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Short Communication

Spectrum of chlorophyll mutations and morphological variations in *Abelmoschus esculentus* L. induced through gamma radiation

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ABSTRACT

Okra [*Abelmoschus esculentus* (L.) Moench], is an economically exploited important traditional vegetable crop of the world. The present investigation examined the variability in induced chlorophyll mutants and other morphological variations in okra. Seeds of two open pollinated popular varieties of okra namely Arka Anamika and Arka Abhay were irradiated with gamma doses of 30, 50 and 80 kR. The treatment 50 kR enhanced plant height, number of fruits per plant, fruit length, single fruit weight and total fruit yield per plant. Spectrum of several chlorophyll mutants were observed in the M_1 generation. Other macro-mutants such as early and late flowering types, dwarf statured plants, leaf and flower mutants were also noticed at different doses of gamma radiation. The total number of visible mutation followed a trend of increasing frequency with the increase in dose of radiation.

Keywords : Chlorophyll mutants, gamma radiation, mutation, okra, variability

INTROCUTION

Okra (*Abelmoschus esculentus* (L.) Moench), family Malvaceae, is one of the most cultivated multipurpose traditional crops of the world grown for its fresh tender fruits in the warm, sub-tropical and tropical areas. The genus *Abelmoschus* consists of thirty-eight species, among which *A. esculentus* is the widely cultivated. Okra fruits are rich in micro nutrients, vitamins, minerals and iodine and are also known for its medicinal values such as anti-inflammatory properties. The inadequacy of resistant genotypes for mitigating the climate change, biotic and abiotic stresses have limiting impacts on the total production and productivity of the crop. The inability to transfer available resistance genes from wild relatives to the cultivated lines as a result of chromosomal variations and faulty meiotic divisions also limits the hybridization programs in okra (Kumar *et al.,* 2019). Under these circumstances, mutation breeding has proven to be a useful technique in crops like okra, for inducing novel genetic variations such as development of mutant okra varieties *i.e.*, Punjab-8, and Parbhani Tillu. The role of mutation breeding in increasing the genetic variability for desired traits in various crop plants has been proved (Kozgar *et al.*, 2012). Chlorophyll mutations can be used as dependable indices for analyzing the efficiency of various physical and chemical mutagens in inducing genetic variability for the crop improvement in several crops (Gupta and Sood, 2019).

The present investigation was carried out to determine the response of okra to the varying doses of gamma radiation in terms of chlorophyll mutations and agro-morphological characters at the College of Agriculture, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga during 2019-2020. The 1000 seeds each of two open-pollinated varieties (Arka Anamika and Arka Abhay) were irradiated with three doses of gamma rays *i.e.*, 30 kR, 50 kR and 80 kR based on the LD_{50} value obtained from other research outcomes. Gamma irradiated seeds of both the varieties were sown with a spacing of 60 × 45 cm in a four meter row in an augmented block design during August 2019 to raise M_1 generation. In every block, untreated Arka Anamika, Arka Abhay, Parbhani Kranti (YVM tolerant) and Pusa Sawani (YVM susceptible) were sown as checks for respective treatments. The recommended package of practices was ensured during the crop period. The observations on twelve quantitative characters were recorded from 40 randomly selected plants in each treatment and averaged. The chlorophyll mutations and other morphological variations were scored and recorded using DSLR camera. Chlorophyll mutants were scored on daily basis from 10th to 31st day after sowing and classified according to Gustafsson (1940) and the identification and description of morphological mutants were followed as proposed by Blixt (1961). The mean values recorded for the characters for each mutant line in all the blocks were subjected to statistical analysis in type 2 modified augmented block design (You *et al.*, 2016) using R programme. The significance of difference among treatments in the M_1 generation of both the varieties were tested using Duncan's multiple range test (DMRT) method (Duncan, 1955).

