RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
54 (5) 1049-1065, 2022
http://doi.org/10.54910/sabrao2022.54.5.8
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

MOLECULAR, PHENOTYPIC MARKER ASSAYS, AND RADIOSENSITIVITY TESTS OF GAMMA-IRRADIATED *CELOSIA ARGENTEA*

E. KURUCZ[1*], A. ZS. ANDRÉ[1], M.G. FÁRI[2], M. SIPOS[1], and G. ANTAL[1]

[1]Institute of Horticultural Science, Food Science and Environmental Management, University of Debrecen, Hungary
[2]Institute of Plant Science, Food Science and Environmental Management, University of Debrecen, Hungary
*Corresponding author's email: era.kurucz@gmail.com
Email addresses of co-authors: zsilane.andre.aniko@gmail.com, miklos0810@gmail.com, siposmarianna@agr.unideb.hu, antal.gabriella@agr.unideb.hu

SUMMARY

Random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), and UPOV phenotype markers were used to study the DNA polymorphism in gamma-ray induced morphological mutants of *Celosia argentea* var. *plumosa* Hungarian variety 'Arrabona.' In the experiments, the study determined the radio sensitivity and the genetic diversity of gamma radiation of *C. argentea* var. *plumosa* 'Arrabona.' Seeds of *C. argentea* var. *plumosa* 'Arrabona' were irradiated with gamma rays to increase their genetic diversity. The irradiation doses consisted of 0, 75, 150, 300, 450, and 600 Grays (Gy). The germination percentage, survival rate, and phenotype of irradiated plantlets underwent evaluation in the first (M1) and second (M2) generations. The investigation of genetic diversity used the ISSR and RAPD primers. Based on the results, the first-generation genetic distance increased as the doses increased. But the trend changed considerably through the generation due to the low condition and fertility of the high doses of gamma-irradiated plants. These individuals did not show at the next mutant generation, changing the population gene pool. In addition, open pollination has also changed genetic diversity. The RAPD and ISSR primers proved proper to evaluate the genetic diversity, nonetheless fewer direct connection occurred between the appearance and the used RAPD or ISSR markers. The LD_{50} dose between 150 and 300 Gray treatments and the radiation between 300-450 Gray induced the median growth reduction in the mutant 'Arrabona' population. Based on these results, the study concluded that both UPOV-based phenotyping and molecular marker analysis revealed appropriate for determining genetic divergence, but detecting greater genetic distance resulted in molecular markers.

Keywords: *Celosia argentea* var. *plumosa*, gamma irradiation, genetic variability, RAPD and ISSR primers, UPOV

Key findings: This paper determined the optimal gamma-radiation dose range to generate new *C. argentea* var. *plumosa* varieties. Results defined the LD_{50} and GR_{50} values as the best adequate data for predicting the irradiation effectiveness for mutant variant induction. The UPOV phenotypic determination method and the RAPD and ISSR primers determined the genetic distance within the M1 and M2 generations.

Communicating Editor: Prof. Dr. Sanun Jogloy

Manuscript received: September 20, 2022; Accepted: November 6, 2022.

To cite this manuscript: Kurucz E, André AZs, Fári MG, M. Sipos M, Antal G (2022). Molecular, phenotypic marker assays, and radiosensitivity tests of gamma-irradiated *Celosia argentea*. *SABRAO J. Breed. Genet.* 54(5): 1049-1065. http://doi.org/10.54910/sabrao2022.54.5.8.

INTRODUCTION

A mutation is a sudden heritable change in the genome of the living cell, which happens randomly and rarely in nature. Mutation breeding is the decided application of artificial/induced mutations in plant breeding by chemical and physical mutagen agents. Mutation breeding is a beneficial opportunity to create new varieties of seedless crops (Pathirana, 2011; Gupta *et al*., 2016). Mutation breeding in ornamental crops is well studied and successful in ornamental plants for decades because of the easy detection of changes in phenotypic characteristics like flower color, shape and size, chlorophyll variegation in leaves, and growth habit (Mohan, 2006; Sood *et al*., 2016; Saika and Hee, 2020). In addition, it easily propagates many ornamental species vegetatively, facilitating the reproduction of the variable mutant and sports lines (Ibrahim *et al*., 2018; Yamaguchi, 2018). At the end of the 1990s, the generation of 'sports families' by mutagenesis has become a routine practice for several important ornamental genera. Breeders became interested in producing new flower color mutants from crossing mutant populations before the original genotype was spread in the market (Broertjes *et al.*, 1980) because former plant breeders' rights regulations allowed the breeder of a sport to take advantage of it commercially. The situation has changed as most countries followed the UPOV convention of 1991 that determined the variety rights of every sport to the breeder of the original cultivar (Schum and Preil, 1998).

Celosia species belong to the family *Amaranthaceae,* whose common name is cockscomb or feathered amaranth. It originates from tropical and subtropical regions. The wild form is *C. argentea,* and cultivars divide into *C. cristata* and *C. plumosa* (Kanu *et al.*, 2017). Each represents three groups of *Celosia* based on the inflorescence size and shape. The plants served bedding purposes, as potted plants or cut flowers (Kováts, 2009). According to Cai *et al.* (2005) and Spórna-Kucab *et al.* (2018), among other bioactive components, *Celosia* species contain betalains (betacyanins, betaxanthins), flavonoids, saponins (celosins), and phenol glycosides (Miguel, 2018; Thorat, 2018).

The wide range of varieties provides a good source for brief breading programs, which can strengthen the genetic stress tolerance and the secondary metabolite content, increasing the medicinal values (Aisyah *et al.*, 2019, 2022; Yudha *et al.*, 2022) besides the ornamental value. Hence, the varieties' uses serve many purposes even under extreme environmental conditions. Hungary had pivotal decorative breeding activity in the 70s, thanks to the dedicated work of Dr. Zoltán Kováts, a famous Hungarian ornamental plant breeder (Fári *et al.,* 2019). Dr. Zoltán Kováts bred the investigated 'Arrabona' variety (named after an ancient Hungarian city), which has received the Fleuroselect Gold Medal and Approved Novelty of Fleuroselect (Naric Research Institute, Hungary). A low-maintenance dwarf variety with a 35-cm height, Arrabona has a long flowering season and tolerates drought and heat. Its feathery flower spikes are orange-red, making it suitable for bedding (edging borders) or as a container plant for parks and landscaping in tropical, subtropical, and continental climates (Fleuroselect, 2022).

Using mutagenesis by gamma radiation (GR) for plant breeding purposes requires the determination of the optimal radiation doses, which can cause a wide range of genetic diversity, such as, the dosage when the population reduction is 50% (median lethal dose, LD_{50}) (Sholihin *et al*., 2019; Aisyah *et al.,* 2021). The adequate parameters, among others, include survival rate, the radiation dose that reduces growth in 50% of the population (median growth reduction, GR_{50}), and the mass or number of germinated specimens (FAO/IAEA, 2015). These parameters depend on the dosage, species, varieties, plant tissue (seed, meristem, callus, etc.), stage of development, and moisture content in the radiation time (Riviello-Flores *et al.*, 2022).

Low radiation can cause a smaller or larger vitality improvement. Meanwhile, the high radiation doses induce radio inhibition by affecting growth regulators and secondary metabolites and, eventually, tissue destruction in most cases (Surakshitha and Soorianathasundaram, 2017; Mostafa *et al.,* 2019). Many authors so far reported a reduction of regenerative capacity and malformation of plant tissues, as well as, tissue destruction due to the high dose of radiation (Chakravarty and Sen, 2001; Yamaguchi, 2018; Andrew-Peter-Leon *et al.*, 2021). Radio sensitivity tests are essential for determining the appropriate radiation dose to induce the highest genetic diversity with the most useful fixed mutations (Songsri *et al.,* 2019; Riviello-Flores *et al.*, 2022). Studies also stated that irradiation with low radiation doses stimulates the plants' vitality, which is beneficial for plant regeneration (Jala and Bodhipadma, 2011; Abubakar *et al*., 2017; Surakshitha and

Soorianathasundaram, 2017; Aisyah *et al.*, 2021). Some studies have reported radiation stimulation by GR in some plant varieties of *Ocimum basilicum* or cowpea (Enkhbileg *et al.*, 2019; Aisyah *et al.*, 2021).

