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Random mutagenesis strategies for the development of gamma-induced mutant populations and discovery of phenotypic spectrum alterations in bread wheat (*Triticum aestivum* L.)

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Abstract

The present investigation was undertaken to evaluate the dose-dependent impact of physical gamma radiation on mutation frequency, mutagenic effectiveness, mutagenic efficiency, and phenotypic spectrum alterations in bread wheat (*Triticum aestivum* L. em. Thell). Pure, uniform seeds of the elite commercial cultivar HUW 510 were exposed to acute gamma radiation at four graded doses: 200 Gy, 250 Gy, 300 Gy, and 350 Gy. Establishing sensitivity parameters was crucial to optimize the induction of high mutational frequencies while minimizing catastrophic biological damage. The genetic and physiological effects of the physical mutagen were systematically evaluated in the M2 generation across distinct seedling, foliar, and reproductive traits. Chlorophyll mutations exhibited a strict linear, dose-dependent relationship with increasing radiation intensities, ranging from 0.00% to 0.225%. Within a large-scale M2 population of 1,10,900 individuals, a total of 823 seedling lethal events and 731 unique macromorphological structural variants were successfully verified. Biometrical analysis demonstrated that mutagenic effectiveness responded non-proportionally to physical energy inputs, with macro-morphological and lethal effectiveness peaking at the lowest exposure threshold of 200 Gy (0.36%). Conversely, mutagenic efficiency for chlorophyll defects increased linearly up to the highest dose of 350 Gy. To prevent excessive population mortality while maximizing the recovery of viable structural modifications, mid-range exposure thresholds (250-300 Gy) are recommended to balance mutational yield and plant survival. The diverse architectural, flag leaf, and ontogenetic flowering variants isolated from this population establish a highly valuable mutant resource block for downstream phenotypic screening to identify novel resistance mechanisms against various stresses.

Keywords: Gamma rays, random mutagenesis, mutagenic effectiveness, mutagenic efficiency, macromorphological variants, bread wheat

1. Introduction

One of the most extensively farmed and consumed crops worldwide, wheat is a staple grain (Tadesse *et al.*, 2019) [14]. In order to meet demand, wheat production must rise by more than 50% over current levels by 2050 as the human population continues to grow (Zhao, 2018) [17]. With an estimated 200 million hectares produced worldwide, bread wheat (*Triticum aestivum* L. Em Thell, 2n = 6x = 42) is a vital food security crop. Approximately 19% of the calories in the average human diet come from eating wheat grains, while about 40% of wheat produced is fed to poultry and livestock. Compared to other major cereals like rice (*Oryza sativa*) and maize (*Zea mays*), wheat grain is higher in protein and richer in carbohydrates. In addition, it has significant amounts of vitamins, phytochemicals, and minerals, including zinc and iron, making it a highly nutritious food crop (Zörb *et al.*, 2018) [18].

Given the shifting global social, economic, and demographic landscape, wheat remains a singular agricultural commodity with significant influence on the global economy and a critical role in maintaining global food security. Approximately 35% of the global population consumes wheat as a secondary or primary cereal staple food crop, contributing 20% of total calories in the daily human diet (Anonymous, 2014) [4]. Grown across a wide variety of latitudes, wheat is renowned for its exceptional ability to adapt to a vast array of environmental factors, accounting for roughly 32% of all global land planted to cereals (Anonymous, 2014) [4].

The generation and management of genetic variability constitute a fundamental component of crop improvement programs. Mutation breeding serves as a pivotal approach enabling geneticists and plant breeders to generate extensive genetic variability through random mutagenesis that is often unattainable through conventional selection or hybridization methods. Induced mutations hold substantial significance for elucidating genetic mechanisms, assessing variation, and expediting the process of plant improvement. The principal objective of induced mutation breeding is the genetic enhancement of well-adapted cultivars by modifying one or two limiting agronomic traits to improve productivity or quality (Novak & Brunner, 1992) ^[9]. Physical mutagens used in plants include ionizing radiation like gamma rays and X-rays. Because they are convenient and can penetrate deeper into tissues, gamma rays are the most commonly used physical mutagen among plant breeders. They cause precise nucleotide changes and small deletions (Sato *et al.*, 2006) ^[12].

The commercial cultivar HUW510 is a high-yielding variety releasing a potential yield of 45-50 quintals per hectare. It is ideal for late-sown irrigated environments, but requires continuous diversification of its structural morphology to avoid yield plateaus. Therefore, the present study was conducted to analyze random mutagenesis profiles and oversee the development of a gamma-induced population to evaluate the frequency, effectiveness, and efficiency profiles of acute gamma radiation across distinct biological parameters in the bread wheat variety HUW510.

