

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(8): 1015-1018
www.biochemjournal.com
Received: 02-06-2026
Accepted: 07-07-2026

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Gamma radiation-induced mutagenesis in barley (*Hordeum vulgare* L.) cv. HUB-113: Radiosensitivity, mutational spectrum, and optimization of mutagenic efficiency

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i8S1.9564>

Abstract

Induced mutagenesis serves as an essential strategy for expanding genetic variability in barley (*Hordeum vulgare* L.) without compromising elite agronomic backgrounds. This study evaluated the biological and genetic impacts of gamma irradiation (350 Gy, 400 Gy, and 450 Gy) on barley cv. HUB-113 across the M₁ and M₂ generations. The optimum lethal dose (LD₅₀) at 400 Gy was determined by radiosensitivity testing in the M₁ generation. A dose-dependent increase in the frequency of chlorophyll mutations (0.131% to 0.455%) was observed in the M₂ generation. Xantha represented the predominant chlorotic phenotype (n = 93), alongside chlorina and tigrina mutants. A broad spectrum of morphological variations was recovered, including altered spike architecture (compact, loose, bent), modified leaf morphology, and difference in flowering phenology. At 350 Gy (n = 110), early-heading variations were mostly recovered, providing valuable resource for the creation of short-duration, climate-resilient cultivars. Standardizing mutagenic parameters revealed an inverse relationship between radiation intensity and mutagenic metrics, with the 350 Gy treatment yielding both the highest mutagenic effectiveness (0.984) and efficiency (174.6). Excessive biological toxicity and fatality (n = 253) were observed for higher radiation doses (450 Gy) without a corresponding increase in efficiency. The ideal gamma radiation dose for HUB-113 was found to be 350 Gy, which effectively balanced a high mutational frequency with little somatic damage. The generated mutant resources provide a foundation for forward breeding, disease resistance screening, and functional gene discovery in barley.

Keywords: Gamma irradiation, mutagenic efficiency, chlorophyll mutants, early heading, crop improvement

1. Introduction

Barley (*Hordeum vulgare* L.) stands as one of the oldest cereal crops in global agriculture (Wang, 2024) [15]. After rice, wheat, and maize, it is considered the fourth most significant grain crop worldwide (Giraldo, 2019) [5]. In terms of total area and production, barley ranks second among Rabi cereals after wheat (Ergun, 2023) [3]. The success of barley is largely attributed to its high ecological adaptability, which enables it to thrive in diverse and extreme environments ranging from sea level to the high-altitude plateaus of the Himalayas and the Andes (Wang, 2024) [15]. Barley (*Hordeum vulgare* L.) is a multi-purpose crop supporting major agricultural and industrial sectors globally, in addition to its role as a regional staple and functional food rich in dietary fiber and β -glucans. Approximately 60% of total production is allocated to animal feed, and 40% to the malting and brewing industries (Ergun, 2023; Giraldo, 2019; Gomez, 2017) [3, 15, 4]. Beyond its commercial worth, barley is considered a key model organism for genetic and genomic research within the Triticeae tribe due to its diploid nature and very small genome (Ohnoutkova, 2019; Wang, 2024) [6, 15]. However, modern barley cultivation often suffers from genetic uniformity, particularly in terms of disease resistance. For example, single-resistance sources such as NDB112 have left cultivars increasingly susceptible to evolving disease pathotypes, including spot blotch (Roy, 2010) [10].

Induced mutagenesis can therefore be used to expand genetic diversity without disrupting elite background traits (Ahumada-Flores, 2021; Ergun, 2023; Tan, 2019) [1, 3, 13].

