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Variability induction in Ox-eye daisy (*Leucanthemum vulgare* Lam.) using gamma rays

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ABSTRACT

The present investigation was aimed to study hormesis, morphological and biochemical variability attributes associated with mutation and purification of novel mutants in Ox-eye daisy. The seeds of *Leucanthemum vulgare* were exposed to 20, 40, 60, 80 and 100 Gy doses of gamma rays (source ^{60}Co). These irradiated seeds were used to raise seedlings and planted in combination with seedlings from non-irradiated seeds (control) in randomized block design. Low doses of gamma irradiation resulted in hormesis and evoked encouraging novelties, whereas, the higher doses elicited higher degree of abnormalities and consequently mortality. The M_2 seeds were seeded to observe new characters and mutations in population in every treatment. The minimum plant survival was 51.60% at 100 Gy gamma rays treatment, which significantly differed from all other treatments. The maximum plant survival (99.67%) was observed in non-irradiated control. It was observed that plant survival significantly declined with the increase in the dose of gamma irradiation. Plants raised from irradiated seeds showed significant delay in flowering over the control. The earliest blooms were observed in control (108.03 days), while the maximum days to bloom (118.30 days) were recorded with 100 Gy treatment. Three promising mutants, *viz*., Spatulate type (L_1) at 40 Gy, Quilled-spatulate type (L_2) at 60 Gy and Quilled type (L_3) at 60 Gy gamma irradiation treatment were labelled, screened and checked for stability of characters in M_2 and M_3 generations. The seeds of M_2 and M_3 generations were raised for observation for variation in morphological characters and stability mutants in every generation.

Key words: *Leucanthemum vulgare*, gamma rays, irradiation, mutation, Ox-eye daisy.

INTRODUCTION

Leucanthemum vulgare Lam. [*Chrysanthemum leucanthemum* Linn.] commonly known as Ox-eye daisy or white weed is a native to Europe and North Asia. It is a leafy, vigorously growing herbaceous, non-woody perennial plant. The species is strictly cross-pollinated due to presence of self-incompatibility. It can be cultivated as a decorative plant in the garden flower beds or as pot plant. Application of mutation breeding has significant role in creating novel genotypes in ornamental crops, like the usually high heterozygosity of the plants that permits direct detection of mutations within the irradiated material, with the intention of improvement in visible characteristics (Broertjes, 3).

Genetically modified ornamental plants do not find market in Europe due to their low acceptance amongst consumers and the ambiguous legal situation. As very little research work has been carried out on *Leucanthemum vulgare*, the current study was therefore aimed to study hormesis, morphological and biochemical variability attributes accompanied with mutation, creation and purification of novel mutants.

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MATERIALS AND METHODS

The experiment was conducted at Model Floriculture Center, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand during 2010 to 2012. The experimental material comprised of the seeds of *Leucanthemum vulgare*. The seeds of the parental line were procured from Thompson & Morgan, Great Britain and were exposed to gamma rays (source ^{60}Co) at 20, 40, 60, 80 and 100 Gy doses at gamma chamber facility of the CSIR-National Botanical Research Institute, Lucknow. The gamma ray-irradiated seeds together with the control (non-irradiated seeds) were seeded on raised nursery beds and transplanted in experimental field in randomized block design with three replications. The plot size was 180 × 100 cm^2 with 12 seedlings/plot with plant spacing of 50 × 30 cm^2. All the recommended package of practices were followed throughout the growing period. Morphological and biochemical parameters were recorded for 13 traits from randomly chosen three plants per treatment per replication. The chlorophyll content (*a*, *b* and total chl) of the leaves was estimated as proposed by Hiscox and Israelstam (9). Visual observations on totally different characters were prepared and the plants showing modification in type of flowers, florets, etc. were critically determined and any abnormality determined within the plants of M_1 generation in the treatments was labelled, screened and recorded. At maturity, every mutant plant was separately harvested and also the seeds were labelled for sowing in the subsequent generations.

