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RESEARCH ARTICLE

Effects of gamma radiation on physio-morphological traits of finger millet (*Eleusine coracana* (L.) Gaertn.)

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Abstract

The present study was carried out to analyse the effects of gamma radiations on physio-morphological traits of seven days old M_1 seedlings of *Eleusine coracana* (L.) Gaertn. (finger millet). The finger millet seeds were irradiated with different doses of gamma rays viz., 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy, 800 Gy, 900 Gy and 1000 Gy. Lower and higher doses of gamma rays induced a substantial increase and decrease in the mean values of physiological and morphological traits respectively. The results revealed a progressive decrease in chlorophyll fluorescence with an increasing dose of gamma rays. Among all the mutagen doses employed, 600 Gy gamma-irradiated seeds showed maximum mean values of physiological and morphological traits in finger millet. Hence, 600 Gy gamma rays may be employed in other related crop species to improve the agro-economic traits.

Keywords

Finger millet, gamma irradiation, photosynthetic pigments, physio-morphological traits

Introduction

Eleusine coracana (L.) Gaertn., commonly known as finger millet is one of the major nutritious food crops with high quantities of mineral nutrients such as calcium, magnesium, phosphorus and iron (1). The finger millet possesses fibre rich starch that enhances digestion and reduces intestinal disorders (2, 3). Millets are least affected by continuous climate change due to their ability to thrive at high temperatures and in low moisture soil (4, 5). Genetic variability is one of the primary prerequisites for crop improvement programmes. Among the breeding strategies, mutation breeding is a coherent tool to increase genetic variability and has been successful in the development of thousands of new varieties in hundreds of crop species (6). Different mutagens have been used by the workers from time to time to accomplish the desired results, however, radiation-induced mutagenesis is mostly used for the improvement of different agro-economic traits (7-10). Among the radiations, gamma rays are widely preferred physical mutagens that affect plant growth and development by altering the biochemical, physiological and morphological traits (11-13). Moreover, the highest number of mutants have been developed via the use of gamma rays due to their ability to induce single and double-stranded DNA breaks (14, 15). Gamma rays interact with plant tissues in two ways: direct and indirect interaction. In direct interaction, gamma rays deposit energy packets on DNA and cause ionisation and excitation of electrons. Depending upon the amount of energy deposition, gamma rays induce single and double-stranded breaks in the DNA that later on result in variations in different agro-economic traits (16, 17). In indirect interaction, gamma rays interact with water molecules and lead to the formation of highly reactive free radicals in a process called radiolysis of water. These free radicals then interact with the DNA and lead to several types of mutations such as substitution, base alterations, base deletions, chromosomal abnormalities and DNA breaks (18-20). The photosynthetic apparatus in plants are reported to be most sensitive part to mutagen induced alterations (21). Gamma rays affect photo pigments in positive or negative ways, depending on the dose and duration of exposure. Therefore, the visualization of fluorescence emitted by photosynthetic pigments in response to mutagen doses is imperative to find out steady-state fluorescence peaks under light illumination (22- 24). The fluorescence spectra in turn depend on chlorophyll contents and the absorbance of the leaves (25). In the present study, we investigated the effects of gamma rays on physio-morphological traits and analysed chlorophyll fluorescence of gamma rays treated M_1 finger millet seedlings.

Materials and Methods

Biological Material

The seeds of finger millet cultivar Paiyur-2 (parent- VL 145 x Selection 10) was procured from Tamil Nadu Agriculture Research Centre, Paiyur, Krishnagiri, Tamil Nadu, India. The experiments were performed at the Department of Botany, School of Life Sciences, Periyar University, Salem, Tamil Nadu, India.

Gamma radiation treatment

The chosen finger millet seeds were cleaned and packed in paper bags and treated with different doses of gamma rays viz., 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy, 800 Gy, 900 Gy and 1000 Gy at Indira Gandhi Centre for Atomic Research, Kalpakkam, Tamil Nadu, India using cobalt 60 as a source of gamma rays. The average measurement rate of uncertainty range of 1-3 Gy/Sec was maintained for this investigation. The calibration of the gamma chamber was accomplished with the Fricke dosimetry system for absorbed doses in line with the guidelines of the International Atomic Energy Agency.

