Morphological changes in colchicine-treated *Pelargonium* × *hortorum* L.H. Bailey greenhouse plants

P. Jadrná[1], O. Plavcová[2], F. Kobza[1]

[1]*Faculty of Horticulture, Mendel University in Brno, Lednice, Czech Republic*
[2]*Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Průhonice, Czech Republic*

Abstract

Jadrná P., Plavcová O., Kobza F., 2010. **Morphological changes in colchicine-treated *Pelargonium* × *hortorum* L.H. Bailey greenhouse plants**. Hort. Sci. (Prague), *37*: 27–33.

Polyploids were effectively pre-selected in colchicine-treated plants of the desirable brown-leaved cultivar Black Velvet Scarlet F1 of the species *Pelargonium* × *hortorum* L.H. Bailey to obtain the basic breeding material for creating new brown-leaved tetraploid cultivars. The green-leaved cultivar Gizela F1 was used for comparison of quantity and quality of response to colchicine treatments. Water solutions of colchicine in the range from 0.1% to 2.5% induced polyploidy in seedlings with treatments repeated each day for 2, 3, 5 or 7 days. Polyploid plants were pre-selected according to their morphological changes and stomata length and density and verified using flow cytometry. Some morphological changes (leaf coloration, flower shapes) in colchiploids differed between the genotypes, others were the same in both cultivars (loss of coloration in mixoploids, failure of blooming).

Keywords: *Pelargonium* × *hortorum*; colchicine; induced polyploidy; tetraploidy; plant breeding

Pelargonium × *hortorum* is a commercially important balcony plant that includes diploid and tetraploid cultivars, mostly with green leaves and variously distinct brown, horseshoe-shaped zones, typical for the parental species *Pelargonium zonale* (Miller 1996). Diploid F1 hybrids of *Pelargonium* × *hortorum* with original blackish-brown leaves and narrow green margins were first created in the Czech Republic (Starý 1999; Plavcová 2007) but corresponding tetraploid brown-leaved cultivars still do not exist. To obtain such plants, existing diploid brown-leaved cultivars can be treated with colchicine to induce polyploidy and the resulting tetraploid genotypes with the desired character and necessary generative fertility can be selected and further bred. Colchicine treatment caused changes in the morphology and fertility of treated plants so that originated plants needed to be evaluated for their morphology, pollen creating and seed set ability. Some specific morphological changes were associated with ploidy levels such as broader leaves in tetraploid *Callistephus chinensis* plants compared to diploid plants (Hanzelka, Kobza 2000) and broader petals in natural tetraploids of *Pelargonium pinnatum* (Gibby et al. 1996). Stomatal size and density were correlated with ploidy in plants species (e.g. Carolin 1954) and was investigated as a potential basis for pre-selecting polyploids in *Pelargonium* × *hortorum*. The aim of this work was to induce polyploidization in diploid *Pelargonium* × *hortorum* L.H. Bailey plants, pre-select the polyploid plants according to their morphological changes and verify the fruitfulness of pre-selection by flow cytometry (FCM).

