QUANTITATIVE AND QUALITATIVE CHARACTERS ASSESSMENT IN THE NEW GLADIOLUS FORMS DERIVED THROUGH GAMMA RADIATION

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Abstract. The assessment of the quantitative and qualitative indices has demonstrated that radiation dose increase results in the prolongation of the phenological stages, namely days before plant germination, days before flowering, days before flowering termination. The phenological stages almost coincide with the phenological stages of the witness at a dose of 30 Gy. Yet, beginning with the dose of 50 Gy, the duration of the stages grows, it being nearly twice as long as in the witness at 150-200 Gy. The dose of 150-200 Gy completely inhibits the development of the flower spike. In addition, a reduction of the flower spike length, the total flower number, the number of the simultaneously opened flowers has been observed. Thus, high doses have a negative impact on the growing processes and inhibit the mitotic activity in the flower shoot apex. The analysis of the perianth size has shown that a correlation between this character and the radiation dose is linear, inversely proportional, thus, the perianth inner and outer circle reduces with the dose increase. The percentage of the yielded corms decreases with the increase of the radiation dose. The average corm weight decreases in comparison with the witness.

Keywords: gladioli, gamma radiation, “in vitro” culture, regenerants.

Rezumat. Evaluarea caracterelor cantitative şi calitative la formele noi de gladiolă obţinute ca rezultat al influenţei radiaţiei gamma. În rezultatul evaluării indicilor cantitativi şi calitativi s-a constatat că, odată cu mărirea dozei de radiaţie, se măreşte perioada fazelor fenologice şi anume numărul de zile până la răsărirea plantei, numărul de zile până la înflorire, numărul de zile până la sfârşitul înfloririi. La doza 30 Gy fazele fenologice aproape coincid cu fazele fenologice ale martorului. Însă începând cu doza 50 Gy durata fazelor creşte, la 150-200 Gy fiind aproape de două ori mai mare comparativ cu martorul. Doza de 150-200 Gy inhibă complet dezvoltarea tijei florale. S-a constatat de asemenea micşorarea lungimii tijei florale, numărului total de flori, numărului de flori deschise simultan. Deci dozele înalte au o influenţă negativă asupra proceselor de creştere, inhibă activitatea mitotică în apexul lăstarului floral. Analizând mărimea periantului s-a constatat că între acest caracter şi doza de radiaţie există o corelaţie liniară, invers proporţională, astfel cercul interior şi exterior al periantului se micşorează odată cu creşterea dozei. Procentul bulbilor recoltaţi descreşte odată cu mărirea dozei radiaţiei. Greutatea medie a unui bulb se micşorează în comparaţie cu martorul.

Cuvinte cheie: gladiole, radiaţii gamma, cultură “in vitro”, regeneranţi.

INTRODUCTION

Flowers have always been a motive of joy for both who gives and receives them. Therefore, people have searched for the tools to expand the flower assortment, to produce flowers in large amounts and of various colors.

Mutagenic techniques can be used in the development of new germ plasma sources to be successfully utilized in flower species breeding (MURIN, 1989; ANTONIUC, 1991; SOSA, 1991). The number of mutant varieties in ornamental species with vegetative propagation is even higher that in those with sexual propagation (NICKE, 1985). This characteristic becomes valuable for some ornamental plants in which mutagenic methods have been applied with a special success. Ornamental plants have been known as the most receptive to the mutagenic impact. Their advantage consists in the fact that some of them are high heterozygous, therefore homozygotization of some mutant recessive genes following a mutagenic treatment is sometimes achieved in the initial generations exposed to selection. This may be a principal reason for which the mutant genotypes of ornamental plants have been developed within a very short period time of 3-4 years, which is impossible in sexually propagated crops. Thus, the advantage of the experimental mutagenesis consists in the possibility to considerably increase the genetic variation of a crop within a relatively short time period (DRYAGINA, 1981).

Mutagenic factors in decorative plants have been first experienced in snapdragon, so many variations being fixed in the first year that before were developed during 12 years (BAUER & STUBBE, 1930; BAUER, 1932).