Table 1 : Analysis of variance for growth and yield parameters in mutant families (M_1) of Arka Anamika variety

Source	Df	DFF	PH	NB	NF	FL	FW	INT	SFW	NS	SWT	TWT	YLD
Blocks	5	26.57**	351.14**	7.31**	18.7**	101.72**	1.18**	55.22**	174.49**	478.29**	3.82**	10.49**	15710.90**
Treatments	123	1.34**	55.6**	2.65**	6.8**	7.18*	3.07**	8.78**	19.88**	217.04**	2.09**	2.83**	1340.08**
Checks	3	17.15**	42.52**	5.59**	7.88**	14.98**	0.01	0.13*	2.78	2.76*	0.64**	5.9**	1173.82**
Mutants	119	1.86**	70.92**	2.81**	6.55**	11.19**	0.51**	11.39**	27.27**	243.15**	2.3**	2.57**	1976.44**
Mutants vs Check	1	23.87**	25.03**	10.73**	125.75**	0.69	21.92**	0.26*	54.18**	138.08**	0.5**	20.85**	3730.09**
Residuals	15	0.28	0.83	0.05	0.03	2.73	0.05	0.04	0.9	0.77	0.03	0.33	55.39
CV		1.06	1.92	4.94	2.74	11.65	4.27	2.19	7.70	1.72	5.02	7.88	10.12
CD (5%) (Mutant v/s Control)		1.35	2.34	0.57	0.42	4.26	0.56	0.5	2.44	2.26	0.47	1.48	19.16
CD (5%) Two Mutants (Different Blocks)		1.77	3.07	0.75	0.55	5.57	0.73	0.65	3.2	2.96	0.62	1.93	25.08

Table 2 : Analysis of variance for growth and yield parameters in mutant families (M_1) of Arka Abhay variety

Source	Df	DFF	PH	NB	NF	FL	FW	INT	SFW	NS	SWT	TWT	YLD
Block	5	38.11**	497.56**	0.52**	11.3**	69.84**	1.21**	16.86**	117.13**	697.16**	6.53**	11.54**	6680.28**
Treatment	123	2.35**	57.22**	1.5**	3.48**	8.02**	3.13**	8.49**	15.53**	165.97**	2.03**	3.75**	616.99**
Check	3	17.15**	42.52**	5.59**	7.88**	14.76**	0.02	0.13*	2.78	2.76*	0.64**	5.9**	946.96**
Mutants	119	2.43**	76.57**	1.43**	3.83**	10.73**	0.63**	9.14**	20.62**	200.23**	2.35**	4.08**	831.68**
Mutants vs Check	1	137.9**	284.07**	0.01	4.82**	0.39	18.64**	40.83**	23.62**	57.97**	0.53**	8.87**	1272.68**
Residuals	15	0.28	0.83	0.05	0.03	2.60	0.05	0.04	0.9	0.77	0.03	0.33	45.88
CV		1.04	2.02	5.76	3.82	11.58	11.72	1.91	8.00	1.75	4.88	7.57	14.45
CD (5%) (Mutant v/s Control)		1.29	2.35	0.56	0.42	4.15	0.55	0.51	2.47	2.26	0.46	1.50	17.43
CD (5%) Two Mutants (Different Blocks)		1.75	3.10	0.75	0.55	5.44	0.72	0.65	3.2	3.04	0.71	1.95	22.83

** at 1% level of significance, * at 5 % level of significance

Analysis of variance for twelve parameters in the M_1 generation of Arka Anamika and Arka Abhay were performed and presented in Table 1 and Table 2, respectively. The ANOVA for both the varieties showed that mean sum of squares within blocks (eliminating treatments), within the treatments (eliminating blocks) and checks vs mutants were significant for all the 12 traits.

Significant differences in the mean values of most of the traits were observed for mutant lines of both okra varieties Arka Anamika and Arka Abhay, in response to varying doses of gamma radiation (Table 3). A lower dose of 30 kR affects most of the traits such as plant height, single fruit weight, number of seeds per fruit, test weight and total fruit yield in mutant lines compared to control in both the varieties.

The treatment 50 kR enhanced the economic yield of okra fruits per plant drastically compared to the other treatments and the control (Fig. 1). Traits such as plant height, number of fruits per plant, fruit length and single fruit weight were observed to be significantly higher in 50 kR treatment in mutants of Arka Anamika and Arka Abhay. Other traits such as number of seeds per plant, seed weight per fruit and test weight were also higher.

In general, a higher dose (80 kR) had detrimental effects on most of the traits such as plant height, number of fruits per plant, fruit length and width, single fruit weight, number of seeds per plant, seed weight per fruit, test weight and total fruit yield. Remarkable increase in the number of days to first flowering was also observed at a higher dose in both the varieties as also reported in number of fruits per plant (Warghat *et al.*, 2011), plant height, fruit length, average fruit weight and total fruit yield (Jadhav *et al.*, 2012) and number of seeds per plant, seed weight per fruit and test weight (Hegazi and Hamideldin, 2010). Mutant lines of 80 kR group recorded more number of branches per plant, increased number of nodes and shorter internodes on the main stem, making the plants looks dwarf and short stature.

