Polyploidization of *Pelargonium* × *hortorum* L. H. Bailey in greenhouse conditions

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ABSTRACT: This study is aimed at induction of polyploidy in the black-leaved cultivar *Pelargonium* × *hortorum* L. H. Bailey Black Velvet Scarlet F1 to obtain basic breeding material for creating new black-leaved tetraploid cultivars. The cultivar Gizela F1 was chosen as a control for the experiment. Tetraploidy was induced in seedlings in the cotyledon stage using various concentrations (from 0.1 to 2.5%) of colchicine water solutions; the treatments were repeated daily for 2, 3, 5 or 7 successive days. The first experiment, done in 2005, was very successful; 17.4% of treated Black Velvet Scarlet F1 plants and 23.7% of treated Gizela F1 plants were tetraploid, and other ploidy levels were also found. However, two other replications from 2006 (involving only five best treatments from the first experiment) were much less successful in comparison with the first one.

Keywords: *Pelargonium* × *hortorum*; zonal pelargonium; Black Velvet Scarlet F1; colchicine; induced polyploidy; flow cytometry

Cultivars of *Pelargonium* × *hortorum* with attractive black leaves with a narrow green margin are relatively new. The first cultivar of this type was Black Velvet Rose F1, bred in Silva Tarouca Research Institute for Landscape and Ornamental Gardening (VÚKOZ) Průhonice in 1996 (the original name was Black Magic Rose F1; later the name of the whole series was changed to Black Velvet). The first cultivars of this diploid black-leaved F1 series were awarded for their originality and quality (Plavcová 2007).

We aimed our work at induction of polyploidy in a cultivar from the Black Velvet series to obtain basic breeding material for creation of new black-leaved tetraploid cultivars with double or full flowers.

Tetraploid cultivars of zonal pelargonium have larger flowers that are mostly double or full. The colours of flowers are often more interesting because of higher amount of genetic information in tetraploid cells.

Nevertheless, the most important advantage of tetraploid cultivars (from a customer's point of view) is lower flower fertility, typical for autotetraploid plants due to disorders in segregation and dislocation of chromosomes in meiosis (Acquaah 2007). This means that the flowers remain blooming for much longer time, whereas after fertilization they would lose their petals and begin creating seeds, which is an unfavourable attribute for ornamental flowering plants. Thus, tetraploid cultivars of zonal pelargonium can be more interesting than diploid cultivars. This character however represents problems for plant breeders that they obtain fewer seeds for their work compared to diploid plants. Even so, it is profitable to have breeding programs for tetraploid pelargoniums because of their market importance (Hofmann 1992).

MATERIALS AND METHODS

Two diploid F1 cultivars of *Pelargonium* × *hortorum* L. H. Bailey, a black-leaved cultivar with orange-red flowers Black Velvet Scarlet F1 and a comparative green-leaved cultivar Gizela F1 with a standard type of leaf zone and bright-red flowers, were chosen to induce polyploidy in their seedlings. Seeds of these cultivars were sown into trays (each tray with

66 units) filled with sowing substrate, covered with a perforated transparent plastic sheet and placed in a greenhouse to germinate.

Germinated seedlings in the cotyledon stage with the first true leaf were treated with drops of colchicine water solutions (colchicine was dissolved in a small amount of 96% ethanol and added to water) in concentrations of 0.1, 0.5, 1.0, 1.5, 2.0, 2.5% in the first experiment (February 2005) and 0.5, 1.0, 1.5% in the second and third replications (September 2006). The drops were applied on the apices of the seedlings each morning (8–10 a.m.) for 2, 3, 5 or 7 days in the first experiment and for 2 and 3 days in the second and third replications. The 1.5% concentration was not applied in the 3-day treatment. The treatments for the second and third replications were chosen on the basis of the results of the first experiment; only concentrations giving the best results were used again as the most suitable variants. After applying the colchicine solutions, we covered the trays of treated seedlings with a perforated transparent plastic sheet to avoid fast vaporization of the drops. Treated plantlets were not washed off.

After the treatment, the plastic sheet was removed from the trays and the plants were further grown according to the common schedule for generatively propagated cultivars (but without using growth regulators).

In August 2005, the stomata of all plants in the first replication were measured. The final selection (to remove diploid plants) was made based on the results of the stomata measurement, focused on bigger leaves, changes in leaf colour and changes in inflorescences and flowers in comparison to the diploid control plants. Selected plants were analyzed using flow cytometry to determine their ploidy levels. In the second and third replication, all treated plants were analyzed using flow cytometry; no habitual selection or stomata measurements were done.

