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Mutagenic Efficiency and Effectiveness in Kodomillet (*Paspalum scrobiculatum* L.)

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Mutagenic effectiveness and efficiency of Gamma ray was studied in two genotypes of Kodomillet (*Paspalum scrobiculatum* L.) *viz*., CO 3 and TNAU 51. Seeds of the two genotypes were subjected to gamma irradiation at a dose of 400, 500, 600, 700 and 800 Gray .The mortality percentage, lethality and injury in M_1 seedlings were found to have linear relationship with the dose of gamma rays. Considering the mortality and mutation frequencies, the dose 500 Gray was found to be highly efficient. Chlorophyll mutants like *Albino, Xantha, Chlorina*, *Striata* and *Viridis* were found to occur in varying proportions in the M_2 generation. The occurrence of more than one type of chlorophyll mutants in the same dose might be due to simultaneous mutations in more than one locus. The mutagenic effectiveness and efficiency were calculated based on biological damage. Both mutagenic effectiveness and efficiency reduced with the increase in dose of irradiation. In the present investigation, 400 Gray dose was observed to be highly efficient in terms of survival reduction for CO3 while for TNAU 51, 800 Gray dose was observed to be highly efficient. With reference to height reduction, 800 Gray dose recorded maximum efficiency. The 800 Gray dose was found to be highly effective for inducing mutation in both the genotypes. But, for optimum recovery of viable mutants, 500 Gray dose was found to be effective dose in kodomillet.

Keywords: Kodomillet, Gamma Rays, Mutation, Effectiveness, Efficiency

Kodomillet (*Paspalum scrobiculatum* L.), a member of the family Poaceae, was domesticated in India some 3,000 years ago (Malleshi and Hadimani, 1994) and is cultivated as agricultural crop in parts of Madhya Pradesh, Maharashtra, Uttar Pradesh, Gujarat, Rajasthan and Tamil Nadu (de Wet *et al*., 1983). It forms the main stay of the dietary nutritional requirements of farmers of marginal and dry lands in many parts of India It occupies an area of 9.08 lakh ha with an annual production of 3.11 lakh tones and average productivity of 342 kg/ha. Among the small millets, productivity per unit area is highest in kodomillet (Ahamed and Yadava, 1996). The crop matures in 3-4 months with average yield varying from 250 to 1000 kg/ha and a potential yield of 2000kg/ha (Harinarayana,1989). It has a 1000 grain weight of 6.7 g.

As genetic variability is essential for any crop improvement programme, the creation and management of genetic variability becomes central base to crop breeding in any crop and more so in crops like kodomillet, in which the available genetic variability is very limited owing to complete self pollination in this crop due to its cleistogamous nature (Harinarayana,1989). Among the approaches to create genetic variability, induced mutation is an important approach. In a mutation breeding experiment, selection of effective and efficient dose of mutagen is very essential to recover high frequency of desirable mutants. Since no information regarding the effective and efficient dose of gamma ray irradiation for kodomillet is available, the present investigation was undertaken to assess the frequency of mutants, effectiveness and efficiency of different doses of gamma ray irradiation.

Materials and Methods

The study was initiated with two Kodomillet genotypes *viz.*,CO3 and TNAU 51. Two samples of 400 seeds each of the above genotypes at a moisture level of eight percent, obtained from Small Millets unit, Department of Millets of Tamil Nadu Agricultural University, Coimbatore were irradiated in 400,500,600,700 and 800 Gray doses of Gamma rays with CO^{60} source in the gamma chamber of Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. The irradiated seeds were immediately raised, during *Kharif*, 2008, in the field and lab in roll towel method in two replications at the rate of 100 seeds per replication along with un-irradiated checks. Observation

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regarding percentage mortality, both under lab and field conditions, were recorded in the M_1 seedlings. Observations were also recorded regarding the relative reduction in survival on 20 days after sowing and reduction in plant height on 30 days after sowing in field condition. The M_1 plants were forwarded on ear to row basis and the resultant M_2 population was sown during *Kharif,* 2009. Observation regarding occurrence of chlorophyll mutants in M_2 was recorded from ten days after sowing. The total number of M_2 seedlings and the seedlings expressing chlorophyll mutation were counted and expressed as mutation frequency. The spectrum of different mutation types were scored according to the system developed by Gustaffson (1940) and their frequency was worked out. Based on the number of mutant plants observed, the mutagenic effectiveness and efficiency of the dose was calculated according to the formula suggested by Konzak *et al*.,(1965).

