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Evaluation of the Effect of Different Doses of Gamma Radiation to Induce Variation in *in vitro* Raised Plants of Chrysanthemum

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Abstract

Variation induction using gamma radiation in *in vitro* raised chrysanthemum is one of the most useful methods in floriculture. Different doses of gamma radiation have a negative effect on the *in vitro* shoot regeneration rate. Irradiation of *in vitro* micro shoots with highest dose 25Gy produced only 3.90% and 9.93% of BRAI Chry-1 and BARI Chry-2 micro shoots. But the lowest dose 5Gy could regenerate the highest percentage of shoots for both varieties. The LD_{50} obtained from the survival percentage of the irradiated *in vitro* raised shoots was found at 9.25 Gy for BARI Chry-1 and 11.19 for BARI Chry-2 variety. Successful mutation can be found around the LD_{50} doses of a specific genotype. During hardening of the M1V5 regenerants of BARI Chry-1 and BARI Chry-2 raised from 15Gy irradiated micro shoots showed highest variation regarding leaf structure, size of the internode and plant height. M1V5 regenerants developed from 10Gy of both varieties produced 20 to 50 percent variations. In future gamma radiation dose between 10-15Gy would be more effective to get variation in this ornamental plant.

Introduction

Chrysanthemum (*Chrysanthemum morifolium* Ramat) is considered as one of the most popular cut flowers and pot plants due to its assorted floral types. It occupies the second place on the world sales list, following the rose (Miler and Jędrzejczyk 2018). As the world flower market is growing, the demand for new varieties has become a primary issue in the flower industry. Despite the great number of cultivars available, customers and breeders are constantly searching for new phenotypes (Tymoszuk and Kulus 2020).

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Mutation induction is regarded as one of the important techniques for producing additional beneficial variation in horticultural crops that are grown vegetatively. By mutation induction, a great number of variations have been created for ornamentals. According to the FAO/IAEA (2021) mutant variety database, the majority of the 465 mutants that were developed among the vegetatively propagated plants, were in the plants used in floriculture. In Bangladesh, floriculture has increased national attention since the late 1970s, with the small-scale cultivation of tuberose. Later, in the middle of the 1980s, Jhikargacha Upazila in the Jashore area began commercially producing a variety of flowers and flowering plants. Now, Jashore, Savar, Chuandanga, Mymensingh, and Gazipur are regarded as primary flower production hubs of our country. Four types of flowers, including roses, tuberoses, marigolds, and gladioli, account for almost 90% of the domestic flower industry's profits. But to meet local demand various flowers, such as Chrysanthemum, tuberose and gladiolus are being imported from India and orchids, gerbera, anthurium and Thai rose from Thailand every year.

There has been considerable success with both physical and chemical mutagens in breeding ornamental plants, as they have produced quite many new varieties (Datta et al. 2005). The induced mutation process has created several new and novel cultivars of chrysanthemums. In vegetatively propagated plants like chrysanthemum, chemical mutagens are not widely used due to their low penetration levels. Physical mutagens are used to induce mutations in chrysanthemum because they are hexaploid plants propagated vegetatively, which makes them difficult to hybridize (Dwimahyani and Widiarsih 2010, Patil et al. 2017).

Physical mutagens like radiations cause mutations in plant cells when certain dosages are applied. Morphological changes were noticed in intact and *in vitro* plants after exposure to the radiation (Hasbullah et al. 2012). Ionizing radiation's gamma rays interact with atoms or molecules to create free radicals in living cells. Depending on the amount of radiation a plant receives, these radicals have been shown to influence the morphology, anatomy, biochemistry, and physiology of plants differently. These impacts include modifications to the plant's cellular structure and metabolism, such as thylakoid membrane dilatation, altered photosynthesis, oxidative system modulation, and phenolic compound accumulation (Kovacs and Keresztes 2002, Wi et al. 2005, Kim et al. 2016). A variety of treatment methods, including direct dosage, split dose, recurring irradiation, and combined treatment have been used for improving the ornamentals. For the practical application of applied mutagenesis, it is also crucial to determine the radiosensitivity of various cultivars of the same crop. Gamma radiation mutation frequency varies depending on cultivar and dose (Baig et al. 2012). Certain cultivars are slightly more sensitive to mutagens than others, while others are resistant to them altogether.