Stomata measurement

Neutral nail enamel was put on the undersides of the leaves. When dry, the film of the enamel, that is an ideal decal of the epidermis cells including the stomata, was removed from the tissue and observed using a binocular microscope at a magnification of 40×. Length of five stomata was measured during observation using a line scale implanted in the ocular. The density of stomata was counted in four particular squares of the microscope vision field.

Flow cytometry (FCM)

Leaf pieces were homogenized (chopped with a blade) in Petri dishes with 0.5 ml of the Otto I buffer (water solution of 0.1M citric acid monohydrate and 0.5% Tween 20) according to the methodology of Doležel (1997) to free the karyons from tissues. After filtration through a 50-μm filter (to remove solid remains of the tissues), 1 ml of Otto II (0.4M $Na_2HPO_4.12H_2O$) containing fluorescent colouring matter that binds to the DNA was added to the suspension in the specified amount, concretely 4´,6-diamino-2-phenylindole (DAPI) – 4 μl/ml of Otto II was added. Partec flow cytometer was used for the analysis of samples.

Because of the high amount of polyphenolic substances in the tissues of the analyzed *Pelargonium* × *hortorum* plants, in some measurements 2 μl/ml of

Table 1. Effects of treatment factors: cultivar, concentration of colchicine solution and length of application in days – on the survival rate of the Black Velvet Scarlet F1 and Gizela F1 plantlets in the first experiment

Concentration of colchicine (%)	Black Velvet Scarlet F1	Gizela F1	Length of application in days	Black Velvet Scarlet F1	Gizela F1
0.1	19.5^{a}	21.75^{a}	2	20.00^{a}	20.5^{a}
0.5	15.0^{abc}	18.5^{ab}	3	17.17^{a}	19.17^{a}
1.0	16.25^{ab}	17.25^{bc}	5	12.83^{b}	15.67^{b}
1.5	11.75^{bc}	15.75^{bcd}	7	7.50^{c}	11.83^{c}
2.0	10.75^{d}	13.5^{e}			
2.5	13.0^{bc}	14.0^{de}	factor cultivar	14.375 ^{a}	16.7917^{b}

Values correspond with number of survival plants (number of plants in each treatment was 22). Each value is the LSD mean of 4 replicates in the case of concentration of colchicine, 6 replicates in the case of length of application in days and 24 replicates in the case of factor cultivar. Multifactor analysis ANOVA was applied after log transformation. Values followed by the same lower-case letters in the same column are not significantly different at the $P \leq 0.05$ level using the *F*-test.

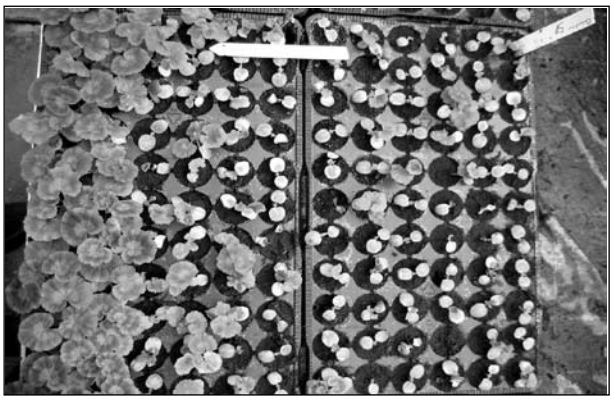

Fig. 1. Stress symptoms of plantlets of *Pelargonium* × *hortorum*, cv. Gizela, caused by colchicine treatment of various concentrations, three weeks after application (spring 2005)

Fig. 2. Phenotypical impact of colchicine treatments on young plants of *Pelargonium* × *hortorum*, cv. Black Velvet Scarlet F1 (summer 2005); on the left side of the picture are leaves with typical colour pattern

2-mercaptoethanol was added to the Otto II buffer to avoid polyphenolics oxidation. To reduce the amount of phenolic substances in the tissues, the leaves were stored in plastic bags in the refrigerator for at least five days before the analysis.