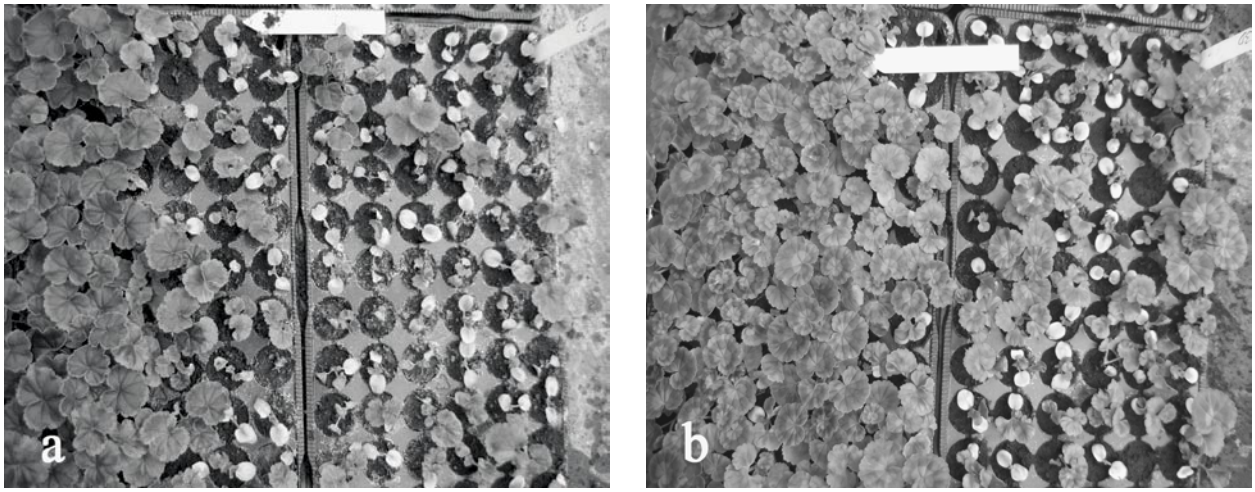

Fig. 1. Seedlings of cv. Black Velvet Scarlet F1 and cv. Gizela F1 7 weeks after colchicine treatment: (a) 5 days of treatment, (b) 3 days of treatment. Concentrations of treatment increase from 0.1% to 2.5% from the left to the right side

MATERIAL AND METHODS

Polyploidy was induced in two diploid F1 cultivars of *Pelargonium* × *hortorum* L.H. Bailey: cv. Black Velvet Scarlet F1 (brown-leaved, orange-red flowers) and for comparison in cv. Gizela F1 (leaves with a typical horseshoe-shaped zone and bright red flowers). Seeds were sowed during February in trays (units with 4 cm diameter; 66 units per one tray) filled with sowing substrate, covered with a transparent plastic pore sheet and placed in a greenhouse to germinate. Colchicine solutions were prepared by dissolving colchicine powder in a small amount of ethanol and then adding water. Solutions of 0.1%, 0.5%, 1.0%, 1.5%, 2.0% and 2.5% concentrations were applied on the apices of seedlings in the first true-leaf stage, each morning (8:00–10:00 a.m.) for 2, 3, 5 or 7 days. In total there were 24 treatment combinations of colchicine concentrations and number of applications for each cultivar. There were 22 replicate plants per treatment including control plants of both cultivars. Immediately after the colchicine application, seedlings were covered with perforated transparent plastic sheet to avoid fast evaporation of solutions. Plants were irrigated twice during the treatment period after solutions had evaporated in the afternoon. After the treatment period, the plastic sheet was removed and seedlings were grown in the same conditions as commercial plants except that no growth regulators were used.

In July stomata lengths and densities were measured in all surviving plants. Neutral nail enamel was put on the undersides of the leaves. When dry, the film (a perfect decal of the epidermis cells including stomata) of the enamel was removed from the tissue and observed using a binocular microscope at 40× magnification power. Five stomata lengths were measured during observation using a line scale implanted in the ocular; the stomata density was counted in four particular squares of the field of vision of the microscope and then extrapolated for 1 mm^2.

During August, plants were pre-selected for possible polyploidy by examining changes in colorations on leaf blades, shapes of blades (including deformations), appearance of inflorescences and single flowers (compactness, vigour, flower colour, wavy or bigger petals, ragged margins of petals) and character of growth (vigour, inhibited growth without flowering, dwarfishness). The morphological changes were evaluated by comparing treated plants with control plants. In November ploidy levels in the preselected plants were determined by FCM (Jadrná et al. 2009) and the results were compared with the previously detected morphological characters of the analysed plants.

RESULTS AND DISCUSSION

Colchicine application was very effective in inducing polyploidy in the treated plants. Many treated plants showed some disorders in the growth and changes of leaf shape or colours. Many plants did not survive the treatment and necrotized in early growth stages because the colchicines treatment caused visible tissue-creation disorders. Survival rate was lower and the growth rate and the rate of development disorders were higher with higher colchicine concentration and more applications of

Table 1. Dependence of stomata lengths and densities on ploidy levels in colchiploids of cv. Black Velvet Scarlet F1 and cv. Gizela F1