The M_2 and M_3 seeds together with the control (non-irradiated seeds) were seeded on raised nursery beds and transplanted in experimental field in randomized block design with three replications, with a plot size of 180 × 100 cm^2 (12 plants per plot) with spacing of 50 × 30 cm^2, following the recommended package of practices. M_2 mutants were sporadically observed right after germination and were labelled for further observations. Any abnormality or variation detected in the plants of M_2 generation was screened and tagged for subsequent observations in M_3 generation. At maturity, every mutant plants were individually harvested and the seeds were labelled and screened, for sowing in the subsequent generation (M_3) and checked for the stability of the characters. The data generated were subjected to the statistical analysis in accordance with the procedure outlined by Gomez and Gomez (7).

The genomic DNA was extracted by using the CTAB method (Doyle and Doyle, 6) with slight alterations. PCR amplification was performed with random decamer primers. Band sharing data was analyzed to get genetic similarities based on Jaccard's similarity coefficient among the isolates by using Numerical Taxonomy and Multivariate Analysis System (NTSYSpc, version 2.2) (Rohlf, 16). UPGMA (Unweighted Pair Group Method using Arithmetical Averages) algorithm was used to determine the genetic relationship of the parent and also the mutants generated in *L. vulgare*.

RESULTS AND DISCUSSION

The observations made on several un-irradiated plants of *L. vulgare* clearly revealed that normal plants grew to a mean plant height of 51.0 cm with plant spread of 38.17 cm (E-W) and 35.17 cm (N-S). The leaves were numerous, green, lacking punctate glandular hairs. Basal leaves were 9.15 cm long, 4.63 cm wide and had 22.62 cm^2 leaf area. Leaves were also long, petiolate with linear or oval cuneately narrow lamina, obtusely toothed, less often shallow lobed. Lower leaves were spatulate, short petiolate, but the upper leaves were sessile, gradually smaller and less divided. Each plant had solitary capitula (25) with ray florets (36) and disc florets (339.17). The diameter of the capitula was 6.87 cm, weighing 1.32 g and disc was 2.05 cm across, borne on a thin (0.475 cm) and long peduncle. Involucre was glabrous with involucre bracts of light-colour or brownish colour with membranous border. The weight of ray florets was 9.20 mg and that of disc florets was 0.92 mg. The ray florets were 2.77 cm long and 0.86 cm broad borne on the flower head, which was 2.10 cm high (Table 2).

Data pertaining to the effect of gamma irradiation on vegetative growth characters, biochemical content and abnormalities are presented in Table 1. The perusal of the data presented in Table 1 and Fig. 1 revealed that the minimum plant survival (51.60%)

Table 1. Effect of gamma irradiation on different characters of *Leucanthemum vulgare* and its mutants.

Trait	Control	Gamma irradiation (Gy)					CD at 5%
		20	40	60	80	100	
Plant survival (%)	99.67	94.67	85.20	76.90	64.83	51.60	7.16
Plant abnormality (%)	0.00	4.11	7.40	12.46	15.33	20.67	1.64
Plant height (cm)	46.33	45.13	42.50	40.87	39.53	36.67	6.12
Plant spread (E-W) (cm)	37.67	34.00	35.00	32.67	31.57	31.20	NS*
Plant spread (N-S) (cm)	34.00	33.13	32.47	31.00	29.83	28.30	3.02
Leaf length (cm)	8.97	8.83	8.47	8.4	7.73	7.67	0.69
Leaf width (cm)	4.53	4.13	4.05	3.82	3.53	3.3	NS
Days to flowering	108.03	110.80	111.57	113.97	117.63	118.30	3.54
Chlorophyll *a* (Chl *a*)	1.782	1.83	1.84	1.599	1.852	1.927	0.07
Chlorophyll *b* (Chl *b*)	0.515	0.531	0.55	0.552	0.564	0.583	0.03
Total chlorophyll (Chl)	2.282	2.345	2.373	2.134	2.399	2.493	0.08
Abnormal leaf (%)	0.00	3.80	7.30	11.87	15.53	21.93	1.28
Abnormal flower (%)	0.00	6.47	11.23	13.56	16.13	18.4	1.57

NS = non-significant; * = significance at 5% level.

Table 2. Morphological characters of *L. vulgare* and its mutants developed through gamma irradiation.

