



Mutagenic-sensitivity and variability in the putative mutants of polyembryonic mango genotypes

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ABSTRACT

Polyembryonic mango genotypes are considered the best resources for developing rootstocks owing to the production of uniform, true-to-type nucellar seedlings with deep tap root systems. However, their use in breeding programmes is often limited by a narrow genetic base due to maternal inheritance. Hence, enhancement in or creation of variability for traits of interest becomes a pre-requisite if rootstock breeding in mango has to be undertaken. In this study, we attempted to induce variability in polyembryonic mango genotypes, Bappakai, Nekkare and Kurukkan, by treating their kernels with different doses of gamma rays ranging from 15 to 35 Gy. The results revealed delayed germination and a decrease in germination percentage with increasing dosage of gamma irradiation. The lethal dose (LD₅₀) values for gamma radiation were determined based on the seed germination percentage using Probit analysis values, which were 22.39 Gy, 19.95 Gy, and 19.95 Gy for Bappakai, Nekkare and Kurukkan, respectively. Other traits like the number of seedlings emerging per kernel, plant height, inter-nodal length, and the number of leaves gradually decreased their mean values with increasing irradiation dosage. The results suggest the effectiveness of induced mutation in bringing about variability in traits like plant height for developing dwarf rootstocks to be used in high-density planting.

Keywords: *Mangifera indica*, Dwarf rootstock, Gamma irradiation, seed germination, LD₅₀

INTRODUCTION

Mango (*Mangifera indica* L.), highly prized for its delicacy, flavour, and nutritional value, and is the best known among the 69 species of *Mangifera*, is the choicest fruit of millions of people in the country (Azam *et al.*, 2). Mango genotypes can be classified as polyembryonic or monoembryonic, depending upon the number of embryos present per seed. Although polyembryonic genotypes are the best resources for the development of mango rootstocks owing to the production of uniform, true-to-type nucellar seedlings with a deep tap root systems, however, the use of these genotypes in breeding programmes are often limited by their narrow genetic base. Thus, the enhancement of existing variability or creation of variability for traits of interest becomes a pre-requisite, if rootstock breeding has to be undertaken in mango. Induced mutations can broaden the genetic base in heterozygous crops like mango creating new combinations of alleles for desirable traits of interest. Dose and rate of mutation and the genetic constitution of the test material greatly influence the efficiency of mutation (Mba *et al.*, 9). Lethal Dose 50 (LD₅₀) is considered the dose resulting in high mutation frequency, while maintaining the biological damages at tolerable levels (Meyer, 10). Hence,

optimization of radiation dose is paramount for successful mutation breeding programme. This study was aimed to determine the LD₅₀ of polyembryonic mango genotypes, Bappakai, Nekkare and Kurukkan and to estimate the variability induced in the treated population by different mutagenic treatments, as the first step in a comprehensive mutation breeding program for mango.

MATERIALS AND METHODS

This study was undertaken at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru during the year 2018-2019. Here, an attempt was made to induce genetic variability in polyembryonic mango genotypes, Bappakai, Nekkare and Kurukkan reported to be tolerant to salinity stress (Nimbolkar, 11) using different doses of gamma irradiation. Fruits of all three genotypes were collected from the trees of respective genotypes maintained at mango germplasm block and stones were extracted from fully ripened fruits and husks were removed, thereafter. The kernels were irradiated with gamma rays using a radioisotope ⁶⁰Co, Cobalt-60 source, at the Gamma chamber-5000 facility, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India. The treatment consisted of five different dosages of gamma irradiation 15Gy (T₁), 20Gy (T₂), 25Gy (T₃) 30Gy (T₄) and 35Gy (T₅) along

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with untreated kernels as control, and a total of 280 kernels per treatment were irradiated. After irradiation, the kernels were sown in polybags (14.5cm × 7.5cm) filled with sand:clay: Arka fermented cocopeat (1:1:1) as rooting media, and shifted to 50% shade net house, where they were maintained for taking different observations.

Initial observations were recorded for germination, and the number of seedlings emerging from each kernel was counted at 4-days interval, until no more variations in the count were observed. Morphological observations on plant height (cm), stem girth (mm), number of internodes, internodal length (cm) and number of leaves, for the mutant progenies of mango genotypes were recorded based on mango descriptor (IPGRI, 4) after 90 days of sowing. Observations on all the parameters were recorded for each individual plant of the putative mutant population. The lethal dose 50 (LD₅₀) values for gamma radiation were determined based on seed germination percentage using Probit analysis (Finney, 3). Descriptive statistics was calculated for all the morphological parameters using standard procedure.

