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In vitro REGENERATION AND POLYPLOIDY INDUCTION IN *PELARGONIUM* × *HORTORUM* L. H. BAILEY

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Abstract

VEJSADOVÁ, H., KUCHTOVÁ-JADRNÁ, P.: *In vitro regeneration and polyploidy induction in Pelargonium × hortorum L. H. Bailey.* Acta univ. agric. et silvic. Mendel. Brun., 2009, LVII, No. 5, pp. 305–312

The objective of this work was to induce *in vitro* shoot regeneration as influenced by plant growth regulators and ascertain an effective method of polyploidy induction using colchicine and oryzalin in two diploid cultivars 'Gizela' and 'Black Velvet Scarlet F_1' of *Pelargonium × hortorum* L. H. Bailey. In both cultivars, benzyladenine (BA) significantly improved shoot regeneration compared to zeatin. The infiltration and the dip methods of explant treatment were used for polyploidy induction. Regenerants were analyzed using the flow cytometry (FCM). In 'Gizela' and 'Black Velvet Scarlet F_1', 10 tetraploids on the level of 2n = 4x were found by the infiltration method. The tetraploidy was determined by the dip method in 4 shoots of 'Gizela' and 11 tetraploids were detected in 'Black Velvet Scarlet F_1'.

Pelargonium × hortorum L. H. Bailey cultivars, *in vitro* shoot regeneration, colchicine, oryzalin, polyploidization

Pelargonium × hortorum L. H. Bailey hybrids (genus *Pelargonium* L.) are universally popular as ornamental plants with a high temperature tolerance, attractive shape and rich blooms. Cultivars of hybrids significant for growers, *P. × hortorum*, *P. × peltatum* and *P. × domesticum*, occur as diploids or tetraploids, that is 2n = 18 or 36, although aneuploidy of both forms is also common, expressed as 2n = 17 or 35. The basic number of chromosomes is x = 9. Because of the cumulative effect of alleles, there are more varieties in the tetraploid range with more degrees of colour intensity, which broadens the possibilities of breeding. The tetraploid plants compared to diploid varieties are characterized by shortened inflorescence-bearing peduncles longer blooming duration and higher regenerative capabilities during vegetative reproduction (BADR and HORN, 1971). If tetraploid genotypes occur in a population of diploid genotypes of the same species, in many plant species the tetraploids are eliminated due to hybridization with diploids and the consequent development of sterile triploids, however, in the case of *Pelargonium× hortorum* there is no hybridization between diploid and tetraploid genotypes (O. PLAVCOVÁ, personal communication).

The commonly used method of polyploidy induction is application of chemical agents inhibiting mitosis (VAN TUYL et al., 1992; BOUVIER et al., 1994). *In vitro* regeneration of explants taken from differentiated organs such as hypocotyls, parts of leaf blades and petioles have been studied in geraniums under various cultivation conditions by MOHAMED-YASSEEN et al. (1995), AGARWAL and RANU (2000), MITHILA et al. (2001). In some plant species polyploidization is possible with growth regulators found in leaf discs (HASSANEIN and DORION, 2005, 2006). The induction of direct organogenesis from already formed plant bases and the influence of various cytokonins on regeneration was studied by WOJTANIA and GABRYSZEWSKA (2001, 2004) who demonstrated a greater effect of the cytokinin *meta*-topolin (*m*-T) in comparison to benzyladenine (BA).

At the Silva Tarouca Research Institute for Landscape and Ornamental Gardening in Průhonice, golden-leaved and brown-leaved varieties of F_1 hybrids have been cultivated and also varieties with

classic green leaves (PLAVCOVÁ, 2007). The brown-leaved 'Black Velvet' series (previously called 'Black Magic') in particular are a big asset to the *Pelargonium × hortorum* cultivar assortment. In diploid cultivars, leaf coloration is marked by an intense brown with sharply defined narrow green leaf margins. However, these brown-leaved forms are completely missing from the assortment of tetraploid cultivars. Polyploidization of geranium cultivars under *in vitro* conditions using chemomutagens is performed at the Pelargonium Fischer Company (unpublished). We did not find any other data about polyploidy induction with colchicine or oryzalin in *P. × hortorum*.

