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Studies on mutagenic effectiveness and efficiency of gamma rays and its effect on quantitative traits in finger millet (*Eleusine coracana* L. Gaertn)

*A.R. Ambavane**, *S.V. Sawardekar, S.A. Sawantdesai, N.B. Gokhale*

Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, MS, India

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ABSTRACT

Dry seeds (12% moisture) of two finger millet cultivar *viz.*, Dapoli-1 and Dapoli Safed were irradiated with four doses of gamma-rays *viz.*, 400 Gy, 500 Gy, 600 Gy and 700 Gy at BARC, Mumbai. In laboratory test, root and shoot lengths of seedlings were decreased with increase in dose of gamma rays. Similarly, germination percentage and survival rate of seedlings were decreased with increase in dose of gamma irradiation during field study. In M_1 generation, three types of chlorophyll mutations *viz.*, *albino*, *xantha* and *viridis* were observed. *Albino* and *xantha* were observed in all treatments, whereas, *viridis* observed only in lower doses *viz.*, 400 Gy and 500 Gy. Based on the chlorophyll mutation frequency on M_1 plants, mutagenic effectiveness and efficiency were computed. In Dapoli-1 variety, two early maturing mutants and three high yielding mutants were isolated from 500 Gy dose and 600 Gy dose, respectively. In M_2 generation, the mutagenic treatments were effective in inducing various types of chlorophyll and morphological macro mutants, few of those show significant change in flowering, maturity and plant height character and few of them have good breeding value.

1. Introduction

Among the major food grains, finger millet (*Eleucina coracana* L. Gaertn) is one of the most nutritious crops. It is an important food crop in South Asia and Africa. The grain of finger millet has fine aroma when cooked or roasted and it is known to have many healthy promising qualities. It is a rich source of calcium and has good amount of magnesium, phosphorous and iron. Finger millet has a favorable amino acid spectrum that includes cysteine, tyrosine, tryptophan and methionine (Rachie, 1975). Genetic improvement of crop depends on the amount of genetic variability present in the population.

Mutation is gene level causes alterations in the structure and position of gene on chromosome called point mutation. This results in the alteration of phenotype of an organism. Changes in basic chromosome number either any addition of loss of any set or parts of them cause appearance of disappearance of new characters. Once the mutation in gene level or chromosomal level is firmly established in populations, they are subjected to natural or artificial selection.

* *Corresponding author.*
E-mail addresses: ajinkyaambavane@gmail.com, ajinkyaambavane@hotmail.com (A.R. Ambavane).
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Mutation breeding is the tool in the hand of breeder to create variability in crop population and to make selection in the population with the view to bring about further improvement in crop. In general mutation breeding has been playing a key role in self-pollinated crop with limit variability. Mutation breeding has been reported by many workers, in castor (Ankineedu, Sharma, & Kulkarni, 1968), in wheat (Swaminathan, 1969), in sesame (Sharma, 1993), in cowpea (Dhanavel et al., 2008), in black gram (Thilagavathi & Mullainathan, 2009) and soybean (Padmavathi, Devi, & Kiranmai, 1992; Pavadai, Girija, & Dhanavel, 2010) developed and improve plant varieties by mutation breeding. Gamma irradiation as mutagen can induce useful as well as harmful mutation in plants (Gupta, 1996; Micke & Domini, 1993). The present investigation was undertaken to study the mutagenic effectiveness and efficiency in M_1 generation and to study effect of gamma rays in quantitative characters of finger millet in M_2 generation and results are discussed.