Modern molecular tools, like a polymerase chain reaction (PCR)-based method, have been given a new opportunity to detect the genetic diversity of a plant's mutant population even before reaching the proper vegetative stage (the appearance of inflorescence or fruit or reaching the final habit). Time-saving and cheap PCR-based methods include inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) used by many scientists to clarify the genetic diversity of mutant populations (Mostafa *et al*., 2014). This study aims to determine the gamma-radiosensitivity of *C. argentea* var. *plumosa* 'Arrabona' varieties and the second-generation genetic diversity to predict the effectiveness of gamma irradiation in the *Celosia* spp. breeding program.

MATERIALS AND METHODS

Gamma-ray irradiation of seeds

Seeds of *Celosia argentea* var. *plumosa* 'Arrabona' underwent irradiation using a 60Co (Cobalt 60) gamma source in ambient conditions, observing all required safety procedures. The standard radiation dose (0-600 Gy) was used during the radiosensitivity test, as determined by the Plant Breeding and Genetics Laboratory, FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications. The seeds were irradiated on 11 July 2018. The seeds (20 seeds per dose in three replications) received irradiation in paper bags and remained in the same bags until the examinations.

Germination and plant growing conditions

Sowing of the treated seeds (M0) proceeded at equal depths in a 58 cm × 38 cm × 12 cm tray filled with soil/compost containing the five treatments in rows of 20 seeds each – including the control and each other treatment, based on the standard operating procedure (Konzak *et al.,* 1967; Van-Harten, 1998). Each test performed three replicates, one tray per replicate (Figure 1). The trays were placed in a greenhouse of the University of Debrecen Faculty of Agriculture, Food Science, and Environmental Management (MÉK) at a temperature of 25°C. Fourteen days after sowing, measuring the seedling height of the M1 population proceeded to determine the Growth Reduction Value 50 (GR50). Seedling height measurement began from the soil level to the tip of the primary leaves. The seed collection of the M1 population took place in September 2018, while the sowing of the collected seeds occurred in March 2019 (M2). Growing the plantlets of the M2 population used pots under semi-covered conditions proceeded in the same place as that of 2018, as extreme environmental conditions affected the tests that year.

Figure 1. The gamma radiosensitivity test of *Celosia argentea* var. *plumosa* 'Arrabona.'
A: 10 days after sowing, the control plants were sown on the right side, the 600 Gy plants were on the left side, B-D: 40-day-old plantlets.

Survival rate calculation

Every irradiated germinated plantlet of the first mutant generation, after the GR50 measurement, underwent transplanting under field conditions in Debrecen, Hungary (GPS: 47°59' E, 21°55' N), except for the individuals surviving from the 600 and 450 Gy irradiated plantlets. Transplanting occurred due to their very low vitality (in the case of 600 Gy) or to improve their health and chance for seed production. Calculating the survival rate of the field population consisted of the rate of transplanted plants to the only remaining plants from the M1 generation.

DNA isolation

Total genomic DNA isolation from three-month-old plants took place, wherein the collection of three plants' young shoots from each subpopulation ensued and mixed for DNA isolation in M1 and one to three plants in M2 populations. Isolation of DNA from the prepared plant sample used the Zymo Quick-DNA Plant/Seed Miniprep Kit (Zymo Research Corp., USA) based on the manufacturer's protocol. The quantity of isolated genomic DNA samples occurred spectrophotometrically (UVS99-Avans Biotechnology Corporation) by measuring absorbance at 260 nm and 280 nm for the OD260/OD280 ratio. Also, visually checking the quality underwent UV light illumination after electrophoresis on 0.8% agarose gel. The stock DNA dilution made the required working solution of 10 ng/µl.

ISSR and RAPD-PCR amplification

The PCR for DNA amplification used the ISSR primers. Comparing the DNA fingerprinting profiles helped evaluate the clonal fidelity and genetic stability. Amplification proceeded in a 50 µL PCR mixture consisting of 47µL DreamTaq Green PCR Mastermix (which contains DreamTaq Green DNA polymerase, dNTP [dATP: dTTP: dCTP: dGTP in 1:1:1:1 parts], and DNA polymerase buffer with $MgCl_2$), 2µL genomic DNA, and 1µL ISSR or RAPD primer, following the methods of Fatinah *et al*. (2012) and Oduwaye *et al.* (2014) to determine the genetic diversity within the Amaranth family (Table 1).

The obtained PCR reactions used a thermal cycler (MJ Research PTC-150 Thermal Cycler) based on the parameters in Table 1. Amplicons were electrophoresed on 1.5% agarose gel and stained with ethidium bromide (5 µL/gel). The DNA marker used 100bp plus DNA Ladder (ThermoFisher Scientific Co.), bands' visualization used a UV light, while photographing them used the Gel Documentation equipment (Bio Rad Laboratories Inc.).

Table 1. The Primers sequences and the PCR steps.

Primer name	Sequence 5'-3'	Melting temp. (°C)	Num. of cycles	Denat. temp. (°C)	Program	End
UBC-807	AGAGAGAGAGAGAGAGT	47	40	94°C-1 min.	94°C-20 sec, a.h-20 sec, 72°C-2 min.	72°C – 6 min.
UBC-810	GAGAGAGAGAGAGAGAT	51	40	94°C-1 min.	94°C-20 sec, a.h-20 sec, 72°C-2 min.	72°C – 6 min.
UBC-818	CACACACACACACACAG	52	40	94°C-1 min.	94°C-20 sec, a.h-20 sec, 72°C-2 min.	72°C – 6 min.
UBC-835	AGAGAGAGAGAGAGAG(CT)C	49	40	94°C-1 min.	94°C-20 sec, a.h-20 sec, 72 °C-2 min.	72°C – 6 min.
UBC-836	AGAGAGAGAGAGAGAGYC	50,2	40	94°C-1 min.	94°C-20 sec, a.h-20 sec, 72°C-2 min.	72°C – 6 min.
UBC-840	GAGAGAGAGAGAGAGAYT	47,4	40	94°C-1 min.	94°C-20 sec, a.h-20 sec, 72°C-2 min.	72°C – 6 min.
UBC-841	GAGAGAGAGAGAGAGAYC	48,5	40	94°C-1 min.	94°C-20 sec, a.h-20 sec, 72°C-2 min.	72°C – 6 min.
UBC-856	ACACACACACACACACYA	52,8	40	94°C-1 min.	94°C-20 sec, a.h-20 sec, 72°C-2 min.	72°C – 6 min.
OPZ-09	CACCCCAGTC	35,8	40	94°C-1 min.	94°C-20 sec, a.h-20 sec, 72°C-2 min.	72°C – 6 min.
OPZ-10	CCGACAAACC	33,2	40	94°C-1 min.	94°C-20 sec, a.h-20 sec, 72°C-2 min.	72°C – 6 min.

Phenotyping (UPOV)

Based on their appearance (plant height, inflorescences or leaf color, and shape), the different radiated populations were divided into three subpopulations. Then, the subpopulations underwent examination according to the UPOV standards (UPOV, 2002) concerning the *Celosia* species (Table 2). Removing some of the traits ensued (as shown in the serial number) because these parameters proved irrelevant to *C. argentea* var*. plumosa* that participated in the data collection. The generated matrix based on the UPOV guidelines proceeded conversion to the NTSYS-PC 2.02j (Rohlf, 1998), similar to what was used in the ISSR and RAPD data in creating the similarity matrixes.

Table 2. Plant features considered in this study based on the UPOV test guidelines for *Celosia* species (UPOV 2002).

1	Plant: height		
	very short	Super Dwarf Kimono Orange	1
	Short	Century Rose	3
	Medium	Martine	5
	Tall	Bombay	7
	very tall		9
2	Stem: thickness		
	Thin	Yellow Flame	3
	Medium	Bombay Gold	5
	Thick	Boscorsun	7
3	Stem: presence of anthocyanin coloration at base		
	Absent	Yellow Flame	1
	Present	Bombay, Purple Martine	9
4	Stem: intensity of anthocyanin coloration at the base		
	very weak	Bombay Yellow, Yellow Flame	1
	Weak	Bombay Gold	3
	Medium	Boscorcass	5
	Strong	Bombay, Bombay Purple	7
	very strong	Enterprise Wine-red	9
5	Stem: color of basal part		
	light green	Enterprise White	1
	medium green		2
	dark green		3
	Yellow	Celrayel, Martine Salmon	4
	Orange	Bombay Salmon, Super Dwarf Kimono Orange	5
	pinkish red	Super Dwarf Kimono Cherry-red	6
	purple red	Celkopured, Enterprise Wine-red	7
6	Stem: color of upper part		
	light green	Bombay Rose, Celrayel	1
	medium green	Martine Salmon	2
	dark green		3
	Yellow		4
	Orange		5
	pinkish red	Celkopured	6
	purple red	Super Dwarf Kimono Red	7
7	Stem: shape in cross section		
	Circular	Enterprise White	1
	Flattened	Boscorcass	2
8	Stem: ribs		
	Absent	Martine Pink, Startrek lilac	1
	Present		9
9	Stem: flowering laterals		
	Absent	Bombay Pink, Boscorsun	1
	Present	Enterprise White, Startrek Lilac	9
10	Petiole: length		
	Short	Celkopured	3
	Medium	Bombay	5
	Long	Enterprise White	7

Table 2. (cont'd).