Ploidy level	Black Velvet Scarlet F1			Gizela F1		
	number of samples	stomata length (μm)	stomata density (mm^{-2})	number of samples	stomata length (μm)	stomata density (mm^{-2})
2x	15	23.03^{a}	38.30^{ab}	27	26.43^{a}	45.39^{a}
4x	92	25.45^{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}
8x	4	27.88^{b}	31.38^{ab}	9	34.83^{b}	16.48^{b}
16x	0	–	–	1	40.00	10.59

Statistical evaluation was not possible – only one sample; each value is the LSD mean of values detected in various amounts of replicates (column Number of samples); multifactor analysis ANOVA was applied after to process data; 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 (Jadrná et al. 2009)

solutions within a treatment. A distinct toxic influence on seedlings (Fig. 1) was observed early after the treatment period (Jadrná et al. 2008). From original 528 treated plants of cv. Black Velvet Scarlet F1 and cv. Gizela F1, 345 plants survived in cv. Black Velvet Scarlet F1 and 403 plants in cv. Gizela F1. Morphological changes of colchicine-treated plants were evaluated by comparing these plants with control plants that had high uniformity in growth and leaf coloration and pre-selection was carried out in summer after which 143 plants of cv. Black Velvet Scarlet F1 and 194 plants of cv. Gizela F1 remained. All these plants were analysed by FCM in the late autumn.

Table 2. Scale of colorations and leaf shape changes in cv. Black Velvet Scarlet F1 – plants after the preselection, divided according to their ploidy level determined by FCM

Ploidy level (by FCM)	Changes in leaf morphology	Plants in total	Numbers of plants in different coloration groups (1–6)*						Dead***
			1	2	3	4	5	6	
Non-measurable**	total	20	13	2	3	2			7
	deformation	15	11	2	1	1			
	change of shape	1	1						
$2n = 2x$	total	15			3 (2)		1	11 (1)	
	deformation	0							
	change of shape	1					1		
$2n = 4x$	total	92	6		36 (10)	15 (2)	2	33 (5)	
	deformation	10	3		2	4		1	
	change of shape	5			3	2			
$2n = 8x$	total	4	3	1					
	deformation	4	3	1					
	change of shape	0							
Mixoploids	total	12	9	1			1	1	
	deformation	10	9	1					
	change of shape	0							
In total	total	143	31	4	42 (12)	17 (2)	4	45 (6)	7
	deformation	39	26	4	3	5	0	1	
	change of shape	7	1	0	3	2	1	0	

*The colouration groups 1–6 represent a coloration scale from absence of coloration (1) to intensive typical coloration matching controls (6); numbers in bracket indicate number of plants with coloration possessing green centres in particular groups; **non-measurable plants were probably mixoploids concerning their characters; ***plants were probably mixoploid and had no coloration, they could not be evaluated due to a very short viability