2. Materials and methods

2.1. Laboratory test

The material for this study comprised of two varieties of finger millet, Dapoli-1 (mid-tall, fully open ear heads with brown seeds) and Dapoli Safed (mid-tall, partially open ear heads with white colour seeds). Dry seeds of both varieties were irradiated with four doses of gamma rays (^{60}Co) *viz.*, 400 Gy, 500 Gy, 600 Gy and 700 Gy at BARC, Mumbai. The experiments to determine the effect of gamma-rays on germination, root and shoot length were conducted on germination paper. Each treatment was replicated five times and for each replication one hundred seeds were sown and tested for their germination, survival, root and shoot length. For determination of LD_{50} observations on germination were recorded on seventh day from the date of sowing. Effect on root and shoot was measured in terms of length of root and shoot respectively on seventh day. In field study, ten seedlings were selected randomly for taking observations. Chlorophyll mutants were scored and classified. Desirable mutants from M_1 generation were selected on the basis of their phenotypical characters and harvested separately.

2.2. Methodology followed to grow M_2 generation

Seed harvested from individual M_1 plants were grown as M_2 generation in factorial randomized block design (FRBD). Two hundred forty seedlings were sown in each plot at spacing of 10×30 cm as well as isolated mutants were also grown individually. All recommended package of practices were followed during growth period of the crop. Newly evolved characters were recorded in M_2 generation. Observation on days to 50 per cent flowering and maturity duration was recorded on plot basis. Observations were recorded on 5 randomly selected plants from each plot of each treatment. In M_2 generation, chlorophyll and morphological macromutants were identified and harvested separately. Mutation frequency was calculated as percentage of M_1 plants and mutagenic effectiveness and efficiency were calculated on the basis of formula suggested by Konzak, Wagner, Nilan, and Foster (1965).

$$\text{Mutagenic Effectiveness} = \frac{M}{\text{Dose of mutagen (krad)}}$$

$$\text{Mutagenic efficiency} = \frac{M}{L} \text{ or } \frac{M}{I} \text{ or } \frac{M}{S}$$

Where,

M = Frequency expressed as percentage of chlorophyll mutation in M_2 generation, estimated on M_1 plant basis.
krad = Kilorad
L = Percentage of lethality or reduction in survival.
I = Percentage of injury or reduction in seedling height.
S = Percentage of panicle sterility.

The data of all characters recorded in M_2 generation statistically analyzed with Statistical Analysis System software (SASs) V. 9.1 (June 2006), SAS Institute.

3. Results and discussion

3.1. Effect on germination and root and shoot length

Noticeable variations were observed in germination percentage after gamma irradiation in paper germination test. But variation was neither proportional to the increase in dosages nor definite pattern was found in both the varieties studied. On field level germination percentage decreased with increase in dose of gamma rays in both varieties (Fig. 1). Similar result have were reported by Ando (1970) in rice, Pathak and Patel (1988), Singh, Richharia, and Joshi (1998), Cheema and Atta (2003), Harding, Johnson, Taylor, Dixon, and Turay (2012), Talebi and Talebi (2012).

LD_{50} was optimized based on reduction in root and shoot length. It was observed that at 500 Gy had 50 per cent in reduction in root and shoot growth as compared to control (Table 1). It was also observed that the root and shoot length decreased with increase in gamma rays dose on approximately linear mode. Similar result was reported by Talebi and Talebi (2012) in rice.

3.2. Spectrum of chlorophyll mutants

Among the treatments, the 600 Gy and 700 Gy gamma rays produced high frequency of *albino* (Table 1). Next common chlorophyll mutant observed was *xantha* in both varieties. *Viridis* mutants were less frequent and found only in certain treatments *viz.*, 400 Gy and 500 Gy. The frequency of chlorophyll mutations varied with the genotype as well as mutagen doses in M_1 generation. Total frequency of chlorophyll mutations was relatively higher in Dapoli-1 than Dapoli Safed. The differential response of genotypes to induction of chlorophyll mutations was possible due to differences in the genetic makeup of the varieties used for mutagenesis. During the present study, *albino* mutant occurred in higher frequency than *xantha* or *viridis*. Several workers also reported a higher frequency of albino mutant in irradiated population, (Ando,