Moreover, the genetic makeup of Chrysanthemum is extremely heterozygous, making them excellent study materials for induced physical mutagenesis. Many researchers have investigated how physical mutagens affect Chrysanthemum and other flowers. Walther and Sauer (1985) examined radio sensitivity for *in vitro* somatic

mutagenesis in *Geberea jamesonii*. They found the higher X-ray doses resulting in greater inhibition of shoot regeneration as regeneration rates were dose dependent. He recommended the inhibition of shoot development on the first cut-off date to estimate the radiosensitivity of gerbera idiotypes.

The effect of various rates of gamma radiation on the frequency of mutation in inflorescence color and type of chimerism in chrysanthemum cv. 'Cherry Dark' was studied by Boersen et al. (2006). A linear decrease in plant height and a quadratic tendency in survival percentage were observed with the increase in mutagen doses (Boersen et al. 2006). The analysis of radio sensitivity of explants is the basic requirement for successful mutation induction. According to Predieri (2001) one of the first steps in mutagenic treatments is the estimation of the most appropriate dose to apply. LD_{50} dose is the dose of gamma radiation which would kill 50% of the treated individuals because recombination will lead to generation of new variability that will be difficult to separate from effects of mutation. LD_{50} is important because the rate of mutation is high at this dose and in most of the mutation induction studies successful mutation induction around this dose. Even though *in vitro* technology has made it possible to rapidly reproduce newly developed mutants, the enormous variation induction potential of tissue culture coupled with *in vitro* mutagenesis is still underutilized (Tymoszuk and Kulus 2020).

The aim of the study was to develop a suitable *in -vitro* mutagenic system for mutation induction and selection of desirable mutants. In the present study, the effect of gamma radiation on *in-vitro* cultures were evaluated following determination of LD_{50} for the *in vitro* raised micro shoots of chrysanthemum. It will facilitate the aim of developing a suitable *in -vitro* mutagenic system for the induction.

Materials and Methods

Two varieties of chrysanthemum, namely, BARI Chrysanthemum-1 (BARI Chry-1) and BARI Chrysanthemum-2 (BARI Chry-2) were used for this experiment. Both varieties were collected from Ornamental and Floriculture Division of the Horticulture Research Center (HRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh. All the mother plants were maintained in the garden of department of Botany, university of Dhaka, Bangladesh for six months before starting the experiment. Sterilized shoot tips were cultured on (MS) medium for explant preparation and collection (Murashige and Skoog 1962, Chowdhury et al. 2021) (Fig. 1A). Two weeks old shoot tip (~1 cm long) cultured on MS medium were the source of young leaves (4th - 5th position) used as the explants for this experiment. Repeated subculture of the shoot tip was done at two weeks interval to ensure supply of sterilized explants in the same medium.

For induction of mutation Co_{60} (cobalt 60) is used as a source of gamma radiation. This part of the experiment was done in the Institute of Food and Radiation Biology situated in Atomic Energy Research Establishment (AERE), Savar, Dhaka, Bangladesh.

About 3-4 weeks old *in vitro* leaf culture (micro shoots) of both chrysanthemum varieties grown on MS medium supplemented with 0.5 mg/l BAP and 2.0 mg/l IAA were exposed to gamma irradiation (Chowdhury at al. 2021) (Fig.1D). Five different doses, namely, 5Gy, 10 Gy, 15 Gy, 20 Gy and 25 Gy were applied. For each variety three replicates were used for each dose along with the control (non-irradiated culture). Every single replication consisted of 10 explants in a conical flask. During the experiment the temperature was 25^0C and the applied doses were estimated using "Fricke Dosimeter". Application of different doses of gamma radiation and estimation of doses were done by the research personnel from Institute of Food and Radiation Biology situated in Atomic Energy Research Establishment (AERE), Savar. The percentage of shoots survival was scored at 14, 28, 42 and 60 days after the application of gamma radiation.