RESULTS AND DISCUSSION

A gradual decrease in per cent germination was observed for genotypes Bappakai and Nekkare with increasing doses of gamma irradiation (Fig.1). However, in Bappakai, percentage of seeds germinated in T₁ (92.14%) and T₂ (87.86%) was higher than control (64.29%), which might be due to the stimulatory effect of lower gamma irradiation doses on cell division rate (Sadegh *et al.*, 13). Further, in the genotype Nekkare, the highest per cent germination was recorded in control (85.71%) which then decreased with increasing doses of irradiation, and was lowest in T₅ (18.93%). This clear trend was not observed for the genotype Kurukkan, where

although the highest germination was observed in the control (85%), no significant variation was observed among the treatments and surprisingly among the treatments, the highest germination was observed for 30 and 35 Gy (T₄ and T₅) (Fig. 1). The LD₅₀ value determined using seed germination percentage was found to be 22.39Gy, 19.95 Gy and 19.95 Gy for Bappakai, Nekkare and Kurukkan, respectively (Fig. 2). Previously the LD₅₀ for seed kernels of polyembryonic mango genotypes Peach and Bappakai treated with gamma rays has been reported to be 27.54 Gy and 23.44 Gy, respectively (Kumar *et al.*, 7). For Arumanis mango (Indonesia), γ -irradiation beyond 60 Gy was found to be lethal (Karsinah *et al.*, 5).

A distinct variation was observed between treatments for the number of seedlings emerging from each kernel. In Bappakai, at lower doses of irradiation, considerably more kernels (than control) produced three (16.28% for 15 Gy and 14.68% for 20 Gy) and four (4.65% for 15 Gy and 4.47% for 20 Gy) seedlings. At the highest irradiation dose (35 Gy), only one seedling emerged from 86.21% kernels, while none of the kernels produced more than two seedlings. In Nekkare, at higher doses of irradiation, none of the kernels produced more than 3 seedlings (Table 1a). In Kurukkan, although none of the kernels beyond 25Gy treatment produced more than 4 seedlings, percentage of kernels producing 3 seedlings were comparatively higher than those from lower doses and control (Table 1a). Number of seedlings emerging from each kernel in three genotypes at different irradiation levels have been presented in Table 1b. In Bappakai, mean number of seedlings emerging from each kernel was more at lower doses of irradiation i.e. 1.94 (15 Gy) and 1.79 (20 Gy) than control (1.42). However, this increase was followed by a marked depression at 35 Gy (1.13). For Nekkare and Kurukkan, this trend was not observed, as they recorded the highest mean number of seedlings for control (1.65 and 2.41, respectively), which decreased with increase in irradiation dose, and was lowest for 35 Gy (1.13 and 2.06, respectively). Spiegel-Roy and Padova (15) reported the highest mean number of seedlings emerging at a lower dosage (0.5 kR) of irradiation more than control, while with increase in radiation dose, the number of seedlings per seed decreased, and was lowest at the highest dose (5kR).

Further, although lower irradiation doses did not have a marked effect, while higher doses delayed germination considerably (Table 2). For Bappakai, Nekkare and Kurukkan, minimum time taken for germination (18.11, 18.87 and 23.18 days, respectively (data represent mean values of time