The objective of this work was to induce *in vitro* shoot regeneration and ascertain an effective method of polyploidy induction using colchicine and oryzalin in two diploid cultivars 'Gizela' and 'Black Velvet Scarlet F_1'of *Pelargonium × hortorum* L. H. Bailey.

MATERIALS AND METHODS

Plant material

Diploid cultivar *Pelargonium × hortorum* from 'Black Velvet Scarlet F_1' (bright orange-red flowers) and the green-leaved variety 'Gizela F_1' with a classic type of leaf zone and bright red flowers were used. Intact leaf blades (cultured abaxial side up) of *in vitro* seedlings were chosen as primary explants (KUCHTOVÁ et al., 2007; VEJSADOVÁ and KUCHTOVÁ-JADRNÁ, 2008). The cultures were cultivated with 16 h photoperiod, at a temperature of 23 / 19°C (day / night) and light intensity of 55 µmol.m^{-2}.s^{-1} supplied from cool white fluorescent tubes.

Nutrient medium composition

The Murashige and Skoog (MS) (MURASHIGE and SKOOG, 1962) media contained an one-half concentration of salts, a modified mixture of vitamins (0.4 mg.l^{-1} thiamin; 0.2 mg.l^{-1} pyridoxine; 0.4 mg.l^{-1} nicotinic acid; 100 mg.l^{-1} myo-inositol; 4 mg.l^{-1} glycine; 500 mg.l^{-1} casein hydrolysate) and 1000 mg.l^{-1} polyvinylpyrrolidone (PVP), 25 g.l^{-1} sucrose; 7.5 g.l^{-1} agar (Cell Culture Tested, Sigma). We employed the following growth regulator combinations: I. 1 mg.l^{-1} IBA + 0.2 mg.l^{-1} zeatin; II. 1 mg.l^{-1} IBA + 0.5 mg.l^{-1} zeatin; III. 1 mg.l^{-1} IBA + 0.2 mg.l^{-1} BA; IV.1 mg.l^{-1} IBA + 0.5 mg.l^{-1} BA. The culture medium was adjusted to pH 5.8 before autoclaving at 120°C and 105 kPa for 20 min. Subculture onto medium with an identical composition was performed every 4 weeks.

Polyploidy induction

Shoots of multiplied *in vitro* cultures of diploid 'Black Velvet Scarlet F_1' and 'Gizela F_1' were used. Two methods of the explant treatment were tested; chemomutagen infiltration from nutrient media (The infiltration method) and dipping of the explants in a chemomutagen solution (The dip method). Colchicine (0.01, 0.02, 0.05, 0.1, 0.2, 0.4%) was dissolved directly in DMSO (dimethyl sulfoxide); while a supply solution was used for oryzalin (10, 20, 40 µmol.l^{-1}). Under sterile conditions, the chemomutagens were quantitively transferred to a hot sterile medium or to sterile water.

The infiltration method was performed on media containing ½ MS solution of salts (macroelements and microelements), a modified MS vitamin mixture, 1000 mg.l^{-1} PVP, 50 mg.l^{-1} casein hydrolysate, 25 g.l^{-1} sucrose, 7.5 g.l^{-1} agar and colchicine or oryzalin in tested concentrations. The pH of the media was adjusted to 5.8 before autoclaving. Chemomutagen was added into the medium with the support, DMSO (1.0–2.5 ml depending on its usage at dissolving of the polyploidization agent). Explant incubation occurred with 16 h photoperiod at a temperature of 23 / 19°C (day / night) and at a light intensity of 55 µmol.m^{-2}.s^{-1}. The infiltration period lasted 7 and 14 d, and then the explants were transferred to media without growth regulators.

In the dip method, explants were submerged in water solutions of colchicine or oryzalin for 20 h, afterwards the explants were rinsed three times with sterile water and placed in nutrient media without growth regulators. The control explants were submerged into sterile distilled water.

The regeneration rate and shoot formation were studied. Ploidy level was determined by the flow cytometry (FCM); samples were prepared using Otto I and Otto II buffer solutions.

