Received: 27 Sep 2023 | Accepted: 31 May 2024 | Online: 03 Jun 2024

Electronic Journal of Plant Breeding

Research Note

Mutation effectiveness and efficiency in kodo millet (*Paspalum scrobiculatum* L.)

M. Vaithiyalingan*, A. Nirmalakumari, and V. Manimozhi Selvi

Centre of Excellence in Millets,Tamil Nadu Agricultural University, Athiyandal, Tamil Nadu – 606 603, India
***E-Mail**: mvaithiyalingan@tnau.ac.in

Abstract
Kodomillet is a staple food grain in semi-arid regions also known for its excessive free radical quenching capacity. Due to the cleistogamous flowering pattern, recombination breeding has limited scope in this crop. This study was formulated to induce genetic variation in kodo millet by induced mutagenesis involving two varieties, ATL 1 and CKMV 1. Seeds were irradiated with five different gamma-ray doses namely., 300 Gy, 400 Gy, 500 Gy, 600 Gy and 700 Gy, respectively and LD50 was determined based on reduction in root and shoot lengths. The gamma ray dosage and root and shoot lengths were observed to be inversely proportional. Chlorophyll mutants namely., *Albino, Xantha, Xantha viridis, Chlorina* and *Striata* were observed in all treatments except 700 Gy in both the genotypes in M_1 generation. Mutagenic effectiveness and efficiency were calculated based on the frequency of chlorophyll mutations in M_1 plants. Two early maturing novel mutants from ATL 1 and three high yielding mutants from CKMV 1 at 400 Gy dose and 500 Gy dose, respectively were isolated. Mutagenic treatments were efficient in creating genetic variation for morphological traits. These mutants would be useful in improvement of kodo millet.

Keywords: Chlorophyll mutants, Gamma rays, Kodo millet, Mutagenic effectiveness, efficiency

The small millets represent six crops, namely, finger millet, kodo millet, foxtail millet, little millet, proso millet and barnyard millet. Among the six crops, kodo millet (*Paspalum scrobiculatum L.*) is indigenous to India. Being a C4 plant, it gains importance as a crop suitable for changing climatic conditions as most of the arable land (69%) in India are arid and dry. Nowadays, as opposed to the main cereals, there is an impetus to develop millets because of the predominant nutritional values in these millets. It is been reported that kodo millet has more free radicals than other millets (Hegde *et al.*, 2005). In addition, it provides protein, minerals and vitamins at low cost in the form of healthy food (Yadava and Jain, 2006).

Genetic variability is essential for any crop improvement programme. The creation and management of genetic variability become fundamental to crop breeding in any crop and more so in crops like kodomillet, in which the available genetic variability is very limited owing to hermaphrodite and complete self-pollination. Due to its cleistogamous nature (Harinarayana,1989), induced mutation is the prudent approach to create genetic variation for important agronomic traits. Mutation is gene level alterations in the structure and position of DNA base pairs on chromosome. Changes within the DNA molecules are referred to as “point mutations” since they occur in a small portion of the DNA but may still have significant effect because they change the “meaning of the code.” This results in the alteration of phenotype of an organism. Physical mutagen like gamma rays can induce useful mutations in plants (Micke and Domini, 1993). Keeping in view, this study aims to induce mutations to isolate useful plant mutants which could be utilized for genetic improvement of kodo millet.

The variety CKMV 1 is characterised by erect growth habit, medium duration, dwarf plant type, irregular arrangement of grains in compact ear heads with dark brown seeds, and the variety ATL 1 is a medium duration, medium to tall plant stature with regular arrangement of grains in compact ear heads with light brown seeds. Three hundred seeds of both the varieties, exposed to five different dosages of gamma irradiation namely 300 Gy, 400 Gy, 500 Gy, 600 Gy, and 700 Gy at the Bhabha Atomic Research Centre (BARC), Mumbai. After irradiation, the treated seeds and untreated ones were sown on germination paper separately. Simultaneously, both irradiated and untreated seeds were raised in field during, *kharif,* 2022. The experiment was carried out at the Centre of Excellence in Millets, Athiyandal, Tamil Nadu .

M_1 generation: Seven days after sowing, germination, root and shoot length were measured in laboratory and germination was measured in field condition. According to Gustafsson's (1940) classification, chlorophyll mutants in M_1 generation were used to assess the mutagenic effect.

Mutation frequency was estimated as a percentage of M_1 plants, and mutagenic effectiveness and efficiency were determined using a formula proposed by Konzak, (1965) and Nilan *et al*. (1965).