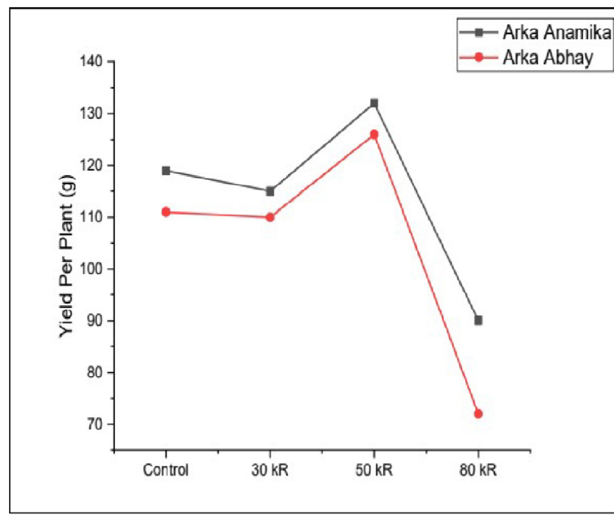


Fig. 1 : Effect of gamma radiation on yield per plant in two okra varieties

Table 3 : DMRT results for different treatments and characters among Arka Anamika and Arka Abhay mutant lines

Variety	Treatment	DFF	PH	NB	NF	FL	INT	SFW	NS	SWT	TWT	YLD
Arka Anamika	30 kR	48.25^{C}	47.17^{AB}	3.82^{BC}	7.32^{C}	13.89^{BC}	7.05^{B}	10.90^{B}	52.67^{A}	3.68^{A}	7.04^{A}	114.27^{AB}
	50 kR	49.35^{B}	51.15^{A}	4.92^{AB}	14.47^{A}	17.05^{A}	9.12^{AB}	14.39^{A}	54.10^{A}	4.00^{A}	7.35^{A}	131.80^{A}
	80 Kr	51.32^{A}	43.10^{B}	5.05^{A}	4.27^{D}	11.76^{C}	10.27^{A}	7.55^{C}	45.40^{B}	3.24^{A}	7.05^{A}	89.95^{B}
	Control	48.76^{BC}	47.01^{AB}	3.62^{C}	10.20^{B}	15.32^{AB}	8.75^{AB}	11.66^{B}	53.23^{A}	3.64^{A}	7.40^{A}	119.48^{AB}
	Mean	49.59	47.13	4.55	9.02	14.28	8.81	12.88	51.24	3.64	7.09	145.34
	CD (5%)	1.35	2.34	0.57	1.50	6.72	0.50	2.26	2.44	0.97	1.48	19.66
Arka Abhay	30 kR	49.27^{c}	46.40^{b}	3.70^{b}	8.57^{ab}	13.25^{bc}	9.12^{ab}	13.00^{a}	53.02^{ab}	4.00^{a}	7.44^{a}	100.72^{ab}
	50 kR	51.37^{b}	50.47^{a}	4.35^{b}	11.90^{a}	16.6^{a}	9.97^{a}	12.40^{a}	55.80^{a}	4.17^{a}	7.74^{a}	126.27^{a}
	80 Kr	56.50^{a}	38.62^{c}	6.05^{a}	3.39^{b}	12.16^{c}	8.98^{b}	8.82^{c}	44.90^{b}	3.16^{a}	7.43^{a}	72.70^{b}
	Control	49.41^{c}	47.01^{b}	3.62^{b}	7.37^{ab}	15.32^{ab}	8.75^{b}	10.00^{ab}	48.23^{ab}	3.64^{a}	7.40^{a}	110.70^{ab}
	Mean	51.01	44.61	4.64	8.62	14.04	10.28	12.29	51.09	3.77	7.53	114.89
	CD (5%)	1.29	2.35	0.56	1.42	6.80	0.51	2.47	2.26	0.46	1.50	17.43

Mean with same superscript within a column do not differ at 5% level of significance

