

NEW CHRYSANTHEMUM CULTIVARS AS A RESULT OF *in vitro* MUTAGENESIS WITH THE APPLICATION OF DIFFERENT EXPLANT TYPES

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Abstract. Induced mutagenesis allows to obtain in relatively short time new cultivars of chrysanthemum differing in single characteristic traits such as the colour or shape and size of inflorescence, which determines their decorative values. The traditional breeding methods as crossing, selection and techniques of genetic transformation face limitations in this species. The object of investigation were three cultivars of *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. – ‘Albugo’, ‘Alchemist’, ‘Satinbleu’. Gamma radiation in dose of 15 Gy was obtained from Co⁶⁰ cobalt source generated by Theratron 780 C. The dose rate was 2.14 Gy × min⁻¹. ‘Albugo’ and ‘Satinbleu’ microcuttings cultured on MS medium were exposed to irradiation. Following the irradiation, single-node explants and leaves were excised from plantlets and subcultured onto MS medium supplemented with 0.6 mg × dm⁻³ BAP and 2.0 mg × dm⁻³ IAA. In ‘Alchemist’ there were irradiated leaf explants with callus regenerated on petioles. A month earlier this explants were placed on the MS medium with 0.6 mg × dm⁻³ BAP and 2.0 mg × dm⁻³ IAA added. The regeneration of adventitious shoots was conducted without subculturing onto fresh medium. Newly formed shoots were transferred onto rooting medium 4 months after the irradiation. Gamma radiation effected the regeneration of shoots on explants. From the mutants obtained in vM₂ generation the most interesting, worth introducing to cultivation five genotypes were selected: ‘Albugo Sunny’, ‘Alchemist Tubular’, ‘Alchemist Golden Beet’, ‘Satinbleu Minty’, ‘Satinbleu Honey’.

Key words: *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam., micropropagation, gamma radiation, mutations

INTRODUCTION

Induced mutagenesis is the most frequently applied chrysanthemum breeding method. Self-incompatibility, high level of ploidy and heterozigosis make crossing and

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selection in that species very difficult. Most mutations go towards the dominant allele to the recessive one, which makes them get identified already in the first mutation generation and it makes it possible to obtain a big number of new cultivars in relatively short time [Schum 2003, Miler 2005]. In chrysanthemum exposed to the effect of mutagen, most often the colour of the inflorescence changes; the trait which mostly determines the decorative value of cultivars. One observes the change in the plant habit or changes in the shape and size of leaves and inflorescences or the number and shape of ligulate florets less frequently [Banerji and Datta 1990, Zalewska 2001, Zalewska 2010].

In mutation breeding the irradiation treatment involves shoot and leaf cuttings *in vivo*, and in the case of *in vitro* propagation small vegetative fragments, such as, e.g.: leaves, leaf petioles, sections of stems, inflorescence peduncle, ligulate florets, as well as callus tissue, suspensions of cells, protoplasts. Additionally, explants *in vitro* are exceptionally convenient material for irradiation. Thanks to the application of cultures *in vitro* on small area, in the environment free from diseases and pests, many irradiated explants could be placed, which ensures a more effective regeneration than under conditions *in vivo* and increases the potential of obtaining mutated plants as well as it allows for a considerable acceleration of all the stages of the breeding program [Jerzy 1997, Zalewska 1995, Zalewska and Jerzy 1997].

The aim of the present paper was to demonstrate that somatic mutagenesis induced *in vitro* with gamma radiation with the application of different explants is a fast and effective way to increase the scope of variation in different chrysanthemum cultivars.

MATERIAL AND METHODS

The experiment was performed from December 2005 to November 2007 at the Laboratory of Biotechnology and in the glasshouse of the Department of Ornamental Plants and Vegetable Crops, the University of Technology and Life Sciences in Bydgoszcz.