Induced mutagenesis involves exposing plant propagules, such as seeds or pollen, to physical or chemical mutagens to generate random, genome-wide variation (Ergun, 2023) [3]. A principal advantage of this approach is its capacity to correct specific target traits, such as disease resistance, or nutritional profile, within an adapted elite cultivar without altering its overall genetic background (Ahumada-Flores, 2021; Gomez, 2017) [1, 4]. Mutation breeding has produced over 3,200 commercial variants in 240 species worldwide by avoiding reproductive barriers and accelerating the attainment of homozygosity (Ohnoutkova 2019; Tan, 2019; Tokhetova, 2020) [6, 13, 14]. In modern crop improvement, pairing induced mutagenesis with reverse-genetic screening platforms like TILLING (Targeting Induced Local Lesions IN Genomes) enables precise identification of novel functional alleles, providing the essential genetic variation required to develop climate-resilient cultivars (Ohnoutkova, 2019; Rajderkar, 2021; Sarada, 2024; Wang, 2024) [6, 8, 12, 15]. Among physical mutagens, gamma rays are the most commonly used due to their high penetration power, ease of use, and ability to cause a wide range of heritable genetic changes, from point mutations and small deletions to chromosomal rearrangements (Ahumada-Flores, 2021; Ergun, 2023; Tan, 2019) [1, 3, 13]. The practical utility of gamma irradiation in barley breeding is well established. Economically important cultivars such as 'Golden Promise' and 'Diamant' (Ohnoutkova, 2019) [6] were developed using gamma irradiation. However, biological responses to ionizing radiation are strictly dose-dependent. Excessive doses cause widespread DNA strand breaks and structural chromosomal aberrations that reduce seed germination, seedling survival, and fertility, whereas moderate doses optimize mutation frequency while minimizing physiological injury (Ahumada-Flores, 2021; Ergun, 2023; Gomez, 2017; Tokhetova, 2020) [1, 3, 4, 14].

The success of a mutation breeding program depends on balancing mutation frequency against biological damage. For assessing changes in the nuclear genome and the impact of treatment, chlorophyll mutations, especially albina, xantha, chlorina, and viridis, serve as standard, readily screenable markers (Ahumada-Flores, 2021; Gomez, 2017) [1, 4]. These phenotypes, when combined with morphological and lethal variations, enable accurate measurement of mutagenic efficiency and mutagenic effectiveness (Rajderkar, 2021; Sarada, 2024) [8, 12]. Identifying a treatment dose that maximizes efficiency while constraining phytotoxicity is therefore essential for downstream mutant selection (Ergun, 2023; Rajderkar, 2021; Sarada, 2024) [3, 12]. Mutagenic efficiency is the ratio of desirable mutations to biological damage such as sterility and lethality and mutagenic effectiveness refers to the mutations induced per unit dose. For downstream mutant selection, it is essential to determine a treatment dose that maximizes effectiveness while limiting phytotoxicity (Ergun, 2023; Rajderkar, 2021; Sarada, 2024) [3, 8, 12].

The current study was conducted to assess the physiological and genetic effects of gamma-ray mutagenesis in barley (*Hordeum vulgare* L.) in order to meet these requirements. In particular, we identified the range of chlorophyll (albina, xantha, chlorina, and viridis), lethal, and morphological mutants in the M₂ generation. We also determined the initial biological responses, such as seed germination rates, median lethal dose (LD₅₀), and visible physiological damage. This study aims to determine the best irradiation therapy for

creating novel genetic variability while limiting biological damage by methodically measuring mutagenesis frequency, efficacy, and efficiency across applied gamma doses.

2. Materials and Methods

2.1 Plant Material and Gamma Irradiation

Barley seeds were obtained from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University. Pure, selfed seeds of uniform size and 12% moisture content of barley (*Hordeum vulgare* L.) cultivar HUB-113 were selected as the primary biological material for physical mutagenesis. Three target dosages of gamma irradiation were applied to seed lots of 200 g each treatment, namely 350 Gy, 400 Gy, and 450 Gy. Irradiation was carried out at the Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Centre (BARC), Mumbai, India, during 2021-2022, using a Cobalt-60 gamma source at a constant dose rate of 0.862 kGy/hr. Based on preliminary biological response evaluations, the median lethal dose (LD₅₀) for cv. HUB-113 was determined to be 400 Gy.

2.2 Field Evaluation and Population Advancement

In order to raise the M₁ generation, the irradiated seeds and untreated controls were sown at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi, India, after mutagenic treatment. At maturity, M₁ plants were harvested individually to advance the population. The resulting M₂ progenies were grown during Rabi 2022-2023. The M₂ population was evaluated using an Augmented Block Design in order to effectively handle the large number of mutant genotypes while ensuring reliable statistical control. Repeated parental checks (cv. HUB-113) were systematically planted throughout the block layout to account for spatial variability in the field.

2.3 Screening and Evaluation of Mutants

Individual plants within the M₂ population were monitored systematically from seedling emergence through harvest. The population was screened for:

- 1. Chlorophyll mutations:** Identified at the early seedling stage and classified according to standard nomenclature (*albina*, *xantha*, *chlorina*, and *viridis*).
- 2. Lethal and morphological mutations:** Assessed throughout the vegetative and reproductive phases to determine the spectrum of macro-mutations induced by each dose.

