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Original Research Article

Induction of Genetic Variability with Gamma Radiation in Some Flowering Ornamental Herbs

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Abstract

The objectives of the present study were to evaluate the variability induced by gamma-irradiation among M_2 plants of three flowering ornamental herbs mutant lines and to identify molecular markers associated with different traits. Mutation induction used to enhance improvement in a short duration in plant varieties. Some mutant lines showed higher values of germination parameters when compared with the original (un-radiated) lines as well as most studied traits. Results revealed that the efficiency of gamma irradiation for inducing genetic variation was high for *Matthiola incana* ssp. *incana* followed by *Dimorphotheca ecklonis*; however, *Dianthus caryophyllus* showed the lowest differences and gave high similarity. The present study might help in explaining different responses and complication to gamma radiation in the three studied ornamental herbs for further study and improvement.

Keywords

DNA marker
Genetic improvement
Mutation
Ornamental herbs
SRAP

Introduction

Genetic improvement of crop and ornamental plants has made a major contribution to the production of food, feed, flowers and biomaterials. Ornamental plants breeding efforts are aimed at increasing flowers yield and economic value by incorporating disease and insect resistance, better flower quality and a shorter growth duration. Genetic variability is the most essential prerequisite for any successful plant improvement program as it provides spectrum of variants for the effective selection, which can be achieved through the processes of hybridization recombination, mutation and selection. Most of cultivated flowering ornamental herbs exhibited a narrow genetic base. Thus, induced mutation is one of the best alternatives for the improvement of ornamental plants as it can help to regenerate and restore the variability, which is generally lost in the process of adaptation to various stresses. Mutagenic treatment of seeds is the most convenient because seeds can be treated in large quantities and are easily handled, stored and shipped. Radiations were used widely for producing useful mutants with improved characteristics in ornamental and many crops (Rehman et al., 1987).

Stock (*Matthiola incana* ssp. *incana*) (2n=14) belongs to Brassicaceae family and Tyrrhenian origin, had a wide use as ornamental plant (Saccardo, 1909). Carnation

(*Dianthus caryophyllus* L.) (Caryophyllaceae) (2n=30) is one of the most important commercial cut flowers of the world due to its excellent keeping quality, also ability to withstand long distance transportation and remarkable ability to rehydrate after continuous shipping. *Dimorphotheca ecklonis* (2n=20), it has beautiful, white ray florets and a dark blue centre. A collection of these plants with other winter annuals creates a stunning show in any garden.

Gamma sources are used to irradiate a wide range of plant materials, like seeds, whole plants, plant parts, flowers, anthers, pollen grains and single cell cultures or protoplasts. Radiations have been used successfully to induce useful mutations for plant breeding. The lower doses concentrations of the mutagenic treatments could enhance the biochemical components, which are used for improved economic characters (Muthusamy et al., 2003). Gamma radiation can induce useful as well as harmful effects on crops so there is need to predict the most beneficial dose for improvement of specific traits of crop plants (Jamil and Khan, 2002). In general irradiation by gamma ray may cause some mutations to the genes of cells through the DNA repair mechanisms within cells (Thacker, 1999). The biological effect of gamma rays is based on the interaction with atoms or molecules in the cell. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level. These effects include changes in the plant cellular structure and metabolism, e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds (Kim et al., 2004; Wi et al., 2005). The irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein, hormone balance, leaf gas-exchange, water exchange and enzyme activity (Hameed et al., 2008). The mutagen treatment breaks the nuclear DNA and during the process of DNA repair mechanism, new mutations are induced randomly and heritable. The changes can occur also in cytoplasmic organelles, and also results in chromosomal or genomic mutations and that enable plant breeders to select useful mutants such as flower colour, flower shape, disease resistance, early flowering types **(**Jain, 2010). Mutation by using radiation has successfully produced quite a large number of new and promising varieties in different seeds and ornamental plants, and is considered to be a most successful tool for breeding ornamental plants (Andrea, 2003).