The research involved three cultivars of *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. (in the past *Dendranthema grandiflora*) of full semi-ball inflorescence – ‘Albugo’ (white), ‘Alchemist’ (dark violet) and ‘Satinbleu’ (dark pink). Gamma radiation at the dose of 15 Gy came from source ⁶⁰Co generated by Theratron 780 C type apparatus. The dose rate was 2.14 Gy × min⁻¹. In ‘Albugo’ and ‘Satinbleu’ the irradiation involved microcuttings growing on a modified Murashige and Skoog [1962] medium (MS medium) without growth regulators. The medium modification covered an increase in the content of calcium and iron by half. The medium, containing 30 g × dm⁻³ of saccharose, was solidified with 8 g × dm⁻³ of agar, and its pH was defined before sterilization as 5.8. After irradiation, on the modified MS medium supplemented with 0.6 mg × dm⁻³ BAP and 2.0 mg × dm⁻³ IAA 100 single-node explants and 100 leaf explants were placed, 5 into each jar. The control consisted of the same number of non-irradiated explants. In ‘Alchemist’ there were irradiated 250 leaf explants with callus regenerated on petioles. A month earlier this explants were placed 5 into each jar on the modified MS medium with 0.6 mg × dm⁻³ BAP and 2.0 mg × dm⁻³ IAA added. Having been irradiated, the regeneration of adventitious shoots from leaf explants was further conducted without

subculturing. The control involved 250 non-irradiated leaf explants. Additionally in each cultivar 25 control shoot tips were placed on the modified MS medium. The shoot regeneration took 4 months, after which they were transferred onto a modified MS rotating medium supplemented with $2.0 \text{ mg} \times \text{dm}^{-3}$ IAA for 2 weeks. Cultures *in vitro* were kept in growth room at the temperature of $24 \pm 2^\circ\text{C}$, exposed to 16-hour photoperiod and the photon flux density of $30\text{--}35 \text{ } \mu\text{mol} \times \text{m}^{-2} \times \text{s}^{-1}$.

For 15 weeks observations were made into the dynamics of regeneration of adventitious shoots on the leaf explants and internodes of single-node explants. Contrary to 'Satinbleu', in 'Albugo' differentiating the axillary shoots from adventitious shoots found on single-node explants was, in practise, impossible due to their intensive growth and regeneration. For that reason their total number was considered. There was also determined the mean number of shoots per one explant inoculated.

The results were statistically verified with the method of the analysis of variance for single-factor experiment in completely randomized design, and the significance of differences was verified with the HSD Tukey's test at the level of significance of $\alpha = 0.05$.

In April 2006 the rooted microcuttings were planted into plastic cuvettes into the peat substrate, earlier mixed with perlite in the volume proportion of 2:1 (v:v) and disinfected with fungicide Benlate 50WP at the concentration of 0.2%. The acclimatization took 14 days. Then the plants were planted out into a permanent place on the tables filled with the peat substrate prepared as above. The row spacing was $5 \times 10 \text{ cm}$. Chrysanthemums were grown exposed to natural photoperiod, applying the standard method.

Growing and flowering of chrysanthemums of the first vegetative mutation generation (vM_1) coincided with the observations to identify the so-called variants (plants of changed morphological traits) and variances corresponding to the traits changed. The frequency of the variants and variances were determined against the number of flowering plants. The variants were selected by defining the colour of the inflorescences of the control plants and the plants irradiated at full flowering stage and the type of inflorescence according to Jerzy [2000]. The colour of the inner side of ligulate florets was determined as agreed and based on the RHSCC catalogue [1966]. Chrysanthemum cultivation in the glasshouse was completed on November 26, 2006.

In June 2007, the shoot cuttings sampled from the stocks were rooted and then brought to flowering following the same procedure as in the first vegetative mutation generation. The colour and the type of inflorescences were again defined as above to demonstrate the stability of the traits changed in generation vM_1 .

Similarly, the qualitative and quantitative composition of pigments in ligulate florets in original cultivars and in selected most interesting, worth introducing to cultivation mutants: 'Albugo Sunny', 'Alchemist Golden Beet', 'Alchemist Tubular', 'Satinbleu Honey', 'Satinbleu Minty' was defined. The research material was made up of fresh fragments of ligulate florets (about 2 cm from the tip), which were sampled at full flowering stage. Carotenoids were extracted following Wettstein's [1957] methodology. Anthocyanins were extracted applying the method by Harborne [1967]. The spectrophotometric analysis of extracts was made in two-beam spectrophotometer UV-VIS 1601-PC SHIMADZU at the wavelength of 330–500 nm for carotenoids and 450–600 nm for anthocyanins. Based on the absorption curves made, there was determined the value of absorbance at the wavelength (λ_{max}) characteristic for each pigment, which facilitated

the calculation of the content of carotenoids and anthocyanins for cyanidin-3-glucoside per 1 g of fresh weight of ligulate florets.

