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Gamma ray induced positive alterations in morphogenetic and yield attributing traits of finger millet (*Eleusine coracana* (L.) Gaertn.) in M_2 generation

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Abstract

Induced mutagenesis by gamma rays plays a potent promising technology to be applied for crop improvement through breeding methods, especially in tiny florets possessing self- pollinated plants such as cereals. Finger millet (*Eleusine coracana* (L.) Gaertn.) which always ensured for valuable nutrients, as well as famine tolerant crop to supply food for global population throughout the year. The present study was performed to assess the spectrum and frequency of macro mutants induced by gamma radiations in M2 generation finger millet. The chlorophyll mutants viz., *albina, xantha, chlorina* and *viridis* and morphological mutants such as tall, dwarf, bushy, brittle stalk and broad leaf were recorded in different doses. Among the mutagen doses 600 Gy dose induced maximum increase in mean values and phenotypic and genotypic coefficients of variation for the plant height (cm), number of leaves per plant, leaf length (cm), number of tillers per plant, number of panicles per plant, panicle length, days to 50% flowering, and 1000 seeds weight. Except for panicle number/plant and 1000 seed weight, all traits showed high heritability in all doses. The results revealed a progressive decrease in mean values of quantitative traits with the increase in doses. The present study provides an idea about the optimum dose of gamma rays from a pool of doses that could be employed in future breeding programmes.

Keywords

genetic advance, genetic variability, heritability, mutations, yield improvements

Introduction

Eleusine coracana (L.) Gaertn. commonly known as finger millet is an important crop with multiple uses in the form of human food, feedstock and industrial food items. Ever-growing population food security, could be promised by this crop in varied stressful uncertain environment. In south Asia and Africa finger millet is a significant food crop. Finger millet grain when cooked or roasted spreads fine aroma and have numerous health promising qualities. It is known to have a rich source of calcium along with considerable amount of phosphorous, magnesium and iron. It is grown predominantly in temperate range of 11 to 27 °C and tropical regions all over the world. In this global changing climatic conditions, the crop requires continuous genetic enrichments and for this purpose, various breeding strategies have been designed and implemented. Among breeding approaches, radiation mutagenesis has proven an effective breeding strategy for crop improvement programmes aimed at increasing variability (1, 2). It is a coherent tool for the creation of genetic variability, an important prerequisite for any crop improvement programmes in a shorter span of time (3-6). Inducing mutations and identifying genetic diversity, which would boost productivity, could be used to improve economically important traits. Radiation mutagenesis have been successful in improving qualitative and quantitative characteristics such as high yield, early flowering, maturity, and stress resistance in several crops (7, 8). Among different radiations, gamma radiation are more preferred for improving yield and yield attributing traits in crops such as rice, maize, beans, cowpea and potato (9-12). In mutagenesis, mutations in chlorophyll are considered reliable indices for evaluating the mutagenic potency and genotypic sensitivity in a variety of legume crops, including cowpea (13), black gram (14) and green gram (15).

Estimates of genetic parameters such as phenotypic coefficient of variability (PCV) and genotypic of coefficient variability (GCV), heritability (h^2) and genetic advance determine the success of breeding strategy (16-18). The genetic variability of quantitative traits is measured using the genotypic coefficient of variation and heritability plays a role in determining variability. Heritability is also critical for selecting elite crops for future generations (18, 19). In general, substantial mutational events in plant breeding might result in large or tiny alterations that lead to the desired genotype. The present study was aimed at evaluating the frequency and spectrum of macro-mutation and mutagenic efficiency and effectiveness in finger millet.

Materials and Methods

Plant Material

Seeds of finger millet cultivar Paiyur-2 were procured from the Tamil Nadu Agriculture Research Centre, Paiyur, Krisnagiri District, Tamil Nadu, India.

Mutagenic treatment

Healthy and dry seeds of finger millet (Paiyur-2) were collected, sterilized and packed in paper bags weighing 25 g each of 10 sets were gamma-irradiated using Cobalt-60 (^{60}Co) source at the Indira Gandhi Centre for Atomic Research, Kalpakkam, Tamil Nadu, India. Seeds were treated with different doses viz., 100 Gy, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 Gy, with unirradiated seeds serving as a control.

Experimental study

The gamma irradiated finger millet seeds and control were sown in a cultivated field plots. Seedlings were raised and transplanted to the field after 21 days, in a randomized block design (RBD) with three replications in a distance of 10 cm in 4m long rows spaced around 30 cm apart to raise M_1 generation. The experiment was conducted at the Centre for Biodiversity Garden, Department of Botany, Periyar University, Periyar, Tamil Nadu, India during the months of June to October 2016. Plant survival was measured from the day of emergence until about 3 weeks later and expressed as a % of control. All the seeds from survived M_1 plants were harvested from both control and gamma irradiated plants. During 2016-2017, seeds from M_1 plants and control were collected and sown to raise M_2 populations in a 3-replication of randomized block design (RBD). All recommended agricultural practices, such as irrigation, weeding and crop protection were followed during the entire growth period of the crop.

From emergence to 3 weeks, chlorophyll and morphological mutations were recorded. Chlorophyll mutations were carefully classified from the seedling stage onwards (20, 21). Three replications of the spectrum and frequency of mutations were calculated per 100 M_2 plant progenies. In M_2 generation, mean values for plant height (cm), number of leaves per plant, leaf length (cm), number of panicles per plant, days to 50% flowering and 1000 seeds weight were recorded (Table 1).

Table 1. Description of chlorophyll and morphological mutants (10 days on onwards) of finger millet (*E. coracana* (L.) Gaertn.) in M_2 generation

Chlorophyll Mutants	**Description**
Albina	Seedling had whitish leaf, and survived for 15 days
Albina-green	Seedlings had whitish leaf tips
Xantha	Seedlings leaves were pale-yellow coloured
Viridis	Seedlings leaves were yellow with green patches
Maculata	Seedlings leaves were green with white spots
Chlorina	Seedlings leaves were pale green coloured
Yellow-viridis	Seedlings leaves were partly green and yellow
Aurea	Seedlings leaves were yellow
Morphological Mutants	**Description**
Tall	Taller than control
Dwarf	Shorter than control
Broadened leaf	Increased leaf width
Slow growing	Grow earth wardly rather upright, flops down due to detection of the gravity pull in a "lazy" manner
More Tillers	Tillers were more in number compared to control
Bushy	Crown like leaves close to ground level
Brittle stalk	Delicate stem compared to control
Panicle with outgrowth	Extra growth of fingers
Curved Panicle	Fingers were curved
More Fingers	Plants possess shorter numerous fingers in addition to normal fingers
Sterile panicle	Plants without seed formation
Sterile plant	Plants without fertile panicles

Chlorophyll fluorescence Spectroscopy

Three replicates of fresh young leaves of finger millet were collected from control, *chlorina, albina* and *xantha* mutants and subjected to fluorescence spectroscopy. Leaves weighing 0.5 g, were homogenized and centrifuged at 2500 rpm for 10 min and supernatant was collected and used for fluorescence spectroscopic examination using a Jasco spectro fluorometer, FP-8200 with a wavelength range of 200 to 750 nm, high sensitivity S/N > 1600 (RMS), dynamic range up to 6 digits and high-speed scanning up to 20000 nm/ min (900 nm optional).

