Biological Forum – An International Journal **14(1): 17-24(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Fixation of Lethal Dose and Study of Physical and Chemical Effect of Mutagenesis on Germination, Pollen Sterility and Plant Survival in M_1 Generation of Wheat (*Triticum aestivum* L.)

Amit Rana[1], Vijay Rana[1,2], Priyanka[1], Shubhanshu Anubhav[1] and Chetan Gupta[1]*
[1]Department of Genetics and Plant Breeding,
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, (Himachal Pradesh), India.
[2]Rice and Wheat Research Centre, Malan,
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, (Himachal Pradesh), India.

(Corresponding author: Priyanka)*
(Received 08 October 2021, Accepted 03 December, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The present study comprises of four wheat varieties *viz.*, HS 490, HPW 89, HPW 360 and HPW 251 which were treated with 12 doses each of gamma rays *viz.*, 25Gy, 50Gy, 75Gy, 100Gy, 125Gy, 150Gy, 175Gy, 200Gy, 225Gy, 250Gy, 275Gy and 300Gy and EMS concentrations *viz.*, 0.05%, 0.15%, 0.25%, 0.35%, 0.45%, 0.55%, 0.65%, 0.75%, 0.85%, 0.95%, 1.05% and 1.15% to study their effect on germination, pollen fertility reduction and plant survival in M_1 generation under both lab and field conditions. The results showed overall reduction in germination percent, plant survival and increased pollen sterility over control in all the mutagenic treatments. LD_{50} values observed using probit analysis of all four varieties ranged from 0.683%-1.169% EMS concentration and 241.41-278.48 Gy. Therefore, concentrations/dose of both mutagens producing less lethality would be suitable for determining varietal effect and inducing large scale mutagenesis in future wheat breeding programmes.

Keywords: Gamma rays, EMS, mutagen, germination, LD_{50}, pollen grains, survival.

INTRODUCTION

Wheat (*Triticum aestivum* L. em Thell) is a hexaploid (2n=6x=42; AABBDD genome), annual and self-pollinated cereal that belongs to tribe Triticeae and family Poaceae. It is an essential staple food of more than two billion people that constitute approx. 36% of the world's population (Pathak and Shrivastav 2015). Being an abundant source of dietary nutrients, it accounts for more than 20% of the food calories consumed globally occupying an area of 220.89 million hectares with production of 775.9 million tonnes and productivity of 3.51 tonnes per hectare (USDA 2020-21).

In plant breeding, improvements can only be possible when sufficient genetic variability is available in the germplasm to the plant breeder (Cheng *et al.*, 2015). Conventionally, hybridization between different varieties, species or genera has been the method to generate variability. However, due to non-availability of desirable parents, infusion of unfavourable traits, occurrence of higher parental types in segregating populations have resulted into bleak possibility for developing desirable variations through hybridization. Therefore, a powerful approach for deciphering the biological functions of genes is to produce mutants through mutagenesis. Mutation breeding offers a great advantage of occurrence of desirable mutants in high yielding varieties of becoming homozygous in M_2 or M_3 generation in comparison to F_5 or F_6 generation through hybridization (Chakraborty and Paul 2013). The chief objective of mutation breeding programmes is to enhance well-adapted popular varieties by altering one or few major agro-morphological traits which limit their yield potential, productivity or quality. It is an effective and efficient tool at the end of plant breeders especially in self-pollinated crops with narrow genetic base (Micke *et al.*, 1990).

Through mutation induction, till now more than 3,365 mutant varieties, including 265 bread wheat varieties, have been produced directly or indirectly (International Atomic Energy Agency 2021). About 80% of the mutant varieties obtained are developed using physical techniques. Mutation induction with radiation has been the most frequently used method to develop direct mutant varieties.

In mutation breeding programmes, both physical and chemical mutagens have been vastly used independently or in combinations to produce genetic variations for genetic and breeding studies. Among radiation techniques, gamma rays particularly is an important physical mutagen due to its ability to induce morphological, cytological and physiological changes in cells and tissues affecting plant growth and development (Thapa 2004; Borzouei *et al*., 2010. Wheat being a polyploid plant, allows higher number of primary induced changes to be transmitted to the subsequent generations. In mutation breeding programmes, determining optimum dose/concentration of the mutagen is most essential for its genetic manipulation through mutagenesis. Contradictory dose usage suitable for creation of new value genotypes between high (Laghari *et al.,* 2012; Mangi *et al*., 2016) and medium dose usage (Shu *et al*., 2013; Nazarenko 2016) remains controversial as high dosage causes physiological injuries which ultimately leads to death of the plant. Therefore, most optimal dose is one which produces maximum variability but not causing more than 50% death of the plants. LC_{50}/LD_{50} dose represent the concentration at which 50% of the population responds. Probit analysis is generally used in toxicology to ascertain the relative toxicity of various concentrations/dose of the chemicals to living organisms. The LD_{50} value of mutagen helps in predicting mutagen sensitivity and subsequent recovery of higher mutation frequency (Harding and Mohamad 2009). Seed germination, pollen sterility and plant survival are some of the common methods to study mutagen sensitivity in plants. Hence, future genetic improvement through induced mutagenesis remains an important aspect and efficient method for the induction of morphological and genetic variabilities in plants.