Trait	Original genotype	Mutants		
		L_1	L_2	L_3
		40 Gy	60 Gy	40 Gy
Plant height (cm)	51.00	39.33	34.67	32.33
Plant spread (E-W) (cm)	38.17	37.33	33.17	32.67
Plant spread (N-S) (cm)	35.17	34.50	33.50	31.50
Leaf length (cm)	9.15	9.77	9.62	8.52
Leaf width (cm)	4.63	3.52	3.35	3.60
Leaf area (cm^2)	22.62	18.39	17.23	16.40
No. of flowers/plant	25.33	21.50	23.00	24.00
Flower dia. (cm)	6.87	7.18	5.83	7.15
Disc dia. (cm)	2.05	2.90	2.47	2.60
No. of ray florets	36.00	39.00	34.00	33.67
No of disc florets	339.17	488.07	415.14	437.58
Head weight (g)	1.32	1.38	1.12	1.37
Ray floret weight (mg)	9.20	10.33	7.55	8.54
Disc floret weight (mg)	0.92	0.91	0.95	1.09
Ray floret length (cm)	2.77	2.80	2.37	2.30
Ray floret width (cm)	0.86	0.43	0.34	0.31
Head height (cm)	2.10	3.04	3.10	3.13
Flower form	Single	Semi-double	Single	Single
Shape of ray florets	Ligulate	Spatulate	Quilled-spatulate	Quilled

at 100 Gy gamma rays treatment was found, which significantly differed from all other treatments. The maximum plant survival (99.67%) was observed in non-irradiated control. It was observed that plant survival significantly declined with the increase in the dose of gamma irradiation. Reduction in plant survival after exposure to gamma rays has been explained to be due to disturbances of auxin synthesis, chromosomal aberration (Gunckel and Sparrow, 8). Similar results were also observed by Kapoor *et al.* (12) in *Chrysanthemum paludosum*. The plant height decreased from 46.33 cm in control with the increase in dose of gamma irradiation to 36.67 cm with 100 Gy treatment. Reduction in plant height was observed with increase in the dose of gamma rays irradiation, which may be due to the fact that inactivation of auxin and decrease in auxin content with increase in radiation doses was responsible for reduction in plant height (Banerji and Datta, 2). Earlier, Misra *et al.* (15) observed reduction in plant height due to gamma irradiation in *C. morifolium*.

Plants arisen from irradiated seeds had significant delay in flowering compared to control. The earliest blooms (108.03 days) were observed in control, while the maximum days to bloom (118.30 days) were recorded with 100 Gy treatment. The delay in bud initiation ultimately resulted in late blooming, which may be due to reduction in the rate of various physiological processes and inhibition of growth and the plant remained in juvenile stage and thus unable to differentiate flower heads due to gamma irradiation. Due to irradiation, many biosynthetic pathways are altered, which are directly and indirectly associated with the flowering physiology (Mahure *et al.*, 14). These results also corroborate with the finding of Dilta *et al.* (5) in *C. morifolium*.

The maximum leaf length (8.97 cm) was observed in control, while minimum (7.67 cm) was in 100 Gy treatments. Significant reduction in size of the leaf with the increasing doses of gamma irradiation was observed. This may be attributed to poor growth of plants due to radiation damage. The chlorophyll content was also influenced significantly by various gamma ray treatments. Increase in the chlorophyll content (*a*, *b* and total chl.) was evidenced with the increase in the doses of gamma irradiation. Datta (4) and Kapoor *et al.* (12) also observed similar results with incite in chlorophyll content as the