Data collection

At the harvest stage, morphological and quantitative characters such as plant height (cm), leaf length (cm), number of leaves per plant, number of tillers per plant, days to 50% flowering, panicle length (cm), number of panicles per plant and 1000 seed weight were recorded.

Statistical analysis

The analysis of variance for the phenotypic characters were investigated using SPSS software. The following equations were used to calculate genotypic and phenotypic coefficients of variation, heritability (h^2), genetic advance (GA) and genetic advance as a % of mean (GAM) and Pearson's correlation was performed in SPSS ver. 21.0 that allowed us to visualize the significance of data.

Co-efficient of variation

Finger millet variability of the Phenotypic and Genotypic co-efficient were computed by the method given by (22).

$$PCV = \frac{(\text{Phenotypic variance})^{1/2}}{\text{General mean}} x100$$

$$GCV = \frac{(\text{Genotypic variance})^{1/2}}{\text{General mean}} x100$$

The range of variation was categorized by the standard method (23) and were classified as (i) more than 20 % - high, (ii) 10-20 % - moderate and (iii) less than 10 % - low.

Heritability

For each character heritability was computed by using the standard formula (24) and were categorized according to (25) as (i) More than 30 % - High, (ii) 10-30 % - Moderate and (iii) Less than 10 % - Low.

$$h^2 = \frac{GV}{PV} x100$$

Where,GV- Genotypic variance, PV- Phenotypic variance

Genetic advance

Genetic Advance =h^2 x бph x K

Genetic advance (GA) for a particular trait was estimated by adopting the standard method (26).

Where, h^2 = Heritability, бph^2 =phenotypic standard deviation, K= Selection differential 2.06 at 5 % level.

Genetic advance as % of mean (GAM)

$$GAM = \frac{GA}{GM} x100$$

Where, GA- Genetic Advance, GM- Genetic Mean,

The Genetic advance as % of mean was categorized as (i) More than 20 % - High, (ii) 10-20 % - Moderate and (iii) Less than 10 % - Low.

Results

Quantitative traits

The mean values of the morphological characters studied in different doses of the M_2 generation of finger millet are furnished in Table 2. The increased plant height (cm), number of leaves per plant, leaf length (cm), number of panicles per plant, days to 50% flowering and 1000 seed weight (g) were recorded in the 400 Gy, 500 Gy and 600 Gy doses compared to the control and other doses. The results revealed a maximum value of quantitative traits in 600 Gy dose. At lower and higher doses, negative shift in mean values were also recorded. In the present study, the maximum plant height (121.86 cm) was recorded in 600 Gy gamma ray treatment. The correlation studies for the above-mentioned morphological characters consider statistically significant. *p< 0. 01* (Table 5).

In this study, the maximum PCV (20.26), GCV (15.25), h^2 (99.83%) and GAM (3.23%) was recorded in 600 Gy gamma ray treatment. However, highest GA was recorded in 500 Gy gamma ray treatment of finger millet. The maximum number of leaves per plant (82.06) was recorded in 600 Gy gamma ray treatment. The maximum PCV (43.48) and GAM (48.25%) was recorded in 600 Gy gamma rays' treatment while highest GCV (39.83) and GA (15.92) was recorded in 500 Gy gamma rays' treatment. The maximum h^2 (99.97%) was recorded in 400 Gy gamma ray treatment. The maximum leaf length (65.96) was recorded in 600 Gy gamma ray treatment. The maximum PCV (28.17), GCV (24.96), h^2 (94.85%) and GAM (19.55%) was recorded in 600 Gy gamma ray treatment. The maximum GA (15.17%) was recorded in 400 Gy gamma ray treatment. The maximum number of tillers (11.90) was recorded in 600 Gy gamma ray treatment. The maximum PCV (99.28), GCV (90.59), GA (37.99%) and GAM (14.52%) was recorded in 600 Gy gamma ray treatment. The maximum h^2 (95.45%) was recorded in 700 Gy gamma ray treatment. The maximum days to 50% flowering (78.63) was recorded in 700 Gy gamma ray treatment. The maximum PCV (15.01), GCV (14.84), h^2 (93.44%) was recorded in 600 Gy gamma ray treatment. The maximum GA (17.99%) and GAM (19.66%) was recorded in 500 Gy gamma ray treatment. The maximum panicle length (7.37) was recorded in 600 Gy gamma ray treatment. The maximum PCV (17.46), GCV (15.11), GA (12.42%) and GAM (9.12%) was recorded in 600 Gy gamma ray treatment. The maximum h^2 (99.02%) was recorded in 300 Gy gamma ray treatment. The maximum panicle number (8.4) was recorded in 600 Gy gamma ray treatment. The maximum PCV (88.34), GCV (85.9) was recorded in 600 Gy gamma ray treatment. The maximum h^2(59.5%) and GA (11.38%) was recorded in 300 Gy gamma ray treatment while as maximum GAM (16.55%) was recorded in 500 Gy gamma ray treatment. The maximum 1000 seed weight (3.08) was recorded in 600 Gy gamma ray treatment. The maximum PCV (13.78), GA (11.13%) and GAM (17.73%) was

Table 2. Effects of different doses of gamma rays on morphological traits of finger millet (*E. coracana* (L.) Gaertn.) in M_2 generation. The data is presented as mean ± SE (standard error)