MATERIAL AND METHODS

Plant material and experimentation. The experimental material comprising of four wheat varieties i.e. HS 490, HPW 89, HPW 360 and HPW 251 which were treated with gamma rays (γ-rays) and ethyl methane sulphonate (EMS) sown under three replications using Split-plot design at Rice and Wheat Research Centre (RWRC), Malan during *Rabi* season 2019-20.

Gamma irradiation. Under gamma rays, 100 seeds treated of all the four wheat varieties for 12 doses *viz.,* 25 Gy, 50 Gy, 75 Gy, 100 Gy, 125 Gy, 150 Gy, 175 Gy, 200 Gy, 225 Gy, 250 Gy, 275 Gy and 300 Gy each irradiated with ^{60}Co gamma rays at BARC, Trombay, Mumbai.

Chemical treatment. For EMS treatment, 100 seeds for 12 doses (0.05%, 0.15%, 0.25%, 0.35%, 0.45%, 0.55%, 0.65%, 0.75%, 0.85%, 0.95%, 1.05% and 1.15%) each for all the four varieties were first pre-soaked in water for 14-16 hours and then treated with respective EMS concentrations for 2 hours at room temperature. The seeds were then thoroughly washed under tap water for about an hour to wash off EMS from the seeds before sowing.

These treatments were then sown both in seedling trays and space planted under field conditions with 10 cm plant to plant spacing and 25 cm row to row spacing along with untreated parent varieties as control and harvested individually to have M_1 generation. The observations were recorded to determine their germination percentage and plant survival reduction (%).

Determination of LD_{50} dose. LD_{50} values of γ-rays and EMS were determined for all the varieties under study based on seedling mortality per cent of seedlings evaluated under natural field conditions using probit analysis. Probit is the inverse cumulative distribution function (CDF), or quantile function associated with the standard normal distribution (Finney 1971; 1978).

The dose concentration was transformed into $\log_{10}$ value and corrected mortality is computed using Abbott (1925) formula. The mortality percentage of seedlings due to treatment doses are rounded to the nearest whole number. Further, the corrected values are converted to probit transformation. A graph was prepared by plotting probit values (Y-axis) against Log_{10} concentration (X-axis) and a best possible straight line was drawn through plotted points, then used this line to estimate the Log_{10} concentration associated with a probit 5. Antilog to the Log_{10} value corresponding to the probit 5 was taken and LD_{50} for the particular mutagen under study was found out.

Corrected mortality (%)

$$= \frac{\text{M observed - M control}}{\text{100 - M control}} \times 100$$

Pollen viability test. Pollen grains from all the varieties for each treatment was taken at the time of flower initiation and were viewed under microscope to observe the effect of mutagens on pollen viability. Pollen grains were then stained with the help of 2% acetocarmine solution. Multiple observations were taken for each treatment and in each field of view, 100 pollen grains were counted in each observation. The pollens which stained were viable and distinguished from unstained non-viable pollen grains.

Pollen viability (%)

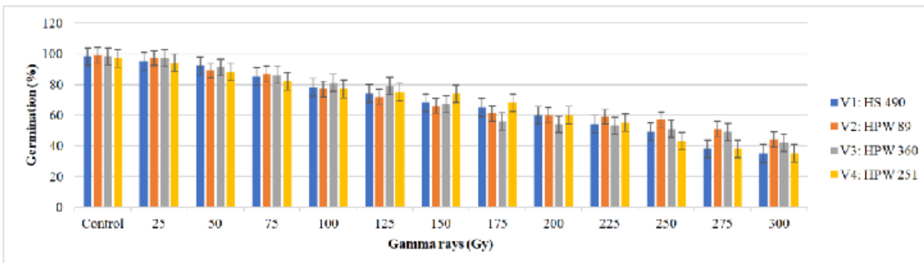
$$= \frac{\text{No. of observed stained pollen grains}}{\text{Total no. of pollen grains in each view}} \times 100$$

RESULT AND DISCUSSION

Effect of induced mutagenesis on Germination (%). Germination percentage of all the four varieties irradiated at 12 different doses *viz.,* 25 Gy, 50 Gy, 75 Gy, 100 Gy, 125 Gy, 150 Gy, 175 Gy, 200 Gy, 225 Gy, 250 Gy, 275 Gy and 300 Gy is presented in Table 1 and Graph 1. The per cent germination was recorded after 15 days of sowing and it was revealed that the reduction in germination per cent over control was noted in all mutagenic treatments of all the four varieties as with the increase in the concentration of the irradiation. At 300 Gy, HS 490 (63%) and HPW 251 (62%) showed highest reduction in germination over control as compared to HPW 360 (56%) and HPW 89 (55%). Probable delay in the one set of mitosis and chromosomal aberration induced enzyme activity leads to reduced germination percentage.

