Genome induced mutation in *Callistephus chinensis* Ness. – evaluation of plant fertility and seed characteristics

P. Hanzelka[1], F. Kobza[2]

[1]*Prague Botanical Garden, Prague, Czech Republic*
[2]*Faculty of Horticulture, Mendel University of Agriculture and Forestry, Brno, Lednice, Czech Republic*

ABSTRACT: Thousand seed weight (TSW, g), achene size (mm) and fertility were evaluated in polyploid plants of C_0 (1999) and C_1 (2000) generations. The fertility of polyploid plants was usually very low (more than 10× compared to diploid plants). Only one plant (genotype *A (TM) 1*) was found as a tetraploid individual with anomalously high fertility, high TSW and large size of achenes. TSW in polyploid plants was 2.6–4.13 g, in diploid plants 2.0–2.3 g. The achene size was mostly about 3.7 mm (diploid plants) and 4.0–4.8 mm (polyploid plants). Achene (seed) size and thousand seed weight (TSW) can be classified among indirect identification methods (size of stomata, number of chloroplasts in guard cells, etc.) of polyploid plants.

Keywords: *Callistephus chinensis*; China aster; polyploidy; plant fertility; seed characteristics

The characteristics of seeds and fertility are very important for evaluation of polyploid plants. Parental fertility is one of the most important qualities especially in annual plants where it is not possible to choose another way of propagation.

Achene size and thousand seed weight (TSW) can be classified among indirect identification methods (size of stomata, number of chloroplasts in guard cells, etc.) of polyploid plants. It is necessary to compare them with the parental diploid genotype because achene size and TSW can be affected by the date of harvest (year). The frequent effect of different ploidy level is a change in polyploid plant morphology. Some changes (shape of leaf blade morphology, shape of involucral bracts) can be used as indirect identification methods of polyploid plants.

MATERIAL AND METHODS

The seeds for sowing (harvested from generation C_0) were divided into groups in dependence of the plant keeping in the field.

The reason of this division was to evaluate how many polyploids would be found in each group (after their sowing). The aim of achene sorting (in C_0) according to their size (a, b, c) was to assess the relevance of this sorting for the subsequent polyploid plant selection (to facilitate the subsequent selection in C_1).

TSW was assessed as a mean value of weighing 3 × 500 achenes in both cultivars. TSW was evaluated in these groups of polyploid plants: G, T, is. (a) (378 achenes), G, T, is. (b) (670 achenes), A, T, is. (a) (490 ache-nes) and A, T, is. (b) (2 × 500 achenes) (C_0 generation).

In C_1 generation 908 achenes in cv. Armida and 3 × 500 achenes in cv. Gerda (+ seeds from plant *A (TM) 1**, 867 achenes) were weighed.

Achene size was evaluated in groups of control plants Armida and Gerda (diploid seeds), A, T, is. (a); A, T, is. (a); G, T, is. (b); G, T, is. (a); (C_0 generation). In C_1 generation seeds from diploid plants and seeds from tetraploid plants were evaluated. In each group a total of 3 × 50 achenes was measured.

The scale F 0–9 was chosen for fertility evaluation in C_0 and C_1 generations. Number 9 corresponded to the quantity of seeds from control (diploid) plants, number 0 – no seeds were harvested.

The seeds from C_0 generation were sorted into groups (as mentioned above) and the number of polyploid (tetraploid) plants was evaluated in each group (C_1 generation). Primary selection was carried out according to changes in leaf blade morphology and involucre morphology, secondary selection was done by FCM (Hanzelka, Kobza 2001a,b).

The fertility of C_0 and C_1 generation was evaluated by the counting of achenes (number of achenes from each plant and comparison with the number of seeds from control diploid plants). Tetraploid plants were sorted into these groups: Gerda, tetraploid, isolated and Gerda, tetraploid, non-isolated (G, T, C_1, is.; G, T, C_1, n-is.), Armida, tetraploid, isolated and Armida, tetraploid, non-isolated (A, T, C_1, n-is.; A, T, C_1, is.).

The number of achenes harvested from all infructescences was recounted per 10 infructescences because of the comparison with control diploid plants. In diploid

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Grant No. 435 100 002.