MS media supplemented with 0.5 mg/l BAP and 2.0 mg/l IAA was used for multiple shoot induction and for their development (before and after irradiation). Subsequent subculture was then carried out at a 3-weeks interval to develop M1V1 to M1V3 shoots. Several shoots died in each subculture. All cultures were sub-cultured regularly at an interval of 3 weeks for maintenance and routinely examined to avoid contamination. During these experiments the survivability of the irradiated shoots M1V3 (60 days after irradiation) were evaluated following determination of LD_{50}. The culture (the conical flasks containing explants) incubated under fluorescent light of 20,000 lux intensity. The light period of the culture room was maintained at 16/8 (light/dark) hours and temperature was 25 ± 2°C with 60-70% relative humidity. After 20 days *in-vitro* regenerated M1V4 irradiated shoots (80 days after irradiation) were developed on MS hormone free medium and formed healthy rooted M1V5 plants which were transplanted in soil (Fig.1E and F). During this study, different mutant lines were selected based on visual observation through morphologic study.

Statistical analysis: The experiments were carried out in a completely randomized design (CRD) with *in vitro* culture of two varieties and their regeneration response towards six doses of gamma irradiation (Table 1). Two-way analysis of variance (ANOVA) and a Tukey's multiple comparison test at a probability level of 0.05 were performed to compare regeneration responses towards six radiation doses. Statistical analysis was also carried out for the observations recorded in experiment to find out whether there exists any significant variation for various parameters among different gamma ray treatments (doses) and find out LD_{50} through regression analysis. All these data were subjected to statistical analysis by using Prism GraphPad 8.0.

Results and Discussion

Effect of radiation on in vitro culture: The comparative study on the effects of five radiation doses (5 Gy,10 Gy, 15 Gy, 20Gy and 25 Gy) on the shoot survival percentage of two varieties of chrysanthemum indicated that highest dose (25 Gy) showed highest lethality as the survival percentage for both varieties was found low (3.90% and 9.93% for BRAI

Chry-1 and BARI Chry-2) (Fig.2 A and B). However, the highest survival percentage was observed for 5 Gy irradiated shoots for both varieties (57.29% for BARI Chry-1 and 64.08% for BARI Chry-2). It was observed that the percentage of survival was different for different varieties and may varied with the genotype involved. Evaluation of the effect of radiation doses and expected effect of creating variation among the plant genotype towards radiation are important steps in the development of an elite mutant line.