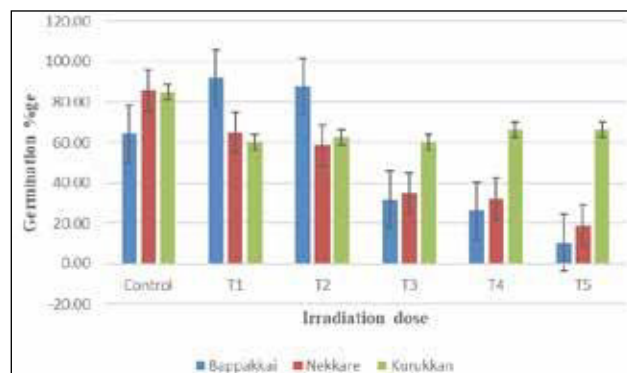
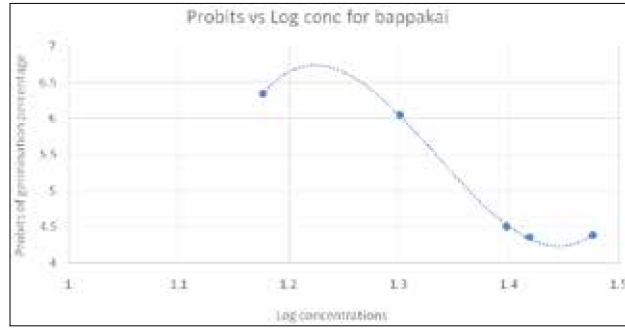
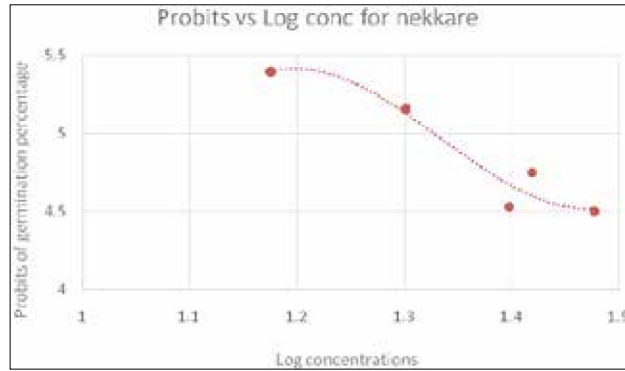


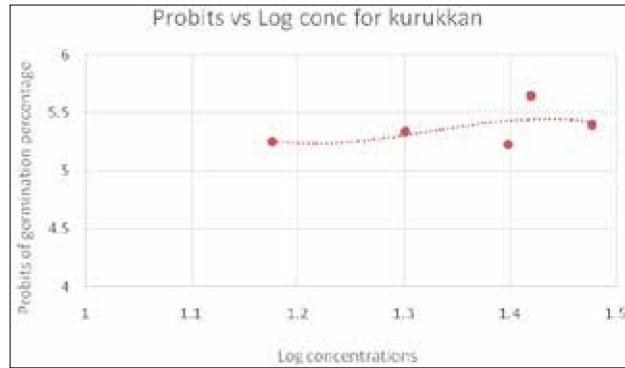
Fig. 1. Effect of gamma irradiation on germination percentage in three polyembryonic mango genotypes.



Bappakai
 Model $Y=15.62-7.75x$
 LD 50 value 22.39
 MSE 0.16
 R² 0.88



Nekkare
 Model $Y=9.13-3.15x$
 LD 50 value 19.95
 MSE 0.02
 R² 0.90



Kurukkan
 Model $Y=4.46+0.67x$
 LD 50 value 19.95
 MSE 0.08
 R² 0.23

Fig. 2. Probit analysis based on mortality percentage of Polyembryonic mango genotypes Bappakai, Nekkare and Kurukkan.

Table 1(a). Effect of gamma irradiation on germination percentage and percentage of kernels with more than one seedling in three polyembryonic mango genotypes.

	Bappakai					Nekkare					Kurukkan							
	Control	T1	T2	T3	T4	T5	Control	T1	T2	T3	T4	T5	Control	T1	T2	T3	T4	T5
% kernels with 1 seedling	71.11	33.72	44.72	85.39	72.97	86.21	45.71	41.79	37.14	29.64	27.14	16.79	35	26.25	27.5	18.75	20	20
% kernels with 2 seedlings	20.00	44.19	36.59	11.24	16.22	13.79	27.14	20.00	16.07	5.00	3.57	1.79	10	15	20	11.25	17.5	28.75
% kernels with 3 seedlings	4.44	16.28	14.63	3.37	10.81	0.00	10.00	2.50	4.64	0.36	1.07	0.36	20	10	8.75	22.5	18.75	11.25
% kernels with 4 seedlings	2.22	4.65	4.47	1.12	0.00	0.00	2.86	0.36	0.36	0.00	0.00	0.00	10	3.75	3.75	7.5	8.75	6.25
% kernels with 5 seedlings	0.00	0.78	0	0.00	0.00	0.00	0.00	0.36	0.00	0.00	0.00	0.00	10	5	2.5	0	0	0

Table 1(b). Effect of gamma irradiation on the number of seedlings emerging per seedling in three polyembryonic mango genotypes.