Statistical analysis

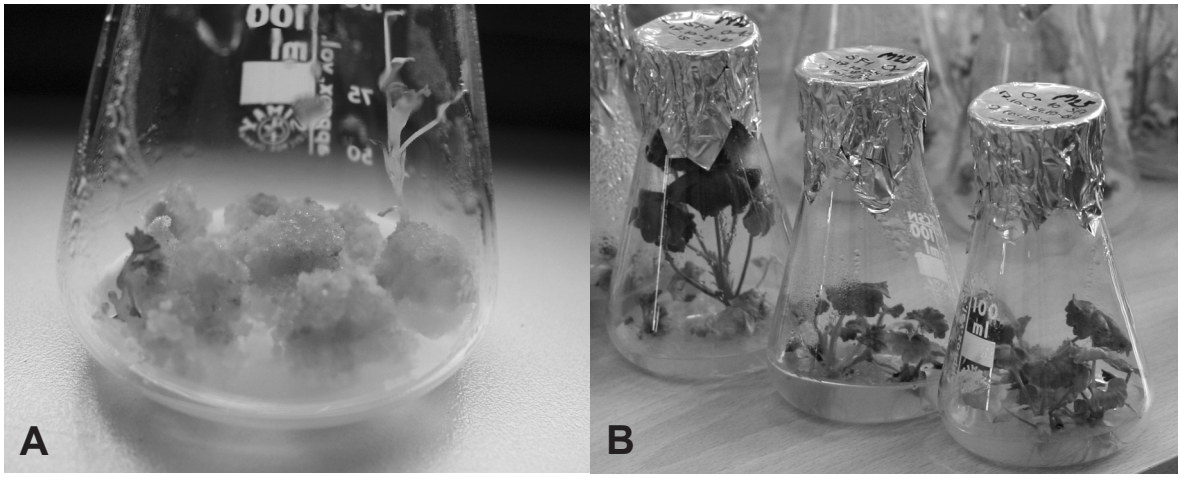
Data were subjected to the analysis of variance (ANOVA) and means were separated using Fisher's least significant difference (LSD) test at P = 0.05 (Tab. IV). Each value represents the means of two repeat experiments with 30 replicates each.

RESULTS AND DISCUSSION

Influence of growth regulators on shoot regeneration

Explants on media with BA or zeatin with the presence of IBA produced shoots within 6–10 weeks (Fig. 1). In both cultivars, a green callus with a significantly higher shoot regeneration was induced with BA (0.5 or 0.2 mg.l^{-1}) as compared to zeatin (Tab. I).

WOJTANIA and GABRYSZEWSKA (2001, 2004) compared the effects of *m*-T and BA on the multiplication of *P. × hortorum* 'Bargpalais'. They found that regenerated shoots after *m*-T treatment showed better quality, having lower number of aberrant shoots compared to shoots induced on media with BA. The multiplication was significantly higher on full-strength MS media containing 1 mg.l^{-1} *m*-T than on a medium containing 0.5 mg.l^{-1} BA. In our experiments, the positive effect of *m*-T and IBA combination on shoot regeneration was observed (VEJSADOVÁ and KUCHTOVÁ-JADRNÁ, 2008). HASSANEIN and DORION (2005) suggested a combination of 0.2 mg.l^{-1} naphthylacetic acid (NAA) with 0.5 mg.l^{-1} BA + 0.5 mg.l^{-1} zeatin as the most suit-



1: *A. Shoot formation after 4 weeks of P. × hortorum 'Gizela F1' explant incubation in dark. B. Multiplication culture of the 'Black Velvet Scarlet F1' cultivar.*

I: *Influence of growth regulators on the shoot regeneration in 'Gizela F_1' and 'Black Velvet Scarlet F_1' after 10 weeks of culture*

Cultivar	Growth regulator (mg.l^{-1})	Shoot regeneration (%)	Mean no. shoots per explant
'Gizela'	none	0	0
	IBA (1.0) + zeatin (0.2)	60	4.9b
	IBA (1.0) zeatin (0.5)	70	5.0b
	IBA (1.0) BA (0.2)	60	3.1c
	IBA (1.0) BA (0.5)	80	6.1a
'Scarlet'	none	0	0
	IBA (1.0) zeatin (0.2)	70	3.1c
	IBA (1.0) zeatin (0.5)	70	2.8c
	IBA (1.0) BA (0.2)	80	5.0ab
	IBA (1.0) BA (0.5)	80	6.8a