Table 1: Effect of gamma rays on germination in different wheat varieties.

Mutagen	V1: HS 490		V2: HPW 89		V3: HPW 360		V4: HPW 251	
Gamma Dose	**Germination (%)**	**Reduction over control (%)**	**Germination (%)**	**Reduction over control (%)**	**Germination (%)**	**Reduction over control (%)**	**Germination (%)**	**Reduction over control (%)**
Control	98	-	99	-	98	-	97	-
25 Gy	95	3	97	2	97	1	94	3
50 Gy	92	6	89	10	91	7	88	9
75 Gy	85	13	87	12	86	12	82	15
100 Gy	78	20	77	22	81	17	77	20
125 Gy	74	24	72	27	79	19	75	22
150 Gy	68	30	66	33	67	31	74	23
175 Gy	65	33	61	38	56	42	68	29
200 Gy	60	38	60	39	54	44	60	37
225 Gy	54	44	59	40	53	45	55	42
250 Gy	49	49	57	42	51	47	43	54
275 Gy	38	60	51	48	49	49	38	59
300 Gy	35	63	44	55	42	56	35	62

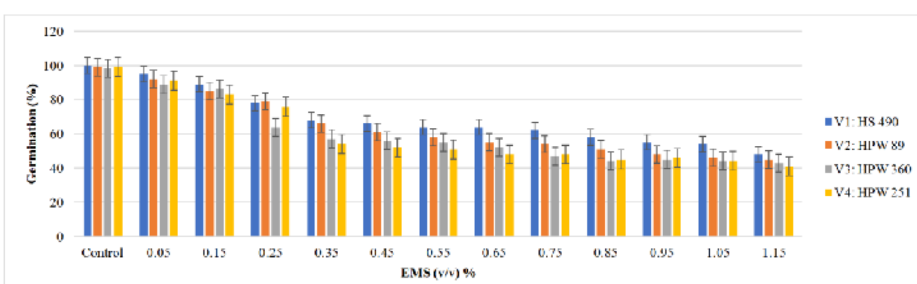


Graph 1. Effect of gamma rays on germination in different wheat varieties.

Per cent germination of EMS treated concentrations *viz.*, 0.05%, 0.15%, 0.25%, 0.35%, 0.45%, 0.55%, 0.65%, 0.75%, 0.85%, 0.95%, 1.05%, 1.15% of all four varieties under study is presented in Table 2 and Graph 2. Here, similar reduction in germination per cent was seen over control for all the mutagenic treatments as with the increase in EMS concentration. At 1.15%, HPW 251 (58%) and HPW 360 (55%) showed highest reduction in germination per cent over control as compared to HPW 89 (54%) and HS 490 (52%).

Table 2: Effect of EMS on germination in different wheat varieties.

Mutagen	V1: HS 490		V2: HPW 89		V3: HPW 360		V4: HPW 251	
EMS Dose (v/v) %	**Germination (%)**	**Reduction over control (%)**	**Germination (%)**	**Reduction over control (%)**	**Germination (%)**	**Reduction over control (%)**	**Germination (%)**	**Reduction over control (%)**
Control	100	-	99	-	98	-	99	-
0.05 %	95	5	92	7	89	9	91	8
0.15 %	89	11	85	14	86	12	83	16
0.25 %	78	22	79	20	64	34	76	23
0.35 %	68	32	66	33	57	41	54	45
0.45 %	66	34	61	38	56	42	52	47
0.55 %	64	36	58	41	55	43	51	48
0.65 %	64	36	55	44	52	46	48	51
0.75 %	62	38	54	45	47	51	48	51
0.85 %	58	42	51	48	44	54	45	54
0.95 %	55	45	48	51	45	53	46	53
1.05 %	54	46	46	53	44	54	44	55
1.15 %	48	52	45	54	43	55	41	58



Graph 2: Effect of EMS on germination in different wheat varieties.

Determination of LD_{50} dose. The LD_{50} is an important criterion for deciding the dose of any mutagen. It is specific dosage of chemicals or radiations at which 50 per cent of test material is killed. It is considered to be the optimum dose that causes high frequency of favourable mutations with minimum damage to the plant since doses lower than LD_{50} favours plant's recovery after treatment, while the use of higher doses increases the probability to induce mutations either in positive or negative direction. The LD_{50} value changes with type of biological material used, nature of treatment applied and environmental conditions (Babaei *et al*., 2010).