Treatment	Bappakai					Nekkare					Kurukkan							
	Mean	St. Err.	St. Dev.	Min	Max	CV	Mean	St. Err.	St. Dev.	Min	Max	CV	Mean	St. Err.	St. Dev.	Min	Max	CV
Control	1.42	0.12	0.78	1.00	4.00	55.06	1.65	0.11	0.82	1.00	4.00	49.68	2.41	0.35	1.46	1.00	5.00	60.55
15 Gy	1.94	0.05	0.87	1.00	5.00	44.85	1.43	0.05	0.66	1.00	5.00	45.98	2.10	0.18	1.28	1.00	5.00	60.62
20 Gy	1.79	0.05	0.85	1.00	4.00	47.68	1.46	0.05	0.68	1.00	4.00	46.28	2.21	0.15	1.10	1.00	5.00	56.48
25 Gy	1.21	0.06	0.55	1.00	4.00	45.60	1.16	0.04	0.40	1.00	3.00	34.24	2.31	0.15	1.06	1.00	4.00	45.62
30 Gy	1.38	0.08	0.68	1.00	3.00	49.08	1.18	0.05	0.47	1.00	3.00	39.51	2.25	0.14	1.05	1.00	4.00	46.47
35 Gy	1.13	0.06	0.34	1.00	2.00	29.87	1.13	0.05	0.39	1.00	3.00	34.81	2.06	0.13	0.93	1.00	4.00	45.15

Table 2. Effect of different doses of gamma irradiation on days to germination in three polyembryonic mango genotypes.

Treatment	Bappakai						Nekkare						Kurukkan					
	Mean	St. Err.	St. Dev.	Min	Max	CV	Mean	St. Err.	St. Dev.	Min	Max	CV	Mean	St. Err.	St. Dev.	Min	Max	CV
Control	18.11	0.39	2.62	12.00	24.00	14.48	18.87	0.38	2.98	15	25.00	15.78	23.18	0.89	3.66	20.00	32.00	15.80
T1	18.57	0.20	3.28	13.00	35.00	17.64	18.36	0.20	2.65	14.00	24.00	14.46	25.80	0.64	4.51	21.00	40.00	17.49
T2	18.40	0.22	3.38	14.00	28.00	18.39	20.37	0.30	3.89	15.00	33.00	19.09	26.92	0.58	4.10	21.00	37.00	15.25
T3	21.01	0.31	3.51	14.00	32.00	16.70	23.34	0.38	3.75	16.00	29.00	16.07	26.90	0.54	3.82	20.00	37.00	14.22
T4	21.88	0.39	3.75	15.00	32.00	17.14	23.29	0.37	3.59	15.00	29.00	15.41	28.04	0.55	3.98	21.00	37.00	14.18
T5	23.62	0.65	4.08	16.00	32.00	17.29	26.42	0.71	5.62	17.00	42.00	21.27	29.00	0.52	3.73	22.00	37.00	12.87

taken for germination) was observed in control, which increased to 23.62, 26.42 and 29 days, respectively, for kernels treated with 35 Gy dose of gamma irradiation (Table 2). Similar trends have been reported in papaya and mango (Mahadevamma *et al.*, 8; Kumar *et al.*, 7).

Considerable variation was observed in the treated population for different morphological traits. A gradual reduction in plant height with increasing dosage of irradiation was recorded for all the genotypes (Table 3; Fig. 3a-c). In Bappakai, maximum and minimum mean plant height was recorded in control (19.25cm) and 35 Gy treatments (13cm) respectively. However, in lower doses of irradiation, plants taller than control were also observed and plant height ranged from 4 to 52cm and 5 to 41cm for 15Gy and 20Gy treatments respectively. At 35Gy, plant height ranged from 4.50 to 28cm compared to control, which ranged from 13 to 32cm. Similarly, for Nekkare and Kurukkan, maximum mean plant height was observed for control (31.18cm and 23.71cm, respectively) and minimum mean plant height was observed for 35 Gy treatment (16.33cm and 13.54cm, respectively). A reduction in plant growth with increasing doses of gamma irradiation has been reported in mango (Karsinah *et al.*, 5), apple (Atay *et al.*, 1) and banana (Kemal *et al.*, 6). Further, a decrease in stem girth with increasing dosage of irradiation was observed in Nekkare and Kurukkan where maximum stem girth was recorded for control (7.33mm and 5.77mm respectively), while minimum was recorded for 30 Gy treatment (5.42mm) and 35 Gy treatment (4.60mm) respectively. However, in Bappakai, stem girth of seedlings was more than control in lower doses of irradiation followed by a marked reduction at 25Gy and 35 Gy (4.59mm and 4.77mm respectively) treatments (Table 3). Average number of internodes present in Bappakai was highest in 20Gy treatment and lowest in control (4.18) while in Kurukkan it was highest in control (3.65) and lowest in 20Gy treatment (3.19). However, in Nekkare highest number of internodes was recorded in 25 Gy treatment (4.22) corresponding to a plant height of 19.37cm and the lowest was recorded for 35 Gy treatment (3.69) with a plant height of 16.33cm (Table 3). For all the genotypes, maximum inter-nodal length was recorded in control, while the minimum inter-nodal length for Bappakai and Nekkare was observed in 25 Gy treatment (2.5 cm and 3.54 cm respectively) and for Kurukkan it was minimum in 30 Gy treatment (2.58cm) (Table 3). A reduction in inter-nodal length is found to be associated with reduced plant height (Rime *et al.*, 12; Singh *et al.*, 14). In this study, a decrease in inter-nodal length was observed with increasing dose of irradiation,

