

Original Research Article

Studies on mutagenic effectiveness and efficiency of gamma rays and EMS in Kodo millet (*Paspalum scrobiculatum*)

Comment [w1]: Title may be more effective

Abstract

Mutation breeding is the defined approach used for crop production and has played a key role in the creation of several varieties. The present research was carried out to induce mutants in Kodo millet variety CO 3 using a physical mutagen (gamma rays) and a chemical mutagen (Ethyl Methane Sulphonate) for creating novelty. Seeds were treated with five doses of gamma-rays viz., 100 Gy, 200 Gy, 300 Gy, 400 Gy, and 500 Gy at BARC, Kalpakam and with three concentrations of EMS viz., 24.15 mM, 32.20 mM and 40.25 mM. In the laboratory test, root and shoot lengths of seedlings were decreased with an increase in the dose of gamma rays and EMS. In field study, the germination percentage and survival rate of seedlings were decreased with an increase in the dose of gamma irradiation and EMS. In M₂ generation, five types of chlorophyll mutations viz., *albino*, *xantha*, *striata*, *chlorina* and *albomaculata* were observed. *Xantha* and *chlorina* were observed in all treatments, whereas, *striata* and *albomaculata* were observed only in 200 Gy. Based on the biological damages on M₂ plants, mutagenic effectiveness and efficiency were estimated. Both mutagenic effectiveness and efficiency reduced with the increase in the dose of irradiation. Regarding height reduction and lethality, the dose of 100 Gy in gamma treatment and 32.20 mM concentration in EMS treatment recorded maximum efficiency. The mutagenic effectiveness was found to be higher at gamma rays irradiated with 100 Gy and in EMS, in 24.15 mM concentration. The 100 Gray dose and 24.15 mM concentration was found to be highly effective for inducing mutation in Kodo millet.

Comment [w2]: Use appropriate term "improvement"

Key words: Kodomillet, Induced mutation, Biological damage, Mutagenic effectiveness

Introduction

Kodo millet, *Paspalum scrobiculatum* L. (2n=4x=40) [12], member of the family Poaceae, is an annual grain that is grown primarily in India. It is widely distributed across the tropics and subtropics of the world in moist environments. The genus *Paspalum* (Gramineae) contains about 400 species and is generally spread in the colder regions of the planet. It was domesticated about 3000 years ago in India, the only country today where it is cultivated as a grain in large amounts, mostly on the Deccan plateau [4]. It is cultivated primarily in India, Philippines, Indonesia, Vietnam, Thailand, and in West Africa. It is grown today from Uttar Pradesh to Bangladesh in the north and Kerala and Tamil Nadu in the south. All over India, small millets occupy an area of 6.74 lakh ha with an annual production of 4.13 lakh tones and average productivity of 613 kg/ha. Among the small millets, Kodo millet has the high productivity per unit area. Apart from its traditional role as a staple food for the poor in the marginal agricultural regions; they are gaining a new role as a nutritional food for the urban high-income people.

Comment [w3]: Use more suitable words instead of grain. May be "millet crop" or "grain crop"

Comment [w4]: Use full stop "." at end of sentence

As genetic variability is indispensable for any crop improvement program, the establishment and management of genetic variability evolves as a central base to crop breeding, in any crop and more so in crops like Kodo millet, in which the available genetic variability is very narrow due to self-pollination in this crop because of its cleistogamous nature. Out of the approaches to generate genetic variability, the induced mutation is a crucial approach. The generation of variations by induced mutagenesis has been a base for strengthening the breeding programs. Various classes of physical and chemical mutagens have been utilized to induce variations

that differ in their nature of inducing mutations and also in the spectrum of mutation-induced. Ionizing mutagens remain the earliest in the order among the mutagens for inducing variability [9,11] and several chemicals like Ethyl Methyl Sulphate (EMS) are equally efficient mutagens [14, 16].