2. Materials and Methods

2.1 Development of gamma-induced mutant population

The field experiment was conducted at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during Rabi 2023-24. Varanasi falls under the Middle Gangetic Plains (Eastern Plains Zone of Uttar Pradesh) agro-climatic region, featuring a humid subtropical climate with an average rainfall of 1110 mm. The initial seed material consisted of the M1 population of the HUW510 parent grown during the previous season. For physical treatment, selfed seeds with uniform size and an optimized internal moisture profile of 12% were exposed to gamma radiation. Irradiation was conducted using a Cobalt-60 source at the Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Center (BARC), Mumbai, India. The continuous dosing rate was maintained at 0.862 kGy/hr across four exposure blocks: 200 Gy, 250 Gy, 300 Gy, and 350 Gy. The harvested seeds of individual M1 spikes were bulked dose-wise to eliminate chimerism and sown to establish the second mutant generation (M2) during the Rabi 2023-24 season.



Plate 1: Experimental trial site during the observation of lethal and chlorotic mutants

A massive population of 1,10,900 M2 seeds was space-sown in plot sizes of 1.0 m x 2.0 m per treatment. For comparison, the untreated HUW510 parent was used as a control row block. The experimental material, along with the control, was grown at a specific geometry of 40 cm row-to-row and 10 cm plant-to-plant distance. The trial was maintained under standard irrigated agronomic practices matching institutional packages.

2.2 Procedure for recording observations

The procedure for recording observations across specific biological parameters was structured as follows:

2.2.1 Lethal Mutants: Seedlings exhibiting early decay, reduced longevity, and premature death relative to the normal survival baseline of adjacent control rows were monitored up to GS 40 (Zadoks *et al.*, 1974) ^[16].

2.2.2 Chlorophyll Mutants: Identified during early seedling growth up to GS 40 and classified into Chlorina (pale green), Xantha (yellow), and Tigrina (transverse green/yellow bands) (Gustafsson, 1940) ^[8].

2.2.3 Morphological Mutants: Monitored continuously until crop maturity. Spikes were classified into degenerated, compact, loose, or bent types. Leaf blade variations were scored into chlorotic, necrotic, crinkled, or curled/rolled configurations. Ontogenetic flowering adjustments were recorded as early heading or late heading against the parent check.

2.3 Statistical analysis

The above observations were recorded to estimate mutagenic frequency, mutagenic effectiveness, and efficiency (Bakshi *et al.*, 2023) ^[5]. The biometrical equations applied are defined below:

Mutagenic effectiveness = [Frequency of mutations in M2/(Radiation dose (Gy) * treatment hrs)] * 100

Mutagenic efficiency = [Frequency of mutations in M2/% lethality] * 100

Mutagenic frequency = [No. of mutants observed in M2/Total M2 population size] * 100

3. Results and Discussion

3.1 Spectrum and Frequency of Chlorophyll Mutations

The range and regularity of chlorophyll mutations found in the M2 generation serve as a highly reliable index for quantifying mutagenic changes. In the current study, a total of 97 specific chlorophyll mutations were recorded, distributed as 76 xantha, 19 chlorina, and 2 tigrina variants. No chlorophyll defects were observed in the untreated parent control.

The overall frequency of chlorophyll mutations increased linearly with higher gamma radiation treatments, moving from 0.00% at 200 Gy up to a peak value of 0.225% at 350 Gy. The mutation spectrum followed a strict hierarchy of Xantha > Chlorina > Tigrina. High physical radiation doses show a high frequency of xantha mutations, which can be attributed to multi-locus gene damage on chromosomes governing key photosynthetic pathways (Bakshi *et al.*, 2023; Balkan *et al.*, 2019) ^[5, 6]. This strict linearity matches the

biological model where higher physical energy tracks increase double-strand DNA breaks. (Riviello-Flores *et al.*, 2022) ^[11].

Table 1: Number and spectrum of lethal mutations induced by gamma mutagenic treatments in M2 Generation

Gamma treatments	No of lethal mutants	No of M2 seedlings	Mutation frequency (%)
200 Gy	201	27700	0.72
250 Gy	150	27600	0.54
300 Gy	205	30800	0.66
350 Gy	215	24800	0.86
Total	823	110900	0.74

3.2 Seedling Lethality Variations

Acute gamma irradiation caused widespread physiological damage and genetic mutations, leading to high seedling lethality before GS 40. A total of 823 lethal plant events were confirmed within the 1,10,900 M2 population. Lethal mutation frequency trends were dose-dependent, starting at 0.725% at 200 Gy, dropping slightly to 0.543% at 250 Gy, and peaking at 0.866% under 350 Gy exposure. Higher physical radiation exposures cause heavy lethality due to severe chromosomal rearrangements, base pair deletions, or the inhibition of essential cellular repair enzymes (Alam *et al.*, 2022) ^[1].