Table 3. Morphological variability induced in three polyembryonic mango genotypes through different doses of gamma irradiation.

Parameter	Kurukkan					Nekkare					Bappakai								
	Control	T1	T2	T3	T4	T5	Control	T1	T2	T3	T4	T5	Control	T1	T2	T3	T4	T5	
Plant height (cm)	Mean	23.71	16.38	14.58	16.83	14.01	13.54	31.18	27.12	25.45	19.37	17.74	16.33	19.25	18.87	18.12	13.72	14.21	13.00
	St. Err.	1.34	0.77	0.60	0.72	0.79	0.63	0.86	0.68	0.69	0.80	0.89	0.77	0.51	0.37	0.38	0.53	0.62	1.01
Stem girth (cm)	Mean	5.53	7.54	5.55	6.53	6.30	5.41	7.77	9.94	10.01	7.87	6.23	7.05	3.40	7.49	6.98	5.14	5.91	5.55
	St. Err.	18.00	4.00	2.00	4.20	2.80	4.00	16.00	6.00	4.45	5.00	4.50	4.28	13.00	4.00	5.00	4.50	3.00	4.50
No. of Internodes	Mean	35.00	37.00	28.00	34.50	29.00	32.00	52.00	50.50	53.00	50.00	30.50	37.00	32.00	52.00	41.00	34.00	31.00	28.00
	St. Err.	23.33	46.03	38.03	38.79	44.93	39.94	24.92	36.66	39.35	40.65	35.12	43.15	17.68	39.69	38.54	37.46	41.56	42.66
Inter length (cm)	Mean	5.77	4.97	4.63	5.21	4.71	4.60	7.33	6.77	6.57	5.71	5.42	5.49	5.32	5.56	5.42	4.59	5.06	4.77
	St. Err.	0.21	0.19	0.18	0.21	0.21	0.17	0.17	0.14	0.14	0.15	0.21	0.20	0.16	0.08	0.09	0.15	0.14	0.27
No. of leaves	Mean	0.88	1.83	1.61	1.88	1.68	1.49	1.54	2.06	1.99	1.50	1.44	1.82	1.06	1.68	1.63	1.46	1.34	1.46
	St. Err.	4.40	2.02	1.68	1.97	1.76	1.90	3.61	1.39	2.49	2.28	2.85	2.87	4.11	2.24	2.09	0.00	1.97	2.18
No. of Internodes	Mean	7.49	9.59	8.62	10.24	8.22	8.25	11.43	11.54	11.80	10.39	7.82	15.50	8.97	11.08	9.85	8.61	8.00	7.54
	St. Err.	15.20	36.72	34.87	36.04	35.65	32.45	21.05	30.38	30.23	26.34	26.60	33.23	19.99	30.22	30.06	31.87	26.53	30.61
No. of Internodes	Mean	3.65	3.37	3.19	3.46	3.36	3.31	4.06	3.90	3.81	4.22	4.15	3.69	4.18	4.28	4.50	4.32	4.22	4.33
	St. Err.	0.17	0.10	0.10	0.12	0.16	0.09	0.13	0.07	0.08	0.10	0.14	0.14	0.17	0.06	0.06	0.12	0.11	0.18
No. of Internodes	Mean	0.70	1.02	0.96	1.08	1.28	0.81	1.14	1.02	1.18	1.02	0.96	1.24	1.15	1.16	1.07	1.18	1.04	0.96
	St. Err.	3.00	1.00	0.00	0.00	0.00	2.00	0.00	1.00	0.00	2.00	2.00	2.00	0.00	0.00	2.00	2.00	2.00	3.00
No. of Internodes	Mean	5.00	7.00	5.00	6.00	5.00	5.00	8.00	7.00	7.00	7.00	6.00	9.00	7.00	8.00	8.00	8.00	6.00	7.00
	St. Err.	19.25	30.33	30.03	31.17	38.01	24.45	28.16	26.11	31.07	24.26	23.08	33.60	27.62	27.05	23.87	27.42	24.54	22.13
No. of Internodes	Mean	4.47	3.21	3.05	3.79	2.58	2.84	5.17	5.08	4.95	3.54	3.61	3.69	3.81	3.49	3.18	2.50	2.76	2.78
	St. Err.	0.31	0.15	0.13	0.26	0.16	0.14	0.18	0.14	0.14	0.18	0.19	0.20	0.15	0.07	0.07	0.10	0.12	0.23
No. of Internodes	Mean	1.28	1.46	1.21	2.37	1.24	1.20	1.64	2.06	2.09	1.73	1.33	1.85	0.98	1.44	1.23	0.94	1.12	1.24
	St. Err.	3.00	0.50	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	2.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00
No. of Internodes	Mean	8.00	7.50	7.00	16.61	5.00	7.00	10.00	13.20	11.00	13.00	6.50	9.00	7.50	12.50	8.00	6.00	6.60	8.00
	St. Err.	28.64	45.44	39.82	62.48	48.02	42.21	31.77	40.55	42.14	48.91	36.75	50.04	25.83	41.22	38.65	37.60	40.66	44.57
No. of Internodes	Mean	13.41	9.85	10.49	11.89	9.94	9.50	15.69	12.72	12.64	10.84	10.20	10.19	14.84	11.20	12.20	10.23	11.88	11.10
	St. Err.	0.40	0.47	0.54	0.62	0.68	0.51	0.36	0.45	0.47	0.52	0.73	0.60	0.39	0.27	0.32	0.51	0.65	1.13
No. of Internodes	Mean	1.66	4.63	4.95	5.60	5.42	4.41	3.22	6.50	6.84	5.09	5.11	5.48	2.61	5.32	5.91	4.99	6.21	6.18
	St. Err.	10.00	1.00	2.00	4.00	2.00	2.00	10.00	2.00	2.00	1.00	2.00	2.00	9.00	2.00	2.00	3.00	3.00	2.00
No. of Internodes	Mean	17.00	20.00	25.00	27.00	24.00	22.00	24.00	30.00	39.00	26.00	30.00	23.00	21.00	31.00	33.00	27.00	40.00	34.00
	St. Err.	12.38	46.98	47.20	47.10	54.50	46.40	20.49	51.07	54.12	46.96	50.09	53.77	17.59	47.52	48.42	48.73	52.26	55.65