LD_{50} values of all the four varieties were determined based on germination percentage after treatment with different doses of EMS and gamma rays evaluated under natural field conditions using probit analysis. The graph pertaining to dose response for EMS treatments is presented in Fig. 1 and gamma rays treatments in Fig. 2. The results indicated that the LD_{50} values under EMS treatment for varieties *viz.*, HS 490, HPW 89, HPW 360 and HPW 251 were found to be 1.169%, 0.885%, 0.725% and 0.683% respectively whereas LD_{50} values for varieties *viz.*, HS 490, HPW 89, HPW 360 and HPW 251 irradiated with gamma rays were found to be 241.41 Gy, 278.48 Gy, 251.27 Gy and 247.96 Gy respectively. Different LD_{50} values for different varieties are due to presence of variations in genotype to genotype and due to different genetic constitution and parentage.

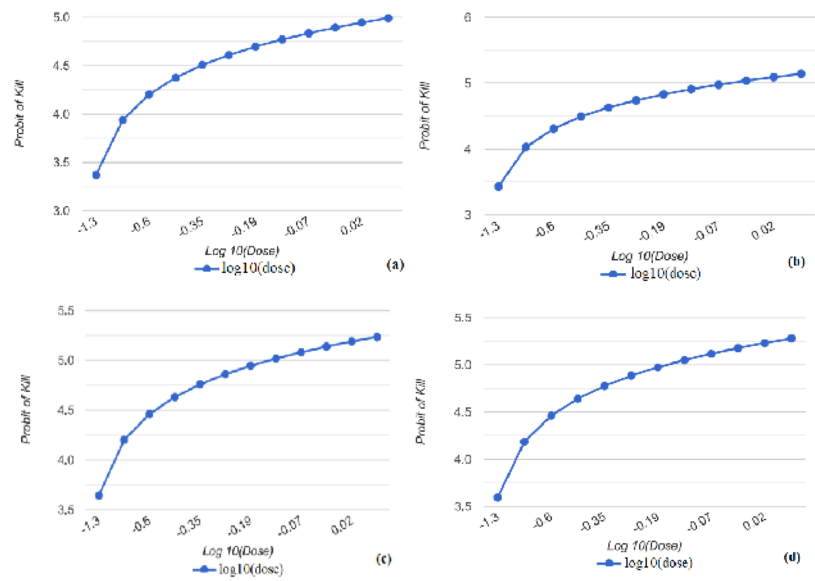


Fig. 1. Dose response curve calculated for calculation of LD_{50} value with EMS treatment using probit analysis (a) HS 490; (b) HPW 89; (c) HPW 360; (d) HPW 251).

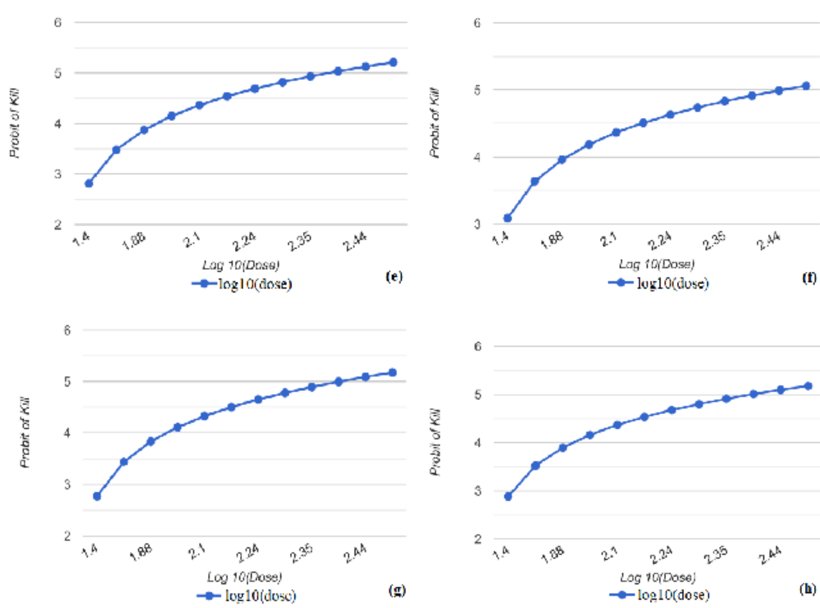


Fig. 2. Dose response curve calculated for calculation of LD_{50} value with gamma irradiation treatment using probit analysis (e: HS 490; f: HPW 89; g: HPW 360; h: HPW 251).

Effect of induced mutagenesis on pollen sterility. Wheat plants at maturity sheds short-lived tricellular pollen grains and identification of viable pollen grains is important to study pollen sterility due to induce mutagenesis. The effect of induced mutagenesis on pollens grains upon treatment with 2% acetocarmine under gamma rays and EMS can be seen in Fig. 3.

The pollen grains which took up the stain were viable and the ones which did not take up stain were classified as inviable. Pollen fertility reduction serves as a measure of effect of increase in concentration of induced mutagens on pollen grains. It was observed that the pollen sterility increased with increasing dose/concentrations of mutagens.

