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Title: Effects of Ethyl methanesulfonate (EMS) on Seedling and Yield contributing Traits in Basmati Rice

Authors list:
Muhammad Rashid, *Nuclear Institute for Agriculture and Biology College, Pakistan Institute of Engineering and Applied Sciences (NIAB-C, PIEAS), Jhang Road, Faisalabad 38000, Pakistan. email:mrashid_niab@yahoo.com*

Areeqa Shamshad, *Nuclear Institute for Agriculture and Biology College, Pakistan Institute of Engineering and Applied Sciences (NIAB-C, PIEAS), Jhang Road, Faisalabad 38000, Pakistan email:areeqa.shamshad71@gmail.com*

Ljupcho Jankuloski, *FAO/IAEA, Plant Breeding and Genetics Laboratories, Vienna, Austria email: L.Jankuloski@iaea.org*

Abstract

Increasing genetic diversity in crop plants has been used for chemical mutagenesis. Through the application of various mutagenic agents, over 430 new varieties have been derived as rice mutants (Oryza sativa L.) Chemical mutagens such as ethyl methane sulphonate (EMS), diepoxybutane derivative (DEB), sodium azide, and gamma ray, x-ray, and quick neutron irradiation have been commonly used to induce a large number of functional variations in rice and others crops. Among chemical mutagens, ethyl methane sulfonate (EMS) is the alkylating agent most widely used in plants because it induces nucleotide substitutions to be extremely frequent, as detected in various genomes. In this study, seeds of potential genotype of the popular variety, (*Oryza sativa* L. Super Basmati variety) were treated with EMS at concentrations of 0.25%, 0.50%, 0.75%, 1% and 1.5%. Various measurements on the M_1 generation determined EMS sensitivity. As concentration of applied EMS increased, will decrease in germination, shoot length, root length, plant height, productive tillers, Panicle Length, Total Spikelet, sterile spikelet and fertility under field conditions were observed in M_1 generation as compared to the non-treatment control. Emergence, shoot length, root length, plant height, productive tillers, Panicle Length, Total Spikelet, sterile spikelet and fertility also decreased with increases in EMS mutagenesis in an approximately linear fashion. The LD_{50} values were observed based on growth reduction of seedlings after EMS treatment with 0.25% and 0.50% on the rice variety (*Oryza sativa* L. spp.).

Keywords: Lethal dose; Chemical mutagenesis; Ethyl methanesulfonate; *Oryza sativa* L.

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Introduction

Irradiation (Gamma rays, X rays, and fast neutrons) and chemical mutagens (EMS, DEB, and sodium azide) have been commonly used to cause a broad range of functional variations in rice. Chemicals primarily cause point mutations, making them suitable for creating missense and nonsense mutations that would result in a sequence of change-of-function mutations. Ionizing radiation, on the other hand, is believed to cause chromosomal rearrangements and deletions[1]. When Auerbach and Robson [2] discovered the mutagenic effects of mustard gas and related compounds during World War II, they discovered the first class of chemical mutagens. Mustard gas, methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), and nitrosoguanidine are all alkylating agents that have different effects on DNA. EMS is the most widely used chemical mutagen in plants due to its potency and ease of use. EMS alkylates guanine bases, causing alkylated G pairs to mispairing with T instead of C, resulting in G/C to A/T transitions [3]. In order to perform EMS mutagenesis in rice, the seeds are soaked in an aqueous solution at a specific concentration (from 0.2 percent to 2.0 percent) for 10 to 20 hours (based on the sensitivity or kill curve of the genotype used). Since EMS creates a large number of non-lethal point mutations (genome-wide), a small mutant population (roughly 10,000) is enough to saturate the genome with mutations. Point mutation density in Arabidopsis can reach four mutations per Mb [4-6].

A significant benefit of using a common mutagen like EMS in forward genetic screens in a variety of organisms is that a large body of literature has confirmed its usefulness in a variety of organisms. The favourite model animal and model plant for mutagenesis studies, respectively, are Drosophila melanogaster and Arabidopsis thaliana. EMS is surprisingly consistent in that these species seem to have reached identical stages of mutagenesis considering their approximately 1 billion-year separation. For example, recessive lethal mutations are thought to occur at similar rates in both cases, with reasonable levels of sterility and lethality caused by EMS doses [7, 8]. In addition, direct estimates show that base substitution rates for Arabidopsis seeds soaked in EMS [9, 10] and EMS-fed Drosophila males [11], are identical, and a reverse genetic screen of zebrafish progeny exposed to N-ethyl-N-nitrosourea (ENU) [12]. Found approximately similar rates. In a number of species, chemical mutagenesis results in a high frequency of nucleotide substitutions.

Since estimates of per gene mutational density observed for Arabidopsis and maize [13], which has a 20-fold larger genome size, genome size does not appear to be a significant factor in EMS mutagenesis. As a consequence, EMS may be the mutagen of choice in plants for TILLING (Target Cause Local Lesion in Genome). However, the toxicity of EMS varies by species, so other mutagens or antioxidant post-treatments may be worth considering [5].

Several new ventures have been undertaken in recent years with the aim of producing EMS-induced rice mutant populations in research institutes [14]. The LD50 dose is first calculated which is then used to determine the best dose for inducing mutations. By skipping this stage, the mutagen dose can result in either a high or low mutation frequency [15]. Chemical doses are measured by adjusting the treatment concentration and length, the solvent used [e.g. dimethyl sulfoxide (DMSO)], or the pH of the solution [16].

Chemical mutagens (EMS, DES, sodium azide) were also used to develop Fusarium wilt-resistant banana shoot tips [17]. Luan et al. used EMS to build salt-tolerant sweet potato (Ipomoea batatas L. callus) lines. The finding was due at the time to variations in the chemical composition of chromosomes near the centromere, which made them more vulnerable to chemical mutagens. Although this may be the case, there are other possibilities. Genes near the centromere, for example, are less likely to be involved in recombination, so mutations in such genes are less likely to be replaced by selection. At least two generations of meiosis with chromosome segregation and recombination are required for mutants. Half of the test species will die at LD50 [18]

Materials and Methods

Plant Materials

Seeds (400) of the Super Basmati rice cultivar (*Oryza sativa* L. spp.) were chosen for EMS-induced mutagenesis in this study.

EMS Mutagenesis

Super Basmati seeds were put in a 500 mL flask, and ultrapure water (100 mL) was added to about 5 cm above the seeds. The seeds were soaked for 20 hours at room temperature overnight. The water was then decanted, and 50 mL of EMS (v/v) concentrations of 0.25 percent, 0.50 percent, 0.75 percent, 1 percent, and 1.25 percent were added. Seeds were incubated at room temperature for 12 hours before being decanted and rinsed with 100 mL ultrapure water (5 times, 4 minutes each) and 200 mL ultrapure water (4 times, 15 minutes each). After that, the seeds were rinsed for four hours under running tap water before being planted in Petri dishes (**Table 1**).

Lethal Dose Study in EMS Mutagenesis

Aside from the untreated control, forty seeds were sown on filter paper soaked in 5 ml of distillate water in petri dishes based on the EMS-induced mutagenesis. Petri dishes were then incubated for 7 days at 25°C in an incubator. The number of seeds that germinated under these conditions was counted after seven days. The grown seeds from each EMS concentration applied, as well as those from the non-treatment control, were transferred to plastic pots and planted in rice field soil. In the green house, the plants were also watered with distillate water (which was only used for research) **(Table 1)**. After two weeks, the shoot and root lengths were measured using the sandwich blotter technique[19]. The treated seeds of each variety were sown in the nursery field and the emergence was reported for each dose in each treated variety after germination. Plant height, active tillers, panicle length, total spikelet, sterile spikelet, and fertility percent were all measured at maturity and registered.