The specification of the M₁ generations assists in analyzing the effectiveness and efficiency of the mutagens, as well as in labeling the plants, in M₂ and M₃ generations, with maximum genetic damage that is probably to carry a high frequency of micro mutations. It is vital to recover the high frequency of the desirable mutations, for any mutation breeding events it is imperative to select effective and efficient mutagen [24]. Hence, the fundamental prerequisite on mutagenic sensitivity, effectiveness, and efficiency of the mutagen, breeding procedure, treatment methods necessary to boost the mutation induction is indispensable for the favorable outcome in any mutation breeding program. Efficiency of a mutagenic agent is of a complex nature, as it does not depend on the reactivity of the agent with the material and on its applicability to the biological system but also on the degree to which physiological damage, chromosomal aberration and sterility are induced in addition to mutations. The mutagenic efficiency gives an idea of the proportion of mutations in relation to other associated undesirable biological effects such as injury, lethality and sterility induced by the mutagen [14]. Efficient mutagens and their treatments are indispensable for the cost-effective use of the mutagen as a tool for the induction of mutations and their direct and indirect utilization in successful breeding programme. The present study was attempted to comprehend the response of Kodo millet variety CO3 to physical (gamma rays) and chemical (EMS) mutagens with the perspective to determine the mutagenic treatment with a high frequency of mutations in M₂ generation.

Comment [w5]: Remove "s" from events

Material and Methods

One promising variety of Kodo millet viz., CO 3 was obtained from the Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. The investigation envisaged on studying the differential sensitivity of Kodo millet variety CO 3 by subjecting it to five doses of gamma-rays viz., 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy of physical (Gamma rays) and three doses viz., 24.15 mM, 32.20 mM and 40.25 mM of chemical mutagen (Ethyl Methane Sulphonate). The treated seeds were raised during Rabi 2017, in the field at the rate of 100 seeds per replication with untreated seeds as check as M₁ generation. The M₂ generation was raised from individual M₁ plants following plant to progeny method in summer 2017. The occurrence of chlorophyll mutants was observed in the seedlings at 2 to 3 leaf stage to assess the effect of mutagen on the biological material. The total number of M₂ seedlings expressing chlorophyll mutation were calculated and expressed as mutation frequency.

Comment [w6]: Pl. check and correct it carefully if treated seeds planting in Rabi 2017 how it is possible to raise the M2 generation in summer 2017.

CHART 1. Types of chlorophyll mutants [10]

Mutant	Description
<i>Albina</i>	White colored seedlings
<i>Xantha</i>	Yellow colored seedlings
<i>Chlorina</i>	Light green colored seedlings
<i>Albomaculata</i>	White dots on green leaves
<i>Striata</i>	Striped leaves

<i>Alboviridis</i>	Initially white and later becomes normal plants
<i>Xanthoviridis</i>	Initially yellow and later become normal plants

The spectrum of distinctive mutation types were recorded [10] and their frequency was worked out, followed by calculating the mutagenic effectiveness and efficiency of the two mutagens namely, gamma rays and EMS taking into account the number of mutant plants observed, according to the formula [14].

Mutagenic effectiveness (%)

$$\text{Gamma rays} = \frac{\text{Mp} \times 100}{\text{kR (or) Gy}}$$

$$\text{EMS} = \frac{\text{Mp} \times 100}{\text{c} \times \text{t}}$$

Comment [w7]: Symbol "x" may be used instead of x wherever in the whole text for sign of multiply

Where,

Mp - Chlorophyll or viable mutation frequency on M₂ plant basis

c - Concentration of the chemical mutagen in mM

t - Duration of treatment with chemical mutagen in hours

Gy - Dose of gamma radiation

Mutagenic efficiency (%)

$$\text{Gamma rays and EMS} = \frac{\text{Mp} \times 100 / \text{L}}{\text{Mp} \times 100 / \text{I}}$$

here,

Mp - Chlorophyll or viable mutation frequency on M₂ plant basis

L - Percentage of lethality *i.e.*, percentage of reduction in survival of seedlings on 20th day

I - Percentage of injury *i.e.*, percentage of height reduction of seedlings on 20th day

Results and Discussion

In any mutation breeding experiment, the mutagenic treatments (mutagens, their doses and method of application) should be effective in inducing genetic changes. Almost all reports on induced mutation studies in different crop plants have shown physiological damage due to mutagenic effects, thereby reducing germination, survival, plant growth and reproductive traits [5]. [23] It is reported that physical and chemical mutagens induce physiological damage (primary injury), factor mutation (gene mutation) and chromosome mutation (chromosomal aberrations) in the biological material.