2.4 Statistical Analysis and Mutagenic Parameters

The experimental data and observations recorded from the generation were utilized to estimate major parameters of mutagenic potential, specifically mutagenic frequency, mutagenic effectiveness, and mutagenic efficiency (Rana *et al.* 2024) [11]. The purpose of these parameters was to ascertain the sensitivity of HUB-113 to the applied gamma-ray doses and to calculate the radiation intensity that would most effectively induce genetic variability. Mutagenic frequency, effectiveness, and efficiency were calculated using standard mathematical formulations (Bakshi *et al.*, 2023) [11]:

- 1. Mutagenic Effectiveness:** This parameter measures the number of mutations induced by a single unit of the mutagenic dose in relation to the treatment duration.

2. **Mutagenic Efficiency:** This evaluates the proportion of desirable mutations relative to the undesirable biological damage, specifically the percentage of lethality induced in the population.

$$\text{Mutagenic effectiveness} = \frac{\text{frequency of mutations in M}_2}{\text{dose} \times \text{treatment (hrs)} \times 100}$$

3. **Mutagenic Frequency:** This indicates the magnitude of mutated plants observed within the population, calculated here in relation to the biological impact

$$\text{Mutagenic frequency} = \frac{\text{no. of mutants observed in M}_2}{\% \text{ lethality}} \times 100$$

4. **Mutagenic Efficiency:** This evaluates the proportion of desirable mutations relative to the undesirable biological damage, specifically the percentage of lethality induced in the population

$$\text{Mutagenic efficiency} = \frac{\text{frequency of mutations in M}_2}{\% \text{ lethality}} \times 100$$

3. Results and Discussion

A basic requirement for crop improvement is the induction of genetic diversity, which provides the raw genetic architecture required to select for superior agronomic qualities. In this study, we assessed the biological response of HUB-113 at three different gamma irradiation doses (350 Gy, 400 Gy, and 450 Gy), describing the spectrum and frequency of induced mutations in the M₂ population as well as physiological damage in the M₁ generation.

3.1 Biological Response and LD₅₀ Determination

Achieving the best possible balance between creating new genetic variety and preserving physiological viability requires standardizing the dosage of mutagens. The LD₅₀ for cv. HUB-113 in the M₁ generation was determined to be 400 Gy. This radiosensitivity threshold differs from that of Ahumada-Flores *et al.* (2021) [1], who reported that bread wheat (*Triticum aestivum* L.) had a lower LD₅₀ of 287.80 Gy. This divergence emphasizes how ploidy level, genomic architecture, seed moisture content, and genotype-specific DNA repair efficiency all have a significant impact on radiotolerance within the Triticeae tribe.

The somatic damage observed in M₁ seedlings could be due to radiation-induced double-strand breaks (DSBs) and structural chromosomal abnormalities that led to the appearance of decreased germination rates, delayed emergence, and stunted root and shoot growth. Large DNA lesions cause checkpoint-mediated cell cycle arrest at higher radiation intensities, which inhibits the transcription of genes crucial for early cellular development and suppresses RNA polymerase activity. Additionally, auxins (indole-3-acetic acid) and gibberellins are oxidatively degraded or synthetically inhibited by gamma irradiation, which directly causes the observed root inhibition and seedling stunting.

3.2 Spectrum and Frequency of Chlorophyll Mutations

Chlorophyll mutations are classical nuclear genetic markers widely employed to assess mutagenic potency, nuclear gene vulnerability, and overall treatment response. Three different kinds of chlorophyll mutants were found in the M₂ generation: tigrine (transversely striped chlorotic/necrotic

leaves), chlorina (yellowish-green seedlings), and xantha (carotenoid-rich, chlorophyll-deficient yellow seedlings). The total frequency of chlorophyll mutations showed a distinct dose-dependent linear response, increasing from 0.131% at 350 Gy to 0.455% at 450 Gy.