Statistical Analysis

The Lethal Dose experiment used a four-replication fully randomized block design with five levels of EMS concentration in the random block. The differences in observed averages of all tested parameters between treatment and non-treatment plants were examined using the least significant difference (LSD) test with P-values less than 0.05. Statistix 8.1 was used to perform the statistical analysis.

Table 1. EMS mutagenesis scheme for rice

400 seeds	Soaking in the 500 mL ultrapure water	Over Night
	Seeds classified in batches of 50 seeds/treatment and placed in the flasks	
0.25%	50 mL was added	12 hours
0.50%		
0.75%	Concentration of EMS (v/v) in water	
1.00%		
1.25%		
0.00%		
rinsing with ultrapure water	100 mL	Repeat 5 times/4 mints

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with 200 mL — Repeat 4 times/15 mints

rinsing under running tap water — 4 hours

40 Seeds/Treatment — Soaked in 5 mL of distillate water — Incubate 7 days at 25˚C

Germination test

Grown seeds/treatment — Planting in pots containing rice field soil — Incubate in the green house

Plants — Watering with distillate water — 2 weeks

The seedling height and root length of plants measured

Results

Effect of EMS-Induced Mutagenesis on Germination

Data analysis of the number of seeds that germinated revealed a decrease in germination in the M_1 generation as EMS concentrations were increased. According to **Figure 1** and **Table 2**, the findings show that an increase in EMS concentration was correlated with a decrease in seed germination (P 0.05).

Effect of EMS Mutagenesis on shoot length and root Length

The average length of the roots and the length of the shoots revealed that EMS-induced mutagenesis had a substantial effect on the shoot length. According to the findings **(Figure 2** and **Table 2)**, shoot length decreased in proportion to the amount of EMS added (P 0.05). In this analysis, **Figure 3** and **Table 2** showed that when the concentration of EMS was increased, the root length decreased when compared to the non-treatment control (P 0.05). After mutagenesis was induced with a 0.25 percent concentration of EMS in Super Basmati rice, the greatest reduction in root length was observed. The control group had the longest shoot range, measuring 5.44 mm. EMS-induced mutagenesis shortened the duration of the shoots. **Table 2** shows that when super basmati rice was handled with a concentration of 0.25 percent, the maximum reduction in shoot duration was observed. Furthermore, the overall root length (6.28 mm) was found to be the greatest among seeds in the control group, followed by the root length and shoot length at 0.25 percent concentration, which were the greatest among the treatment group. When a 1% concentration of EMS concentration was applied, the shortest root length was reported. When treated with EMS concentrations greater than 1%, no readings for seed germination, root length, or seedling height were observed for the genotype under consideration

Effect of EMS Mutagenesis on yield contributing attributes:

(Plant height, Productive tillers, Panicle Length, Total Spikelet, Sterile Spikelet and Fertility):

Plant height, active tillers, Panicle Length, Total Spikelet, sterile spikelet, and fertility data were collected at maturity. According to the findings (**Figure 4** and **Table 2**), plant height decreased in proportion to the amount of EMS added (P 0.05). When super basmati rice was handled with a concentration of 0.25 percent, the maximum reduction in plant height (104.57 cm) was observed.

When the concentration of EMS was increased, the active tillers, panicle duration, and total spikelet (**Figures 5–7** and **Table 2**) decreased when compared to the non-treatment control (P 0.05). After mutagenesis was induced with 0.25 percent concentration of EMS in Super Basmati rice, the maximum reduction in active tillers, panicle duration, and total spikelet (3.67, 26.9cm, 111.9) was observed. In this study, **Figure 8** and **Table 2** showed that the proportion of sterile spikelets decreased as the applied EMS concentration increased (P 0.05). When super basmati rice was treated with a concentration of 0.25 percent, the largest reduction in sterile spikelets (85.2) was observed. Figure 9 indicates that as the applied EMS concentration was increased, fertility decreased (P 0.05). In the control group, the highest fertility rate (13.12 percent) was observed. **Table 2** shows that when Super Basmati rice was handled with a concentration of 0.25 percent, the highest decrease in fertility (11.48 percent) was observed. When 1 percent EMS concentration was applied, the lowest plant height, productive tillers, panicle length, total spikelet, sterile spikelet, and fertility were reported. When treatment with EMS concentrations greater than 1% was used for the genotype in question, no readings were observed. LD50 values for seed germination (0.069%), shoot length (0.625%), root length (0.6%), plant height (1.125%), active tillers (1.125%), panicle length (1.125%), complete spikelet (1.126%), sterile spikelet (1.06%), and productivity (1.05%) for Super Basmati rice variety (Figure 1a, 2a, 3a, 4a, 5a, 6a, 7a, 8a, 9a).

Discussion

Effect of EMS Mutagenesis on Germination

EMS mutagenesis resulted in a major reduction in germination in field conditions, as can

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be shown. As the EMS concentration was increased, there was a substantial decrease (P 0.05) in seed germination. EMS has been shown to be the most potent of the chemical mutagens and alkylating agents. Polyploids are more tolerant than diploids, according to previous research [20]. According to **Figure 1a**, the current research results showed that after EMS treatment was applied, seed germination was decreased significantly with increasing EMS ($P < 0.05$).

Although the mutagenic reaction is more or less linear with dosage, polyploids are more tolerant than diploids. According to Figure 1a, seed germination was significantly reduced with increasing EMS (P 0.05) after EMS treatment was applied. Plant survival to maturity is dependent on the type and extent of chromosomal damage, according to a previous study on radiation mutation [21], Germination inability, plant growth, and survival can be reduced as the occurrence of chromosomal damage increases with growing radiation dose. Gamma ray treatments were linked to changes in germination percentage in another study [22].

Furthermore, genes close to the centromere are more sensitive to mutagenic treatment than genes further apart. Chlorophyll mutants were found regularly in the EMS treatment group but were uncommon in the physical mutagens treatment group [20, 23]. The activation of RNA or protein synthesis may be responsible for the stimulating effect of physical mutation on germination. It can happen after the seeds have been handled during the early stages of germination [24, 25].

Effect of EMS Mutagenesis on Shoot Length and Root Length

Shoot length is widely used as an index to classify the biological effects of various physical and chemical mutagens in M_1 [1]. Shoot duration and the dosage of physical or chemical mutagens have been shown to have a linear relationship. In line with this observation, our findings show that increases in EMS concentration caused decreases in shoot length. Our findings revealed that when the rice variety Super Basmati was handled with EMS, the shoot length decreased significantly (p 0.05) when compared to the control group. The concentration of applied EMS had an important impact (p 0.05) on the root length of Super Basmati rice. Every subsequent increase in EMS concentration resulted in a reduction in root duration. Enhancement or inhibition of germination, shoot length, and other biological responses are frequently observed in low or high dose treated plants [26, 27].

