Polyploidy effects on frost tolerance and winter survival of garden pansy genotypes

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ABSTRACT: This study was conducted to interpret the differences in frost tolerance and winter survival between 8*x*, 10*x*, 12*x*, 14*x*, and 16*x* ploidy levels of garden pansy (*Viola* × *wittrockiana* Gams) genotypes grown in the field conditions. Plants of each genotype were analyzed for their ploidy levels using flow cytometry. The chlorophyll fluorescence parameters were measured with portable chlorophyll fluorometer in the greenhouse and in the field at different time intervals. Increased frost stress generally reduced the fluorescence values in all genotypes. However, the genotypes differed significantly in their responses to frost as they were exposed to minimum temperatures of 1°C to –7.7°C in the field. Based on the percentage reduction in F_V/F_M values against –7.7°C temperature the hexadecaploids were ranked as sensitive to intermediate followed by 12*x* (sensitive), and genotypes with 10*x* and 14*x* ploidy levels were tolerant as the controls. The winter survival rate of hexadecaploids was by 7 to 9% lower than in the controls followed by the genotype with 12*x* and both genotypes with 10*x* and 14*x* ploidy levels were about equal to the controls. On the other hand, the content of photosynthetic pigments (chlorophyll *a*, *b* and total carotenoids) was the highest in hexadecaploids and tended to increase with increasing ploidy level. Further, the results gave insight that chlorophyll fluorescence could be applied directly in the field conditions to screen genotypes and select plants having higher frost tolerance in combination with improved aesthetic qualities.

Keywords: *Viola* × *wittrockiana*; garden pansy; induced polyploidy; photosynthetic pigments; flow cytometry; chlorophyll fluorescence; frost tolerance

Pansies (*Viola* × *wittrockiana* Gams) are a commercially important ornamental plant, grown and marketed during autumn and spring (Adams et al. 1997).

Two hexadecaploid (16*x*) genotypes were obtained by induced polyploidy in octoploid garden pansy cultivars (Ajalin et al. 2002). The hexadecaploids were selfed in advanced generations (Lagibo, Kobza 2004a,b) and used also for further breeding in order to exploit the induced desirable traits such as improved compactness and general vigour effect in plant height and in flower size. As a result, the genotypes with 2*n* = 10*x*, 2*n* = 12*x*, and 2*n* = 14*x* ploidy levels were produced from crosses and backcrosses which are included in this study.

According to Levin (1983), polyploidy, induced or natural, may greatly alter the cytological, genetic and physiological characteristics, and it often alters resistance to cold. Moreover, the effect of polyploidy on frost tolerance may vary from species to species and in some plants it has been demonstrated to show an increase or decrease with higher ploidy level. For instance, in *Brassica campestris*, autotetraploids were more resistant to frost than their diploid counterparts (Choudhury et al. 1968). On the other hand, Goral et al. (1964) found lower resistance of tetraploids to frost in *Trifolium repens* and higher resistance of tetraploids in *Trifolium pratense*.

In this study, in addition to induced polyploidy, since pansies are commonly planted in autumn for spring flowering, frost tolerance is one of the most important factors to be assessed in new genotypes. Therefore, this study was conducted to identify

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Table 1. Flow cytometric analysis of garden pansy genotypes

Genotype*	Ploidy level	CV (%)	Flower colour
PW	$2n = 8x$	2.56	pure white
LB	$2n = 8x$	2.59	light blue
MPW	$2n = 16x$	2.30	pure white
MLB	$2n = 16x$	2.29	light blue
H5BC1	$2n = 10x$	2.17	pure white
H3M3	$2n = 12x$	2.27	pure white
H7BC1	$2n = 14x$	2.37	mixture

*PW – Pure White cultivar, LB – Light Blue cv., both standard controls; MPW – pure white, MLB – light blue, both hexadecaploids (16*x*) of M_4 generation; H3M3 – offspring from the cross between 16*x* and 8*x*; H5BC1 and H7BC1 are progenies from backcrosses, CV – coefficient of variation in percent

differences in frost tolerance and winter survival between 8*x*, 10*x*, 12*x*, 14*x*, and 16*x* ploidy levels of garden pansy genotypes grown in field conditions and thereby to acquire information as to their usefulness for breeding purposes and also for practical use.