From Table 3 and 4, it can be noted that highest pollen viability reduction can be seen in HPW 251 under both gamma rays at 300 Gy and EMS at 1.15% for up to 11.5% and 8.7% respectively as compared to other varieties over control (depicted in Graph 3 and 4). In most cases meiotic abnormalities are mostly responsible for reduction in the pollen fertility. For both gamma rays and EMS treatments, subsequent reduction in pollen fertility is seen in HPW 360 for up to 10% and 7.7% followed by HPW 89 up to 6.2% and 6.8% and later HS 490 up to 5.5 % and 3.9% over control at highest dose 300 Gy and 1.15% concentration under study.

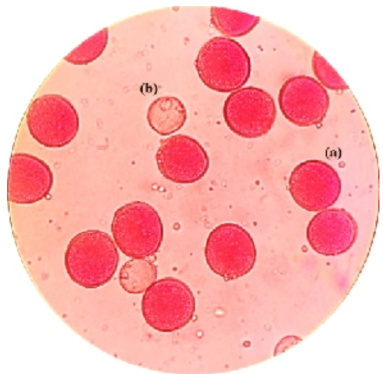
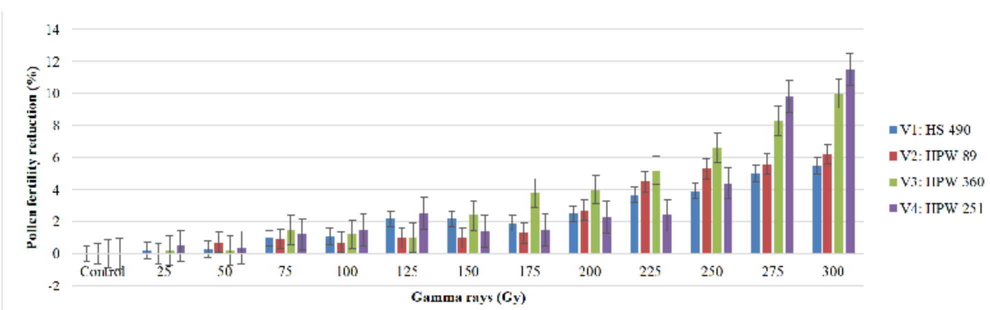


Fig. 3. Microscopic view (400x) of pollen grains of induced mutagenic treatments (a) stained: viable and (b) unstained: inviable.

Table 3: Effect of gamma rays on pollen fertility in different wheat varieties.

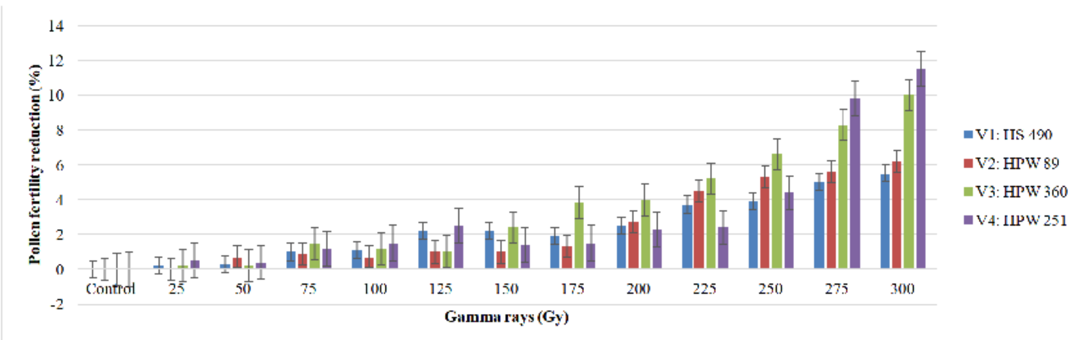
Mutagen	V1: HS 490		V2: HPW 89		V3: HPW 360		V4: HPW 251	
Gamma Dose	Pollen fertility (%)	Reduction over control (%)	Pollen fertility (%)	Reduction over control (%)	Pollen fertility (%)	Reduction over control (%)	Pollen fertility (%)	Reduction over control (%)
Control	99.7	-	99.8	-	99.9	-	99.9	-
25 Gy	99.5	0.2	99.8	0	99.7	0.2	99.4	0.5
50 Gy	99.4	0.3	99.1	0.7	99.7	0.2	99.5	0.4
75 Gy	98.7	1	98.9	0.9	98.4	1.5	98.7	1.2
100 Gy	98.6	1.1	99.1	0.7	98.7	1.2	98.4	1.5
125 Gy	97.5	2.2	98.8	1	98.9	1	97.4	2.5
150 Gy	97.5	2.2	98.8	1	97.5	2.4	98.5	1.4
175 Gy	97.8	1.9	98.5	1.3	96.1	3.8	98.4	1.5
200 Gy	97.2	2.5	97.1	2.7	95.9	4	97.6	2.3
225 Gy	96	3.7	95.3	4.5	94.7	5.2	97.5	2.4
250 Gy	95.8	3.9	94.5	5.3	93.3	6.6	95.5	4.4
275 Gy	94.7	5	94.2	5.6	91.6	8.3	90.1	9.8
300 Gy	94.2	5.5	93.6	6.2	89.9	10	88.4	11.5



Graph 3: Effect of Gamma rays on pollen fertility reduction in different wheat varieties.

