ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 109, No. 4 (2022), p. 373–382
DOI 10.13080/z-a.2022.109.048

Induction and analysis of polyploids in daylily (*Hemerocallis* L.) plants

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Abstract

Daylily (*Hemerocallis* L.) is a popular herbaceous perennial ornamental plant. The American Hemerocallis Society (AHS) currently has over 96,000 cultivars registered. Of these, half are tetraploids (4x), and the other half are diploids (2x). To enrich the breeding lines of tetraploid daylilies, colchicine is the most widely used. During the experiment, oryzalin (ORZ) and colchicine (CLC) were used for the polyploidisation of daylilies. Germinating seeds derived from diploid and tetraploid daylily cultivars as maternal plants were exposed to solutions of ORZ and CLC at different concentrations. Ploidy of seedlings was determined after six months by flow cytometry, and the obtained results were related to the size of the stomata. Chimerism of the young side shoots developing on treated plants was assessed three years after polyploidisation by checking the ploidy using a flow cytometer. It has been found that whole-genome duplication is not common in daylilies during polyploidisation of germinating seeds – triploids (3x), pentaploids (5x), hexaploids (6x), and heptaploids (7x) were obtained. The optimal concentration of CLC for induction higher ploidy was 125–250 µmol when exposed to seeds of the diploid maternal plant (DMP) and 250–500 µmol when exposed to the tetraploid maternal plant (TMP) one. In the case of ORZ, optimal treatments were 10 µmol for germinating DMP seeds and 40 µmol for TMP seeds, respectively. Stomata size can preliminarily determine daylily ploidy. A flow cytometry should be used for a more accurate determination of ploidy. Individuals treated with antimitotic agents are prone to chimerism. The most extensive ploidy variation observed in individuals with uneven ploidy is usually in the direction of even ploidy. Therefore, the ploidy of clones used for breeding should be checked regularly.

Keywords: polyploidy, flow cytometry, stomata size, chimerism.

Introduction

Daylily (*Hemerocallis* spp.) is a herbaceous perennial ornamental plant widely cultivated worldwide (Gulia et al., 2009). The genus *Hemerocallis* is native to East Asia, with 15 to 19 species (Li et al., 2021). It is assumed that the genetic diversity of modern breeds has constantly been through breeding using interspecific crosses and changing the ploidy level. The species are usually diploid, except for some triploids, which are typically sterile (Tomkins et al., 2001).

During the experiment, germinating seeds were for the first time exposed to oryzalin (ORZ) in addition to colchicine (CLC). So far, this method has used only CLC to polyploidise daylilies. CLC is highly toxic to animal cells, has anticancer effects and is used in chemotherapy to prevent the growth of cancer cells in medicine (Sivakumar et al., 2017). ORZ is used as an alternative to CLC – it is less toxic to animals than CLC. Its action depends more on binding plant tubulins (Miguel, Leonhardt, 2011). Antimitotic agents can act as mutagens and induce various genetic and epigenetic variations resulting in morphological and cytological changes. These changes may be related to the breakdown of metabolites during synthesis or by rearrangement to form secondary metabolites during potential mutations (Samadi et al., 2022).

In nature, daylily species are usually diploid and have 22 chromosomes (Stout, 1934; Brennan, 1992; Plodeck, 2002; Zhiwu et al., 2009). Wild daylilies evaluated in China's Tai Gang Mountain region had diploid and triploid sets of chromosomes (Zhang et al., 2013b); wild tetraploid daylilies have not been found. Tetraploid (4x = 44 chromosomes) daylilies were obtained by doubling the chromosome sets of diploid individuals using antimitotic agents such as CLC (Tomkins et al., 2001; Zhiwu et al., 2009) or trifluridine (Li et al., 2018). When comparing diploid and induced tetraploid daylilies, it is observed that tetraploid scapes are more compact and have fewer flower buds, but the scapes are thicker

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Misiukevičius E., Stanys V. 2022. Induction and analysis of polyploids in daylily (*Hemerocallis* L.) plants. Zemdirbyste-Agriculture, 109 (4): 373–382. https://doi.org/10.13080/z-a.2022.109.048

and more robust. Tetraploids are characterised by larger flowers. They have wider and longer leaves and a higher concentration of chlorophyll *a* than diploid plants (Zhang et al., 2013c; Podwyszyńska et al., 2015).

Current breeding programs focus on crossing tetraploid daylily using a limited number of genotypes (Tomkins et al., 2001; Zhiwu et al., 2009). As a result, the crossbreeding results do not exhibit a large variety of unique characteristics (Sakhanokho et al., 2004; Zhiwu et al., 2010). The most effective way to assess polyploidisation is seed treatment with antimitotic agents, but seeds must be at the germination stage with the maximum number of dividing cells (Griesbach et al., 1963). Because of the asynchrony of mitosis in meristematic tissues, antimitotic agent exposure to the whole plant usually results in chimeric individuals (Arisumi, 1964). Daylilies are now polyploidised *in vitro*, and homogeneous tetraploids, mixoploids, and partial chimeras are obtained (Podwyszyńska et al., 2011; 2015).

The aim of the study is to determine the response of daylily plants of different ploidy to the exposure to antimitotic agents.

Material and methods

Plant material. The experiment was carried out at the Lithuanian Research Centre for Agriculture and Forestry in 2018–2021. For the research, daylily (*Hemerocallis* L.) seeds of diploid (2x) maternal plants (DMP) and tetraploid (4x) maternal plants (TMP) were used. Before the exposure to antimitotic agents, seeds of diploid ('Carnelian Chameleon' × various diploid parental individuals) and tetraploid ('Tom and Doug' × different tetraploid parental individuals) daylilies were stratified for four months in a refrigerator (2–4°C). In the experiment, three replications were used. Approximately 600 DMP and 600 TMP seeds were used. Germinating seeds were batched in three replications with the same number of seeds in each treatment, because not all seeds were viable and germinated unevenly.