11	Petiole: presence of anthocyanin coloration		
	Absent	Bombay Rose, Celrayel	1
	Present	Caripe, Celkopured	9
12	Leaf blade: length		
	Short	Bombay Fire	3
	Medium	Martine	5
	Long	Bombay Rose, Caripe	7
13	Leaf blade: width		
	Narrow	Bombay Fire	3
	Medium	Bombay, Caripe, Martine, Salmon	5
	Broad	Bombay Rose, Enterprise White	7
14	Leaf blade: shape		
	Narrow elliptic	Sharon	1
	Elliptic	Bombay Rose	2
	Ovate	Bombay Purple	3
	broad ovate		4
15	Leaf blade: shape of apex		
	Acute	Caripe, Sharon	1
	short acuminate	Bombay Salmon	2
	long acuminate	Celkopured	3
16	Leaf blade: color		
	light green	Bombay Salmon, Enterprise White	1
	medium green		2
	dark green	Celkopured	3
	greenish red	Flamingo Feather	4
	red purple	5	
17	Leaf blade: presence of anthocyanin coloration of the main vein		
	absent	Enterprise White	1
	present	Celkopured	9
18	Leaf blade: blistering		
	absent or very weak	Bombay Pink	1
	weak	Celrayel, EnterpriseWine-red, Startrek Lilac	3
	medium	Bombay Rose, Celkopured	5
	Strong	Enterprise White	7
	very strong		9
19	Leaf blade: undulation of margin		
	absent	Bombay Rose, Enterprise White	1
	present		9
20	Leaf blade: the curvature of the longitudinal axis		
	Upwards		1
	Straight		2
	downwards		3
21	Inflorescence: main shape		
	Spicate	Enterprise Wine-red, Flamingo Feather	1
	Plumose	Hiryu no.2, Kimono Cherry-red	2
	paniculate	Gerana Orange	3
	Cristate	Bombay Rose, Martine	4
22	Inflorescence: length of main inflorescence		
	Short	Enterprise Salmon, Martine Pink	3
	Medium	Bombay Salmon	5
	Long	Caripe	7
23	Inflorescence: width of main inflorescence		
	Narrow	Caripe, Enterprise Wine-red	3
	Medium	Bombay Fire, Martine Pink	5
	Broad	Bombay Salmon, Boscorcur	7

Table 2. (cont'd).

24	Inflorescence: color		
	white	Enterprise White	1
	green		2
	yellow	Martine Yellow	3
	orange	Super Dwarf Kimono Orange	4
	orange pink		5
	pink	Bombay Rose	6
	red	Red Chief	7
	purple		8
28	Tepal: shape		
	elliptic	Enterprise White, Enterprise Wine-red	1
	ovate	Martine, Martine Scarlet	2
30	Stamen: color of filament		
	white	Enterprise White, Martine Scarlet	1
	green		2
	yellow		3
	orange		4
	orange pink	Boscorkir	5
	pink	Bombay Orange, Canaima	6
	red		7
	purple	Bombay Purple, Boscorcass	8
31	Pistil: color of style		
	white		1
	green		2
	yellow	Martine Yellow, Yellow Flame	3
	orange		4
	orange pink	Bombay Salmon, Bombay Velvet	5
	pink	Martine Salmon, Martine Scarlet	6
	red		7
	purple	Bombay Purple	8
32	Pistil: color of stigma		
	white		1
	green		2
	yellow		3
	orange		4
	orange pink		5
	pink		6
	red		7
	purple		8

Statistical analysis

The PhylElph software analyzed the amplified PCR products. The scoring of binary matrixes for band presence or absence for each accession consisted of presence = 1 and absence = 0, with the Microsoft Excel program designing the binary data matrix in a separate datasheet for each primer. Primer banding characteristics, such as, the number of total bands (TB), the number of polymorphic bands (PB), and the percentage of polymorphic bands (PPB) resulted. Summarizing all the data in one data sheet proceeded its conversion to NTSYS-PC 2.02j (Rohlf, 1998) software, used to determine the genetic relationships among the *C. argentea* var. *plumosa* 'Arrabona' and its mutants. Generating the pairwise similarity matrices used Jaccard's coefficient of similarity (Jaccard, 1908). This matrix underwent the unweighted pair-group method for the average arithmetic analysis (UPGMA) procedure to create a dendrogram using the average linkage procedure.

RESULTS

Irradiation effects on the germination of *C. argentea* var*. plumosa*

In the first mutant generation (M1 from M0 seeds) the gamma irradiation had no negative effect on the germination percentage (Figure 1/A; Figure 2). In contrast with the M2 generation, the rate of germinated seeds decreased dramatically among the mutant populations from 83.3% to 61.6% to 35% in

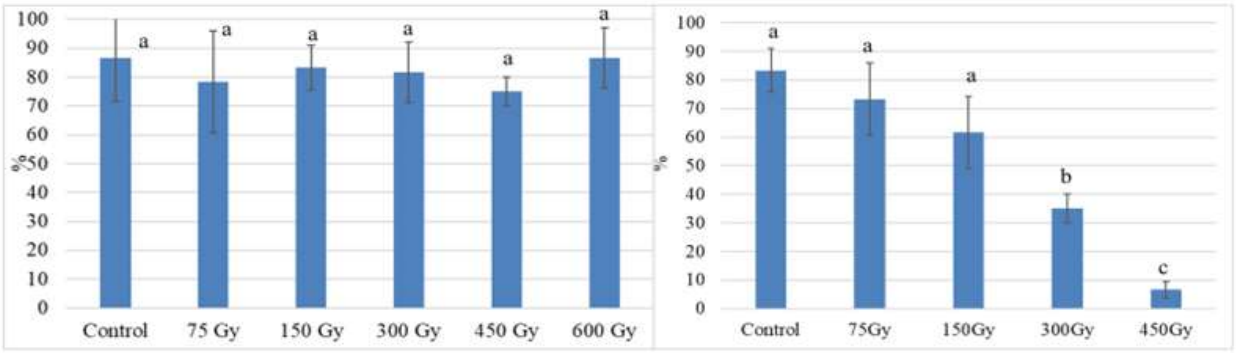

Figure 2. The germination percentage of the M0 (left) and M1 (right) generations of gamma-irradiated *Celosia argentea* var. *plumosa* 'Arrabona' population. The different letters at the top of the column represent the significant differences, which were determined with Dunnett's test at a probability level of 5%.