Growth conditions

The control and treated seeds (n = 3) were kept moistened in germinating paper inside glass petri dishes (9 × 3 cm). Seedlings were grown individually for all doses in a greenhouse environment. Seven days old seedlings were used to determine the chlorophyll fluorescence (CF) intensity and content of photosynthetic pigments viz., chlorophyll-*a,* chlorophyll-*b* and carotenoids. Seedlings were raised in a nursery bed for three weeks followed by subsequent transplantation in the field beds and sown in randomized block design with three replications. All recommended agricultural practices (irrigation, fertilizers and weeding) were followed throughout the field study.

Chlorophyll extraction

Young leaves (100 mg) collected from seven days old seedlings were ground in 1 ml of 80 % acetone with pestle and mortar. The leaf extract was then spun at 2500 revolutions per minute (rpm) for 10 min at 20 °C in a centrifuge (Remi R 24). The homogenate was re-extracted with 80 % acetone until the green colour disappeared in the residue and the extract was pooled and made up to 2 ml with 80 % acetone. Then 2 ml of extract was transferred into a cuvette and the absorbance was read at 663, 645 and 480 nm in a UV spectrometer (Elico), against 80 % acetone as blank. Chlorophyll *a*, Chlorophyll *b* and carotenoid contents were estimated as per the following formula (26, 27).

Chlorophyll *a* (µg/ml) = (12.7*A663) - (2.69*A645)

Chlorophyll *b* (µg/ml) = (22.9*A645) - (4.68*A663)

Carotenoids (µg/ml) = A480 + (0.114* A663) – (0.638 * A645)

Where, A645 = absorbance at 645 nm; A663 = absorbance at 663 nm; A480 = absorbance at 480 nm.

Chlorophyll fluorescence

Chlorophyll fluorescence was determined in the leaves of both control and gamma-irradiated seedlings using a fluorescence spectrophotometer (Jasco, FP-8200 and Japan). The data interval (0.5 nm), scan rate (200 nm/min) and wavelengths Ex / Em (excitation/emission) were kept in a range from 400 to 700 nm. The wavelengths used for emission/excitation could reveal the variations after exposure to different doses of gamma rays.

Morphological and yield parameters

Regular field evaluations were carried out to collect the data on morphological and yield traits 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 panicle per plant and 1000 seed weight.

Statistical analysis

The gamma irradiated samples were analyzed with three replications in petri dish studies on 7-days old M_1 seedlings. One-way Analysis of Variance (ANOVA) and Pearson's correlation was performed in SPSS ver. 21.0 that allowed us to visualize the significance of data

Results

Lethal Dose (LD50)

Based on 50% mortality, the radio sensitivity of seedlings to different doses of gamma rays was evaluated. The apparent variation of 50% reduction in germination was observed at 600 Gy gamma rays treatment (25.33%) as compared to control (45.30%). The seedling survival depicted a negative correlation with increasing doses of radiation given in Fig. 1.

Effects of gamma rays on photosynthetic pigments

Our results revealed a progressive decrease in the contents of the photosynthetic pigments viz., chlorophyll *a* and chlorophyll *b* with increasing doses of gamma rays. The maximum decrease in photosynthetic pigments was

