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A Study on Physiological and Phytochemical Changes in Gamma Radiation-Induced Seeds of *Panicum miliaceum* L.

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Abstract

Panicum miliaceum L. (family Poaceae), commonly known as proso millet is cultivated throughout the tropical regions of the world including India. The seeds are small and gluten-free, containing high amount of protein compared to other grains (10-12%) and significantly rich in essential amino acids. The present investigation was carried out to determine the effects of induced gamma irradiation in proso millet (*Panicum miliaceum*) variety CO(PV) 5 on physiological parameter such as germination and growth. Dry seeds of proso millet are exposed to gamma source - Cobalt-60 (^{60}Co) at doses ranging from 50, 100, 150 and 200 Gray (Gy). The results showed that the final germination percent and seedling (radicle and plumule) length was observed to be high in radiated seeds of 50 Gy and 100 Gy respectively, when compared to control (non-irradiated). Phytochemical differences based on protein content revealed that seeds irradiated at 150 Gy contained high protein than control. The study concluded that the results were dependent on time and dose of gamma radiation.

Key words: Induced gamma radiation, Gamma source, Gray, Physiological parameter, Proso millet

The physical, chemical, and biological characteristics of any material or matter can be modified using ionizing radiation which is highly advantageous in the agricultural sector, where it plays a crucial role in crop development, plant growth and breeding. [1]. Gamma radiation has been reported as an environmentally important ionizing radiation with very shorter wavelength (~10^{-3} to 1 Å), high frequency and high-energy photons [2]. Gamma sources are useful for irradiating a wide range of plant materials: seeds, whole plants, flowers, anthers, pollen grains and single cell cultures or protoplasts [3].

Panicum miliaceum L. (proso millet) of family Poaceae (according to Bentham and Hooker classification) is a warm-season herbaceous crop which cultivated annually. Due to its high water-use efficiency, it is well adapted to many soil and climatic conditions [4-6]. An attempt was made in a study to promote the public's daily use of this extremely nutritious, readily digestible, and gluten-free proso millet, not only as a means of curing illnesses but also as a means of sustaining good health [7]. Growing use of proso millet and its derivatives has been linked to important functional elements and health advantages, such as lowered risk of chronic conditions including raised blood cholesterol, type II diabetes, cardiovascular disease, liver injury and the potential for anti-cancer, anti-oxidant, and anti-proliferative actions [8-14]. The current study concentrates on the advantageous impacts of induced gamma radiation in proso millet on physiological (seed germination and seedling growth) and phytochemical (protein content) parameters.

MATERIALS AND METHODS

Plant material

The seeds of *Panicum miliaceum* Linn. (panivaragu / proso millet) variety CO (PV) 5 were obtained from Centre of Excellence in Millets (CEM), Tamil Nadu Agricultural University (TNAU), Athiyandal, Tamil Nadu, India.

Gamma irradiation

Irradiation of proso millet seeds was performed with Gamma Chamber 5000 using a Cobalt 60 (^{60}Co) gamma source in ambient conditions at Indian Council of Agricultural Research-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru, India. The doses of exposure were 50, 100, 150 and 200 Gy whereas non-irradiated seeds served as control.

Study of germination and seedling growth

After irradiation, 10 seeds from each treatment with five replicates were placed uniformly by forceps in each petri dish containing cotton bed with filter paper and regular spraying of distilled water. Petri dishes were placed in dark at room conditions and the number of germinated seeds was recorded for 7 days from the day of sowing. The final germination percent (FGP) was calculated by dividing the number of germinated seeds after 7 days to the total number of seeds.

$$FGP = \frac{\text{Number of germinated seeds after 7 days}}{\text{Total number of seeds}} \times 100$$

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The length of radicle and plumule of the grown seedlings (in cm) were recorded and tabulated for the evaluation of the effects of gamma irradiation on proso millet seeds, seven days after sowing. A set of two replicates were maintained for treated and control.