S.No	Gamma rays	Plant height	No. of leaves per plant	Leaf length	No. of tillers/ Plant	Days to 50% flowering	Panicle length per plant	Panicle number per plant	1000 seeds weight
	Control	90.11±0.73	12.05±0.64	15.93±0.99	3.5±0.26	80.01±1.34	7.11±0.49	1.96±0.24	2.26±0.04
2	100 Gy	92.63±0.68	14.06±0.59	28.46±0.78	4.33±0.60	80.03±0.50	7.12±0.45	2.16±0.33	2.37±0.08
3	200 Gy	94.16±0.39	17.1±0.94	36.03±0.59	6.03±0.34	80.33±1.44	7.14±0.34	2.53±0.32	2.40±0.09
4	300 Gy	95.96±0.42	25.03±1.26	40.23±0.89	6.53±0.43	80.39±1.43	7.19±1.00	2.69±0.38	2.45±0.23
5	400 Gy	98.03±0.65	35.33±0.82	45.96±1.20	7.83±0.34	80.43±0.94	7.21±0.21	3.76±0.24	2.64±0.09
6	500 Gy	101.1±0.66	51.63±1.17	58.4±1.48	8.76±0.45	82.7±0.99	7.25±0.99	5.1±0.74	2.92±0.14
7	600 Gy	121.86±4.52	82.06±6.72	65.96±2.90	11.9±0.67	81.63±2.04	7.37±0.67	8.4±0.36	3.08±0.05
8	700 Gy	112.53±1.94	57.03±2.76	38.03±1.55	6.03±0.80	78.63±1.34	7.15±0.71	1.93±0.26	2.58±0.07
9	800 Gy	89.13±1.68	21.5±2.35	28.93±1.44	4.1±0.67	82.3±0.67	7.21±0.67	1.41±0.28	2.36±0.08
10	900 Gy	53.91±3.58	11.86±0.68	19.06±1.06	2.5±0.44	85.13±0.47	6.37±0.71	0.76±0.34	2.35±0.04
11	1000 Gy	37.63±1.85	5.9±0.94	12.28±1.03	1.26±0.40	88.53±0.73	5.81±0.81	0.75±0.17	1.82±0.08

recorded in 600 Gy gamma ray treatment. The maximum GCV (10.17) was recorded in 500 Gy gamma ray treatment while as highest h^2 (95.64%) was recorded in 300 Gy gamma ray treatment (Table 3). Using these quantitative features, h2 analysis outperformed for GCV, PCV, GA and GAM.

Chlorophyll mutants

In determining micro and macro-mutants of finger millet, the selection of mutagen dose / concentration is crucial. The extent of mutagenic effects may be assessed by evaluating the frequency of chlorophyll mutants. In the present study,

Table 3. Effect of gamma rays on phenotypic (PCV), genotypic (GCV) coefficient of variation, heritability (h^2) and genetic advance (%) and genetic advance percent of mean in M_2 generation of finger millet (*E. coracana* (L.) Gaertn.)

S,No	Doses	Genetic Parameters	Plant height (cm)	No of leaves / plant	leaf length (cm)	No of tillers / plant	Days to 50% flowering	Panicle length /plant (cm)	Panicle number / plant	1000 seeds weight
		PCV	10.80	15.27	10.21	10.68	3.76	5.29	28.35	6.28
		GCV	8.87	12.03	7.25	9.11	4.32	4.38	25.55	2.82
1	100 Gy	h^2	7.09	8.67	7.26	3.53	8.72	5.69	2.48	9.90
		GA	12.99	13.57	14.22	13.89	15.71	10.18	10.58	10.24
		GAM (%)	3.23	25.38	14.85	9.94	6.88	2.58	6.99	1.10
		PCV	11.08	28.67	16.11	31.09	7.95	8.08	35.50	7.11
		GCV	8.87	25.59	13.09	28.29	5.81	7.77	32.37	5.24
2	200 Gy	h^2	5.36	5.83	6.23	3.65	5.06	5.43	3.22	2.22
		GA	1.37	4.43	3.14	2.19	9.37	1.51	1.67	1.12
		GAM (%)	1.45	5.95	11.72	3.34	11.67	7.19	6.49	5.08
		PCV	12.17	33.17	18.30	52.17	9.29	9.17	41.17	8.17
		GCV	10.99	30.64	15.30	49.04	7.68	8.17	39.90	5.15
3	300 Gy	h^2	71.73	30.01	5.46	79.23	26.62	99.02	59.5	95.64
		GA	11.66	10.69	10.14	11.84	10.68	12.30	11.38	8.22
		GAM (%)	1.73	1.72	1.14	1.92	1.70	2.39	1.44	2.31
		PCV	15.32	41.41	20.63	74.25	10.47	11.06	55.32	9.87
		GCV	13.17	39.1	18.63	72.65	9.27	13.76	51.11	7.23
4	400 Gy	h^2	78.66	99.97	85.96	78.77	91.26	85.56	12.60	18.3
		GA	12.10	12.37	15.78	11.81	16.76	10.51	10.03	10.09
		GAM (%)	12.14	10.04	12.57	10.13	18.40	17.16	13.03	13.73
		PCV	18.16	42.11	25.44	88.64	13.02	14.91	78.93	12.15
		GCV	15.08	39.83	22.02	85.63	12.85	12.66	75.33	10.17
5	500 Gy	h^2	92.57	91.04	85.21	88.93	93.44	90.20	42.43	36.96
		GA	40.19	15.92	15.58	12.83	17.99	10.66	10.82	10.22
		GAM (%)	14.13	11.46	19.55	32.32	19.66	19.12	16.55	17.73

		PCV	20.26	43.48	28.17	99.28	15.01	17.46	88.34	13.78
		GCV	15.25	38.45	24.96	90.59	14.84	15.11	85.90	9.35
6	600 Gy	h^2	99.83	99.75	94.85	95.03	93.26	80.98	52.02	24.59
		GA	13.09	13.59	12.53	14.52	7.87	12.42	11.11	11.13
		GAM (%)	23.17	48.25	15.97	37.99	19.64	15.77	13.23	14.45
		PCV	12.82	34.09	21.82	81.00	12.17	15.35	78.25	11.57
		GCV	10.79	30.00	19.13	79.08	11.99	14.06	75.11	10.11
7	700 Gy	h^2	99.16	98.62	83.12	95.45	93.18	87.20	15.67	37.80
		GA	3.38	6.33	5.09	4.74	7.81	1.55	1.08	1.23
		GAM (%)	11.89	2.64	3.40	7.67	9.94	7.81	4.47	9.01
		PCV	11.29	31.37	19.43	68.23	10.95	13.55	45.11	8.00
		GCV	9.29	30.27	15.43	66.23	9.85	12.52	42.11	5.38
8	800 Gy	h^2	8.41	8.07	3.02	4.12	3.94	3.33	2.40	3.90
		GA	1.65	1.76	1.87	1.11	1.58	1.46	1.51	1.07
		GAM (%)	1.82	4.00	2.67	1.40	5.57	6.69	3.99	3.07
		PCV	10.00	16.81	18.11	58.51	9.70	12.48	37.08	7.35
		GCV	9.97	14.81	15.93	55.84	8.39	11.20	30.19	2.85
9	900 Gy	h^2	9.69	7.59	7.46	3.46	7.62	8.83	3.94	3.98
		GA	2.15	3.18	1.22	1.87	1.72	1.64	1.66	1.28
		GAM (%)	1.08	2.87	2.63	1.50	1.37	1.15	6.70	1.10
		PCV	9.41	10.91	15.41	45.21	8.76	10.60	25.04	5.15
		GCV	7.32	8.89	12.08	41.21	7.48	9.35	22.52	2.91
10	1000 Gy	h^2	9.77	2.71	2.92	3.26	2.09	3.61	3.81	2.28
		GA	9.77	1.69	1.92	2.25	8.02	2.51	2.51	2.21
		GAM (%)	2.24	1.69	1.67	1.73	1.59	1.14	1.02	1.96