The mutagenic efficiency of physical mutagen depends not only on the properties of the physical agent, but also on the genotype. Published data indicate that different species and even cultivars may respond differently. PCR-based DNA marker techniques seem to provide the means for generating useful information on polymorphism, genetic relatedness and diversity. The PCR-based random amplified polymorphic DNA (RAPD) markers are dominant markers and are extensively used in genetic mapping (Siddiqui et al., 2010) and for the identification of markers linked with useful traits (Bai et al., 2003). Due to its technical simplicity and speed, RAPD methodology has been used for diversity analyses in several crops (Priolli et al., 2010). However, SRAP not used for studding ornamental herbs, to our knowledge this is the first report. The aim of this investigation was to study the effects gamma irradiation on the possibility of inducing molecular variations based on SRAP markers in three ornamental herbs.

Materials and methods

Plant materials and gamma radiation

Field experiment was carried out at the Experimental Farm, Faculty of Agriculture, Kafr El-Sheikh University during the two successive seasons 2013/2014 and 2014 /2015. Seeds of local variety of Stock, *(M. incana* ssp. *incana*), Carnation, (*D. caryophyllus*) and (*D. ecklonis*) were used. Gamma rays were generated from Cobalt-60 source in Gamma in the Irradiation Laboratory at Middle East Regional Radio – Isotope Center for the Arab Countries at Dokki, Giza, Egypt. The dose rate was 150 rad/ sec. Batches of 100 seeds were treated with one dose of Gamma-irradiation then were sown in trays filled with a medium of peat moss and vermiculite "2:1 by volume" in 1st October of each season where germination was started after one week of sowing. Six weeks after sowing, seedlings were transplanted into open field experiment in a silty clay soil on November 15th in plots 2.5 x 1.5m with three rows at 50 cm apart in each plot and 50 cm between the seed hills within the row as two plants/ hill as every plot contained 15 hills / plot. With the onset of the first flower, flowering date was recorded and when fifty percent of the flowers on plant were opened, plant height, branches number per plant, aerial parts fresh and dry weights and flowers number per plant were recorded. Selected improved plants with the best traits collected separately and planted in next year as (second mutant generation) M_2

plants. Different plants of M_2 were examined daily to isolate the variations on the basis of vegetative growth and panicles which concluded leaf changes (color and shape), branching and flowering changes of each treatment to determine the accessions which exhibited abnormal phenotypes.

DNA extraction and SRAP-PCR condition

Nine selected plants fresh leaves of each treated herbs were used for DNA isolation by CTAB method comparing with control (non irradiated plants) according to Doyle and Doyle (1990). PCR reactions with ten SRAP combinations were used in this study as shown in Table 1. The reactions were optimized and mixtures were prepared (in total volume of 25 µl) contained 10 ng DNA, 200 µM dNTPs, 1 µM primer, 0.5 units of *Taq* polymerase and 10-X *Taq* polymerase buffer. PCR cycling was carried out as the following program, one cycle at 95 °C for 5 min., followed by 30 cycles were performed as follows: 1 min. at 95 °C for denaturation, 45 sec. at 50°C for annealing and 2 min. at 72°C for extension. Reaction was incubated at 72°C for 7 min. and then kept at 4°C. The PCR products were separated by electrophoresis using 1.2% agarose gel in 1 x TAE buffer against 100 bpp DNA Ladder as a size marker. Bands were detected with ethidium bromide staining and documented on Gel Documentation.

Table 1. Primers name and sequences of SRAP*

Primer No.	Primer name	5˝→3˝ Primer sequences
1	me1	TGAGTCCAAACCGGATA
2	me2	TGAGTCCAAACCGGAGC
3	me3	TGAGTCCAAACCGGAAT
4	me4	TGAGTCCAAACCGGACC
5	me5	TGAGTCCAAACCGGAAG
6	em1	GACTGCGTACGAATTAAT
7	em2	GACTGCGTACGAATTTGC

*All possible 10 combinations (me1+em1, me1+em2, me2+em1, me2+em2, , me3+em1, , me3+em2, me4+em1, me4+em2, me5+em1 and me5+em2)

Data analysis

Amplification profiles for studied genotypes as a result of SRAP were compared with each other and DNA fragments were scored as a binary data, where (1) means presence and (0) means absence. Data were used to estimate genetic similarity on the basis of number of shared amplification products. The electrophoresis patterns of the reproducible banding patterns of each primer which produced by SRAP were chosen for analysis. Pairwise comparisons between individuals were made to calculate the Jaccard coefficient using PAST program (PAleontological Statistics, Version 1.94b) adapted by Hammer et al. (2001). Cluster analysis was performed to produce a dendrogram using unweighted pair-group method with arithmetical average (UPGMA).

