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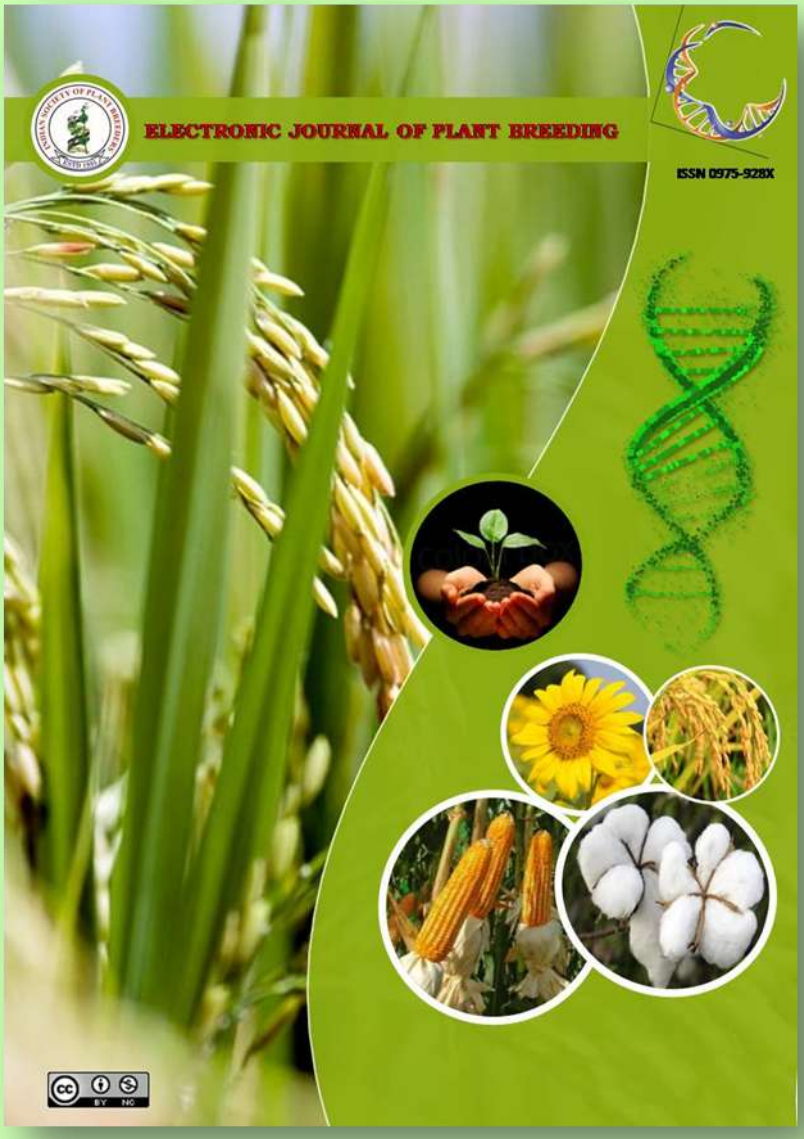

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Research Article

Mutagenic effectiveness and efficiency in barnyard millet (*Echinochloa frumentacea*) using physical, chemical and combination of mutagens

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Abstract
The present investigation was undertaken to study the frequency and spectrum of Chlorophyll mutations along with the mutagenic effectiveness and efficiency of different doses of EMS, Gamma ray and combination of both, in barnyard millet variety CO(Kv). 2. In M2 generation, no chlorophyll mutations were observed in the control population. The treatments behaved differently in the frequency of occurrence of chlorophyll mutations. The spectrum of chlorophyll mutations (chlorina, xantha, albino, viridis, and striata) were observed and grouped. Except striata, remaining four kinds of mutations *viz*., xantha, chlorina, viridis and albino were observed more frequently. The overall mutation spectrum showed that chlorina (0.47%) occurred with the highest frequency, followed by albino (0.37%), xantha (0.26%) viridis (0.19%) and Striata (0.08%). The mutagenic effectiveness decreased with the increase in dose of mutagen in both mutagens, indicating that negative relationship between effectiveness and dose of mutagen. Mutagenic efficiency (mutation rate in relation to M_1 damage) in both mutagens was highest at the lowest dose and it decreased with the increase in dose. The study concluded that Barnyard millet, Co(Kv).2 millet responds well to EMS, Gamma ray and combination of EMS and Gamma ray treatments.