MATERIALS AND METHODS

Seeds of garden pansy genotypes with different polyploidy levels were chosen and used as plant material. Two hexadecaploids ($2n = 16x$) which were obtained from induced chromosome doubling and developed by selfing in subsequent generations, and genotypes with 10*x*, 12*x*, and 14*x* ploidy levels produced by crosses and backcross, and their corresponding two standard octoploid (8*x*) cultivars were used as controls (Table 1). Seeds were sown on August 15, 2003, in seed trays filled with a standard substrate, for spring flowering. Then the flats were placed in a greenhouse with a day temperature of 18–22°C and 12–16°C at night. The substrate was kept moistened until the seedlings emerged, and chemical protection against fungi and pests was carried out twice per week in the greenhouse. When the first 2 to 3 true leaves appeared, seedlings were transplanted into multi-cell trays, one seedling per cell. On October 7, well-established seedlings with 8 to 12 leaves were transplanted into the field. Three replications of 30 plants per plot and 90 plants per genotype were planted in a randomized block design.

The field preparation and cultural practices were conducted according to commonly accepted recommendations for garden pansy. Fertilizers were applied on the basis of soil sample test results.

Ploidy levels were analyzed and determined by flow cytometry (Partec PAS, Germany) at the Institute of Experimental Botany of the Czech Academy of Sciences at Olomouc. The procedures for sample preparation and analysis according to Doležel (1997) and Galbraith et al. (1998) were used. Unstressed leaves were collected from seedlings of each genotype in the greenhouse, wrapped in moistened paper tissue and put into plastic bags. For the isolation of nuclei, pieces of leaf stalks (approximately 20 mg) were chopped using razor blades in a Petri dish containing 0.5 ml of Otto I buffer (0.1M citric acid, 0.5% Tween 20). The homogenate suspension was filtered through a 50-µm nylon mesh filter to remove fragments and large tissue debris from samples. Then 1 ml of Otto II buffer (0.4 M Na_2HPO_4) containing 4 µg/ml DAPI (4.6-diamidino-2-phenylindole) was added to stain nuclear DNA. Prior to analysis, the gain of the cytometer was adjusted so that the G_1 peak of nuclei isolated from control octoploid plants was located on channel 100. This setting was kept constant during the analysis of samples and checked periodically. At least 2000 nuclei were counted for each sample and peaks representing G_1 nuclei (dominant peaks) were applied to determine the expected ploidy levels of the genotypes listed in Table 1.

The chlorophyll fluorescence parameters, minimal fluorescence (F_O), maximal fluorescence (F_M), the ratio of variable to maximal fluorescence (F_V/F_M which is equal to $(F_M-F_O)/F_M$) were measured using a portable chlorophyll fluorometer OS-30 (Opti-Sciences, Inc., USA), first in the greenhouse under (relatively) optimum conditions before transplanting and then in the field as the plants were exposed to low temperature regimes. Thirty plants from each genotype were randomly selected, marked to make repeated measurements at different time intervals on the same plant and to ensure comparability of measurements. The conditions and procedures for chlorophyll fluorescence measurements were described by Smillie and Hertherington (1990), Maxwell and Johnson (2000) and a quick reference guide of the manufacturer for OS-30 was also consulted and followed. To perform the measurements, leaves were dark adapted for thirty minutes using lightweight plastic leaf clips. The F_M values were measured at a saturating light intensity of 3,000 µmol/m^2/s. The data obtained from dark adapted leaves in the greenhouse and in the field were saved in the memory of OS-30 and then transferred to the computer for further analysis. The ratios of F_V/F_M were applied to quantify and identify the differences in frost tolerance between the genotypes according to Hakam et