Polyploidisation using antimitotic agents. Antimitotic agents used for polyploidisation were oryzalin (ORZ) and colchicine (CLC) at concentrations of 5, 10, 20, and 40 µmol and 62.5, 125, 250, and 500 µmol, respectively. The seeds were germinated in Petri dishes on moistened filter paper. The seeds were treated with antimitotic agents after the appearance of the coleoptile (Figure 1).

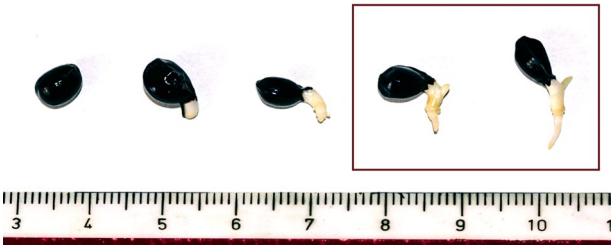


Figure 1. The stage of seed germination suitable for antimitotic agents

Exposure to antimitotic agent solutions was for 25 hours. After that, the seeds were washed under running water for 4 hours and planted one by one in pots filled with a substrate consisting of sand, 3 mm perlite, and garden soil (5:5:1). When planting, only the primary root was covered with the substrate leaving the root neck level with the substrate layer in the pot. Seedlings were grown in a greenhouse at a temperature of 20 ± 2°C, and after the threat of frost was over, the plants were planted in beds outside. Morphological parameters – the length and number of leaves – were assessed one and six months after exposure to antimitotic agents.

Determination of ploidy. To determine the ploidy of individuals, the flow cytometry method with Partec (Germany) equipment was used. Then 0.05 g of fresh leaves was weighed and chopped using a razor-sharp knife in small Petri dishes after pouring 500 µL of CyStain UV (Sysmex, Germany) dye. Another 1500 µL of dye were added and kept at room temperature for 5 minutes. The mixture was filtered through 50 µm filter meshes removing unbroken tissue particles and used for ploidy analysis. For each analysis, approximately 15,000 cells were counted. Individuals of the DMP and TMP seedlings that were not subjected to the treatment were used as the control. Flow cytometer readings were standardised to diploid, tetraploid, and pentaploid (5x) cultivars of known ploidy. For others, ploidy was determined by peak shift. The chimerism of polyploidised daylily seedling clones was determined by assessing the ploidy of all shoots of each clone in leaf tissue after three years since the treatment.

Determining the size of the stomata. The apical part of the leaf was studied. The epidermis layer is peeled from the lower side of the leaf. The tissue on an objective slide covered with a coverslip in a drop of water was evaluated with a microscope Nikon Y-IDP (Japan) with 30x magnification and an eyepiece with a scale. Stomata were measured in two fields of view of five stomata each measuring the length and width.

Statistical analysis. The data of the observations were evaluated using the Microsoft Office package Excel. Means and standard deviations ($\bar{x}$ ± SD) of seedling growth and development along with stomata size were calculated. The results of seedling survival were evaluated by variance analysis of qualitative characteristics using ANOVA (from the package Excel). To determine the tendency of stomata size and ploidy, the program DARwin.6 (Perrier, Jacquemoud-Collet, 2006) of difference principal coordinate analysis (PCA) was used.

Results and discussion

Effects of antimitotic agents. Under the influence of antimitotic agents, survival decreased and the plant's length of leaves changed. However, the number of leaf changes was insignificantly different. It was observed that different antimitotic agents acted differently, and their action depended on the ploidy of the plant. ORZ was less toxic than CLC (Table 1).

One month after exposure, 75% of the DMP seedlings survived. After using CLC, this percentage decreased. After a few months of exposure, all DMP seedlings exposed to solutions of the highest CLC concentration died. This concentration for TMP was also toxic with more than 8% of seedlings surviving. The survival rate of DMP seedlings exposed to ORZ in the first month was similar to the control. After two months, it steadily decreased to 29.4%. After two and six months,

Table 1. The survival rate of daylily seedlings exposed to antimitotic agents

Antimitotic agent	Concentration µmol	Plants examined	Survival %		
			after 1 month	after 2 months	after 6 months
Diploid maternal plants (DMP) seedlings					
Colchicine (CLC)	62.5	34	47.1 g	29.4 fg	17.6 gh
	125	34	47.1 g	17.6 hi	17.6 gh
	250	32	43.8 h	12.5 ij	12.5 hi
	500	32	62.5 f	0.0 k	0.0 j
Oryzalin (ORZ)	5	32	75.0 e	31.3 fg	31.3 f
	10	34	76.5 e	23.5 gh	17.6 gh
	20	34	64.7 f	29.4 fg	23.5 fg
	40	34	64.7 f	35.3 f	17.6 gh
Control	Water	32	75.0 e	56.3 e	50.0 e
Tetraploid maternal plants (TMP) seedlings					
Colchicine (CLC)	62.5	42	95.2 ab	71.4 cd	71.4 c
	125	46	73.9 e	56.5 e	52.2 e
	250	48	83.3 d	25.0 gh	20.8 g
	500	48	54.2 g	8.3 j	8.3 i
Oryzalin (ORZ)	5	46	100.0 a	87.0 b	82.6 b
	10	46	91.3 bc	69.6 d	65.2 cd
	20	46	87.0 cd	65.2 d	65.2 cd
	40	46	95.7 ab	78.3 bc	60.9 d
Control	Water	44	100.0 a	95.5 a	90.9 a

Note. Data are presented as means; numbers in the same column followed by different letters are significantly different at $p \leq 0.01$.

the TMP seedlings exposed to ORZ survived significantly more.