chlorophyll mutants identified in the seedling stage of this investigation included *albina-green*, *albina*, *xantha*, *viridis* and *chlorina* (Table 4) (Fig. 1). Among the mutants *albina* isolated in 300 Gy, 400 Gy, 500 Gy and 700 Gy treated plants were small and survived for 20 days only after germination. *Xantha* mutants were isolated in 300 Gy, 400 Gy, 600 Gy and 700 Gy treated plants showed delayed development and survived for 30-35 days. Early on the dark green *viridis* seedlings isolated in 700 Gy and 800 Gy treated plants turned to a typical green colour in the following

Table 4. Frequency of chlorophyll and morphological mutants in M_2 generation of finger millet (*E. coracana* (L.) Gaertn.

S.No	Doses	100Gy	200Gy	300Gy	400Gy	500Gy	600Gy	700Gy	800Gy	900Gy	1000Gy
	No of plants studied	389	391	378	381	357	374	378	391	371	378
1	*Albina*	-	-	3	2	4	5	2	1	-	-
2	*Xantha*	2	1	4	4	5	5	1	-	-	-
3	*Viridis*	-	-	2	5	6	4	2	1	1	-
4	*Chlorina*	-	-	1	4	3	2	1	1	-	-
5	Tall	-	-	-	2	3	7	2	-	-	-
6	Dwarf	-	1	1	1	2	2	2	1	1	-
7	Brittle stalk	-	1	1	2	1	1	2	3	4	1
8	Bushy	-	-	-	2	3	5	2	1	-	-
9	Slow growing	-	-	-	1	1	-	1	2	1	-
10	Tillers more	-	1	1	1	2	4	2	1	1	-
11	Fingers more in number	-	-	1	2	3	3	3	1	1	
12	Early flowering	-	2	2	3	4	3	2	-	-	-
13	Late flowering	-	-	-	1	2	2	3	3	1	-
14	Broadened leaf	-	-	-	-	1	2	1	1	-	-
	Mutation frequency	0.514	1.534	4.232	7.874	11.204	12.032	6.878	4.092	2.645	0.264

Fig. 1. Chlorophyll mutants of finger millet (*E. coracana* (L.) Gaertn.).. (*4x magnification). (**A**) Albino-green (**B**) Xantha (**C**) Viridis (**D**) Maculata (**E**) Chlorina (**F**) Yellow-viridis (**G**) Aurea (H) Albino.*

days. A *chlorina* mutant with a bright green colour was isolated in 200 Gy, 300 Gy and 400 Gy treated plants. Among the various frequencies (500 Gy, 600 Gy) of chlorophyll mutants, viridis seedling stage achieved green coloration early, followed by *chlorina, xantha* and albina.

Morphological mutants

The morphological mutants of finger millet such as bushy were isolated in 400 Gy dose. Tall mutants and mutants with more fingers were isolated in 600 Gy dose, whereas early flowering, late flowering, brittle stalk mutants were isolated in 500 Gy dose. Few mutants were isolated in higher doses for instance, slow growing and dwarf mutants in 900 Gy and broad stem in 800 Gy dose (Table 4) (Figs. 2, 3). The frequencies of such mutants were also evaluated and maximum value was recorded between 500 and 600 Gy doses. Among the various levels of therapy, mutants treated with 300 Gy, 400 Gy, 500 Gy and 600 Gy had the best morphological traits compared to other doses of treatment.

Fluorescence spectrometric studies

Young leaves from the *chlorina, xantha* and *albina* chlorophyll mutants of finger millet were subjected to fluorescence spectrometric analysis. The difference in absorption spectra between the chlorophyll mutants (*chlorina, xantha, albina* and control) (Fig. 4) may be revealed by the emission/excitation wavelength. The excitation spectra of control at 679 nm showed a fluorescence peak value of 6276.19. When comparing control with the mutants, the

Table 5. Pearson's correlation coefficients for morphological and yield-related traits of M_2 generation finger millet (*E. coracana* (L.) Gaertn.)

Correlations									
		Plant height	Number of leaves per plant	Leaf length	Tillers per plant	Days to 50% flowering	Panicle length	Number of panicles per plant	1000 Seed weight
Plant height	Pearson Correlation	1	.605**	.766**	.715**	-.038	.768**	.663**	.631**
	Sig. (2-tailed)		.000	.000	.000	.486	.000	.000	.000
	N		330	330	330	330	330	329	330
Number of leaves per plant	Pearson Correlation		1	.676**	.605**	-.059	.404**	.611**	.494**
	Sig. (2-tailed)			.000	.000	.286	.000	.000	.000
	N			330	330	330	330	329	330
Leaf length	Pearson Correlation			1	.828**	-.067	.586**	.821**	.679**
	Sig. (2-tailed)				.000	.225	.000	.000	.000
	N				330	330	330	329	330
Tillers per plant	Pearson Correlation				1	-.058	.530**	.751**	.570**
	Sig. (2-tailed)					.291	.000	.000	.000
	N					330	330	329	330
Days to 50% flowering	Pearson Correlation					1	-.027	-.018	.008
	Sig. (2-tailed)						.619	.749	.891
	N						330	329	330

Panicle length	Pearson Correlation	1	.497**	.494**
	Sig. (2-tailed)		.000	.000
	N		329	330
Number of panicles per plant	Pearson Correlation		1	.578**
	Sig. (2-tailed)			.000
	N			329
1000 Seed weight	Pearson Correlation			1
	Sig. (2-tailed)			
	N			

**. Correlation is significant at the 0.01 level (2-tailed).

Fig. 2. Morphological mutants of finger millet (*E. coracana* (L.) Gaertn.). showing variations in morphological traits. control (**A**) Plant height mutants (Tall, Dwarf, Control) (**B**) Early flowering (**C**) Bushy (**D**) Broad leaf (**E**) Slow growing (**F**) Sterile (*4x magnification).*

Fig. 3. Morphological mutants of finger millet (*E. coracana* (L.) Gaertn.). showing variations in yield associated traits. (**A**) More Tillers (**B**) More fingers (**C**) Curved Panicle (**D**) Panicle with outgrowth (**E**) Sterile Panicle (**F**) Brittle stalk (*4x magnification).*