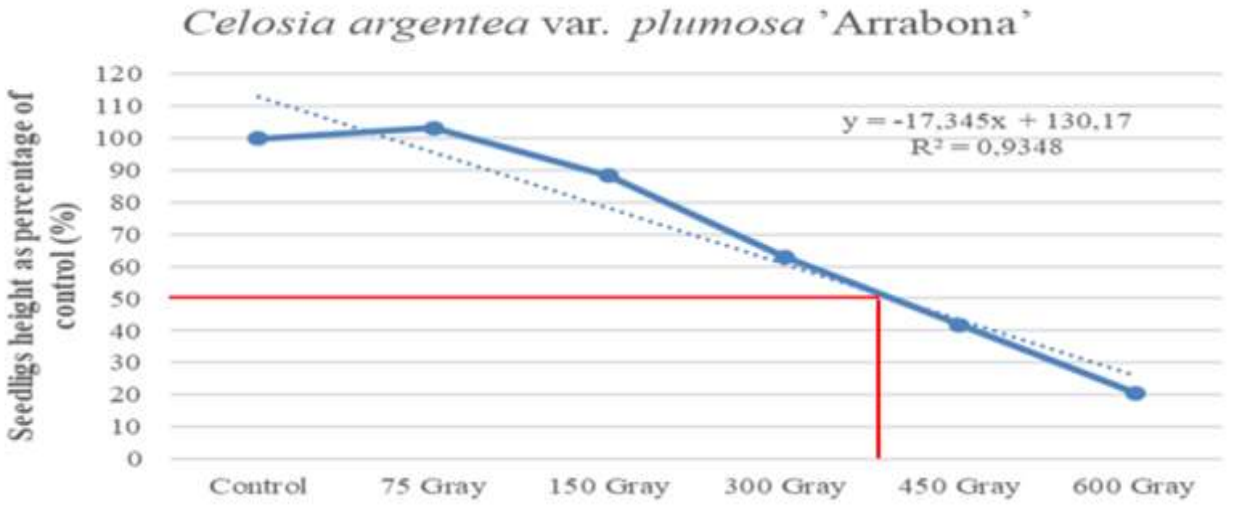

Figure 3. The seedling height changes among the radiation doses in M1 generation of gamma-irradiated *Celosia argentea* var. *plumosa* 'Arrabona.'

the case of 300 Gy and 6.6% within the 450 Gy irradiated plants (Figure 2). This reduction could be due to the low fertility of the first generation caused by the low vitality of the pollen or the less developed inflorescent within higher, even moderate dosage. In the case of okra, Jadhav *et al.* (2012) reported that first-generation pollen sterility increased in line with the increasing gamma irradiation (150–600 Gy), but it reduced in the second generation to the control level (2%–3%). The plants treated with the highest dosage did not produce seeds at all. This tendency results from the co-effect of high irradiation damage and extreme climate conditions during the vegetation phase, which harmed the vitality. The proponents also considered the negative effect of the extreme environmental conditions in the field during the data analysis. Most M1 mutant plants treated with the higher dose (450-600 Gy) did not produce seeds in general. In the case of the 450 Gy treatment, only two plants had developed vital seeds, grown in a 12-cm diameter pot.

Irradiation effect on 14-day-old plantlets of *C. argentea*

Based on the FAO/IEAE protocol in general, the effective dosage caused a 50% height reduction in the 40-day-old plantlets compared with the control plants. In the case of the *C. argentea* var. *plumosa* 'Arrabona,' the effective mutation induction dosage showed between 300 and 450 grays (Figure 3). The lower dose of gamma irradiation had some increasing

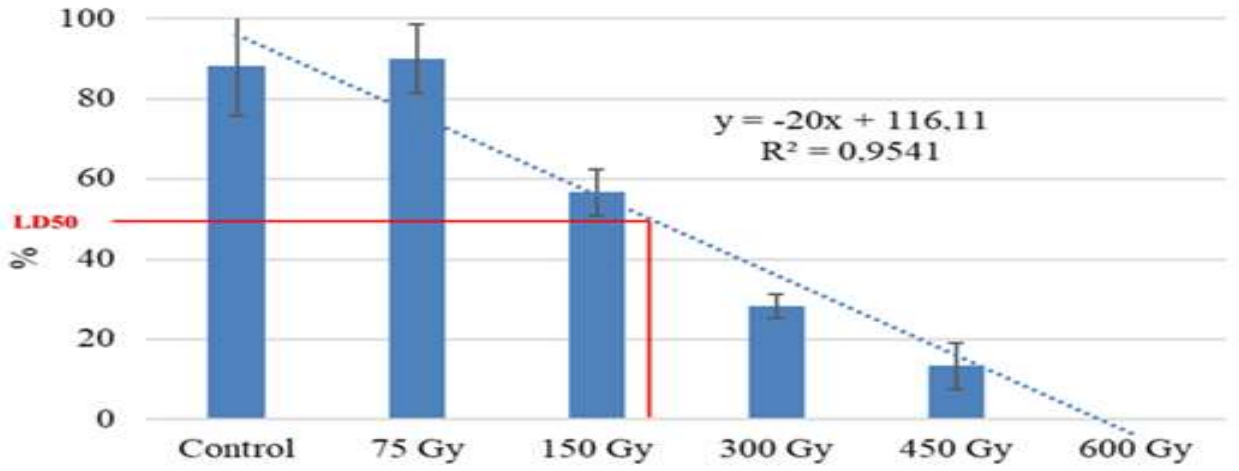

Figure 4. The survival rate of M1 generation of gamma-irradiated *Celosia argentea* var. *plumosa* 'Arrabona.'

Table 3. The information value of different primers in different mutant generations.

M1	Total number of bands	Polymorphic bands	P%	M2	Total number of bands	Polymorphic bands	P%
UBC-807	8	8	100	UBC-807	8	2	25
UBC-818	6	0	0	UBC-818	6	3	50
UBC-836	13	2	15	UBC-836	13	11	85
UBC-835	11	10	91	UBC-835	10	8	80
UBC-840	7	1	14	UBC-840	6	3	50
UBC-841	7	0	0	UBC-841	12	10	83
UBC-856	9	3	33	UBC-856	7	4	57
OPZ-09	5	2	40	OPZ-09	7	2	29
OPZ-10	10	10	100	OPZ-10	6	0	0
OPI-10	6	4	67				

Note: M1: first mutant generation.

effect on the plants' height—within 75 Gy treatment, the plant height ranged from 103%–115% of the control. According to Fehr (1978) the objective is to use dosage and rate in which 50% of the M1 seeds will germinate and produce seed for the next generation. The 300–400 gray gamma irradiation proved effective based on the abovementioned expectations.

The survival rate of the M1 mutant population

The mutant plants irradiated with higher dosage had poor vitality and condition, thus reaching vegetation shortly after the transplantation, only 13% of 450 Gy treated plants survived, while no crucial plants stemmed in the 600 Gy dosages treated population (Figure 4.). Given the low survival rate of the higher dosage, the 200–300 Gray gamma irradiation should be suitable for breeding *Celosia ssp*. The LD_{50} dosage ranged between 150 and 300 Gray of gamma irradiation.

Genetic diversity and ISSR and RAPD profile of the mutant populations

The ISSR and RAPD marker efficiencies varied in the different mutant populations. The M1 generation derived 82 bands, with 40 polymorphic (46%). The second mutant generation (M2) resulted in 67 total bands during the PCR reactions. One of the RAPD primers could not generate assessable products (Table 3). Some of the primers had identified higher polymorphism (more polymorphic bands) in the first generation (UBC-807, UBC-835, OPZ-10), and some primers revealed most effective in the second generation in identifying genetic diversity

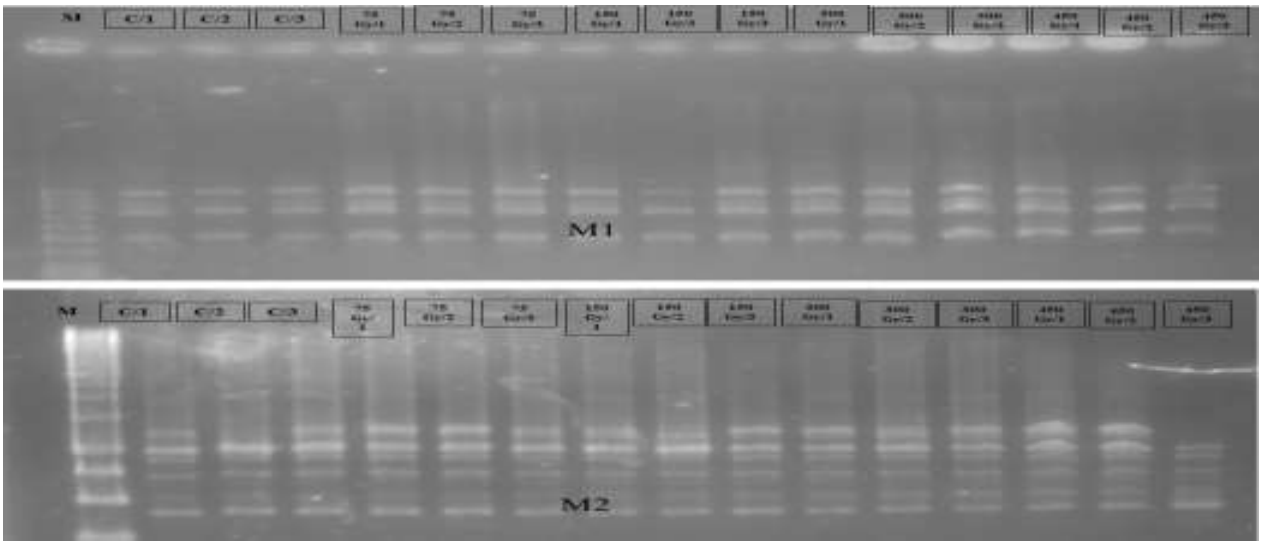

Figure 5. The agarose gel electrophoresis results of the OPZ-09 primer.