Table 4: Effect of EMS on pollen fertility in different wheat varieties.

Mutagen	V1: HS 490		V2: HPW 89		V3: HPW 360		V4: HPW 251	
EMS Dose (v/v) %	**Pollen fertility (%)**	**Reduction over control (%)**	**Pollen fertility (%)**	**Reduction over control (%)**	**Pollen fertility (%)**	**Reduction over control (%)**	**Pollen fertility (%)**	**Reduction over control (%)**
Control	99.8	-	100	-	99.9	-	99.8	-
0.05 %	99.4	0.4	99.7	0.3	99.5	0.4	99.8	0
0.15 %	99.8	0	99.8	0.2	99.4	0.5	99.4	0.4
0.25 %	99.5	0.3	99.8	0.2	98.4	1.5	99.4	0.4
0.35 %	98.8	1.0	99.5	0.5	99.2	0.7	98.5	1.3
0.45 %	97.9	1.9	97.4	2.6	98.1	1.8	97.4	2.4
0.55 %	98.5	1.3	98.8	1.2	98.5	1.4	97.1	2.7
0.65 %	96.4	3.4	97.1	2.9	97.5	2.4	96.4	3.4
0.75 %	97.4	2.4	96.6	3.4	98.2	1.7	96.5	3.3
0.85 %	97.4	2.4	96.8	3.2	98.4	1.5	95.4	4.4
0.95 %	97.2	2.6	95.4	4.6	97.7	2.2	96.2	3.6
1.05 %	96.3	3.5	96.2	3.8	96.4	3.5	95.7	4.1
1.15 %	95.9	3.9	93.2	6.8	92.2	7.7	91.1	8.7

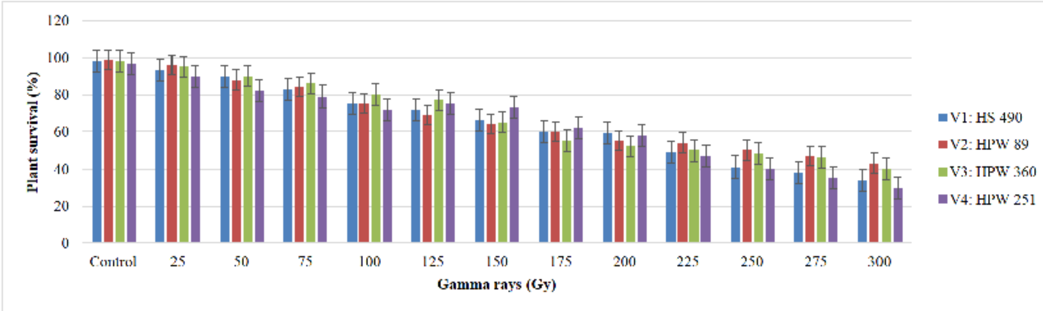


Graph 4: Effect of EMS on pollen fertility reduction in different wheat varieties.

Effect of induced mutagenesis on plant survival (%). Different concentrations of γ-rays and EMS have directly influenced the plant survival percentage with the corresponding increase in concentrations of mutagens in all the four varieties of wheat. The effect of induced mutagenesis for both mutagens is presented in Table 5, 6 and depicted in Graph 5 and 6. Under gamma rays, highest plant survival reduction percentage was observed at 300 Gy in HPW 251 (67%) followed by HS 490 (64%), HPW 360 (58%) and later HPW 89 (56%) in comparison to their controls whereas under EMS treatments, highest survival reduction was observed at 1.15% concentration in HPW 89 (63%) followed by HPW 251 (62%), HPW 360 (60%) and later HS 490 (53%) over their respective controls. The decrease in survival percentage was associated with increases in the dose/ concentration of the mutagens in all the varieties.

Table 5: Effect of gamma rays on plant survival in different wheat varieties.