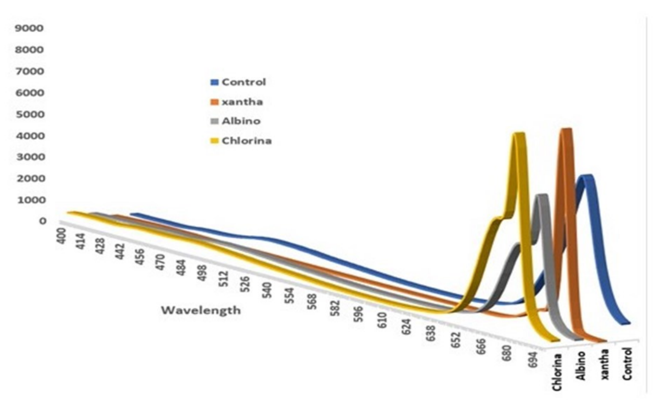

Fig. 4. Finger millet (*E. coracana* (L.) Gaertn.). - Fluorescence spectrum in fresh young leaves of chlorophyll mutants in M_2 generation.

lowest peak value detected in *albina* was 5658.94 at 680 nm. The excitation spectra of the *chlorina* mutant peak were 8204.4 at 680 nm. The spectra excitation of the *xantha* mutant was 8254.04 at 680 nm. When compared to control, the *albina* mutant had low fluorescence excitation spectra since it was devoid of chlorophyll pigment, whereas *chlorina* and *xantha* revealed maximum excitation spectra.

Discussion

Induced mutagenesis is an effective method for improving agronomic traits and genetic variability of important crops such as cowpea (27, 28) lentil (29, 30) faba bean (31, 32), chickpea (33, 34) urd bean (35, 36), mung bean (37), rice (38, 39) black cumin (40, 41), fenugreek (42), and finger millet (43). In the present study on finger millet (Paiyur-2), all the studied morphological and quantitative traits increased in mutagenized population treated with 400 Gy to 600 Gy doses. These findings were in agreement with the results of previous studies in crops such as black gram (44), maize (45), chick pea (46) cowpea (47, 48). In the present study, 600 Gy gamma irradiated finger millet plants revealed substantial increase in physio-morphological and yield related traits. Therefore, 600 Gy gamma ray dose could be used in crop improvement programs aimed at increasing agronomic traits and genetic variability. Moreover, we also evaluated the correlation of different yield and yield attributing traits as determining the correlation is critical for the selection of desired phenotypes in mutation breeding programmes. This was supported by earlier studies (33) that also reported a strong and positive correlation among various quantitative traits in the chickpea mutagenized population. In this investigation of finger millet more fertile tillers and panicle / plant demonstrated a significant relationship with yield, leading to direct selection of traits for desired characters. Also, manifold increase in genetic parameters such as genetic coefficient of variability, heritability and genetic advance were recorded in gamma irradiated population.

Chlorophyll mutants

In the present study of finger millet different chlorophyll mutants, viz., *chlorina, albina, xantha* and *viridis* were recorded and categorized as per the classification by (49). Chlorophyll mutants developed using mutagenesis have been reported in several crops such as soybean (50) and cowpea (51). Due to its better precision in scoring, the frequency of chlorophyll mutants is considered as a reliable index to evaluate the mutagenic potency and genetic effects of mutagens. This study revealed both viable and lethal chlorophyll mutations in the M_2 generation. *Albina* mutants were slow growing and died due to a lack of chlorophyll, however other mutants, viz. *chlorina, xantha* survived few days longer.

Morphological mutants

The variations of Paiyur – 2 finger millet in several morphological traits such as plant habit, leaf, spike attributes and days to maturity were recorded in the present study. These morphological mutants were named on the basis of recording a continuous variation in a specific trait and represent a valuable genetic resource for future breeding programmes. In the present study, morphological mutants such as dwarf, tall, early maturity, brittle stalk, bushy, broad stem and maculata were recorded in different dosages of gamma radiation. In general, morphological mutants serve as stock of genetic resources that could be exploited in future breeding programmes. The morphological mutations may be attributed to gamma rays induced chromosome abnormalities, small deletions or duplications and most likely gene mutations (52). Several researchers have reported the morphological mutations in several crops such as cowpea (53), chickpea (54), faba bean (55) and urdbean (56, 57).

Chlorophyll fluorescence

The chlorophyll fluorescence (CF) provides a vivid image of photosystem function and enhances our understanding of the photosynthesis mechanism in finger millet (43). In addition, in this study chlorophyll fluorescence could be used to evaluate gamma radiations induced stress damage in chlorophyll mutants. A fraction of the light energy detected by chlorophyll pigments in the chloroplast would be released as far-red light (fluorescence) and red light. This method had been widely used to study photosynthetic processes in vivo for many years (58-62). Fluorescence studies have been proven to be a sensitive way to compare the effects of various stresses on different genotypes and has emerged as a powerful analytical tool to investigate stress-related damage processes (63-66). When compared to control (6276.19 at 679 nm), *chlorina* (8204.4 at 680 nm) and *xantha* (8254.04 at 680 nm) showed maximal excitation spectra, indicating stress damage. The *albina* (5658.94 at 680 nm) has the most stress effects due to its absence of chlorophyll pigment.

Effect of gamma irradiation on genetic variability

The genotypic coefficient of variation, genetic advance and heritability were recorded in quantitative traits of finger millet such as number of tillers / plants, panicle number / plant, and 1000 seeds weight in the M_2 generation. The results showed a significant increase in the genotypic coefficient of variation, genetic advance and heritability for all the studied quantitative traits in the M_2 generation. Heritability and genetic advance are essential aspects to consider when deciding whether or not to continue with the selection process (67). The present study on finger millet revealed high heritability and genetic advance in all of the above-mentioned quantitative traits at 600 Gy of gamma rays in M_2 generation. Several researchers also reported increase in genotypic coefficient of variation, genetic advance and heritability for quantitative traits in crops such as lentil (68), urdbean (69, 70), Sorghum (71) and faba bean (72-74). The present research demonstrates a wide range of variability for a variety of factors, as well as substantial genetic advancement and heritability for crucial yield qualities, which will be useful for effective selection. In the next generation, this genetic variability stability should be recognized and examined, and true breeding economic features can be applied to improve finger millet.