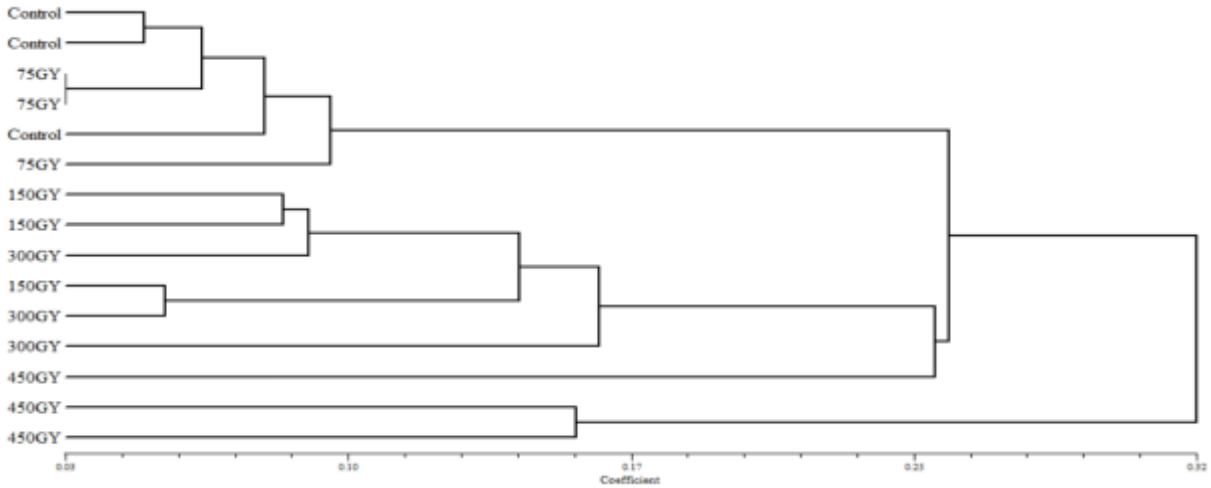

Figure 6. Dendrogram of M1 population based on ISSR and RAPD primer.

(Figure 5). The different levels of radiosensitivity within the varieties result in divergent gene pools in the following generations in the case of the *C. argentea* var*. plumosa* 'Arrabona.' Determining the ideal dose and optimizing it for the types should be beneficial information for future breeders.

The genetic diversity changed from 0.32 to 0.03 in the first generation, which remained at the same level as the second generation, but the cluster analysis showed gene pool realignment. In the first generation, the genetic distance has grown together with the radiation dosage. Meanwhile, the next generation with lower irradiation shows greater genetic distance. It is due to the mutation fixation by self-pollination of the low-fertility mutated plants. The genetic distance of the third mutant generation of soybean was 15% compared with the control plants, and the M3 generation had divided into two groups (Anwar *et al.*, 2021). Based on the clustering analysis, the first generation subdivided into two groups, which had a 0.32 genetic distance. One of the groups included the higher dosage mutant (450 Gy), and the other group with 0.27 genetic distance underwent further division into two subgroups. This group undergoes further subdividing into subclusters. As the dendrogram shows (Figure 6), the genetic

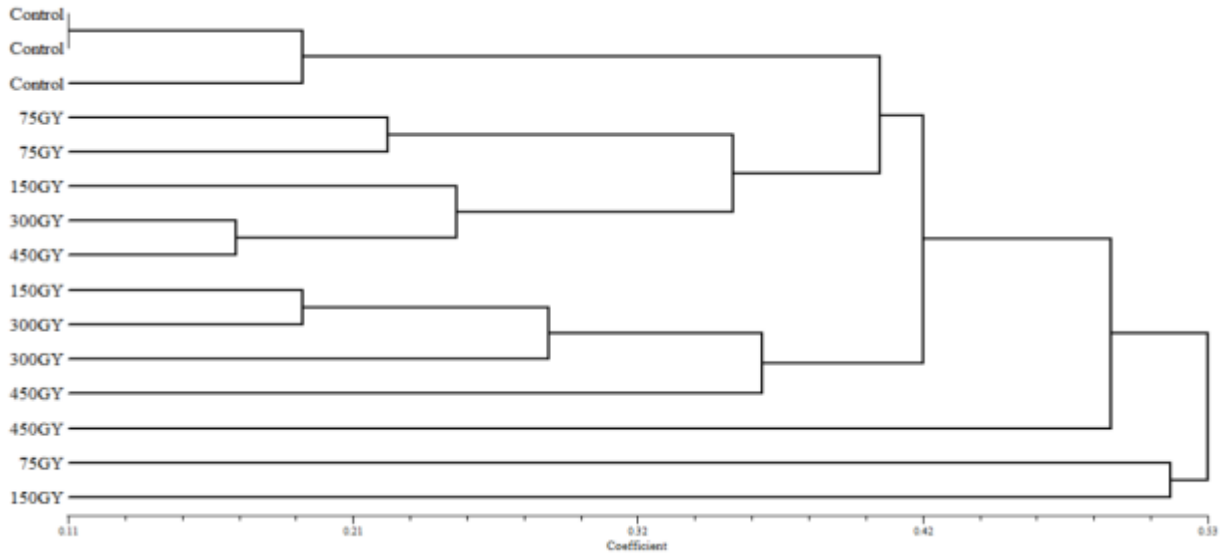

Figure 7. Dendrogram of M2 population based on ISSR and RAPD primer.

relationship between the mutant populations proved parallel to the irradiation dosage. Thus, the higher dose of gamma irradiation resulted in a higher genetic distance in the first mutant generation (0.32–0.25), except for the 75 and 150 Gy dosages, where the genetic similarity was better than other treatments (100%–90%).

The dendrogram of the M2 population depicts that the genetic relations have changed, proving the mutation fixing from generation to generation (Figure 7). Based on the ISSR and RAPD markers, the genetic distance between the 75 and 150 Gy displayed greater (0.53 dissimilarity) compared with control in the second-generation plants. The lower dose of irradiation could nearly result in a higher genetic distance. The dendrogram shows more defined groups, and some with lower dosage treatments seem to have a genetically higher genetic distance. However, the plants irradiated with higher dosages kept the higher genetic distance (Figure 7).

Phenotypic marker assay based on UPOV guidelines

The generated dendrogram, based on the matrix (Tables 4 and 5) according to the UPOV formula, shows that the genetic distance based on the phenotypic marker occasionally correlated with the results of the ISSR and RAPD primers. Most often, the plants' height, habit, inflorescence size, and/or color tend to change with the irradiation dosage. Given the generated dendrogram based on UPOV standards, the first irradiated generation (M1) undertook division into two main groups, where one included the control and a lower dose treatment (Figure 8). On the other hand, the second group contained a higher dose of GR that caused malformation of the stems and inflorescences, resulting to poor vitality and fertility at the first generation (Figure 9). These individuals did not pass on their mutations, so these mutants did not appear in the subsequent breeding program. In some cases, the leaf morphology and anthocyanin content can also change. Observation revealed that the simple PCR method effectively detected the genetic distance, even if these differences did not refer to the proper phenotypic traits. The M2 generation displayed quite a uniform appearance among and within the treatments. Most plants' phenotypes resemble the control ones, but one or two within the same treatments were completely different. The second generation formed two main clusters, the 300 Gy/2 represented the principal group and the rest of the subpopulation was divided into four groups, the largest group including the control (Figure 10). The appearance of the M2 generation showed similarity to the control, except for two individuals, which produced pink flowers (75 Gy), and two plants had shorter internodes (300 and 150 Gy) (Figure 11).

Table 4. The phenotypic matrix of M1 generation based on *Celosia* UPOV standards and descriptions.