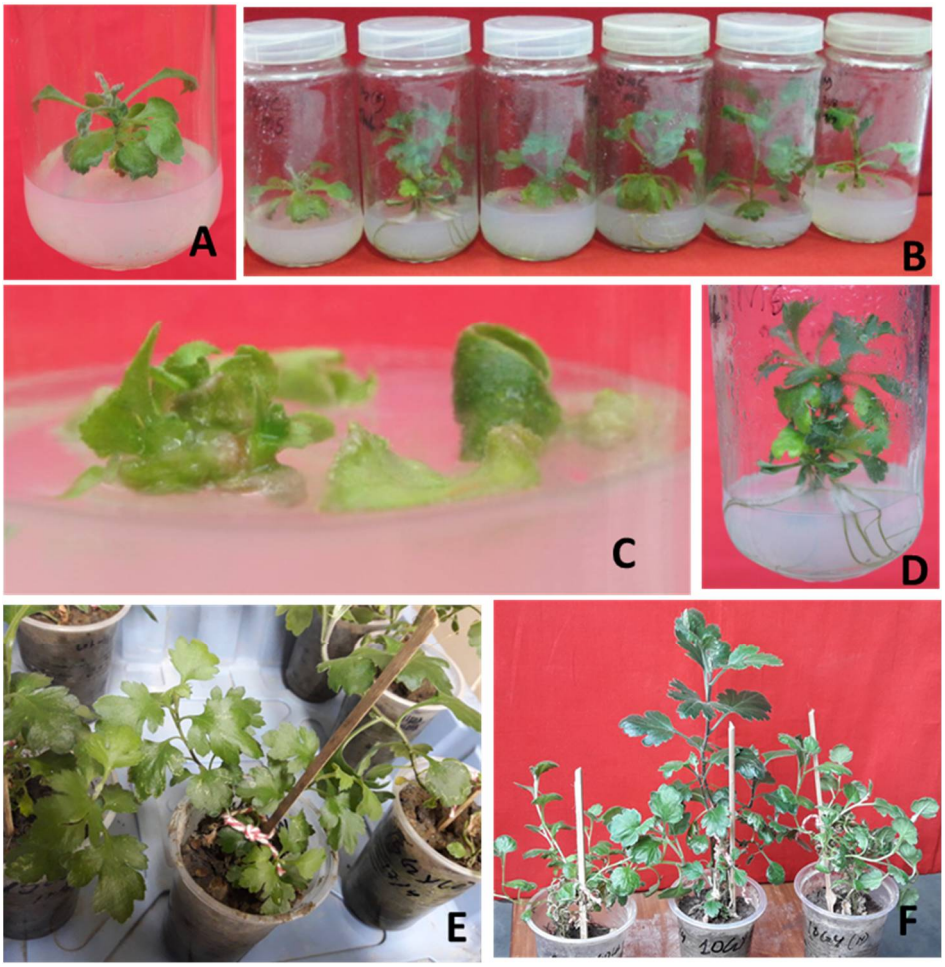

Fig. 1. Development of plantlets from leaf explants and hardening of plantlets of BARI Chry-2: (A) Shoot developed from sterilized shoot tip collected from field in MS medium after 2 weeks, (B) *In vitro* grown plants used as a source of leaf explants used during *in vitro* regeneration on MS medium after 4 weeks, (C) Direct regeneration started from leaf explants on MS medium supplemented with 0.5 mg/l BAP and 2.0 mg/l IAA after 2 weeks and after 1 more week it was exposed to gamma radiation, (D) Rooted plant on MS medium after 4 weeks of inoculation, (E-F) Acclimatized healthy plants ready to transplant in the field showing morphological variations (leaf structure, height and internode size).

Determination of LD50 for two Chrysanthemum varieties: Using the percentage of *in vitro* survival of Chrysanthemum shoots, LD_{50} dose for both varieties were determined. To obtain the LD_{50} (50% lethal dose) the data presented in figure 2 were plotted as shown in Fig. 3 following regression analysis. The LD_{50} obtained from Fig. 3 was found at 9.25 Gy for BARI Chry-1(Fig. 3a) and 11.19 for BARI Chry-2 variety (Fig. 3b).

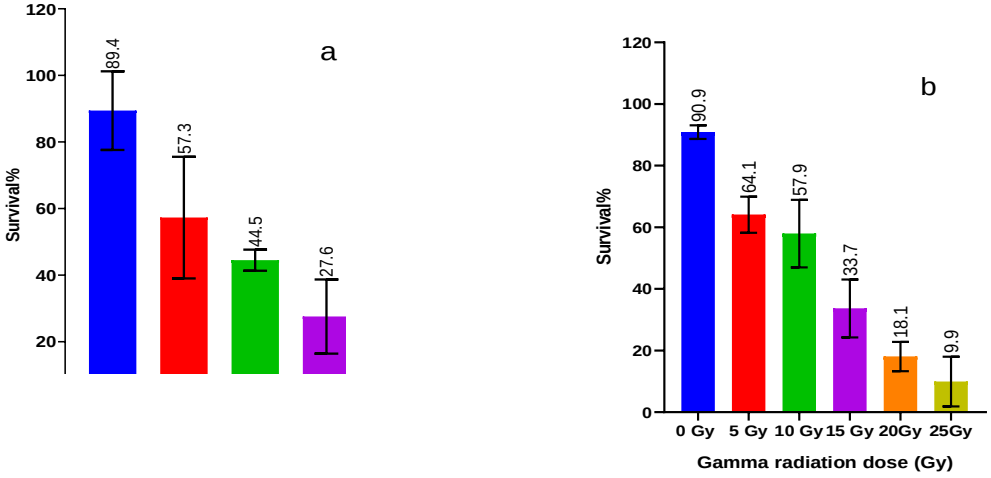

Fig. 2 Effect of gamma radiation on survival percentage at *in vitro* shoot regeneration (60 days after gamma irradiation). (a) The percentage of survival of *in vitro* shoots % of BARI Chry-1, (b) Same as for BARI chry-2.