Conclusion

One of the varieties of finger millet (*E. coracana* (L.) Gaertn.). Paiyur 2 was selected for present study which is cultivated predominantly in the north western zone of Tamil Nadu. Induced mutagenesis is the most efficient way to increase genetic variation on variety level in a short period of time. The varietal differences of the Paiyur -2 finger millet somewhat better than other varieties of finger millet, however our objective is to improve the yield and calcium improvement in Paiyur -2 variety. In the M_2 generation, a wide range of chlorophyll, and morphological mutants were detected that represent valuable genetic resource and could serve as source of elite genes. Optimal gamma radiation dosages (500 Gy, 600 Gy and 700 Gy) induced a substantial genetic variability in the treated population compared to other gamma radiation doses. The high genetic parameters like PCV, GCV, heritability, genetic advance, genetic advance as % of mean (GAM) was recorded in 600 Gy in the traits of number of tillers per plant and panicle number per plant. It also confirmed the gamma radiation proved the agronomic traits increased in 600 Gy, it will help for finger millet crop improvement for the beneficial trait. Higher mutation frequency was observed in 500 and 600 Gy doses**.** However, treatment with 600 Gy of gamma rays, resulted in mutants with the highest mean values of morphological and quantitative traits. Such mutants could be utilized to further improve agronomical traits of finger millet (*E. coracana* (L.) Gaertn.).

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Authors contributions

LS and AD contributed for Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing- original draft, Writing-review and editing. AG Analyzed and methodology construction. AR reviewed this manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None.

References

1. Raina A, Laskar RA, Khursheed S, Amin R, Tantray YR, Parveen K, Khan S. Role of mutation breeding in crop improvement-past, present and future. Asian Research Journal of Agriculture. 2016;2(2):1-3. https://doi.org/10.9734/ARJA/2016/29334
2. Raina A, Sahu D, Parmeshwar K, Laskar RA, Rajora N, Soa R, Khan S, Ganai RA. Mechanisms of genome maintenance in plants: playing it safe with breaks and bumps. Frontiers in Genetics. 2021;12:861. https://doi.org/10.3389/fgene.2021.675686
3. Raina AA, Khursheed S, Khan S. Optimisation of mutagen doses for gamma rays and sodium azide in cowpea genotypes. Trends Biosci. 2018;11(13):2386-89.
4. Arulbalachandran D, Mullainathan L, Velu S. Screening of mutants in black gram (*Vigna mungo* (L.) Hepper) with effect of DES and COH in M2 generation. J Phytol. 2009; 1(4):213-18.
5. Devi AS, Mullainathan L. Effect of gamma rays and ethyl methane sulphonate (EMS) in M3 generation of black gram (*Vigna mungo* (L.) Hepper). Afr J Biotechnol. 2012; 11(15):3548-52. https://doi.org/10.5897/AJB10.1773
6. Tshilenge -Lukanda L, Kalonji-Mbuyi A, Nkongolo KK, Kizungu RV. Effect of gamma irradiation on morpho-agronomic characteristics of groundnut (*Arachis hypogaea* L.). Am J Plant Sci. 2013; Nov 13; 4 (11): 2186. https://doi.org/10.4236/ajps.2013.411271
7. Rajput MA, Sarwar G, Siddiqui KA. Development of high yielding mutants in lentil. 2001.
8. Sadiq MS, Haidar S, Haq MA, Abbas G. A high yielding and disease resistant mutant of lentil developed through seed irradiation of an exotic germplasm. Can J Appl Sci. 2008; May: 411.
9. Jayawardena SD, Peiris R. Food crops breeding in Sri Lanka-achievements and challenges. BIO News. 1988; 4(2):22-34.
10. Donini P, Sonnino A. Induced mutation in plant breeding: current status and future outlook. In: Somaclonal Variation and Induced Mutations in Crop Improvement Dordrecht: Springer. 1998; 255-91. https://doi.org/10.1007/978-94-015-9125-6_14
11. Schum A. Mutation breeding in ornamentals: an efficient breeding method? In: XXI International Eucarpia Symposium on Classical versus Molecular Breeding of Ornamentals-Part I. 612 2003; Aug 25 pp. 47-60. https://doi.org/10.17660/ActaHortic;2003.612.6
12. Yoon KE, Im BG, Park YH. Effect of gamma radiation on seed germination and androgenesis in *Nicotiana tabacum* L. Korean Journal of Breeding (Korea R.). 1990.
13. Kumar VA, Vairam N, Amutha R. Effect of physical mutagen on expression of characters in arid legume pulse cowpea (*Vigna unguiculata* (L.) Walp.). Electron J Plant Breed. 2010; 1(4):908-14.
14. Selvam YA, Elangaimannan R, Venkatesan M, Karthikeyan P, Palaniraja K. Chemically induced mutagenesis in blackgram (*Vigna mungo* (L.) Hepper). Electron J Plant Breed. 2010; Jul; 1 (4):921-24.
15. Sangsiri C, Sorajjapinun W, Srinives P. Gamma radiation induced mutations in mung bean. Sci Asia. 2005;31:251-55. https://doi.org/10.2306/scienceasia1513-1874.2005.31.251
16. Wani AA. Induced polygenic variability for quantitative traits in chickpea var. Pusa-372. Comun Sci. 2011;2(2):100-16.

17. Wani MR, Kozgar MI, Tomlekova N, Khan S, Kazi AG, Sheikh SA, Ahmad P. Mutation breeding: A novel technique for genetic improvement of pulse crops particularly chickpea (*Cicer arietinum* L.). Improvement of crops in the era of climatic changes. 2014; 217-48. https://doi.org/10.1007/978-1-4614-8824-8-9.

18. Kozgar MI, Kozgar IM. Mutation Breeding in Chickpea. De Gruyter Open Poland. 2014; Sep 1.

19. Tabasum A, Saleem M, Aziz I. Genetic variability, trait association and path analysis of yield and yield components in mungbean (*Vigna radiata* (L.) Wilczek). Pak J Bot. 2010; Dec 1; 42 (6):3915-24.

20. Gustafsson Å. The mutation system of the chlorophyll apparatus. Kungliga Fysiografiska Sallskapets i Lund Handlingar. 1940;51(11).

21. Blixt S. Quantitative studies of induced mutations in peas. V. Chlorophyll mutations. Agri Hort Genet. 1961; Jan 1;19.

22. Burton GW. Qualitative inheritance in grasses. Vol. 1. In: Proceedings of the 6th International Grassland Congress, Pennsylvania State College. 1952; Aug pp. 17-23.

23. Sivasubramanian J, Madhavamenon P 1973. Genotypic and phenotypic variability in rice. Madras Agric J. 12: 15-16.

24. Lush JL. Intra-sire correlations or regressions of offspring on dam as a method of estimating heritability of characteristics. Journal of Animal Science. 1940 Dec 1; 1940(1):293-301.

25. Robinson HF. Quantitative genetics in relation to breeding on centennial of Mendelism. In Indian Journal of Genetics and Plant Breeding. 1966; Jan 1 p. 171. Indian Agriculture Res Inst, New Delhi-110 012, India: Indian Soc Genet Plant Breed.