Means in columns with the same letter are not significantly different at the P = 0.05 level by Fisher's test. Each treatment consisted 30 explants.

able growth regulator for organogenesis induction in *P.* 'Panaché Sud' leaf explants. Our previous results (KUCHTOVÁ and VEJSADOVÁ, 2006; KUCHTOVÁ et al., 2007) have not confirmed any significant effect of NAA on *in vitro* regeneration of *P.* × *hortorum*. In the present work, BA increased shoot regeneration at half-nutrient strength MS media in both cultivars.

The effect of chemomutagens on polyploidy induction

Shoots after colchicine treatment proved stress symptoms, in particular at the higher polyploidogen concentration and longer duration of treatment. In both cultivars, explant regeneration was significantly reduced (to 20%) by a higher concentration of chemomutagens (Tab. II). In the 'Gizela F_1', the highest number of polyploid plants was found after 14-day-treatment of the explants with 0.05% colchicine – tetraploidy was determined in 5 shoots, and mixoploidy in 3 shoots. Oryzalin had no effect on ploidy induction in this cultivar. On the other hand, 'Black Velvet Scarlet F_1' explants were more responsive to oryzalin than colchicine; with 10 tetraploids and 7 mixoploid shoots after 7 and 14 d of 10 µmol.l^{-1} oryzalin infiltration, when even octoploidy appeared in 3 microshoots (Fig. 2).

When 'Black Velvet Scarlet F_1' explants were dipped in a solution of 20 µmol.l^{-1} oryzalin, the explant regeneration was not significantly reduced compared to control (Tab. III). Colchicine significantly decreased shoot regeneration and polyploidy was induced only in one shoot, whereas oryzalin induced tetraploidy in 11 explants and mixoploidy in 5 shoots. Oryzalin was also more effective in

II: *Shoot regeneration after 6 weeks and polyploidy induction using the infiltration method in 'Gizela F_1' and 'Black Velvet Scarlet F_1'*

Cultivar	Treatment	Shoot regeneration (%)	Mean no. shoots per explant	No. tetraploids 2n = 4x	No. mixoploids 2n = 2x, 4x	No. octoploids 2n = 8x
	Colchicine % / d					
'Gizela'	0.02 / 7	90	1.1 a	1	9	0
	0.02 / 14	50	0.7 b	2	0	0
	0.05 / 7	30	0.7 b	2	6	0
	0.05 / 14	20	0.3 cd	5	3	0
'Scarlet'	0.02 / 7	80	0.9 ab	1	0	0
	0.02 / 14	30	0.4 c	2	0	0
	0.05 / 7	40	0.8 b	0	0	0
	0.05 / 14	20	0.2 d	0	2	0
	Oryzalin µmol.l^{-1} / d					
'Gizela'	10 / 7	90	1.2a	0	0	0
	10 / 14	80	0.8b	0	0	0
	20 / 7	50	0.6bc	0	0	0
	20 / 14	30	0.4c	0	0	0
'Scarlet'	10 / 7	80	1.1 a	4	2	2
	10 / 14	40	1.2 a	4	3	0
	20 / 7	40	0.6 bc	1	1	1
	20 / 14	20	0.3 cd	1	1	0
'Gizela'	Control	100	1.0 a	0	0	0
'Scarlet'	Control	90	0.9 a	0	0	0