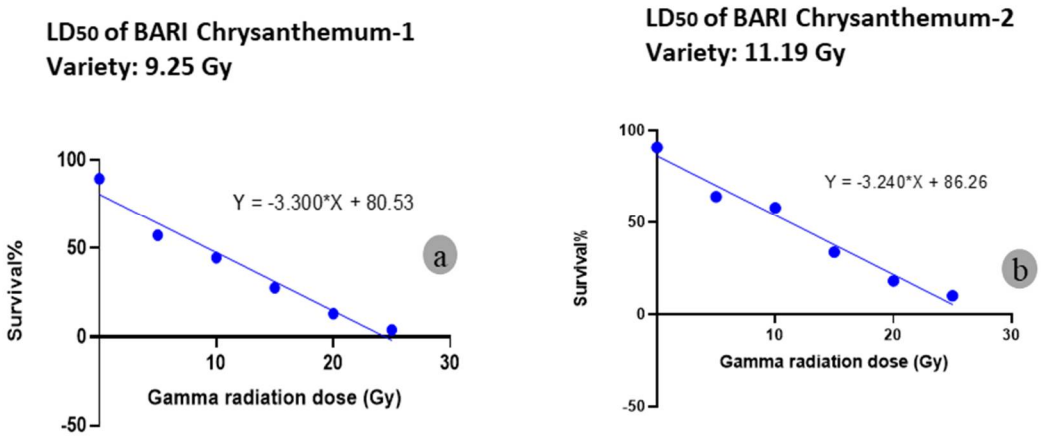

Fig. 3. Determination of LD_{50} dose for both varieties of chrysanthemum. (a) LD_{50} dose for BARI Chry-1, (b) LD_{50} dose for BARI Chry-2.

Response of the in-vitro grown irradiated shoots towards variation induction: The M1V5 Plantlet (rooted shoots) were transplanted to soil and kept in growth room for 2 weeks to acclimatize and variation in leaf structure, internode size and height of the plant were observed (Fig. 1E-F). The frequency of morphological variation at different radiation

doses was measured as the index reflecting the effect of radiation on plant morphological changes. According to the formula (Walther, 1969), variation frequency (%) = (No. of variations/No. of treated plants) × 100%, frequency of morphological variation was calculated (Table 1 and 2). Highest variation frequency (75-80%) regarding the changes in leaf shape, size of the internode and plant height were found for BARI Chry-1 variety from dose 15 Gy. Whereas dose 5Gy, 10 Gy and 15 Gy produced remarkable variation frequency % for BARI chry-2. None of the 20 Gy treated shoot could survive in the elongation or rooting phage, so variation frequency regarding all four measured aspects were 0% for both varieties. It was also noticed that variation frequency is not directly increasing with the increased radiation doses.

Table 1. Effect of different doses of gamma radiations on M1V5 plants of both varieties towards variation induction rate

Radiation Dose (Gy)	Variation rate of BARI Chry-1 (%)			Variation rate of BARI Chry-2 (%)		
	Leaf shape change	Internode size	Plant height	Leaf shape change	Internode size	Plant height
0	0	10	25	0	0	0
5	5	10	5	35	75	80
10	25	20	25	50	50	50
15	80	75	75	40	75	50
20	0	0	0	0	0	0
25	10	10	10	0	0	0

[According to Walther, 1963, Formula for physiological variation frequency % = No. of plants showing variation / No. of treated plants * 100%] Here n=20]