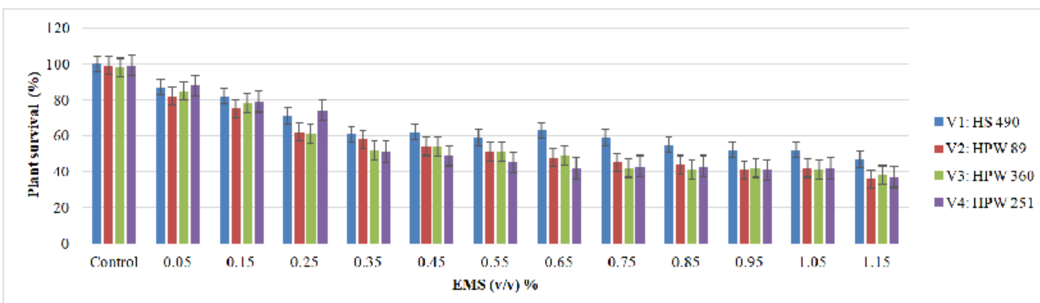
Mutagen	V1: HS 490		V2: HPW 89		V3: HPW 360		V4: HPW 251	
Gamma Dose	**Plant Survival (%)**	**Reduction over control (%)**	**Plant Survival (%)**	**Reduction over control (%)**	**Plant Survival (%)**	**Reduction over control (%)**	**Plant Survival (%)**	**Reduction over control (%)**
Control	98	-	99	-	98	-	97	-
25 Gy	93	5	96	3	95	3	90	7
50 Gy	90	8	88	11	90	8	82	15
75 Gy	83	15	84	15	86	12	79	18
100 Gy	75	23	75	24	80	18	72	25
125 Gy	72	26	69	30	77	21	75	22
150 Gy	66	32	64	35	65	33	73	24
175 Gy	60	38	60	39	55	43	62	35
200 Gy	59	39	55	44	52	46	58	39
225 Gy	49	49	54	45	50	48	47	50
250 Gy	41	57	50	49	48	50	40	57
275 Gy	38	60	47	52	46	52	35	62
300 Gy	34	64	43	56	40	58	30	67



Graph 5: Effect of Gamma rays on plant survival in different wheat varieties.

Table 6: Effect of EMS on plant survival in different wheat varieties.

Mutagen	**V1: HS 490**		**V2: HPW 89**		**V3: HPW 360**		**V4: HPW 251**	
EMS Dose (v/v) %	**Plant Survival (%)**	**Reduction over control (%)**	**Plant Survival (%)**	**Reduction over control (%)**	**Plant Survival (%)**	**Reduction over control (%)**	**Plant Survival (%)**	**Reduction over control (%)**
Control	100	-	99	-	98	-	99	-
0.05 %	87	13	82	17	85	13	88	11
0.15 %	82	18	75	24	78	20	79	20
0.25 %	71	29	62	37	61	37	74	25
0.35 %	61	39	58	41	52	46	51	48
0.45 %	62	38	54	45	54	44	49	50
0.55 %	59	41	51	48	51	47	45	54
0.65 %	63	37	48	51	49	49	42	57
0.75 %	59	11	45	54	42	56	43	56
0.85 %	55	45	44	55	41	57	43	56
0.95 %	52	48	41	58	42	56	41	58
1.05 %	52	48	42	57	41	57	42	57
1.15 %	47	53	36	63	38	60	37	62



Graph 6: Effect of EMS on plant survival in different wheat varieties.

CONCLUSION

In the present study, effect of induced mutagenesis on germination percentage were observed in four wheat varieties using gamma rays and EMS mutagens. Irradiation with gamma dose at 300 Gy, HS 490 (63%) and HPW 251 (62%) showed highest reduction in germination over control whereas at 1.15% EMS treatment, HPW 251 (58%) and HPW 360 (55%) showed highest reduction in germination per cent over control indicating reduction in germination per cent in all the mutagenic treatments with increase in the concentration of the mutagens. Selection of appropriate mutagen and its dose plays a pivotal role in success of any mutation breeding programme. The LD_{50} value of mutagen helps in predicting mutagen sensitivity and subsequent recovery of higher mutation frequency. Hence, it is vital to optimize lethal dose of mutagens to isolate desired utilizable mutations (Hazra *et al.*, 2021). LD_{50} values observed under EMS and gamma irradiation treatment for varieties *viz.*, HS 490, HPW 89, HPW 360 and HPW 251 were found to be 1.169%, 0.885%, 0.725%, 0.683% and 241.41 Gy, 278.48 Gy, 251.27 Gy and 247.96 Gy respectively using probit analysis. The effect of induced mutagenesis on pollen grains revealed increased pollen sterility with increasing concentrations of mutagens. Highest pollen viability reduction were observed in HPW 251 under both gamma rays at 300 Gy and 1.15% EMS for up to 11.5% and 8.7% respectively as compared to other varieties over control. Highest plant survival reduction percentage was observed at 300 Gy in HPW 251 (67%) whereas at 1.15% EMS concentration, HPW 89 (63%) over their respective controls.

Therefore, medium dose/concentrations of both mutagens produced less biological damage and would be suitable for inducing desirable attributes in wheat establishing varietal effect of mutagens like gamma rays and EMS in different wheat varieties to be used in inducing large scale mutagenesis in wheat breeding programmes.

