



Gamma radiation induced variations in corn marigold (*Glebionis segetum*) and their RAPD-based genetic relationship

MANISH KAPOOR¹, PRABHAT KUMAR² and SHANT LAL³

College of Agriculture, G B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand 263 145

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ABSTRACT

The present investigation was conducted during the *kharif* season of 2010-2012 to study hormesis, morphological and biochemical attributes associated with mutation and purification of novel types in corn marigold. The seeds of *Glebionis segetum* were treated with gamma rays (Source ⁶⁰Co) at the dose of 20, 40, 60, 80 and 100 Gy were sown in the field along with control (un-irradiated seeds). Low doses of gamma irradiation resulted in hormesis and induced encouraging novelties, while the higher doses induced higher degree of abnormalities which led to mortality. The M₂ seeds were sown to observe their characters and mutants in each population. Five promising mutants, viz. S₁-S₃ at 20 Gy and S₄-S₅ at 40 Gy gamma irradiation treatments were tagged, screened and checked for stability of characters for genetic study and possible uses of the traits. RAPD molecular marker technique was used for the study of genetic divergence and establishment of distinctiveness or similarity between the mutants developed as a result of mutagenic treatment.

Key words: Gamma rays, *Glebionis segetum*, Irradiation, Mutation, Mutants, RAPD

Glebionis segetum (L.) Fourr. (syn *Chrysanthemum segetum* L.) (2n=2x=18) also called corn marigold and is native to Eastern Mediterranean and North Africa. It is a leafy, vigorously growing herbaceous annual, glabrous plant, easily distinguished by grayish green toothed leaves. Stems simple to somewhat branched. Capitula are solitary with golden yellow florets, usually not in corymbs. The species is strictly cross pollinated due to self-incompatibility. It is an important ornamental plant grown in the garden as tall border planting and also as cut flower.

There is an ample room for flower breeders to improve the species. Mutation techniques in *G. segetum* are useful in creating genetic variability and obtaining novel traits. In floriculture industry, there is always a craze for developing new novelties to replace older varieties with newer ones, since in ornamentals, a variety cannot maintain interest for a long time and people have the desire for newer forms through various methods of breeding. The possibilities of mutation breeding are favourable for various reasons such as the usually large heterozygosity of the material which allows direct detection of mutations in the irradiated material, with the intention of improvement in visible characteristics (Broertjes 1968).

Scientific interest in breeding using mutagens has drastically decreased during the last few decades as the interest has shifted towards development and application of molecular techniques in breeding, since they allow more directed approach in pursuance of breeding goals. Molecular techniques generate high developmental costs and require sophisticated equipments and a highly trained staff, also the investment in such expensive methods does not seem adequate in case of ornamental crops as compared to agricultural crops (Schum 2003). As very little research work has been done on *G. segetum*, the present study was aimed to study hormesis, morphological and biochemical changes associated with mutation, creation and purification of novel mutants in *G. segetum*.

MATERIALS AND METHODS

The experiment was conducted at the Model Floriculture Center, G B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand during 2010-12. The experimental material comprised the seeds of *Glebionis segetum*. The seeds of the parental line were obtained from Horti Flora Seed Farms, Patiala (Punjab) and were exposed to 20, 40, 60, 80 and 100 Gy of gamma rays doses using the gamma irradiator facility of National Botanical Research Institute (NBRI), Lucknow. The gamma irradiated seeds (M₁) along with the control (un-irradiated seeds) were sown on raised nursery beds and transplanted in experimental field in randomized block design with three replications. The plot size was 100 cm × 180 cm with 12 seedlings/plot at a

¹ e mail: jdmanishkapoor@yahoo.com, Department of Botany, Punjabi University, Patiala, Punjab 147 002; ² Division of Floriculture and Landscaping, Indian Agricultural Research Institute, New Delhi; ³ Department of Horticulture

spacing of 50 cm × 30 cm. All the recommended package of practices were followed throughout the growing period. Morphological observations were recorded for eighteen traits from randomly selected three plants per treatment per replication. The chlorophyll content (chlorophyll *a*, chlorophyll *b* and total chlorophyll) of the leaves was estimated as suggested by Hiscox and Israelstam (1979). The data was booked for both the years (2010 to 2012) and average was computed. Any abnormality or variation observed in the plants of M₁ generation in different treatments was also recorded.