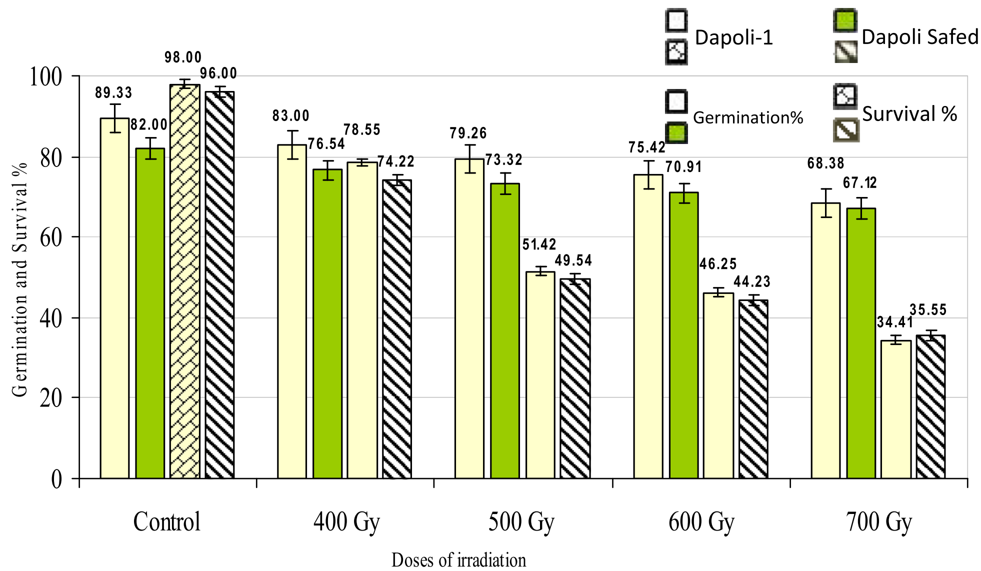

Fig. 1 – Germination and survival percentage affected by gamma rays irradiation in two varieties of finger millet (M_1 generation).

1970; Chakravarti, Singh, Kumar, Lal, and Vishwakarma, 2013 in rice; Cheema & Atta, 2003; Singh et al., 1998; Subramanian, Nirmalakumari, and Veerabadhiran, 2011 in kodo millet).

3.3. *Mutagenic effectiveness and efficiency*

Mutagenic effectiveness and efficiency (mutations per unit dose) varies with doses in different genotypes given in Table 2. It was also observed that, the mutagenic efficiency were lowest at 600 Gy and 700 Gy dose in both variety, because maximum panicle sterility was observed in 700 Gy dose followed by 600 Gy dose producing sterile panicle which resulted in decrease in the mutagenic efficiency in 700 Gy and 600 Gy doses. In Dapoli-1, maximum mutagenic efficiency was recorded in 500 Gy dose (1.16) followed by 400 Gy dose (1.13). Whereas, 400 Gy dose (1.10) recorded maximum mutagenic efficiency in Dapoli Safed.

It was also noticed that, mutagenic effectiveness decreased with increase in strength of gamma rays in both the genotypes. Similar observations of general decrease in effectiveness with increasing doses of gamma rays irradiation was reported in finger millet by Muduli and Misra (2007), in Mung bean by Solanki and Sharma (1994) and in foxtail millet by Gupta and Yashvir (1975).

3.4. *Effect on yield contributing characters*

Mean value for quantitative characters are presented in Table 3. In most of the treatments, average increase in number of days to first flowering was observed than control in both varieties. The maximum increase in number of days was seen at 700 Gy (120 days) in Dapoli Safed, while minimum increase in number of days for first flowering at 400 Gy in both the varieties compared to untreated seed. But two early mutants (85 and 86 days to first flowering) were also observed in Dapoli-1 variety at 600 Gy dose of gamma irradiation. This result is in agreement with results obtained by Nirmalakumari et al., (2007) in little millet (*Panicum sumatrense*). The maximum

Table 1 – Root and shoot length and spectrum of chlorophyll mutation.