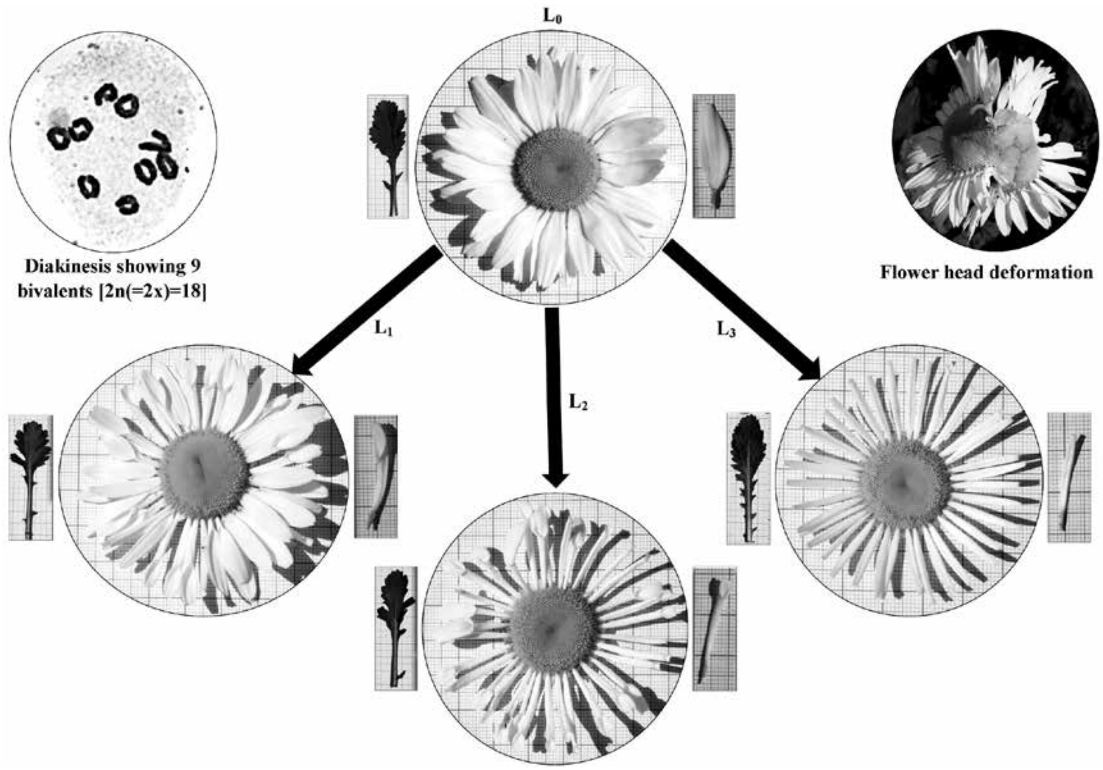

Fig. 1. Capitula of *Leucanthemum vulgare* (L_0) and its gamma irradiation induced mutants (L_1, L_2 and L_3)

gamma irradiation dose increased and reported that its basic cause may be associated with the physiological disturbances, change in enzyme activity and breakage of metabolites with the increase in irradiation doses.

The per cent abnormal plants increased significantly with the increase in gamma rays treatment over the control. Among the different gamma rays treatments, maximum deformed plants (20.67%) were recorded with 100 Gy treatment and none in control. This significant induction of abnormalities might be due to radiation damage of the irradiated plants particularly chromosomal breakage, which causes physiological, morphological and cytological disturbances. Misra *et al.* (15) also recorded similar trends in chrysanthemum variety Pooja. Per cent abnormal leaves increased significantly with the increasing gamma ray irradiation doses compared to control. The different types of leaf abnormalities recorded included change in leaf shape and size, margins, apex, fission and fusion after irradiation. There were no dose specific abnormalities in leaves. Earlier, Banerji and Datta (1) also observed similar results in *Dendranthema* cv. Surekha. Significant increase in plants with flower head fasciation/ asymmetrical flower heads due to irradiation was noted, which was not specific to the doses. Flower heads became fasciated in different forms (Fig. 1). The formation of fascinated heads after irradiation was also observed by Kapoor *et al.* (11). These abnormalities are genotype dependent and damage within the organism may be due damage to plant parts (Datta, 4).

Visual observations on traits were made and the plants showing change in form of flowers, florets *etc*. (Fig. 2 a-d), were critically observed and the type of forms other than normal ones were tagged and recorded. The plants were also observed for chimera formation, which could be maintained through vegetative propagation or through tissue culture techniques. The change in flower form was also recorded by Kumari *et al.* (13) on *C. morifolium*.

Three mutants, *viz*., Spatulate type (L_1) at 40 Gy, Quilled-Spatulate type (L_2) and Quilled type (L_3) at 60 Gy were screened, tagged and checked for the stability of the characters. The observations were recorded on morphological characters of the mutants developed after gamma irradiation in M_1, M_2 and M_3 generations and the pooled mean values are presented in Table 2 and Fig. 2. Mutation in flower head shape/ size has also been reported earlier in annual chrysanthemum (Jain *et al*., 10). Some of the prominent mutants identified were as listed below.