Mutagenic Effectiveness = M/Dose of mutagen(krad)

Mutagenic Efficiencv = $M/LM/L$ (or) $M/I\ M/I$ (or) $M/SM/S$

Where,
M = Frequency expressed as percentage of chlorophyll mutation in M_1 generation, estimated on M_1 plant basis.
Krad = Kilo rad
L = Percentage of lethality or reduction in survival.
I = Percentage of injury or reduction in seedling height.
S = Percentage of panicle sterility.

M_2 generation: M_1 generation plants were harvested individually to rise M_2 generation. Two hundred M_2 seedlings of both the genotypes were planted with a spacing of 30 x 10 cm. Standard package of practices were followed throughout the crop period as per the TNAU crop production guide to raise a healthy crop. Biometric observation on days to 50 % flowering (days), days to maturity (days), plant height (cm), number of tillers per plant, panicle length (cm) and single plant grain yield (g) in all the plants in each treatment were recorded according to a descriptor (IBPGR, 1985).

Determination of LD_{50}, Mutagenic effectiveness and efficiency: Induced mutation is a powerful method to create genetic variability. Before embarking on mutation-breeding program, information on the relative effectiveness of the mutagens is essential to determine the correct dose/concentration of the mutagens.

In M_1, germination percentage, root length, shoot length, days to germination were recorded and vigour indices was calculated. Based on reduction in root and shoot length, the LD_{50} for ATL 1 and CKMV was determined as 500 Gy and 400 Gy, respectively (**Table 1**). Further, it was observed that the root and shoot length declined with increasing gamma ray exposure in a linear way.

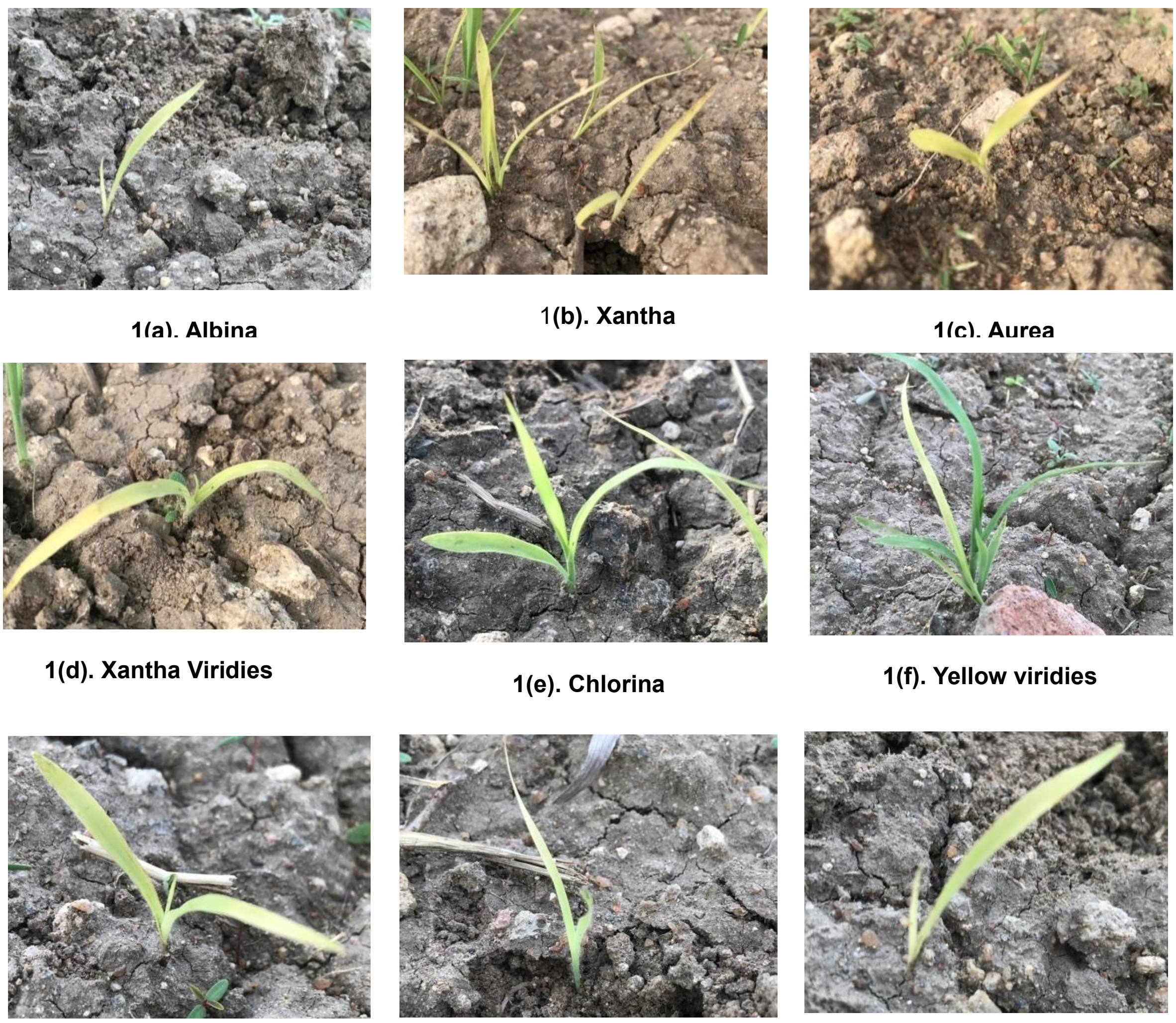
Mutagenic effectiveness and efficiency was observed to be genotype dependent, while at the dose of 700, there was no germination in both the varieties. The maximum panicle sterility was observed at the dose of 600 Gy, which resulted in the lowest mutagenic efficiency in both the genotypes. Maximum mutagenic efficiency and effectiveness were noted at the dosage of 300 Gy in both the varieties. Moreover, for both genotypes