*plant *A (TM) 1* – tetraploid individual with anomalously high fertility, high TSW and large size of achenes

Table 1. Groups of plants in C_0 generation

Name of plant group	Used abbreviations
Gerda, tetraploid, isolated (a)*	G, T, is. (a)
Gerda, tetraploid, non-isolated (a)	G, T, n-is. (a)
Gerda, tetraploid, isolated (b)**	G, T, is. (b)
Gerda, mixoploid, non-isolated (a)	G, T, n-is. (a)
Gerda, mixoploid, non-isolated	G, M, n-is.
Gerda, mixoploid, isolated	G, M, is.
Gerda, mixoploid, from the growth (from the growth of tetraploid individuals)	G, M, p.
Gerda, tetraploid, non-isolated	G, T, n-is.
Armida, tetraploid, isolated (a)	A, T, is. (a)
Armida, tetraploid, isolated (b)	A, T, is. (b)
Armida, tetraploid, non-isolated (a)	A, T, n-is. (a)
Armida, mixoploid, non-isolated	A, M, n-is.
Armida, mixoploid, from the growth (from the growth of tetraploid individuals)	A, M, p.
Armida, tetraploid, non-isolated	A, T, n-is.
Armida, mixoploid, isolated	A, M, is.
Armida, mixoploid, isolated (c)***	A, M, is. (c)

*(a) – considerably larger seeds (compared to control plants)
** (b) – noticeably larger seeds (compared to control plants)
*** (c) – seeds were only slightly larger than control plants
Seeds without signification (a, b, c) were not possible to differentiate from control (visual evaluation)

Table 2. Scale of fertility

Fertility scale	0	1	2	3	4	5	6	7	8	9
Quantity of seeds compared with control (%)	0	< 1	1–5	5–10	10–20	20–30	30–50	50–70	70–90	> 90

plants 10 infructescences were always harvested in each 20 plants in both cultivars.

The significance of differences between diploid and tetraploid plants (C_0 and C_1) was tested by analysis of variance with $\alpha = 0.05$ (*) and 0.01 (**).

RESULTS AND DISCUSSION

Relatively high fertility of some plants of C_0 generation, analysed as tetraploid individuals, was connected with problematic appearance of chimerical plants in this

Table 3. Fertility of C_0 generation

Group/plant number	Fertility scale (F 0–9) with the number of individuals									
	0	1	2	3	4	5	6	7	8	9
G, T, n-is./14	–	8 (3)*	4	1	1	–	–	–	–	–
G, T, is./31	–	27 (8)	4	–	–	–	–	–	–	–
G, M, p./33	–	12 (4)	10	5	3	1	1	1	–	–
G, M, is./10	–	5 (1)	2	2	–	1	–	–	–	–
G, M, n-is./15	–	5 (1)	3	–	3	1	–	1	1	1
A, T, is./33	2	20 (7)	6	1	–	2	–	–	2	–
A, T, n-is./30	–	8 (3)	6	5	2	3	1	–	1	4
A, M, is./9	–	4 (2)	2	2	–	1	–	–	–	–
A, M, n-is./51	–	10 (1)	3	6	9	4	3	6	7	3
A, M, p./23	1	4 (1)	3	3	1	2	1	1	4	3
Total/249	3	103 (31)	43	25	19	15	6	9	15	11

*Numbers in brackets show the number of individuals where less than 10 achenes/plants were harvested

Table 4. Fertility of C_1 generation

Group/number of plants	Fertility scale									
	0	1	2	3	4	5	6	7	8	9
A, T, C1, is./32	**13**	**15** (14)	**4**	0	0	0	0	0	0	0
A, T, C1, n-is./25	**4**	**9** (8)	**9**	**1**	**2**	0	0	0	0	0
A (TM) 1/1	0	0	0	0	0	0	**1**	0	0	0
G, T, C1, is./39	**3**	**13** (13)	**16** (4)	**2**	**1**	0	0	0	0	0
G, T, C1, n-is./85	**13**	**11** (11)	**37** (6)	**20**	**4**	0	0	0	0	0
Sum/182	33	48	66	23	7	0	1	0	0	0

*Numbers in brackets show the number of individuals where less than 10 achenes/plants were harvested

Table 5. Achenes size in C_0 generation

Group	Size (mm ± 3 × standard deviation)
G, control	3.78 ± 3 × 0.22 mm
A, control	3.75 ± 3 × 0.29 mm
G, T, is. (a)	4.46 ± 3 × 0.36 mm**
G, T, is. (b)	4.22 ± 3 × 0.29 mm**
A, T, is. (a)	4.31 ± 3 × 0.38 mm**
A, T, is. (b)	3.9 ± 3 × 0.26 mm