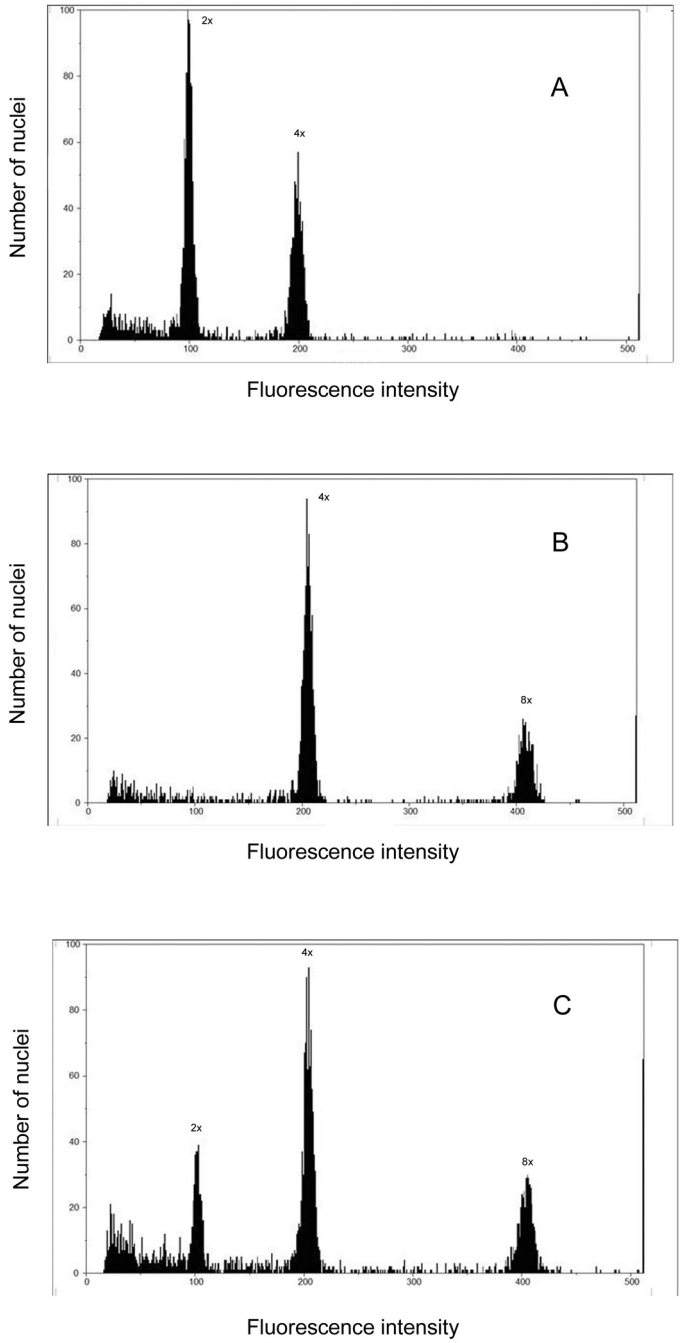
Means in columns with the same letter are not significantly different at the P = 0.05 level by Fisher's test. Each treatment consisted 30 explants.

III: *Shoot regeneration after 6 weeks and polyploidy induction using the dip method in 'Gizela F_1' and 'Black Velvet Scarlet F_1'*

Cultivar	Treatment	Shoot regeneration (%)	Mean no. shoots per explant	No. tetraploids 2n = 4x	No. mixoploids 2n = 2x, 4x
	Colchicine %				
'Gizela'	0.2	30	0.3 cd [a]	0	2
	0.4	0	0	0	0
'Scarlet'	0.2	60	0.7 b	0	1
	0.4	60	0.7 b	1	2
	Oryzalin µmol.l^{-1}				
'Gizela'	20	50	0.8 b	2	2
	40	30	0.4 c	2	0
'Scarlet'	20	80	1.0 a	11	5
	40	0	0	0	0
'Gizela'	Control	90	1.0 a	0	0
'Scarlet'	Control	90	1.0 a	0	0

[a] Means in columns with the same letter are not significantly different at the P = 0.05 level by Fisher's test. Each treatment consisted 30 explants.

the 'Gizela F_1' (4 tetraploids), but none after colchicine treatment. 'Black Velvet Scarlet F_1' explants proved to be more responsive to dipping in oryzalin solutions than 'Gizela F_1'. However, 'Gizela F_1' explants showed to be more responsive to colchicine infiltration than 'Black Velvet Scarlet F_1'. Lateral shoots formed in control plants as well, in which slight mortality was recorded, most likely as a result of dipping explants in water which might lead to stress. Colchicine in concentration of 0.4% was too



2: *Representative flow cytometric histograms of nuclei isolated from microshoots treated by oryzalin in the 'Black Velvet Scarlet F1' cultivar. A. Tetraploid 2n=4x. B. Octoploid 2n=8x. C. Mixoploid 2n=2x,4x.*

strong for 'Gizela F_1' explants (no regeneration) but 30% of explants regenerated at 0.2% colchicine. Using FCM analysis performed on 'Black Velvet Scarlet F_1' responsive explants, 13 tetraploids was detected with the infiltration method and 12 tetraploids was found with the dip technique (total tetraploid no.).