Results and Discussion

Kodomillet being small millet with maximum productivity in an unit area (Ahamed and Yadava, 1996) is a best suited crop for farmers of marginal and tribal lands for sustenance of nutritional and livelihood security. Since this crop is highly self pollinated and cleistogamous, the variability available for breeding program is very less. To induce variability, mutagenesis using gamma rays was attempted.

Table 1 . Effect of gamma rays on growth parameters in M_1 generation

Sl. No.	Dose (Gy)	Mortality percentage under field condition		Mortality percentage under lab condition		Survival reduction on 20th day (L)		% reduction of plant height on 30th day (I)	
		CO3	TNAU 51	CO3	TNAU 51	CO3	TNAU 51	CO3	TNAU 51
1	Control	10.6	9.0	2.8	5.2	-	-	-	-
2	400	23	29.7	18.3	15.7	16.3	22.6	21.2	26.1
1	500	45	54.4	48.3	58.1	42.4	44.4	28.3	28.6
2	600	56	63.4	67.9	61.3	55.2	63.0	38.2	35.5
3	700	65	71.0	84.2	79.8	76.0	76.1	39.1	43.6
4	800	86.3	92.0	95.1	91.3	84.9	87.4	41.9	45.7

Physical mutagens induce gene mutations and chromosomal aberrations in biological materials in the M_1 generation (Gaul,1970). The efficiency of mutagens can be estimated by quantitative determination of M_1 damages and this can be done by analyzing reduction in germination, survival (Lethality) and seedling height at various stages of plant growth (injury) (Boureima *et al*.,2009) and these were considered in the present study. Among the doses tested, it was observed that the reduction in germination ranged from 23 percent in 400 Gray to 86.3 percent in 800 Gray for variety CO3. In case of pre release culture TNAU 51, it ranged form 29.7 to 92.0 percent (Table 1). In both the genotypes, increase in dose corresponds to reduction in germination. Such dose dependent inhibition was reported in Ragi by Ayyamperumal (1977), in Tenai by Gowda (1977) and in Sunflower by Raja Ramesh Kumar and Venkat Ratnam (2010). The reduction in germination was more pronounced in TNAU 51 compared to CO3 at comparable dose levels. Such heterogeneity in radiation tolerance among species and among genotypes of the same species has been reported by Sparrow (1966) and it is attributed to the difference in DNA content per nucleus and the chromosome volume at the time of irradiation.

Chlorophyll mutants are used as markers in genetic, physiological and bio chemical investigations and are under the control of nuclear and cytoplasmic genes (Van Harten, 2007). The frequency of chlorophyll mutants in the M_2 generation is mainly used as a dependable measure of genetic effects of mutagens (Nilan *et al.,*1963) and is suggested to provide a reliable index of mutation rate because of greater accuracy in scoring (Gaul,1964). Among the different measures of estimation of mutation rate, like number of mutations per 100 M1 plants, number of mutations per 100 M_1 spikes and number of mutants per 100 M_2 seedlings, estimation based on M_2 seedlings is better than the other since it is independent of variations in progeny and size of mutated sector. In the present study, the frequency of chlorophyll mutants ranged from 9 to 23 in case of genotype CO3 and 12 to 77 in TNAU51 (Table 2). The mutagen effectiveness in terms of

Table 2. Effectiveness of gamma irradiation in Kodomillet on M_2 seedling basis

Genotype	Dose (Gray)	M_2 seedling basis: No. seedlings studied	No. plants segregating Chlorophyll mutants	Mutants per 100 M_2 seedlings	Mutagen effectiveness based on M_2 seedlings
CO 3	Control	280	0	0	0
	400	120	23	19.17	47.93
	500	129	34	26.35	52.70
	600	114	20	17.54	29.23
	700	54	14	29.95	42.78
	800	16	9	56.25	70.31
TNAU 51	Control	342	0	0	0
	400	157	12	7.64	19.1
	500	149	37	24.83	49.67
	600	135	32	23.72	39.54
	700	164	49	29.87	42.67
	800	119	77	64.70	80.87