Results and discussion

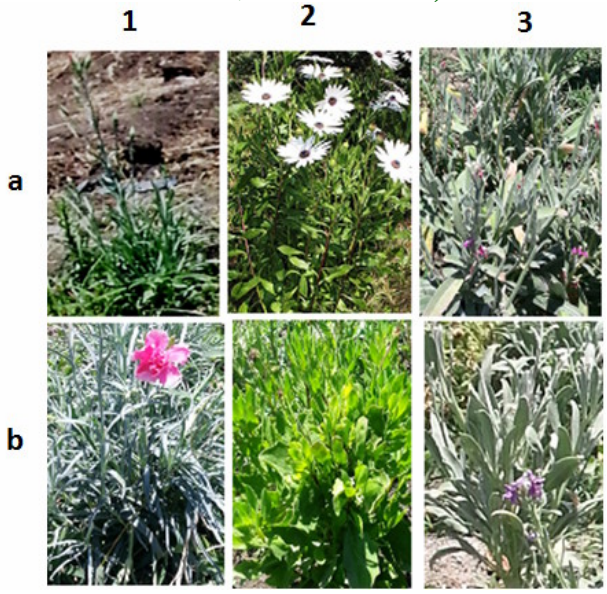
Effect of gamma irradiation on germination and growth parameters

Data in Table 2 showed that, the three plants featured various percent of positive variability for gamma-irradiation treatment. As the treatment delay the emergence in both *Dimorphoteca ecklonis* and *Mathiola incana* plants (15.80 and 12.47 days) whereas accelerate it in *Dianthus caryophyllus* plant (14.28 days) compared to control (13.73, 8.59 and 20.76 days, respectively). For the three treated plants, Control treatment surpassed treated one in germination percentage as record 55.63 vs. 43.22% for *Dimorphoteca ecklonis*, 65.48 vs. 58.29% for *Mathiola incana* and 60.90 vs. 49.57% for *Dianthus caryophyllus*. It was noticed that, plants phenotype seemed the most affected, where this obvious evident through there vegetative growth, such as treated plants were shortest than control one as record 37.86, vs. 41.52 cm for *Dimorphoteca ecklonis*, 21.61 vs. 26.80 cm for *Mathiola incana* and 40.11 vs. 47.95 cm for *Dianthus caryophyllus*. Also, gamma irradiated plants gave the highest branches and leaves numbers in both seasons compared to control (Fig. 1). The widest leaves over control resulted from both *Mathiola incana* which gave 46.22 cm^2 vs. 19.57 cm^2 for control and *Dianthus caryophyllus* which gave 4.83 cm^2 vs. 2.10 cm^2, respectively Inhibition of seed germination after gamma radiation could be due to the damage in seed tissue, chromosomes and mitotic division (Datta, 2009). Whereas, control treatment surpassed treated one in the leaf area for *Dimorphoteca ecklonis* plant as record 3.07 cm^2 vs. 2.89 cm^2 for treated plants (Rehman et al., 1987; Hameed et al., 2008).

Table 2. Effect of gamma irradiation on days to emergence, germination percentage, plant height, branches and leaves number and leaf area of M_2 plants.

Treatment		Days to emergence	Germination %	Plant height (cm)	Branch No.	Leaves No.	Leaf area (cm^2)
Dimorphoteca ecklonis	Control	13.72	55.63	41.52	1.74	53.29	3.07
	G R	15.80	43.22	37.86	12.61	126.31	2.89
Mathiola incana	Control	8.59	65.48	26.80	1.07	38.41	19.57
	G R	12.47	58.29	21.61	5.13	67.24	46.22
Dianthus caryophyllus	Control	20.76	60.90	47.95	4.62	179.57	2.10
	G R	14.28	49.57	40.11	10.25	385.31	4.83

Fig. 1: Effect of gamma rays (a) un-irradiated plants (b) irradiated plants. (1. *Dianthus caryophyllus*;2. *Dimorphoteca ecklonis*; 3. *Mathiola incana*)