In both tested methods of chemomutagen treatments, we observed growth stress effects on the explants; their growth was slower in comparison with the control or is completely stopped. Oryzalin significantly decreased shoot regeneration than colchicine but polyploidy induction was dependent on cultivar and chemomutagen concentration. Polyploidization using chemomutogens resulted in greater phenotype homogenity of induced polyploids. Growing tetraploid individuals of the green-leaved 'Gizela F_1' are characterized with more robust growth and richer flowers. In the 'Black Velvet Scarlet F_1' tetraploid plants, the unique dark brown leaf blades have been preserved.

IV: *Effects of treatment (T), explant type (E), cultivar (C) and plant growth regulator (PGR) on Pelargonium × hortorum regeneration*

	Shoots per explants					
	Tables[a]					
	I			II+III		
Variation source	df	SE	P	df	SE	P
T	1	0.48915	*	16	0.72522	*
E	1	0.70252	*	1	0.52436	*
C	2	0.61747	*	2	0.48715	*
PGR	5	0.54331	*	–	–	–
T × E × C	2	1.78852	*	32	1.72386	*
PGR × C	10	1.15693	*	–	–	–

[a] No. Tables in Results and Discussion
[b] Standard errors and *P* values for differences of shoot no. means (Fisher's test). *Significant at P = 0.05.

SUMMARY

The objective of this work was to induce *in vitro* shoot regeneration and ascertain an effective method of polyploidy induction using colchicine and oryzalin in two diploid cultivars 'Gizela' and 'Black Velvet Scarlet F_1' of *Pelargonium × hortorum* L. H. Bailey. Cultivars of hybrids significant for growers, *P. × hortorum*, *P. × peltatum* and *P. × domesticum*, occur as diploids or tetraploids, that is 2n = 18 or 36, although aneuploidy of both forms is also common, expressed as 2n = 17 or 35. The basic number of chromosomes is x = 9. If tetraploid genotypes occur in a population of diploid genotypes of the same species, in many plant species the tetraploids are eliminated due to hybridization with diploids and the consequent development of sterile triploids, however, in the case of *Pelargonium× hortorum* there is no hybridization between diploid and tetraploid genotypes.

At the Silva Tarouca Research Institute for Landscape and Ornamental Gardening in Průhonice, golden-leaved and brown-leaved varieties of F_1 hybrids have been cultivated and also varieties with classic green leaves. The brown-leaved 'Black Velvet' series (previously called 'Black Magic') in particular are a big asset to the *Pelargonium × hortorum* cultivar assortment. In diploid cultivars, leaf coloration is marked by an intense brown with sharply defined narrow green leaf margins. However, these brown-leaved forms are completely missing from the assortment of tetraploid cultivars. Diploid cultivar *Pelargonium × hortorum* from 'Black Velvet Scarlet F_1' (bright orange-red flowers) and the green-leaved variety 'Gizela F_1' with a classic type of leaf zone and bright red flowers were grown from seeds and used in this work. Intact leaf blades (cultured abaxial side up) of *in vitro* seedlings were used as primary explants. The Murashige and Skoog (MS) media contained an one-half concentration of salts, a modified mixture of vitamins (0.4 mg.l^{-1} thiamin; 0.2 mg.l^{-1} pyridoxine; 0.4 mg.l^{-1} nicotinic acid; 100 mg.l^{-1} myo-inositol; 4 mg.l^{-1} glycine; 500 mg.l^{-1} casein hydrolysate) and 1000 mg.l^{-1} polyvinylpyrrolidone (PVP), 25 g.l^{-1} sucrose; 7.5 g.l^{-1} agar (Cell Culture Tested, Sigma), pH 5.8. We employed the following growth regulator combinations: I. 1 mg.l^{-1} IBA + 0.2 mg.l^{-1} zeatin; II. 1 mg.l^{-1} IBA + 0.5 mg.l^{-1} zeatin; III. 1 mg.l^{-1} IBA + 0.2 mg.l^{-1} BA; IV.1 mg.l^{-1} IBA + 0.5 mg.l^{-1} BA. In both cultivars, benzyladenine (BA) significantly improved shoot regeneration compared to zeatin.