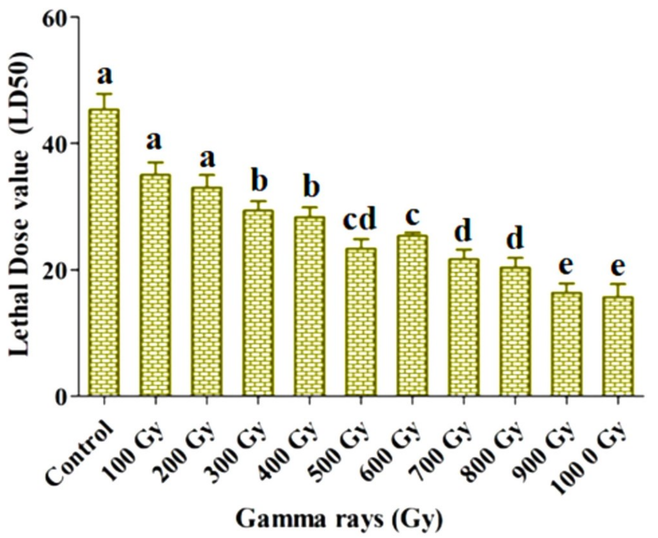

Fig. 1. Evaluation of LD_{50} value in seven day old M_1 seedlings of finger millet treated with different doses of gamma rays. Mean within columns followed by the same letter is not different at the 1% level of significance based on Duncan's Multiple Range Test.

recorded in 1000 Gy gamma rays treatment (chlorophyll *a*: 0.52 chlorophyll *b*: 0.16 mg g^{-1} FW) as compared to control (chlorophyll *a*: 1.54 chlorophyll *b*: 0.20 mg g^{-1} FW). The results also revealed a dose-dependent significant increase in carotenoid contents (Car: F= 270.65, P< 0.01). The maximum increase in carotenoids contents was observed in 1000 Gy gamma rays treatment (3.88 mg g^{-1} FW) as compared to control (1.31 mg g^{-1} FW) shown in Table 1.

Table 1. Effects of different doses of gamma rays on chlorophyll *a*, chlorophyll *b* and carotenoid content (mg g^{-1} FW) in 7 days old M_1 finger millet seedlings. The data is presented as mean ± SE (standard error) (n=3). Mean within columns followed by the same letter is not different at the 1% level of significance based on Duncan's Multiple Range Test.

Gamma rays	Chl *a*	Chl *b*	Carotenoids
Control	1.54±0.01c	0.20±0.00de	0.98±0.00e
100 Gy	2.67±0.28a	0.29±0.00cde	1.06±0.12e
200 Gy	2.75±0.09a	0.30±0.00cde	1.30±0.04d
300 Gy	2.65±0.14a	0.33±0.04cd	1.38±0.01d
400 Gy	2.85±0.17a	0.36±0.04c	1.46±0.02cd
500 Gy	2.74±0.18a	0.61±0.15a	1.64±0.03c
600 Gy	3.53±0.16a	0.86±0.07a	2.12±0.00b
700 Gy	2.10±0.00b	0.63±0.03a	2.62±0.04a
800 Gy	1.69±0.23c	0.49±0.01bc	2.96±0.11a
900 Gy	1.11±0.00d	0.26±0.08cde	3.48±0.14a
1000 Gy	0.52±0.05e	0.16±0.01e	3.88±0.07a

The statistical analysis revealed a significant enhancement of the pigments at optimum doses (300 Gy, 400 Gy, 500 Gy, 600 Gy) compared to control (Chl *a*: F= 50.94, P<0.01) (Chl *b*: F= 18.71, P<0.01).

Spectrofluorometric studies

The spectra of chlorophyll fluorescence were recorded within excitation wavelength varied from 400 to 700 nm. The peak value of 7529.67 obtained in the 400 Gy leaf extract sample revealed a maximum excitation spectra at 677 nm compared with a peak value of 6352.97 in control. The results revealed a progressive decrease in fluorescence intensity with the increasing dose of gamma rays depicted in Fig. 2.

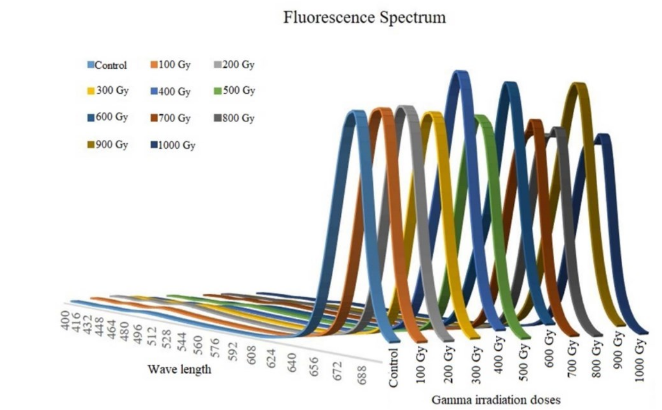

Fig. 2. Fluorescence spectrum of seven days old M_1 finger millet seedlings treated with different doses of gamma rays.