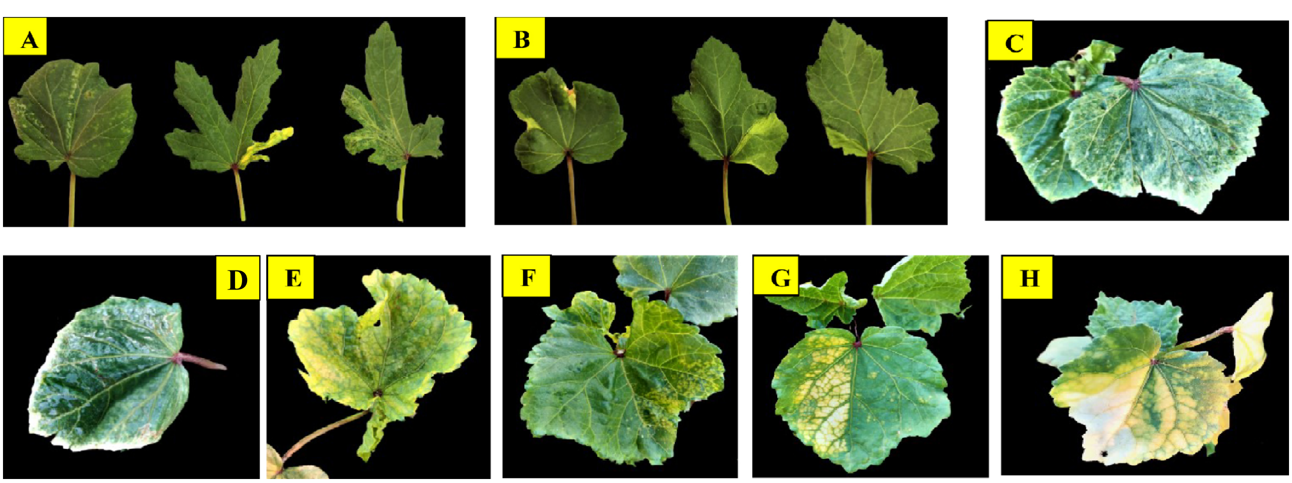
DFF-days to first flowering, PH-plant height, NB-number of branches, NF-number of fruits, FL-fruit length, INT-number of internodes, NS-number of seeds, SWT-seed weight, TWT-test weight, SFW-single fruit weight, YLD-yield per plant

Point mutations in the gene sequences of active photosynthetic parts of plants are the major source of chlorophyll mutations. Spectrum of various chlorophyll mutants in the M_1 generation of Arka Anamika and Arka Abhay are summarized in Table 4. Various chlorophyll deficient mutants such as chlorina, xantha, albina green, albino and viridis types were observed among the mutant plants, following the irradiation with gamma radiation (Fig. 2). Similar mutants were also documented by Mohite *et al.* (2019) in okra. The number of chlorina type of mutants was higher followed by xantha and viridis type. The appearance of chlorina, xantha and viridis types in a larger frequency implies the involvement of polygenic nature of chlorophyll formation (Gaul, 1964). It was also noticed that the frequency and spectrum of different chlorophyll mutations were higher at lower dose (30 kR) and was least at highest dose (80 kR). Kolar *et al*. (2011) opined that strong mutagens such as gamma radiations reach their saturation point even at lower or moderate doses and further increase in the dose may not yield higher frequency of mutants. An increase in the dose beyond a threshold level might generate more toxic effects with the strong mutagens which in fact exactly substantiate this research outcome.

The number of various visible mutants in the M_2 generation were recorded and presented in Table 5. Plants were significantly shorter in the highest dose (80 kR) which may be attributed to the sensitivity of plants to the extreme radiation dose. Number of dwarf plants were considerably high at extreme dose (10 and 8 for Arka Anamika and Arka Abhay, respectively), whereas, taller mutant plants was a peculiar observation in 50 kR treatment. The plants in 30 kR were almost synchronized with control plants for the onset of flowering, whereas, plants in 50 kR were early to flower in both the varieties. However, the extreme dose (80 kR) delayed flowering.

Table 4 : Spectrum of induced chlorophyll mutants by different doses of gamma radiation in M_1 generation

Type of mutation	Arka Anamika					Arka Abhay				
Chlorophyll mutants	Control	30 kR	50 kR	80 kR	Total	Control	30 kR	50 kR	80 kR	Total
Chlorina	-	4	2	1	7	-	3	3	1	7
Xantha	-	3	2	1	6		3	2	0	5
Viridis	-	3	0	0	3	-	3	1	0	4
Albina green	-	3	0	0	3	-	2	0	0	2
Albino	-	1	0	0	1	-	0	0	0	0