Fresh and dry mass of gamma-irradiation plants were the heaviest compared to control ones (Table 3). The extensive variance among treated plants and control especially with *Dimorphoteca ecklonis* (87.38 and 12.69g fresh mass and 28.73 and 4.21g dry mass per plant) then *Dianthus caryophyllus* (82.60 and 36.50g fresh mass and 29.62 and 11.80g dry mass per plant) may be due to the induced variability by gamma-irradiation among a population of M_2. Also, roots number and both roots fresh and dry weights of treated plants take the same trend as these plants record highest roots number and heaviest fresh and dry roots than control. On the contrary, gamma-irradiation *Dimorphoteca ecklonis* and *Mathiola incana* plants record the shortest roots, while, *Dianthus caryophyllus* control plants roots were the longest. Roots number was increased in adverse of roots length. These results are in agreement with those of Venkatachalam and Jayabalan (1997) which found on *Zinnia elegans* cv. crimsom red that, gamma rays produced more significant morphological changes. Recently, Ramesh et al. (2010) stated that, plant sensitivity depends on the genetic constitution, dose-employed, DNA amount, moisture content, and stage of development and genotype.

Effect of gamma irradiation on some flowering measurements and total green color

Gamma-irradiation *Dimorphoteca ecklonis*, *Mathiola incana* and *Dianthus caryophyllus* plants gave the highest number of flowers and greenest plants compared to untreated ones (Table 4). On the contrary, Gamma-irradiation treatment was the most influential in delay flowering and reducing flower diameter of all treated plants. This may be due to irradiation and many others biosynthetic pathways are altered which are directly and indirectly associated with the flowering physiology (Mahure et al., 2010). These results also corroborate with the finding of Dilta et al. (2003); Muthusamy et al. (2003) and Kim et al. (2004).

Table 3. Effect of gamma irradiation on fresh and dry mass and roots number, length, fresh and dry weight of M_2 plants.

Treatment		Fresh mass / plant (g)	Dry mass / plant (g)	Roots No.	Roots length (cm)	Roots F. W. (g)	Roots D.W. (g)
Dimorphoteca ecklonis	Control	12.69	4.21	4.75	22.60	2.53	0.95
	G R	87.38	28.73	87.22	18.51	15.37	10.34
Mathiola incana	Control	29.37	12.42	2.06	23.06	8.26	2.84
	G R	48.87	19.20	5.81	21.80	12.40	4.28
Dianthus caryophyllus	Control	36.50	11.80	5.10	20.83	7.99	2.88
	G R	82.60	29.62	9.43	22.55	12.65	4.59

Table 4. Effect of gamma irradiation on flowers number, days to flowering, flower diameter and total green color on M_2 plants.

Treatment		Flowers No.	Days to flowering	Flower diameter (cm)	Total green color (SPAD)
Dimorphoteca ecklonis	Control	6.91	88.37	3.41	25.77
	G R	11.75	104.22	2.96	37.31
Mathiola incana	Control	1.00	41.81	0.45	44.50
	G R	5.03	58.44	0.37	61.82
Dianthus caryophyllus	Control	28.22	94.11	4.72	65.88
	G R	56.32	131.32	4.20	68.42

Molecular characterization based on SRAP

Amplifications were successful for all SRAP primer pair combinations. Out of 10 primer pairs, eight primer pairs resulted in informative and polymorphic products. Table 5 summarizes the results obtained based on the analysis of selected nine individuals of the three studied herbs (total numbers of bands, polymorphic bands and polymorphic %) using the polymorphic SRAP and their control (un-irradiated). The number of alleles varied widely among these loci and the used herbs. A total of 62, 40, and 52 alleles were observed among for *M. incana* ssp. *incana*, Carnation (*D. caryophyllus*) and *D. ecklonis*, respectively. The number of alleles ranged from one using primer pairs me1+em1 on all three herbs to 10 using primer pairs me1+em2 on *M. incana*. Also as shown in Figs. 2, 3 and 5. Moreover, the highest poylorphism percentage was 88.8% using primer pairs , me1+em5 on *M. incana* followed by 71.4% using same primer pairs on *D. ecklonis*. On the other hand, the lowest polymorphism % was 33.3% and recorded by me2+em5 primer pairs on *D. caryophyllus.*

Table 5. DNA polymorphism using SRAP using three flowering herbs.