Determination of protein content

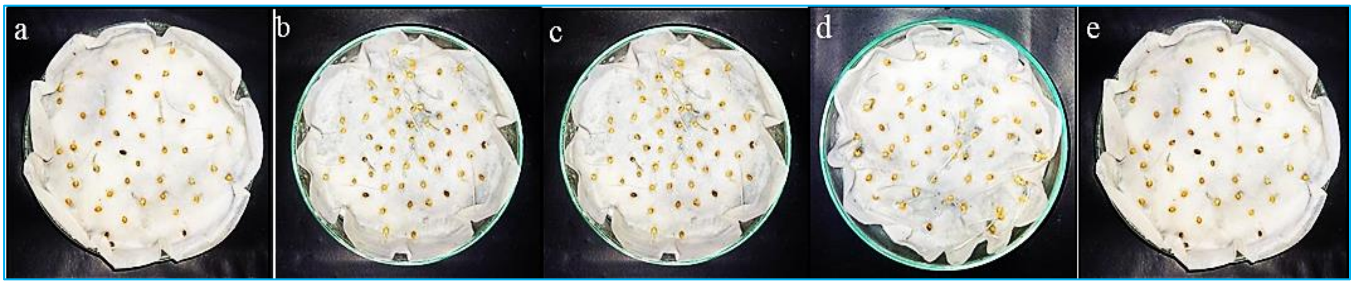
The protein content in proso millet seeds was determined following the method of Bradford [15]. Samples (250 mg) of each irradiated and non-irradiated (control) proso millet seeds were ground using pre-chilled mortar and pestle and then homogenized in 750 µl of potassium phosphate buffer (0.1 M, pH 7.5) containing 10 µl of 0.1 M Na_2-EDTA (2- sodium ethylenediamine tetra acetic acid) and 1% polyvinylpolypyrrolidone (PVPP) at 4ºC. The homogenate was centrifuged at 10000 rpm for 10 minutes at 4 °C. The cell free supernatant was collected and used as a protein source. To 10 µl of buffer, 990 µl of distilled water was added followed by 5 ml of Bradford reagent and taken as blank. To 10 µl of seed extract, 990 µl of distilled water was added followed by 5 ml of Bradford reagent. It was mixed well and read at 595 nm in spectrophotometer (UV-1650PC, Shimadzu, India). The protein content of the samples was calculated using a standard graph constructed with BSA.

Statistical analysis

Experimental data were subjected to one-way analysis of variance (one-way ANOVA) to determine the differences in average of all tested parameters between irradiated and non-irradiated groups. Statistical analysis was performed in Microsoft Excel 2010 with comparison of means using XLSTAT (2018).

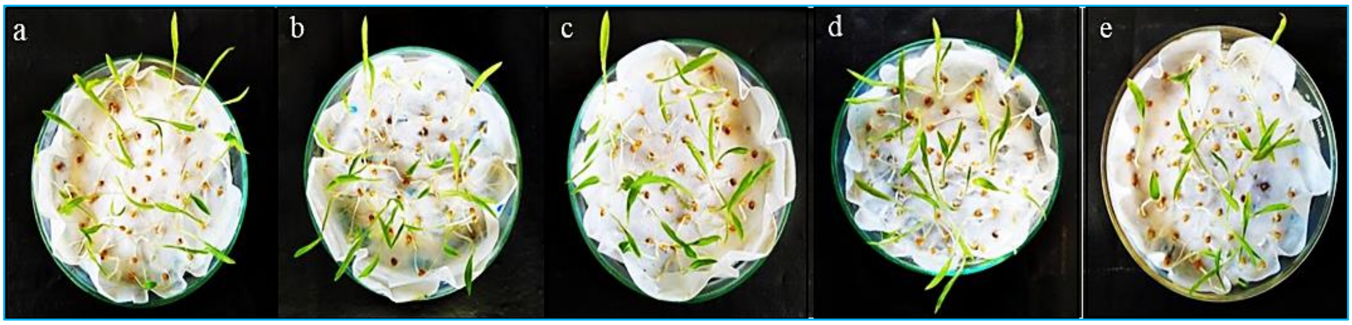
RESULTS AND DISCUSSION

Results of germination study of irradiated and non-irradiated *Panicum miliaceum* seeds revealed that the final germination percent (FGP) varied between control, 50, 100, 150 and 200 Gy irradiated seeds (Fig 1, Table 1). The final germination percent significantly decreased along with increase in gamma doses as well as delay in germination time was more observed in irradiated seeds than control. The maximum percentage of germination was observed in seeds exposed to 50 Gy (76%), followed by 100 Gy (71%) and 150 Gy (69%) than control (62%). Decrease in percentage of germination was observed in seeds exposed to 200 Gy (55%). Gamma radiation had significant effect on final germination percentage. These results were in accordance with the germination studies carried out in *Atropa belladonna* L. [16], *Oryza sativa* L. [17], *Helianthus annus* L. [18] and *Sesamum indicum* L. [19]. Thus, increasing frequency of chromosomal damage with increasing radiation dose may be responsible for less germinability [20].