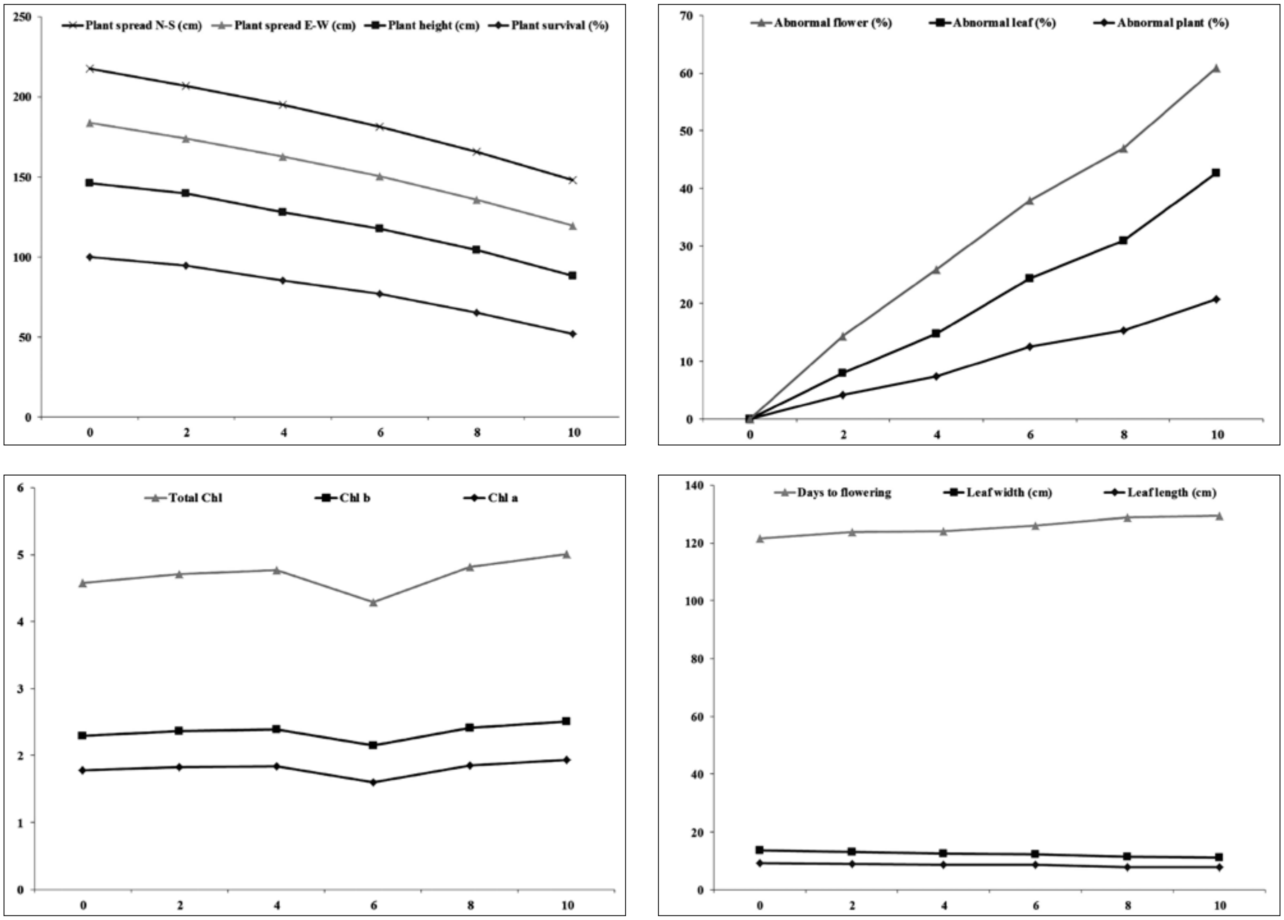

Fig. 2 **(a)** Effect of gamma irradiation on vegetative characters, **(b)** Effect of gamma irradiation on per cent abnormalities, **(c)** Effect of gamma irradiation on chlorophyll content, **(d)** Effect of gamma irradiation on days to flowering and leaf size.