Key words
Barnyard Millet • Induced mutagenesis • EMS • Gamma rays • Chlorophyll mutations

Introduction

Barnyard millet (*Echinocloa frumentacea* L.) is the fastest growing millet producing yield in 6 weeks, having the highest fibre and iron content among all the millets, widely cultivated as minor cereal across India and semi-arid tropics of Asia and Africa. Presently, in India, barnyard millet is the second important small millet after finger millet having production and productivity 87 thousand tonnes and 857 kg/ha, respectively. The primary objective of the plant breeder is to produce crops that perform better, usually in terms of yield and quality, than existing cultivars and this is dependent on the availability of genetic variation, preferably in the primary gene pool (Festus *et al.*, 2016). Where genetic diversity is insufficient, new material needs to be accessed or new variation created through induced mutation. Mutation induction has become an established tool in plant breeding to supplement existing germplasm and to improve cultivars for certain specific traits.

Induced mutagenesis has been successfully used to generate wider variability, portioning for isolating mutants with desirable characters of economic importance such as superior dwarf plant types for non-lodging, synchronous maturity, high tillers, high grain yield, larger seed size and desirable seed colour etc. (Ganapathy.*et al.*, 2008).

Before going for genetic improvement of a crop through mutation breeding, a thorough knowledge of mutagenic effectiveness and efficiency of mutagens to be used is essential to identify the useful mutagens as well as doses/ concentrations for effective breeding programme. Efficient mutagenesis means the production of maximum desirable changes accompanied by the least possible undesirable changes.

The chlorophyll mutation frequency in M_2 generation is the most dependable index for evaluating the genetic effects of mutagenic treatments (Raveendran, 1976; Sarkar and Sharma, 1989). Improvement in the frequency and spectrum of mutations in a predictable manner and thereby achieving desired plant characteristics for their either direct or indirect exploitation in the breeding program is an important goal of mutation research.

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Morphological mutation affecting different plant parts can be of having immense practical utility and many of them have been released directly as crop varieties (Mehraj-ud-din *et al.,* 1999; Gupta and Yashvir, 1975). The usefulness of a mutagen in mutation breeding depends not only on its mutagenic effectiveness (mutations per unit dose of mutagen), but also on its mutagenic efficiency (mutation in relation to undesirable changes/damage like sterility, lethality, injury *etc*.). The selection of effective and efficient mutagen(s) is very essential to recover a high frequency and spectrum of desirable mutations (Sharma, 1990; Solanki and Sharma, 1999). Of physical mutagens that include different types of Electromagnetic irradiations, the gamma irradiation is known to be the most effective in inducing a wide range of mutations (Bado *et al.,* 2015). Gamma-rays penetrate deeply into target tissues than other radiations (Mba *et al.,* 2012) and it is less destructive, whereas other radioactive rays causes translocations, chromosome losses and large deletions (Sikora *et al.,* 2011) Gamma rays are belonging to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and affect different morphology, anatomy, biochemistry and physiological characters in plants, mainly depending on the level of irradiation. These effects could cause changes in plant, the cellular structure and metabolism, like dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative and accumulation of phenolic compounds (Waghmare and Mehra, 2001; Kitch 1998).

Among the chemical mutagens, EMS have clastogenic (chromosome damaging) effects on plants via reactive oxygen-derived radicals (Yuan and Zhang 1993). These effects can occur both spontaneously and artificially following induction by mutagens. (Girija *et al*.,2013)Combination of both the mutagens, Gamma ray and EMS were also accepted to find out the effectiveness and efficiency. The present investigation was undertaken to study the frequency and spectrum of chlorophyll mutations along with the mutagenic effectiveness and efficiency of different doses of EMS, Gamma rays and combination of both in Co. (Kv) 2 variety.

Materials and Methods

The experimental materials for the present study comprised of prominent Barnyard millet variety, CO 2. Three hundred dry seeds with moisture content of 10- 12% were treated with EMS doses (60 mM, 70 mM and 80mM) and Gamma rays at doses 700, 800 and 900 Gray and the combination of both (70 mM + 700Gy, 70 mM + 800Gy and 70 mM + 900Gy). Equal number of seeds, at the rate of 300 per each dose was treated with corresponding mutagens and doses at room temperature. The treated seeds along with control (untreated seeds) were sown immediately in the field with the spacing of 45cm X 15 cm. to raise the M_1 generation. The experiment was conducted during crop season *Kharif* 2016 at Agricultural College & Research Institute, Madurai. (latitude; 11º North; longitude: 78º.8 East; altitude: 426.72 meter MSL).