With 93 cases in the assessed sample, xantha was the most common trait among the observed mutational spectrum. The high frequency of xantha mutants reveals locus vulnerability in the pathways leading to the biosynthesis of tetrapyrrole and the development of chloroplasts. Chlorophyll and leaf-pattern mutants, especially lesion mimic and variegated varieties, are useful genetic tools for analyzing disease resistance pathways and programmed cell death (PCD) in cereals in addition to being markers of mutagenesis efficiency. To clarify the regulatory functions of cytochrome P450 monooxygenases in regulating reactive oxygen species (ROS) dynamics and innate immune responses in barley, for example, lesion mimic mutants like *nec3* have been effectively employed (Ameen *et al.*, 2021; Rosignoli *et al.*, 2022) [2, 9].

3.3 Lethal and Morphological Variations

The induction of lethal mutations provides a direct physical measure of genomic damage and somatic instability resulting from gamma irradiation. A total of 430 lethal mutations were found in the assessed M₂ population of cv. HUB-113. Although the maximum treatment dose of 450 Gy (n = 253) resulted in the largest absolute mutant count, the mutation frequency peaked at 350 Gy (1.97%), as opposed to 1.12% at 400 Gy. According to this non-linear distribution, moderate dosages successfully create lethal structural changes at high relative frequencies among surviving M₁ progenies before severe sterility or non-viable embryo development limits recovery, whereas acute doses result in widespread mortality.

The range of morphological mutations was extremely wide, affecting developmental phenology, leaf morphology, and inflorescence architecture. Degenerated, compact, loose, bent inflorescences make up the spike architecture. Foliage alterations include tightly curled or rolled leaf blades, crinkled flag leaves, and necrotic and chlorotic leaf diseases. Phenological Shifts: Both early- and late-heading lines result from noticeable changes in blooming time. Early heading was the most common trait among all morphological variations, and it was most common at the 350 Gy dosage (n = 110). The high recovery of early-maturing variants at moderate irradiation levels suggests that, without causing fatal chromosomal rearrangements, 350Gy efficiently induces point mutations or fine structural changes in regulatory gene networks controlling photoperiodic or vernalization pathways (such as *Ppd* or *Vrn* homologs). For the purpose of producing short-duration, climate-resilient cultivars suited to late-sown circumstances, these early-heading lines constitute significant germplasm.

3.4 Mutagenic Effectiveness and Efficiency

The practical success of a mutation breeding program lies on maximizing useful mutational frequency while limiting concurrent biological damage. In cv. HUB-113, there was a negative association between increasing gamma radiation dose and both mutagenic effectiveness (the rate of caused mutations per unit dose) and mutagenic efficiency (the ratio of mutant frequency to treatment-induced biological harm, such as fatality or sterility). Mutagenic Effectiveness: The

effectiveness of morphological features was highest at 350 Gy (0.984) and lowest at 400 Gy (0.334). Mutagenic Efficiency: At 350 Gy (174.6), efficiency peaked, but at 450 Gy, it significantly decreased to 117.8.

These patterns are consistent with findings by Ahumada-Flores *et al.* (2021)^[1] and Prashantha *et al.* (2026)^[7], who showed that moderate doses consistently produce superior efficiency because, at higher radiation intensities, biological damage accelerates at a disproportionately faster rate than mutation frequency. For barley cv. HUB-113, 350 Gy is the appropriate treatment threshold because it strikes the perfect balance between creating high-frequency, new genetic diversity and preserving enough population survival for efficient phenotypic selection.

4. Conclusion

This study demonstrates the efficacy of gamma irradiation in inducing novel genetic variability in barley cv. HUB-113. An LD₅₀ threshold of 400 Gy was determined by measuring physiological damage in the M1 generation. A broad range of chlorophyll (xantha, chlorina, and tigrina) and morphological alterations impacting inflorescence structure, leaf morphology, and developmental time were found in the M2 population. Early-heading variations were found at high frequencies among these, especially at 350 Gy (n = 110), providing useful material for the creation of short-duration, climate-resilient cultivars. By optimizing useful mutation frequency while reducing severe somatic toxicity and lethality, standardizing mutagenic measures determined that 350 Gy was the ideal radiation intensity, resulting in the highest mutagenic effectiveness (0.984) and efficiency (174.6).

The mutant population generated in this study serves as a valuable resource for barley genetic improvement and functional genomics. In order to stabilize desired macro-mutations, future research will have to concentrate on propagating certain M₂ lines through successive generations, in addition to screening for grain quality traits, yield components, and spot blotch resistance. Furthermore, integrating these mutant resources with genomic screening techniques, such as TILLING, will facilitate the identification and characterization of novel functional alleles underlying key agronomic traits.

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