Establishment of M_1V_5 mutant lines in field and morphological evaluation for selection of variant lines: During these series of experiments, plants showing vegetative variations were grown in the field till flowering and noted performance in field condition along with the control plants. Observation and selection were made for desirable variants at flowering time. Changes in flower color, form and shape were observed in plants treated with gamma rays of 5, 10, 15, and 25 Gy for both chrysanthemum varieties. None of the 20 Gy treated shoot could survive in the elongation or rooting phage, so no variation frequency regarding flower structure were available for both varieties. Mutation frequency regarding flower form (structure) and flower colour were found highest in plants developed from 10 Gy irradiated plants for both BARI Chry-1 (25%) and BARI Chry-2 (35%) (Table 2). During this screening several variant lines from both BARI Chry-1 and BARI Chry-2 were noted. These mutants need to evaluate one more year to confirm their stable status of mutation. This result showed the highest frequency of variation around the LD_{50} dose determined for both varieties in the present study. The growth

stage of the *in vitro* grown culture is important for induction of mutation through gamma radiation. The present experiment was conducted using *in vitro* grown shoot micro shoots for both chrysanthemum varieties. It was found that this stage of the explant (micro shoots) was very effective as it produced many shoots after overcoming the lethal effects of different radiation doses.

Table 2. Flower form and colour mutation frequency in M1V5 of yellow and BARI Chry-2 chrysanthemum variety in field during flowering

Radiation dose (Gy)	No. of plant Investigated	Flower form and colour mutation (%)	
		BARI Chry-1	BARI Chry-2
5	20	0	20
10	20	25	35
15	20	10	15
20	0	0	0
25	20	5	0

Lethal effects are common after irradiation during mutation induction. A comparative study on the effects of five radiation doses (5Gy,10Gy,15Gy, 20Gy and 25 Gy) on the shoot survival percentage of two varieties of Chrysanthemum indicated that highest dose (25 Gy) showed highest lethality as the survival percentage for both varieties was found low (3.90% and 9.93% for BARI Chry-1 and BARI Chry-2). However, the highest survival percentage was observed for 5Gy irradiated shoots for both varieties (57.29% for BARI Chry-1 and 64.08% for BARI Chry-2). Similar results were reported by others working on Chrysanthemum mutation induction through gamma radiation (Wang et al. 2020, Nasri et al. 2021). The lethal dose (LD_{50}) for 50% of the regenerating explants in irradiated explants was estimated during the study. In the case of Chrysanthemum, LD_{50} was calculated as 9.25 Gy for BARI Chry-1 and 11.19 for BARI Chry-2 variety. Hasbullah et al. 2012 reported 25Gy but Lamseejan et al. 2000 observed 14Gy as LD_{50} for Chrysanthemum. These differences regarding LD_{50} may be due to use of different genotype and culture conditions using different explants. It is also noted that the variation in LD_{50} values for different genotypes of the same species is a common observation in mutation studies depending upon the biological materials, their size, maturity, hardness, and moisture content at the time of treatment (Babaei et al. 2010, Tabasum et al. 2011). Lethal dose also depends on the age of the explant used for irradiation, rate of irradiation, genotype, and culture condition. Mutation breeding studies using vegetatively propagated ornamentals including *C. morifolium* and *G. jamesonii*, the dose chosen should result in the highest survival of irradiated explants and a low inhibition of the rate of production of new shoots would give the highest efficiency in recovering useful mutants (Laneri et al. 1990, Hasbullah et al. 2012). Kumari et al.

(2013) found 10Gy and 15Gy gamma radiation doses were good for induction of color and shape mutations in chrysanthemum flowers. Kumar et al. (2012) found 20 Gy as optimal dose of gamma radiation for *in vitro* mutations and selected resistant plants of chrysanthemum toward *Septoria obesa*, a leaf spot pathogen. The growth of the regenerated shoots became slow and required more time for regeneration after irradiation during the present study. A considerable time was needed to recover multiple shoots of M1V3 after irradiation. Several shoots died in each subculture and M1V4 shoots were developed during the third subculture on hormone free MS medium. It was observed that the growth response reduced with the increase of the radiation doses. Similar responses were also reported by other researchers (Ibrahim 1969, Datta et al. 2005, Soliman et al. 2014).