This non-proportional deviation indicates that a moderate physical treatment (250 Gy) triggers intracellular stress sensors (such as ATM/ATR kinase complexes) that temporarily pause the cell cycle, allowing native enzymatic repair pathways to repair single-strand cuts (Preuss & Britt,

2003) ^[10]. In contrast, the high-energy photon tracks at 350 Gy generate multiple overlapping double-strand breaks that bypass these cellular repair checkpoints. When processed by error-prone non-homologous end-joining (NHEJ) systems, these breaks cause dicentric formations and significant deletions (Shu *et al.*, 2012; Van Harten, 1998) ^[13, 15]. These structural rearrangements prevent normal spindle attachments during mitotic division, leading to cell arrest and high seedling mortality during initial growth.

3.3 Macromorphological Mutation Spectrum Analysis

A broad range of 731 individual structural morphological mutations were verified, establishing a cumulative frequency of 0.659%. Altered spike mutants were concentrated in the moderate (300 Gy) to high (350 Gy) treatment ranges. Degenerated and compact spike variants recorded overall frequencies of 0.051% and 0.064%, respectively. Loose spikes (59 total plants) and bent spikes (43 total plants) were recorded at frequencies of 0.053% and 0.038%.

For leaf structural phenotypes, chlorotic mutations peaked at 300 Gy (19 plants), while necrotic mutations were most frequent at 350 Gy (17 plants). Crinkled flag leaves were most abundant at 250 Gy (16 plants), and curled/rolled leaves peaked at 300 Gy (15 plants). Early-heading variants (129 lines) were highly abundant at lower doses (200-250 Gy), showing an individual trait mutation frequency of 0.228%. Late-heading mutants peaked at 300 Gy (22 lines), confirming that higher radiation exposure causes growth retardation and delays heading (Albokari *et al.*, 2014; Dorrani-Nejad *et al.*, 2022; Shu *et al.*, 2012) ^[2, 7, 13].

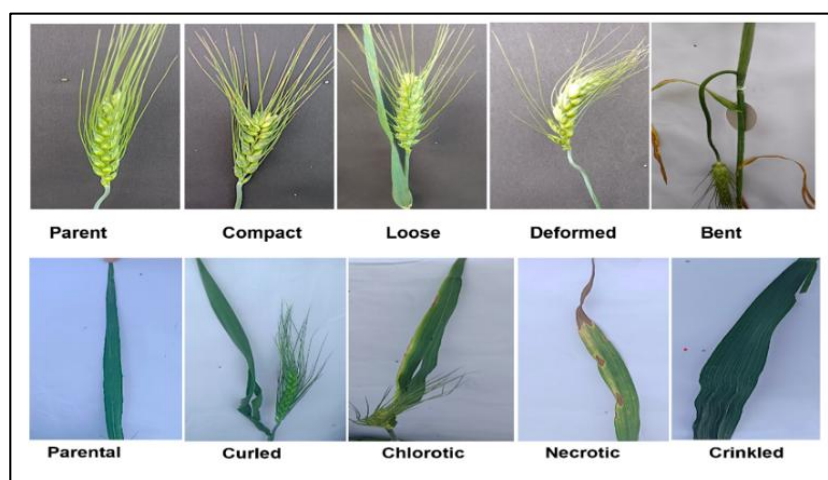


Plate 2: Spectrum of spike and leaf morphological mutants identified in experimental study

Table 2: Number and spectrum of morphological mutations induced by mutagenic treatments in M2 generation in HUW510

Types of mutant	200Gy	250Gy	300Gy	350Gy	Mutation frequency (%)
Degenerated spike	9	5	10	33	0.051
Compact spike	15	7	19	31	0.064
Loose spike	3	6	21	29	0.053
Bent spike	4	7	14	18	0.038
Chlorotic	6	12	19	13	0.040
Necrotic	2	7	9	17	0.020
Crinkled flag leaf	12	16	13	15	0.050
Curled/Rolled	6	10	15	13	0.039
Late heading	14	11	22	15	0.055
Early heading	129	60	41	23	0.228
Total mutant	200	141	183	207	Cumulative: 731
M2 population	27700	27600	30800	24800	Total: 110900
Mutagenic frequency	0.722%	0.511%	0.594%	0.835%	Overall: 0.659%

3.4 Mutagenic Effectiveness

Mutagenic effectiveness calculations showed non-proportional responses to increasing physical radiation doses. Based on chlorophyll mutations, effectiveness peaked at 250 Gy (0.11) and fell to 0.00 at 200 Gy. However, based on structural, morphological, and lethal parameters, effectiveness was highest at the lowest dose of 200 Gy (0.36), decreasing at higher doses.