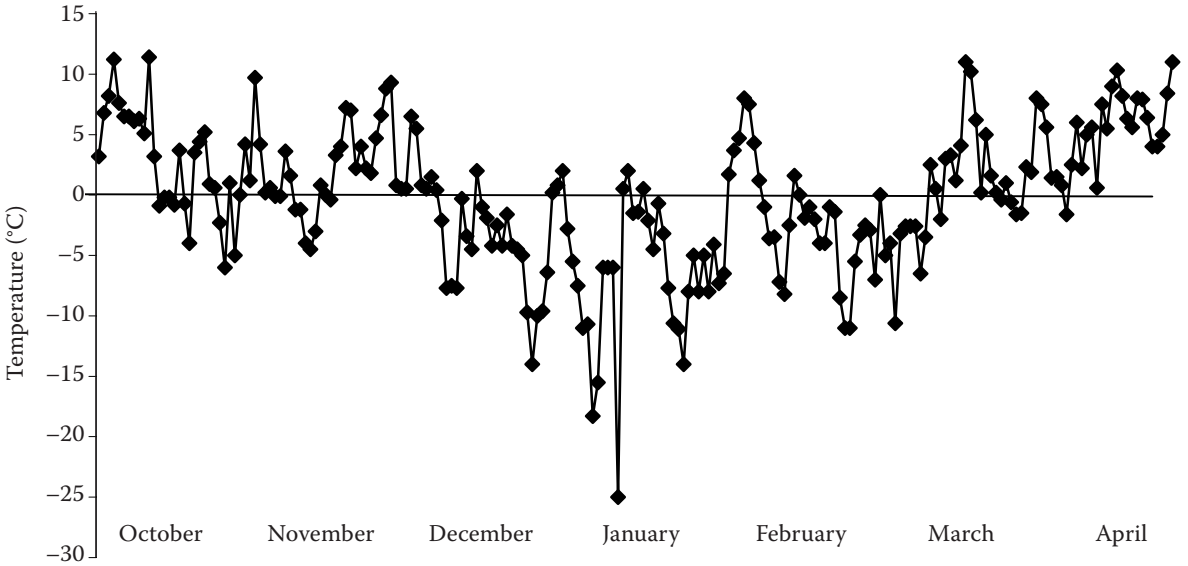

Fig. 1. Daily minimum temperatures during the field experiment conducted from October 2003 to April 2004 (Source: Mendel Research Station, Lednice) (temperatures measured with sensors located at 5 cm above the ground level)
Altitude 176 m a.s.l., longitude 16°48´E, latitude 48°40´N

al. (2000), Percival and Faser (2001). In addition, plants were assessed by a commonly used technique, visual rating before winter and in spring to estimate frost tolerance of the genotypes. Each plant was rated on a zero (0) to 5 scale where: no visible frost injury on plant leaves = 0 (very tolerant); 1–20% leaf injury = 1 (tolerant); 21–40% = 2 (intermediate to tolerant); 41–60% = 3 (intermediate); 61–80% = 4 (intermediate to sensitive) and above 81% = 5 (sensitive). Finally, the mean values for each genotype were compared with their corresponding control cultivar and the significance of differences was tested by analysis of variance (ANOVA) at $\alpha = 0.05$ (*) and $\alpha = 0.01$ (**).

Content of chlorophyll (Chl) *a*, *b* and total carotenoids were determined on a Jenway 6100 spectrophotometer from pigment extracts in 80% acetone according to Lichtenthaler (1987). The samples were filtered, homogenized and the absorbance spectra of the extract solution samples were measured at 662 nm for Chl *a*, at 644 nm for Chl *b* and at 440 nm for carotenoids, then the results were calculated in order to determine the photosynthetic pigment concentration in the genotypes.

Plants were counted in the field on December 8 before winter and on March 26 in spring to determine the survival rate. Moreover, the genotypes were evaluated for flowering time, plant height and dimensions, compactness, flower size and overall appearance according to common methods in the peak growing period in spring to early summer.

RESULTS AND DISCUSSION

The ploidy levels of the genotypes were analyzed and determined by the analysis of the relative nuclear DNA content using flow cytometry (Table 1). The nuclei isolated from control octoploid plants (with known ploidy level) were used as a reference or standard. Peaks representing G_1 nuclei (dominant peaks) were applied to verify ploidy levels. Histograms of the flow cytometric analysis of relative nuclear DNA content in garden pansy genotypes are shown in Fig. 2. There was no important variation in the relative nuclear DNA content and in the histograms obtained from both standard octoploid cultivars and also both hexadecaploid genotypes. Thus, the two octoploid controls and two hexadecaploids are represented by one histogram in Fig. 2. Further, flow cytometry was found to be a suitable tool and provided a rapid and accurate analysis of expected ploidy levels in garden pansy genotypes.

Temperatures were recorded with thermometer located at 5 cm above the ground level. The daily minimum temperatures during the field experiment period are shown in Fig. 1.