RESULTS AND DISCUSSION

Prior to the start of radiation breeding, the selection of the kind of radiation and determination of its dose are essential [Jerzy and Lubomski 1991]. According to Zalewska [1995] as well as Zalewska and Jerzy [1997], in chrysanthemums it is most effective to use gamma radiation giving a greater range of variation than X radiation. As reported by Banerji and Datta [1990], the dose of 15–20 Gy of gamma radiation is optimal. Similarly Jerzy and Lubomski [1991] report on the dose of 15 Gy being most suitable since high doses of 20–25 Gy, despite increased probability of the occurrence of mutation, limit or inhibit regeneration. With the above in mind, the choice of the dose of 15 Gy of gamma radiation in the present experiment seems justifiable.

Gamma irradiation did not affect the numbers of the adventitious and axillary shoots on single-node explants in 'Albugo', adventitious shoots on leaf explants in 'Alchemist', or the axillary shoots on single-node explants in 'Satinbleu'. However it limited the regeneration of adventitious shoots on single-node explants in 'Satinbleu' and inhibited it completely on leaf explants in 'Albugo' and in 'Satinbleu' (tab. 1).

As reported by Broertjes and Lock [1985], the irradiation with the dose of 5–8 Gy decreased the number of adventitious shoots regenerated on the explants cut off from the peduncle in 'Accent', 'Janancy', 'Parliament' and in 'Regoltime', however, it increased in 'Clinstar'. In 'Super White' the capacity for the regeneration of adventitious shoots was observed only after irradiation, whereas in 'Refour' and 'Winter Westland' such capacity was demonstrated neither before nor after irradiation. No effect of gamma radiation on the growth of axillary shoots in nodes could have been due to the fact that axillary buds were already formed before the application of the mutagenic factor. However, as reported by Broertjes et al. [1976], in the case of leaf explants *in vitro* of the same cultivar, doses 4–10 Gy limited the number of regenerated shoots by as much as half. Higher doses demonstrated a negative effect on the regeneration of adventitious shoots even more considerably, and when applying the dose of 20 Gy, the process was completely inhibited. Zalewska [1995] in 'Red Nero' observed a drastic decrease in the capacity for regeneration noted in adventitious shoots on leaf explants *ex vitro* after the irradiation with the dose of 20 Gy of X and gamma radiation, and after the application of the dose of 25 Gy, the regeneration of shoots did not occur. A considerable role of the cultivar specificity in the process of regeneration of adventitious shoots on leaf explants is reported by Jerzy and Lubomski [1991]; depending on the cultivar, the mean number of adventitious shoots regenerated on the leaf exposed ranged from 0.13 to 2.66 and decreased after irradiation. A significant effect on inhibiting the process of regeneration of adventitious shoots on irradiated leaf explants in 'Albugo' and 'Satinbleu' was observed for the mutagenic factor applied, as well as the cultivar specificity. However, one cannot exclude the effect of other factors. According to Roest and Bokelman [1975], the formation of adventitious shoots *in vitro* depends also on such factors as: the leaf age and its position on the stem, the presence or absence of the leaf petiole as well

Table 1. Effect of gamma radiation on the number of shoots per one explants inoculated in the cultivars tested
 Tabela 1. Wpływ promieniowania gamma na liczbę pędów przypadającą na jeden wyłożony eksplantat u badanych odmian