Quantitative traits

In this study, mean performances of different quantitative traits were recorded in all doses in M_1 generation. The optimum doses (300 Gy, 400 Gy, 500 Gy and 600 Gy) increased the mean performance of quantitative traits. The maximum increase in plant height (130.89±4.75 cm) was recorded in 600 Gy gamma rays treatment as compared to control (90.84±0.47 cm). Plant height decreased significantly with increasing dose of gamma rays (F= 453.53, P<0.01) and a maximum reduction was noted in 1000 Gy gamma rays treatment (37.93±1.90 cm) shown in Table 2. The maximum increase in the number of leaves per plant was recorded in 300 Gy gamma rays treatment (83.9±6.54). The results showed a decreasing trend in the number of leaves per plant with the increasing dose of gamma rays. The highest decrease in the number of leaves per plant was recorded in 1000 Gy gamma rays treatment (6.1±1.00) as compared to control (13.20±0.34) given in Table 2. The maximum leaf

Table 2. Effects of different doses of gamma rays on morphological traits of finger millet in M_1 generation. The data is presented as mean ± SE (standard error) (n=3) Mean within columns followed by the same letter is not different at the 1% level of significance based on Duncan's Multiple Range Test

Gamma rays	Plant height	No. of leaves / plant	Leaf length	Tillers/plant
Control	90.84±0.47e	13.20±0.34gh	14.66±0.52h	3.40±0.17h
100 Gy	93.57±0.41de	14.80±0.49fg	25.71±1.43f	4.40±0.57f
200 Gy	95.48±0.13cd	16.70±0.84f	33.78±1.12e	6.10±0.24e
300 Gy	96.09±0.39bc	83.90±6.54e	64.91±0.81d	6.80±0.37d
400 Gy	98.55±0.16b	45.6±1.03d	56.91±0.94c	7.80±0.26c
500 Gy	100.90±0.34a	42.1±0.65c	43.13±0.88b	9.40±0.50b
600 Gy	130.89±4.75a	25.5±0.77a	37.25±0.32a	11.80±0.46a
700 Gy	102.72 ±10.93a	21.2±2.81b	37.25±0.32d	5.90±0.77e
800 Gy	90.18±1.42e	18.3±2.30e	27.20±1.35f	3.70±0.54g
900 Gy	54.60±3.69f	12.20±0.60g	18.07±1.07g	1.90±0.55i
1000 Gy	37.93±1.90g	6.10±1.00h	13.77±0.42h	1.0 0±0.35j

length was recorded in 300 Gy gamma rays treatment (64.91± 0.81). The mean performance of leaf length per plant exhibited gradual reduction as the gamma rays dose increases. A higher reduction in plant leaf length was recorded in 1000 Gy gamma rays treatment (13.77±0.17cm) as compared to control (14.66±0.52). The number of tillers per plant showed more reduction in 1000 Gy gamma rays treatment (1.0±0.35) as compared to control (3.40±0.17). Increased mean tillers per plant were recorded in 600 Gy gamma rays treatment (11.80±0.46) shown in Table 2.

The maximum reduction of panicle number was recorded in 1000 Gy gamma rays treatment (0.5±0.17) as compared to control (2.0±0.27). The minimum day to 50% flowering was recorded in 600 Gy gamma rays treatment (77.4±0.54) as compared to control (81.2±0.14) (Table 3). The maximum increase in days to 50% flowering was recorded in 1000 Gy gamma rays treatment (89.4±0.17). Our results revealed maximum panicle length in 600 Gy gamma rays treatment (66.02±1.17) as compared to control (21.84±1.27) depicted in Table 3.

Table 3. Effect of different doses of gamma rays on yield related traits of finger millet in M_1 generation. The data is presented as mean ± SE (standard error) (n=3). Mean within columns followed by the same letter is not different at the 1% level of significance based on Duncan's Multiple Range Test.