Primer	*Matthiola incana*			*Dianthus caryophyllus*			*Dimorphotheca ecklonis*		
	Total no. of band	Poly. bands	Poly. %	Total no. of bands	Poly. band	Poly. %	Total no. of band	Poly. bands	Poly. %
1, me1+em1	1	0	0.0%	1	0	0.0%	1	0	0.0%
2, me1+em2	11	7	63.6%	4	1	25.0%	2	1	50.0%
3, me1+em3	5	3	60.0%	5	2	40.0%	7	3	42.8%
4, me1+em4	7	4	57.1%	4	2	%	5	2	40.0%
5, me1+em5	9	8	88.8%	6	3	50.0%	7	5	71.4%
6, me2+em1	7	3	42.8%	5	2	40.0%	6	2	33.3%
7, me2+em2	8	5	62.5%	6	1	16.6%	7	2	28.5%
8, me2+em3	2	1	50.0%	2	1	50.0%	6	3	50.0%
9, me2+em4	2	0	0%	1	0	0.0%	4	2	50.0%
10, me2+em5	10	6	60.0%	6	2	33.3%	7	3	42.8%
Total	62	37		40	14		52	23	

Fig. 2: SRAP patterns generated following PCR amplification of total DNA *of Matthiola incana* ssp. *incana.* a: me2+em1 and b: me5+em1, M: 100 bp ladder, c : control and 1-9 radiated individuals.

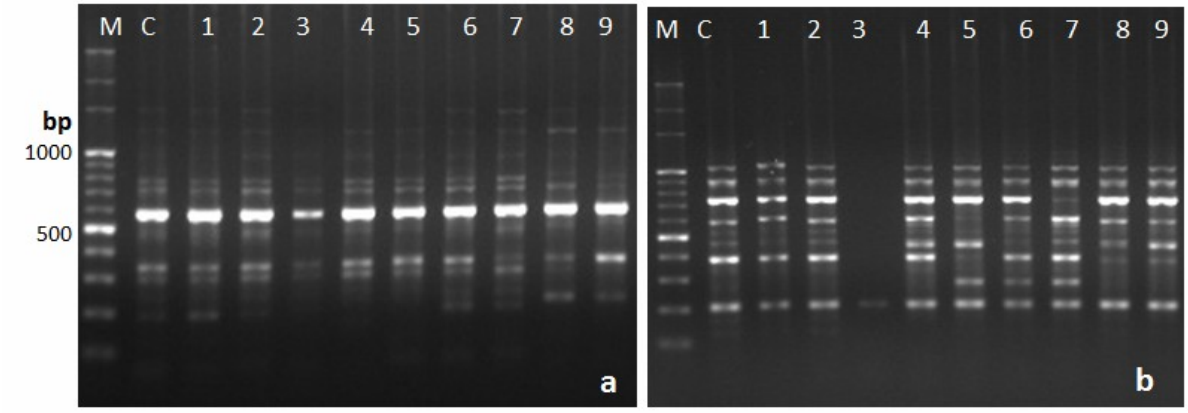


Table 6. Similarity index based on Jaccard for *Matthiola incana* ssp. *Incana*.

Control	M1	M2	M3	M4	M5	M6	M7	M8	0.00
0.57	0.53	0.64	0.31	0.64	0.64	0.56	0.53	0.85	M9
0.71	0.67	0.79	0.46	0.79	0.79	0.69	0.65	1.00	M8
0.63	0.69	0.69	0.31	0.69	0.69	0.81	1.00		M7
0.79	0.86	0.73	0.43	0.86	0.86	1.00			M6
0.77	0.71	0.71	0.50	0.85	1.00				M5
0.92	0.85	0.85	0.50	1.00					M4
0.55	0.50	0.50	1.00						M3
0.92	0.85	1.00							M2
0.92	1.00								M1

Fig. 3: SRAP patterns generated following PCR amplification of total DNA of Carnation (*Dianthus caryophyllus*).
a: me5+em1and b: me5+em2, M: 100 bp ladder, c : control and 1-9 radiated individuals.