26. Johnson HW, Robinson HF, Comstock RE. Estimates of genetic and environmental variability in soybeans. Agronomy Journal. 1955;47(7):314-https://doi.org/10.2134/agronj1955.00021962004700070009x

27. Raina A, Laskar RA, Tantray YR, Khursheed S, Samiullah Khan. Characterization of induced high yielding cowpea mutant lines using physiological, biochemical and molecular markers. Sci Rep. 2020; 10:3687. https://doi.org/10.1038/s41598-020-60601-6

28. Raina A, Laskar RA, Wani MR, Jan BL, Ali S, Khan S Comparative mutagenic effectiveness and efficiency of gamma rays and sodium azide in inducing chlorophyll and morphological mutants of cowpea. Plants. 2022;11:1322. https://doi.org/10.3390/plants11101322

29. Laskar RA, Khan S, Deb CR, Tomlekova N, Wani MR, Raina A, Amin R. Lentil (*Lens culinaris* Medik.) diversity, cytogenetics and breeding. In: Advances in plant breeding strategies: Legumes. Springer, Cham. 2019; pp. 319-69. https://doi.org/10.1007/978-3-030-23400-3_9

30. Wani MR, Laskar RA, Raina A, Khan S, Khan TU. Application of chemical mutagenesis for improvement of productivity traits in lentil (*Lens culinaris* Medik). Annals of Biology. 2021; 37(1): 69-75.

31. Khursheed S, Laskar RA, Raina A, Amin R, Khan S. Comparative analysis of cytological abnormalities induced in *Vicia faba* L. genotypes using physical and chemical mutagenesis. Chromosome Sci. 2015;18(3):47-51.

32. Khursheed S, Raina A, Khan S. Improvement of yield and mineral content in two cultivars of *Vicia faba* L. through physical and chemical mutagenesis and their character association analysis. Archives Current Res Int. 2016;4(1):1-7. https://doi.org/10.9734/ACRI/2016/24802

33. Laskar RA, Khan S, Khursheed S, Raina A, Amin R. Quantitative analysis of induced phenotypic diversity in chickpea using physical and chemical mutagenesis. J Agron. 2015;14:102. https://doi.org/10.3923/ja.2015.102.111

34. Raina A, Laskar RA, Khursheed S, Khan S, Parveen K, Amin R. Induce physical and chemical mutagenesis for improvement of yield attributing traits and their correlation analysis in chickpea. International Letters of Natural Sciences. 2017;61. https://doi.org/10.18052/www.scipress.com/ILNS.61.14

35. Goyal S, Wani MR, Laskar RA, Raina A, Khan S. Mutagenic effectiveness and efficiency of individual and combination treatments of gamma rays and ethyl methanesulfonate in black gram [*Vigna mungo* (L.) Hepper]. Advances in Zoology and Botany. 2020;8 (3): 163-68. https://doi.org/10.13189/azb.2020.080311

36. Goyal S, Wani MR, Laskar RA, Raina A, Khan S. Performance evaluation of induced mutant lines of black gram (*Vigna mungo* (L.) Hepper). Acta fytotechn zootechn. 2020; Jun 18;23(2):70-77. https://doi.org/10.15414/afz.2020.23.02.70-77

37. Wani MR, Dar AR, Tak A, Amin I, Shah NH, Rehman R, et al. Chemo-induced pod and seed mutants in mung bean (*Vigna radiata* (L.) Wilczek). SAARC J Agric. 2017;15:57-67. https://doi.org/10.3329/sja.v15i2.35161

38. Raina A, Khan S. Increasing rice grain yield under biotic stresses: mutagenesis, transgenics and genomics approaches. In: Rice Research for Quality Improvement: Genomics and Genetic Engineering. 2020; pp. 149-78. Springer, Singapore. DOI: 10.1007/978-981-15-5337-0_8. https://doi.org/10.1007/978-981-15-5337-0_8

39. Raina A, Parmeshwar K, Khan S. Increasing Rice Grain Yield Under Abiotic Stresses: Mutagenesis, Transgenics and Genomics Approaches. In: Aryadeep C (Editor) Rice Research for Quality Improvement: Genomics and Genetic Engineering. Springer. 2020; 753-77. https://doi.org/10.1007/978-981-15-4120-9_31

40. Tantray AY, Raina A, Khursheed S, Amin RU, Khan SA. Chemical mutagen affects pollination and locule formation in capsules of black cumin (*Nigella sativa* L.). Intl J Agric Sci. 2017;8(1):108-17.

41. Amin R, Wani MR, Raina A, Khursheed S, Khan S. Induced morphological and chromosomal diversity in the mutagenized population of black cumin (*Nigella sativa* L.) using single and combination treatments of gamma rays and ethyl methane sulfonate. Jordan Journal of Biological Sciences. 2019; Mar 1;12(1).

42. Hassan N, Laskar RA, Raina A, Khan S. Maleic hydrazide induced variability in fenugreek (*Trigonella foenum-graecum* L.) cultivars CO1 and Rmt-1. Res Rev J Bot Sci. 2018; 7(1):19-28.

43. Sellapillaibanumathi L, Dhanarajan A, Raina A, Ganesan A. Effects of gamma radiations on morphological and physiological traits of finger millet (*Eleusine coracana* (L.) Gaertn.). Plant Sci Today. 2022; Jan 1;9(1):89-95. https://doi.org/10.14719/pst.1142

44. Arulbalachandran D. Physical and chemical mutagenesis in black gram (*Vigna mungo* (L.) Hepper) Ph.D [thesis], Annamalai University; 2006.

45. Gnanamurthy S, Dhanavel D, Bharathi T. Effect of chemical mutagenesis on biochemical activity and variability, heritability and genetic advances in *Zea mays* (L.). International Journal of Current Science. 2013;(5):57-61.

46. Khan S. Genetic variability and correlations studies in chickpea mutants. J Cytol Genet. 2005; 6:155-60.

47. Dhanavel D, Gnanamurthy S, Girija M. Effect of gamma rays on induced chromosomal variation in cowpea *Vigna unguiculata* (L.) Walp. International Journal of Current Science. 2012;2012:245-50.

48. Vanmathi S, Arulbalachandran D, Soundarya V. Effects of gamma radiation on quantitative traits and genetic variation of three successive generations of cowpea (*Vigna unguiculata* (L.) Walp.). Plant Sci Today. 2021;8(3):578-88. https://doi.org/10.14719/pst.2021.8.3.1054

49. Saroj SK, Poudel PP, Singh MN. Induced genetic variability with EMS and studies on frequency and spectrum of chlorophyll

mutations in pigeon pea. Electron J Plant Breed. 2016; Sep 12;7 (2):209-14. https://doi.org/10.5958/0975-928X.2016.00029.6

50. Geetha K, Vaidyanathan P. Studies on induction of chlorophyll mutations in soyabean through physical and chemical mutagens. Agric Sci Digest. 2000; 20(1):33-35.