Table 1. Root and shoot length and spectrum of chlorophyll mutation in of kodo millet through gamma irradiation

Dose	Variety	Root length (cm)	Shoot length (cm)	No. of mutant seedlings	*Albino*	*Xantha*	*Chlorina*	*Striata*	*Xantha Viridis*
Control	ATL 1	7.3	6.52	0	0	0	0	0	0
	CKMV 1	8.6	7.51	0	0	0	0	0	0
300 Gy	ATL 1	6.1	4.6	32	0	12	7	4	9
	CKMV 1	7.0	5.1	26	0	11	4	3	8
400 Gy	ATL 1	5.2	3.75	24	1	8	4	5	6
	CKMV 1	6.2	4.2	22	0	11	3	1	7
500 Gy	ATL 1	3.8	3.1	20	8	2	6	2	2
	CKMV 1	4.3	2.8	12	5	1	3	2	1
600 Gy	ATL 1	2.6	1.4	7	3	0	2	1	1
	CKMV 1	3.2	1.6	4	3	0	1	0	0
700 Gy	ATL 1				Not germinated				
	CKMV 1								

mutagenic efficacy was observed to decrease as gamma ray strength increased. Mutagenic efficiency was low at 700 Gy and 600 Gy dose in both the varieties. In studies on finger millet, Ambavane *et al*. (2014), Ganapathy *et al.* (2021) and Surashe *et al.* (2022) in sorghum revealed similar observations of a gradual decline in efficacy with higher levels of gamma ray irradiation.

Frequency and spectrum of Chlorophyll mutants: Different types of chlorophyll mutants namely Albina,xantha, Aurea, Xantha-Viridis, Chlorina, Yellow viridis, viridis, albina green, and striata (**Fig. 1**) were observed in all the treatments in M_1 generation. Among the treatments, 500 Gy and 600 Gy gamma irradiation dose produced high frequency of albino in both the varieties. In ATL 1, 500 Gy produced maximum number of albino mutants (20 mutants) (**Table 2**). The most common chlorophyll mutants discovered in the ATL 1 genotype were Xantha, Xantha viridies, Chlorina, and Striata. On the other hand, Xantha and Xantha viridis mutants were less common, and were discovered in response to the 300 Gy and 400 Gy treatments in CKMV 1. The genotype and mutagenic doses in M_1 generation both affected the frequency of chlorophyll mutations. ATL 1 had a slightly greater frequency of chlorophyll mutations than the CKMV1. It was made conceivable by the differences in the genetic make-up of the varieties that different genotypes responded to induction of chlorophyll mutations in different ways. In the current investigation, *albino* mutants were more common than *chlorina* and *striata*. Similar findings were reported earlier by Subramanian *et al.* (2011) in kodo millet; Ambavane *et al.* (2015) and Ganapathy *et al.* (2021) in finger millet; Ramesh *et al.* (2019) and Karthikeyan *et al.* (2022) in barnyard millet, Francis *et al.* (2022) in proso millet; Nirmalakumari *et al.* (2007) in little millet and Surashe *et al*. (2022) in sorghum.
Isolation of mutants for yield contributing traits: The mean