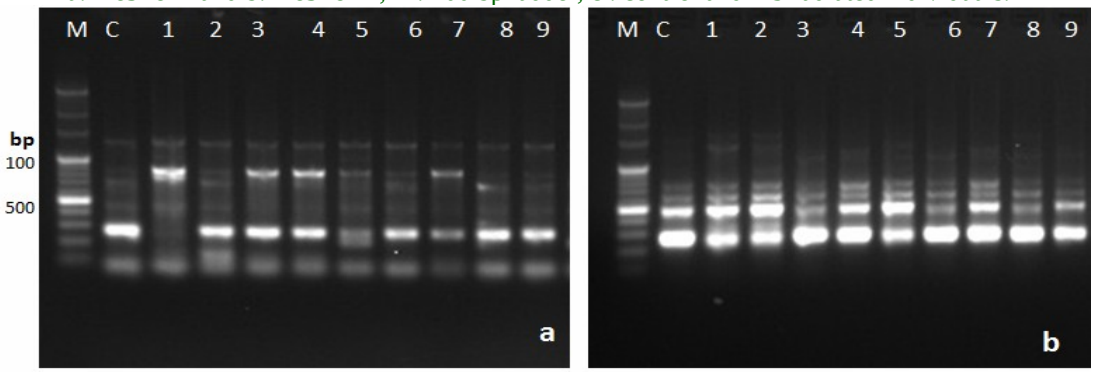


Table 7. Similarity index based on Jaccard for Carnation, *(Dianthus caryophyllus*)

Control	M1	M2	M3	M4	M5	M6	M7	M8	0.00
0.63	0.70	0.67	0.44	0.44	0.60	0.67	0.70	0.67	M9
0.75	0.80	1.00	0.56	0.56	0.89	1.00	0.80	1.00	M8
0.60	1.00	0.80	0.45	0.45	0.90	0.80	1.00		M7
0.75	0.80	1.00	0.56	0.56	0.89	1.00			M6
0.67	0.90	0.89	0.50	0.50	1.00				M5
0.71	0.45	0.56	1.00	1.00					M4
0.71	0.45	0.56	1.00						M3
0.75	0.80	1.00							M2
0.60	1.00								M1

Fig. 4: Phylogenetic tree of (a) *Matthiola incana* ssp. *incana,* (b) *Dianthus caryophyllus* and (c) *Dimorphotheca ecklonis.*

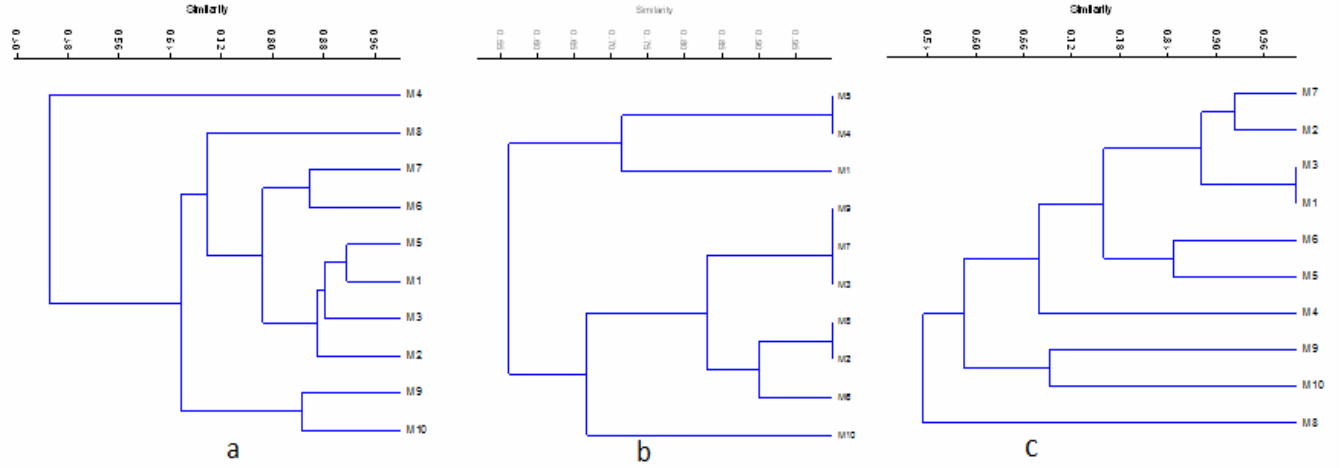


Fig. 5: SRAP patterns generated following PCR amplification of total DNA *of Dimorphotheca ecklonis.*
a: me5+em1and b: me3+em2, M: 100 bp ladder, c : control and 1-9 radiated individuals.