A and B: Chlorina, C and D: Albina green, E and F: Viridis, G and H: Xantha

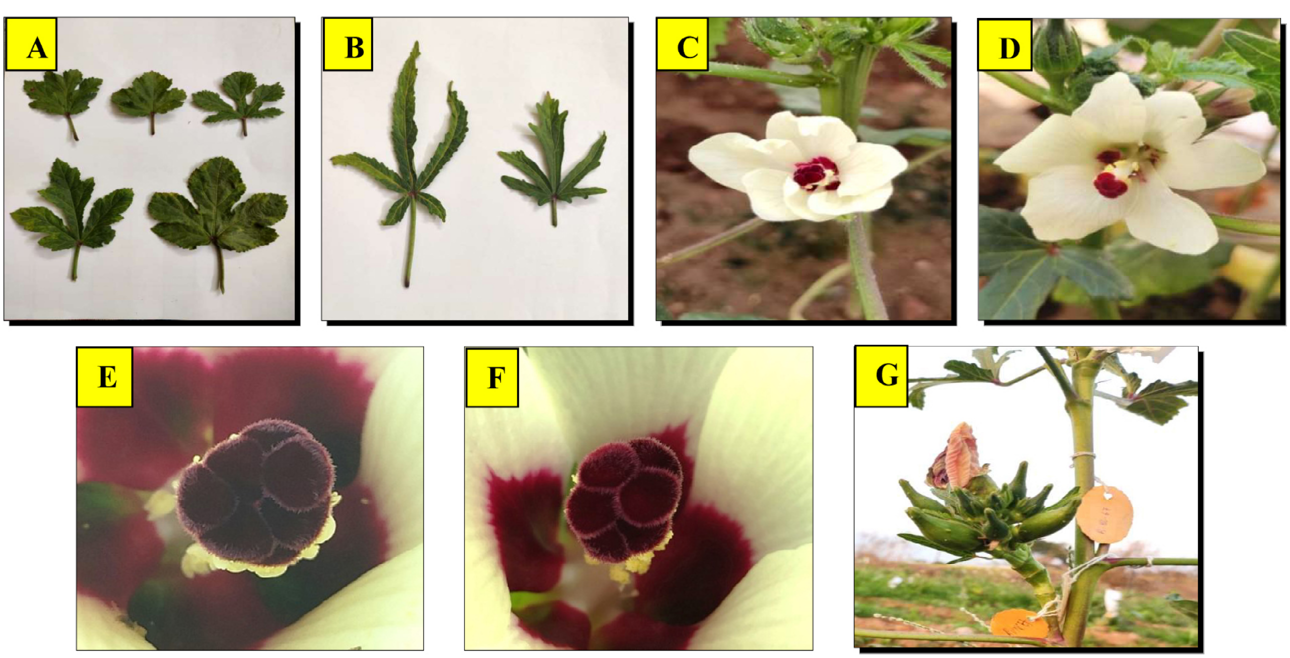
Fig. 2 : Spectrum of chlorophyll mutations induced by gamma radiation

Table 5 : Spectrum of induced visible mutations by different doses of gamma radiation in M_2 generation

Type of mutation	Arka Anamika					Arka Abhay				
	Control	30 kR	50 kR	80 kR	Total	Control	30 kR	50 kR	80 kR	Total
Dwarf	-	1	1	8	10	-	0	0	8	8
Tall	-	1	3	0	4	-	0	4	0	4
Early flowering	-	0	3	0	3	-	1	5	0	6
Late flowering	-	1	1	5	7	-	1	2	6	9
Leaf mutants	-	3	2	4	9	-	2	3	6	11
Flower mutants	-	0	0	2	2	-	0	0	1	1
Pod mutants (Increased ridge number)	-	4	6	15	25	-	6	7	12	25