RESULTS AND DISCUSSION

The first experiment was highly successful in inducing polyploidy and other changes in the treated plants. The negative influence of the treatment was observed several weeks after the treatments, when plants treated for more days and with higher concentrations of solutions showed symptoms of stress, such as termination of growth and increased mortality (Fig. 1). Many plants died in the early stages of their growth (Table 1) or had inhibited growth due to visible colchicine-induced disorders in tissue development (Fig. 2). Some statistically significant influences of the treatment factors (cultivar, length of application in days and concentrations of the colchicine solutions) on the survival rate of the plants of both cultivars in the first replication (Table 1) were found using a multifactorial ANOVA analysis. However, in the second and third replication, the toxic stress on plantlets planted in September 2006, such as the lethality, was distinctly lower (Figs. 3 and 4) in comparison to the first replication. Plantlet growth during the 2nd and 3rd replications was slightly slower than in the control plants; this variance disappeared throughout the growth period. A statistical difference in lethality between the first replication and the other two replications was found using a multifactorial ANOVA analysis.

The stomata measurement completed in July 2005 on all viable plants of the first experiment showed no significant differences among the stomata length and density, parameters that could help in the determination of diploid and tetraploid plants. This could be due to toxic stress called "colchiploidy". Hassenein and Dorion (2006), however, mention that water stress causes a significant increase in sto-

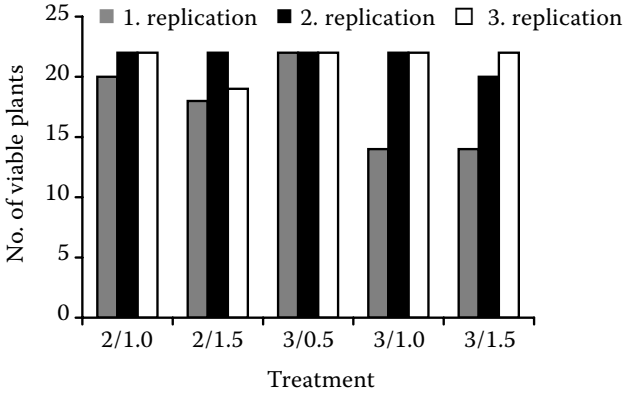

Fig. 3. No. of viable plants after colchicine treatment, cv. Black Velvet Scarlet F1 (22 plants in treatment)

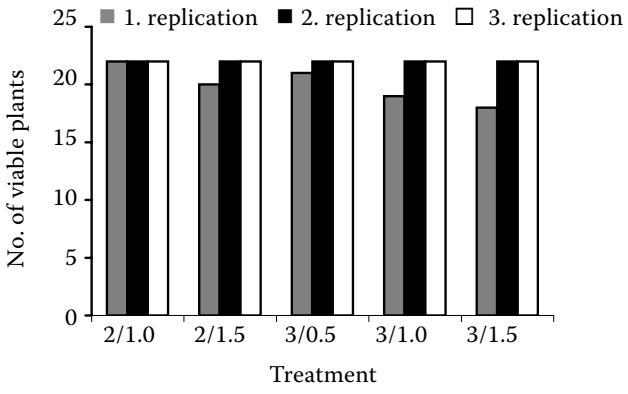

Fig. 4. No. of viable plants after colchicine treatment, cv. Gizela F1 (22 plants in treatment)

Table 2. Dependence of stomata length and stomata density on ploidy level in colchicine-treated plants of cultivars Black Velvet Scarlet F1 and Gizela F1

Ploidy level	Black Velvet Scarlet F1			Gizela F1		
	No. of replicates	Length of stomata (μm)	Density of stomata*	No. of replicates	Length of stomata (μm)	Density of stomata*
2	15	23.03^{a}	38.30^{ab}	27	26.43^{a}	45.39^{a}
4	92	25.46^{b}	31.40^{a}	128	27.89^{a}	37.95^{a}
Mixoploids	7	23.93^{ab}	43.54^{b}	22	28.34^{a}	36.97^{a}
8	4	27.88^{ab}	31.38^{ab}	9	34.83^{b}	16.48^{b}
16	0	–	–	1	40.00^{b}	10.59^{ab}

*Number of stomata per 1 mm^2 of the leaf blade area
Values are the LSD means of various numbers of replicates (see the table). One-way ANOVA was applied after log transformation. Values followed by the same lower-case letters in the same column are not significantly different at the $P \leq 0.05$ level using the *F*-test.

mata density on leaves of *Pelargonium* × *hortorum,* cv. Féerie Orange and Improved Rubin. Another possible reason for not using the stomata measurement method is that during the FCM analysis endopolyploidy was found to be a native property of *Pelargonium* × *hortorum* but the histological study of Li (2005) testifies against this hypothesis by finding that the epidermis of the *Pelargonium* × *hortorum* species possesses one level of ploidy, whereas cells of deeper laid somatic tissues of the plant exist in two ploidy levels, which is recognized by the FCM as endopolyploidy.