In vitro regenerate shoots of diploid 'Black Velvet Scarlet F_1' and 'Gizela F_1' were used for polyploidy induction. Two methods of the explant treatment were tested; chemomutagen infiltration from nutrient media and dipping of the explants in a chemomutagen solution. Colchicine (0.01, 0.02, 0.05, 0.1, 0.2, 0.4%) was dissolved directly in DMSO (dimethyl sulfoxide); while a supply solution was used for oryzalin (10, 20, 40 µmol.l^{-1}). Under sterile conditions, the chemomutagens were quantitively transferred to a hot sterile medium (the infiltration method) or to sterile water (the dip method). The regeneration medium was supplemented with ½ MS solution of salts, a mixture of MS vitamins, 1000 mg.l^{-1} PVP, 50 mg.l^{-1} casein hydrolysate, 25 mg.l^{-1} sucrose and 7.5 g.l^{-1} agar (pH 5.8). The regeneration rate and shoot formation were determined. The ploidy level was ascertained by the flow cytometry (FCM) method. Data were subjected to the analysis of variance (ANOVA) and means were separated using Fisher's test at P = 0.05.

In the 'Gizela F_1', the highest number of polyploid plants was found after 14-day-treatment of the explants with 0.05% colchicine – tetraploidy was determined in 5 shoots, and mixoploidy in 3 shoots. Oryzalin had no effect on ploidy induction in this cultivar. On the other hand, 'Black Velvet Scarlet F_1' explants were more responsive to oryzalin than colchicine; with 10 tetraploids and 7 mixo-

ploid shoots after 7 and 14 d of 10 µmol.l^{-1} oryzalin infiltration, when even octoploidy appeared in 3 microshoots. Polyploidization using chemomutogens resulted in greater phenotype homogenity of induced polyploids. Growing tetraploid individuals of the green-leaved 'Gizela F_1' are characterized with more robust growth and richer flowers. In the 'Black Velvet Scarlet F_1' tetraploid plants, the unique dark brown leaf blades have been preserved.

SOUHRN

In vitro regenerace a indukce polyploidie u *Pelargonium × hortorum* L. H. Bailey

Cílem této práce bylo indukovat *in vitro* regeneraci výhonů a zjistit účinnou metodu indukce polyploidie pomocí kolchicinu a oryzalinu u dvou diploidních kultivarů *Pelargonium × hortorum* L. H. Bailey: 'Gizela' a 'Black Velvet Scarlet F_1'. Kultivary pěstitelsky významných hybridů, *P. × hortorum*, *P. × peltatum* a *P. × domesticum,* se vyskytují jako diploidní a tetraploidní, respektive 2n = 18 nebo 36, častá je však i aneuploidie obou forem, tedy 2n = 17 nebo 35. Základní počet chromozomů je x = 9. Pokud se tetraploidní genotypy vyskytují v populaci diploidních genotypů téhož druhu, dojde u mnoha rostlinných druhů k eliminaci tetraploidů díky křížení s diploidy a následnému vzniku sterilních triploidů. V případě *Pelargonium× hortorum* však ke křížení diploidních genotypů s tetraploidními nedochází.

Ve Výzkumném ústavu Silva Taroucy pro krajinu a okrasné zahradnictví, v.v.i. v Průhonicích byly kromě odrůd s klasickými zelenými listy vyšlechtěny i zlatolisté a hnědolisté odrůdy typu F_1 hybridů. Především hnědolistá série 'Black Velvet' (dříve označovaná jako 'Black Magic') je velkým přínosem v sortimentu kultivarů *Pelargonium × hortorum*. Vybarvení listů u diploidních kultivarů se vyznačuje intenzivní hnědou barvou s ostře ohraničenými tenkými zelenými okraji listů. V sortimentu tetraploidních kultivarů však tyto hnědolisté formy zcela chybí.