Chlorophyll fluorescence parameters were measured on dark adapted leaves in the greenhouse and in the field at different time intervals. Mean values for the ratio of variable to maximal fluorescence (F_V/F_M) are contained in Table 2. The mean F_V/F_M values ranged from 0.61 to 0.78 in the greenhouse (12°C) and in the field as the temperature rose (6°C, 11°C) in spring. Thus, during these periods, when the

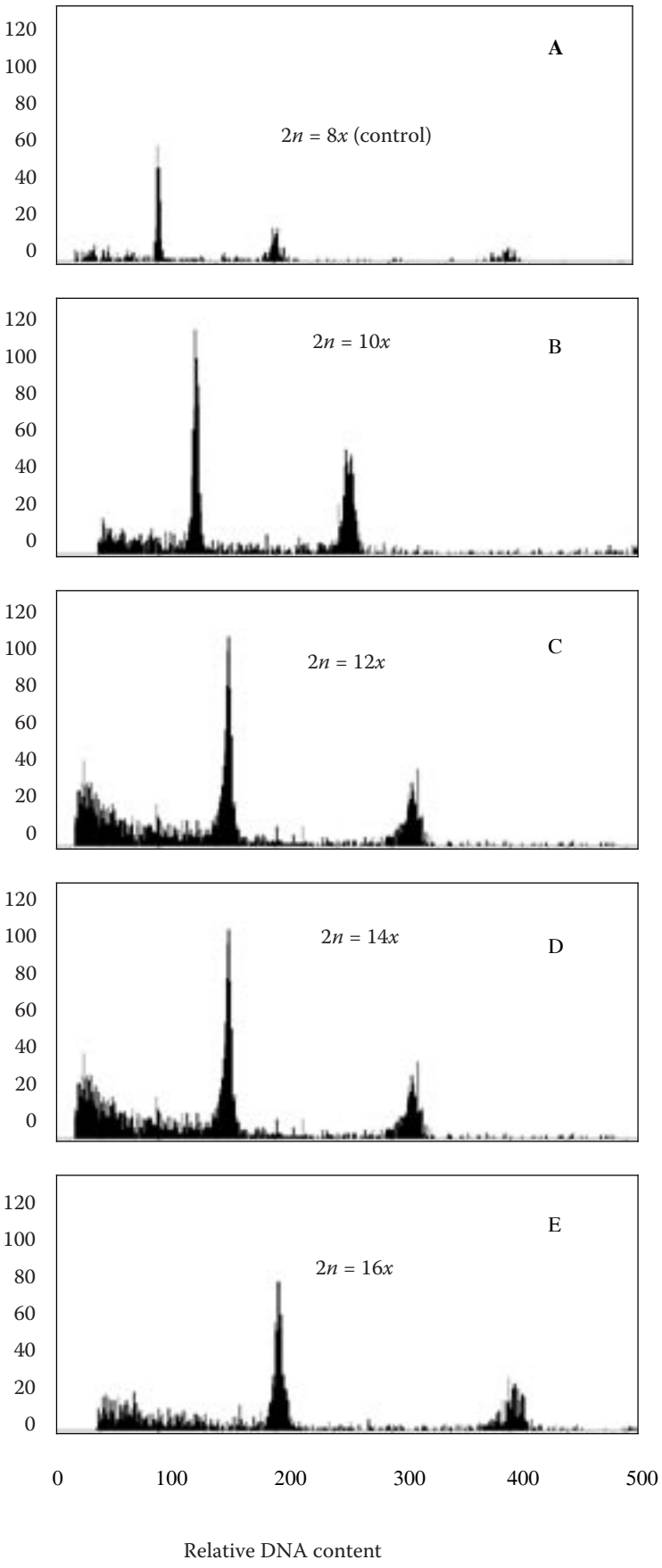

Fig. 2. Histograms of flow cytometric analyses of relative nuclear DNA content in garden pansy genotypes (A – controls PW and LB; B – genotype H5BC; C – genotype H3M3; D – genotype H7BC1; E – hexadecaploid genotypes MPW and MLB)

plants were in relatively optimum conditions in the greenhouse and as the temperature rose in spring, there was no significant difference between the genotypes. The F_V/F_M ratio decreased gradually as the plants were exposed to low or below freezing temperatures of 1°, –0.5°, –1°, –3°, and –7.7°C (Table 2). In general, low to below freezing temperatures caused an obvious reduction in the F_V/F_M values in all genotypes. However, the genotypes differed significantly ($p \leq 0.01$) as the plants were exposed to low non-freezing to below freezing temperatures between 1°C and –7.7°C. The two control cultivars are known as frost tolerant and there was no significant difference between the mean F_V/F_M values except for the measurement on November 13 ($p = 0.043$). The mean F_V/F_M values were used to identify the differences in frost tolerance between the genotypes. To simplify the comparison, only the mean F_V/F_M values with significant differences which were measured at low or below freezing temperatures of 1°, –0.5°, –1.2°, –3°, and –7.7°C were considered in Table 2. The scale for ranking according to Percival and Faser (2001) is shown in Table 3. The mean F_V/F_M values of control genotypes were used as a base and the mean F_V/F_M values of other genotypes were converted into percentage in order to compare the reduction from the control (Table 4). Based on the percentage reductions in F_V/F_M values against –7.7°C, below freezing temperature on December 8, hexadecaploid genotype MLB showed the highest percentage reduction (31%) from control genotype LB followed by genotype H3M3 (30%) and genotype H5BC1 showed the lowest re-