Table 6. TSW in C_0 generation

Group of seeds	Number of achenes	Weight (g)	TSW (g)
G, control	3 × 500	3.49	2.33
G, T, is. (a)	378	1.1	2.91**
G, T, is. (b)	670	1.73	2.58*
A, control	3 × 500	3.44	2.29
A, T, is. (a)	490	1.51	3.08**
A, T, is. (b)	2 × 500	2.67	2.67**

Table 7. Seed size in C_1 generation (2000)

Group of seeds	Achene size ± 3 × standard deviation
G, control	3.7 ± 3 × 0.29 mm
G, T, C1	4.394 ± 3 × 0.317 mm**
A, control	3.59 ± 3 × 0.369 mm
A, T, C1	4.08 ± 3 × 0.21 mm**
A (TM) 1	***4.596 ± 3 × 0.27 mm*****

Table 8. TSW in C_1 generation (2000)

Group of seeds	Number of achenes	Weight (g)	TSW (g)
G, control	3 × 500	3.164	2.11
G, T, C1	3 × 500	4.19	2.79 **
A, control	3 × 500	2.958	1.97
A, T, C1	908	2.986	3.29 **
A (TM) 1	***867***	***3.58***	***4.13 *****

generation. Some plants, first analysed as tetraploids, were likely mixoploids or diploids in reality. Reanalysis of some plants of C_0 generation done by FCM confirmed this theory. FCM reanalysis was carried out in 40 randomly selected plants, first analysed as tetraploids, in the phase of "milky" seed ripeness. Involucre bracts and achenes were evaluated as samples (Fig. 1).

LAPTEV (1988) reported that the enlargement of seed size was found by many authors. Larger seeds were found in polyploid forms of *Panicum* sp., *Cannabis* sp., *Brassica* sp., *Nicotiana* sp., *Phaseolus* sp., *Fagopyrum* sp., etc. The weight of tetraploid forms of *Oryza sativa* was about 50% higher than in diploid forms. A decrease in fertility belongs amongst the characteristics that are usually typical of most polyploid plants, too. It is described in cotton-plant, sugar beet, *Trifolium pratense*, tomatoes, lettuce and other plants. The reason for lower fertility is usually problems in meioses and in division of chromosomes into gametes and gamete imbalance. Fertility usually increases in the subsequent generation and in crossing of polyploid plants.

A decrease in fertility in C_0 and C_1 generations of *Amaranthus caudatus* and *A. hypochondriacus* was reported by MOHINDER and KHOSHOO (1977). LAPINS (1975) found out similar results in an apricot autotetraploid form. SEIDLER-LOZYKOVSKA (pers. commun.) mentioned that TSW of the tetraploid cultivar of *Chamomila recutita* (L.) Rausch. Zloty Lan was 0.1 g, this is about 100% higher than in the original, diploid form. FRYDRYCH (1968) wrote about the possibilities to sort the seeds of cole crops according to the ploidy level.

The fertility decrease in many ornamental polyploid plants and enlargement of seed size were reported by MATVEYEVA (1962) and UHLÍK (1981), too.

Larger seeds of tetraploids usually bring about higher robustness and vitality of tetraploid seedlings. But the more intensive growth of seedlings cannot continue until maturity (LEVIN 1983). The higher weight of *Dactylis glomerata* seeds was mentioned by BRETAGNOLLE and LUMARET (1995). TSW also increased in trefoil (MÁLEK, PROCHÁZKA 1976).