The mutants were periodically observed right after germination and were tagged for subsequent observations. The data was recorded on characters and number of the mutants. At maturity, each mutant plant was individually harvested. The data generated were subjected to the statistical analysis in accordance with the procedure outlined by Gomez and Gomez (1984).

RAPD molecular marker technique was used for the studies of genetic divergence and establishment of distinctiveness/similarity between the mutants developed as a result of the mutagenic treatment of *Glebionis segetum*. The genomic DNA was extracted by using the CTAB method (Doyle and Doyle 1990) with slight modifications. PCR Amplification was performed (William *et al.* 1990) with arbitrary decamer primers.

Band sharing data was analyzed to obtain genetic similarities based on Jaccard's similarity coefficient (Jaccard 1908) among the isolates by using Numerical Taxonomy and Multivariate Analysis System (NTSYSpc, Version 2.2) (Rohlf 2009). UPGMA (Unweighted Pair Group Method using Arithmetical Averages) algorithm was employed to determine the genetic relationship of the parent and the

mutant genotypes in *G. segetum*.

RESULTS AND DISCUSSION

The observations made on several unirradiated plants of *G. segetum*, raised in the experimental field are presented in Table 1; indicate the different parameters pertaining to morphology. The normal plants of *G. segetum* grew to a mean plant height of 68.83 cm with plant spread of 65.33 cm (E-W) and 60.17 cm (N-S). The leaves were grayish green, lack punctate glandular hairs, fleshy to somewhat succulent, petiolate narrowed at base, sessile, oblong to oblanceolate and irregularly coarse toothed to shallow pinnatilobate. The mean leaf length 6.40 cm, leaf width 3.27 cm and leaf area of 11.18 cm² was recorded. Each plant had large number of solitary radiate capitula (55.17) with yellow ray florets (21.17) and disc florets (339.67). The average diameter of the radiate capitula was 5.97 cm, weighing 2.06 g and disc was 2.01 cm across, borne on a long, 0.458 cm thick peduncle. The weight of ray florets was 25.37 mg and that of disc florets was 2.02 mg. The golden yellow ligulate ray florets were 2.35 cm long and 1 cm broad borne on flower head which was 1.82 cm in height.

Complete plant survival was recorded in control, which declined with increase in dose of gamma rays, with minimum (51.50%), when exposed to 100 Gy. Reduction in plant survival after exposure to gamma rays may be explained to be due to disturbances of auxin synthesis (Gordon 1957) and chromosomal aberration (Gunckel and Sparrow 1961). Similar results were also obtained by Banerji and Datta (1992) in *C. morifolium* cv Jaya.

Significant reduction in plant height was observed with increase in the dose of gamma rays irradiation. The

Table 1 Effect of gamma irradiation on different characters of *Glebionis segetum*