According to Khan et al. [28], low dose irradiation induces growth stimulation by modifying the hormonal signaling network in plant cells or growing the cells' anti-oxidative ability. Plants can easily withstand everyday stress factors such as light intensity and temperature variations in the growth environment [29]. The cell cycle arrest at G2/M process during somatic cell division and/or various damages in the entire genome have been attributed to the high dose treatment that induced growth inhibition [30]. Variability was assessed in this analysis by the mean values of shoot and root lengths, both of which decreased as the concentration of EMS increased. When radiation is sufficient to reduce rooting percentages, the root lengths do not exceed a few millimeters in length, according to a physical mutation analysis by Chaudhuri [31], As a result of the seeds' metabolic disorders following radiation therapy, they are unable to germinate [32].

Effect of EMS Mutagenesis on yield contributing attributes:

(Plant height, Productive Tillers, Panicle Length, Total Spikelet, Sterile Spikelet and Fertility)

Seeds treated with EMS developed a variety of mutants in this sample. This may be due to the pleiotropic impact of mutated genes or mutations on various genome loci (Basu 2008) [33]. A number of morphological mutations in legume plants have been identified [34],], and some of these mutations have been shown to affect multiple characters. In the EMS-treated plants, higher dose and treatment period combinations resulted in higher death and lower yield in the plant attributes. Similar findings were made with EMS-treated fenugreek seeds, where no callus cultures developed when treated with EMS concentrations greater than 1% (Jain and Agarwal 1994)[35]. In this analysis, LD50 values for yield contributing traits plant height (1.125%), active tillers (1.125%), panicle length (1.125%), complete spikelet (1.126%), sterile spikelet (1.06%), and fertility (1.05%) were found in seed treated with 0, 0.25, 0.5, 0.75, 1, and 1.5 percent EMS, resulting in an inverse association between all of these yeilding traits. (13, 14, 15, 16, 17 and 18) [36]. The efficacy of the current study decreased as the concentration of EMS increased. This was confirmed by Vanniarajan's (1989) findings in blackgram, Jebaraj and Marappan's (2006) findings in cowpea, and Packiaraj's findings in cowpea [37, 38].

The variation in LD50 for the Super basmati rice variety at different EMS percent concentrations has been observed in mutation studies, and it is thought to be due to the biological material, its scale, maturity, hardness, and moisture content at the time of exposure of breeding material [39]. There is proof that the radiation-induced sterility of M_1 panicles is passed on to subsequent generations [40]. Physiological damage induces a significant portion of sterility, which is not passed on to the next generation. With increasing doses of mutagen treatments, induced panicle sterility increased panicle sterility in this research. These findings are consistent with those of previous researchers [41, 42], who found that gamma ray treatment caused rice plants to become highly sterile.

Conclusion

Seed germination, shoot length, root length, plant height, active tillers, panicle length, complete spikelet, sterile spikelet, and fertility emergence of the M_1 generation were all assessed in the field to assess the Lethal Dose. Quantitative measurements were used on a daily basis in this experiment. The following information was collected and recorded: seed germination, shoot length, root length, plant height, active tillers, panicle length, total spikelet, sterile spikelet, and fertility percent. Variability was measured based on observed means. Seed germination, shoot length, root length, plant height, active tillers, panicle length, complete spikelet, sterile spikelet, and fertility were all significantly influenced by variations in EMS treatment concentrations (p 0.05). As a result, LD50 values for the variety super basmati rice were determined based on seedling growth reduction after treatments with 0.25 percent and 0.50 percent EMS concentrations.

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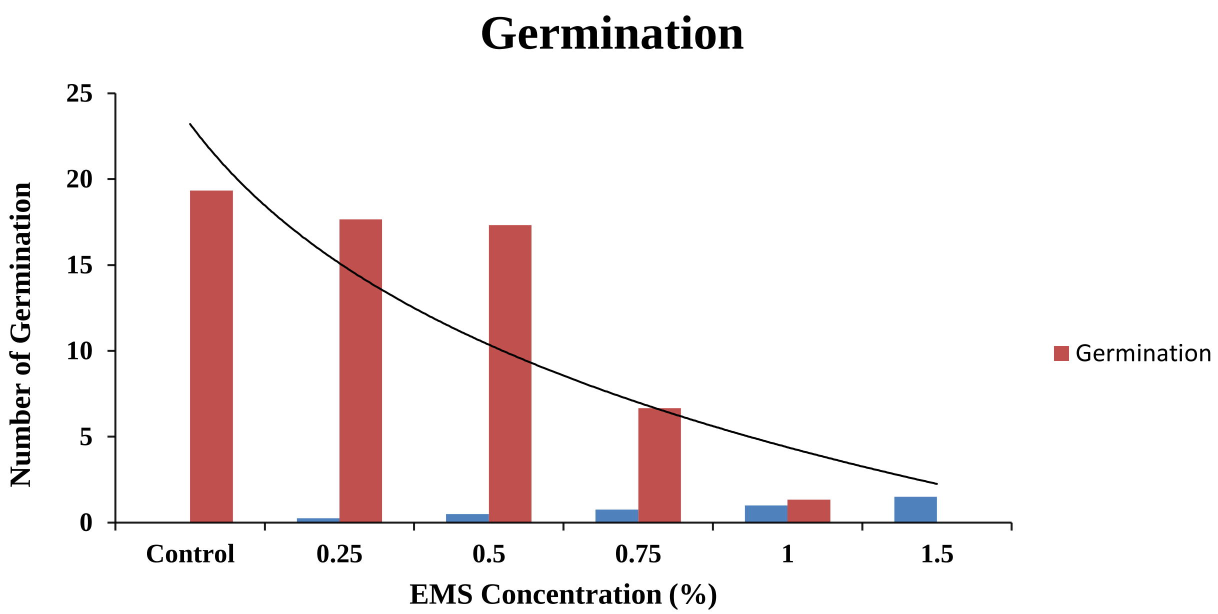

Figure 1a. Effect of different concentration of EMS mutagenesis on seed germination

