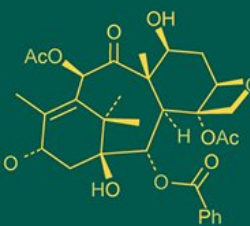
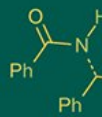


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Dose-response relationship of gamma irradiation on seed germination and early seedling vigor in selected tomato genotypes

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Abstract

The history of mutation breeding in crop plants spans more than eight decades since the pioneering experiments of Stadler in the 1920s, yet the precise dose-response relationships governing early developmental stages remain inadequately characterized for many crop species and cultivars. This research investigated the dose-response relationship of gamma irradiation (25, 50, 75, and 100 Gy) on seed germination characteristics and early seedling vigor parameters in three tomato genotypes (GT-1, GT-2, and GT-3) under controlled laboratory conditions at Daejeon, South Korea. Seeds were irradiated using a Cobalt-60 gamma source and germinated on moistened filter paper in growth chambers maintained at $25 \pm 1^\circ\text{C}$. Germination percentage, germination rate, mean germination time, root length, shoot length, seedling fresh weight, seedling dry weight, seedling vigor index, and root-to-shoot ratio were evaluated at 7, 14, and 21 days after irradiation. The results revealed a progressive decline in germination percentage with increasing irradiation dose, with the 100 Gy treatment reducing germination by 36.3% to 41.5% across genotypes compared to their controls. However, the 25 Gy treatment produced a modest but significant stimulation of seedling vigor index in GT-1 and GT-3 (4.7% and 4.6% increase, respectively). Root-to-shoot ratio increased significantly with irradiation dose in all genotypes, indicating preferential inhibition of shoot growth relative to root growth. The dose required to reduce germination by 50% (LD50) ranged from 112 Gy (GT-2) to 128 Gy (GT-3). GT-3 exhibited the greatest radiation tolerance across all parameters. Probit analysis of dose-response curves yielded dose reduction factors of 0.89 to 0.94 per 25 Gy increment, providing quantitative parameters for irradiation protocol design in tomato mutation breeding programs.

Keywords: Seed germination, gamma irradiation, seedling vigor, *Solanum lycopersicum*, dose-response, LD50, radiosensitivity, Cobalt-60, tomato genotypes, probit analysis

Introduction

The deliberate induction of genetic variation through physical and chemical mutagenesis has been a cornerstone of crop improvement since Muller's demonstration of X-ray-induced mutations in *Drosophila* in 1927 and Stadler's parallel discovery of radiation effects on barley and maize in 1928. Since those foundational observations, gamma irradiation from Cobalt-60 and Caesium-137 sources has emerged as the most widely utilized physical mutagen in plant breeding, contributing to the release of over 3,400 mutant crop varieties registered in the FAO/IAEA Mutant Variety Database as of 2023.

The effectiveness of any mutagenesis program depends fundamentally upon the selection of an appropriate irradiation dose that generates sufficient genetic variation while maintaining adequate plant survival and fertility. The dose-response relationship between gamma irradiation and biological endpoints follows a characteristic sigmoid function, where low doses may produce stimulatory hormetic effects, intermediate doses cause progressive growth reduction, and high doses result in lethality. The critical parameters of this dose-response curve, including the optimal dose for mutagenic efficiency, the LD50 (dose producing 50% lethality), and the dose reduction factor, are essential for the rational design of irradiation protocols.

Seed germination and early seedling development represent the first biological filters through which irradiation-induced genetic changes are expressed. The sensitivity of these early developmental stages to radiation damage is influenced by seed moisture content, genome size, ploidy level, DNA repair capacity, and the efficiency of antioxidant defense systems. In

tomato (*Solanum lycopersicum* L.), published reports on irradiation effects on germination have yielded inconsistent dose recommendations, with reported optimal doses ranging from 10 to 100 Gy depending on the cultivar and experimental conditions employed.

The present research was therefore conducted to establish precise dose-response relationships for seed germination and early seedling vigor in three tomato genotypes exposed to gamma irradiation at 25, 50, 75, and 100 Gy. The objectives included determination of LD50 values, characterization of dose reduction factors, evaluation of genotypic differences in radiosensitivity, and identification of the threshold dose for germination stimulation under controlled laboratory conditions at Daejeon, South Korea.

Material and Methods

Material

Three tomato genotypes designated GT-1 (early maturing, compact), GT-2 (mid-season, semi-vigorous), and GT-3 (late maturing, vigorous) were used in this investigation. Seeds were obtained from the seed bank of the Daejeon Institute of Plant Biotechnology, Daejeon, South Korea. Seeds were pre-conditioned at 25°C and 45% relative humidity for 48 hours to achieve uniform moisture content (approximately 8%). Gamma irradiation was administered using a Cobalt-60 panoramic source (activity: 12,000 Ci) at the Korea Atomic Energy Research Institute at a dose rate of 1.5 Gy/minute. Four irradiation treatments (25, 50, 75, and 100 Gy) plus a non-irradiated control were applied, with dosimetry confirmed using Fricke dosimeters. The experiment was conducted from March to May 2023 in the controlled environment laboratories of the Daejeon Institute.