Physical mutagens induce gene mutations and chromosomal aberrations [5] whereas chemical mutagen *viz.*, EMS alkylates guanine bases and leads to mispairing, causing transitions [2] in biological materials in the

M₂ generation. The efficiency of mutagens can be assessed by quantitative calculation in the M₂ loss and this can be achieved by evaluating the reduction of germination, survival (lethality), and seedling height (injury) at various stages of plant development [3] and these were included in the present analysis.

Relative percentage of chlorophyll mutations

The present study on induced mutations with gamma rays and EMS involved in the determination of frequency and spectrum of chlorophyll mutants in M₂ generation of CO 3. The frequency of chlorophyll mutations varied with the mutagen doses in M₂ generation. The frequency and spectrum of induced chlorophyll mutations treated with gamma rays and EMS revealed that, several chlorophyll mutants like *albino*, *xantha*, *striata*, *chlorina* and *albomaculata* were observed in the M₂ generation (Fig. 3), among the five classes of chlorophyll mutants the frequency of *chlorina* was found to be maximum with a frequency of 2.83 in gamma irradiation and 2.54 in EMS. This result clearly indicated that the maximum frequency of *chlorina* was higher than other four classes of chlorophyll mutants (Table 1). Among the treatments, the 200 Gy, 400Gy and 32.20 mM produced high frequency of *chlorina*. Next common chlorophyll mutant observed was *xantha* in both mutagens. Albino mutants were less. The least reported mutant was *albomaculata* which was visible in 100Gy 200 Gy, 24.15 mM and 32.20 mM (Fig. 1& 2). The frequency of chlorophyll mutations was higher in physical mutagen *viz.*, gamma rays than EMS [26]. Several workers also reported a higher frequency of chlorophyll mutant in irradiated population; in kodo millet [26] and in finger millet [1, 8, 15, 17, 21, 25 and 28] induction of similar type of chlorophyll mutations like *albino*, *xantha*, *chlorina*, *striata* and *viridis* were reported.

The proportion of *albino* and *xantha* was high in 100 Gy in physical mutagen and the proportion of *xantha* and *chlorina* was high in 32.20 mM in chemical mutagen. The manifestation of more than one class of chlorophyll mutants in the same dose may be due to occurrence of concurrent mutation in more than one locus [26] in Kodo millet. In both the mutagens there was no unequivocal connection between the frequency of mutation and the dose of the mutagen.

The hike in the mutation frequency was shown to be accompanied by the damages in M₁ plants [13]. To comprehend, the increase in chlorophyll mutation frequency accompanied an increase in dosage up to certain extent, further which it displayed a dropdown. This exhibits a bottleneck at certain dosage. This decrease at higher dose level was associated to the accuracy of diplontic and haplontic selections in the treated materials [27]. This is because in the post-irradiation treatment, of a multicellular tissue the mutated cell has to challenge with unaffected cells and, by eradication due to haplontic or diplontic selection [6].

Mutagenic effectiveness and efficiency

The practical usability of any mutagen would rely on its effectiveness and efficiency [14]. The term “effectiveness” is a measure of gene mutations in relation to dose and “efficiency” as an estimate of biological effects induced such as lethality and injury. With reference to height reduction and lethality, the dose 100 Gy (seedling height-2.56, survival reduction-12.61) in gamma treatment and 32.20 mM (seedling height-2.90, survival reduction-2.94) concentration in EMS treatment was observed to record maximum efficiency. The mutagenic effectiveness was found to be higher at gamma rays irradiated with 100 Gy (1.39) whereas in EMS concentration of 24.15 mM (6.92) (Table 2). It was also noticed that mutagenic effectiveness decreased with an increase in the strength of gamma rays and EMS. Similar observations of a general decrease in effectiveness

with increasing doses of gamma rays irradiation were reported in Kodo millet [26], in finger millet [1, 16] and in foxtail millet [9]. A similar observation has been recorded in pulses [7, 22]. Thus, mutagenic effectiveness and efficiency play an essential role in any mutation breeding program for choosing the best dose or dose concentration while working with physical and chemical mutagens.