M1	C	C	C	75/1	75/2	75/3	150/1	150/2	150/3	300/1	300/2	300/3	450/1	450/2	450/3
Plant: height	5	5	5	5	5	7	5	5	5	5	3	5	3	1	3
Stem: thickness	5	5	5	5	5	5	5	5	5	5	3	5	3	3	3
Stem: presence of anthocyanin coloration at base	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Stem: intensity of anthocyanin coloration at base	5	5	5	5	5	7	5	5	7	5	5	7	5	5	5
Stem: color of basal part	5	5	5	5	5	6	5	5	6	5	5	6	5	5	5
Stem: color of upper part	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Stem: shape in cross section	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Stem: flowering laterals	9	9	9	9	9	9	9	9	9	9	9	9	9	9	1
Petiole: length	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Petiole: presence of anthocyanin coloration	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Leaf blade: length	5	5	5	5	5	5	5	3	5	5	5	5	3	3	3
Leaf blade: width	5	5	5	5	5	5	5	5	5	5	5	5	5	3	3
Leaf blade: shape	2	2	2	2	2	2	2	2	2	2	2	1	2	1	1
Leaf blade: shape of apex	2	2	2	2	2	2	1	2	2	1	1	2	1	1	1
Leaf blade: color	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Leaf blade: presence of anthocyanin coloration of main vein	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Leaf blade: blistering	7	7	7	7	7	7	7	7	7	9	7	7	9	9	9
Leaf blade: undulation of margin	1	1	1	1	1	1	1	1	1	9	1	1	9	9	9
Leaf blade: curvature of longitudinal axis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Inflorescence: main shape	2	2	3	2	2	2	2	2	1	2	2	2	1	1	1
Inflorescence: length of main inflorescence	5	5	5	5	5	5	5	3	5	5	5	3	5	5	3
Inflorescence: width of main inflorescence	5	5	5	5	5	5	5	5	5	5	3	5	5	5	3
Inflorescence: color	7	7	7	7	7	6	7	7	7	7	7	7	7	7	7

Note: M1: first mutant generation, C: Control.

Table 5. The phenotypic matrix of M2 generation based on Celosia UPOV standards and descriptions.

M2	C	C	C	75GY/1	75GY/2	75GY/3	150GY/1	150GY/2	150GY/3	300GY/1	300GY/2	300GY/3	450GY/1	450GY/2	450GY/3
Plant: height	5	5	5	5	5	7	5	5	5	5	5	5	5	5	5
Stem: thickness	5	5	5	5	5	5	5	5	5	5	3	5	5	5	5
Stem: presence of anthocyanin coloration at base	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Stem: intensity of anthocyanin coloration at base	5	5	5	5	5	7	5	5	5	5	5	5	5	5	5
Stem: color of basal part	5	5	5	5	5	6	5	5	6	5	5	6	5	5	5
Stem: color of upper part	5	5	5	5	5	5	5	5	3	5	5	5	5	5	5
Stem: shape in cross section	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Stem: flowering laterals	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Petiole: length	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Petiole: presence of anthocyanin coloration	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Leaf blade: length	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Leaf blade: width	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Leaf blade: shape	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2
Leaf blade: shape of apex	2	2	2	2	2	2	1	2	2	1	1	2	2	2	2
Leaf blade: color	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Leaf blade: presence of anthocyanin coloration of main vein	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Leaf blade: blistering	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Leaf blade: undulation of margin	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Leaf blade: curvature of longitudinal axis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Inflorescence: main shape	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2
Inflorescence: length of main inflorescence	5	5	5	5	5	5	5	3	5	5	5	3	5	5	5
Inflorescence: width of main inflorescence	5	5	5	5	5	5	5	5	5	5	3	5	5	5	5
Inflorescence: color	7	7	7	7	7	7	7	5	7	7	7	7	7	7	7

Note: M2: second mutant generation, C: Control.

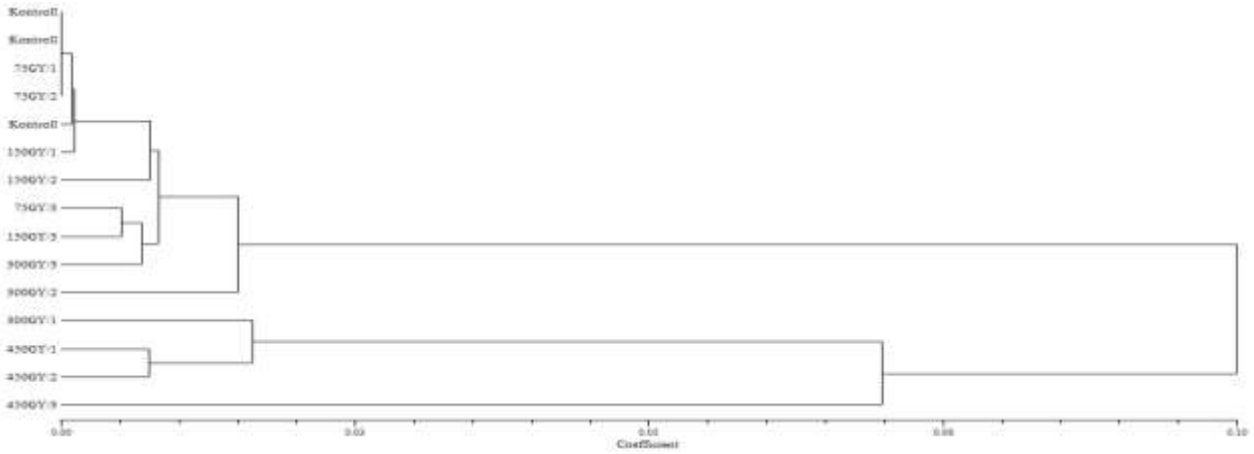

Figure 8. Dendrogram of M1 population based on phenotypic markers.

Figure 9. M1 mutant population under field condition in 2018, in Debrecen (Hungary).
A: The mutant population 22.08.2018; B-G: The mutant population 14.09.2018; C: 450 Gy mutant; D: 300 Gy mutant; E: 300 Gy mutant; F: 150 Gy mutant; H: 75 Gy mutant.

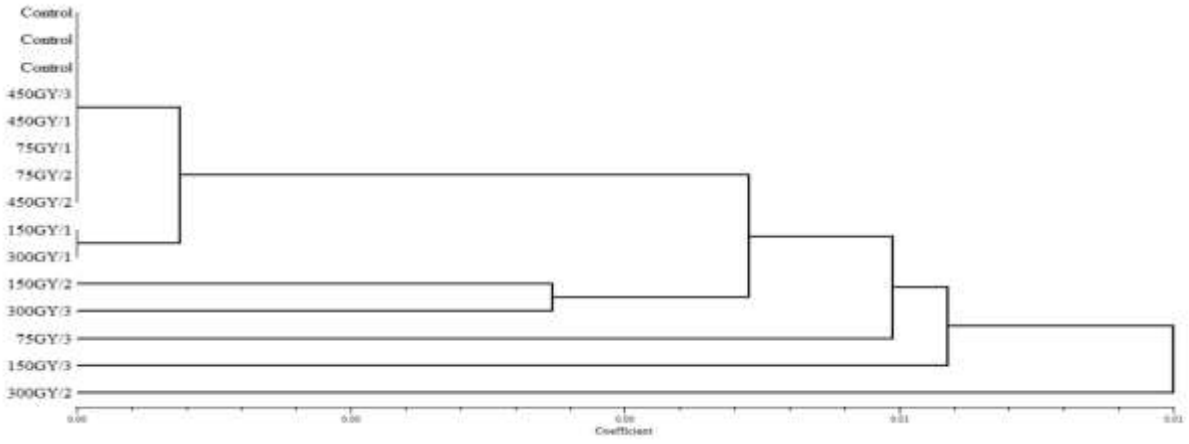

Figure 10. Dendrogram of M2 population based on phenotypic markers.

Figure 11. The M2 mutant generation of gamma-irradiated *Celosia argentea* var. *plumosa* 'Arrabona' (2009, Debrecen, Hungary).