In M1V5 of Chrysanthemum, the frequency of morphological variation at different radiation doses was recorded. Highest variation percentage regarding leaf shape, internode size and plant height were found at the regenerants developed from 15Gy dose treated micro shoots of both varieties. None of the 20Gy treated shoots of both varieties could survive on the elongation or rooting phage, so no variation was observed regarding all three measured aspects. It was noticed that variation frequency is not directly increasing with the increased radiation doses. Some reports support this point for mutation induction in ornamentals as well as some crop plants (Hell 1983, Walther and Sauer 1985, Wang et al. 1988, Cheng et al. 1990, Shen et al. 1990, Charbaji and Nabuls 1999, Predieri and Gatti 2000, Datta et al. 2005, Soliman et al. 2014). Whereas, in case of flower structure, frequency of variation was found highest for the plants developed from 10 Gy irradiated microshoots. It was observed for both varieties that 10-15 Gy would be more appropriate dose for mutation induction. In the present study, LD_{50} was successfully determined and for both varieties in M1V5 mutant lines showed the highest variation frequency around the dose. In future this LD_{50} dose could be beneficial for mutation induction in other varieties of chrysanthemum grown in Bangladeshi.

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References

Babaei A, Nematzadeh GA, Avagyan V and **Hashemi-Petrodi** SH (2010) Radio sensitivity studies of morpho-physiological characteristics in some Iranian rice varieties (*Oryza sativa* L.) in M1 generation. African J. Agricul. Res. **5**(16): 2124-2130.

Baig MMQ, Hafiz IA, Abbasi NA, Yaseen M, Akram Z and **Donnelly DJ** (2012) Reduced-stature Rosa species through in vitro mutagenesis. Canadian J. Plant Sci. **92**(6): 1049-1055.

Boersen AM, Tulmann NA, Latato RR and **Santos PC** (2006) Dose effect gamma irradiation in obtaining colour mutants of inflorescence of chrysanthemum. *Revista brasileira* Horticult. Ornamental. **12**(2): 126-133.

Charbaji T and **Nabulsi I** (1999) Effect of low doses of gamma irradiation on *in vitro* growth of grapevine. Plant Cell, Tissue and Organ Cult. **57**(2): 129-132.

Cheng XY, Gao MW, Liang ZQ and **Liu KZ** (1990) Effect of mutagenic treatments on somaclonal variation in wheat (*Triticum aestivum L.).* Plant Breeding. **105**(1): 47-52.

Chowdhury J, Hoque MI and **Sarker RH** (2021) Development of an efficient *in vitro*regeneration protocol for chrysanthemum (*Chrysanthemum morifolium* Ramat). Plant Tissue Cult. Biotech. **31**(2): 161-171.

Datta SKD and **Janakiram T** (2015) Breeding and genetic diversity in Chrysanthemum morifolium in India: A review. Indian Journal of Agricultural Sciences. **85**(10): 1379-1395.

Dwimahyani I and **Widiarsih** S (2010) The effects of gamma irradiation on the growth and propagation of in-vitro chrysanthemum shoot explants (cv. Yellow Puma). Atom Indonesia. **36**(2): 45-49.

Hasbullah NA, Taha RM, Saleh A and **Mahmad N** (2012) Irradiation effect on *in vitro* organogenesis, callus growth and plantlet development of *Gerbera jamesonii.* Horticultura Brasileira. **30**: 252-257.

Hell KG 1983. Survival of Nicotiana tabacum L. cv. Wisconsin-38 plants regenerated from gamma-irradiated tissue cultures. Environmental and Experimental Botany. **23**(2): 139-142.

Ibrahim RK (1969) Normal and abnormal plants from carrot root tissue cultures. Canadian J. Bot. **47**(5): 825-826.

Kim YS, Sung SY, Jo YD, Lee HJ and **Kim SH** (2016) Effects of gamma ray dose rate and sucrose treatment on mutation induction in chrysanthemum. Eur. J. Hortic. Sci. **81**(4): 212-218.

Kovacs E and **Keresztes A** (2002) Effect of gamma and UV-B/C radiation on plant cells. Micron. **33**(2): 199-210.