Table 2. Mean values for the ratio of variable to maximal fluorescence ($F_V/F_M = (F_M–F_O)/F_M$) as an indicator of frost tolerance in garden pansy genotypes

Year/Date 2003/2004	Min. temp. (°C)	Ratio of variable to maximum fluorescence (F_V/F_M)						
		Genotypes						
		PW[1] (8*x*)	LB[2] (8*x*)	H5BC1 (10*x*)	H3M3 (12*x*)	H7BC1 (14*x*)	MPW (16*x*)	MLB (16*x*)
October 05[g]	12.0	0.78 ns	0.77 ns	0.77 ns	0.77 ns	0.78 ns	0.77 ns	0.78 ns
November 09	–1.2	0.58 ns	0.57 ns	0.57 ns	0.49**	0.58 ns	0.53**	0.53**
November 13	–3.0	0.48*	0.46*	0.46 ns	0.41**	0.46 ns	0.45**	0.42**
December 08	–7.7	0.30 ns	0.29 ns	0.29 ns	0.21**	0.28 ns	0.24**	0.20**
March 27	–0.5	0.47 ns	0.45 ns	0.45 ns	0.41**	0.46 ns	0.42**	0.39**
April 02	1.0	0.56 ns	0.56 ns	0.53*	0.51**	0.55 ns	0.55 ns	0.51**
April 14	6.0	0.62 ns	0.62 ns	0.61 ns	0.61 ns	0.63 ns	0.61 ns	0.61 ns
April 30	11.0	0.75 ns	0.76 ns	0.77 ns	0.77 ns	0.76 ns	0.76 ns	0.76 ns

[1] and [2] – octoploid controls used for comparison: PW – control for MPW, H5BC1, H3M3; LB – control for MLB, H7BC1; * and ** – significant difference between the means at $p \leq 0.05$ and $p \leq 0.01$, respectively; ns – not significant by ANOVA, [g] – the mean F_V/F_M values measured in the greenhouse before transplanting

Table 3. Scale for the ranking of frost tolerance of garden pansy genotypes based on the percentage reduction in F_V/F_M values from the control

Percentage reduction in F_V/F_M values from control (%RC)[x]	Frost tolerance rank (TR)
0 (zero percent reduction from control)	0 = very tolerant (VT)
1–5	1 = tolerant (T)
6–10	2 = intermediate to tolerant (I-T)
11–15	3 = intermediate (I)
16–20	4 = intermediate to sensitive (I-S)
≥ 21	5 = sensitive (S)

[x] – control cultivars in Table 2 considered as frost tolerant and the percentage reduction in F_V/F_M values from the control used for the ranking of frost tolerance of the other genotypes

Table 4. Ranking of frost tolerance of garden pansy genotypes based on the percentage reduction in F_V/F_M values from the control

Genotype	Temperatures (°C)										Mean %RC	Total TR
	–7.7		–3.0		–1.2		–0.5		1.0			
	%RC	TR	%RC	TR	%RC	TR	%RC	TR	%RC	TR		
PW[1]	(0.30)	T	(0.48)	T	(0.58)	T	(0.47)	T	(0.56)	T	(0)	T
MPW	20.0	I-S	6.3	I-T	8.6	I-T	10.6	I	1.8	T	9.5	I-T
H5BC1	3.3	T	4.2	T	1.7	T	4.3	T	5.4	T	3.8	T
H3M3	30.0	S	14.6	I	15.5	I-S	12.8	I	8.9	I-T	16.4	I-S
LB[2]	(0.29)	T	(0.46)	T	(0.57)	T	(0.45)	T	(0.56)	T	(0)	T
MLB	31.0	S	8.7	I-T	7.0	I-T	13.3	I	8.9	I-T	13.8	I
H7BC1	3.5	T	0.0	VT	(+1.8)	VT	(+2.2)	VT	1.8	T	0.26	T