Radiation dose Dawka promieniowania (Gy)	Albugo			Alchimist			Satimbleu		
	propagation method <i>in vitro</i> sposób rozmnażania <i>in vitro</i>								
	adventitious shoots on leaf explants pędy przybyszowe na eksplantatach liściowych	adventitious and axillary shoots on single-node explants pędy przybyszowe boczne na jednowęzłowych fragmentach pędu	adventitious shoots on leaf explants pędy przybyszowe na eksplantatach liściowych	adventitious shoots on leaf explants pędy przybyszowe na eksplantatach liściowych	adventitious shoots on leaf explants pędy przybyszowe na eksplantatach liściowych	adventitious shoots on single-node explants pędy przybyszowe na jednowęzłowych fragmentach pędu	adventitious shoots on single-node explants pędy boczne na jednowęzłowych fragmentach pędu	axillary shoots on single-node explants pędy boczne na jednowęzłowych fragmentach pędu	
0 Control Kontrola	1.82*	3.92a	0.31a	3.13a	3.13a	4.09a	2.42a		
15	0b	3.48a	0.45a	0b	0b	0.46b	2.33a		

* Means in columns followed by the same letter do not differ significantly at $\alpha = 0.05$

* Wartości w kolumnach oznaczone tą samą literą nie różnią się istotnie między sobą przy $\alpha = 0,05$

as the composition of the medium. Besides, as demonstrated by the results of the present experiment, to produce adventitious shoots, it is more effective to irradiate the leaf explants with the callus induced already before on petioles than to excise leaf from irradiated microcuttings.

Besides, Rademaker and Jong [1990] claim that the main factors affecting the regeneration cover the genotype, the type of explants and the medium composition. Within a given cultivar, the formation of adventitious buds depends on the explant type applied and the combination and concentration of growth regulators in the medium. 'Royal Purple' in the experiment reported by Lu et al. [1990] produced more adventitious shoots on the explants derived from internodes than on leaf explants. Similarly Himstedt et al. [2001] claim that in most of the 19 chrysanthemum cultivars researched, the regeneration was more successful on stem than leaf explants. In the present experiment the explants were placed on the MS medium supplemented with growth regulators at the amount of $0.6 \text{ mg} \times \text{dm}^{-3}$ BAP and $2.0 \text{ mg} \times \text{dm}^{-3}$ IAA. The irradiated leaf explants were also placed on the medium of the same composition by Jerzy and Zalewska [1996] in 'Richmond' and Zalewska [1995] in 'Red Nero'.

In 'Albugo' the appearance of the first shoots on control single-node explants were observed in the 3rd week of culture and on the irradiated ones in the 4th week (fig. 1). In 'Alchemist' the regeneration of adventitious shoots started in the 6th week both on control and on the irradiated leaf explants (fig. 2). 'Satinbleu' regenerated adventitious shoots, respectively, in the 5th and the 6th week of culture on control and irradiated internodes (fig. 3). The regeneration on control leaf explants occurred in 'Albugo' in the 4th, and in 'Satinbleu' in the 5th week. The gamma radiation also affected the time needed for the regeneration of adventitious shoots on leaf explants, as reported by Zalewska and Lema-Rumińska [2004]. The authors observed the formation of adventitious shoots in 'Lady Apricot' and in 'Lady Vitroflora' on control explants in the 5th week of culture and on the irradiated ones – two and three weeks later, respectively.

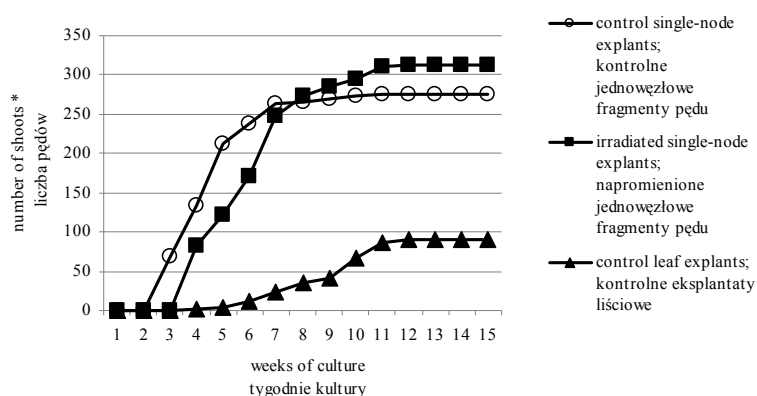


Fig. 1. Dynamics of shoots regeneration in 'Albugo': *on 60 control single-node explants; 83 irradiated single-node explants; 35 control leaf explants