Plant survival was recorded from emergence till the age of three weeks after germination and expressed as percentage of control. The damage was computed as the reduction in plant height (I-Injury), plant survival (L- Lethality), and seed sterility (S) for each treatment. Surviving plants with sufficient seeds in different treatments including control were harvested and threshed individually, and their seed yield was recorded. Consequently, seed sterility (S) for each treatment in both the genotypes was expressed as the reduction in seed fertility in relation to control.

All the surviving M_1 plants were harvested individually. The first formed seeds in the middle portion of the main tiller inflorescence alone were taken from all plants and raised M_2 population along with comparable controls (untreated seeds) during 2016 -2017. Necessary cultural practices were adopted to raise a healthy crop. The control and progenies were screened for lethal chlorophyll mutations and recorded right from emergence till the age of three weeks after germination, when the seedlings were at four leaf stage in the field as per identification and classification recommended by Ambli and Mullainathan, (2015). Different kinds of chlorophyll mutations (albina, chlorina, xantha, viridis and striata) and morphological mutations affecting different features of the plants (growth habit, plant height, maturity and pollen sterility) were grouped according to the modified classification proposed by Waghmare and Mehra.,2001. Mutation frequency was calculated as the percentage of mutated progenies and plants. Both mutagenic effectiveness and efficiency were determined as per the formulae suggested by Konzak, 1965.

Mutagenic effectiveness (Physical mutagens) = Mf×100/ Gy.
Mutagenic effectiveness (Chemical mutagens) = Mf×100/ mM.
Mutagenic effectiveness (Combined mutagens) = Mf×100/mM + krad.
Mf = Mutation frequency for 100 M_2 plants

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C = Concentration of mutagen in mM in percent,

Gy = Dose of mutagenic radiation
L = Percentage of lethality (or) survival reduction
I = Percentage of injury (or) reduction in seedling size.
S= Percentage of Sterility (or) reduction in Fertility of seeds

Results and Discussion

Chlorophyll mutations are considered as the most dependable indices for evaluating the efficiency of different mutagens in indicating the genetic variability for crop improvement and are also used as genetic markers in basic and applied research (Ambli and Mullainathan, 2015). The occurrence of chlorophyll mutations after treatments with physical and chemical mutagens have been reported in several crops. The chlorophyll mutation observed in the M_2 generation were scored at the seedlings stage in the field and expressed on M_1 panicle family basis as well as on M_2 seedlings basis (Table 1).

In the field trial, no chlorophyll mutations were observed in the control population. The two mutagens behaved differently in the frequency of occurrence of chlorophyll mutations. No definite relationship however, was discernible between the rate of chlorophyll mutations and types of treatments. On an average, the frequency of mutations was 32.68% for EMS alone, 27.06% for Gamma rays and 22.65% for combination of both mutagens indicating that EMS treatment has expressed maximum frequency than the other treatments, when estimated on M_1 panicle family basis.

Before going for genetic improvement of a crop through mutation breeding, a thorough knowledge of mutagenic effectiveness and efficiency of mutagens to be used is essential to identify the useful mutagens as well as doses/ concentrations for effective breeding programme. Efficient mutagenesis means the production of maximum desirable changes accompanied by the least possible undesirable changes. Among both the mutagens tested, EMS induced maximum frequency of chlorophyll mutations than gamma ray, indicating their greater effectiveness, which was also reported by Ganapathy *et al.,* (2008) and Ambli and Mullainathan, (2015). The mutation frequency showed a decreasing trend with increase in the dose or concentration of mutagens for EMS, gamma ray and combined treatments, which was also observed by Girija and Dhanavel (2009).

The frequency of chlorophyll and viable mutants observed in M_2 generation is mainly used as a dependable measure of genetic effect in mutagen. With reference to M_2 seedlings basis, it was 2.02% for EMS alone, 1.73% for gamma rays alone and 1.43% for combination of both mutagens. Frequencies of mutations were generally higher in EMS alone (2.02%) followed by gamma rays (1.73%) and their combinations (1.43%). This rise was due to recovery of more chlorophyll and viable mutation in EMS than gamma rays and their combinations which occurred in accordance with the findings of (Jayakumar and Selvaraj, 2003).