Lower fertility is often typical of the "young" generation of polyploid plants (UHLÍK 1981; SUN et al. 1994; GRESENS 1996; RAMULU et al. 1976; NIIZEKI, KITA

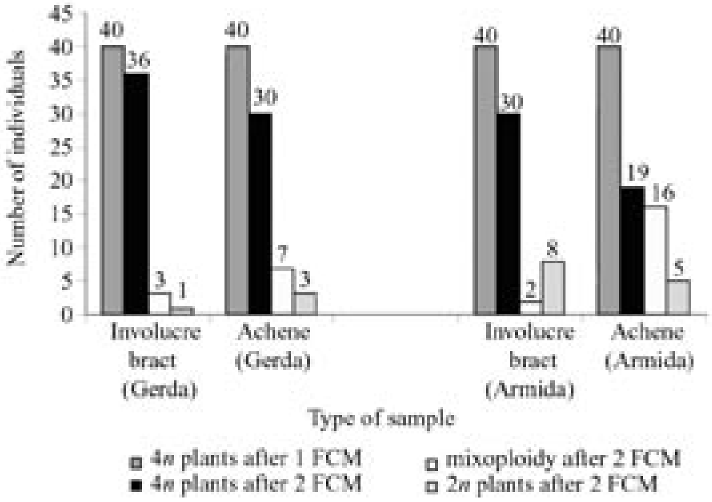

Fig. 1. Reanalysis of tetraploid plants by flow-cytometry (FCM)

1975) and is one of the most important problems for plant propagation. Fertility can increase in the older plant generation after colchicine treatment (JAKEŠOVÁ pers. commun.; MOHINDER, PANDEY 1983). BURTON and HUSBAND (2000) found that diploid progeny of *Chamaenerion angustifolium* (syn. *Epilobium angustifolium*) produced more seeds than tetraploids and triploids. DNYANSAGAR and SUDHAKARAN (1970) mentioned markedly lower fertility in tetraploid plants of *Catharanthus roseus*. The fertility problems were also mentioned by LAPINS (1975) in apricots. PLAVCOVÁ et al. (1975) found that colchicine treatment markedly affected the number of fruits and seeds in tulips. The treated plants produced a considerably lower number of capsules and seeds compared to control plants.

Fig. 2. The seed size of *Callistephus chinensis* (from the left diploid Gerda, tetraploid Gerda, tetraploid Armida */G (TM) 1 = A (TM) 1/*)

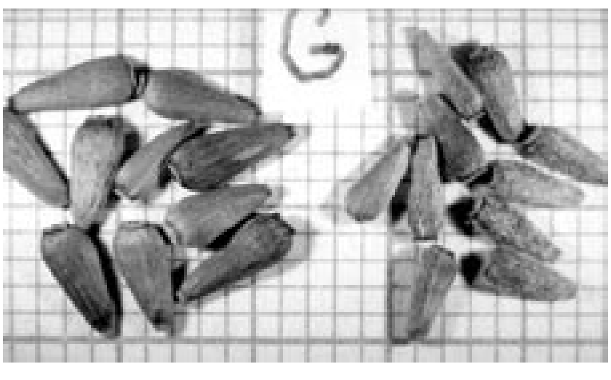

Fig. 3. The seed size of tetraploid (left) and diploid (right) cultivar Gerda

CONCLUSION

The quality characteristics of polyploid (tetraploid) seeds were significantly different in the experiments. Highly significant differences were found in achene size and in TSW (in both cultivars). Fertility decreased in tetraploid generations C_0 and C_1 considerably. It is usual in many polyploid plants at the beginning of polyploidy breeding. A special genotype of cv. Armida (*A (TM) 1*) was identified in C_1 generation, with relatively high fertility, almost 2× higher weight of seeds (TSW) and about 25% larger size of achenes.

It was proved that the seed sorting (according to achene size and TSW) could successfully help in subsequent selection of polyploid plants (especially in C_1).

References

BRETAGNOLLE F., LUMARET R., 1995. Bilateral polyploidization in *Dactylis glomerata* L. subsp. *lusitanica*: occurrence, morphological and genetic characteristics of first polyploids. Euphytica, *84*: 197–207.

BURTON T.L., HUSBAND B.C., 2000. Fitness differences among diploids, tetraploids and their triploid progeny in *Chamerion angustifolium*: mechanisms of inviability and implications for polyploid evolution. Evolution, *54*: 1182–1191.

DNYANSAGAR V.R., SUDHAKARAN I.V., 1970. Induced tetraploidy in *Vinca rosea* Linn. Cytologia, *35*: 227–241.

FRYDRYCH K., 1968. Možnosti mechanického třídění anisoploidního osiva v heterozním šlechtění košťálovin. Genet. a Šlecht., *4*: 63–67.

GRESENS I., 1996. Polyploidisierung durch Colchicinierung von *Satureja hortensis* L. und *Origanum majorana* L. Drogenreport Jg., *9* (15).