The range of the effectiveness estimations based on the frequency of morphological mutations was 0.19-0.36%. According to the frequency of morphological alterations, the effectiveness estimations were, in turn, 0.20% at 250 Gy, 0.19% at 300 Gy, 0.23% at 350 Gy, and 0.36% at 200 Gy. Whereas based on lethal mutations, the effectiveness estimates were, in order, 0.36% at 200 Gy, 0.21% at 250 Gy, 0.22% at 300 Gy, and 0.24% at 350 Gy based on the frequency of lethal modifications. The details are given in

Table 3. Flowering time variants exhibited an inverse distribution pattern across treatments.

Early-heading mutants (129 plants) were highly abundant at lower doses (200-250 Gy), achieving an individual trait frequency of 0.228%. In contrast, late-heading plants (22 lines) were concentrated within the higher 300-350 Gy treatments. This pattern indicates that lower radiation levels induce precise changes in vernalization (Vrn) or photoperiod (Ppd) pathways, whereas higher exposures cause growth retardation and delayed heading (Albokari *et al.*, 2014; Dorrani-Nejad *et al.*, 2022) ^[2, 7]. This high effectiveness at 200 Gy demonstrates that lower physical doses are highly efficient at inducing stable, viable structural modifications, whereas higher doses induce multi-locus deletions that cause background damage and mask useful mutations (Bakshi *et al.*, 2023; Shu *et al.*, 2012; Van Harten, 1998) ^[5, 13, 15].

Table 3: Mutagenic effectiveness based on chlorophyll and morphological mutations

Mutagenic treatments (Gy)	Chlorophyll Frequency	Chlorophyll Effectiveness (%)	Morphological Frequency	Morphological Effectiveness (%)
Control and 200	0.000	0.00	0.722	0.36
250	0.029	0.11	0.510	0.20
300	0.107	0.03	0.594	0.19
350	0.225	0.06	0.835	0.23

3.5 Mutagenic Efficiency

The present investigation estimates the efficiency of gamma-rays using the frequency of morphological mutations, chlorophyll and lethal mutants. As the dose of mutagenic treatments was increased, the study's findings demonstrated that mutagenic efficiency did not rise proportionately, a non-linear biological trend frequently observed in hexaploid cereal crops subjected to physical radiation triggers (Bakshi *et al.*, 2023; Balkan *et al.*, 2019) ^[5, 6]. Utilizing a seedling lethality percentage as the primary indicator of physical cellular injury, the dynamic mutagenesis efficiency was determined (Table 4).

This indicates that the 300 Gy threshold is optimal for

inducing precise point mutations within complex chlorophyll synthesis pathways without causing excessive, catastrophic background genetic damage. This exposure configuration strictly below the 350 Gy treatment level induces single nucleotide variations and small structural modifications while avoiding the extensive double-strand chromosomal breaks that cause extensive cell-cycle arrest and high population mortality (Preuss & Britt, 2003; Shu *et al.*, 2012) ^[10, 13]. This operational safety margin provides highly viable, structurally stable mutant lines that are perfectly optimized for downstream agronomic selection and field evaluation programs (Albokari *et al.*, 2014; Van Harten, 1998) ^[2, 15].

Table 4: Mutagenic efficiency based on chlorophyll and morphological mutations of Gamma-ray's treatments

Gamma rays (Gy)	Chlorophyll Freq.	Lethal Freq. (%)	Chlorophyll Efficiency (%)	Morphological Freq.	Morphological Efficiency (%)
200	0.000	0.72	0.00	0.722	99.50
250	0.028	0.54	5.33	0.511	93.99
300	0.107	0.66	16.09	0.594	89.26
350	0.225	0.86	26.04	0.835	96.27

4. Conclusion

Induced mutations drive novel genetic variation, forming the baseline of modern crop improvement programs. This investigation will lead to knowing the optimum dose of mutagen; this can be the basis of many studies for crop improvement. A dose-dependent reduction was observed, and the optimum dose was found to be 300 Gy. Considering the limited published information on mutagen sensitivity in HUW 510, which is still a farmer-accepted variety of the north-eastern plain zones, farmers can utilize these optimized dosing thresholds in an efficient manner to maximize mutant recovery while safeguarding population survival. The mutagenic effectiveness and efficiencies were other parameters dealt with in this investigation. The macromutations were observed in the population, which could be base of various breeding programs. In the present investigation, mutagenic profiles of physical gamma

radiation in bread wheat (*Triticum aestivum* L.) were determined based on structural marker reduction across graded concentrations. A dose-dependent relationship was recorded across chlorophyll features, enabling precise estimation of physiological parameters. The identified low-dose configurations offer a practical reference point for inducing adequate mutation frequency while maintaining sufficient population size for effective selection. These results will facilitate the development of structured mutation breeding strategies aimed at improving economically important traits in bread wheat and may serve as a foundation for further genetic enhancement studies in underexploited cereal crops.

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6. Competing Interests

The authors have declared that no competing interests exist.

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