Characters	Control	Gamma irradiation (Gy)					SEM±	CD (5%)	CV
		20	40	60	80	100			
Plant survival (%)	100.00 (90.00)*	80.33 (63.70)*	71.83 (57.95)*	63.67 (52.95)*	59.67 (50.58)*	51.50 (45.86)*	1.11	3.50	2.71
Plant abnormality (%)	0.00 (0.00)*	6.20 (14.41)*	8.72 (17.17)*	13.51 (21.56)*	17.97 (25.08)*	21.70 (27.76)*	0.45	1.42	6.88
Plant height (cm)	67.67	65.63	64.07	62.50	59.00	54.00	1.80	5.67	5.02
Plant spread (E-W) (cm)	65.00	61.67	57.83	55.07	54.77	52.53	2.52	7.94	7.55
Plant spread (N-S) (cm)	58.00	55.70	54.13	52.17	51.77	50.87	1.05	3.30	3.37
Leaf length (cm)	4.63	4.50	4.27	4.07	4.00	3.83	0.17	NS	6.97
Leaf width (cm)	2.20	2.17	2.00	1.93	1.86	1.74	0.15	NS	13.11
Days to flowering	140.77	141.13	143.27	144.67	145.47	146.77	1.50	NS	1.81
Chlorophyll <i>a</i> (<i>Chl a</i>)	0.962	1.161	1.270	1.277	1.304	1.368	0.033	0.105	4.712
Chlorophyll <i>b</i> (<i>Chl b</i>)	0.220	0.290	0.316	0.326	0.341	0.373	0.015	0.048	8.525
Total chlorophyll (<i>Chl</i>)	1.176	1.443	1.577	1.594	1.634	1.730	0.031	0.098	3.531
Abnormal leaf (%)	0.00 (0.00)*	4.83 (12.67)*	9.94 (18.34)*	13.63 (21.65)*	16.96 (24.31)*	25.63 (30.39)*	1.06	3.34	15.54
Abnormal flower (%)	0.00 (0.00)*	8.63 (17.07)*	12.43 (20.58)*	14.15 (22.10)*	16.43 (23.91)*	20.43 (26.86)*	0.87	2.73	12.50

* Angular transformed values

maximum plant height in control (67.67 cm) and the minimum in 100 Gy (54 cm) were recorded. Inactivation of auxin and decrease in auxin content with increase in radiation doses is responsible for reduction of plant height (Banerji and Datta 2002). Misra *et al.* (2009) observed similar observations in *C. morifolium*. Significant reduction in plant spread (E-W and N-S) was recorded in *G. segetum* with increase in the dose of gamma irradiation. Zargar *et al.* (1998) also observed similar results. Non-significant delay in flowering over the control was observed, with earliest blooms in control (140.77 days), while the maximum days to bloom (146.77 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.* 2010). These results also corroborate with the finding of Diltia *et al.* (2003).

Reduction in size of the leaf was observed non-significant, with increase in dose of gamma irradiation. This may be due to the poor growth of plants due to radiation damage (Gaul 1970). In an earlier investigation, gamma rays also significantly reduced the leaf length and width in *Chrysanthemum* varieties Sonar Bangla, Satish Modi and Flirt (Zargar *et al.* 1998).

The chlorophyll content was influenced significantly by various gamma rays treatments. Increase in the chlorophyll content [chlorophyll 'a' (*Chl a*), chlorophyll 'b' (*Chl b*) and total chlorophyll content (*Chl*)] was observed with increase in the gamma irradiation dose. Datta (1988) also observed similar results and reported that the basic cause of abnormalities is associated with physiological disturbances of growth substances, change in enzyme activity, variation in ascorbic acid concentration, breakage of phosphate metabolism, accumulation of free amino acids, etc.; incited by X-rays and colchicine.

The per cent abnormal plants significantly increased with the increase in gamma rays treatment over the control. Among the different gamma rays treatments, maximum deformed plants (21.70%) were recorded with 100 Gy gamma rays treatment and none in control. This significant production of abnormalities may be due to radiation damage of the irradiated plants particularly chromosomal breakage (Gaul 1970), which causes physiological, morphological and cytological disturbance by gamma radiation. Misra *et al.* (2009) also recorded similar trends in *Chrysanthemum* variety Pooja. Per cent abnormal leaves significantly increased with the increase in gamma rays dose over the control. The different types of leaf abnormalities included change in leaf shape and size, margins, apex, fission and fusion were recorded after irradiation. There was no dose specific or variety specific abnormalities in leaves. Banerji and Datta (2001), observed the similar results in *Dendranthema cv Surekha*. Significant increase was also

observed in plants with flower head fasciation/asymmetrical flower heads due to irradiation and these abnormalities were not dose specific. Flower heads became fasciated in different forms. These abnormalities are genotype dependent and mechanism may be involved in the repair of radiation induced damage within the organism (Datta 1984).