chlorophyll mutants in M_2 generation ranged from 29.23 to 70.31 percent for CO3 and 19.1 to 80.87 percent for TNAU 51 (Table 2). In both the varieties, in the M_2 generation, the chlorophyll mutation frequency and the dose followed a linear relationship up to 500 Gray beyond which the relationship followed a non-linear pattern. This could be due to elimination of some mutatnts by rigor of haplontic and diplontic selections as reported by Swaminathan (1961) and Rameshwar kumar (2009). Several chlorophyll mutants like Albino, Xantha, Chlorina, Striata and Viridis were observed in the M2 plants. In the dose of 500 Gray, the relative percentage of Viridis type mutants was higher compared to other types in CO3 while in TNAU 51, the proportion of Albino was high (Table 3) at the same dose. The occurrence of more than one type of chlorophyll mutants in the same dose may be the result of simultaneous occurrence of mutations in more than one locus. In both the varieties, there was no direct relationship between the dose and the frequency of mutations.

Table 3. Spectrum of chlorophyll mutants observed in M_2 generation

Dose	Genotype	No. M_2 seedlings studied	Spectrum of chlorophyll mutants (Relative percentage)											
			Albino		Xantha		Chlorina		Striata		Viridis		Total	
			No	%	No	%	No	%	No	%	No	%	No	%
CO3	Control	280	-	-	-	-	-	-	-	-	-	-	-	-
	400	120	0	0	8	6.7	15	12.5	0	0	0	0	23	19.2
	500	129	5	3.9	8	6.0	8	6.0	1	0.8	12	9.3	34	26.4
	600	114	8	7.0	3	2.6	7	6.1	0	0	2	1.8	20	17.5
	700	54	11	20.4	0	0	3	5.6	0	0	0	0	14	26.0
	800	16	9	56.3	0	0	0	0	0	0	0	0	9	56.3
TNAU 51	Control	342	-	-	-	-	-	-	-	-	-	-	-	-
	400	157	0	0	5	3.2	5	3.2	0	0	2	0.1	12	7.6
	500	149	17	11.4	6	4.0	8	5.4	3	2.0	3	2.0	37	24.8
	600	135	9	6.7	4	2.9	11	8.2	2	1.5	6	4.5	32	23.7
	700	164	38	23.2	0	0	11	6.7	0	0	0	0	49	29.9
	800	119	69	60	0	0	9	7.6	0	0	0	0	77	64.7

The usefulness of any mutagen would depend on its effectiveness and efficiency (Konzak *et al*.,1965). In the present study, the dose 400 Gray was observed to be highly efficient in terms of survival reduction for CO3 while for TNAU 51, the dose 800 Gray was observed to be highly efficient. With reference to height reduction, the dose 800 Gray was observed to record maximum efficiency. The dose 800 Gray was found to be highly effective for inducing mutation in both the genotypes (Table 4). Although the mutagen effectiveness was high for both the genotypes at doses of 800 Gray, most of the seedlings were albinic and could not survive in field till flowering.

Table 4. Mutagenic effectiveness and efficiency in M_2 population of Kodomillet varieties

Treatment	Survival reduction (%) (L)	Height reduction (% (I)	Mutant per 100 M2 seedlings (Mp)	Effectiveness (Mp/dose in Gray)	Efficiency	
					Mp/L	Mp/I
CO3						
400	16.3	21.2	19.17	4.79	117.6	90.42
500	42.4	28.3	26.35	5.27	62.14	93.11
600	55.2	38.2	17.54	2.92	31.77	45.92
700	76.0	39.1	29.95	4.29	39.4	76.60
800	84.9	41.9	56.25	7.03	66.3	134.25
TNAU 51						
400	22.6	26.1	7.64	1.91	33.80	29.27
500	44.4	28.6	24.83	4.97	56.43	86.82
600	63.0	35.5	23.72	3.95	37.65	66.82
700	76.1	43.6	29.87	4.27	39.25	68.51
800	87.4	45.7	64.70	8.09	74.03	141.58

A brief perusal of literature reveals a paucity of data regarding the effective and efficient dose of gamma radiation for kodomillet. The dose of 500 Gray was reported as the LD 50 dose for kodomillet by Subramanian *et al.*, 2009. Based on the results of the study, it is concluded that, for optimum recovery of viable mutants in kodomillet, the dose 500 Gray would be suitable. Further evaluation of the surviving mutants will produce many useful macro and micro mutations which could be exploited in kodomillet breeding programme in future.

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