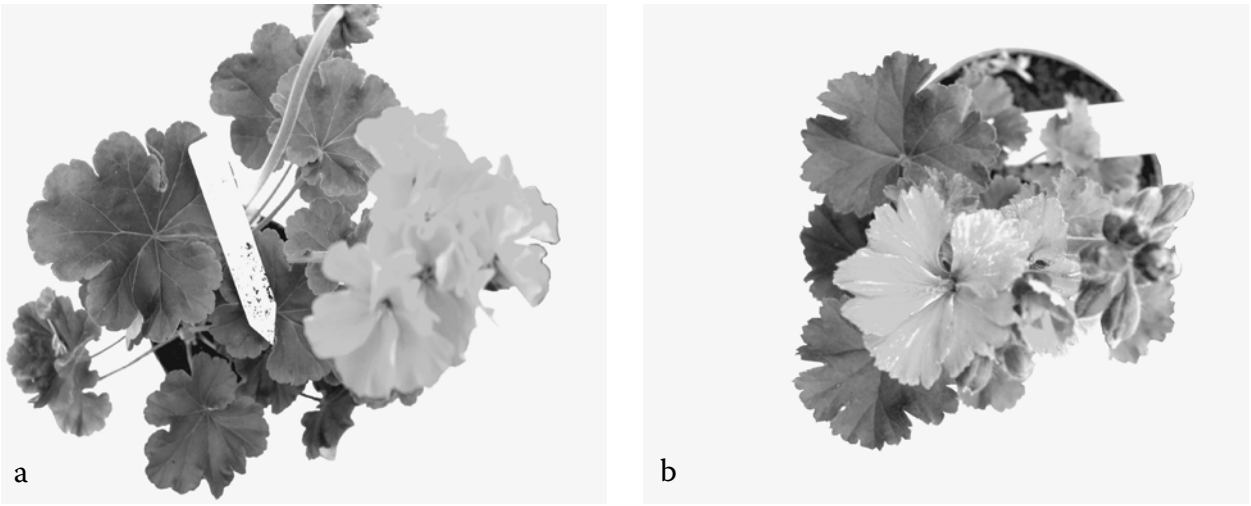

Fig. 2. Examples of colours and shapes of habitus in tetraploids raised from cv. Black Velvet Scarlet F1 showing change of leaf blade morphology (a, b) and ragged flowers (b)

Stomata measurement

Stomata measurements (Table 1) were performed in summer (before pre-selection) on 118 plants of cv. Black Velvet Scarlet F1 and 187 plants in cv. Gizela F1. The remaining selected plants were retarded in growth so that taking samples was not possible. Analysis showed no clear border between diploid and tetraploid samples for either cultivar with the exception of octoploid and mixoploid samples containing octoploid cells, which were distinctly different from samples with lower ploidy levels. Thus, without clearly determined stomata size or density groups, this method was inconclusive for use in pre-selection. The results were consistent with partially overlapping Gaussian curves measured by Li (2005) in stable model genotypes of *Pelargonium* × *hortorum* with various ploidy levels. The determination of ploidy level using stomata measurement was not reliable in stable genotypes of this species and therefore unpromising for more complicated colchiploid plants.

Impacts of polyploidization on plant phenotype

Changes in flower morphology. The investigated cultivars differed in morphological responses to colchicine treatments. The effects on cv. Gizela F1 were slight, consisting of just three plants with "burnt" flowers (flowers that had violet petals and white, most likely "burnt", margins and that dropped off very early after flowers opened). Many plants of cv. Black Velvet Scarlet F1 produced flowers with ragged petals (Fig. 2b). Later FCM analysis proved that all these plants were tetraploid and accounted for more than 15% of all tetraploids raised from this cultivar. Two of these types of flowers had sectorial chimerism (petal with white stripe). Flowers with ragged petals had non-adhesive stigmas and could not bear seeds. The results were opposite to those previously obtained by Plaschil (1997) with vegetatively propagated *Pelargonium* × *hortorum* cv. Led-

Table 3. Occurrence (%) of plants with "ragged" flowers in cv. Black Velvet Scarlet F1 after different colchicine treatments (days/concentration)

Treatment	2/0.1	2/0.5	2/1.0	2/1.5	2/2.0	2/2.5	3/0.1	3/0.5	3/1.0	3/1.5	3/2.0	3/2.5
Selected plants	0	36.4	31.8	50.0	31.8	31.8	13.6	77.3	40.9	27.3	31.8	36.4
Ragged petals	0	13.6	13.6	9.09	13.6	0	0	18.2	0	4.55	0	0
Non-flowering	0	9.1	4.6	18.2	13.6	18.2	0	40.9	22.7	18.2	9.1	18.2
	5/0.1	5/0.5	5/1.0	5/1.5	5/2.0	5/2.5	7/0.1	7/0.5	7/1.0	7/1.5	7/2.0	7/2.5
Selected plants	31.8	18.2	45.5	13.6	0	40.9	22.7	22.7	18.2	13.6	4.55	9.09
Ragged petals	0	0	9.09	0	0	0	9.09	0	4.55	4.55	0	0
Non-flowering	4.6	4.6	18.2	4.6	0	22.7	0	13.6	4.6	4.6	0	4.6