Visual observations on different characters were made and the plants showing change in form of flowers, florets etc. (Fig 1), were critically observed and the type of form other than normal were tagged and recorded. The plants were also observed for any chimera which could be used for chimera management through vegetative propagation or through tissue culture techniques. The change in flower form was also recorded by Lamseejan *et al.* (2003) in *C. morifolium*.

Five mutants, viz. (S_1 - S_3) at 20 Gy and (S_4 - S_5) at 40 Gy were screened, tagged and checked for the stability of the characters. The observations were recorded on

Table 2 Morphological characters of mutants developed after gamma irradiation in *Glebionis segetum*

Characters	Original	Mutants of <i>G. segetum</i>				
		S_1 20 Gy	S_2 20 Gy	S_3 20 Gy	S_4 40 Gy	S_5 40 Gy
Plant height (cm)	68.83	66.50	62.48	61.92	53.50	58.18
Plant spread (E-W) (cm)	65.33	64.33	61.67	60.25	50.75	52.32
Plant spread (N-S) (cm)	60.17	58.45	57.75	55.92	46.58	49.10
Leaf length (cm)	6.40	6.12	5.93	5.52	5.53	5.70
Leaf width (cm)	3.27	3.16	3.08	2.98	2.93	3.01
Leaf area (cm ²)	11.18	10.67	9.76	8.81	8.69	9.17
No. of flowers/plant	55.17	54.55	52.83	52.17	45.18	47.17
Flower diameter (cm)	5.97	6.43	6.83	6.48	7.90	6.48
Disc diameter (cm)	2.02	2.13	2.52	2.72	2.97	2.32
No. of ray florets	21.17	20.33	23.67	22.25	21.58	18.23
No. of disc florets	339.67	365.03	432.31	466.67	509.61	397.96
Head weight (g)	2.06	2.22	2.36	2.28	2.73	2.24
Ray floret weight (mg)	25.37	25.52	26.33	26.37	27.90	26.04
Disc floret weight (mg)	2.02	1.84	1.99	1.54	2.11	1.91
Ray floret length (cm)	2.35	2.30	2.28	2.38	2.53	2.12
Ray floret width (cm)	1.00	0.82	0.83	1.05	0.65	1.09
Head height (cm)	1.82	2.63	2.52	2.44	2.82	2.76



Fig 1 Capitula of *Glebionis segetum* (S_0) and its five mutants gamma irradiation induced mutants (S_1 - S_5)

morphological characters of the mutants developed after gamma irradiation in M_1 and M_2 generations and the pooled mean values of the two years are presented in Table 2. Mutation in flower head shape/size in *Chrysanthemum* have also been earlier reported in annual *Chrysanthemum* (*C. coronarium*), by Rana (1965); Jain and Mazumdar (1957) and Jain *et al.* (1961).

Mutant S_1

This mutant developed in *G. segetum* at 20 Gy gamma rays irradiation treatment and differed in many characters than the original *G. segetum* plants and attained marginally lesser plant height and plant spread. The leaf length, width and area were lesser and the number of flower per plant was marginally lesser. Flower size, flower head diameter, disc diameter was more with more ray florets and disc florets. The flower head weight and ray floret weight were more, while disc floret weight was marginally lesser. The ray floret length and width were marginally lesser but the flower had more flower head height. No change was recorded in flower form in this mutant, except for the change in the colour of the disc to brown from yellow.