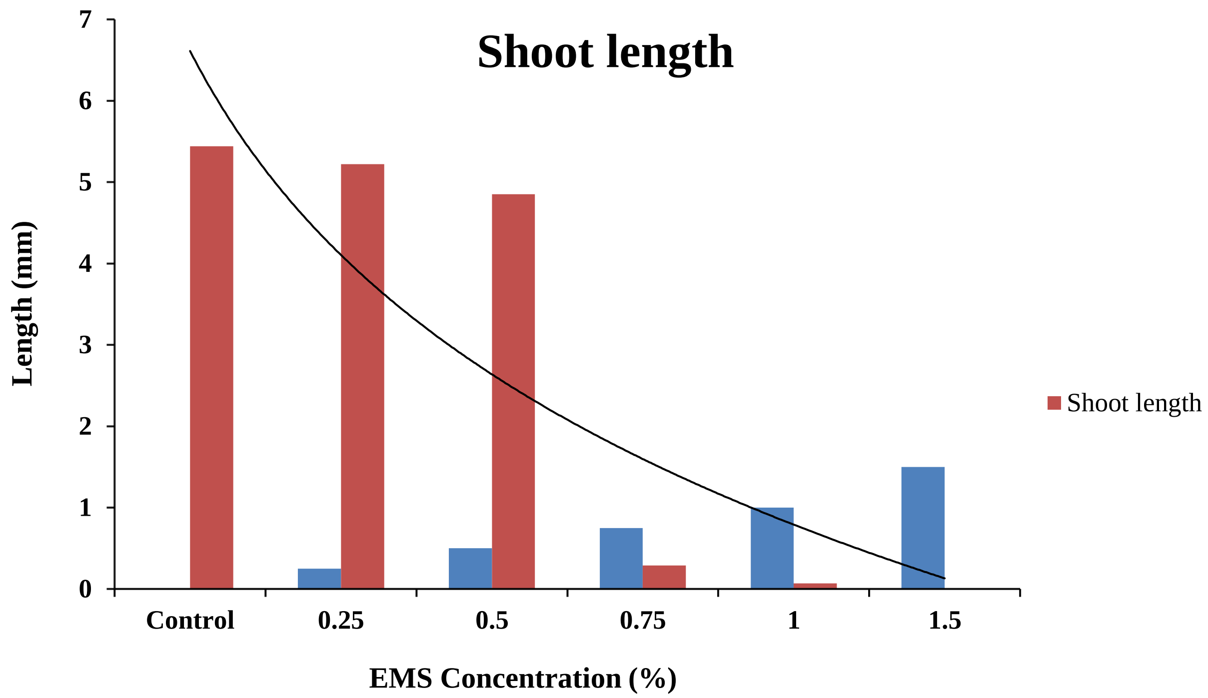

Figure 2a. Effect of different concentration of EMS mutagenesis on shoot length

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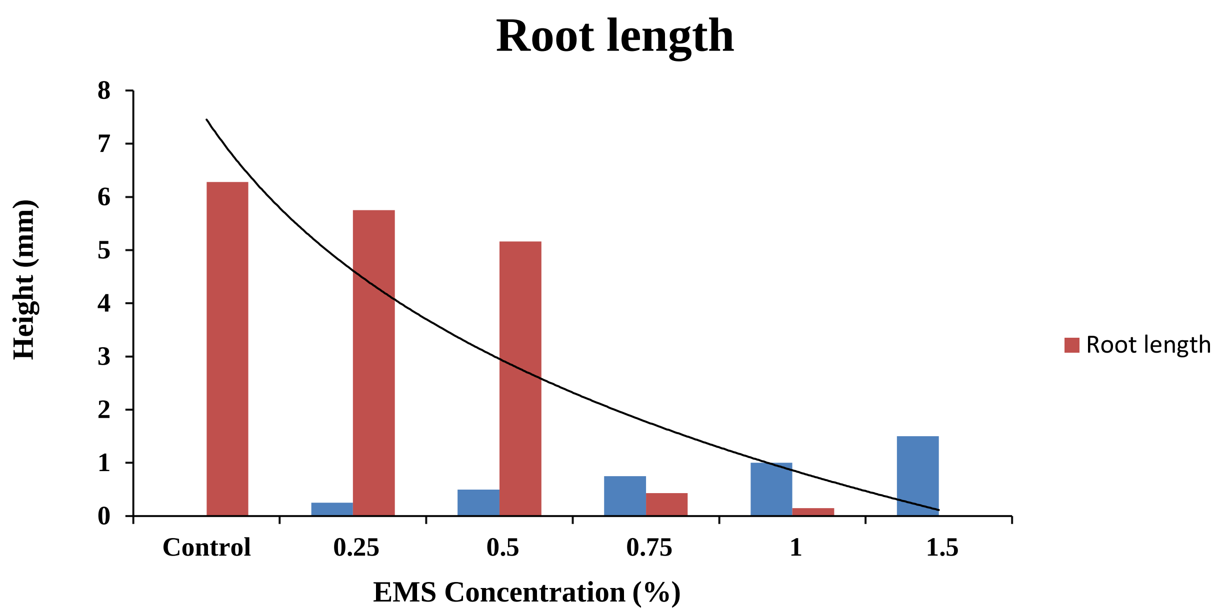