Doses	Variety	Root length (cm)	Shoot length (cm)	Mutant seedlings	*Albino*	*Xantha*	*Viridis*
Control	Dapoli-1	5.0	4.64	–	–	–	–
	Dapoli Safed	7.06	3.75	–	–	–	–
400 Gy	Dapoli-1	3.8 (24.00)	3.10 (33.19)	27	55.55	33.33	11.11
	Dapoli Safed	4.75 (32.72)	2.43 (35.20)	21	57.14	33.33	9.52
500 Gy	Dapoli-1	2.6 (48.00)	2.25 (51.51)	21	52.38	33.33	14.28
	Dapoli Safed	3.58 (49.29)	1.95 (48.00)	19	78.94	15.78	2.26
600 Gy	Dapoli-1	1.35 (73.00)	1.60 (65.52)	18	72.22	27.77	–
	Dapoli Safed	3.45 (51.33)	1.95 (48.00)	17	76.47	23.52	–
700 Gy	Dapoli-1	1.2 (76.00)	1.45 (68.75)	16	87.50	25.00	–
	Dapoli Safed	3.25 (53.96)	1.48 (60.53)	15	80.00	20.00	–

Figures in parenthesis indicate per cent reduction.

Table 2 – Mutagenic effectiveness and efficiency in M_1 generation.

Doses	Mutagenic effectiveness		Mutagenic efficiency	
	Dapoli-1	Dapoli Safed	Dapoli-1	Dapoli Safed
Control	–	–	–	–
400 Gy	0.675	0.525	1.13	1.10
500 Gy	0.420	0.380	1.16	1.05
600 Gy	0.300	0.283	1.00	1.00
700 Gy	0.283	0.214	1.00	1.00

mean value for days to maturity was recorded at 700 Gy dose in both cultivar. Significant difference was observed in plant height at 400 Gy, 500 Gy and 600 Gy for Dapoli-1 whereas in Dapoli Safed cultivar significant difference was observed at 700 Gy dose of gamma rays. The maximum number of tillers at 600 Gy dose of gamma rays (6.58 and 3.95) in both cultivar as compare to control (1.85 and 1.56). All the mutagenic treatments increase the number of tillers when compared with control. The number of panicles were increased at all doses of gamma rays treatment. Significantly superior difference was observed in all doses of gamma rays treatments for fingers panicle^{-1} as compared to Dapoli-1 and Dapoli Safed. The maximum fingers were observed at 500 Gy (9.13 and 8.93) plant^{-1} for Dapoli Safed and Dapoli-1, respectively. The maximum finger length (8.23 cm) was observed at 600 Gy in Dapoli-1, whereas, Dapoli Safed recorded maximum finger length (7.59 cm) at 500 Gy. Similar results were also reported by Muduli and Misra (2008) in finger millet. It indicates that effect of doses of gamma rays varies from genotype to genotype. All the mutagenic treatments showed increase in weight of panicle plant^{-1} as compared to control. The maximum weight of panicle (2.81 g) was recorded at 600 Gy in Dapoli-1, whereas Dapoli Safed recorded maximum weight (2.86 g) at 500 Gy.

The macro mutations were recorded in grain density cm^{-1} and yield plant^{-1} in both varieties of finger millet. The maximum grain density (57.95 grains cm^{-1}) was observed in Dapoli-1 at 600 Gy, whereas Dapoli Safed recorded maximum grain density (47.80 grains cm^{-1}) at 500 Gy. The maximum grain yield plant^{-1} (5.18 g) was observed at 600 Gy in Dapoli-1 and 4.80 g in Dapoli Safed at 500 Gy. The increase in grain yield plant^{-1} is inversely related to intensity of radiation doses. These results are in agreement with Chakravarti et al., (2013) in rice and Hayat, Khan, Sadiq, Elahi, and Shakoor (1990) in sorghum cultivars. A number of morphological mutations have been reported in cereal plants and several of these mutations have been shown to exhibits modification in more than one character.