Ryc. 1. Dynamika regeneracji pędów u odmiany 'Albugo': *na 60 kontrolnych jednowęzłowych fragmentach pędu; 83 napromienionych jednowęzłowych fragmentach pędu; 35 kontrolnych eksplantatach liściowych

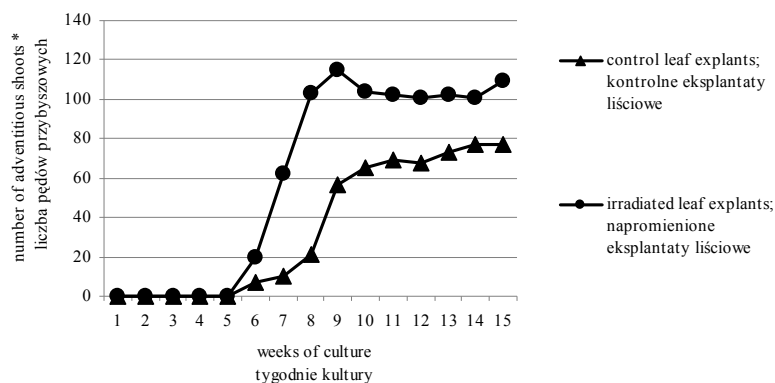


Fig. 2. Dynamics of adventitious shoots regeneration in 'Alchemist': *on 53 control leaf explants; 68 irradiated leaf explants

Ryc. 2. Dynamika regeneracji pędów przybyszowych u odmiany 'Alchemist': *na 53 kontrolnych eksplantatach liściowych; 68 napromienionych eksplantatach liściowych

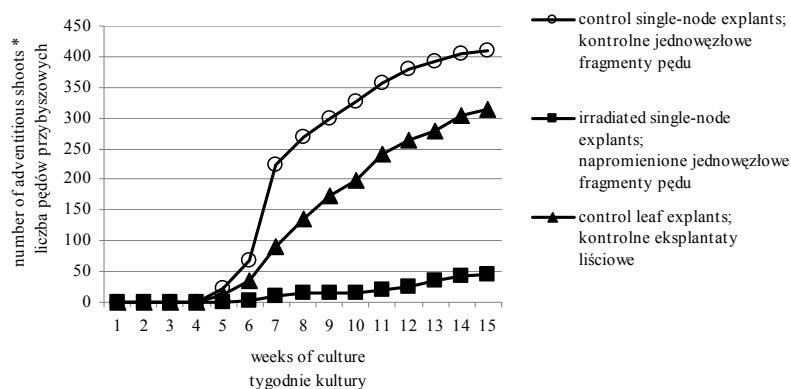


Fig. 3. Dynamics of adventitious shoots regeneration in 'Satinbleu': *on 100 control single-node explants; 16 irradiated single-node explants; 84 control leaf explants

Ryc. 3. Dynamika regeneracji pędów przybyszowych u odmiany 'Satinbleu': *na 100 kontrolnych jednowęzłowych fragmentach pędu; 16 napromienionych jednowęzłowych fragmentach pędu; 84 kontrolnych eksplantatach liściowych

In generation vM₁ in white cultivar 'Albugo' there were observed variants of decorative inflorescences; yellow ('Albugo Sunny') and the plant being a chimera of the decorative inflorescence, white-yellow. The semi-ball, dark violet inflorescence in 'Alchemist' turned into tubular, silver violet ('Alchemist Tubular'), pale violet ('Alchemist

Bright'), decorative ('Alchemist Aster') and decorative golden beet ('Alchemist Golden Beet'). In dark pink 'Satinbleu' there appeared pale pink ('Satinbleu Minty'), salmon ('Satinbleu Honey'), pink ('Satinbleu Rose') and dark violet ('Satinbleu Violet') variants. The inflorescence of 'Satinbleu Minty' chrysanthemums were of decorative type (tab. 2, phot. 1–3). The mutations involving a change in the inflorescence colour occur most frequently among pink and violet chrysanthemums, which was confirmed in the experiment with violet cultivar 'Bravo' [Stepczyńska et al. 1980] in which there were recorded 4 mutations of the colour as well as the experiment with a violet pink cultivar 'Richmond' [Jerzy and Zalewska 1996] in which there were obtained 21 colour mutations from white to honey brown. Similarly in the present experiment a greater spectrum of changes in inflorescence colour occurred in dark violet 'Alchemist' and dark pink 'Satinbleu' than in white 'Albugo'.