Fig. 3a Bappakkai Control (left) and putative mutants derived by 35 Gy gamma irradiation



Fig. 3b Kurukkan Control (left) and putative mutants derived by 35 Gy gamma irradiation



Fig. 3c Nekkare Control (left) and putative mutants derived by 35 Gy gamma irradiation

which was in congruence with the corresponding decrease in plant height. Irrespective of the genotype, number of leaves was highest in control (Table 3). Although, the number of leaves reduced in the entire gamma irradiated population, the reduction was not so profound within the treatment, albeit it was considerably reduced as compared to control.

Mutation breeding although an efficient tool for creation of genetic variation, has not yet been exploited in mango. The results of this investigation suggest the effectiveness of induced mutation using gamma irradiation in bringing about variability especially for traits like plant height. This is particularly useful as dwarf rootstocks are highly demanded in mango for taking up high-density plantation to increase productivity. The LD 50 value for mango genotypes Bappakai, Nekkare and Kurukkan are determined to be 22.39 Gy, 19.95 Gy and 19.95 Gy, respectively. This information will be useful for future mutation studies in mango since determination of mutagenic-sensitivity is the pre-requisite for any mutation breeding programme.

AUTHORS' CONTRIBUTION

Conceptualization of research (MRD and MS); Designing of the experiments (NP, MRD and MS); Contribution of experimental materials (MRD and MS); Execution of field/lab experiments and data collection (NP); Analysis of data and interpretation (RV and NP); Preparation of the manuscript (NP).

DECLARATION

The authors declare that there is no conflict of interest

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