The spectrum of chlorophyll mutations obtained in the present study induced different types, *viz.*, albina, chlorina, xantha, viridis and striata were grouped (Table 2). These types of mutations in Cow pea (Girija *et al.*,2013) and albina, xantha, chlorina, and viridis in lentil (Solanki and Sharma, 1994) have been reported earlier. The overall mutation spectrum showed that chlorina (0.59%) occurred with the highest frequency, followed by xantha (0.54%), albino (0.29%) viridis (0.19%) and Striata (0.12%). Except Striata, remaining four kinds of mutations *viz.*, chlorina, albino, xantha and viridis were more frequently, which was in line with the findings of Ganapathy *et al.,* (2008). The overall mutation spectrum showed that chlorina (0.85%) occurred with the highest frequency at 60mM EMS, followed by xantha (0.68%) also by 60mM EMS, albino (0.37%) at 70 mM EMS, viridis (0.22%) at 700 Gy gamma ray and Striata (0.18%) at 70mM EMS + 800 Gy gamma ray.

EMS was noticed to be higher superior to gamma rays with higher frequency and wider spectrum of chlorophyll mutations in M_2 generation, which is in accordance with the findings of Ambli and Mullainathan., (2015) and Swaminathan, (1970).

The usefulness of a mutagen in mutation breeding depends not only on its mutagenic effectiveness, but also on its mutagenic efficiency. The selection of effective and efficient mutagens is very essential to recover a high frequency and spectrum of desirable mutations (Sharma, 1990; Solanki and Sharma,1999). Mutagenic Effectiveness means the rate of mutation induction as dependent upon the mutagenic does and to be expressed simply, it is a measure of the frequency of mutations induced per unit dose of mutagen. Mutagenic efficiency is indicative of the proportion of mutations as against associated undesirable biological effects such as gross chromosomal aberrations, lethality and sterility, induced by the mutagen in question and usually a measure of damage (Wani, 2011). Mutagenic effectiveness and efficiency of mutagen was estimated on the basis of relative proportion of families segregating for macro-mutations. Some definite pattern regarding the biological damage,

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i.e. reduction in plant survival, plant height reduction and seed sterility were observed. (Table 3 & 4). In order to obtain high effectiveness and efficiency, the mutation effect must greatly surpass other effects in the cell such as chromosomal aberrations, physiological and toxic effects, which reduce cell survival and eliminate the mutation (Girija and Dhanavel.,2009).

On M_1 plant basis, EMS was found to be more effective than gamma rays and combined treatments in inducing mutation. In M_1, the maximum mutagenic effectiveness was observed at 60 mM of EMS (15.63) and the minimum mutagenic effectiveness was observed at 70 mM + 900Gy of combined treatments (1.48). In M_2, the maximum mutagenic effectiveness was observed at 60 mM of EMS (0.921) and the minimum mutagenic effectiveness was observed at 70 mM + 900Gy of combined treatments (0.115). Similar trend of results were recorded by Girija and Dhanavel.,(2009) in Cowpea; Gautam *et al*. (1998) and Deepalakshmi and Anandakumar, (2003) in mung bean and Solanki and Sharma, (1994) and Solanki, (2005) in lentil; Yadava *et al*. (2003) in Kodo millet; and Jabee and Ansari, (2005) in chickpea.

Dose-dependent relationship for biological damage was observed in all treatments in M_1 generation. In general the effectiveness decreased with increasing dose or concentration. The reduction in biological criteria (Plant height and survival) may be attributed to a drop in the auxin level (Gordon and Webber, 1955), inhibition of auxin synthesis (Skoog, 1935), Chromosomal aberrations (Sparrow, 1961) or due to decline of assimilation mechanism (Quastler and Baer, 1950). With increasing doses of EMS or Gamma rays the values obtained for all the biological criteria for M_1 generation were decreased, which was also noticed by Kavithamani *et al.,* (2008). General decrease in effectiveness with increasing doses of gamma rays irradiation was reported in Samai (Ganapathy *et al.,* 2008), foxtail millet (Mba *et al.,* 2015); in lentil (Sikora *et al.,* 2011) Efficiency of a mutagenic agent is of a complex nature, as it depends on the degree to which physiological damage, chromosomal aberration and sterility are induced in addition to mutations (Girija.M., and D. Dhanavel.2009; Dhanavel, 2008). 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 (Shah *et al.,* 2006).