Antimitotic agents at lower concentrations did not strongly affect plant growth, while higher concentrations had a lethal impact with CLC. ORZ was less toxic than CLC, and plants restored homeostasis more easily and quickly after exposure to the antimitotic agents. The more significant toxicity of CLC can be explained by its sufficiently pronounced mutagenic properties (Sattler et al., 2016). The primary mechanism of CLC is binding to alpha and beta tubulin dimers, which inhibit microtubule polymerisation in plant cells during mitosis and stop chromatid migration during anaphase (Manzoor et al., 2019). The exact chromosome mechanism is still unclear (Zhou et al., 2017). Effective concentrations of ORZ were 50–250 times lower than those of CLC (Eng, Ho, 2019).

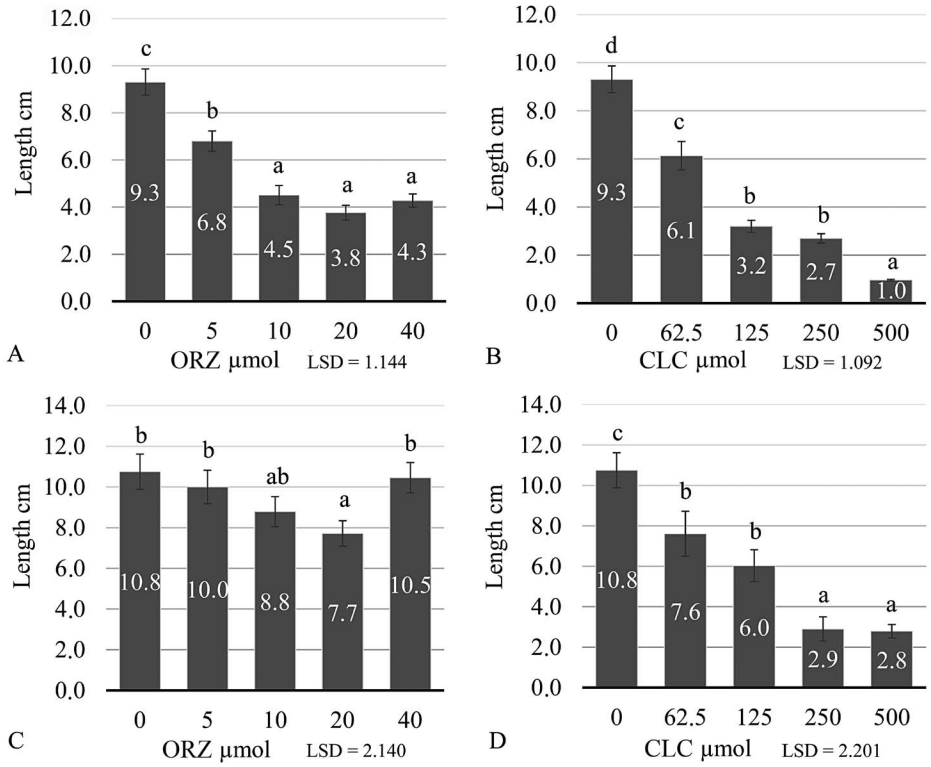
One month after exposure, CLC-exposed plants developed shorter leaves in both DMP and TMP seedling groups (Figure 2B and 2D). The effect of ORZ on the length of leaves of both DMP and TMP groups of plants was uneven. Under the influence of ORZ, the length of the leaves of the group of DMP plants was more clearly shortened than that of TMP (Figure 2A and 2C). The leaf length of DMP individuals exposed to 10 µmol ORZ has significantly shortened (Figure 2A). The appearance of TMP seedlings and the morphological parameters observed even when exposed to the highest concentration of 40 µmol ORZ did not differ from the control variant or the lowest concentration (Figure 2C). This may be due to gene redundancy, which protects polyploids from the deleterious effects of mutations (Comai, 2005). Antimitotic agents did not affect the number of leaves.

Six months after the exposure (at the end of vegetation), when the seedlings were already growing in beds in the field, the stimulating effect of antimitotic agents on the length of the leaves became evident (Figure 3). The number of leaves varied within the margin of error. Significant changes in leaf length compared to the control seedlings were observed when the DMP seedlings were exposed to a concentration of 10 µmol ORZ (Figure 3A), the TMP seedlings were exposed to a concentration of 40 µmol ORZ (Figure 3C), and the TMP seedlings were exposed to a concentration of 62.5 µmol CLC (Figure 3D). A marked negative effect was observed in the DMP seedlings at a concentration of 125 µmol CLC (Figure 3B).

Changes in ploidy. After examining the ploidy of daylily seedlings exposed to antimitotic agents, 106 plants with altered ploidy from 234 polyploidised seedlings were identified (Table 2). Tetraploids were obtained from the DMP seedlings under the influence of the lowest (62.5 µmol) CLC concentration – 4 tetraploids, 10 µmol ORZ – 2 tetraploids, and 40 µmol ORZ – 4 tetraploids. By treating diploids with antimitotic agents, 18 triploids (3x) were obtained.