A mutant L_1 (spatulate type) was selected after treatment with 40 Gy gamma rays irradiation treatment and was found significantly different from mother plants, *i.e.* dwarf and less spreading. The leaves were long, while width and area were lesser and the number of flowers per plant was marginally lesser. Flowers were of bigger size, flower head diameter, disc diameter were more with ray florets and disc florets. The flower head and ray floret weights were more, while disc floret weight was marginally less. The ray floret length was marginally more, while width was lesser but the flower had more flower head height. Change was recorded in flower form to semi-double, along with the change in the shape of the ray florets from ligulate with laciniate shape of tip and keeled upper surface to spatulate (Table 2).

The traits of the mutant L_2 (Quilled-Spatulate type) is shown in Table 2. It is a dwarf mutant developed with 60 Gy gamma rays irradiation. The plant spread was also reduced in both the directions. The leaf length was though more, while leaf width and leaf area were lesser. The number of flowers per plant reduced marginally, but the flowers were smaller in size, more disc diameter and lesser number of ray florets of lesser weight. The disc florets were more in number (415.14) and weight (0.95 mg). The length and width of the ray florets were lesser than the original species. The flower had more flower head height. No change was recorded in flower form, except for the change in the shape of the ray florets from ligulate with laciniate shape of tip and keeled upper surface to quilled-spatulate.

A third potential mutant L_3 (Quilled type) was developed with 40 Gy gamma rays irradiation treatment and differed in several traits from the original plants, *viz.*, plants were very dwarf with lesser plant spread. The leaf length (8.52 cm), leaf width (3.60 cm) and leaf area (16.40 cm^2) were less and the number of flowers per plant also got reduced. Mutant L_3 had slightly bigger flower with higher flower head diameter, disc diameter and lower number of ray florets and more number of disc florets. The flower head weight was more, while ray florets weight was lesser and disc florets weight was more. The ray floret size was lesser and flower head height was more than that of the original. No change was recorded in flower form, except for the change in the shape of the ray florets from ligulate with laciniate shape of tip and keeled upper surface to quilled (Table 2).

PCR amplification of DNA with random primers of which LC-94 and LC-86 showed sufficient polymorphism (93.33 to 90.91%) with an average polymorphic percentage of 92.12%. A total number of 26 loci were amplified (Fig. 3). This gave an average of 13 loci per primer. Polymorphic information content (PIC) value was 0.57 for primer LC-94 and 0.59 for primer LC-86 with an average of 0.58 for both the primers (Table 3).

The dendrogram generated using SAHN cluster analysis and UPGMA method illustrated in Fig. 4 and the matrix of the Jaccard's similarity coefficient of the mutants of *L. vulgare* based on RAPD markers (Table 4) reveal that the dendrogram separated the original species of *L. vulgare* and its three mutants into two major clusters A and B, at the demarcation of approximately 37% genetic similarity. Cluster A consisted of the original species and its 2 mutants, while the cluster B had only mutant L_3. Cluster A was further categorized into two sub-clusters I and II, at the demarcation of approximately 58% genetic similarity. Sub-cluster I had the original species and its mutants L_1 with approximately 68% genetic similarity. Sub-cluster II had only one mutant L_2.