Figure 3a. Effect of different concentration of EMS mutagenesis on root length

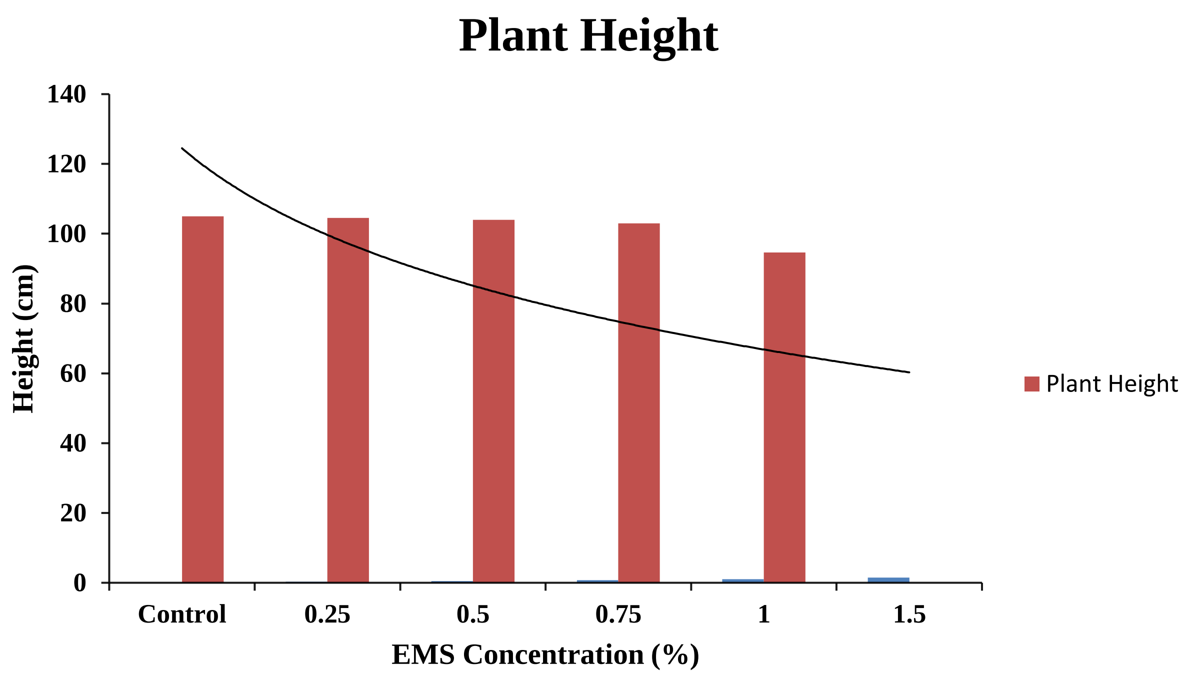

Figure 4a. Effect of different concentration of EMS mutagenesis on plant height

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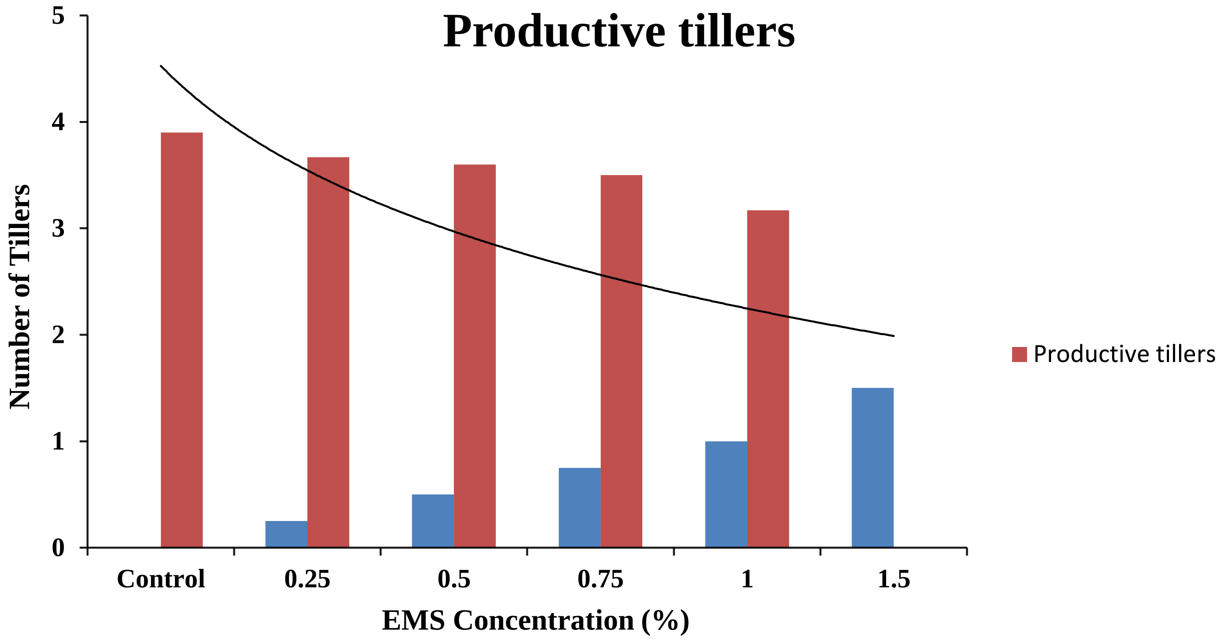

Figure 5a. Effect of different concentration of EMS mutagenesis on productive tillers

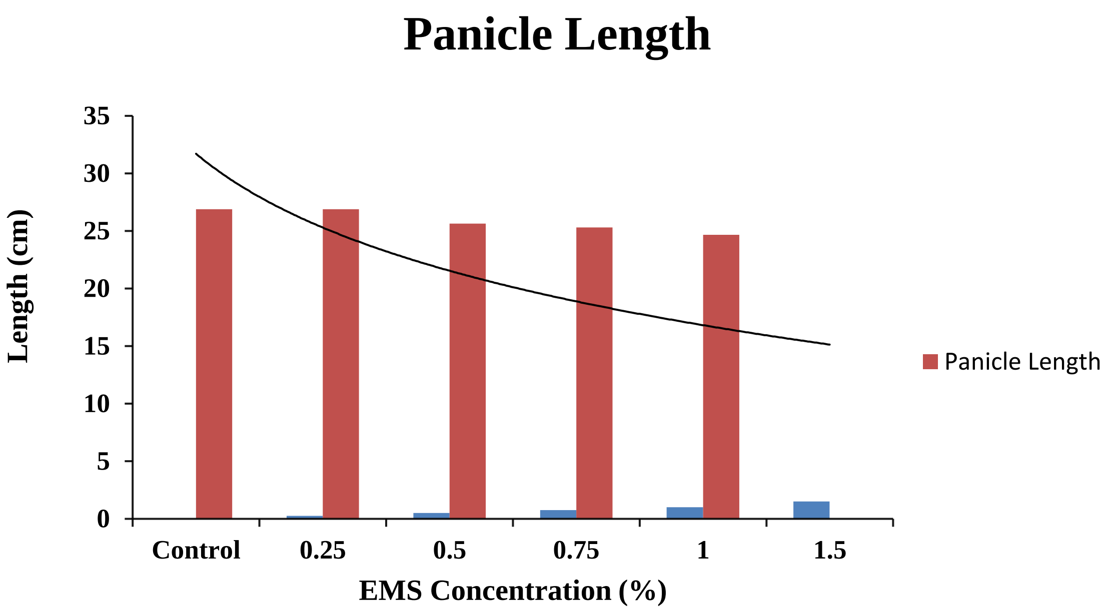

Figure 6a. Effect of different concentration of EMS mutagenesis on panicle length

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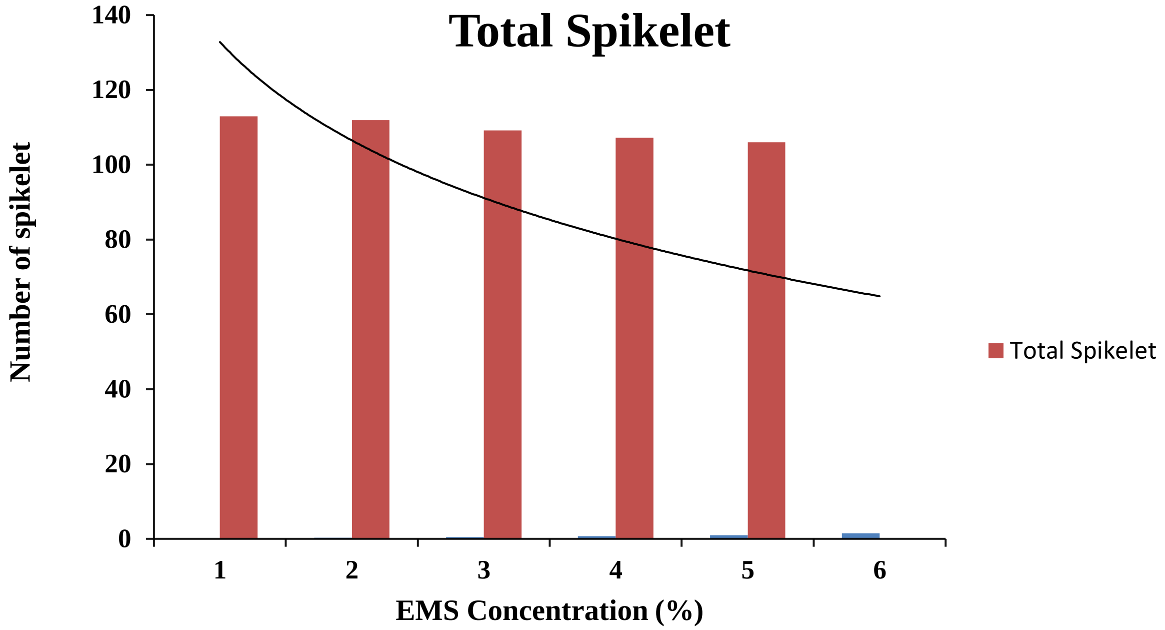

Figure 7a. Effect of different concentration of EMS mutagenesis on total spikelet