In the present experiment in 'Albugo' there were obtained 8 variants of the same yellow decorative inflorescences ('Albugo Sunny'), and in 'Satinbleu' 6 variants of pale pink decorative inflorescences ('Satinbleu Minty'). The appearance of identical mutants was also observed by Zalewska [1995] who claims that it might have been due to the existence of some specific variation trends. Broertjes et al. [1976] report on mutants of the phenotypic characters changed the same way originating from the same explants; the researchers point to a possibility of a very fast growth of the initial cell and, as a result, the formation of the multimeristem giving rise to more than one shoot.

The frequency of variants and mutants was highest among the chrysanthemums obtained as a result of regeneration of adventitious shoots in 'Satinbleu' and in 'Alchemist'. The lowest frequency was observed among the plants derived from axillary shoots (tab. 3). Such results could be accounted for referring to the theory by Broertjes and Keen [1980] who demonstrated that the formation of the adventitious meristem involves a single cell. Thus the plant developing from a single mutated cell is made up of genetically homogenous tissues and it is a stable mutant. The irradiated chrysanthemums, especially those derived from lateral shoots, could have been periclinal chimeras only with a changed external layer of tissues and additionally the occurrence of the diplontic selection could have resulted in a lower frequency of variants and variations as compared with adventitious shoots [Zalewska 1995].

Changes in the colour and shape of inflorescences observed in generation vM_1 reoccurred in generation vM_2 , except for chimera in 'Albugo', which thus also justifies the selection of the breeding method. Changes in the colour in chimera in generation vM_1 could have been a result of the modification caused by an environmental factor and not by mutation [Jerzy et al. 1994]. From the mutants obtained there were selected five interesting, worth introducing to cultivation genotypes: 'Albugo Sunny', 'Alchemist Tubular', 'Alchemist Golden Beet', 'Satinbleu Minty' and 'Satinbleu Honey'.

The spectrophotometric measurement of the absorbance of pigment extracts from ligulate florets is a method which is simple, universal and cheap and, most importantly, it ensures the objectivity and accuracy of the measurements, and thus it is applicable to demonstrate the separate character of mutants as compared with original cultivars and their full identification [Lema-Rumińska and Zalewska 2004]. The present spectrophotometric analysis showed that the inflorescences of mutants 'Albugo Sunny', 'Alchemist Golden Beet' and 'Satinbleu Honey', in contrary to the inflorescences of original cul-

Table 2. Variants, variances and mutants and mutations observed in cultivars tested
 Tabela 2. Zaobserwowane warianty, wariacje oraz mutanty i mutacje u badanych odmian chryzantem

Original cultivar Odmiana wyjściowa	Inflorescence Kwiatostan	Propagation <i>in vitro</i> Rozmnażanie <i>in vitro</i>	Variant/Mutant Wariant/Mutant	Number of variants/mutants Liczba wariantów/ mutantów	Variances/mutations of inflorescence Wariacje/mutacje kwiatostanu
Albugo	semiball, white półkulisty, biały (155D) ^{1/}	adventitious and axillary shoots pędy przybyszowe i boczne	Albugo Sunny	8/8	decorative, yellow dekoracyjny, żółty (9C)
			chimera	1/0	decorative, white-yellow dekoracyjny, biało-żółty (155D/9C)
Alchimist	semiball, dark violet półkulisty, ciemnofioletowy (77A)	adventitious shoots pędy przybyszowe	Alchimist Tubular	1/1	tubular, silver violet igielkowy, srebrzysto-fioletowy (77C)
			Alchimist Bright	1/1	pale violet jasnofioletowy (77B)
			Alchimist Aster	1/1	decorative – dekoracyjny
			Alchimist Golden Beet	1/1	decorative, golden beet dekoracyjny, buraczkowo-żółty (60C)
Satinbleu	semiball, dark pink półkulisty, ciemnoróżowy (75B)	adventitious shoots pędy przybyszowe	Satinbleu Minty	6/6	decorative, pale pink dekoracyjny, jasnoróżowy (75D)
			Satinbleu Honey	1/1	salmon – lososiowy (58A)
			Satinbleu Rose	1/1	pink – różowy (75C)
			Satinbleu Violet	1/1	dark violet ciemnofioletowy (81C)