Some plants regarded as polyploid after stomata measurements were later detected as diploid after the FCM analysis was completed in November 2005. This difference resulted from the genomic instability of the plant material after treatments. Using the FCM to measure *Humulus lupulus* L., Koutoulis et al. (2005) found that the measured plants could be mistakenly classified as tetraploid when analyzing the root tissues, whereas leaf tissue analysis of the same genotypes proved them to be mixoploid (2×, 4×). Using the one-way ANOVA, a significant statistical difference in stomata density was found between tetraploids and chimeras and in stomata length among diploid, tetraploid and octoploid plants in cv. Black Velvet Scarlet F1. A significant statistical difference in stomata density was also found among octoploids, diploids and tetraploids and in stomata length between octoploids, hexadecaploids, tetraploids, diploids and chimeras in cv. Gizela F1 (Table 2).

FCM analysis was completed on plants selected on the basis of their appearance and stomata measurement. Using this analysis, we observed that karyons exist naturally in two ploidy levels in the tissues of all plants, including the control plants (Fig. 5), meaning that natural endopolyploidy is a feature of the species *Pelargonium* × *hortorum*. Endopolyploidy is not rare among plant species. For example, FCM analysis also detected this character in the garden pansy, but the G_1 stages always represented dominant peaks (Lagibo et al. 2005), while the peaks of *Pelargonium* × *hortorum* measurements were variable in their dominance. Endopolyploidy was also detected for *Kochia scoparia* (Chodová et al. 2004).

The total effectiveness of the colchicine treatments was distinctly higher in the first treatment than in the other two treatments. When evaluating treatments done for all replications (i.e., 2/1.0; 2/1.5; 3/0.5; 3/1.0 and 3/1.5 – days/concentration of colchicine solution), 30% of the Black Velvet Scarlet F1 plants (Fig. 6) were tetraploid in the first replication, 5.5% in the second and 7.3% in the third. For Gizela F1 plants, 33.6% were tetraploid in the first replication, 10.0% in the second and 5.5% in the third (Fig. 7). The occurrence of octoploids and mixoploid plants was low (below 5.0%) and similar for all replications. The differences between the first and the other two replications were most probably caused by the diverse microclimate conditions (humidity and solar radiation intensity) in the greenhouse on the different treatment dates. If we consider all treatments in the first experiment – from 2 days with 0.1% colchicine solution concentration to 7 days with 2.5% colchicine solution concentration, 17.4% of the total number of tetraploids originated from the cultivar Black Velvet Scarlet F1 and 23.7% from Gizela F1.