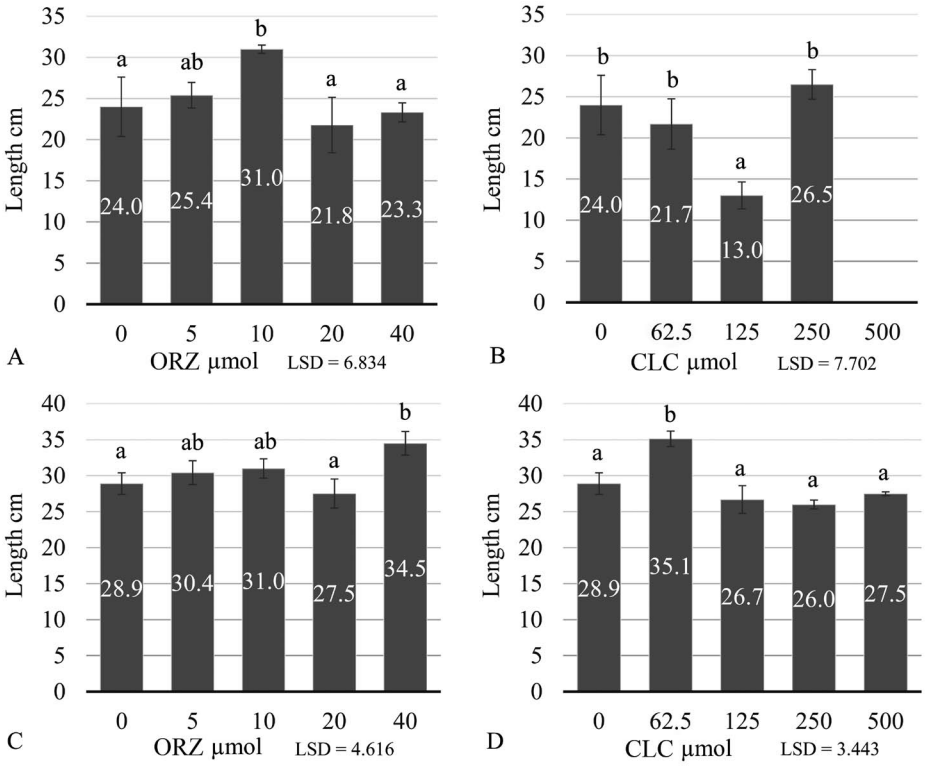
All the control plant seedlings obtained from the crossing combination of two diploid cultivars had a diploid number of chromosomes. In a tetraploid cross family, 90% of the plants were tetraploids and 10% were pentaploids. This indicates that gametogenesis disorders occur more frequently in plants with higher ploidy. Therefore, a greater diversity of plants is likely when polyploidising the seeds of plants of higher ploidy according to the level of ploidy. In the current experiment, 38 pentaploids from 68 individuals were obtained by exposing tetraploids to different CLC concentrations accounting for 55.9%. When tetraploids were exposed to ORZ solutions, 6 triploids, 40 pentaploids, and 2 hexaploids (6x) were obtained from 124 affected seedlings, which accounted for 32.3%. When tetraploid seeds were exposed to a 5 µmol ORZ, 2 hexaploids were obtained. In the ORZ treatments, 6 triploids were found. From the TMP seeds, most pentaploids were obtained by exposure to the highest (40 µmol) ORZ concentration – 12, and to the lowest (62.5 µmol) CLC concentration – 16.

In the current study, individuals of odd ploidy were observed next to plants of even ploidy. Their formation from DMP or TMP seedlings remains unexplained during the experiment. Individuals with



Note. DMP individuals exposed to oryzalin (ORZ) (A) and colchicine (CLC) (B), TMP individuals exposed to ORZ (C) and CLC (D); data are presented as means ± SD; numbers in the columns followed by different letters are significantly different at $p \leq 0.05$.

Figure 2. Leaf length ($\bar{x}$ ± SD) of antimitotic agent-exposed diploid (DMP) and tetraploid (TMP) maternal plants of daylily individuals one month after exposure



Note. DMP individuals exposed to oryzalin (ORZ) (A) and colchicine (CLC) (B), TMP individuals exposed to ORZ (C) and CLC (D); data are presented as means ± SD; numbers in the columns followed by different letters are significantly different at $p \leq 0.05$.

Figure 3. Leaf length ($\bar{x}$ ± SD) of antimitotic agent-exposed diploid (DMP) and tetraploid (TMP) maternal plants of daylily individuals six months after exposure

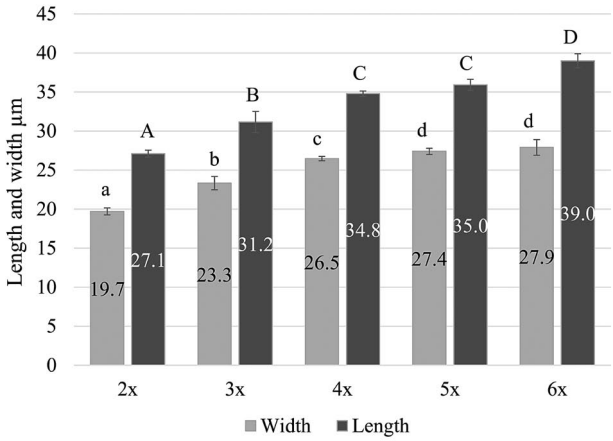
Table 2. Distribution of daylily seedlings affected by antimitotic agents according to ploidy (number of plants/ percent)

Antimitotic agent	Concentration µmol	Plants examined	Ploidy level				
			2x	3x	4x	5x	6x
Diploid maternal plants (DMP) seedlings							
Colchicine (CLC)	62.5	4			4/100		
	125	6	2/33.3	4/66.7			
	250	4	2/50	2/50			
	500	0					
Oryzalin (ORZ)	5	10	6/60	4/40			
	10	6		4/66.7	2/33.3		
	20	6	4/66.7	2/33.3			
	40	6		2/33.3	4/66.7		
Control	Water	16	16/100				
Tetraploid maternal plants (TMP) seedlings							
Colchicine (CLC)	62.5	30			14/46.7	16/53.3	
	125	24			14/58.3	10/41.7	
	250	10			2/20	8/80	
	500	4				4/100	
Oryzalin (ORZ)	5	36			24/66.7	10/27.8	2/5.5
	10	30		4/13.3	18/60	8/26.7	
	20	30		2/6.7	18/60	10/33.3	
	40	28			16/57.1	12/42.9	
Control	Water	40			36/90	4/10	

