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Irradiation effect of gamma rays on growth and SDS-PAGE analysis in *Panicum sumatrense* Roth Ex.

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Key words: *Panicum sumatrense*, Gamma rays, Growth parameters, SDS-PAGE analysis, Protein banding pattern

Abstract

An attempt was made to find out the effect of gamma radiation on *Panicum sumatrense*. The seeds of little millet cv. ATL-1 and CO-2 were exposed to different doses of gamma rays, like 100, 200, 300, 400, 500, and 600 Gy. The seeds treated with gamma rays were sown in plastic pots with red soil to analyze the LD50 value and growth parameters. The lethal dose 50% value was observed at 400 Gy and 500 Gy for CV ATL-1 and CO-2, respectively. In both cultivars, all the growth parameters, such as percentage of germination, percentage of survival, shoot length, root length, fresh weight, and dry weight, showed a decreasing trend towards control with increasing doses of gamma rays, and the reduction was maximum at 600 Gy and minimum at 100 Gy. The cv.ATL-1 seedlings treated with gamma radiation were subjected to estimation of total protein content, and this protein content showed a decreasing trend compared to the control. The number of protein bands and kDa values of cv. ATL-1 were observed by using SDS-PAGE analysis. The kDa value of 132.8 was present only in 400, 500, and 600 Gy. The growth attributes of cultivar ATL-1 were unique compared to cultivar CO-2.

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Introduction

Millets which are members of the family Poaceae (Gramineae), are thought to be the first Cereal crop cultivated thousands of years ago (Lu *et al*., 2009). Millet grains are also known as "famine reserves" because they stored for two years or more (Ashoka *et al*., 2020). Little millet is grown indigenously in the tropics and subtropics. It is a drought-tolerant crop and requires less water to complete its life cycle. Hence, it can provide us with food security in unfavorable climatic conditions. In addition, it is rich in vitamin B and minerals like potassium, phosphorus, iron, zinc, and magnesium. Therefore, it can address nutritionally sensitive agriculture, which aims at nutritional enhancement to compact the present scenario of micronutrient malnutrition (Arunachalam *et al*., 2005).

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) is commonly used for the identification and quantification of newly generated proteins as well as for the determination of protein molecular weights and the identification of numerous unknown proteins. For both initial genotype identification and later genotype verification, electrophoresis has shown to be a more efficient and trustworthy alternative method for characterizing plant leaf or seed storage proteins (Mohammed 2000).

Among general physical mutagens, gamma radiation has been frequently employed for mutation induction in both seed and vegetative propagated crops (Jain 2010). The most energetic form of electromagnetic radiation is gamma rays. Their energy level range between 10 and several hundred kilo electron volts, and they are thought to be the mostpenetrating of all radiations (Kovacs *et al*., 2002). Gamma radiation can be employed to change physiological characteristics (Kiong *et al*., 2008).

Material and methods

Seed material

Mature, healthy and uniform seeds of *Panicum sumatrense* Roth Ex cv-ATL-1 were obtained from the Centre of Excellence in Millets, Athiyandal, Thiruvanamalai, and the cv-CO-2 variety was obtained from Agriculture University Coimbatore (Dt), Tamil Nadu, India.

Gamma Irradiation

The seeds were irradiated with six different dose levels, such as 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, and 600 Gy, and these doses were delivered from a Caesium-137 source cell installed at a National Research Centre for Banana, Somarasempettai-Thogaimalai Road, Thayanur, Trichy, Tamil Nadu, 620 102, India. The gamma source was stable and its irradiations were done at a dose rate of 3200 Rads/min by a movable down a cylindrical gasket carrying the seeds. The irradiated seeds of both the verities were sown in mud pots containing red soil in triplicates. The following parameters were measured and calculated on percentage of germination (7^{th} day), percentage of survival (15^{th} day), shoot length, root length, fresh weight and Dry weight. The growth parameters were measured in all the three replicates and taken as average.

LD50%

The formula proposed by Finney (1971) was used by the OPSTAT software to calculate the probit analysis.

Calculation

1. Percentage of germination

$$= \frac{\text{Number of seeds germinated in each mutagenic treatment}}{\text{Number of seeds germinated in control}}$$

2. The shoot and root length were measured individually and taken as average

3. The fresh weight and dry weight measured individually and taken as average.

4. The protein bands were calculated based on Rf value.

Protein estimation

The Lowry (1951) method has been employed for protein estimation, while the Laemmli (1970) method was performed for SDS-PAGE analysis.