^{1/} colour of the inner part of ligulate florets according to the RHSCC catalogue [1966]

^{1/} barwa wewnętrznej strony kwiatów języczkowatych wg katalogu RHSCC [1966]



Photo 1. 'Albugo' cultivar (from the left) and its variant, 'Albugo Sunny', and chimera
 Fot. 1. Odmiana 'Albugo' (od lewej) i jej wariant 'Albugo Sunny' oraz chimera



Photo 2. 'Alchimist' cultivar (from the left) and its variants, 'Alchimist Tubular', 'Alchimist Bright', 'Alchimist Aster' and 'Alchimist Golden Beet'
 Fot. 2. Odmiana 'Alchimist' (od lewej) i jej warianty 'Alchimist Tubular', 'Alchimist Bright', 'Alchimist Aster' i 'Alchimist Golden Beet'

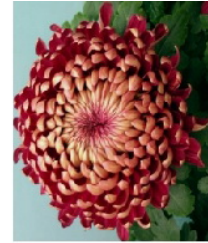


Photo 3. 'Satinbleu' cultivar (from the left) and its variants, 'Satinbleu Minty', 'Satinbleu Honey', 'Satinbleu Rose' and 'Satinbleu Violet'
 Fot. 3. Odmiana 'Satinbleu' (od lewej) i jej warianty 'Satinbleu Minty', 'Satinbleu Honey', 'Satinbleu Rose' i 'Satinbleu Violet'



Table 4. Absorbance of the extracts of pigments and the content of pigments in mg per 1 gram of fresh weight of ligulate florets in original cultivars and their mutants

Tabela 4. Absorbancja ekstraktów barwników oraz zawartość barwników w mg na 1 gram świeżej masy kwiatów języczkowatych odmian wyjściowych oraz ich mutantów

Original cultivars and mutants Odmiany wyjściowe i mutanty	Absorbance Absorbancja		Content of pigments Zawartość barwników	
	carotenoids karotenoidy $\lambda = 440 \text{ nm}$	anthocyanins antocyjany $\lambda = 530 \text{ nm}$	carotenoids karotenoidy	anthocyanins antocyjany
Albugo ^{1/}	-	-	-	-
Albugo Sunny	0.38	-	0.18	-
Alchemist ^{1/}	-	0.76	-	0.60
Alchemist Golden Beet	0.19	0.74	0.09	0.55
Alchemist Tubular	-	0.84	-	0.70
Satinbleu ^{1/}	-	0.14	-	0.10
Satinbleu Honey	0.22	0.08	0.10	0.05
Satinbleu Minty	-	-	-	-