Each treatment involved 22 plants

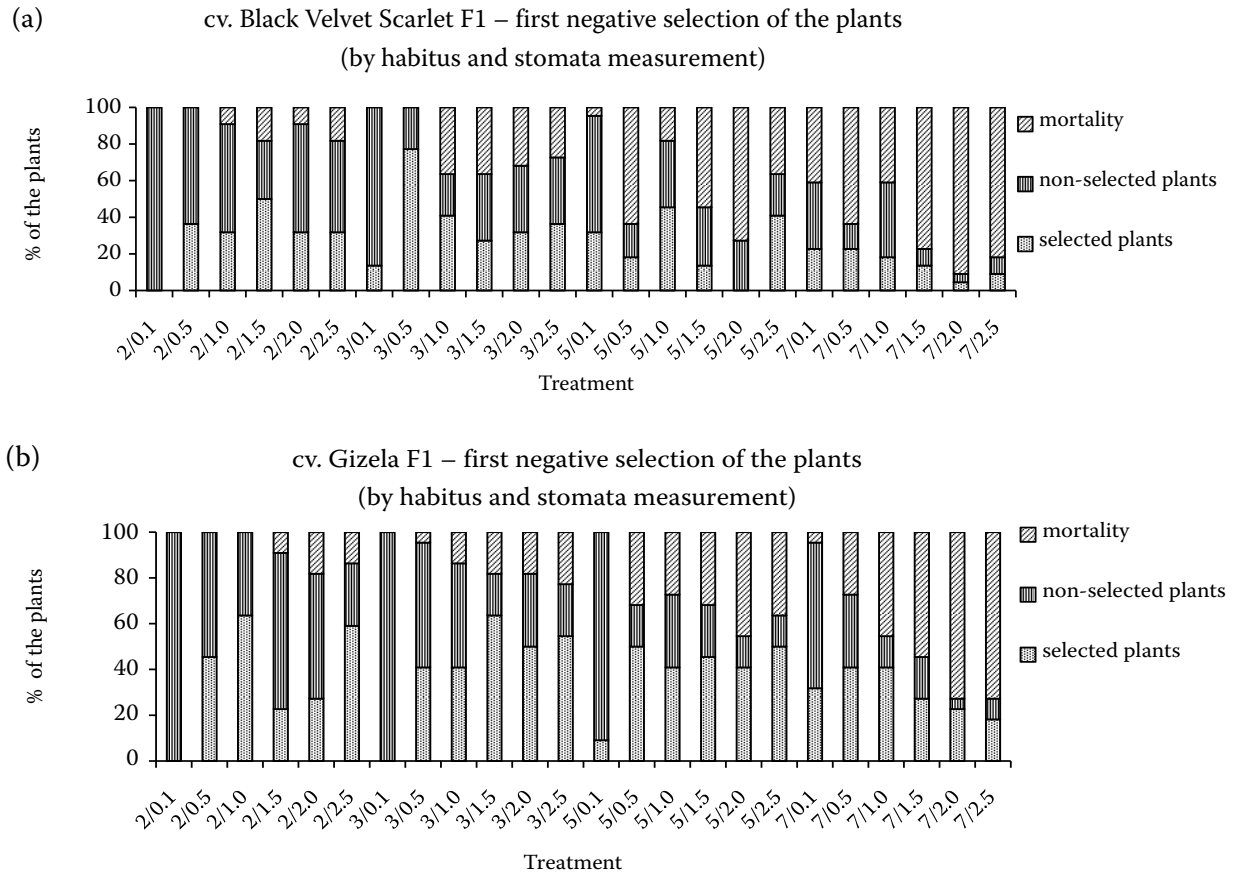

Fig. 3. Survival rates in cv. Black Velvet Scarlet F1 (a) and Gizela F1 (b) after colchicine treatment and rate of pre-selection in particular treatments

nice, which had ragged flowers with non–adhesive stigmas and could not be pollinated. In the present study, colchicine treatment created normally shaped flowers with functional adhesive stigmas that could be pollinated and bear seeds. The reasons for these differences are unclear but may be associated with our observations of non-fertile, ragged flowers.