To expand the genetic variability of the *Freesia hybrida* assortment*,* bulbotubers were X-irradiated at certain doses. A variability of some characters that presented an interest for breeding was established in the first generation, including the number of stem ramification, stem length, the flower number per inflorescence, the sepal number, flower length, and flowering time (DATTA, 1991; 1995).

An essential range of genetic variability has been obtained in gerbera (*Gerbera jamesonii*) under the influence of ionizing radiation (WALTHER et al., 1990; 1991). “In vitro” propagation is an efficient and rapid method to isolate mutants including those of *Cryzantemum* derived from γ-irradiated meristems (AHLOOWALIA, 1992).

The action of different γ-radiation doses on tulip bulbs has shown that the maximal dose is 1,000 P (for diploid varieties), 2,000 P (for triploid varieties). The findings of the experiments have demonstrated that these doses induce a maximal range of genetic variability (MURIN, 2000).

For the first time, the works involving these studies were initiated in the Republic of Moldova in the early ’70-ies. It has been found that irradiation of gladiolus seeds and corms with a dose of 5 kP stimulates the growth and development of vegetative offsprings, while corm and seed irradiation leads to the appearance of various mutants with improved decorative characters.

It is noteworthy, that gladiolus irradiation results in both hereditary alterations – mutants and chimaeras and temporary modifications – radiomorphoses. The fractionated radiation dose action has a higher effect on the variability range in comparison with the doses of the same value but administered integrally.

Thus, the practical achievements due to experimental mutagenesis demonstrate that the utilization of these methods have prospects in breeding of both farm and decorative crops.

Presently, about a hundred of new names belonging to more than twenty species cultivated in different countries of the world have been added through the experimental mutagenic method.

As mentioned before, induced mutagenesis, the method of cell and tissue culture have become more and more useful in genetic research and breeding (GHIORGHIȚĂ, 1990; ARORA, 1995; KAMO, 1997).

MATERIAL AND METHODS

Characterization of the material used in the research.

The *Gladiolus* genus comprises about 250 spontaneous and cultivated species, more than 8 thousand varieties that belong to the *Iridaceae* family.

The wild species present a decorative interest and especially through their light bright inflorescence. The flower spike is short and the flowers are small, the characters that need an essential improvement for the decorative purpose.

The decorative gladioli fall into two groups: those with a winter-spring development (*G. x colvillei, G. x nanus, G. x tubergenii, G. x haarlemensis*) and those with a summer development (*G. x primulinus, G. x hybridus hort*.).

Decorative gladioli originate from African species and need a rest period in the North conditions, which is more favorable during winter because of the weak resistance to wintering. Therefore, corms are dug up in the autumn and planted again in the spring.

Gladioli belong to the neutral plant group for the photoperiodic reaction due to the origin area and a high amount of nutrients in corms. Gladioli can flower under the conditions of reduced illumination up to 8-10 hours and even form inflorescences in the dark.

The *Gladiolus* genus is composed of the species that have a different number of chromosomes in 2n=30 up to 2n=120. Of 53 studied varieties, half have a chromosome number in somatic cells making 2n=30 and are diploids (27 varieties), the rest of them are polyploids: triploids (2n=45), tetraploids (2n=60), pentaploids (2n=75), hexaploids (2n=90), and octaploids (2n=120).

The gladioli varieties Aniutinî glazki and Jreț 458, were used as an object material for this research set.

Aniutinî glazki variety

According to the vegetation beginning-flowering period, this variety is assessed as one with a medium vegetation period. It makes 53-81 days depending on the environmental conditions. The basic flower color is white (00). The flower spike is long (95-102 cm). The inflorescence is formed of 19-21 flowers, 7-9 of which blossom simultaneously. The propagation capacity is good.

Jreț 458 variety

It is a variety derived from crossing the Little Rock variety (458) and the 6-102 (558) form. According to the vegetation duration, it is a medium period variety. The basic flower color is dark cherry (04). The flowers are large. The flower spike is 91-110 cm long. According to the vegetation-flowering duration, it is considered an early variety, the duration period making 71-74 days. The inflorescence is formed of 18-19 flowers, 7-8 of them blossom simultaneously.