^{1/} Original cultivars – Odmiany wyjściowe

tivars, contained carotenoids. Mutant ‘Alchemist Golden Beet’ included less and mutant ‘Alchemist Tubular’ – more anthocyanins in the inflorescence than the original cultivar ‘Alchemist’. In the inflorescences of mutants obtained from ‘Satinbleu’ there was reported a decrease in the content of anthocyanins (tab. 4). Changes in the colour in the present research were thus a result of both quantitative and qualitative differences in the content of respective pigments. Lema-Rumińska and Zalewska [2005] obtained, from violet pink original cultivar ‘Richmond’, containing anthocyanins, mutants in which there was identified the presence of carotenoids or no anthocyanins at all in ligulate florets. In the mutants derived from ‘Lilac Wonder’ there was noted a decrease or increase in the content of anthocyanins [Lema-Rumińska and Zalewska 2004]. The colour mutations were noted as a result of changes in the content of respective pigments or the occurrence of a new type of pigment as compared with the original cultivar [Datta and Gupta 1981 after Heslot 1968]. Many chrysanthemum cultivars have a single dominant allele of a gene which is responsible for an inhibition of a carotenoid biosynthesis. A destruction of DNA in the area of this allele, e.g. as a result of the effect of the mutagen, can lead to the biosynthesis of carotenoids in radiomutant [Langton 1989, Lema-Rumińska and Zalewska 2004]. The ionization irradiation can result in a partial or complete inactivation of the genes encoding the enzymes of pathway of the biosynthesis of anthocyanins. The mutations of that type which concern single genes result in the accumulation of intermediate compounds and, as a result, a change in colour. Mutations can also occur in the genes responsible for the production of proteins (GS-X) taking part in the transport of anthocyanidin pigments by membranes to vacuole where they are accumulated [Lema-Rumińska and Zalewska 2005, Onozaki et al. 1999, Kobayashi et al. 2001].

CONCLUSIONS

1. Gamma radiation at the dose of 15 Gy can be successfully applied *in vitro* as a mutagenic factor in chrysanthemum breeding. Induced mutagenesis is very effective chrysanthemum breeding method, as confirmed in the past by many other authors.
2. New chrysanthemum cultivars were obtained by using single-node explants from irradiated microcuttings or by irradiating leaf explants with callus regenerated earlier on leaf petiole.
3. Quantitative and qualitative changes in the content of pigments in inflorescences of the cultivars obtained were a result of mutagenic gamma radiation.

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NOWE ODMIANY CHRYSANTEM UZYSKANE NA DRODZE MUTAGENEZY *in vitro* Z ZASTOSOWANIEM RÓŻNYCH TYPÓW EKSPLANTATÓW

Streszczenie. Indukowana mutageneza pozwala uzyskać w stosunkowo krótkim czasie nowe odmiany chryzantem o zmienionych pojedynczych cechach jak barwa lub kształt i wielkość kwiatostanu, które decydują w głównej mierze o wartości dekoracyjnej. U tego gatunku bardzo utrudnione jest stosowanie tradycyjnych metod hodowli, takich jak krzyżowanie, selekcja oraz technik transformacji genetycznej. Badaniom poddano trzy odmiany *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. – ‘Albugo’, ‘Alchimist’, ‘Sa-

tinbleu'. Promieniowanie gamma zastosowane w dawce 15 Gy pochodziło ze źródła ^{60}Co aparatu typu Theratron 780 C. Moc pochłoniętej dawki wynosiła $2,14 \text{ Gy} \cdot \text{min}^{-1}$. U odmian 'Albugo' i 'Satinbleu' napromienieniu poddano mikrosadzonki rosnące na pożywce MS. Po napromienieniu – na pożywkę MS uzupełnioną $0,6 \text{ mg} \times \text{dm}^{-3}$ BAP i $2,0 \text{ mg} \times \text{dm}^{-3}$ IAA wyłożono jednowęzłowe fragmenty pędu i liście. U odmiany 'Alchemist' napromieniano eksplantaty liściowe z kalusem zregenerowanym na ogonkach. Miesiąc wcześniej eksplantaty te wyłożono na pożywkę MS z dodatkiem $0,6 \text{ mg} \times \text{dm}^{-3}$ BAP i $2,0 \text{ mg} \times \text{dm}^{-3}$ IAA. Regenerację pędów przybyszowych prowadzono bez pasażu na tej samej pożywce. Po 4 miesiącach od napromienienia uzyskane pędy przeniesiono na pożywkę ukorzeniającą. Promieniowanie gamma wpłynęło na regenerację pędów na eksplantatach. Spośród uzyskanych mutantów w pokoleniu vM_2 wyłoniono pięć interesujących, godnych wprowadzenia do uprawy genotypów: 'Albugo Sunny', 'Alchemist Tubular', 'Alchemist Golden Beet', 'Satinbleu Minty', 'Satinbleu Honey'.

Słowa kluczowe: *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam., mikrorozmnażanie, promieniowanie gamma, mutacje

Accepted for print – Zaakceptowano do druku: 28.02.2011