Conclusion

The dependability and outcome of any mutation breeding rely upon the effectiveness and efficiency of the mutagen. Induction of chlorophyll mutations, in general, is considered as a measure to assess the effectiveness of mutagen and mutability of the variety. A quick review of the literature shows a shortage of evidence on the safe and efficient dosage of gamma radiation for kodo millet. *Chlorina* mutants occurred at higher levels in both the treatments relative to other forms of chlorophyll mutants. The dose of 311Gy for gamma rays and 32.20 mM was recorded as the LD₅₀ dose for kodo millet [19, 20]. Based on the experimental outcomes of this study we come to a denouement that, for getting viable mutants in kodo millet, the doses from 100 Gy to 300 Gy would be suitable [18] and with respect to chemical mutagen the concentration 32.20 mM would be an ideal one to achieve useful mutants. More investigation of the extant mutants would yield several helpful macro and micro mutations that could be used in the kodo millet breeding programmes in the future

Comment [w8]: Use full stop "." at the end of sentence

References

1. Ambavanae AR, Sawardekar SV, Sawantdesai SA, Gokhale NB. Studies on mutagenic effectiveness and efficiency of gamma rays and its effect on quantitative traits in finger millet (*Eleusine coracana* L. Gaertn). J of Rad Res and Appl Sci.2014; 1-6.
2. Bhat R, Upadhyaya N, Chaudhury A, Raghavan C, Qiu F, Wang H, Wu J, McNally K, Leung H, Till B. Chemical and irradiation-induced mutants and TILLING. Rice Fun Genomics. 2007; 148-180.
3. Boureima S, Diouf M, Silme RS, Diop T, Van Damme P, Cagiran MI. Radio sensitivity of African Sesame cultivars to gamma rays. Turkish J Field Crops. 2009; 14:181-190.
4. de We JMJ, Rao KEP, Mengesha MH, Brink DE. Diversity in kodo millet, *Paspalum scrobiculatum*. Econ Bot. 1983; 37:159-163.
5. Gaul H. Mutagen effects observable in the first generation. In: Manual on mutation breeding, International Atomic Energy Agency, Vienna, 1970; 119:85-89.
6. Gaul H. Studies on diplontic selection after X-irradiation of barley seeds. In: Effects of ionizing radiation on seeds. Proc. IAEA/FAO Symp. 1961. pp. 117-138.
7. Girija M, Dhanvel D. Mutagenic Effectiveness and Efficiency of gamma rays, EMS and their combined treatments in cowpea (*Vigna unguiculata* L. Walp.). Global J Mol Sci. 2009; 4:68-75.
8. Gowda MB. Studies on mutation frequency and rectification of defects in four finger millet cultivars following gamma irradiation. Mysore J Agric Sci. 1987; 21(1):93-94.
9. Gupta PK, Yashvir. Induced mutations in foxtail millet (*Setaria italica* Beauv.). Chlorophyll mutations induced by gamma rays, EMS and DES. Theoretical and Applied Genetics. 1975; 45(6): 242-249.
10. Gustafsson A. The mutation system of the chlorophyll apparatus. Lond Univ. Arsskr., 1940; 36:1-40.
11. Hayat K, Khan A, Sadiq M, Elahiand F, Shakoor A. Gamma radiation induced variation in sorghum cultivar. Pak J of Agri Sci. 1990; 11(1): 13-16.
12. Hiremath SC. and Dandin SB. Cytology of *Paspalumscrobiculatum* Linn. Curr Sci. 1975; 44:20-21.