In M_1, On the basis of lethality, the highest mutagenic efficiency was recorded at 60 mM of EMS (76.97) and the lowest mutagenic efficiency was observed at 70 mM + 900Gy of combined treatments (27.33). On the basis of injury, the maximum mutagenic efficiency was observed at 60 mM of EMS (308.59) while the lowest being at 70 mM + 900Gy of combined treatments (57.76). On the basis of sterility, the maximum mutagenic efficiency was observed at 60 mM of EMS (234.28) while the lowest being at 70 mM + 900Gy of combined treatments (45.53).

In M_2, on the basis of lethality, the highest mutagenic efficiency was recorded in 60 mM of EMS treatments (4.54) and the lowest mutagenic efficiency was observed at 70 mM + 900Gy of combined treatments (2.11). On the basis of injury, the maximum mutagenic efficiency was observed at 60 mM of EMS treatments (18.19) while the lowest being at 70 mM + 900Gy of combined treatments (4.46). On the basis of sterility, the maximum mutagenic efficiency was observed at 60 mM EMS (13.81) while the lowest being at 70 mM + 900Gy of combined treatments (3.52). The same trend was observed in Chick pea (Girija. and Dhanavel.2009) and Cow pea (Dhanavel, 2008).

The average mutagenic effectiveness was higher at lower doses of gamma-rays and EMS and vice versa, as also experienced by (Ganapathy 2008; Anju Pathania and Sood 2007; Mba *et al.,* 2012; Sikora *et al.,* 2011 and Mudibu *et al.,* 2012). . This may be due to the fact that saturation point (dose/ concentration at which all mutable loci get mutated) occur at lower doses of mutagens and further increase in dose does not result in any change in mutation rate (Anju Pathania and Sood, 2007: Kaul and. Bhan, 1977).

Mutagenic efficiency (in relation to M_1 damage and M_2 seedling basis) is high in EMS treatments than gamma rays which matches with the findings of Anju Pathania and Sood, (2011); Kavithamani *et al* (2008); and Gautam *et al*. (1992) and Solanki and Sharma, (1994) in Lentle; Yadava *et al*. (2003) in Khodo millet and Jayakumar and Selvaraj, (2003) in sunflower. Hence, EMS is widely used as an efficient mutagen due to the reason that, they form adducts with nucleotides, causing them to mispairing with their complementary bases, thus introducing base changes after replication (Girija et al., 2013).

From this experiment, it is found that the frequency of chlorophyll mutants was concentration/doses dependant. The increase in chlorophyll and mutation frequency was recorded with increased concentration/doses of all mutagens. Mutagenic effectiveness and efficiency decreased with increase in doses. The mutations isolated in the

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present study might have been due to small deletions or point mutations. In conclusion, the genotype Co(Kv).2 Barnyard millet has responded well to physical and chemical mutagens and offers scope for the isolation of economic mutants with higher yield.

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Table 1. Chlorophyll mutation frequencies in M_2 generation

Mutagens (Dose/Conc.)	No. of M_1 plants		No.of M_2 seedlings		Mutation frequency (%)	
	Plants forwarded	Segregating	Studied	Chlorophyll mutants	M_1 plant basis	M_2seedling basis
Gamma rays						
Control	70	0	350	0	0	0
EMS						
60 mM	168	63	7644	169	37.50	2.21
70 mM	148	47	6530	133	31.76	2.04
80 mM	132	38	5386	97	28.79	1.80
EMS Mean					**32.68**	**2.02**
Gamma ray			0			
700 Gy	155	48	7366	134	30.97	1.82
800 Gy	140	37	6280	110	26.43	1.75
900 Gy	122	29	4970	81	23.77	1.63
Gamma ray Mean					**27.06**	**1.73**
EMS + Gamma rays			0	0		
70mM + 700 Gy	146	38	6345	95	26.03	1.50
70mM + 800 Gy	131	32	5416	78	24.43	1.44
70mM + 900 Gy	120	21	4586	62	17.50	1.35
EMS + Gamma rays Mean					**22.65**	**1.43**