%RC – percentage reduction in F_V/F_M values from the control, TR – frost tolerance rank (see Table 3); [1] and [2] – octoploid controls with their mean F_V/F_M values from Table 2 considered as a base for percentage conversion and comparison for the other genotypes below them

Table 5. Visual evaluation of garden pansy genotypes for frost tolerance

Genotype	06 Dec. 2003 (–2.3°C)		26 Mar. 2004 (–0.1°C)	
	Rating[x]	TR	Rating[x]	TR
PW	1.34[ns]	T	1.31[ns]	T
LB	1.41[ns]	T	1.36[ns]	T
H5BC1	1.31[ns]	T	1.35[ns]	T
H3M3	4.09**	I-S	4.20**	I-S
H7BC1	1.27[ns]	T	1.21[ns]	T
MPW	3.81**	I-S	4.01**	I-S
MLB	4.03**	I-S	4.51**	S

[x] – rating on a zero to 5 scale (see Materials and Methods), TR – frost tolerance rank; ** significant difference between the means at $p \leq 0.01$; ns – not significant by ANOVA

Table 6. Winter survival in garden pansy genotypes[a]

Genotype	Ploidy level	Number of plants		Mean (%) WS[b]
		before winter	after winter	
PW[c]	$2n = 8x$	90	87	96.7[ns]
LB[c]	$2n = 8x$	90	85	94.4[ns]
MPW	$2n = 16x$	90	81	90.0*
MLB	$2n = 16x$	90	78	86.7*
H5BC1	$2n = 10x$	90	86	95.6[ns]
H3M3	$2n = 12x$	90	79	87.8*
H7BC1	$2n = 14x$	90	86	95.6[ns]

[a] – plants were counted before and after winter in the field; [b] – winter survival rate (WS) plot means in percent compared for significance of difference; * statistically significant; ns – not significant at $p \leq 0.05$ by ANOVA; [c] – both octoploid controls compared with each other

duction (3.3%) from control genotype PW. Based on the percentage reduction in F_V/F_M values from the control against –7.7°C (the lowest temperature of all measurements) in Table 2, genotype MLB was more sensitive followed by genotype H3M3 (sensitive), MPW (intermediate to sensitive) and genotypes H5BC1 and H7BC1 were tolerant as the control (Table 4). The percentage reduction for genotype H7BC1 against –3.3°C temperature on November 13 was zero percent indicating exactly the same tolerance as the control and even +2% against –1.2°C and –0.5°C temperatures possibly having higher frost tolerance than the control.

Autumn (2003) was characterized by mild and fluctuating temperatures followed by a slight frost especially in late autumn (–1 to –5°C). Winter started without snow, but low temperatures in December up to –15°C and even the temperature drops as low as –25°C and snow cover up to 15 cm were recorded in January. Pansies tolerate some frost, however mild or warm temperatures in late autumn, followed by a slight frost (–1 to –2°C) often severely damage or kill plants in the field (Armitage 1994). In seed catalogues and in magazines for growers it is also usually stated that slight frost can be more dangerous if the plants are not protected by mulch or covered by adequate snow in the field. In fact, autumn planted pansies can tolerate frost if temperatures in autumn decrease gradually to allow them better hardening off for winter survival.

In the presented field experiment study, plants survived the temperature drops as low as –25°C in January and overwintered well in the field. However, genotypes varied in winter survival rates. Mean winter survival rates ranged from 86.7% for genotype MLB (16*x*) to 96.7% for octoploid control genotype PW (Table 6). The difference in winter survival rates between hexadecaploid genotypes and their corresponding octoploids was statistically significant ($p < 0.05$) whereas the difference between the two control octoploid genotypes was not significant. Information on winter survival and general performance of induced polyploids, especially for higher ploidy levels, above hexaploids and for advanced generations is too limited in literature. According to Smith (1946) Lewis (1980) reported for *Sedum pulchellum* that hexaploids had the best survival rates in a competitive plot experiment, the diploids had the poorest survival rate and the tetraploids were intermediate. However, the experiment was based on the comparison of the cytotypes over a limited period of time and the winter survival was not mentioned.