Pro experimenty byla vybrána F_1 diploidní odrůda *Pelargonium × hortorum* L. H. Bailey ze série 'Black Velvet' pojmenovaná 'Black Velvet Scarlet F1' (oranžovočervené zářivé květy) a zelenolistá odrůda 'Gizela F1' s klasickou zonální kresbou na listech a zářivě červenými květy. Jako primární explantáty byly použity listové čepele *in vitro* semenáčů. Murashige a Skoog (MS) média obsahovala poloviční koncentraci solí, modifikovanou směs vitaminů (0,4 mg.l^{-1} thiamin; 0,2 mg.l^{-1} pyridoxin; 0,4 mg.l^{-1} kys. nikotinová; 100 mg.l^{-1} myo-inositol; 4 mg.l^{-1} glycin, 500 mg.l^{-1} kasein hydrolyzát), 1000 mg.l^{-1} polyvinylpyrolidyn (PVP), 25 g.l^{-1} sacharózu, 7,5 g.l^{-1} agar Sigma, pH 5,8. Použily jsme následující kombinace růstových regulátorů: I. 1 mg.l^{-1} IBA + 0,2 mg.l^{-1} zeatin; II. 1 mg.l^{-1} IBA + 0,5 mg.l^{-1} zeatin; III. 1 mg.l^{-1} IBA + 0,2 mg.l^{-1} BA (benzyladenin); IV.1 mg.l^{-1} IBA + 0,5 mg.l^{-1} BA. U obou kultivarů, benzyladenin (BA) průkazně zvýšil regeneraci výhonů ve srovnání se zeatinem.

Pro indukci polyploidie byly použity *in vitro* regenerované výhony u diploidních kultivarů 'Black Velvet Scarlet F1' a 'Gizela F1'. Byly testovány dvě metody ošetření explantátů: prostřednictvím infiltrace z média obsahujícího chemomutagen a máčením explantátů v jeho roztoku. Kolchicin (0,01, 0,02, 0,05, 0,1, 0,2, 0,4%) byl rozpouštěn přímo v DMSO (dimethylsulfoxid), pro oryzalin (10, 20, 40 µmol.l^{-1}) byl použit zásobní roztok. Ve sterilních podmínkách byly chemomutageny kvantitativně převedeny do horkého sterilního média (metoda infiltrace) či sterilní vody (metoda máčení). Regenerační médium obsahovalo ½ MS roztok solí, směs vitaminů MS, 1000 mg.l^{-1} PVP, 50 mg.l^{-1} kasein hydrolyzát, 25 g.l^{-1} sacharóza, 7,5 g.l^{-1} agar, pH 5,8. Byla sledována míra regenerace a tvorba výhonů. Stupeň ploidie byl zjištěn metodou průtokové cytometrie (FCM). Pro statistické vyhodnocení výsledků byla použita analýza variance ANOVA a srovnávací Fisherův test (F-test) na hladině pravděpodobnosti P = 0.05.

U kultivaru 'Gizela F_1', byl zjištěn nejvyšší počet polyploidních rostlin po čtrnáctidenním ošetření explantátů 0,05% kolchicinem – tetraploidie byla stanovena u pěti a mixoploidie u tří výhonů. Oryzalin neměl u 'Gizela F_1' na indukci ploidie žádný efekt. Na druhé straně, byly explantáty 'Black Velvet Scarlet F_1' vice citlivější k oryzalinu než kolchicinu – bylo nalezeno deset tetraploidních a sedm mixoploidních výhonů po sedmidenní a čtrnáctidenní infiltraci oryzalinu, kdy byla u tří výhonů objevena oktoploidie. Polyploidizace pomocí chemomutagenů měla za následek větší fenotypovou vyrovnanost získaných polyploidů. Dopěstovaní tetraploidní jedinci kontrolní zelenolisté odrůdy 'Gizela F1' se vyznačují statnějším vzrůstem a bohatším kvetením. Tetraploidní rostliny 'Black Velvet Scarlet F_1' si zachovaly unikátní tmavě hnědé zbarvení listových čepelí.

kultivary *Pelargonium × hortorum* L. H. Bailey, *in vitro* regenerace výhonů, kolchicin, oryzalin, polyploidizace

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