Irradiation of the biologic material

The irradiation of the material under study was made using an RXM-γ-20 installation and the radiation source was the Co^{60} isotope. The irradiation was single with an irradiation flow of 20 R/min. The doses administered were as follows: 5, 10, 20, 30, 40, 50, 100, 150, 200, 230, 250, 300, 400, 500, 600 Gy. We mention that the 200 Gy dose was lethal.

RESULTS AND DISCUSSION

The research aimed at the assessment of variability frequency and range in gladioli.

To accomplish this aim the following objectives were set forward:

Appreciation of radiosensitivity and radioresistance at the tissue and vegetative organ (corm) level.

- Identification of efficient γ-radiation doses to start the mutation and recombination process for diversification of the genetic variability range in the tissue culture.
- Estimation of the frequency and range of chromosomal aberrations in the regenerant offsprings derived through callus γ-irradiation.
- Studies on the genetic variability range in the regenerants derived from the tissue culture and γ-radiation utilization through a study of DNA molecule polymorphism.
- Studies on the regenerants derived through tissue culture and γ-radiation for a number of quantitative and qualitative characters.

The following indices were used as a criterion of radiosensivity: "cormel germination percentage" at the intact organ level; "callus weight dynamics", "somatic embryogenesis frequency", "plantlet regeneration percentage", "rooting percentage", and "root growth dynamics" at the tissue level.

The γ-radiation within the dose limits of 5-30 Gy has been found to have a weak stimulating effect at the intact organ level. It is characteristic of the both varieties studied - Jreț and Aniutinî glazki. The γ-radiation impact on the cormel germination percentage has an inhibiting effect within the dose limits of 50-150 Gy. It has been found that the dose of 200 Gy has a lethal effect on cormel germination in the two studied varieties (Fig. 1).

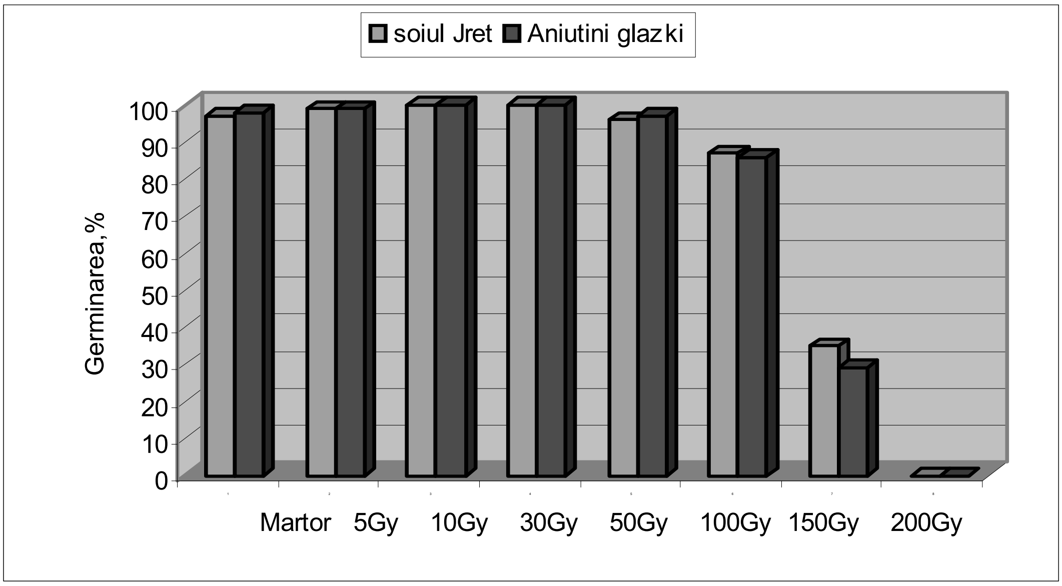

Figure 1. Radiosensitivity of gladiolus cormels at the action of gamma radiation.
Figura 1. Radiosensibilitatea bulbililor de gladiole sub acțiunea radiației gamma.

The limits of stimulating, inhibiting, and lethal doses have been estimated according to the distribution curve of the γ-radiation dose effect on the indice of cormel germination (Table 1).