Table 2. Frequency of different types of chlorophyll mutants in M_2 generation

Mutagens (Dose/ Conc.)	Total chlorophyll mutants in M_2 generation	Relative percentage of chlorophyll mutants (%)									
		Albino		Xantha		Chlorina		Viridis		Striata	
		Nos	%	Nos	%	Nos.	%	Nos.	%	Nos.	%
Control	Nil										
EMS											
60 mM (T1)	7644	26	0.34	52	0.68	65	0.85	16	0.21	10	0.13
70 mM (T2)	6530	24	0.37	42	0.64	48	0.74	14	0.21	5	0.08
80 mM (T3)	5386	15	0.28	30	0.56	38	0.71	10	0.19	4	0.07
EMS Mean			0.33		0.63		0.76		0.20		0.09
Gamma rays											
700 Gy (T4)	7366	25	0.34	43	0.58	43	0.58	16	0.22	7	0.10
800 Gy (T5)	6280	21	0.33	36	0.57	36	0.57	12	0.19	5	0.08
900 Gy (T6)	4970	15	0.30	25	0.50	25	0.50	10	0.20	6	0.12
Gamma rays Mean			0.33		0.55		0.55	0	0.20		0.10
EMS + Gamma rays											
70mM + 700 Gy (T7)	6345	15	0.24	29	0.46	30	0.47	10	0.16	11	0.17
70mM + 800 Gy (T8)	5416	10	0.18	25	0.46	25	0.46	8	0.15	10	0.18
70mM + 900 Gy (T9)	4586	9	0.20	18	0.39	21	0.46	7	0.15	7	0.15
EMS + Gamma ray Mean			0.21		0.44		0.46		0.15		0.17
Overall Mean			**0.29**		**0.54**		**0.59**		**0.19**		**0.12**

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Table 3. Mutagenic Effectiveness and Efficiency (Chlorophyll mutants) M_1 family basis

Mutagens (Dose/ Conc.)	Percent survival reduction on 30th day (Lethality)	Percent height reduction on 30th day (Injury)	Seed fertility reduction (Sterility) (S)	Mutants 100 M_1 Plants (Mp)	Effectiveness (%)	Efficiency (%)		
						Lethality	Injury	Sterility
EMS								
60 mM (T1)	48.72	12.15	16.01	37.50	15.63	76.97	308.59	234.28
70 mM (T2)	55.64	15.12	23.77	31.76	11.34	57.08	210.02	133.59
80 mM (T3)	60.90	17.86	29.79	28.79	9.00	47.27	161.15	96.62
Gamma rays								
700 Gy (T4)	51.71	14.36	21.10	30.97	4.42	59.89	215.64	146.80
800 Gy (T5)	56.99	16.48	26.73	26.43	3.30	46.38	160.35	98.87
900 Gy (T6)	62.58	21.12	30.43	23.77	2.35	37.99	112.53	78.10
EMS + Gamma rays								
70mM + 700 Gy (T7)	55.79	24.69	28.71	26.03	2.66	46.65	105.42	90.66
70mM + 800 Gy (T8)	60.67	28.02	32.54	24.43	2.26	40.26	87.19	75.08
70mM + 900 Gy (T9)	64.02	30.30	38.44	17.50	1.48	27.33	57.76	45.53

Table 4. Mutagenic Effectiveness and Efficiency (Chlorophyll mutants) M_2 plant basis

Mutagens (Dose/Conc.)	Percent survival reduction on 30th day (Lethality)	Percent height reduction on 30th day (Injury)	Seed fertility reduction (Sterility) (S)	Mutants 100 M_2 Plants (Mp)	Effectiveness (%)	Efficiency (%)		
						Lethality	Injury	Sterility
EMS								
60 mM (T1)	48.72	12.15	16.01	2.21	0.921	4.54	18.19	13.81
70 mM (T2)	55.64	15.12	23.77	2.04	0.727	3.66	13.47	8.57
80 mM (T3)	60.90	17.86	29.79	1.80	0.563	2.96	10.08	6.04
Gamma rays								
700 Gy (T4)	51.71	14.36	21.10	1.82	0.260	3.52	12.67	8.62
800 Gy (T5)	56.99	16.48	26.73	1.75	0.219	3.07	10.63	6.55
900 Gy (T6)	62.58	21.12	30.43	1.63	0.181	2.60	7.72	5.35
EMS + Gamma rays								
70mM + 700 Gy (T7)	55.79	24.69	28.71	1.50	0.153	2.68	6.06	5.22
70mM + 800 Gy (T8)	60.67	28.02	32.54	1.44	0.133	2.37	5.14	4.43
70mM + 900 Gy (T9)	64.02	30.30	38.44	1.35	0.115	2.11	4.46	3.52

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