(a) Control, (b) 50 Gy, (c) 100 Gy, (d) 150 Gy, (e) 200 Gy

Fig 1 Germination studies of irradiated and non-irradiated *Panicum miliaceum* CO (PV) 5 seeds



(a) Control, (b) 50 Gy, (c) 100 Gy, (d) 150 Gy, (e) 200 Gy

Fig 2 Growth studies of proso millet seedlings seven days after sowing

Results reveal that gamma radiation imposed a significant effect on both radicle and plumule length (Fig 2, Table 1). The maximum length of radicle as well as plumule was observed in 100 Gy seedlings (7.67 cm and 5.20 cm respectively) when compared to other irradiated concentrations and control. The radicle length was measured to be 5.80 cm in 50 Gy, followed by a significant increase in 200 Gy with 6.29 cm and 150 Gy with 7.33 cm. On the contrary, the control seedlings showed the minimum radicle length of 5.25 cm. The plumule length was measured to be 5.03 cm in 150 Gy. The treatment of 50 Gy and 200 Gy radiations as well as control had meagre differences with minimum plumule length ranging from 4.42 cm, 4.83 cm and 4.57 cm respectively. These results highlight the optimal gamma radiation dose for promoting seedling growth, with 100 Gy being the most effective in enhancing both radicle and plumule lengths compared to other doses and the control. Study on gamma radiation effects in *Lactuca sativa* L. (lettuce) seeds by Marcu *et al*. [21] revealed an enhancement of root and hypocotyl length, as compared to untreated plants; whereas a study by Toker *et al*. [22] in chickpea seeds showed 200 Gy seedlings had significant increase in shoot length, but 400 Gy showed depression in shoot length stating that the changes observed were dose- dependent similar to the present study.

Phytochemical differences based on protein content (Table 1) revealed that seeds irradiated at 150 Gy contained high protein of 9.15 mg. g^{-1} fw than control (7.35 mg.g^{-1} fw). A quantitative increase was observed in protein content of seeds with 200 Gy exposure (7.56 mg.g^{-1} fw) and 50 Gy (7.95 mg.g^{-1} fw) exposure. The minimum protein content was observed in case of seeds with 100 Gy exposure (5.85 mg.g^{-1} fw). Kanemaru *et al*. [23] observed that the protein content of irradiated wheat flour was not affected by irradiation dose (0.5, 1.0 and 2.0 kGy). Therefore, gamma radiation had influence over the protein content in proso millet seeds, as negligible variations with respect to the different gamma doses over control were observed. Therefore, the percentage of germination and other seedling parameters linearly decreased with increase in strength of gamma rays which might be due to irradiation of gamma rays that interrupt or slow down metabolic activity of seedling and inhibit synthesis of protein during germination [24]. The percentage of germination and other seedling parameters were observed to decrease linearly with increasing gamma radiation strength, likely due to the interruption or slowing down of metabolic activities and the inhibition of protein synthesis during germination caused by gamma rays.

Table 1 Effects of gamma radiation on final germination percentage (FGP), radicle length, plumule length and protein content of *Panicum miliaceum* L.

Parameter	Radiation Dosage (Gy)				
	Control	50	100	150	200
FGP (%)	62	76	71	69	55
Radicle length (cm)	5.25 ± 0.35*	5.80 ± 0.34*	7.67 ± 0.39*	7.33 ± 0.31*	6.29 ± 0.35*
Plumule length (cm)	4.57 ± 0.17**	4.42 ± 0.19**	5.20 ± 0.18**	5.03 ± 0.12**	4.83 ± 0.08*
Protein content (m g.g^{-1} fw)	7.35 ± 0.01**	7.95 ± 0.01**	5.85 ± 0.02***	9.15 ± 0.02***	7.56 ± 0.01**

± indicates standard deviation
*Significant difference ($p < 0.05$); **Highly significant difference ($p < 0.01$); ***Extremely significant difference ($p < 0.001$)

CONCLUSION

The outcomes of the current study show that different levels of gamma radiation have diverse effects. It is evident γ-radiation enhances protein content, stimulates germination, and promotes seedling growth. The seeds that were exposed to 150 Gy had a high protein content, while the seedlings that were developed from seeds exposed to 100 Gy showed the maximum length of radicle and plumule. On the other hand, the 50 Gy exposure increased the proportion of *P. miliaceum* seeds that sprouted. There are still many beneficial applications for gamma radiation, hence making it a potentially helpful tool to agriculture.

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