Changes in leaf morphology in diploids and tetraploids. Leaf coloration changes occurred in response to colchicine treatment depending on the phenotype of original cultivars. In cv. Gizela F1 zonal coloration varied continuously from non visible to very distinct. In cv. Black Velvet Scarlet F1 differences occurred in the intensity and shape of the leaf blade coloration. Leaf blade coloration in cv. Black Velvet Scarlet F1 was visually evaluated and plants were divided into qualitative groups according to their intensities of colour and presence of green centres (Table 2). The group of diploids tended to retain color intensity of the original plant material. Tetraploids showed the highest variability in colour intensities and shapes of coloration. Mixoploids, non-measurable plants, and octoploids mostly showed coloration loss and deformed leaf tissues. Many plants had smaller or larger green blade centres instead of having the whole leaf surface brown as in the original. Such leaf coloration changes were mainly observed in successfully tetraploidized plants, but also occurred in diploids (Table 2). The described leaf coloration changes occurred, to a certain extent, also in further seed generations of genotypes coming from cv. Black Velvet Scarlet F1, which were used for the breeding programme. Grieger (2007) induced tetraploidy in *Pelargonium* × *hortorum* by applying trifluralin solutions on buds of adult plants and reported changes in leaf coloration as a relatively certain character of successful tetraploidization with the most effective concentration of 0.075% trifluralin producing 8% tetraploids. Some changes in leaf blade shape also occurred in some colchiploids (Fig. 2) but due to the high variability these could not be divided into groups.

Morphology of mixoploids, octoploids and hexadecaploids. Many plants in both cultivars, Black Velvet Scarlet F1 (Table 3) and Gizela F1, failed to blossom at all. These non-flowering plants had typically thickened and irregularly created so-