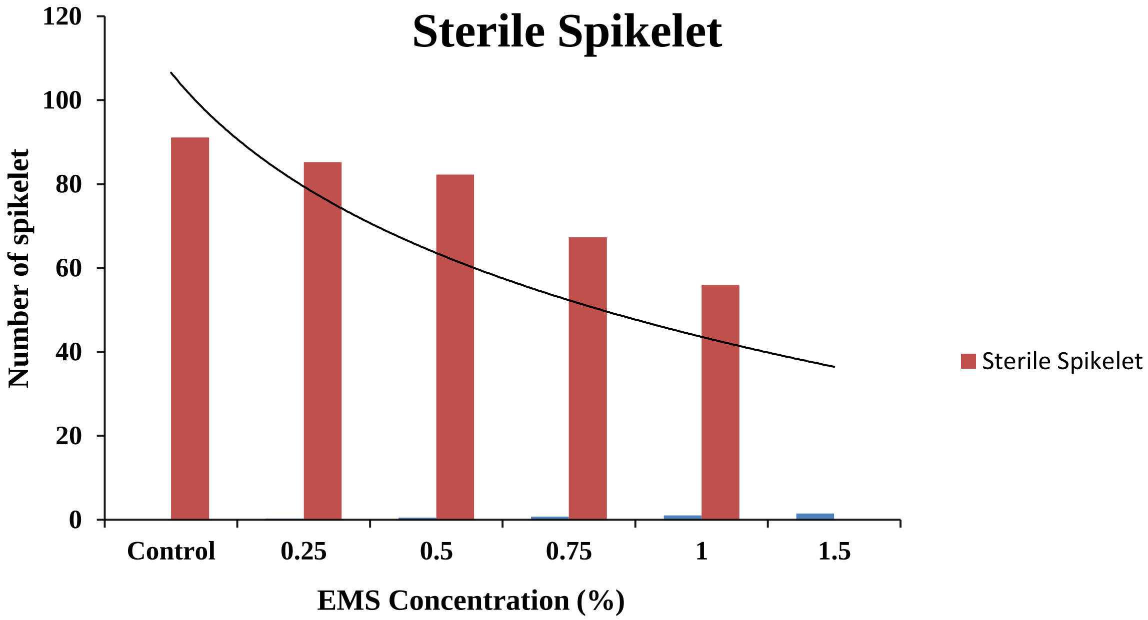

Figure 8a. Effect of different concentration of EMS mutagenesis on sterile spikelet

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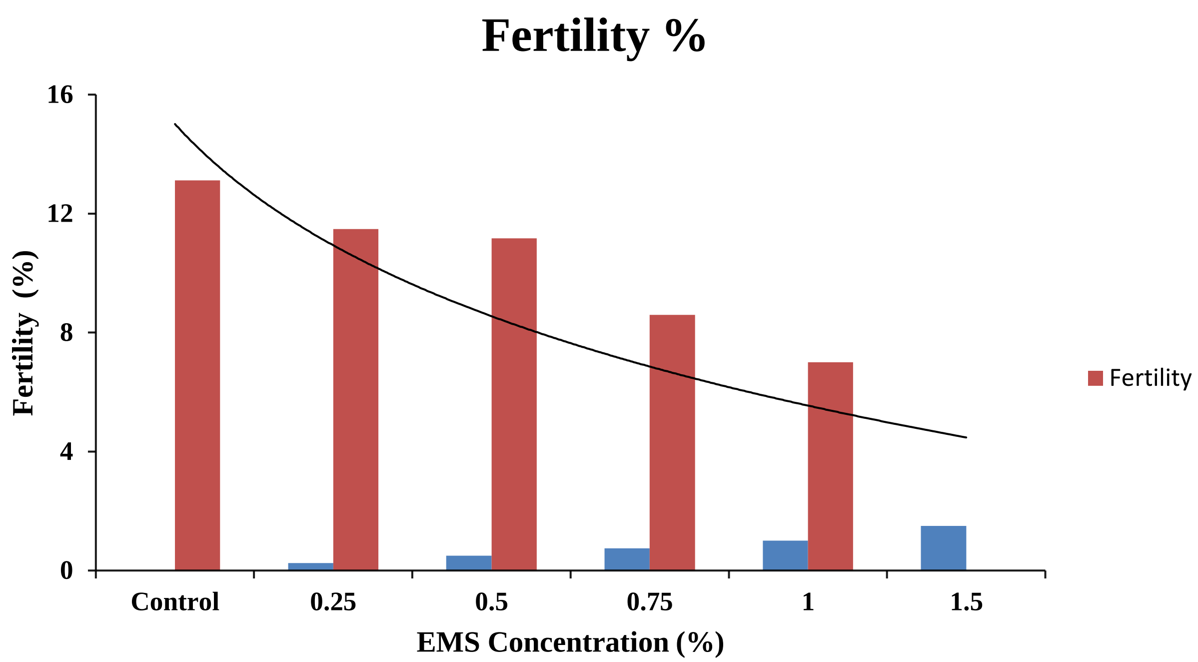

Figure 9a. Effect of different concentration of EMS mutagenesis on fertility

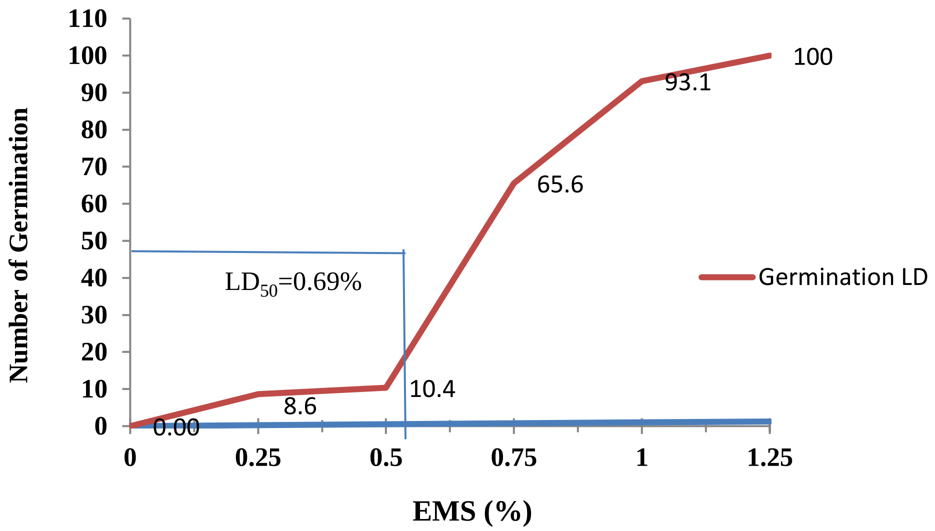

Figure 10: *LD_{50}* of chemical mutagen (EMS) for super Basmati

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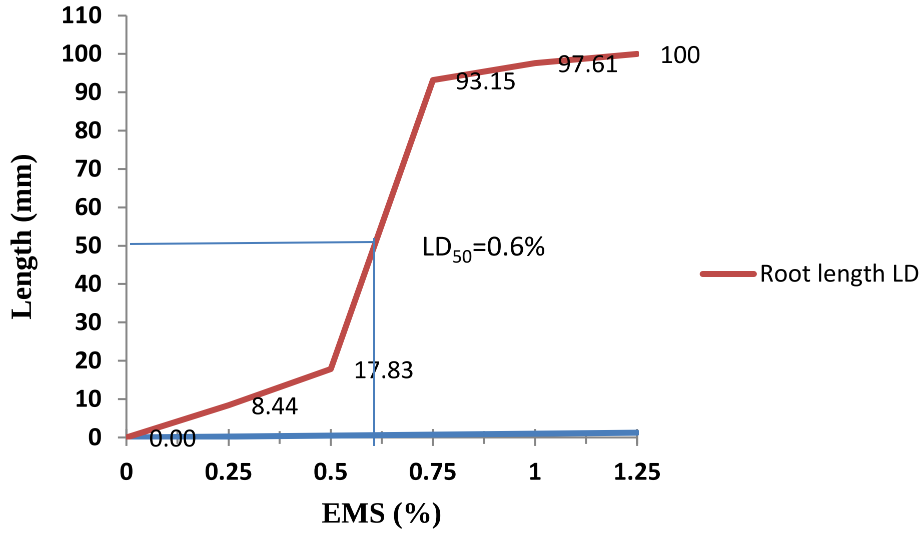