DISCUSSION

Based on the results, the gamma irradiation did not affect germination in the first generation in the case of *C. argentea* var. *plumosa*, as confirmed by other studies dealing with the irradiation effect on germination (Melki and Marouani, 2010). The germination percentage of seeds from the next generation reduced significantly due to the poor condition of the mother plants from the previous generation. Based on the study of Melki and Marouani (2010), the lower dosage of gamma irradiation had a positive effect on the germination percentage of hard wheat, and Beyaz *et al.* (2016) demonstrated that the *Lathyrus chrysanthus* was insensitive to even the highest doses (100-150 Gy). In addition, in the case of cowpea, the germination was not inhibited by even 500 Gy of gamma rays (Bind and Dwivedi, 2014). However, the high dosage (100-1000 Gy) harmed the germination percentage in several plant species (maize, okra, and groundnut) (Mokobia and Anomohanran, 2005; Um *et al.,* 2017).

Based on study observations, the higher irradiation (450-600 Gy) affected the plants' health and the survival rate undesirably. Meanwhile, the 75-150 Gy resulted in the highest genetic diversity, with no vital degradation observed. The results are in line with other studies that have also reported negative effects of higher irradiance (Sood *et al.*, 2016; Mahla *et al.,* 2018; Majeed *et al.*, 2018), but the results of this study show better resistance in the case of *C. argentea* var. *plumosa*. Based on the study of Aisyah *et al.* (2021), the differences between varieties are quite large and need further investigation. In the case of *in vitro* culture, the condition can enhance the radiosensitivity of *Celosia* species (Hayati and Aisyah, 2016), but such a method is very expensive and time-consuming for an annual ornamental species.

The genetic diversity in the mutant generation varied among the irradiation doses in the first generation. Generally, it is higher within the increased mutagen dosage, even if it is a physical or chemical mutagen agent. Single primer-based PCR techniques like ISSR and RAPD can determine genetic diversity (Fatinah *et al*., 2012; Chikmawati, 2019; Ho and Tu, 2019), but the results should only be interpreted in conjunction with the phenotyping results. The inheritance of the effective mutation depends on the species and the generative-specific features. Related species sometimes have different radiosensitivity, i.e., *C. cristata*, which has lower radiosensitivity. Even the 75 Gray dosages radically reduced the plant height and changed some characteristics—traits inherited by the second and third generations (Hayati and Aisyah, 2016). The clustering results based on the morphological data demonstrated very complicated genetic relationships. The study of Lefebvre *et al*. (2001) about the peppers, grapevine by Martínez *et al.* (2003), and rice by Andrew-Peter-Leon *et al.* (2021), generated a better-divided dendrogram based on the morphological characteristics between the mutants. The recent experiment observed a

very low connection between the morphological and ISSR and RAPD marker data. The low correlations between morphological and molecular marker data have been similarly reported in pepper (Lefebvre *et al*., 2001; Kwon *et al*., 2005; Kim *et al*., 2011).

This experiment verified the hypothesis that UPOV morphological traits could be objectively measured and utilized in genetic diversity and cultivar identification studies. The ornamental plants have a critical role in providing an alternative method for faster and inexpensive genetic diversity measurements compared with molecular marker tools. However, the study detected a low correlation between UPOV morphological and ISSR marker genetic similarities, as shown in other studies, including the pepper (Lefebvre *et al.,* 2001; Kwon *et al.,* 2005), wheat (Marić *et al.,* 2004), and grapevine (Martínez *et al.,* 2003).

CONCLUSIONS

The recent study aimed to enhance academic understanding of the factors affecting the change in gene pool composition through the gamma-irradiated *C. argentea* var. *plumosa* 'Arrabona.' Results showed that low dosages of gamma radiation (75-300 Gy) cause valuable genetic differences. Consequently, identifying new dedicated ornamental varieties of *C. argentea* var. *plumosa* 'Arrabona' based on UPOV morphological data requires objective facts for statistical analysis. These include the removal of environmentally influenced morphological data or repeated measurements conducted in many recurrent plants under varied environmental conditions. Non-visible traits, like drought tolerance, disease resistance, etc., marker-assisted breeding could be beneficial.

ACKNOWLEDGMENTS

This work proceeded with the support of the Pannon Breeding program number GINOP 2.2.1-15-2017-00042, "Genetic utilization of plants from the Pannonian region."

REFERENCES

Abubakar A, Falusi AO, Daudu OAY (2017). Morphological and phenotypic effects of fast neutron irradiation (FNI) on Lagos spinach (*Celosia argentea* L.). *Radiat. Sci. Technol.* 3(5): 47-53.

Aisyah S, Buchori A, Nurcholis W (2021). Improving the morphology of *Celosia argentea* var. *plumosa* through induced mutation by gamma-ray irradiation. *Acta Hortic.* 1334: 63-70.

Aisyah SI, Muhallilin I, Sukma D, Nurcholis W (2019). The morphological and phytochemical studies on the effect of acute and recurrent irradiation in *Celosia cristata* seeds. *Biodivers. J.* 20(12): 3766-3771.

Aisyah SI, Yudha YS, Sukma D, Nurcholis W (2022). Phenotypic variation and the polyphenols content alteration of *Celosia cristata* due to chronically induced mutation using ethyl methane sulphonate. *J. Southwest Jiaotong Univer.* 57(3): 221-230.

Andrew-Peter-Leon MT, Ramchander S, Kumar KK, Muthamilarasan M, Pillai MA (2021). Assessment of the efficacy of mutagenesis of gamma-irradiation in plant height and days to maturity through expression analysis in rice. *PLOS ONE* 16: e0245603.

Anwar S, Lukiwati DR, Kusmiyati F (2021). Genetic diversity based on RAPD markers of third-generation (M3) soybean mutant at saline soil. *IOP Conf. Ser.: Earth Environ. Sci.* 803 012017.

Beyaz R, Kahramanogullari CT, Yildiz C, Darcin ES, Yildiz M (2016). The effect of gamma radiation on seed germination and seedling growth of *Lathyrus chrysanthus* Boiss. under *in vitro* conditions. *J. Environ. Radioactiv.* 162: 129-133.

Bind D, Dwivedi VK (2014). Effect of mutagenesis on germination, plant survival, and pollen sterility in M3 soybean mutant at saline soil. *Indian J. Agric. Res.* 48(5): 398-401.

Broertjes C, Koene P, Van Veen JWH (1980). A mutant of a mutant of a mutant of a...: Irradiation of progressive radiation-induced mutants in a mutation-breeding program with *Chrysanthemum morifolium* Ram. *Euphytica* 29: 525-530.

Cai YZ, Sun M, Corke H (2005). Characterization and application of betalain pigments from plants of the *Amaranthaceae*. *Trends Food Sci. Tech.* 16(9): 370-376.

Chakravarty B, Sen S (2001). Enhancement of regeneration potential and variability by γ-irradiation in cultured cells of *Scilla indica*. *Biol. Plant.* 44(2): 189-193.

Chikmawati T (2019). Genetic diversity and population structure analyses in *Baccaurea angulata* accessions using ISSR primers. *SABRAO J. Breed. Genet*. 51(4): 390-404.

Enkhbileg E, Fenyvesi A, Bíró B, Fári MG, Kurucz E (2019). Mutation induction in sweet basil (*Ocimum basilicum* L.) by fast neutron irradiation. *Int. J. Hortic. Sci.* 25(1-2): 30-38.

FAO/IAEA (2015). Protocol for X-ray mutagenesis of plant material: Seed. *The Plant Breeding and Genetics Laboratory (PBGL) of the FAO/IAEA Manual*: pp. 1-13.

Fári M, Kisvarga S, Hlaszny E, Zsila-André A, Koroknai J, Kurucz E, Antal G (2019). New

methodological possibilities in the outdoor herbaceous ornamental plant breeding and technical innovation in Hungary with special regard to market opportunities and the effects of climate change-an overview. *Hung. Agric. Res.* 28(2): 31-37.

Fatinah AA, Arumingtyas EL, Mastuti R (2012). Genetic diversity study among six genera of amaranth family found in Malang based on RAPD marker. *J. Trop. Life Sci.* 2(3): 81-86.

Fleuroselect (2022). *Celosia argentea plumosa* 'Arrabona' https://www.fleuroselect.com/awarded-varieties/variety/arrabona/

Gupta N, Sood S, Singh Y, Sood D (2016). Determination of lethal dose for gamma rays and ethyl methane sulphonate induced mutagenesis in okra (*Abelmoschus esculentus* L. Moench.). *SABRAO J. Breed. Genet*. 48(3): 344-351.

Hayati D, Aisyah SI (2016). Radiosensitivity levels of *in vitro* cultured *Celosia cristata* plantlets by γ-Ray irradiation. *J. Trop. Crop Sci.* 3: 61-65.