Table 1. The effective dose in the γ-radiation of gladiolus cormels.
Tabel 1. Doza-efect la iradierea gamma a bulbililor de gladiole.

Variety	γ-radiation dose, Gy		
	Stimulating	**Mutational**	**Lethal**
Jreț	5-30	50-150	200
Aniutinî glazki	5-30	50-150	200

The effect of γ-radiation on the regenerants has been evaluated by appreciating the quantitative indices of the genotypes. The analysis of the indices has shown that the radiation dose increase extends the period of phenological stages, and namely, days before plant germination, days before flowering, days before flowering termination (Table 2). At the dose of 30 Gy, the phenological stages almost coincide with those of the witness. Yet, the stage duration extends beginning with the dose of 50 Gy, at 150-200 Gy, it is almost twice as long as in the witness. The dose of 150-200 Gy completely inhibits the development of the flower spike.

An important quantitative character for gladioli is the flower number per inflorescence. The analysis of the modifications in the plant height and quantitative characters of inflorescence has established that the indices of the quantitative characters decrease with the radiation dose increase in comparison with the witness.

Thus, the plant height differs slightly in comparison with the witness in the both varieties studied at 30 Gy, yet it is considerably lower at 50-200 Gy. The analysis of the inflorescences has also revealed a decrease in the flower spike length, the total number of buds, the number of simultaneously opened flowers (Table 3). This is explained by the fact that high doses have a negative influence on the growing processes reducing the mitotic activity in the flower shoot apex.

Analyzing the perianth dimensions, we have found that the correlation between this character and the radiation dose is linear, inversely proportional, thus the dimension of the inner and outer circle in the perianth reduces with the dose increase (Table 4). It has been concluded from the findings that the doses of 150-200 Gy have an inhibiting impact on the plant growth and development.

The mutations that affected the leaf, inflorescence, and flower shape, flower stem ramification were dominant. The highest number of changes was observed at the doses of 50 Gy and 100 Gy, which was significantly lower in the witness and at the dose of 30 Gy.

The irradiation has acted on the productivity of vegetative propagation, as well (Table 5). The percentage of the cormels yielded decreases with the increase of the radiation dose. The average weight of a corm reduces in comparison with the witness. The number of cormels yielded per a corm also demonstrates a negative action of the radiation on the vegetative propagation in the first generation (R_1) after the regenerants have been produced.

Table 2. The influence of γ-radiation on the duration of gladiolus phenological stages (M_1R).
Tabel 2. Influența radiației-γ asupra duratei fazelor fenologice la gladiole (M_1R).

NN	Variety name	γ-radiation dose, Gy	Days before plantlet germination	Days before flowering	Days before flowering termination
1.	Jreț	Witness	19±0.35	83±0.12	95±1.33
2.		30	19±0.17*	86±0.35**	104±1.01**
3.		50	20±0.12**	97±1.02**	107±4.34*
4.		100	23±0.15**	99±1.0***	111±2.35***
5.		150	25±0.23***	-	-
6.		200	27±1.35***	-	-
7.	Aniutinî glazki	Witness	20±0.34	84±0.32	96±0.55
8.		30	20±1.95	86±0.95*	102±0.79*
9.		50	23±1.35*	98±3.35***	109±3.37***
10.		100	24±3.38*	101±4.34***	116±2.48***
11.		150	26±3.25**	-	-
12.		200	28±2.9***	-	-

Legend: *,**, *** significant at P≤0.05; P≤0.01 and P≤0.001, respectively

Table 3. The influence of γ-radiation on the gladiolus quantitative characters (M_1R).
Tabel 3. Influența radiațiilor-γ asupra caracterelor cantitative la gladiole (M_1R).