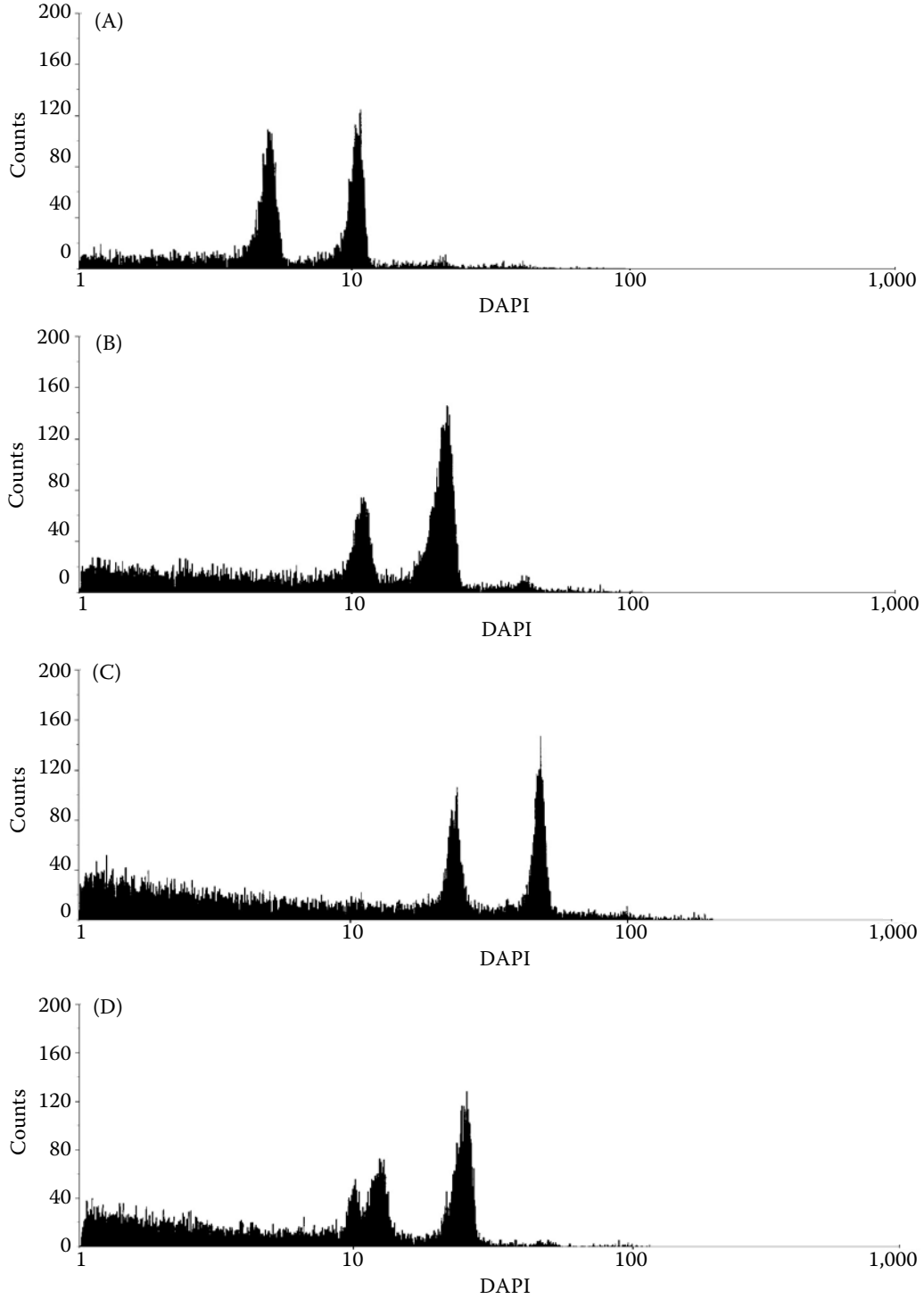

Fig. 5. Ploidy levels of *Pelargonium* × *hortorum* detected with FCM: (A) diploid plant; (B) tetraploid plant; (C) octoploid plant; (D) an example of a chimeric plant

CONCLUSION

The application of colchicine drops (0.1–2.5% for 2–7 days) on apices of plantlets proved to be effective for creation of tetraploid genotypes of *Pelargonium* × *hortorum* L. H. Bailey, cultivars Black Velvet Scarlet F1 and Gizela F1. More tetraploids in the first experiment originated from Gizela F1 (23.7%) than from Black Velvet Scarlet F1 (17.4%). When comparing the same treatments of the first replication with the other two replications, a distinctly lower percentage of polyploids including tetraploids was found in the other two treatments. Tetraploids raised from Black Velvet Scarlet F1 with desired characters will be used as basic

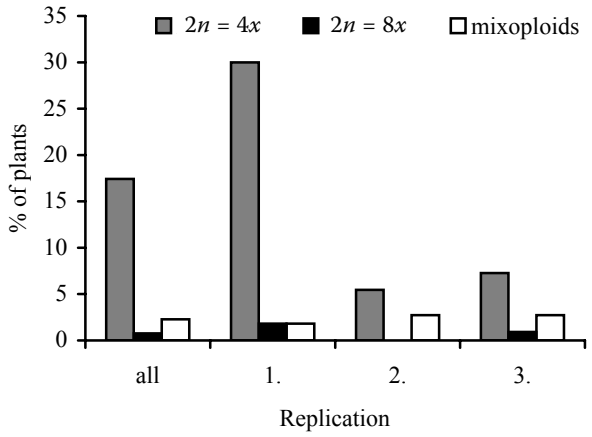

Fig. 6. Percentage of polyploids in particular replications, cv. Black Velvet Scarlet F1