1(a). Albina **1(b). Xantha** **1(c). Aurea**

1(d). Xantha Viridies **1(e). Chlorina** **1(f). Yellow viridies**

1(g). Viridies **1(h). Albina green** **1(i). Striata**

Fig. 1. Different types of Chlorophyll mutants

Table 2. Chlorophyll mutation frequencies in M_1 generation of kodo millet through gamma irradiation

Dose	Genotype	Number of M_1 plants		Number of M_2 seedlings		Mutation frequency (%)	
		Studied	Chlorophyll mutants	Studied	Chlorophyll mutants	M_1 plant basis	M_2 seedling basis
Control	ATL 1	50	0	466	0	0	0
	CKMV 1	50	0	416	0	0	0
300 Gy	ATL 1	196	32	1377	52	16.32	3.77
	CKMV 1	190	26	1230	46	13.61	3.73
400 Gy	ATL 1	191	24	1000	30	12.56	3.0
	CKMV 1	182	22	987	28	12.08	2.83
500 Gy	ATL 1	174	20	950	25	11.49	2.63
	CKMV 1	179	12	827	21	6.70	2.53
600 Gy	ATL 1	82	7	144	2	8.53	1.38
	CKMV 1	67	4	79	1	5.97	1.26

Table 3. Mutagenic effectiveness and efficiency of gamma irradiation in kodo millet

Doses	Mutagenic effectiveness (%)		Mutagenic efficiency (%)	
	ATL 1	CKMV 1	ATL 1	CKMV 1
300 Gy	1.25	1.24	0.55	0.42
400 Gy	0.75	0.70	0.29	0.27
500 Gy	0.52	0.50	0.21	0.10
600 Gy	0.23	0.21	0.10	0.07
700 Gy	Lethal dose			

Table 4. Effect on yield contributing characters in M_2 generation of kodo millet (ATL 1 and CKMV 1)

Dose Characters	Genotypes	Control	300 Gy	400 Gy	500 Gy	600 Gy
Days to 50 % flowering (days)	ATL 1	80	72	60	65	66
	CKMV 1	75	65	61	50	55
Days to maturity (days)	ATL 1	125	112	110	105	97
	CKMV 1	120	109	105	100	92
Plant height (cm)	ATL 1	82	76	71	67	63
	CKMV 1	68	64	62	55	59
No. of productive tillers per plant	ATL 1	17	20	23	30	17
	CKMV 1	8	11	15	16	17
Panicle length (cm)	ATL 1	8.0	8.9	9.7	10.3	8.7
	CKMV 1	8.5	9.2	9.3	9.6	9.0
1000 grain weight (g)	ATL 1	5.0	5.2	5.5	5.4	5.1
	CKMV 1	5.2	5.3	5.6	6.0	5.8
Single plant grain yield (g)	ATL 1	17.8	19.3	20.7	22.4	18.1
	CKMV 1	20.5	21.3	22.8	26.0	19.4

performance of M_2 generation mutants for different yield contributing traits is furnished in **table 4**. In ATL 1 mutants, the days to 50 % flowering ranged from 66 DAS at 600 Gy to 60 DAS at 400 Gy dose. Substantial difference in plant height was observed at 600 Gy, and maximum number of tillers (30 numbers) was observed at 500 Gy. When compared to untreated control, the 600 Gy mutants had the maximum mean value for days to maturity (97 days). In case of the variety CKMV1, the dose 600 Gy, produced a novel mutant with maximum number of productive tillers

(17 nos), short duration mutant (92 days). Interestingly, two early mutants were observed at dose of 500 Gy in the varieties ATL 1 (97 days) and CKMV 1 (92 days), respectively. The findings from Nirmalakumari, *et al*. (2007) in little millet agree with this result.

In both genotypes, under all mutagenic treatments, the number of productive tillers was observed to increase in comparison to the control. While CKMV 1 recorded the maximum panicle length (9.6 cm) at 500 Gy, ATL1 recorded the maximum panicle length (10.3 cm). The effects of gamma radiation exposures were shown to vary according on genotype. With respect to bold grain types, ATL 1 mutants exhibited the maximum test weight (5.5 g) at 400 Gy, CKMV 1 recorded the maximum 1000 grain weight (6.0 g) at 500 Gy.

The study indicated that ATL 1 and CKMV 1, responded favourably to treatment with gamma irradiation. It was observed that 500 Gy was the effective and efficient gamma irradiation dose for creating kodomillet genetic variants in ATL 1 and CKMV 1 . The two early mutants with better performance for yield-contributing traits in the M_2 generation could be used for kodomillet breeding after conducting confirmative trails.