Ho VT, Tu NT (2019). Genetic characterization of mango accessions through RAPD and ISSR markers in Vietnam. *SABRAO J. Breed. Genet*. 51(3): 252-265.

Ibrahim R, Ahmad Z, Salleh S, Hassan AA, Ariffin S (2018). Mutation Breeding in Ornamentals. In: J. Van Huylenbroeck (ed) Ornamental Crops. Handbook of Plant Breeding, vol 11. Springer, Cham. https://doi.org/10.1007/978-3-319-90698-0_8.

Jaccard P (1908). Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaudoise Sci. Nat.* 44: 223-270.

Jadhav PA, Kalpande HV, Kathale MN, Dahale GP (2012). Effect of gamma rays and ethyl methane sulphonate on germination, pollen viability and survival of okra (*Abelmoschus esculentus* L. Moench). *J. Crop Weed* 8(2): 130-131.

Jala A, Bodhipadma K (2011). Low doses of acute gamma radiation promote root formation and leaf canopy in common cockscomb (*Celosia argentea* var. *cristata*). *J. King Mongkut's Univer. Techn. North Bangkok* 21(3): 503-507.

Kanu CL, Owoeye O, Imosemi IO, Malomo AO (2017). A review of the multifaceted usefulness of *Celosia argentea* Linn. *Eur. J. Pharm. Med. Res.* 4(10): 72-79.

Kim GJ, Song YH, Gi GY, Kim ST, Lee JH, Han TH (2011). Application of UPOV data for the analysis of genetic variation in rose cultivars. *Hort. Sci. Technol. 29*(3): 240-246.

Konzak CK, Mikaelson K, Sigurbjorersson B, Burtescher A (1967). Recommended standard procedure for irradiating, cultivating, and measuring cereal seeds to determine the effect of neutron irradiation in the neutron-seed-irradiation program. In Neutron irradiation of seeds (technical reports series, No. 76), IAEA, Vienna pp. 103-107.

Kováts Z (2009). Breeding of outdoor ornamental plants adapting well to climatic changes. *Agric. Res. Hung.* 18(3-4): 4-7.

Kwon YS, Lee JM, Yi GB, Yi SI, Kim KM, Soh EH, Kim BD (2005). Use of SSR markers to complement tests of distinctiveness, uniformity, and stability (DUS) of pepper (*Capsicum annuum L*.) varieties. *Mol. Cell* 19(3): 428-435.

Lefebvre V, Goffinet B, Chauvet JC, Caromel B, Signoret P, Brand R, Palloix A (2001). Evaluation of genetic distances between pepper inbred lines for cultivar protection purposes: Comparison of AFLP, RAPD, and phenotypic data. *Theor. Appl. Genet.* 102(5): 741-750.

Mahla HR, Sharma R, Bhatt RK (2018). Effect of gamma irradiations on seed germination, seedling growth and mutation induction in cluster bean (*Cyamopsis tetragonoloba* [L.] Taub.). *Indian J. Genet. Plant Breed.* 78(2): 261-269.

Majeed A, Muhammad Z, Ullah R, Ali H (2018). Gamma irradiation I: Effect on germination and general growth characteristics of plants–a review. *Pak. J. Bot.* 50(6): 2449-2453.

Marić S, Bolarić S, Martinčić J, Pejić I, Kozumplik V (2004). Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficients of parentage. *Plant Breed.* 123(4): 366-369.

Martínez L, Cavagnaro P, Masuelli R, Rodriguez J (2003) Evaluation of diversity among Argentine grapevine (*Vitis vinifera* L.) varieties using morphological data and AFLP markers. *Electr. J. Biotechnol.* 6: 244-253.

Melki M, Marouani A (2010). Effects of gamma rays irradiation on seed germination and growth of hard wheat. *Environ. Chem. Lett.* 8(4): 307-310.

Miguel MG (2018). Betalains in some species of the *Amaranthaceae* family: A review. *Antioxid.* 7(4): 53. doi: 10.3390/antiox7040053.

Mohan JS (2006). Mutation-assisted breeding for improving ornamental plants. *Acta Hortic*. 714: 85-98.

Mokobia C, Anomohanran O (2005). The effect of gamma irradiation on the germination and growth of certain Nigerian agricultural crops. *J. Radiol. Prot.* 25(2): 181-188.

Mostafa G, Alfrmawy A, El-Mokadem H (2014). Induction of mutations in *Celosia argentea* using dimethyl sulphate and identification of genetic variation by ISSR markers. *Int. J. Plant Breed. Genet.* 8(2): 44-56.

Mostafa G, Alfrmawy A, El-Mokadem H (2019). The diversity of morphological characteristics and chemical content of *Celosia cristata* plantlets due to gamma-ray irradiation. *Biodivers. J.* 20(3): 862-866.

Oduwaye OA, Ojo DK, Popoola AR, Daniel IO, Baránek M, Čechová J (2014). Genetic diversity assessment in amaranth germplasm using AFLP and ISSR markers. *J. Crop Imp*. 28(4): 518-529.

Pathirana R (2011). Plant mutation breeding in agriculture. *CABI Rev.* (2011): 1-20.

Riviello-Flores ML, Cadena-Iñiguez J, Ruiz-Posadas LM, Arévalo-Galarza M, Castillo-Juárez I, Soto Hernández M, Castillo-Martínez CR (2022). Use of gamma radiation for the genetic improvement of underutilized plant varieties. *Plants* 11(9): 1161.

Rohlf FJ (1998). NTSyS-p.c. Numerical Taxonomy and Multivariate Analysis System (Version 2.0). Setauket., Exeter Software Publishers Ltd.

Saika A, Hee JL (2020). Mutation breeding using gamma irradiation in the development of ornamental plants: A review. *Flower Res. J.* 28: 102-115.

Schum A, Preil W (1998). Induced mutations in ornamental plants. In Somaclonal variation and induced mutations in crop improvement. Springer, Dordrecht, pp. 333-366.

Sholihin, Noerwijati K, Mejaya MJ (2019). Genotypic variability in cassava (*Manihot esculenta* Crantz) mutants (M1V4) using gamma irradiation. *SABRAO J. Breed. Genet*. 51(2): 107-116.

Songsri P, Jogloy S, Holbrook C, Puangbut D (2019). Determination of lethal dose and effect of gamma rays on growth and tuber yield of *Jerusalem artichoke* mutant. *SABRAO J. Breed. Genet*. 51(1): 1-11.

Sood S, Jambulkar S, Sood A, Gupta N, Kumar R, Singh Y (2016). Median lethal dose estimation of gamma rays and ethyl methane sulphonate in bell pepper (*Capsicum annuum* L.). *SABRAO J. Breed. Genet.* 48(4): 528-535.

Spórna-Kucab A, Milo A, Kumorkiewicz A, Wybraniec S (2018). Studies on polar high-speed counter-current chromatographic systems in the separation of amaranthine-type betacyanins from *Celosia* species. *J. Chromatography B*. 1073: 96-103.

Surakshitha NC, Soorianathasundaram K (2017). Determination of mutagenic sensitivity of hardwood cuttings of grapes 'Red Globe' and 'Muscat' (*Vitis vinifera* L.) to gamma rays. *Sci. Hortic.* 226: 152-156.

Thorat BR (2018). Review on *Celosia argentea* L. Plant. *Res. J. Pharmacogn. Phytochem.* 10(1): 109-119.

Um M, Kang SY, Lee JW, Lee OR (2017). Effect of gamma-ray on germination, growth, and antioxidant activity of Senna tora. *Korean J. Med. Crop Sci*. *25*(5): 290-295.

UPOV (2002). Celosia L. International union for the protection of new varieties of plants, TG/188/1 (2002), Guidelines for the conduct of tests for distinctness, uniformity and stability, https://www.upov.int/edocs/mdocs/upov/en/tg/tg_188_1_proj1.pdf.

Van-Harten AM (1998). Mutation Breeding: Theory and Practical Applications. Cambridge University Press, Cambridge, UK.

Yamaguchi H (2018). Mutation breeding of ornamental plants using ion beams. *Breed. Sci.* 68: 71-78.

Yudha YS, Aisyah S, Sukma D, Nurcholis W (2022). Phenolic, flavonoid and antioxidant capacities evaluation of *Celosia cristata* resulted from induced mutation using ethyl methane sulphonate. *Pak. J. Biol. Sci.* 25(5): 380-386.