regard to shoot multiplication rates in *Cornus florida*, Kaveriappa et al. [1997] reported that the highest mean number of shoots per explant (between 5.5 and 6.0) was achieved with 1.0 mg·dm^{-3} BA.

The pH medium little affected the proliferation rate in micropropagation of smoke bush which regenerated fairly well in pH range 4.8–6.8. However, explants regenerated best on MS medium with pH 5.8, forming also the highest number of shoots per explant. Different acidity also affected proliferation of cranberry microshoots *in vitro* [Staniené and Stanyté 2007].

Numerous studies have been carried out to optimize conditions for the *in vitro* regeneration and multiplication of woody species. Unfortunately, the effectiveness of some culture conditions appears to be highly genotype specific. There is a need for more protocols on the tissue culture of woody plants and an understanding of the processes involved. The results of studies on smoke bush presented here confirm that composition and concentration of macro- and micronutrients, the presence of growth regulators in the medium and the medium pH are decisive for the regeneration process efficiency.

CONCLUSIONS

1. The medium type and a concentration of the cytokinin significantly affected the regeneration rate of explants, the number, length and weight of axillary shoots as well as callus growth in smoke bush.

2. The MS medium containing full strength of mineral salts is most suitable for regeneration of smoke bush.

3. High shoot proliferation (100%) and the highest number of shoots per explant (4.6) were obtained due to the use of 1.0 mg·dm^{-3} BA in combination with 0.1 mg·dm^{-3} NAA .

4. The acidity of the medium little affected the shoot proliferation in smoke bush.

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WPŁYW ZESTAWU SOLI MINERALNYCH, pH POŻYWKI I REGULATORÓW WZROSTU NA REGENERACJĘ PĘDÓW *Cotinus coggygria* Scop. 'ROYAL PURPLE' *in vitro*

Streszczenie. Perukowiec podolski (*Cotinus coggygria* Scop.) jest popularnym i poszukiwanym na rynku krzewem ozdobnym, którego rozmnażanie może sprawiać problemy. Celem badań było określenie wpływu zestawu soli mineralnych, stężenia regulatorów wzrostu oraz pH pożywki na regenerację i wzrost *in vitro* perukowca podolskiego *C. coggygria* Scop. 'Royal Purple'. Wierzchołkowe fragmenty pędów wykładano na pożywki wg Andersona (AN), Murashige i Skoog (MS) (pełny zestaw, ½ lub ¼), Nitsch i Nitsch (NN), Quorin i Lepoivre (QL) oraz Lloyd i McCown (WPM). Pożywki wzbogacano w 6-benzyloadeninę (BA) w różnych stężeniach w kombinacji z kwasem naftylo-1-

-octowym (NAA). Wykazano istotny wpływ rodzaju pożywki i stężenia zastosowanej cytokininy na liczbę, długość i świeżą masę zregenerowanych pędów i wzrost kalusa. Najlepsze efekty uzyskano na pożywce MS o pełnym składzie makroskładników i pH 5,8. W obecności 1,0 mg·dm^{-3} BA i 0,1 mg·dm^{-3} NAA eksplantaty wierzchołkowe regenerowały w 100% i odznaczały się najwyższym współczynnikiem rozmnażania (4,6).

Słowa kluczowe: AN, mikrorozmnażanie, NN, QL, perukowiec podolski, WPM

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