this ploidy were unstable. Triploids were inherently unstable due to an odd number of sets of chromosomes and have the lowest levels of homozygosity and high mismatches during meiosis (Reis et al., 2022). Triploids can be obtained from interspecific crossing (Hamn et al., 1990; Ollitrault et al., 2007; Blasco, Badenes, 2015) or in studies using an inhomogeneous starting material in terms of ploidy. In the current experiment, 4 pentaploids were detected in the tetraploid control. Such a result could have been determined by using plants of different ploidy for crossing. It was noticed that not all of them were tetraploids when studying the registered cultivars. Almost 30% of individuals show a lower level of ploidy (Zhang et al., 2013a). When using cultivars with unknown ploidy, triploids and pentaploids can be expected when crossing tetraploids. Therefore, it is likely that triploidy arose not only due to antimitotic agents but also due to extensive selection works in the past.

Comparison of stomata size and ploidy. After analysing the length and width of stomata and dividing them according to the studied ploidy, an increase in the length of the stomata was observed as the ploidy of the plant increased (Figure 4). Differences in the stomata width were noted between diploids, triploids, tetraploids, and pentaploids. However, the difference between pentaploids and hexaploids was within the margin of error. Differences in the length of stomata was seen between diploids, triploids, tetraploids, and hexaploids. The stomata length of tetraploids and pentaploids agree within the error limits. To determine the ploidy of the plant preliminarily, the size of the stomata is measured (Xie et al., 2015). Therefore, in the current experiment, determining ploidy based on the stomata size can only identify diploids, triploids, and tetraploids. More accurate data can be obtained by examining ploidy with a flow cytometer.

Analysing the correlation of ploidy with the size of the stomata, a regular distribution of plants was observed according to the dimensions of the stomata (Figure 5). When the control individuals were evaluated by the method of PCA, diploid and tetraploid variants

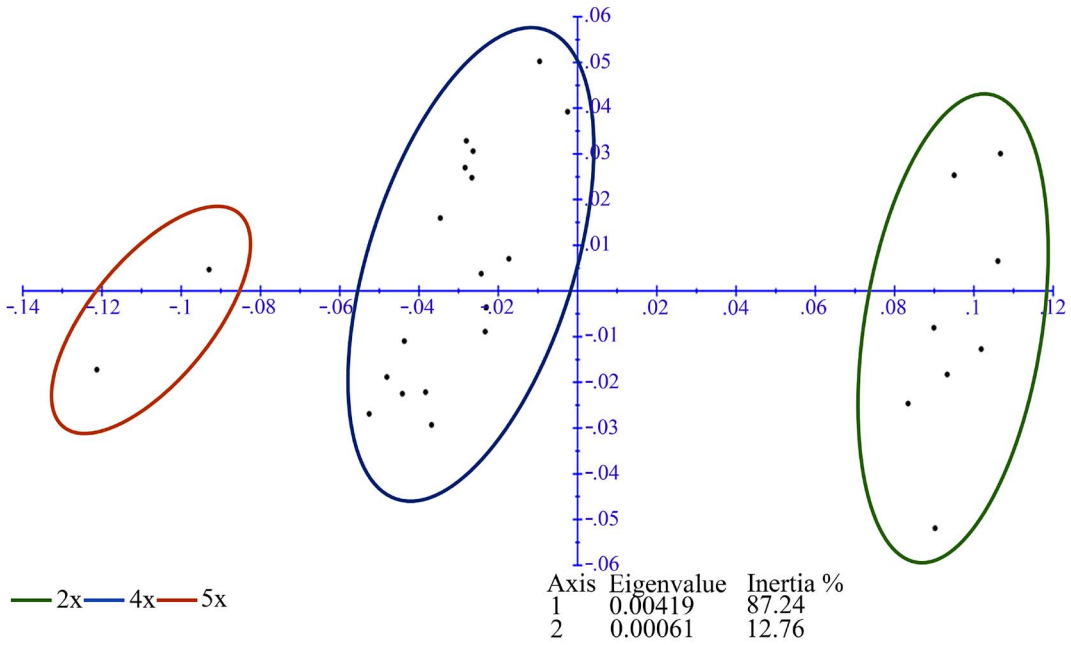


Note. Data are presented as means ± SD; numbers in the same column followed by different letters (upper letters for stomatal length, lower letters for width) are significantly different at $p \leq 0.01$.