Several studies suggest that chlorophyll fluorescence parameters provide a rapid and reliable technique of detecting and quantifying plant tolerance to environmental stress, to temperatures (heat, cold or frost, freezing, chilling), water stress (drought or water logging), salinity, etc. Chlorophyll fluorescence is widely used to screen and to compare the chilling tolerances of different species, cultivars or crops, for example maize (*Zea mays* L.), cassava (*Manihot esculenta*), rice (*Orzya sativa* L.), peanut (*Arachis hypogaea* L.), cucumber (*Cucumis sativus*) and under conditions of continuous chilling, fluorescence eventually declines to zero (Smillie, Hertherington 1990). Chlorophyll fluorescence has been used to detect stress in pot plants (Harbinson 1995), to assess chilling tolerance in rose genotypes (Hakam et al. 2000), to identify differences in salinity and freezing tolerance between *Crataegus* species (Per-

Table 7. Content of photosynthetic pigments in garden pansy genotypes

Genotype		Pigments in (μg/g/ FW)[x]		
		Chl *a*	Chl *b*	carotenoids
PW	mean	828 ± 0.005	359 ± 0.005	459 ± 0.019
(2*n* = 8*x*)	SD	0.027	0.025	0.107
LB	mean	832 ± 0.010	367 ± 0.003	447 ± 0.022
(2*n* = 8*x*)	SD	0.056	0.018	0.119
MPW	mean	914 ± 0.004**	548 ± 0.009**	570 ± 0.007**
(2*n* = 16*x*)	SD	0.023	0.052	0.036
MLB	mean	892 ± 0.008**	490 ± 0.006**	530 ± 0.024**
(2*n* = 16*x*)	SD	0.044	0.031	0.132
H5BC1	mean	870 ± 0.008**	377 ± 0.001*	482 ± 0.008[ns]
(2*n* = 10*x*)	SD	0.024	0.005	0.024
H3M3	mean	885 ± 0.003**	382 ± 0.003**	490 ± 0.001*
(2*n* = 12*x*)	SD	0.009	0.01	0.004
H7BC1	mean	886 ± 0.009**	394 ± 0.001**	496 ± 0.004**
(2*n* = 14*x*)	SD	0.028	0.004	0.013

[x] – mean values for pigment concentration in μg per gram of fresh weight (FW) of leaves; * and ** – significant difference between the means at $p \leq 0.05$ and $p \leq 0.01$, respectively; ns – not significant by ANOVA; SD – standard deviation; ± standard error

CIVAL, FASER 2001), and to identify drought tolerant woody perennials (PERCIVAL, SHERIFFS 2002).

Because polyploidy alters biochemistry and also influences photochemical efficiency of a plant, it was of interest to know the status of photosynthetic pigments in the genotypes. The content of photosynthetic pigments (Chl *a*, *b* and carotenoids) was the highest in both hexadecaploid genotypes and tended to increase with increasing ploidy level (Table 7). WARNER et al. (1987) investigated changes in leaf anatomy and biochemical activities in the photosynthetic cells of *Panicum virgatum*, and the chlorophyll content in octoploid plants was 40 to 50% higher than in tetraploid plants.

In this study, the genotypes with 16*x* ploidy level had significantly lower frost tolerance ($p < 0.01$) and lower winter survival rates ($p < 0.05$) than their corresponding octoploid cultivars. Lower frost tolerance of hexadecaploid genotypes confirms the results of GORAL et al. (1964), who reported lower frost resistance of tetraploids in *Trifolium repens* as compared to their diploid counterparts. Although hexadecaploids (2*n* = 16*x*) were employed as parent lines in the crossings and backcross, the polyploids used in this study were not produced in the same way. Thus, the gene flow from parents to offspring and the effects would not be expected to be strictly similar to the other referred studies. An increase or decrease in frost tolerance and winter survival rates did not follow the range of ploidy levels in assessed genotypes. When overall frost tolerance was evaluated from mean percentage reductions in F_V/F_M values, genotype H3M3 (12*x*) was ranked as intermediate to sensitive followed by genotype MLB (intermediate) and MPW (intermediate to tolerant). The genotypes with 10*x* and 14*x* ploidy levels were tolerant as the controls. Lower frost tolerance and also lower winter survival rate that occurred in genotype H3M3 (12*x*) might be due to a hybridization effect and on the contrary, a possible reason for higher frost tolerance and higher winter survival rates in genotypes with 10*x* and 14*x* ploidy levels could be explained as an indication of restored or improved performance through consecutive crossing and backcross effects.