Figure 11: ***LD_{50}*** **of chemical mutagen (EMS) for super Basmati**

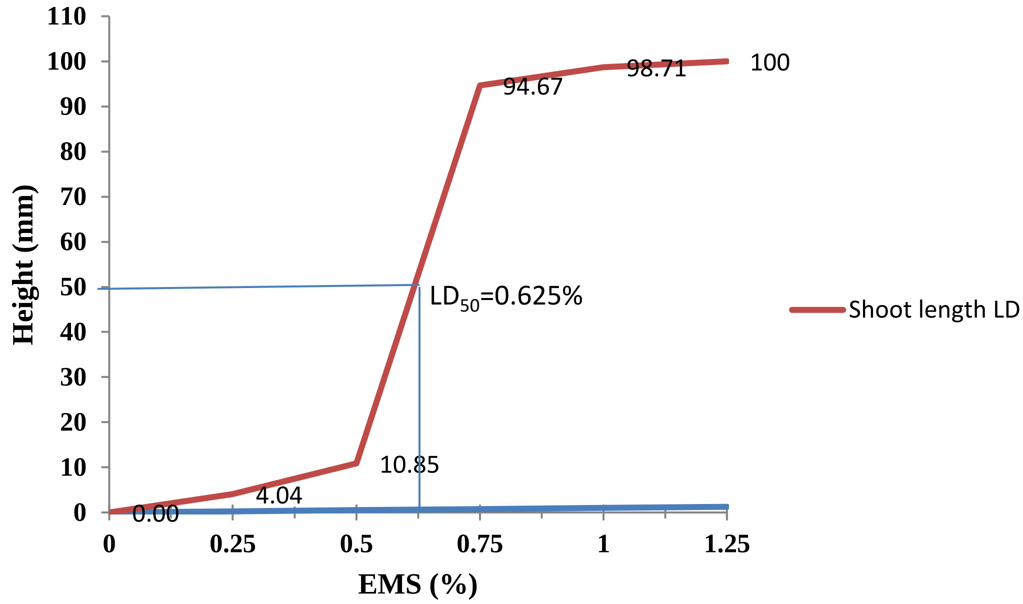

Figure 12: ***LD_{50}*** **of chemical mutagen (EMS) for super Basmati**

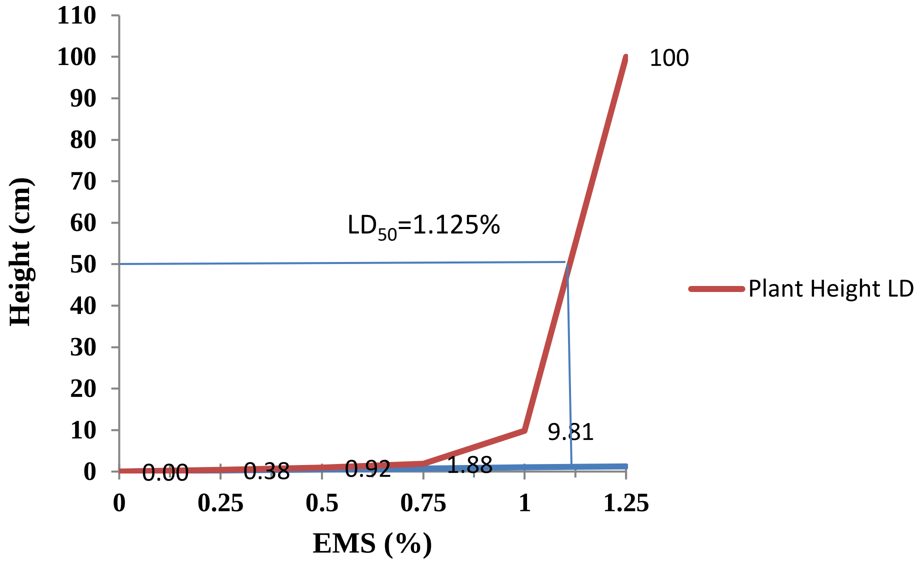

Figure 13: ***LD*** $_{50}$ **of chemical mutagen (EMS) for super Basmati**

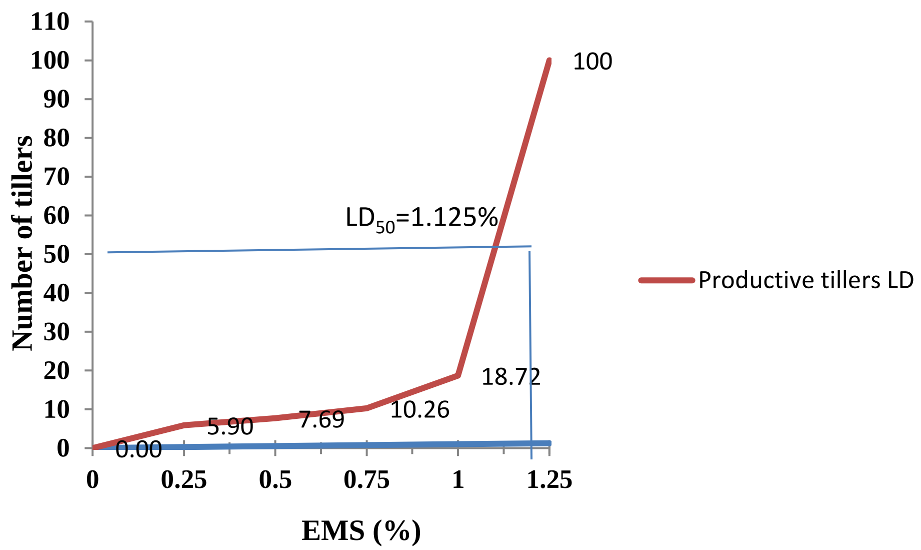

Figure 14: ***LD*** $_{50}$ **of chemical mutagen (EMS) for super Basmati**

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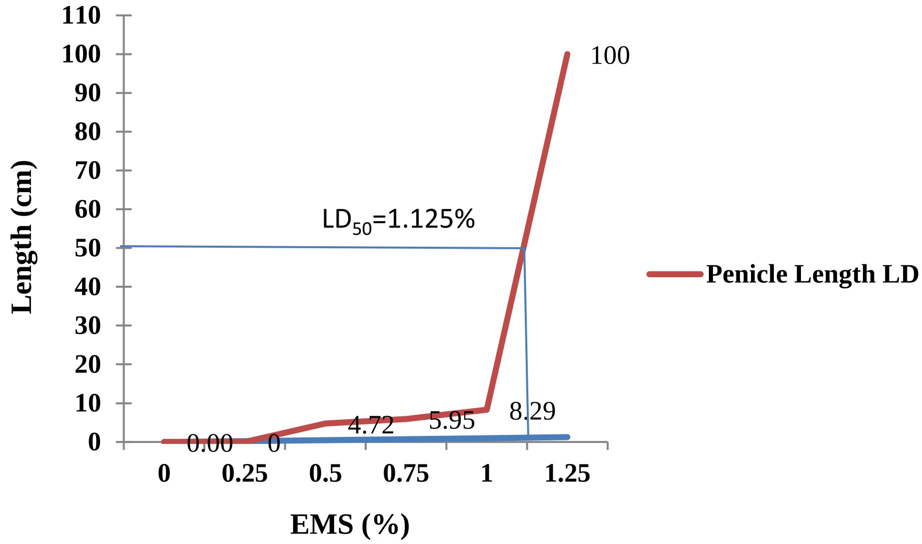