Mutant S_2

This mutant also developed at 20 Gy gamma rays irradiation treatment and was dwarfed than the original

species. The plant spread also reduced. The leaf length, width and area were lesser. The number of flowers per plant reduced marginally but the flowers were of bigger size and more weight. The florets were more in number and weight. No change was recorded in flower form, except for the change in the colour of the disc to light brown from yellow and the ray floret colour to white from yellow.

Mutant S_3

This mutant also developed at 20 Gy gamma rays irradiation treatment and differed in many characters from the original *G. segetum* plants, attaining a marginally more plant height but lesser plant spread. The leaf length, width and area were lesser and the number of flowers per plant also reduced. Mutant S_3 had bigger flower with more flower head diameter, disc diameter and number of ray florets and disc florets. The flower head weight and ray floret weight were more, while disc floret weight was lesser. The ray floret size and flower head height was more than that of the original. No change was recorded in flower form in mutant S_3 from the single original form, except for the change in the colour of the disc to brown from yellow and the floret colour to white from yellow.

Mutant S_4

This mutant developed at 40 Gy gamma rays irradiation treatment of the seeds and differed in many characters than the control. This mutant was dwarf with lesser plant spread. The leaf length, width and area also reduced and the number of flowers per plant also reduced. Mutant S_4 had bigger flower with more flower head diameter, disc diameter and number of ray florets and disc florets, with more flower head weight, ray floret weight and disc floret weight. The ray florets were longer and slimmer. The flower had more flower head height with no change in flower form, except for the change in the colour of the floret to white from yellow.

Mutant S_5

This mutant also developed in *G. segetum* at 40 Gy gamma rays irradiation treatment and differed in many characters than the original *G. segetum* parent plants. This mutant was marginally dwarfed with lesser plant spread. The leaf length, width and area were lesser with reduced number of flowers per plant and bigger flower with more flower head diameter and disc diameter but lesser number of ray florets and more number of disc florets. The flower head weight and ray floret weight were more, while disc floret weight lesser than the control. The ray florets were shorter and broader. The flower had more flower head height. The ray floret colour turned bicoloured with the upper part of floret turned white from yellow and the lower part remained yellow.

From the present analysis of original species and their gamma rays induced mutants, it has been found that in addition to change in flower colour, significant changes in some morphological and biochemical characters had

Table 3 Details of RAPD primers used for the molecular characterization of mutants of *Glebionis segetum*

Code	Primer Sequence (5' to 3')	<i>Glebionis segetum</i>								
		%GC	MMB	PMB	% Poly	PIC	H _i	Rp	D	D _L
LC-94	5'GTCGCCGTCA3'	70	3	4	57.14	0.41	0.15	2.3	0.43	0.27
LC-86	5'GTTGCGATCC3'	60	1	8	88.89	0.46	0.33	5	0.50	0.29
	Average		2	6	73.02	0.43	0.24	3.7	0.46	0.28

MMB - Monomorphic bands, PMB - Polymorphic bands, %Poly - % Polymorphism, PIC - Polymorphic Information Content, H_i - Average expected gene diversity, Rp - Resolving power, D - Discrimination power, D_L - Discriminating power

occurred in the mutants. This suggests that the changes were perhaps induced at several independent loci. However, pleiotropic or epistatic effects of a mutant gene controlling different characters cannot be ruled out. Gamma irradiation induced new flower colour mutants of the present experiment may find very useful in future practical breeding programme and the new mutants can also be used directly for floriculture industry/trade.

PCR amplification of DNA extracted from the mutants and the original species of *Glebionis segetum* was performed with two random primers (LC-94 and LC-86). The PCR products run on agarose gel were scored manually. The amplification profile generated by each primer was compared and the relative molecular size of each band was examined by comparing with DNA size marker.

The number of RAPD loci scored and polymorphism detected by the two primers in the mutants and the original species of *Glebionis* are presented in Table 3.