Gamma rays	Days to 50% flowering	Panicle length	No. of panicles/ plant	1000 seed weight
Control	81.20 ±0.14ef	21.84±1.27gh	2.0±0.27f	2.47±0.01g
100 Gy	81.00±0.22ef	22.73±0.17fg	2.0±0.27f	2.67±0.02f
200 Gy	81.50±0.82de	24.40±0.16e	2.7±0.22e	2.76±0.01e
300 Gy	79.90±0.48g	27.10±0.70d	3.2±0.34d	2.87±0.04d
400 Gy	80.60±0.70fg	36.07±1.13c	3.6±0.17c	2.95±0.03c
500 Gy	81.50±0.23def	49.90±1.43b	4.7±0.22b	3.18±0.02b
600 Gy	77.40±0.57h	66.02±1.17a	8.8±0.14a	3.40±0.03a
700 Gy	82.00±1.11d	23.27±0.46ef	2.0±0.27f	2.69±0.08ef
800 Gy	85.50±0.17c	21.01±0.19h	1.5±0.17g	2.37±0.03h
900 Gy	86.50±0.32b	13.61±0.77i	0.7±0.22h	2.17±0.03i
1000 Gy	89.40±0.17a	7.86±1.21j	0.5±0.17h	1.72±0.08j

However, it decreased in seedlings raised from seeds treated with 700 Gy to 1000 Gy gamma rays with a maximum reduction in 1000 Gy gamma rays treatment (7.86±1.21). The results revealed a maximum reduction in seed weight in 1000 Gy gamma rays treatment (1.72±0.08 g) as compared to control (2.47±0.01 g). The maximum increase in 1000 seed weight was recorded in 600 Gy gamma rays treatment (3.4±0.03 g). Pearson's correlation coefficients for phenotypic and yield-related traits of finger millet in different doses of gamma irradiation are given in Table 4. The correlation between the morphological and yield traits was significant and positive. (Table 4)

Discussion

Lethal Dose

The induction of mutations in plant breeding is a well-established tool to accomplish the objectives of crop improvement programmes (28). Prior to induction of mutations, the evaluation of LD_{50}, a mutagen dose that induces 50% reduction in seed germination is imperative. It allows the breeders to assess the mutagenic efficiency in relation to the mutagen induced biological damage (29). In the present study, the LD_{50} was determined in seven days old finger millet M_1 seedlings. The maximum damage induced was recorded at higher gamma rays treatments. This may be attributed to the deleterious effects of higher doses of gamma rays on seed meristematic tissues that led to chromosomal aberrations, growth regulator disturbances, DNA damage and lethality. The reduced germination may also be due to inhibitory effects of gamma rays on vital functions of cells leading to cell and embryo fatality (30). A reliable indicator for the optimization of radiation dose requires the evaluation of LD_{50} and the effects of gamma rays on physio-morphological traits (31). The previous results of LD50 in the range of 599 - 731Gy was reported earlier in pearl millet (32). A paper germination test to optimize LD_{50} depends upon germination percentage and reduction in root and shoots length. As compared to control, a reduction in root and shoot length has been previously reported in higher doses of gamma rays treatments (33). In the present investigation, higher doses of gamma rays affected seedling growth parameters and similar findings were also reported (34). The destructed cell components in seeds treated with higher doses of gamma rays lead to chromosomal damage and reduced seedling growth. Therefore, in induced mutagenesis, the determination of LD50 is important for the overall success of the breeding programme.