Figure 15: ***LD_{50}*** **of chemical mutagen (EMS) for super Basmati**

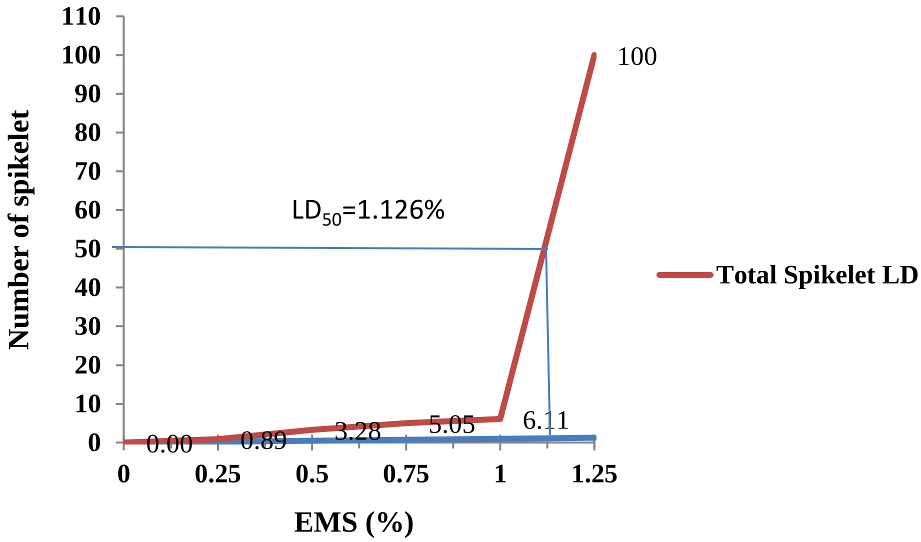

Figure 16: ***LD_{50}*** **of chemical mutagen (EMS) for super Basmati**

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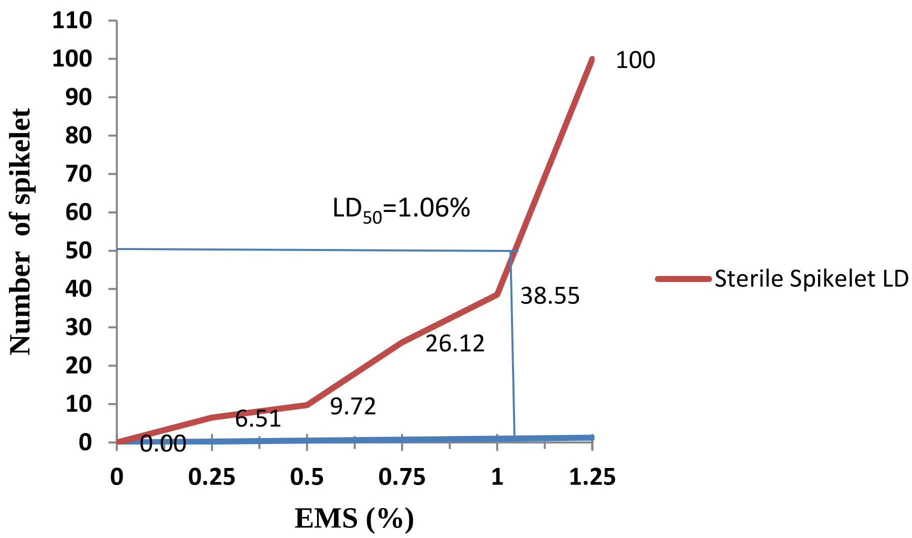

Figure 17: ***LD_{50}*** **of chemical mutagen (EMS) for super Basmati**

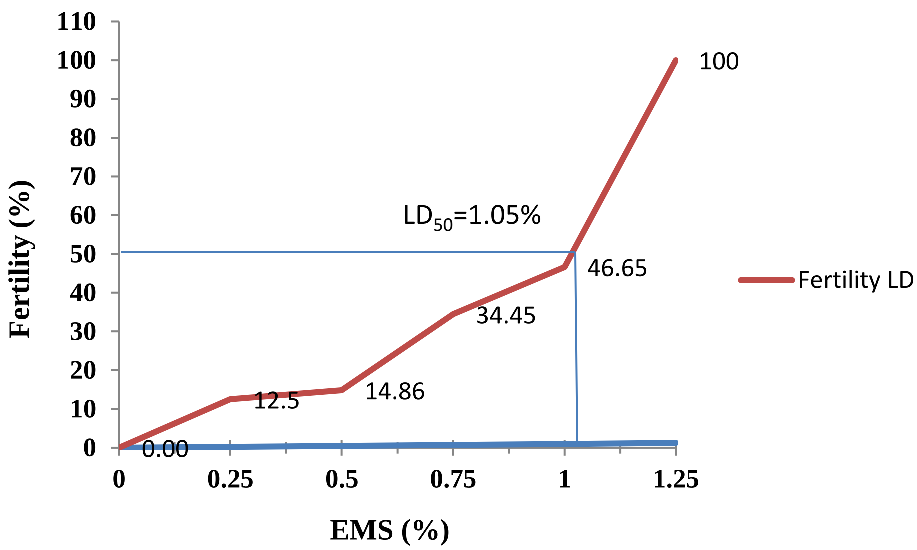

Figure 18: ***LD_{50}*** **of chemical mutagen (EMS) for super Basmati**

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Table 2. Mean value of germination, shoot length, root length, plant height, productive tillers, panicle length, total spikelet, sterile spikelet, fertility following EMS mutagenesis.

Treatment	Germination		Root length (cm)		Shoot length (cm)		Plant Height (cm)		Productive tillers		Panicle Length (cm)		Total Spikelet		Sterile Spikelet		Fertility (%)	
	Actual	%Control	Actual	%Control	Actual	%Control	Actual	%Control	Actual	%Control	Actual	%Control	Actual	%Control	Actual	%Control	Actual	%Control
Control	19.33	100	6.28	100	5.44	100	104.97	100	3.90	100	26.9	100	112.9	100	91.13	100	13.12	100
0.25	17.67	91.4	5.75	91.56	5.22	95.96	104.57	99.62	3.67	94.10	26.9	100	111.9	99.11	85.2	93.49	11.48	87.5
0.50	17.33	89.6	5.16	82.17	4.85	89.15	104	99.08	3.60	92.31	25.63	95.28	109.2	96.72	82.27	90.28	11.17	85.14
0.75	6.66	34.4	0.43	6.85	0.29	5.33	103	98.12	3.50	89.74	25.3	94.05	107.2	94.95	67.33	73.88	8.6	65.55
1	1.33	6.9	0.15	2.39	0.07	1.29	94.67	90.19	3.17	81.28	24.67	91.71	106	93.89	56	61.45	7	53.35
1.25	0	**0**	0	0	0	0	0	0	0.00	0	0	0	0	0	0	0	0	0
LSD%	0.94		0.16		0.53		4.93		0.52		0.90		42.23		5.26		0.99	
C.V%	5.07		3.01		11.34		3.25		9.87		2.35		27.72		4.65		6.50	