Kumar B, Kumar S and **Thakur M** (2012) *In vitro* mutation induction and selection of Chrysanthemum (*Dendranthema Grandiflora Tzelev*) lines with improved resistance to *Septoria Obesa* Syd. Inter. J. Plant Res. **2**(4):103-107.

Kumari K, Dhatt KK and **Kapoor M** (2013) Induced mutagenesis in *Chrysanthemum morifolium* variety 'Otome Pink'through gamma irradiation. The Bioscan. **8**(4): 1489-1492.

Laneri U, Franconi R and **Altavista A** (1990) Somatic Mutagenesis of *Gerbera Jamesonii* Hybr. Irradiation and *In Vitro* Culture. International Symposium on *In Vitro* Culture and Horticultural Breeding Acta Horticulturae. **280**: 395-402.

Miler N and **Jędrzejczyk I** (2018). Chrysanthemum plants regenerated from ovaries: A study on genetic and phenotypic variation. Turkish Journal of Botany. **42**(3): 289-297.

Murashige T and **Skoog F** (1962) A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. Physiologia Plantarum. **15**(3): 473-497.

Nasri F, Zakizadeh H, Vafaee Y and **Mozafari AA** (2018) Callus Induction and Plant Regeneration of *Chrysanthemum morifolium* and *C. coccineum* via Direct and Indirect Organogenesis and Genetic Fidelity Analysis Using IRAP, ISSR and SCoT Molecular Markers. J. Ornamen. Plants. **8**(4): 265-284.

Patil UH, Karale AR, Katwate SW and **Patil MS** (2017) Mutation breeding in chrysanthemum (*Dendranthema grandiflora* T.). J. Pharmacogn. Phytochem. **6**(6): 230-232.

Predieri S and **Gatti E** (2000) Effects of gamma radiation on microcuttings of plum (*Prunus salicina Lindl*.)'Shiro'. Advances in Horticultural Science. 7-11 pp.

Predieri S and **Gatti E** (2000) Effects of gamma radiation on microcuttings of plum (*Prunus salicina Lindl*.) 'Shiro'. Advances in Horticultural Science. 7-11 pp.

Shen XS, Wan JZ, Luo WY and **Ding XL** (1990) Preliminary results of using *in vitro* axillary and adventitious buds in mutation breeding of Chinese gooseberry. Euphytica. **49**(1): 77-82.

Soliman T, Lv S, Yang H, Hong B, Ma N and **Zhao L** (2014) Isolation of flower color and shape mutations by gamma radiation of *Chrysanthemum morifolium* Ramat cv. Youka. Euphytica. **199**(3): 317-324.

Tabasum A, Cheema AA, Hameed A, Rashid M and **Ashraf M** (2011) Radio sensitivity of rice genotypes to gamma radiation based on seedling traits and physiological indices. Pak. J. Bot. **43**(2): 1211-1222.

Tymoszuk A and **Kulus D** (2020) Silver nanoparticles induce genetic, biochemical, and phenotype variation in Chrysanthemums. Plant Cell, Tissue and Organ Cult. **143**: 331-344.

Walther F and **Sauer A** (1985) Analysis of radiosensitivity - a basic requirement for *in vitro* somatic mutagenesis. I. *Prunus Avium* L. Acta Hortic. **169**: 97-104.

Walther F and **Sauer A** (1985). Analysis of radiosensitivity - a basic requirement for *in vitro* somatic mutagenesis. I. *Prunus Avium* L.. Acta Hortic. **169**: 97-104.

Wang GZ, Xue X, Wang TC, Xu XL and **Li JL** (1988) Study in breeding and cytogenetics of *octoploid triticales* with different cytoplasms. Acta Genetica Sinica. **15**(5): 340-347.

Wang L, Wu J, Lan F and **Gao P** (2020) Morphological, cytological and molecular variations induced by gamma rays in *Chrysanthemum morifolium* 'Donglinruixue'. Folia Horticulturae. **32**(1): 87-96.

Wi SG, BY Chung, JH Kim, MH Baek, DH Yang, JW Lee and **JS Kim** (2005) Ultrastructural changes of cell organelles in Arabidopsis stem after gamma irradiation. J. Plant Biol. **48**: 195-200.

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