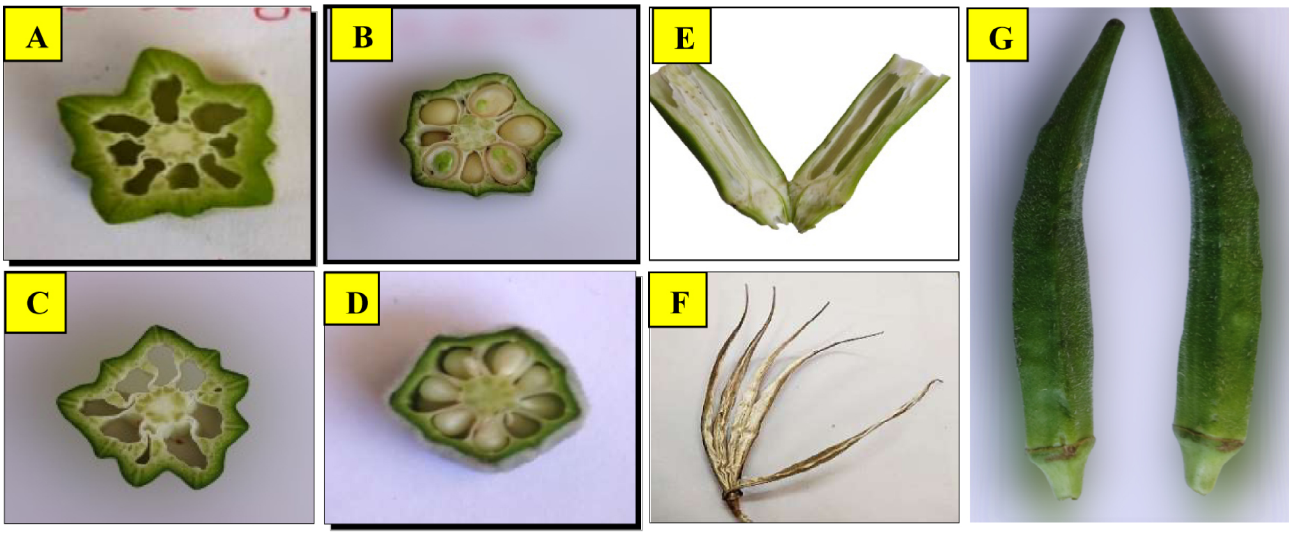
Different leaf shape mutants such as rosette, round and lobed types were also observed (Fig. 3). Leaf mutants were scored based on the sizes, shapes and colours. Although the leaf mutants were identified in all treatments, comparatively a higher number was recorded in 80 kR. Leaves were smallest in 80 kR treatment and also varied for the shapes such as rosette. Similar types of leaf mutants were also observed in okra (Surendran and Udayan, 2017; Gupta and Sood, 2019). The abnormalities observed in leaves may be attributed to various causes such as mutations in phytochromes, disturbances in chromatin material, mineral deficiencies, disturbance in DNA synthesis or mitotic division, enlargement of palisade, spongy and mesophyll cells (Gupta and Sood, 2019).

Flower mutants with six and more number of petals and altered aestivation were noticed at higher doses of gamma radiation. Considerable amount of sterility was also noticed. Record of different flower mutants following the gamma irradiation in okra is available (Surendran and Udayan, 2017). A mutant line with a bunch of fruits in a single node (Fig. 3; G) was isolated. Extremely short as well as long fruited mutants were recovered among different family. The number of ridges and locules per fruits increased from



A: round leaves, B: rosette leaves, C and D: increased number of petals, E and F: increased number of stigmata, G: multiple flower buds in a bunch

Fig. 3 : Morphological mutations for leaves and flowers induced by gamma radiation



A, B C and D: increased number of ridges and locules, E and F: sterile/ absence of seeds, G: hard pubescence

Fig. 4 : Morphological mutations for fruits, ridges and locules induced by gamma radiation

five up to eight, altering the normal ridge shape (Fig. 4). Pods with sterile seeds or miniature, shrunken seeds were also identified. The total number of visible mutations followed a trend of increasing frequency with the increase of the dose of the mutagen. A maximum number of viable and morphological mutations were obtained at a moderate dose in the study. Mohite *et al.* (2019) reported that the number of total lethal mutations increases corresponding to the increase in doses in mesta plant.

CONCLUSION

The present study, using gamma radiation confirmed the creation of genetic variability by exhibiting wide range of macro and micro mutations. The frequency and spectrum of different chlorophyll mutations such as chlorina, xantha, viridis and albino green were higher at lower dose (30 kR) and kept decreasing as the dose increased. Prominent changes in leaf morphology, including notable alterations in shape, size, increased surface roughness, and enhanced trichome sharpness and visibility were observed in higher dosage. On the other hand, the total number of visible agro-morphological mutations followed a trend of increasing frequency with the increase dose of gamma radiations. Economic traits such as total yield per plant, average fruit weight, number per plant, fruit length and seed yield per fruit were found highest at 50 kR followed by control set. Dose 50 kR can be suggested to improve agronomic characters in okra and superior mutant lines can be identified and stabilized during next generation of M_2.

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