13. Kawai T, Sato H. Some factors modifying the effects of radiation in seed treatment in rice. In: Mutation in Plant Breeding, IAEA, Vienna, pp. 1996; 151-171.
14. Konzak CP, Wagner RA, Nilan J, and Foster RJ. Efficient chemical mutagenesis. Radiation Botany, 5(Suppl.), 1965; 49-70.
15. Kumar B, Mahto JL, Haider ZA. Induced mutation studies in finger millet (*Eleusine coracana* Gaertn.). Indian J Genet. 1996; 56(4):526-532.
16. Muduli KC, Misra RC. 2007. Efficacy of mutagenic treatments in producing useful mutants in finger millet (*Eleusine coracana* Gaertn.). Indian J of Genet and Plant Breed. 2007; 67(3): 232-237.
17. Parida D, Mohapatra BK. Micromutational variability induced by gamma rays, EMS and NG in finger millet. In: National seminar on small millets-current research trends and future priorities as food, feed and in processing for value addition held at Univ. of Agric. Sci., Bangalore, India, April 23-24; 1997:56.
18. PoornimaJency J, Ravikesavan R, Roshan KS, Raveendran M, Jeyakumar P, Muthamilarasan M, Manoj P, Jeeva G. Induced Mutagenesis Enhances Lodging Resistance and Photosynthetic Efficiency of Kodomillet (*Paspalum Scrobiculatum*). Agronomy. 2020; 10: 227.
19. PoornimaJency J, Ravikesavan R, Sumathi P, Raveendran M. Determination of lethal dose and effect of physical mutagen on germination percentage and seedling parameters in kodo millet variety CO 3. Electron J Plant Breed. 2016; 7: 1122–112.
20. PoornimaJency J, Ravikesavan R, Sumathi P, Raveendran M. Effect of chemical mutagen on germination percentage and seedling parameters in Kodo millet variety CO 3. Int J Chem Std. 2017; 5: 166–169.
21. Prusty K. Induced genetic variability in ragi: Study of M₁ and M₂ generations. M.Sc. (Ag.) Thesis, QUAT, Bhuvanesar; 1999.
22. Sharma SK, Sood R, Pandey DP. Studies on mutagen sensitivity, effectiveness and efficiency in urdbean (*Vignamungo* (L.) Hepper). Indian J Genet. 2005; 65:20-22.
23. Singh B, Mohapatra BK. Prediction of M₂ mutation frequency based on M₁ estimates in black gram. Legume Res. 2004; 27(2): 137-139.
24. Solanki IS, Sharma B. Mutagenic effectiveness and efficiency of gamma rays, ethyleneimine and N-nitroso-Nethyl urea in macrosperma lentil (*Lens culinaris* Medik.). Indian J of Genet and Plant Breed. 1994; 54(1):72-76.
25. Sreekantaradhya R, Menon PM. Rosette habit an induced physiological mutation in ragi or finger millet (*Eleusine coracana* (L.) Gaertn.) with gamma rays and EMS-II Chlorophyll mutation frequency spectrum. Environ Exp Bot. 1979; 19(3):123-126.
26. Subramanian A, Nirmalakumari A, Veerabhadhiran P. 2011. Mutagenic efficiency and effectiveness in kodo millet (*Paspalum scrobiculatum* L.). Madras Agric J. 2011; 98(1-33): 22-25.
27. Swaminathan MS. 1961. Effect of diplontic selection on the frequency and spectrum of mutations induced in polyploids following seed irradiation. In: Effects of Ionizing Radiations based on seed, IAEA, Vienna; 1961. pp. 279-288.
28. Tikka SBS. Mutation research in finger millet. Sarada Publishing Academy, Sidhapur, Gujarat; 1985.

Table 1 Frequency and spectrum of chlorophyll mutants in M₂ generation of Kodo millet variety CO 3

Mutagen	Classes of chlorophyll mutants					Number of chlorophyll mutants	Number of plants observed	Relative percentage (frequency) of chlorophyll mutants					Mutagenic frequency
	A	X	S	C	Al			A	X	S	C	Al	
Gamma rays (Dosage)													
Control	0	0	0	0	0	0	1000	0	0	0	0	0	0
100Gy	2	6	0	4	0	12	861	0.23	0.70	0	0.46	0	1.39
200Gy	6	13	3	18	3	43	2658	0.23	0.49	0.11	0.67	0.11	1.62
300Gy	0	1	0	1	0	2	216	0	0.47	0	0.47	0	0.93
400Gy	0	1	0	2	0	3	162	0	0.62	0	1.23	0	1.85
						60		0.46	2.28	0.11	2.83	0.11	5.79
EMS (Concentration)													
Control	0	0	0	0	0	0	1000	0	0	0	0	0	0
24.15mM	11	22	4	27	2	66	3975	0.28	0.55	0.10	0.68	0.05	1.66
32.20mM	12	28	1	38	1	80	3963	0.30	0.70	0.03	0.98	0.03	2.04
40.25mM	8	15	1	20	0	44	2274	0.35	0.66	0.04	0.88	0	1.93
						213		0.93	1.91	0.17	2.54	0.08	5.63