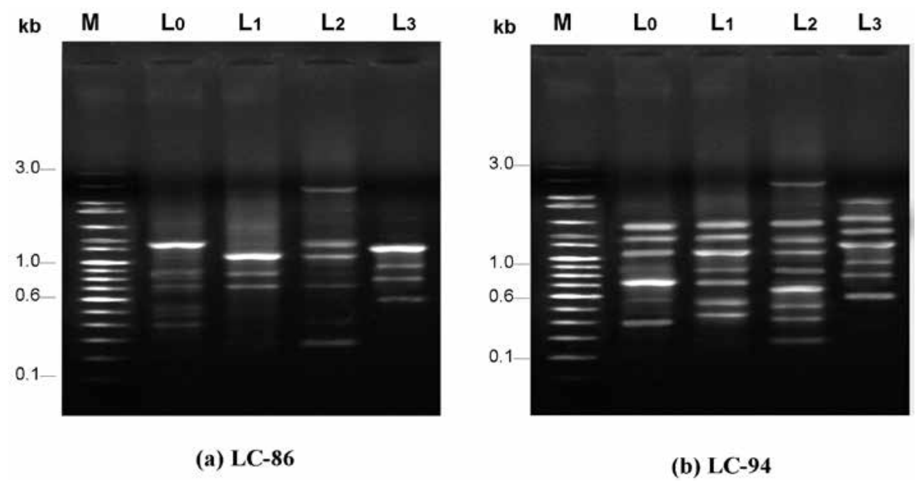

Fig. 3 Molecular diversity generated among *Leucanthemum vulgare* (L_0) and its three mutants (L_1, L_2 and L_3) by RAPD primer LC-86 and LC-94.

Table 3. Characterization of mutants of *Leucanthemum vulgare* using RAPD primers.

Code	Primer sequence (5′ to 3′)	% GC	MMB	PMB	Poly (%)	PIC	H_i	Rp	D	D_L
LC-94	’GTCGCCGTCA’	70	1	14	93.33	0.57	0.30	8	0.63	0.37
LC-86	’GTTGCGATCC’	60	1	10	90.91	0.59	0.33	7	0.66	0.38
Av.			1	12	92.12	0.58	0.31	7.5	0.65	0.37

MMB = monomorphic bands, PMB = polymorphic bands, % Poly = Per cent polymorphism, PIC = Polymorphic Information content, H_i = Average expected gene diversity, Rp = Resolving power, D = Discrimination power, D_L = Discriminating power.

Table 4. Jaccard's similarity coefficient of *L. vulgare* and its mutants based on RAPD markers.

Genotype	*L. vulgare*	L_1	L_2	L_3
L. vulgare	1.000			
L_1	0.667	1.000		
L_2	0.482	0.667	1.000	
L_3	0.407	0.444	0.259	1

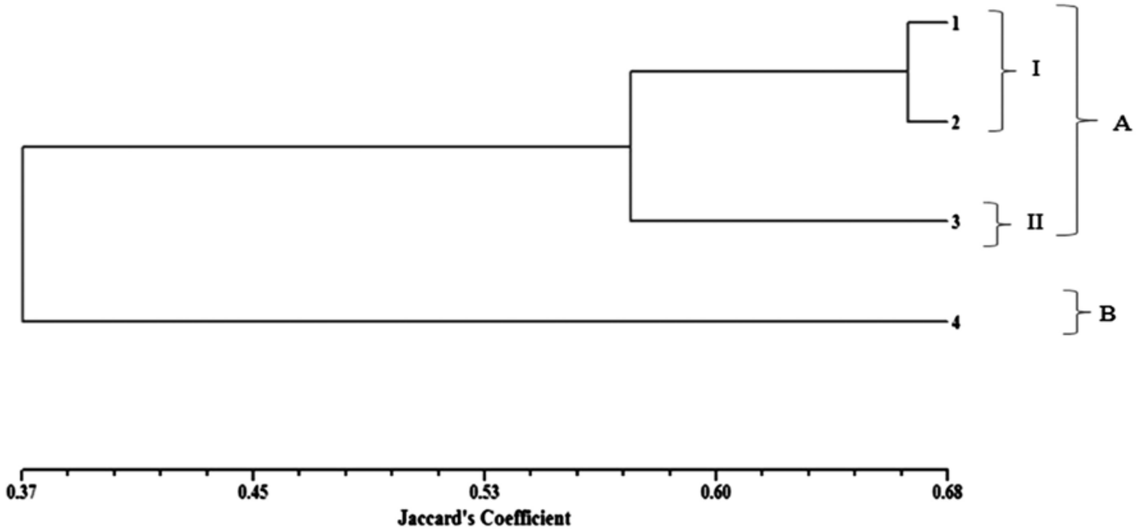

Fig. 4 Dendrogram depicting the classification of *Leucanthemum vulgare* and its three mutants based on RAPD. 1. *L. vulgare*, 2 to 3. Mutants of *L. vulgare* L_1 to L_3.

From the present investigation, it has been empirically perceived that in addition to change in flower shape, cogent changes in some morphological characters had occurred in the mutants. Gamma irradiation induced new flower shape appearance mutants, screened in the present investigation may find very advantageous in future practical breeding programmes and can also be used directly for cultivation.

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