Acknowledgements: The author(s) gratefully acknowledge Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Centre (BARC), Mumbai, Maharashtra, India, Rice and Wheat Research Centre (RWRC), Malan, Himachal Pradesh, India and Department of Genetics and Plant Breeding, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India for providing the research facilities for this research.

Conflict of Interest. None.

REFERENCES

Abbott, W. S. (1925). A Method of Computing the Effectiveness of an Insecticide. *Journal of Economic Entomology, 18*(2): 265-267.

Babaei, A., Nematzadeh, G. A., Avagyan, V., Hamidreza, S. and Petrodi, H. (2010). Radio sensitivity studies of morpho-physiological characteristics in some Iranian rice varieties (*Oryza sativa* L.) in M_1 generation. *African Journal of Agricultural Research, 5*(16): 2124-2130.

Borzouei, A. M. Kafi, Khazaei, H., Naseriyan, B. and Majdabadi, A. (2010). Effects of gamma radiation on germination and physiological aspects of wheat (*Triticum aestivum* L.) seedlings. *Pakistan Journal of Botany, 42*(4): 2281-2290.

Shah, T. M, Atta, B. M., Mirza, J. I. and Haq, M. A. (2012). Radiosensitivity of various chickpea genotypes in M_1 generation II-field studies. *Pakistan Journal of Botany, 44*(2): 631-634.

Chakraborty, N. R. and Paul, A. (2013). Role of Induced Mutations for Enhancing Nutrition Quality and Production of Food. *International Journal of Bio-resource and Stress Management, 4*(1): 91-96.

Cheng, X., Chai, L., Chen, Z., Xu, L., Zhai, H., Zhao, A., Peng, H., Yao, Y., You, M., Sun, Q. and Ni, Z. (2015). Identification and characterization of a high kernel weight mutant induced by gamma-radiation in wheat (*Triticum aestivum* L.). *BMC Genetics, 17*: 112-118.

Finney, D. J. (1971). Probit analysis. Cambridge, England, Cambridge University Press.

Finney, D. J. (1978). Statistical method in biological assay. Charles Griffin & Co.

Harding, S. and Mohamad, O. (2009). Radiosensitivity test on two varieties of Terengganu and Arab used in mutation breeding of roselle (*Hibiscus sabdariffa* L.). *African Journal of Plant Science, 3*(8): 181-183.

Hazra, S., Gorai, S., Umesh Kumar, V., Bhattacharya, S., Maji, A., Jambhulkar, S. and Chattopadhyay, A. (2021). Optimization of gamma radiation dose for induction of mutations in okra. *International Journal of Vegetable Science, 27*(6): 574-584.

International Atomic Energy Agency (2021). Mutant varieties database. [Online] Vienna: IAEA.

Laghari, K. A., Sial, M. A., Arain, M. A., Khanzada, S. D. and Channa, S. A. (2012). Evaluation of stable wheat mutant lines for yield and yield associated traits. *Pakistan Journal of Agriculture, Agriculture Engineering, Veterinary Sciences, 28*(2): 124-130.

Mangi, N., Baloch, A. W., Arain, S. M., Baloch, M., Kandhro, M. N., Abro, T. F., Baloch, S. N. and Mari, S. N. (2016). Evaluation of advance mutant genotypes and interrelationship analysis of yield and yield associated traits in bread wheat genotypes. *Sindh University Research Journal (Science Series), 48*(3): 783-786.

Micke, A., Donini, B. and Maluszynski, M. (1990). Induced mutations for crop improvement. *Mutation Breeding Review, 7*: 1-41.

Nazarenko, M. (2016). Parameters of winter wheat growing and development after mutagen action. *Bulletin of Transilvania University of Brasov - series II – Forestry, Wood Industry, Agricultural, Food Engineering, 9*(2): 109-116.

Pathak, V. and Shrivastav, S. (2015). Biochemical studies on wheat (*Triticum aestivum* L.). *Journal of Pharmacognosy and Phytochemistry, 4*(3): 171-175.

Shu, Q. Y., Forster, B. P. and Nakagava, H. (2013) Plant mutation breeding and biotechnology. Vienna: CABI publishing, p. 301-326.

Thapa, C.B. (2004). Effect of acute exposure of gamma rays on seed germination and seedling growth of *Pinus kesiya* Gord and *P. wallichiana* A.B. Jacks. *Our Nature, 2*: 13-17.

USDA (2020-21). World agricultural production-Foreign Agricultural Service (Circular Series) p. 23.

How to cite this article: Rana, A.; Rana, V.; Priyanka ; Anubhav, S. and Gupta, C. (2022). Fixation of Lethal Dose and Study of Physical and Chemical Effect of Mutagenesis on Germination, Pollen Sterility and Plant Survival in M_1 Generation of Wheat (*Triticum aestivum* L.). *Biological Forum – An International Journal, 14*(1): 17-24.