Results and discussion

LD_{50}

The LD50 value for both cultivars was determined by probit analysis. The irradiated seeds of little millet were

used for calculating the LD_{50} value. The LD_{50} values were determined based on a 50% decrease in germination counts on the 15th day. The lethal 50% values of cv ATL-1 and CO-2 were shown at 400 Gy and 500 Gy respectively (Table 1). The results of the current investigation, showed that the little millet germination percentage dropped as gamma radiation exposure levels raised. Like that of our present study, the same trend was supported by Ramkumar and Dhanavel (2021) in little millet and Vannirajan *et al.* (2021) in barnyard millet.

Table 1. Probit analysis for LD50 of gamma rays in *Panicum sumatrense* – CO-2 and ATL-1.

Conc.	Tot No	No. Kills	%Mort.	Log (Dose)	Exp. Prop	Emp Probit	Exp Probit	Work. Probit	Weight
CO-2									
Control	100.00	-	-	-	-	-	-	-	-
100Gy	100.00	12.000	0.120	2.000	0.119	-1.175	-1.179	1.175	37.768
200Gy	100.00	28.000	0.280	2.301	0.240	0.583	0.707	0.578	52.959
300Gy	100.00	30.000	0.300	2.477	0.333	-0.524	0.431	-0.522	59.480
400Gy	100.00	33.000	0.330	2.602	0.407	-0.440	0.236	-0.434	62.388
500Gy	100.00	50.000	0.500	2.699	0.467	0.000	0.084	0.000	63.499
600Gy	100.00	55.000	0.550	2.778	0.516	0.126	0.040	0.125	63.625
ATL-1									
Control	100.00	-	-	-	-	-	-	-	-
100Gy	100.00	5.000	0.050	2.000	0.009	-1.645	-2.382	-0.613	6.407
200Gy	100.00	8.000	0.080	2.301	0.103	-1.405	-1.266	-1.393	34.773
300Gy	100.00	10.000	0.100	2.477	0.270	-1.282	-0.613	-1.127	55.475
400Gy	100.00	50.000	0.500	2.602	0.441	0.000	-0.149	0.001	63.149
500Gy	100.00	60.000	0.600	2.699	0.583	0.253	0.210	0.253	62.647
600Gy	100.00	75.000	0.750	2.778	0.693	0.674	0.504	0.667	58.018

Percentage of germination and Survival of cv. ATL-1 and CO-2

Gamma rays caused changes in the percentage of germination of the cv. ATL-1 seeds. The percentage of germination in the control group was 91.12. The percentages of germination in the seedlings exposed to gamma rays were 79.96, 69.94, 68.82, 66.67, 61.00, and 51.00 in 100, 200, 300, 400, 500, and 600 Gy respectively. There were variations in the percentage of survival for the cv ATL-1 seedlings after gamma radiation treatment. The survival rate in the group under control was 90.10. The percentages of survival in the gamma-treated seeds were 78.90, 68.90, 67.80, 65.60, 60.00, and 49.00 respectively in 100, 200, 300, 400, 500, and 600 Gy.

The control group's germination rate in CO-2 was 86.60 percent. In seeds exposed to gamma rays, values of 76.60, 69.92, 58.84, 47.70, 42.20, and 36.69 were discovered in the ranges of 100, 200, 300, 400, 500, and 600 Gy. The trend for all of the treatment was downward. Gamma radiation treatment caused changes in the percentage of survival in cv CO-2 seedlings. The survival rate for the control group was 85.51 percent. The percentages of seedlings that survived gamma radiation treatments at 100, 200, 300, 400, 500, and 600 Gy were 75.53, 68.82, 57.75, 46.66, 41.13, and 35.57 respectively.

Shoot, Root length of cv. ATL-1 and CO-2

In ATL-1, the control shoot length was 10.70 cm, whereas the shoot lengths at 100, 200, 300, 400, and 600Gy were 8.84, 7.75, 6.27, 5.14, and 4.27 cm respectively. Shoot length was found to decrease with increasing gamma radiation doses. Gamma radiation treated seeds generally had shorter roots than control seedlings, which had an average root length of 8.36 cm. The highest decreases were 7.37, 5.87, 4.98, 4.15, 3.85, and 2.55cm in 100, 200, 300, 400, 500, and 600Gy.

The shoot length in CO-2 was 8.53 in the control compared to 6.94, 6.35, 5.86, 5.22, 4.96 and 4.27cm in the 100, 200, 300, 400, 500 and 600Gy cases respectively. In all of the treatments compared to control, there was a decrease in shoot length. The root length in the control seeds was 5.56 cm, however a reduction in root length compared to the control was seen in seeds treated with gamma radiation. Maximum reductions occurred in 100, 200, 300, 400, 500, and 600 Gy at 5.47, 5.30, 5.25, 5.00, 4.24, and 2.73 cm.

