Original Research Article

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

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 M_2 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 M_2 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. .

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

Introd**uction**

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.

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 M_1 generations assists in analyzing the effectiveness and efficiency of the mutagens, as well as in labeling the plants, in M_2 and M_3 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 depends 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 M_2 generation.

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 M_1 generation. The M_2 generation was raised from individual M_1 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 M_2 seedlings expressing chlorophyll mutation were calculated and expressed as mutation frequency.

CHART 1. Types of chlorophyll mutants [10]

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

Alboviridis	Initially white and later becomes normal plants
Xanthoviridis	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 (%)

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

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

Where,

Mp - Chlorophyll or viable mutation frequency on M_2 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 M_2 plant basis

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

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

M_2 generation. The efficiency of mutagens can be assessed by quantitative calculation in the M_2 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 M_2 generation of CO 3. The frequency of chlorophyll mutations varied with the mutagen doses in M_2 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 M_2 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 M_1 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 LD_{50} 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

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Table 1 Frequency and spectrum of chlorophyll mutants in M_2 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
Gamma rays (Dosage)	*A*	*X*	S	*C*	Al			A	X	S	C	Al	
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

Table 2. Mutagenic effectiveness and efficiency based on chlorophyll mutations in M_2 generation of Kodomillet

Mutagen	Seedling Height (cm) (I)	% survival reduction (L)	Mutation frequency (M)	Effectiveness (M x 100) / Gy or C x t	Efficiency	
					(M x 100) / I	(M x 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

Fig 1 Relative percentage (frequency) of chlorophyll mutants observed in M_2 plants treated with gamma rays

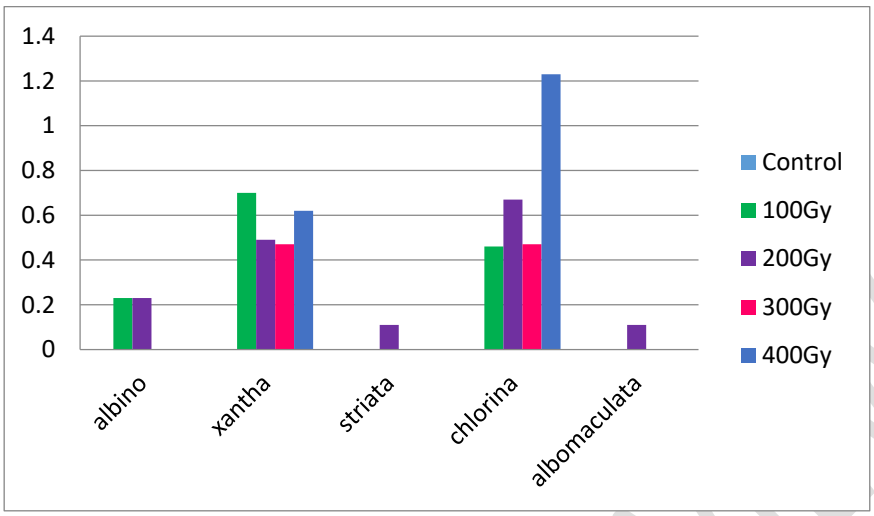


Fig 2 Relative percentage (frequency) of chlorophyll mutants observed in M_2 plants treated with Ethyl Methyl Sulphate (EMS)

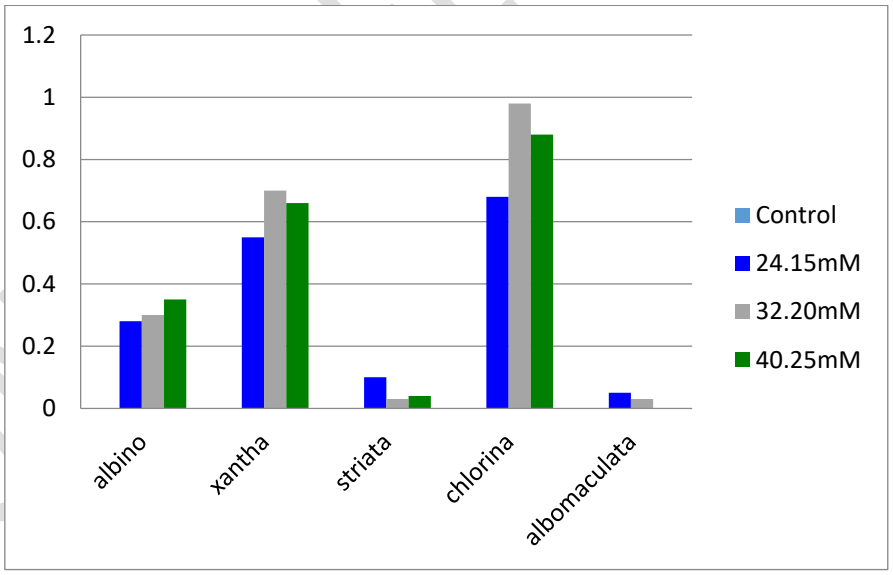
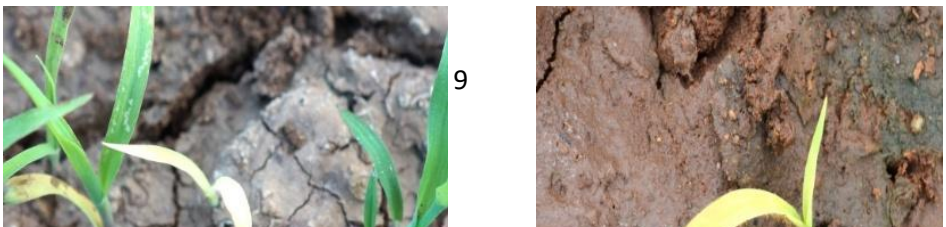


Fig. 3 Chlorophyll mutants observed in M_2 generation



albino *xantha*

striata *albomaculata*

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Control