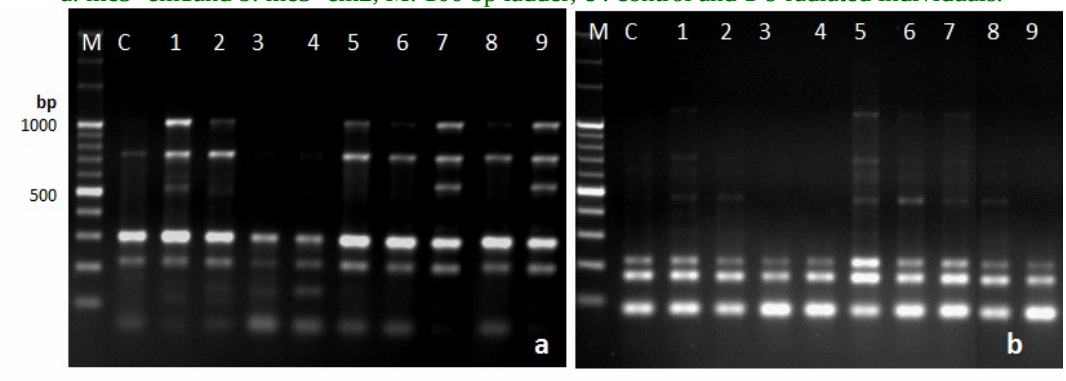


Table 8. Similarity index based on Jaccard for *Dimorphotheca ecklonis*

Control	M1	M2	M3	M4	M5	M6	M7	M8	0.00
0.57	0.53	0.57	0.46	0.60	0.57	0.50	0.31	0.69	M9
0.69	0.64	0.69	0.58	0.60	0.57	0.60	0.50	1.00	M8
0.62	0.69	0.62	0.50	0.53	0.40	0.64	1.00		M7
0.85	0.92	0.85	0.62	0.86	0.71	1.00			M6
0.69	0.64	0.69	0.73	0.85	1.00				M5
0.85	0.79	0.85	0.62	1.00					M4
0.73	0.67	0.73	1.00						M3
1.00	0.92	1.00							M2
0.92	1.00								M1

From aforementioned results it seems that using the radiation *M. incana* was the most affected because it gave the highest polymorphism % and showed lowest similarity based on *Jaccard's Similarity Index* the similarity was 0.31 among the plant No. 3 and 6, 7 and 9, in the same manner the plant number was the most different than control and show less similarity 0.55 compared to other plants.

Gamma irradiation has the potential for developing new varieties of *Curcuma alismatifolia* with improved commercial properties suitable for the flower industry, the use of DNA markers facilitate the exploration of genetic variability among treated and non-treated plants and will help to distinguish the plants showing differences in morphological characters (Taheri et al., 2014).

Based on the similarity indices shown in Tables 6, 7 and 8, and phylogenetic trees in Fig. 4, *M. incana* is the most affected plant with radiation which have less similarities and highly diverged as observed from the phylogenetic tree. However, *D. caryophyllus* have less affected by radiation with the same dose and still similar and have no variation as observed from its phylogenetic tree. On the other hand, *D. ecklonis* have moderate affect in between both other lines.

The number of absent or extra bands after gamma irradiation suggested that the DNA damage may be serious in the majority of plant cells; molecular analyses have revealed that gamma rays could induce DNA rearrangements in the genome (Vizir and Mulligan, 1999; Dhakshanamoorthy et al., 2011). However, it is difficult to interpret the current data on herbs after radiation via gamma rays with models and parameters conditions of previous experiments have varied greatly.

In addition, the frequency of mutations highly varied from one plant to another, the used dose, the species/variety (Turuspekov et al., 2002; Kim et al., 2009). The choice of the radiation dose to be applied for the highest mutant rescue is often left to the breeder's experience with the specific plant material, its genetics, and its physiology. Finally, the present study might help in explaining different responses and complication to gamma radiation in the three studied ornamental herbs for further study and improvement.

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