Fresh weight and Dry weight of cv. ATL-1 and CO-2

The fresh weight of the seeds in the control group was 1.88g in ATL-1; however, in the gamma rays treated seeds, a reduction in fresh weight relative to the control plants was seen. 1.73, 1.40, 1.26, 0.43, 0.35, and 0.21g in 100, 200, 300, 400, 500, and 600 Gy, respectively, were the decreasing values. The dry weight of the control seeds was 0.34 in ATL-1, but the gamma-treated seeds had a dry weight that was lower than the control. In 100, 200, 300, 400, and 500 Gy, the decrease in weight was 0.25, 0.23, 0.22, 0.20, 0.18, and 0.13 g, with a maximum in 600 Gy. In CO-2, seeds that had been exposed to gamma rays had a fresh weight of 1.81g. Fresh weight over control was found to have decreased. In 100, 200, 300, 400, and 500, the drop was 1.65, 1.36, 1.14, 0.21, 0.13, and 0.10g, and it was at its greatest in 600Gy. Compared to seeds treated with gamma rays, the dry weight of CO-2 in the control was 0.25g. Dry weight above control was found to have decreased. In 100, 200, 300, 400, and 500, the drop was 0.20, 0.18, 0.15, 0.13, 0.11, and 0.09g, and it maximum in 600Gy (Table 2).

Table 2. Effect of gamma rays on growth characteristics of two cultivars of little millet *Panicum sumatrense*.

Treatment	Germination Percentage			Survival Percentage			Shoot length (cm)			Root length (cm)			Fresh weight (g)			Dry weight (g)		
	ATL-1	CO-2	Mean	ATL-1	CO-2	Mean	ATL-1	CO-2	Mean	ATL-1	CO-2	Mean	ATL-1	CO-2	Mean	ATL-1	CO-2	Mean
Control	90.12	86.60	88.35	90.10	85.51	87.80	10.70	8.53	9.60	8.36	5.56	6.90	1.88	1.81	1.84	0.34	0.25	0.29
100Gy	79.91	76.60	78.25	78.90	75.53	72.20	8.84	6.94	7.85	7.37	5.47	6.20	1.73	1.65	1.69	0.25	0.20	0.22
200Gy	69.94	69.92	69.50	68.90	68.82	68.85	7.75	6.35	7.00	5.87	5.30	5.20	1.40	1.36	1.38	0.23	0.18	0.20
300Gy	68.82	58.84	63.80	67.80	57.75	62.75	7.36	5.86	6.55	4.98	5.25	5.15	1.26	1.14	1.20	0.22	0.15	0.18
400Gy	66.67	47.70	57.15	65.60	46.66	56.10	6.27	5.22	5.70	4.15	5.00	4.55	0.43	0.21	0.28	0.20	0.13	0.16
500Gy	61.00	42.20	51.60	60.00	41.13	50.55	5.14	4.96	5.00	3.88	4.24	4.00	0.35	0.13	0.25	0.18	0.11	0.14
600Gy	51.00	36.69	43.80	49.00	35.57	42.25	4.27	4.27	4.20	2.55	2.73	2.60	0.21	0.10	0.20	0.13	0.09	0.11
C.D.	-	-	13.71	-	-	13.33	-	-	1.45	-	-	N\A	-	-	0.16	-	-	0.03
SE(m)	-	-	3.88	-	-	3.70	-	-	0.41	-	-	0.74	-	-	0.04	-	-	0.00
SE(d)	-	-	5.50	-	-	5.34	-	-	0.58	-	-	1.05	-	-	0.06	-	-	0.01
CV	-	-	8.50	-	-	8.40	-	-	0.90	-	-	21.23	-	-	6.74	-	-	6.34

In this experiment work, all the growth attributes showed a decreasing trend compared to control. Similarly, Kiong *et al.* (2008) observed that the reduction in the percentage of germination caused by the high doses of radiation applied may have resulted in a reduction in the amount of internal growth regulators, depending on the radiation. The decrease in seed germination induced by mutagenic treatment may be due to damage to constituents at the molecular level or altered enzyme activity (Khan and Goyal, 2009). A reduction in seed germination may be due to the effect of mutagen on the meristematic tissues of the radical or plumule (Talebi *et al.*, 2012). One of the physiological effects caused by treatment with these mutagens, particularly chemical mutagens, might be because of the disturbances in the formation of enzymes involved in the germination process (Deepika *et al.*, 2016). Similar inhibitory effects on seed germination by the different mutagenic treatments were reported earlier in onion Kulkarani (2011). If the higher dose was imposed, growth reduction would be the result in plants. Joshi *et al.* (2011).