Table 4. Pearson's correlation coefficients for morphological and yield-related traits of M_1finger millet seedlings

	Plant height	No. of leaves/ plant	Leaf length	Tillers/ plant	Days to 50% flowering	Panicle length	No. of panicles/ plant	1000 seed weight
Plant height	1							
No. of leaves/ plant	.744**	1						
Leaf length	.703**	817**	1					
Tillers/ plant	.725**	.728**	.907**	1				
Days to 50% flowering	-.739**	-.497**	-.641**	-.663**	1			
Panicle length	.666**	.812**	.922**	.876**	-.670**	1		
No. of panicles/ plant	.620**	.764**	.864**	.831**	-.672**	.924**	1	
1000 seed weight	.787**	.696**	.877**	.859**	-.798**	.857**	.810**	1

Chlorophyll content

In the present investigation, the chlorophyll content gradually decreased with the increase in doses of gamma rays. This may be due to the enhanced production of free radicals at higher doses. In contrast, carotenoid contents increased linearly with the increase in gamma rays doses. The results were in agreement with the previous findings (35, 36) that also reported augmented carotenoid contents in mutant lines. The significant stable and steady increase in carotenoid contents indicate its potent role in photo protection. The enhanced carotenoid contents in higher doses may be attributed to their vital role in free radical scavenging activity during stress induced by radiations (37).

Chlorophyll fluorescence

Chlorophyll fluorescence is extensively utilized to evaluate the relative influence of environmental stress on photosynthetic properties (38-44). From photosystem II the maximum amount of excited energy is converted into chemical energy and then utilized in carbon fixation. Even though the excess energy is converted into heat and a very little fraction is lost as fluorescence (45). Evaluation of CF provides insight into photosystem functioning. CF values observed in the present study show maximum peak values at 600 Gy gamma rays treatment.

Quantitative traits

The results revealed a progressive decrease in the mean values of yield and yield attributing traits with the increase in gamma-ray doses. The diminution of mean values of yield attributes may be due to mutagen induced physiological and biochemical disturbances, and destruction of auxin and ascorbic acid content that may lead to inhibition in cell division and cell elongation (35, 36). The results were in agreement with the findings of the earlier studies (11, 46, 47). Our results revealed a progressive decrease in root and shoot length with an increase in gamma rays doses. The results were in agreement with previous findings in rice (48). Our findings showed that plant height correlated significantly in a positive direction with other agronomic traits such as number of leaves per plant, leaf length, number of tillers per plant, days to 50% flowering, panicle length, number of panicles per plant and 1000 seeds weight. These findings correlated with the results in rice that also reported plant height, flowering time and yield are the significant agronomic traits (49). The results also revealed that plant height, number of leaves per plant, leaf length, number of tillers per plant, number of panicles per plant, panicle length and 1000 seed weight decreased as the dose increased. The results were in line with the findings in sesame treated with ethyl methanesulphonate that showed a linear decrease in seedling emergence, seedling survival and delayed 50% flowering and maturity with the increase in gamma rays doses (50). The present study also revealed delayed seed germination and plant growth at higher radiation doses which may be attributed to late flowering in finger millet. Days to 50% flowering showed a significant positive correlation with the number of panicles per plant. The results were in agreement with previous studies that reports a significant positive correlation of pods with the days to flowering in *Brassica juncea*, *Brassica napus* and *Brassica campestris* (51). Similarly, in linseed, plant height, seed yield, number of branches during primary and secondary stages, capsule number per plant were positively correlated (52). The results of the present study revealed that optimum doses of gamma rays stimulated growth regulators and enzymes associated with growth, yield and 1000 seed weight. This may be attributed to gamma rays induced morphogenetic, cytological, biochemical and physiological changes in cells and tissues (7, 53).

Conclusion

Eleusine coracana (L.) Gaertn. (Finger millet) seeds irradiated at 600 Gy gamma rays dose revealed positive significant effects on physio-morphological and yield traits as compared to higher doses. Similarly, gamma irradiation also increased photosynthetic pigment contents at optimum doses (300 Gy, 400 Gy, 500 Gy and 600 Gy) compared to control. Therefore, it is concluded that the gamma-irradiated finger millet seeds with optimum doses improved the yield attributes of finger millet. In conclusion, lower doses of gamma rays treatment could be employed for the improvement of finger millet and other related crops.

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Authors contributions

LS conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing original draft, writing review and editing. AD conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing original draft, writing review and editing. AG analysed and methodology construction. After the original draft was framed AR rewrote the article, analyzed and interpreted the data.

Compliance with ethical standards

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

Ethical issues: None.

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