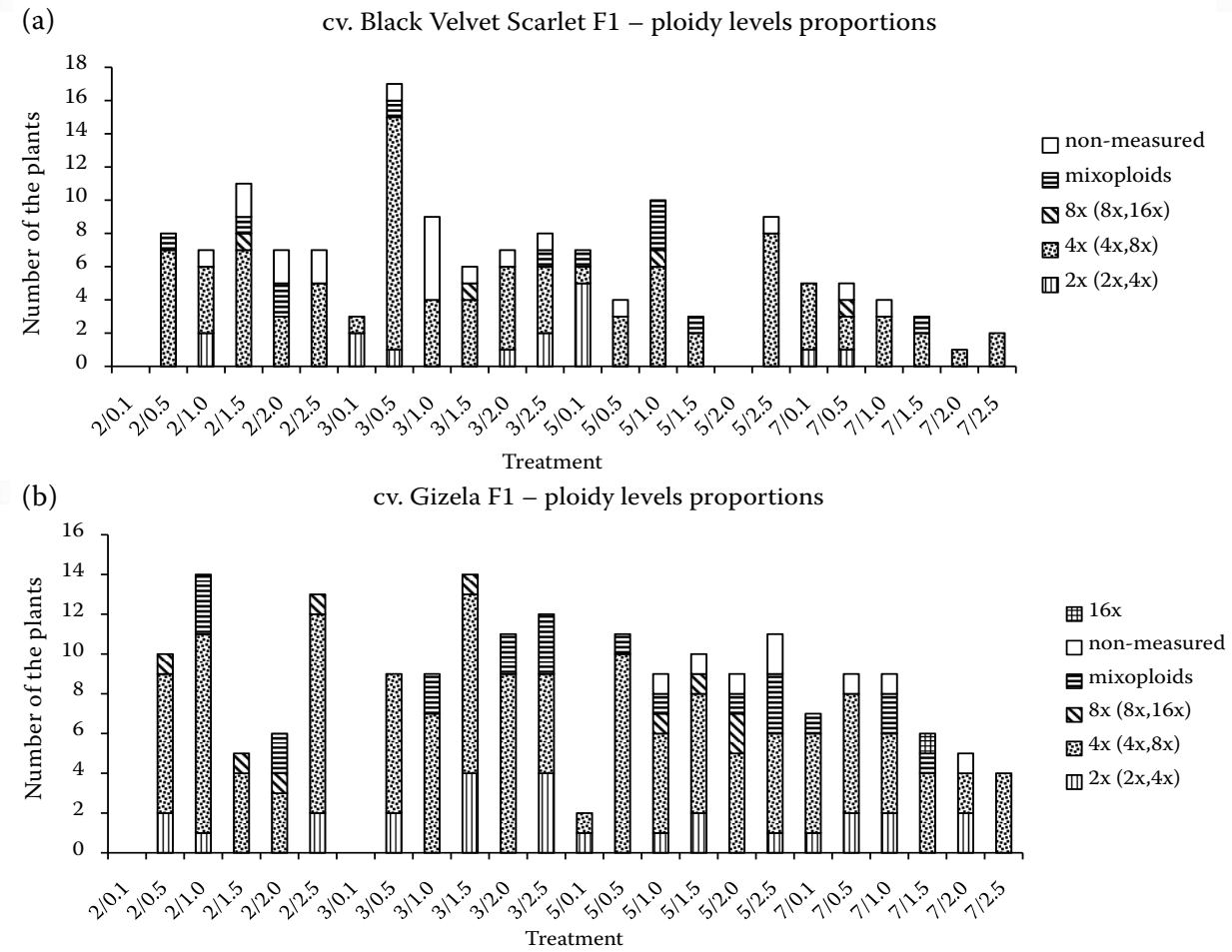

Fig. 4. Ploidy level proportions of cv. Black Velvet Scarlet F1 (a) and Gizela F1 (b) after colchicine treatment of pre-selected plants

matic tissues visible as leaf deformations with loss of leaf coloration. Some plants created inflorescences where many of the single flowers in their inflorescences aborted in the early bud stages or never created pollen and possessed non-adhesive stigmas so they could never be pollinated and set seeds. These deformations occurred in all plants determined by FCM as mixoploid (usually $2n = 2x$, $4x$, $8x$) or octoploid and in one detected hexadecaploid. Such plants never created flowers with ragged petals in cv. Black Velvet Scarlet F1.

Preselection of probable polyploids. Pre-selection among surviving plants was carried out on the basis of changes in morphology and stomata measurements in the colchicines-treated generation. Because polyploidy was assumed in these plants, 65.3% of the plants in cv. Black Velvet Scarlet F1 and 76.3% in cv. Gizela F1 (Fig. 3) were pre-selected. Ploidy levels of pre-selected plants were confirmed by FCM which showed high rates of polyploids in most treatments, from which the best were obtained with cv. Black Velvet Scarlet F1 (3/0.5) with treatment giving 63.6% of desired tetraploids including dead plants, and the best with cv. Gizela F1 were the treatments were 2/1.0, 2/2.5 and 5/0.5, which each gave 45.4% desired tetraploids. In total, 17.4% tetraploids were obtained in cv. Black Velvet Scarlet F1 and 23.7% tetraploids were obtained in cv. Gizela F1 regardless of the treatment (Fig. 4). The accuracy of polyploid preselection assessed by comparison with ploidy levels determined by FCM was 75.5% in cv. Black Velvet Scarlet F1 and 82.1% in cv. Gizela F1. The proportion of tetraploids was 64.3% in cv. Black Velvet Scarlet F1 and 65.6% in cv. Gizela F1. These tetraploids were the most frequent polyploidy detected and the only group of polyploids suitable for further breeding. Plants which could not be measured showed characteristics of mixoploids (Table 4).

Selection for desired characters in genotypes originating from cv. Black Velvet Scarlet F1 (leaf coloration, flower size) was first carried out in the first seed generation obtained after pollinating fertile tetraploids with their blended pollen.

Table 4. Percentage of various ploidy levels in the amount of plants remaining in the colchiploid generation after preselection, determined by FCM analysis

Ploidy level	Black Velvet Scarlet F1	Gizela F1
Diploid	10.5	13.8
Tetraploid	64.3	65.6
Polyploid in total	75.5	82.1
Non-detected*	14.0	4.1

*% of plants unable to be detected involves samples which could not be analysed by FCM, they had mostly mixoploid-like habitus. 195 selected plants of cv. Gizela F1 and 143 plants of cv. Black Velvet Scarlet F1 were analyzed

CONCLUSIONS

Applying colchicine solutions in concentrations of 0.1%–2.5% on the apices of cotyledons for 2 to 7 days was very successful in inducing polyploidy apart from extremely mild or severe combinations of treatment. Tetraploids were the most frequent of all enhanced ploidy levels obtained (tetraploids, octoploids, hexadecaploids, mixoploids). Pre-selection made on the basis of subjective evaluation of morphological changes in combination with results of stomata measurement was relatively effective as confirmed by FCM. Tetraploid genotypes of cv. Black Velvet Scarlet F1 with retained fertility were used in further breeding of new brown-leaved tetraploid cultivars of *Pelargonium* × *hortorum*.