The total number of 16 loci was amplified from two primers. This gave an average of 8 loci per primer. The polymorphism percentage ranged from 88.89% to 57.14%, with an average polymorphic percentage of 73.02%. Based on polymorphism percentage and unique band amplification, all the primers were considered highly informative primers. PIC value was 0.41 for primer LC-94 and 0.46 for primer LC-86 with an average of 0.43 for both the primers.

The dendrogram generated using SAHN cluster analysis and UPGMA method illustrated in Fig 2 and the matrix of the Jaccard's similarity coefficient of the mutants of *Glebionis segetum* based RAPD markers (Table 4) reveal

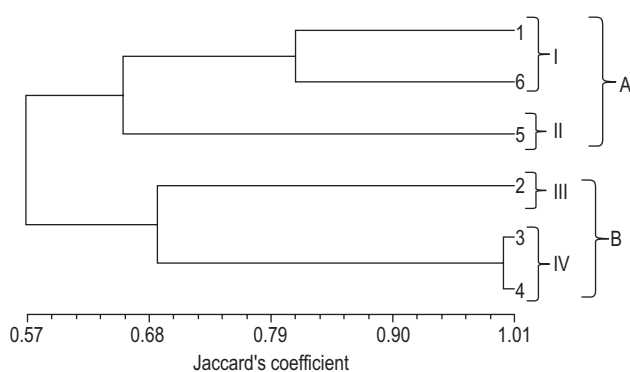


Fig 2 Dendrogram depicting the classification of *Glebionis segetum* and its five mutant based on RAPD. 1: *G. segetum* (S₀), 2-6: Mutants of *G. segetum* (S₁-S₅)

the following information:

The dendrogram separated the original species of *G. segetum* and its 5 mutants into two major clusters A and B, at the demarcation of approximately 57% genetic similarity. Cluster A consisted of the original species and its 2 mutants while the cluster B of its 3 mutants. Cluster A was further categorized into two sub-clusters I and II, at the demarcation of approximately 65% genetic similarity. Sub-cluster I had the original species and its mutants S₅ with approximately 80% genetic similarity. Sub-cluster II had only one mutant S₄. Cluster B was further categorized into two sub-clusters III and IV, at the demarcation of approximately 68% genetic similarity. Sub-cluster III consisted of only one mutant S₁, while sub-cluster IV had 2 mutants S₂ and S₃, having approximately 98% genetic similarity.

The UPGMA dendrogram based on RAPD analysis indicated that most of the mutants were fairly distant from the parents and also among themselves. Knowledge of the genetic relationship between different mutants, contributing to genetic diversity can greatly aid the development of efficient germplasm utilization and management strategies. The variability that is introduced by mutation breeding includes flower colour and form variation which is quite divergent when compared to the parent. The percentile variations in the mutated population were studied to decipher the extent of variation the mutagen has brought about at the molecular level. Molecular markers provide the knowledge of the genetic relationships and genetic diversity between different mutants where these are unable to distinguish by morphological data. In this context DNA based markers are suggested as key strategy to determine the cultivars purity leading to improve IPR mechanism (Riek 2001).

Our present study clearly indicate that RAPD markers can be efficiently used for genetic diversity studies among the radiation induced mutants and the original species at

Table 4 Matrix of Jaccard's similarity coefficient of *Glebionis segetum* and its mutants based on RAPD markers

	1	2	3	4	5	6
1	1					
2	0.688	1				
3	0.625	0.688	1			
4	0.625	0.688	1	1		
5	0.625	0.563	0.500	0.500	1	
6	0.813	0.500	0.563	0.563	0.688	1

1 - *G. segetum*, 2 to 6 - Mutants of *G. segetum* S₁ to S₅

genomic level. The results obtained suggest that by using RAPD molecular marker, the newly evolved *Glebionis* mutants in different species can be easily differentiated from their parent. This would be a very useful tool in identifying and protecting them from possible infringements and for protecting the Plant Breeders' Rights in future.

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