Figure 4. Stomata length and width of daylily plants of different ploidy

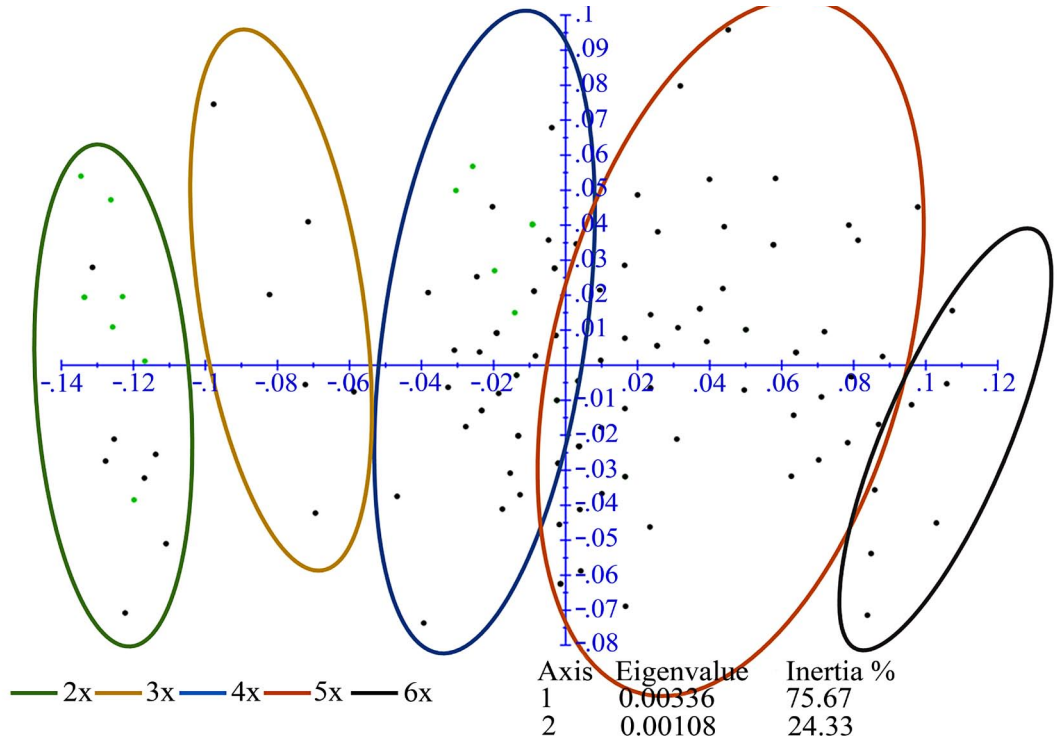
tended to be located. The 2 pentaploid individuals detected in the tetraploid control differ from the tetraploids in terms of stomata size. The dependence of stomata length on ploidy was greater than that of width.

When evaluating all seedlings used during the experiment (control and antimitotic agent-treated), their ploidy and shoot size depended on each other. Analysing the PCA obtained data of differences, it was observed that the control seedlings of known ploidy (green dots) and the seedlings induced by antimitotic agents were distributed in a tendentious manner (Figure 6). Individuals of the diploid control are on the left, and towards the y (1) axis is the tetraploid control. The individuals furthest to the right are pentaploids and hexaploids. Between diploid and tetraploid, there are triploid individuals. The observed trend between the stomata size and ploidy in each variant along the x (2) axis would allow a preliminary determination of plant ploidy by stomata microscopy.



2x – diploid, 4x – tetraploid, 5x – pentaploid

Figure 5. Principal coordinate analysis (PCA) of daylily ploidy and stomatal size differences in evaluating diploid and tetraploid control variants



Note. 2x – diploid, 3x – triploid, 4x – tetraploid, 5x – pentaploid, 6x – hexaploid; green dots represent control plants.

Figure 6. Principal coordinate analysis (PCA) of daylily plants ploidy and stomatal size differences

A cytological method is often used to determine ploidy where the number of chromosomes is counted in the metaphases of the root tip (Ley et al., 2013). However, this method only allows the ploidy of one histological layer to be seen. Flow cytometry uses leaf tissues that cover all three histological layers (LI, LII, and LIII) resulting in more reliable mixoploid data (Brummer et al., 1999; Schepper et al., 2001). When registering cultivars, the breeder records the ploidy of the AHS only according to the ploidy of the parent plants used. Those working with autotetraploids and their hybrids usually measure pollen size. However, this indicator varies greatly and cannot be relied upon. It would be more reliable and faster to estimate the size and amount of stomata of individuals per unit area, which determines the ploidy of most plants.

Chimerism of polyploidised clones. Three years later after additional shoots were grown on plants

exposed to antimitotic agents, the ploidy of each division was reassessed. This way, the chimerism of plants of different ploidy groups was evaluated. In the group of diploid plants, 40% was found. Tetraploid shoots with 59% altered ploidy (Figure 7). The ploidy of hexaploids did not change during the experiment. The chimerism of triploids reached 86% and even 92% of pentaploids. This shows that clones of odd ploidy were more likely to change the ploidy of the plant.

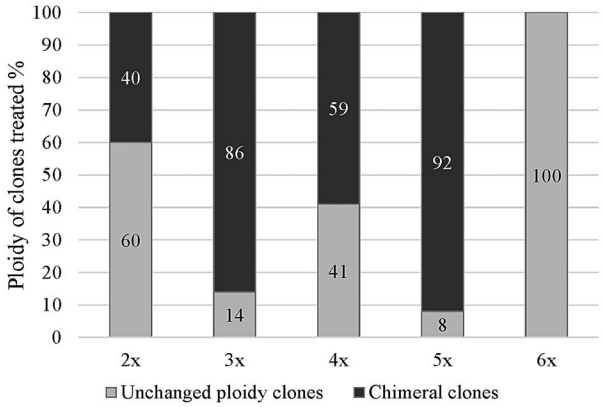
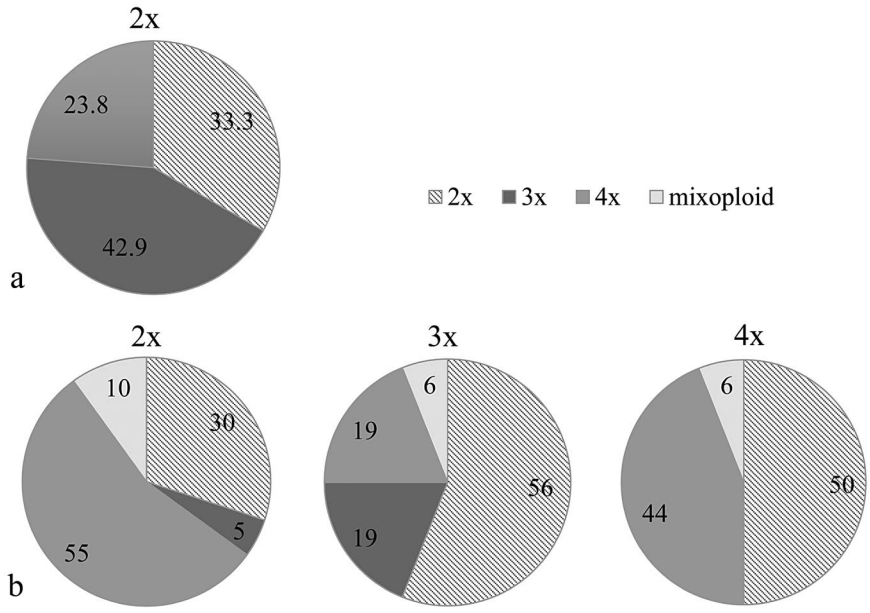