Acknowledgments

The authors are grateful to Prof. Dr. L. Hendriks and Prof. Dr. G. Schroeder from the Geisenheim Horticultural Research Center, Germany, and Doc. Ing. J. Doležel, DrSc., and Mgr. P. Suchánková from the Institute of Experimental Botany of AV ČR in Olomouc for permission to use the flow cytometry laboratories and for their helpful consultations.

References

Carolin R.C., 1954. Stomatal size, density and morphology in the genus Dianthus. Kew Bulletin, *9*: 251–258.

Gibby M., Hinnah S., Marais E.M., Albers F., 1996. Cytological variation and evolution within *Pelargonium* section *Hoarea* (*Geraniaceae*). Plant Systematics and Evolution, *203*: 111–142.

Grieger P., 2007. Untersuchungen zur Züchtung variegater *Pelargonium* × *zonale* Hybriden auf tetraploider Stufe (Investigation of breeding the variegated *Pelargonium* × *zonale* hybrids at the tetraploid level). [Ph.D. Thesis.] Berlin, Humboldt University: 129.

Hanzelka P., Kobza F., 2000. Results of indirect identification methods in polyploid plants of *Calistephus chinensis* NESS. Roczniki Akademii Rolniczej w Poznaniu 323, *1*: 39–44.

Jadrná P., Plavcová O., Kobza F., 2008. Toxicity of colchicine in polyploidization of *Pelargonium* × *hortorum* plants. In: Hejnák V., Skalický M. (eds.), Proceedings of the 1st Prague Plant Scientific Workshop, Prague: 62–65.

Jadrná P., Plavcová O., Kobza F., 2009. Polyploidization of *Pelargonium* × *hortorum* L.H. Bailey in greenhouse conditions. Horticultural Science (Prague), *1*: 32–38.

Li M., 2005. Anatomische, cytologische und histologische Untersuchungen zur somatischen Variation in verschiedenen Teilklonen von *Pelargonium zonale* `Kleiner Liebling`(Anatomical, cytological and histological investigation of somatic variation of the different clones of *Pelargonium zonale* 'Kleiner Liebling'). [Ph.D. Thesis.] Berlin, Humboldt University: 96.

Miller D., 1996. Pelargoniums. London, B.T. Batsford Ltd.: 175.

Plaschil S., 1997. Vergleichende Untersuchungen zur histogenetisch bedingten Sternmusterbildung in der Petalenfärbung bei *Camellia* L., *Myosotis* L., *Pelargonium* L'Herit., *Phlox* L., *Rhododendron* L., *Saintpaulia* H. Wendl., *Verbena* L. (Comparing proves of histogenetically determined pinwheel patterns in petals colourings of *Camellia* L., *Myosotis* L., *Pelargonium* L'Herit., *Phlox* L., *Rhododendron* L., *Saintpaulia* H. Wendl., *Verbena* L). [Ph.D. Thesis.] Berlin, Humboldt University, *21*: 33–35.

Plavcová, O. (2007). Leaf zonation of Pruhonice's brown leaved F1 varieties of *Pelargonium zonale* hort. In: Proceedings Strom a květina – součást života (The Tree and Flower – a Part of Life), September 4–5, 2007. VÚKOZ, v.v.i., Průhonice: 257–259.

Starý F., 1999. *Pelargonium*. Zahradnický slovník naučný (N–Q) (Horticultural Dictiontary). Prague, Ústav zemědělských a potravinářských informací: 243–244.

Received for publication July 8, 2009
Accepted after corrections December 8, 2009

Corresponding author:

Ing. Otka Plavcová, CSc., Silva Tarouca Research Institute for Landscape and Ornamental Gardening, (VÚKOZ, v.v.i.), Květnové náměstí 391, 252 43 Průhonice, Czech Republic
Phone: + 420 296 528 253, fax: + 420 267 750 023, e-mail: plavcova@vukoz.cz