HANZELKA P., KOBZA F., 2001a. Genome induced mutation in *Callistephus chinensis* Nees. I.: effect of colchicine application on the early plant development. Hort. Sci. (Prague), *28*: 15–20.

HANZELKA P., KOBZA F., 2001b. Genome induced mutation in *Callistephus chinensis* Nees. II.: results of colchicine application – efficiency of polyploidisation. Hort. Sci. (Prague), *28*: 21–27.

LAPINS K.O., 1975. Polyploidy and mutations induced in apricot by colchicine treatment. Can. J. Genet. Cytol., *17*: 591–599.

LAPTEV J.P., 1988. Heteroploidia v šľachtení rastlín. Bratislava, Príroda: 288.

LEVIN D.A., 1983. Polyploidy and novelty in flowering plants. Amer. Natur., *122*: 1–25.

MÁLEK J., PROCHÁZKA J., 1976. Vzcházivost a další vlastnosti tetraploidní formy jetele lučního. Úroda, *24*: 499–500.

MATVEYEVA T.S., 1962. Polyploidija u děkorativnych rastěnij. In: BARANOV P.A., Polyploidija u rastěnij. Moskva, Izdateľstvo Akaděmiji Nauk SSSR: 376.

MOHINDER P., KHOSHOO T.N., 1977. Evolution and improvement of cultivated *Amaranthus*, VIII. Induced autotetraploidy in grain types. Z. Pfl.-Zücht., *78*: 135–148.

MOHINDER P., PANDEY R.M., 1983. Decrease in quadrivalent frequency over a 10 year period in autotetraploids in two species of grain amaranths. Cytologia, *47*: 795–801.

NIIZEKI M., KITA F., 1975. Effect of different chromosome number of tobacco pollen on plant formation by anther culture and fertilization. Jap. J. Breed., *25*: 258–264.

PLAVCOVÁ O., PETROVÁ E., HOLITSCHER O., 1975. Šlechtění polyploidních tulipánů jako výchozího materiálu pro další šlechtění. [Závěrečná zpráva 1971–1975.] Průhonice, VŠÚOZ.

RAMULU K.S., DEVREUX M., ANCORA G., LANERI U., 1976. Chimerism in *Lycopersicum peruvianum* plants regenerated from *in vitro* cultures of anthers and stems internodes. Z. Pfl.-Zücht, *76*: 299–319.

SUN Y., CHENG S.Q., LIANG G.H., 1994. Induction of autotetraploid plants of *Sorghum versicolor*. Cytologia, *59*: 109–114.

UHLÍK J., 1981. Kompendium pro postgraduální studium genetiky a šlechtění. Praha, VŠZ: 105–195.

Received for publication October 10, 2003
Accepted after corrections January 19, 2004

Indukovaná mutageneze genomu *Callistephus chinensis* Nees. – hodnocení fertility rostlin a vlastností osiva

ABSTRAKT: U polyploidních rostlin generací C_0 a C_1 byla hodnocena fertilita, velikost nažek (mm) a hmotnost tisíce semen (HTS). Fertilita polyploidních rostlin byla obecně velmi nízká, většinou více než 10× nižší ve srovnání s diploidními rostlinami. Byla ale nalezena i výjimečná tetraploidní rostlina (genotyp *A (TM) 1*) s neobvykle vysokou fertilitou, výrazně vyšší HTS a rovněž nápadně větší velikostí nažek. HTS se u polyploidních rostlin pohybovala v rozmezí 2,6–4,13 g, kdežto u diploidních rostlin mezi 2,0–2,3 g. Velikost nažek byla u diploidních rostlin blízká hodnotě 3,7 mm, kdežto u polyploidů mezi 4,0–4,8 mm. Velikost nažek a hmotnost tisíce semen lze přiřadit mezi nepřímé identifikační metody polyploidních rostlin podobně jako např. velikost průduchů a počet chloroplastů ve svěracích buňkách.

Klíčová slova: *Callistephus chinensis*; astra čínská; polyploidie; fertilita rostlin; vlastnosti osiva

Corresponding author:

Ing. PETR HANZELKA, Ph.D., Pražská botanická zahrada, Nádvorní 134, 171 00 Praha 7-Troja, Česká republika
tel.: + 420 233 540 299, fax: + 420 233 542 629, e-mail: p.hanzelka@botgarden.cz