Comment [w9]: Mutagens instead of Mutagen

Table 2. Mutagenic effectiveness and efficiency based on chlorophyll mutations in M₂ generation of Kodomillet

Mutagen	Seedling Height (cm) (I)	% survival reduction (L)	Mutation frequency (M)	Effectiveness	Efficiency	
				$(M \times 100) / C \times t$ / Gy or	$(M \times 100) / I$	$(M \times 100) / L$
Gamma rays (Dosage)						
Control	86	0	0	0	0	0
100Gy	54.14	11.02	1.34	1.39	2.56	12.61
200Gy	66.63	22.60	1.62	0.81	2.43	7.16
300Gy	63.19	40.68	0.93	0.31	1.47	2.28
400Gy	58.83	73.45	1.85	0.46	3.14	2.52
EMS (Concentration)						
Control	86	0	0	0	0	0
24.15mM	71.86	60.00	1.66	6.92	2.31	2.77
32.20mM	70.31	68.00	2.04	6.38	2.90	2.94
40.25mM	74.65	71.00	1.93	4.83	2.59	2.72

Comment [w10]: Symbol "x" may be used instead of x wherever in the whole text for sign of multiply

UNDER PEER REVIEW

Fig 1 Relative percentage (frequency) of chlorophyll mutants observed in M₂ plants treated with gamma rays

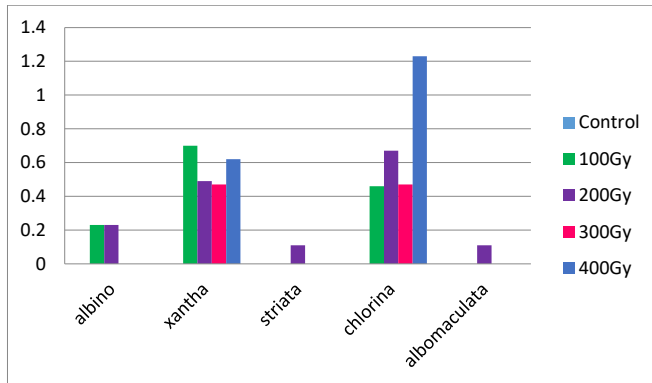


Fig 2 Relative percentage (frequency) of chlorophyll mutants observed in M₂ plants treated with Ethyl Methyl Sulphate (EMS)

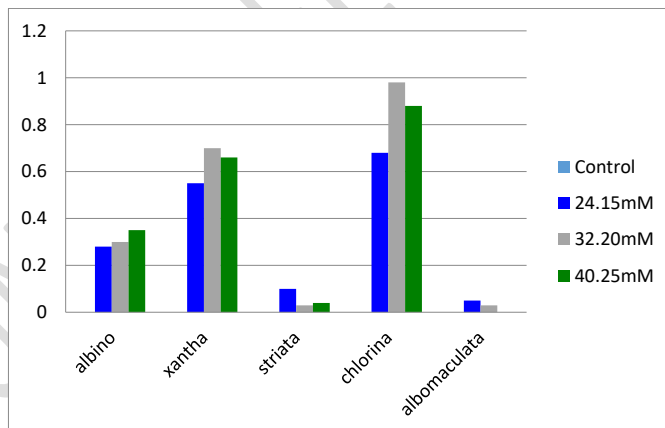


Fig. 3 Chlorophyll mutants observed in M₂ generation



albino



xantha



striata



albomaculata



Control

UNDER PEER REVIEW