51. Raina A, Laskar RA, Wani MR, Jan BL, Ali S, Khan S. Gamma rays and sodium azide induced genetic variability in high yielding and biofortified mutant lines in cowpea [*Vigna unguiculata* (L.) Walp.]. Frontiers in Plant Sciences. 2022b; 13:911049. https://doi.org/10.3389/fpls.2022.911049

52. Usharani KS, Kumar CA. Induced viable mutants in urd bean (*Vigna mungo* (L.) Hepper). Bioscan. 2015; Aug 14; 10(3):1103-08.

53. Rasik S, Raina A, Laskar RA, Wani MR, Reshi ZA, Khan S, Ndhlala AR. Lower doses of Sodium azide and Methyl methane sulphonate improved yield and pigment contents in vegetable cowpea [*Vigna unguiculata* (L.) Walp.]. South African Journal of Botany. 148:727-36. https://doi.org/10.1016/j.sajb.2022.04.034.

54. Raina A, Khan S, Laskar RA, Wani MR, Mushtaq W. Chickpea (*Cicer arietinum* L.) cytogenetics, genetic diversity and breeding. In: Al-Khayri JM et al (Editors) Advances in Plant Breeding: Legumes. Springer, Cham. 2019; pp. 53-112. https://doi.org/10.1007/978-3-030-23400-3_3

55. Khursheed S, Raina A, Parveen K, Khan S. Induced phenotypic diversity in the mutagenized populations of faba bean using physical and chemical mutagenesis. J Saudi Society Agric Sci. 2019;18 (2):113-19. https://doi.org/10.1016/j.jssas.2017.03.001

56. Goyal S, Wani MR, Laskar RA, Raina A, Khan S. Assessment on cytotoxic and mutagenic potency of Gamma rays and EMS in *Vigna mungo* (L.) Hepper. Biotecnología Vegetal. 2019;19(3):193-204.

57. Goyal S, Wani MR, Laskar RA, Raina A, Amin R, Khan S. Induction of morphological mutations and mutant phenotyping in black gram [*Vigna mungo* (L.) Hepper] using gamma rays and EMS. Vegetos. 2019;32(4):464-72. https://doi.org/10.1007/s42535-019-00057-w

58. Kautsky H, Hirsch A. Chlorophyll fluoreszenz und kohlensäure assimilation. I.D as fluoreszenzverhalten grüner pflanzen. Biochem Z. 1934; 274:423-34.

59. Lavorel J, Etienne AL. *In vivo* chlorophyll fluorescence. In: Primary Processes in Photosynthesises. (Editor, J Barber). 1977; pp. 203-68.

60. Krause GH, Weis E. Chlorophyll fluorescence as a tool in plant physiology. Photosynth Res. 1984 Jun;5(2):139-57. https://doi.org/10.1007/BF00028527

61. Briantais JM, Dacosta J, Goulas Y, Ducruet JM, Moya I. Heat stress induces in leaves an increase of the minimum level of chlorophyll fluorescence, Fo: a time-resolved analysis. Photosynthesis Research. 1996;48(1):189-96. https://doi.org/10.1007/BF00041008

62. Renger GE, Schreiber UL. Practical applications of fluorometric methods to algae and higher plant research. Light emission by plants and bacteria. 1986;587-619. https://doi.org/10.1016/B978-0-12-294310-2.50025-1

63. Smillie RM, Nott R. Salt tolerance in crop plants monitored by chlorophyll fluorescence *in vivo*. Plant Physiol. 1982; Oct;70 (4):1049-54. https://doi.org/10.1104/pp.70.4.1049

64. Schreiber U, Bilger W. Rapid assessment of stress effects on plant leaves by chlorophyll fluorescence measurements. In: Plant Response to Stress; Springer, Berlin, Heidelberg. 1987; pp. 27-53. https://doi.org/ 10.1007/978-3-642-70868-8_2

65. Baker NR. Chlorophyll fluorescence quenching during photo inhibition. Photo Inhibition. 1988.

66. Strand M, Oquist G. Effects of frost hardening, dehardening and freezing stress on *in vivo* chlorophyll fluorescence of Scots pine seedlings (*Pinus sylvestris* L.). Plant Cell Environ. 1988;11:231-38.https://doi.org/10.1111/j.1365-3040.1988.tb01141.x

67. Ogunniyan DJ, Olakojo SA. Genetic variation, heritability, genetic advance and agronomic character association of yellow elite inbred lines of maize (*Zea mays* L.). Niger J Genet. 2014; Jul 1; 28 (2):24-28. https://doi.org/10.1016/j.nigjg.2015.06.005

68. Laskar RA, Wani MR, Raina A, Amin R, Khan S. Morphological characterization of gamma rays induced multi podding mutant (mp) in lentil cultivar Pant L 406. International Journal of Radiation Biology. 2018b; 94(11):1049-53. https://doi.org/10.1080/09553002.2018.1511927

69. Goyal S, Wani MR, Raina A, Laskar RA, Khan S. Quantitative assessments on induced high yielding mutant lines in urd bean [*Vigna mungo* (L.) Hepper]. Legume Science. 2021a.: e125.https://doi.org/10.1002/leg3.125

70. Goyal S, Wani MR, Raina A, Laskar RA, Khan S. Phenotypic diversity in mutagenized population of urdbean (*Vigna mungo* (L.) Hepper). Heliyon. 2021b; May 1;7(5): e06356. https://doi.org/10.1016/j.heliyon.2021.e06356

71. Unche PB, Misal MB, Borgaonkar SB, Godhawale GV, Chavan BD, Sawant DR. Genetic variability studies in sweet sorghum (*Sorghum bicolor* L. Moench). Int J Plant Sci. 2008; 3(1):16-18.

72. Khursheed S, Raina A, Laskar RA, Khan S. Effect of gamma radiation and EMS on mutation rate: their effectiveness and efficiency in faba bean (*Vicia faba* L.). Caryologia. 2018;71(4):397-404. https://doi.org/10.1080/00087114.2018.1485430.

73. Khursheed S, Raina A, Khan S. Physiological response of two cultivars of faba bean using physical and chemical mutagenesis. International Journal of Advance Research in Science and Engineering. (IJARSE) 2018;7(4):897-905.

74. Khursheed S, Raina A, Amin R, Wani MR, Khan S. Quantitative analysis of genetic parameters in the mutagenized population of faba bean (*Vicia faba* L.). Research on Crops. 2018;19(2). https://doi.org/10.5958/2348-7542.2018.00041.4

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