4. Conclusion

The cultivar Dapoli-1 and Dapoli Safed responded more and more number of viable and economic mutants for higher productivity observed at 500 Gy and 600 Gy, respectively than other mutagenic treatments. The present investigation revealed that, the isolation of early maturing mutants with

Table 3 – Mean values of quantitative characters in M_2 generation of variety Dapoli-1 and Dapoli Safed.

Sr. No	Character	Control		400 Gy		500 Gy		600 Gy		700 Gy		SEm ±		CD at 5%	
		DPL1	DS	DPL1	DS	DPL1	DS	DPL1	DS	DPL1	DS	DPL1	DS	DPL1	DS
1)	Days to first flowering	93.66	99.54	100.50	105.20	105.21	108.91	106.40	113.74	115.57	120.57	0.19	0.60	0.75	2.37
2)	Days to first panicle maturity	127	135.42	135.72	143.42	138.04	150.32	145.01	152.24	150.13	160.13	0.85	0.44	3.36	1.76
3)	Plant height (cm)	36.28	36.39	30.07	34.05	30.67	33.45	29.66	33.25	33.13	28.57	0.95	1.44	3.74	5.66
4)	Tillers/plant	1.85	1.56	4.48	1.94	4.53	2.86	6.58	3.95	3.05	2.7	0.21	0.43	0.82	1.68
5)	Panicles/plant	1.15	1.06	4.08	3.35	4.05	2.63	5.76	2.60	3.26	2.43	0.61	0.29	2.38	1.13
6)	Fingers/panicle	6.35	6.66	8.40	8.06	8.93	9.13	7.53	9.05	8.66	5.68	0.39	0.65	1.54	2.54
7)	Finger length (cm)	5.77	5.4	8.16	7.02	7.91	7.59	8.23	6.15	7.70	5.94	0.41	0.44	1.61	1.75
8)	Panicle Weight/plant (gm)	1.84	1.56	1.93	2.12	1.91	2.86	2.81	2.56	2.25	1.90	0.47	0.17	1.86	0.65
9)	Grain density/cm	46.66	35.83	44.95	38.23	48.9	47.80	57.95	45.33	45.15	35.7	3.58	4.75	14.06	18.66
10)	Yield per plant (gm)	3.121	3.926	4.2526	3.3606	4.7494	4.8096	5.1817	4.3951	4.2972	4.115	0.24	0.07	0.94	0.27

DPL1 – Dapoli-1, DS – Dapoli Safed.

high yield and yield component characters is possible in 500 Gy and 600 Gy doses of gamma irradiation in finger millet.

5. Definitions

5.1. *A gene mutation or point mutation*

A point mutation or single base substitution, is a type of mutation that causes the replacement of a single base nucleotide with another nucleotide of the genetic material (DNA or RNA).

5.2. LD_{50}

The amount of a toxic agent (as a poison, virus or radiation) that is sufficient to kill 50 per cent of a population, usually within a certain time.

5.3. *Macro mutants*

A mutants http://dictionary.reference.com/browse/mutation having profound effect on the regulatory gene that controls the expression of many structural genes.

5.4. *Gy*

The gray (symbol: Gy) is a derived unit of ionizing radiation dose in the International System of Units (SI). It is a measure of the absorbed dose and is defined as the absorption of one joule of radiation energy by one kilogram of matter.

6. Future issues

As a source of variability, induced mutations supplement naturally occurring variation. When specific mutants are selected following mutagenic treatments it is highly likely that a number of mutational changes will have occurred in the selected genotype. Hence, although most of the mutant varieties released, so far have resulted from mutation and direct selection, the future trend will be for increasing use of mutants in association with recombination. Mutations in combination with other techniques of genetic engineering will constitute the tools of the plant breeders of the future. The present role of mutation in plant breeding has been established. Mutation breeding has advantages in certain situations, disadvantages in others. Greater understanding will lead to their more widespread use.

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