Variety	Quantitative character indices	Witness	Γ-radiation doses, Gy				
			30	50	100	150	200
Jreț	Plant height (cm)	100.88±4.52	93.13±5.58	82.34±7.05*	60.0±5.01*	42.63±50.1***	20.31±3.34***
Inflorescence analysis							
	Flower spike length (cm)	60.4±8.3	63.8±6.02	58.5±4.59	45±5.0*	-	-
	Total flower bud number	20.1±1.34	18.7±1.34	18.95±0.99*	13±0.12**	-	-
	Number of simultaneously opened flowers	5.05±1.02	4.84±1.01	4.1±0.73	3.0±0.95*	-	-
Aniutinî glazki	Plant height (cm)	99.3±3.53	99.51±4.39	78.3±3.35*	55.0±4.57*	40.1±3.81***	35.0±2.35***
Inflorescence analysis							
	(cm) Flower spike length (cm)	54.5±1.35	53.4±1.58	50.3±2.51	39.4±4.32***	-	-
	Total flower bud number	18.2±1.71	16.3±3.25	15.1±2.38**	13.3±3.41***	-	-
	Number of simultaneously opened flowers	4.8±1.39	4.5±2.11	4.0±1.95	3.5±0.91*	-	-

Legend: *,**, *** significant at P≤0.05; P≤0.01 and P≤0.001, respectively

Table 4. Analysis of the dimensions of the perianth outer and inner circle under the influence of γ-radiation.
Tabel 4. Analiza dimensiunilor cercului exterior şi interior al periantului sub influența radiațiilor gamma.

Variety	Indices	Witness	γ-radiation dose, Gy				
			30	50	100	150	200
Jreț	Perianth outer circle	11.34±1.20	10.27±1.27	9.2±1.01*	8.0±1.0*	-	-
	Perianth inner circle	3.52±0.65	2.52±0.51*	2.5±0.63*	2.3±0.95*	-	-
Aniutinî glazki	Perianth outer circle	10.9±1.23	10.3±1.31	9.3±1.31	2.0±1.02***	-	-
	Perianth inner circle	2.78±1.03	2.5±1.04	2.0±0.95**	1.95±1.32**	-	-

CONCLUSIONS

The γ-doses of 50, 100, and 150 Gy provoke in gladioli essential disorders in the mitotic process of the *"in vitro"* yielded regenerants. The resistance of the varieties to the radiation action is different.

The "in vitro" regenerants differ essentially in the quantitative and qualitative characters from the varieties of mutagenic origin. The regenerants are characterized by an increase in the duration of the phenological periods of germination, germination-flowering, flowering beginning-termination. The duration of the phenological periods extends with the increase of the radiation dose used to irradiate explants.

The gamma radiation at a dose higher than 50 Gy induces in gladiolus regenerants a regress of the quantitative characters – plant height, flower spike length, total number of flower buds, the number of simultaneously opened flowers, the perianth diameter, the corm number, weight, and viability.

Table 5. The action of γ-radiation on the vegetative propagation capacity in gladioli (M_1R), the mean of the experimental results for the years of 1999-2000.
Tabel. 5. Acțiunea radiației γ asupra capacității de multiplicare vegetativă la gladiole (M_1R), media rezultatelor experimentale pentru anii 1999-2000.

NN	Variety name	γ-radiation dose, Gy	Number of cormels planted, unit	Number of the corms yielded	% of cormel viability	Average weight of a corm, g	Number of the cormels yielded per a corm, unit	Average weight of a cormel, g
1.	Jreț	Witness	30	25	83.3	57.3±5.31	50.5±5.31	0.544±0.20
		30	30	27	90.0	49.3±1.75*	49.3±1.75	0.488±0.08
		50	25	18	72.0	36.2±3.54***	12.2±2.31***	0.155±0.10
		100	30	20	66.7	21.5±2.88***	7.33±1.50***	0.161±0.11*
		150	20	10	50.0	11.8±1.94***	3.66±2,16***.	0.096±0.05*
		200	20	7	35.0	9.3±0.81***	-	-
2.	Aniutinî glazki	Witness	30	26	86.7	52.0±2.36	46.0±3.52	0.332±0.23
		30	30	26	86.7	45.6±3.50*	44.16±4.16	0.294±0.12
		50	30	20	66.7	31.2±3.43***	10.33±3.88**	0.155±0.10
		100	20	11	55.0	26.0±3.40***	7.0±2.36**	0.048±0.03*
		150	20	9	45.0	10.3±3.07***	4.0±3.84***	0.060±0.03*
		200	20	5	25.0	6.5±2.88***	-	-

Legend: *, *** significant at P≤0.05 and P≤0.001, respectively

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