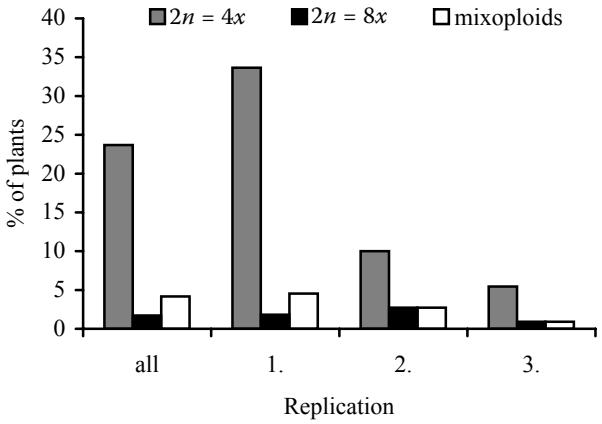

Fig. 7. Percentage of polyploids in particular replications, cv. Gizela F1

breeding material for creation of new tetraploid black leaved cultivars of *Pelargonium* × *hortorum*.

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References

ACQUAAH G., 2007. Principles of Plant Genetics and Breeding. Oxford, Blackwell Publishing: 218–220.

CHODOVÁ D., MIKULKA J., JOKEŠ M., 2004. Changes in Kochia (*Kochia scoparia* /L./ Schrad.) ploidy in relation to resistance to acetolactate synthase inhibitors. In: Proceedings of the XVI. Slovak and Czech Plant Protection Conference. Acta Fytotechnica et Zootechnica, *7* – Special Issue. Nitra, Slovak Agricultural University: 104–106.

DOLEŽEL J., 1997. Application of flow cytometry for the study of plant genomes. Journal of Applied Genetics, *38*: 285–302.

HASSENEIN A., DORION N., 2006. Determining morphological and physiological parameters for the selection of drought-tolerant geraniums (*Pelargonium* × *hortorum* L. H. Bailey). Journal of Horticultural Science and Biotechnology, *81*: 707–714.

HOFMANN CH., 1992. Schöne Pelargonien (Geranien). Stuttgart, Eugen Ulmer: 57.

KOUTOULIS A., ROY A.T., PRICE A., SHERIFF L., LEGGETT G., 2005. DNA ploidy level of colchicine-treated hops (*Humulus lupulus* L.). Scientia Horticulturae, *105*: 263–268.

LAGIBO A.D., KOBZA F., SUCHÁNKOVÁ P., 2005. Polyploidy effects on frost tolerance and winter survival of garden pansy genotypes. Horticultural Science (Prague), *32*: 138–146.

LI M., 2005. Anatomische, cytologische und histologische Untersuchungen zur somatischen Variation in verschiedenen Teilklonen von *Pelargonium znale* Kleiner Liebling. [Dissertation.] Berlin, Humboldt University: 44.

PLAVCOVÁ O., 2007. Leaf zonation of Pruhonice's brown leaved F1 varieties of *Pelargonium zonale* hort. In: Strom a květina – součást života. Sborník z vědecké konference, 4.–5. 9. 2007, Průhonice. Průhonice, VÚKOZ, v.v.i.: 257–259.

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Polyploidizace *Pelargonium* × *hortorum* L. H. Bailey ve skleníkových podmínkách

ABSTRAKT: Cílem práce bylo indukovat polyploidii u cenné černolisté odrůdy *Pelargonium* × *hortorum* L. H. Bailey, Black Velvet Scarlet F1, za účelem získání výchozího šlechtitelského materiálu pro šlechtění nových černolistých

tetraploidních kultivarů. Jako srovnávací odrůda byla použita Gizela F1. Tetraploidie byla indukovaná u semenáčů ve fázi děložních lístků pomocí vodného roztoku kolchicinu o různých koncentracích (od 0,1 do 2,5 %), ošetření byla opakována každý den po dobu dvou, tří, pěti a sedmi dnů. První opakování, jež proběhlo v roce 2005, bylo velmi úspěšné; 17,4 % ošetřených rostlin u odrůdy Black Velvet Scarlet F1 a 23,7 % ošetřených rostlin u odrůdy Gizela F1 bylo tetraploidních, byly zjištěny i vyšší úrovně ploidie. Další dvě opakování z roku 2006 (po šesti variantách, které byly v prvním opakování nejperspektivnější) byla ve srovnání s prvním opakováním výrazně méně úspěšná.

Klíčová slova: *Pelargonium× hortorum*; pelargonie páskatá; Black Velvet Scarlet F1; kolchicin; indukovaná polyploidie; průtoková cytometrie

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