Figure 7. Chimerism of tissue ploidy of daylily clones affected by antimitotic agents three years after the treatment

More than half (55%) of diploid individuals whose ploidy was unchanged during the first year after exposure became tetraploids, 5% became triploids, and 10% became mixoploids, which had more than one peak when checked by using a flow cytometer; 30% of diploids remained unchanged in ploidy (Figure 8 b). The ploidy of the shoots of chimeric plants more often changed to the side of even (2x, 4x, and 6x) ploidy. Most of the triploids (56%) became diploids, 19% of tetraploids and 6% of mixoploids were detected, and 19% of individuals remained unchanged (Figure 8). Half (50%) of the tetraploids became diploids, and 6% became mixoploids. Triploids were not detected, and 44% remained unchanged (Figure 8).

Among the plants grown from the seeds of TMP treated by antimitotic agents, there was a part of triploid plants during the first ploidy assessment. In the third year of cultivation, most (38%) of their shoots became hexaploids and mixoploids (38%) (Figure 9b). The ploidy of 13% of individuals changed to tetraploids and pentaploids; however, no triploid individuals were identified. In the groups of tetraploid individuals, the ploidy of 22% of adventitious shoots remained unchanged, 7% of shoots became triploids and 9% pentaploids. Hexaploids were found as predominant (38%). A heptaploid (7x) and 21% of mixoploids were detected. In the group of pentaploid plants, 7% of triploid adventitious



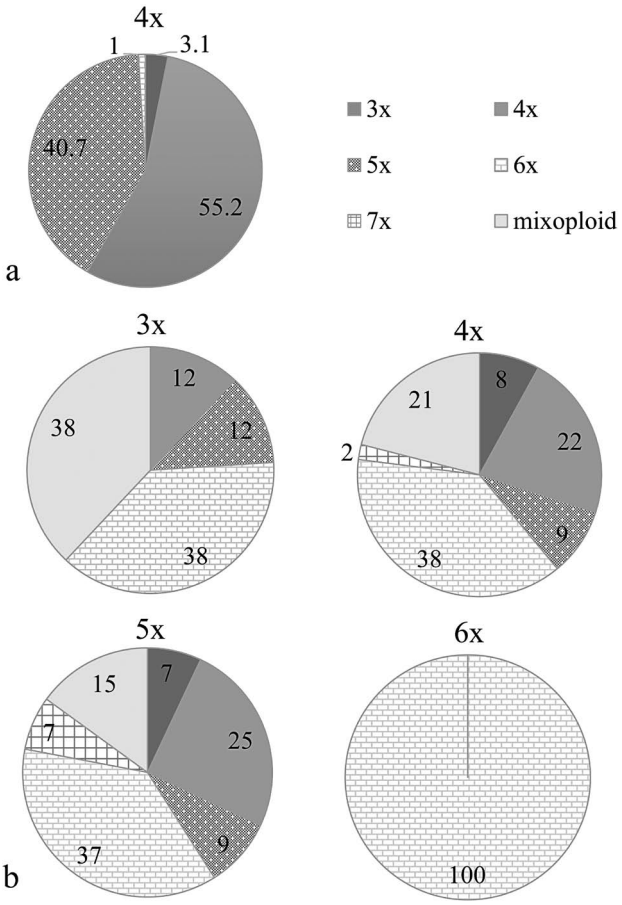
Note. 2x – diploid, 3x – triploid, 4x – tetraploid; mixoploid – plant that represents two types of ploidy on the same tissue.

Figure 8. Ploidy chimerism in each daylily plant division of treated diploid maternal plant (DMP) individuals: plant ploidy changes six months after treatment (a), after three years and sorted in the previously analysed ploidy groups (b)

shoots and 25% of tetraploid ones were identified in the second year of growth. In this group, 37% of hexaploid, 7% of heptaploid, and 15% of mixoploid shoots were placed. The ploidy of only 9% of the divisions remained unchanged; however, while treating natural tetraploid plants, the whole genome duplication occurs resulting in octoploids and mixoploids (having tetraploid and octoploid cells) (Eng et al., 2021).

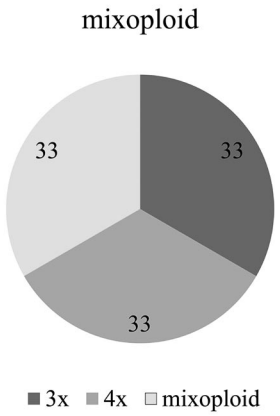
The ploidy of mixoploid individuals changed in equal parts (33% each) to triploids and tetraploids, and the remaining 33% remained unchanged (Figure 10).

A doubling of the cell genome is likely to result in a doubling of the size of the nucleus; however, cell and nuclear volume increases were not uniform (Iannicelli et al., 2020; Fajkus et al., 2021; Ghasemi et al., 2021). This can disrupt the balance between chromosomes and nuclear components. This imbalance can cause various disorders during mitosis and meiosis (Comai, 2005; Madlung, 2013; Bharadwaj, 2015; Zhang et al., 2019) and promote ploidy instability. Pinheiro et al. (2000) suggested that an unequal migration of chromosomes during mitotic anaphase-telophase might result in



Note. 2x – diploid, 3x – triploid, 4x – tetraploid, 5x – pentaploid, 6x – hexaploid, 7x – heptaploid; mixoploid – plant that represents two types of ploidy on the same tissue.