The results of the F_V/F_M value based ranking for frost sensitivity also highly agreed with visual assessment of frost injury on leaves made before winter and in spring, by rating the plants in the field (Table 5). Slight variations in the frost tolerance ranking of the genotypes observed in Tables 5 and 4, between visual assessment and F_V/F_M value based ranking, could be due to the subjectivity of visual rating. Chlorophyll fluorescence helped to assess, quantify and determine the differences in frost tolerance between the genotypes with increased certainty. The suitability of chlorophyll fluorescence to detect stress tolerance or sensitivity was confirmed and suggested by

many authors. The advent and recent introduction of portable fluorometers have led to an upsurge in the use of chlorophyll fluorescence directly in the field conditions while the measurements are non-destructive, rapid and reliable to screen genotypes for frost tolerance (Maxwell, Johnson 2000). In this study, the decrease in F_V/F_M values of all genotypes with decreasing temperatures is similar to other crops reported by Smillie and Hertherington (1990). In general, the results agree with the description made by Hakam et al. (2000), who reported the suitability of chlorophyll fluorescence to quantify and compare differences in chilling tolerance between rose genotypes and similarly Percival and Faser (2001), who applied the F_V/F_M value based ranking in *Crataegus* species.

SUMMARY

The effect of polyploidy on frost tolerance and winter survival was assessed in garden pansy genotypes with 8*x*, 10*x*, 12*x*, 14*x*, and 16*x* ploidy levels grown in the field conditions. Chlorophyll fluorescence parameters were measured in a greenhouse and in the field at different time intervals in order to determine the frost tolerance of the genotypes. The mean F_V/F_M values of the plants in the greenhouse remained at a level of 0.78 and the difference between the genotypes was not significant. The F_V/F_M values decreased gradually during late autumn to the winter onset and increased stepwise in spring in the field conditions. In general, very low to below freezing temperatures caused an obvious reduction in the F_V/F_M values in all genotypes. However, the genotypes varied significantly in their responses to frost as they were exposed to minimum temperatures of 1° to –7.7°C in the field. Based on the reduction in F_V/F_M values against –7.7°C temperature the hexadecaploids were ranked as sensitive to intermediate followed by genotype 12*x* (sensitive). Genotypes with 10*x* and 14*x* ploidy levels were tolerant as the controls. In addition to this, the increase in F_V/F_M values differed significantly between the genotypes in spring as the plants started to recover from frost damage. The winter survival rate of hexadecaploids was by 7 to 9% lower than in the octoploid controls followed by the genotype with 12*x* ploidy level and genotypes with 10*x* and 14*x* ploidy levels were the same as the controls. On the other hand, the content of photosynthetic pigments (Chl *a*, *b* and carotenoids) was the highest in the hexadecaploids and tended to increase with increasing ploidy level. Furthermore, the results suggested that chlorophyll fluorescence could be applied directly in the field conditions to screen genotypes and select plants having better frost tolerance in combination with desirable aesthetic qualities.

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Vliv polyploidie na odolnost zahradních macešek vůči mrazu a na přezimování

ABSTRAKT: Studie je zaměřena na prezentaci rozdílů v odolnosti k mrazu a přezimování genotypů zahradních macešek (*Viola* × *wittrockiana* Gams) v polních podmínkách s 8*x*, 10*x*, 12*x*, 14*x*, a 16*x* úrovní ploidie. Rostliny z každého genotypu byly analyzovány na úroveň ploidie pomocí průtokové cytometrie. V časových intervalech byly měřeny hodnoty chlorofylové fluorescence pomocí přenosného chlorofylového fluorometru ve skleníku a na polním záhonu. Zvýšený mrazový stres obecně snížil hodnotu fluorescence u všech genotypů. Přesto genotypy průkazně odlišně reagovaly na mráz po vystavení teplotám od 1 °C do –7,7 °C v polních podmínkách. Při vycházení z redukce hodnot F_V/F_M byly hexadecaploidní genotypy hodnoceny jako citlivější a středně citlivé a byly následovány 12*x*; genotypy s 10*x* a 14*x* úrovní ploidie byly v odolnosti blízké kontrolním (8*x*). Na druhé straně obsah fotosyntetických pigmentů (chlorofylu *a, b* a karotenoidů) byl nejvyšší u hexadecaploidů se zřetelnou tendencí zvyšování s narůstající hodnotou ploidie. Výsledky získané měřením fluorescence chlorofylu mohou být aplikovány přímo v polních podmínkách pro srovnání genotypů a selekci rostlin s vyšší mrazovou tolerancí v kombinaci s hodnotou estetické kvality.

Klíčová slova: *Viola* × *wittrockiana*; zahradní maceška; indukovaná polyploidie; fotosyntetické pigmenty; průtoková cytometrie; fluorescence chlorofylu; mrazuvzdornost genotypů

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