REFERENCES

Ambavane, A.R., Sawardekar, S.V., Sawantdesai, S.A. and Gokhale. N.B. 2015. Studies on mutagenic effectiveness and efficiency of gamma rays and its effect on quantitative traits in finger millet (*Eleusine coracana* L. Gaertn). *Journal of Radiation Research and Applied Sciences*, **8**(1): 120-125. [Cross Ref]

Francis, N., Ravikesavan, R., Iyanar, K., Raveendran, M., Chitdeshwari, T. and Senthil, A. 2022. Dose optimization, mutagenic effectiveness and efficiency of ems in proso millet (*Panicum miliaceum* L.). *Madras Agricultural Journal*, **109**(march (1-3)): 1.

Ganapathy, K.N., Patro, T.S., Palanna, K.B., Das, I.K., Elangovan, M., Prashant, B. and Tonapi, V.A. 2021. Development of improved mutants for grain yield and related traits in finger millet (*Eleusine coracana* L Gaertn.) through gamma mutagenesis. *International Journal of Plant Soil Science*, **33**(18): 225-235. [Cross Ref]

Gustafsson, A. 1940. The mutation system of the chlorophyll apparatus. *Lunds. Univ. Arrskr. N.F. Adv*, **36**: 1-40.

Harinarayana, G. 1989. Origin, evolution and systematics of Minor cereals. In: Seetharam, A., Riley, K.W., Harinarayana, G. (Eds) Small Millets in Global Agriculture (Ist Edn), Oxford and IBH Publishing Company, Delhi, India, pp 5-6.

Hegde, P.S. and Chandra, T.S. 2005. ESR spectroscopic study reveals higher free radical quenching potential in kodo millet (*Paspalum scrobiculatum*) compared to other millets. *Food Chem*. **92:** 177-182. [Cross Ref]

Karthikeyan, M., Vanniarajan, C., Vetriventhan, M., Nirmalakumari, A., Sundaram. R.S., Sivakumar, T. and Srinivasan, G. 2022. Mutagenic effectiveness and efficiency of chemical mutagens in barnyard millet (*Echinochloa frumentacea*). *Biological Forum An International Journal*, **14**(1): 470-476.

Konzak, C.F. 1965. Efficient chemical mutagenesis. In: The use of induced mutations in plant breeding. Report of the FAO/IAEA technical meeting organized by the food and agriculture organization of the United Nations and the International Atomic Energy Agency in cooperation with the European Association for Research on Plant Breeding, Rome, Italy: FAO

Micke, A. and Domini, B. 1993. Induced Mutations. In. M.D. Hyward, N.O. Bosemark. And I. Ramagosa (Eds.), Plant breeding principles and prospects (pp. 52-62). London: Chapman and Hall. [Cross Ref]

Nilan, R.A., Konzak, C.F., Wagner, J. and Legault, R.R. 1965. Effectiveness and efficiency of radiations for inducing genetic and cytogenetic changes. The Use of Induced Mutations in Plant Breeding. *Radiation Botany*, **5**: 71-91.

Nirmalakumari, A., Arulselvi, S., Ganapathy, S., Souframaniyan, J., Senthil, N. and Devan, P. 2007. Gamma ray induced variation for lodging resistance and its associated characters in little millet (*Panicum sumatrense* Roth ex. Roem and Scultz.) *Madras Agricultural Journal*, **94**(7-12): 151-155. [Cross Ref]

Ramesh, M., Vanniarajan, C., Ravikesavan, R., Aiyan, K.E. and Mahendran, P.P. 2019. Determination of lethal dose and effect of EMS and gamma ray on germination percentage and seedling parameters in barnyard millet variety Co (KV) 2. *Electronic Journal of Plant Breeding*, **10**(2): 957-962. [Cross Ref]

Subramanian, A., Nirmalakumari, A. and Veerabadhiran, P. 2011. Mutagenic efficiency and effectiveness in kodomillet (*Paspalum scrobiculatum* L.). *Madras Agricultural Journal*, **98**(1-3): 22-25. [Cross Ref]

Surashe, S. M., Kalpande, H. V., Ashok Badignnavar, Ambika More and Ganapathi, T. R. 2022. Mutation induced phenotypic variation for yield traits in sorghum [*Sorghum bicolor* L. Moench].*Electronic Journal of Plant Breeding,* **13**(2): 312-318. [Cross Ref]

Yadava, H.S. and Jain, A.K. Advances in kodo millet research ISBN: 81-7164-062-1 Directorate of Information and Publications of Agriculture. Indian Council Agricultural Research, 2006.