Figure 9. Ploidy chimerism in each daylily plant division of treated tetraploid maternal plant (TMP) individuals: plant ploidy changes six months after treatment (a), after three years and sorted in the previously analysed ploidy groups (b)



Note. 3x – triploid, 4x – tetraploid; mixoploid – plant that represents two types of ploidy on the same tissue.

Figure 10. Ploidy chimerism from the first ploidy test in each daylily plant division of treated tetraploid maternal plant (TMP) individuals that expressed a mixoploid type of ploidy after three years since treatment

different ploidy numbers and pointed out that an induced set of somatic cells would result in plants with altered chromosome numbers.

Most of the polyploidy studies cover only whole genome duplication with chimerism and aneuploidy, while we found uneven patterns of chromosome arrangements. It is still unclear why uneven ploidy (3x, 5x, and 7x) plants were induced, and ploidy switch was on such a high level. Due to the chimerism of polyploidised daylily plants, the clones used for selection should be checked regularly, not only the ploidy of the plant but also the ploidy of different shoots. The duplication of all genomes of the organ cells under investigation by exposure to the antimitotic agents in young seedlings is very low, and the formation of chimeras is widespread (Chen et al., 2021). After flowering, daylilies produce at least several lateral shoots, which may differ in ploidy due to primary chimerism. For the dechimerisation of such plants, it would be necessary to clone them dividing them into individual shoots, cross them with compatible partners, and look for individuals of the desired ploidy in the offspring. However, it is not known what ploidy offspring can be expected when crossing chimeric daylilies.

Conclusions

1. Different antimitotic agents act differently in daylilies, and the initial ploidy of the plant influences the action itself. As the colchicine (CLC) concentration increases, its negative effect increases exponentially, and the length of the leaves in affected diploid (DMP) and tetraploid (TMP) maternal plants seedlings decreases. The highest (40 µmol) oryzalin (ORZ) concentration did not reduce the viability of TMP seedlings.

2. The polyploidisation of diploid seedlings resulted in plants with a doubled number of chromosomes, while the polyploidisation of TMP seedlings did not result in a complete doubling of chromosomes. From 234 polyploidised seedlings, 114 polyploids were induced. Most pentaploids from tetraploid seeds were exposed to the highest (40 µmol) ORZ and the lowest (62.5 µmol) CLC concentration.

3. Plant ploidy can be preliminarily determined by relying on stomatal size measurements, but more accurate data can be obtained by flow cytometry. When polyploidising daylily germinating seeds, almost half of the plants grow into chimeras. A more significant proportion of chimeras is obtained by polyploidising germinating seeds of higher ploidy (tetraploid) plants.

Acknowledgements

This research plant material (seeds) was received by donors of The American Hemerocallis Society (AHS) members and hybridisers John Kulpa, Claudia Conway, and Mike Derrow. I wish to acknowledge the help provided by Dr Gražina Statkevičiūtė, Dr Kristina Jaškūnė, and Dr Rita Armonienė, the head of the Laboratory of Genetics and Physiology, Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry for being allowed to use the ploidy evaluation equipment.

Received 09 08 2022
Accepted 21 10 2022

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Viendienės (*Hemerocallis* L.) augalų poliploidų indukcija ir analizė

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Santrauka

Viendienė (*Hemerocallis* L.) yra populiarus žolinis daugiametis dekoratyvinis augalas. Šiuo metu Amerikos viendienių augintojų asociacijoje (American Hemerocallis Society, AHS) yra užregistruota daugiau kaip 96 000 veislių, iš jų pusė yra tetraploidai, kita pusė – diploidai. Siekiant papildyti tetraploidinių viendienių selekcines linijas, plačiausiai naudojamas kolchicinas. Tyrimo metu viendienių poliploidizacijai pirmą kartą naudotas orizalinas ir poliploidizuoti ne tik diploidiniai, bet ir tetraploidiniai individai. Dygstančios skirtingo ploidiškumo viendienių sėklos paveiktos skirtingų koncentracijų orizalino ir kolchicino tirpalais. Ploidiškumas nustatytas po 6 mėnesių tėkmės citometrijos metodu, gauti rezultatai susieti su žiotelių dydžiu. Chrimeriškumas vertintas praėjus dviem metams nuo poliploidizacijos kiekvieno augalo skirtingų ūglių ploidiškumą tikrinant tėkmės citometru. Nustatyta, kad poliploidizuojant viendienes visiškas genomo duplikavimas nėra dažnas – gauta triploidų, pentaploidų, heksaploidų ir heptaploidų. Optimali didesnį ploidiškumą indukuojanti kolchicino koncentracija buvo 125–250 µmol veikiant diploidinius ir 250–500 µmol veikiant tetraploidinius individus, orizalino – atitinkamai 10 µmol diploidus ir 40 µmol tetraploidus. Preliminariai viendienių ploidiškumą gali nusakyti žiotelių dydis. Siekiant tiksliau nustatyti ploidiškumą, reikėtų taikyti tėkmės citometrijos metodą. Poliploidizuoti individai yra linkę į chimeriškumą. Didžiausias ploidiškumo kitimas nustatytas nelyginio ploidiškumo individuose. Jis dažniausiai kito lyginio ploidiškumo kryptimi, dėl to selekcijai naudojamų klonų ploidiškumas turėtų būti reguliariai tikrinamas.

Reikšminiai žodžiai: poliploidija, tėkmės citometrija, žiotelių dydis, chimeriškumas.