Estimation of total protein of ATL-1

From seedlings exposed to various doses of gamma radiation, including 100, 200, 300, 400, 500, and 600 Gy, as well as control seedlings, the protein content was assessed. In all gamma ray treatments, the protein content was consistently lower than the control. In the control, protein concentration was measured at 26.73 mg/g FW. The amount of protein content decreased with higher doses of gamma irradiation due to this. The protein content was 25.72, 22.84, 21.17, 19.39, 17.68, and 15.36 in units of 100, 200, 300, 400, 500, and 600Gy respectively. In our present study, the estimation of total protein content was observed in the cultivar of ATL-1. In all the protein content was showed decreasing trend than control.

Total number of protein banding pattern of ATL-1

In ATL-1, the total number of protein bands in control was 5, with kDa values of 17.2, 26.9, 41.3, 57.6, and 82.5. In 100 and 200 Gy, the total number of bands was 4, with kDa values of 26.9, 43.3, 57.6, 82.5, and 26.9, 43.3, 57.6 and 82.5 respectively. In 300 Gy, the total number of bands

was 5, with kDa values of 17.2, 26.9, 41.3, 57.6, and 82.5. In 400 Gy, the total number of protein bands was 6, with kDa values of 17.2, 26.9, 41.3, 57.6, 82.5, and 132.8. In 500 and 600 gy, the total number of bands is 5.0 with kDa values of 26.9, 41.3, 57.6, 82.5, and 132.8, whereas kDa values of 26.9, 41.3, 57.6, 82.5, and 132.8 The kDa value of 17.2 was present in control, 300, and 400 Gy only. The kDa value of 132.8 was present only in 400, 500, and 600 Gy (Table-3 and Fig.-1).

Table 3. Effect of gamma rays on protein banding pattern and protein content *in Panicumsumatrense*.

S.NO	kDavalue	Control	100Gy	200 Gy	300Gy	400 Gy	500 Gy	600Gy
1	17.2	+	-	-	+	+	-	-
2	26.9	+	+	+	+	+	+	+
3	41.3	+	+	+	+	+	+	+
4	57.6	+	+	+	+	+	+	+
5	82.5	+	+	+	+	+	+	+
6	132.8	-	-	-	-	+	+	+
7	Protein content	26.73	25.72	22.84	21.17	19.39	18.68	15.36

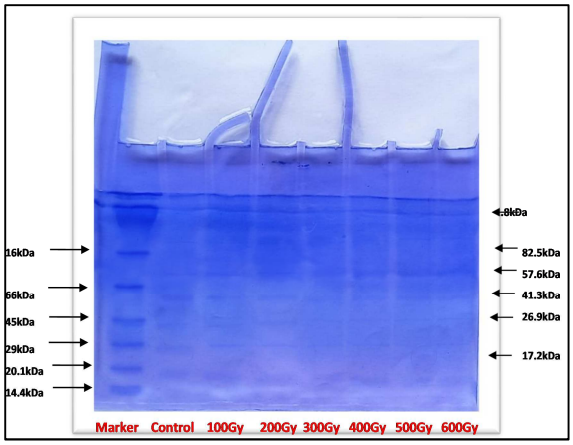

Fig. 1. Effect of gamma rays on SDS-PAGE analysis of little millet seedling cvATL-1.

On seedlings made from mutagens, morphological characteristics and SDS-PADE studies were carried out in the study's present part. Similar to this, Hegazi and Hamideldin (2010) observed that gamma irradiation caused variations in the number of protein bands in two types of okra. They discovered that there are fifteen polymorphic bands between the two kinds under consideration. Various therapies in Sabahia caused modifications to the protein pattern. According to (Sabahia and Balady), the higher gamma irradiation doses (400 and 500Gy) resulted in the most alterations. Like that of present study, Cholakova *et al.* (2003) observed alterations in the electrophoretic patterns of the soluble seeds proteins of isogenic pepper lines developed after gamma irradiation. Several authors reported SDS-PAGE analysis some important mutagen treated plants SDS-PAGE analysis was performed on samples of M1 generation okra leaves that had been exposed to gamma radiation by Manivel *et al.* (2022). The SDS-PAGE analysis of the treated population and control using the M1 generation leaf sample revealed the emergence and disappearance of several protein bands in the okra sample. It has been shown that gamma rays can modify protein patterns by causing some protein bands to appear or vanish. This conclusion was attained by Rashed *et al.* (1994). Like that of our study, Muniappan *et al.* (2022) studied the effect of dimethyl sulphate on SDS-PAGE analysis in mature embryos of groundnut (*Arachis hypogaea* L.) after seed treatment. The electrophoretic analysis showed differences in number of bands per samples and molecular weight of the band (kDa).

Conclusion

The present investigation, different doses of gamma radiation reduce the seedling growth, protein content and number of protein banding patterns. This investigation is useful to initiate mutation breeding programme in little millet.

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Conflict of interest

The authors declare that there is no conflict of interests.

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