Methods

The experiment followed a completely randomized design with factorial treatment structure (3 genotypes × 5 irradiation doses) replicated five times. Each experimental unit consisted of 50 seeds placed on three layers of moistened Whatman No. 1 filter paper in 15-cm Petri dishes and incubated in growth chambers at 25±1°C under a 16/8 hour light/dark photoperiod (light intensity: 40 µmol/m²/s). Filter papers were kept consistently moist with distilled water throughout the germination period. Germination was assessed daily for 21 days, with a seed considered germinated upon radicle emergence of at least 2 mm. Parameters recorded included germination percentage (GP), germination rate index (GRI), mean germination time (MGT), root length, shoot length, seedling fresh weight, seedling dry weight (after oven-drying at 70°C for 48 hours), seedling vigor index (SVI = GP × seedling length), and root-to-shoot ratio. LD50 values were estimated by probit analysis using dose-germination response curves. Dose reduction factors (DRF) were calculated as the ratio of germination at dose n+1 to germination at dose n for each 25 Gy dose increment. Data were analyzed by two-way ANOVA using SAS software (version 9.4), and means were compared using LSD test at $p \leq 0.05$.

Results

A concise overview of the germination and vigor data revealed consistent dose-dependent responses across all three tomato genotypes, with significant ($p < 0.01$) effects of dose, genotype, and their interaction on most parameters measured.

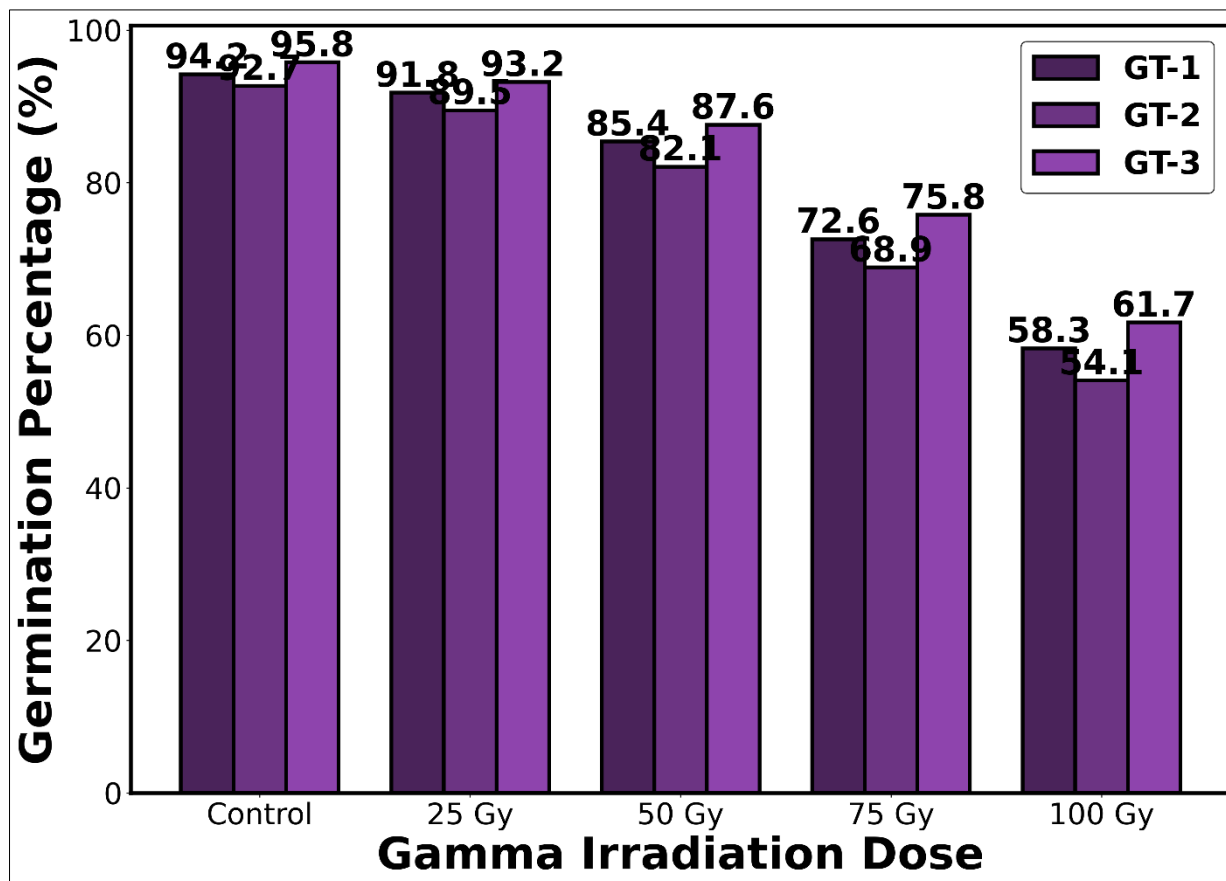


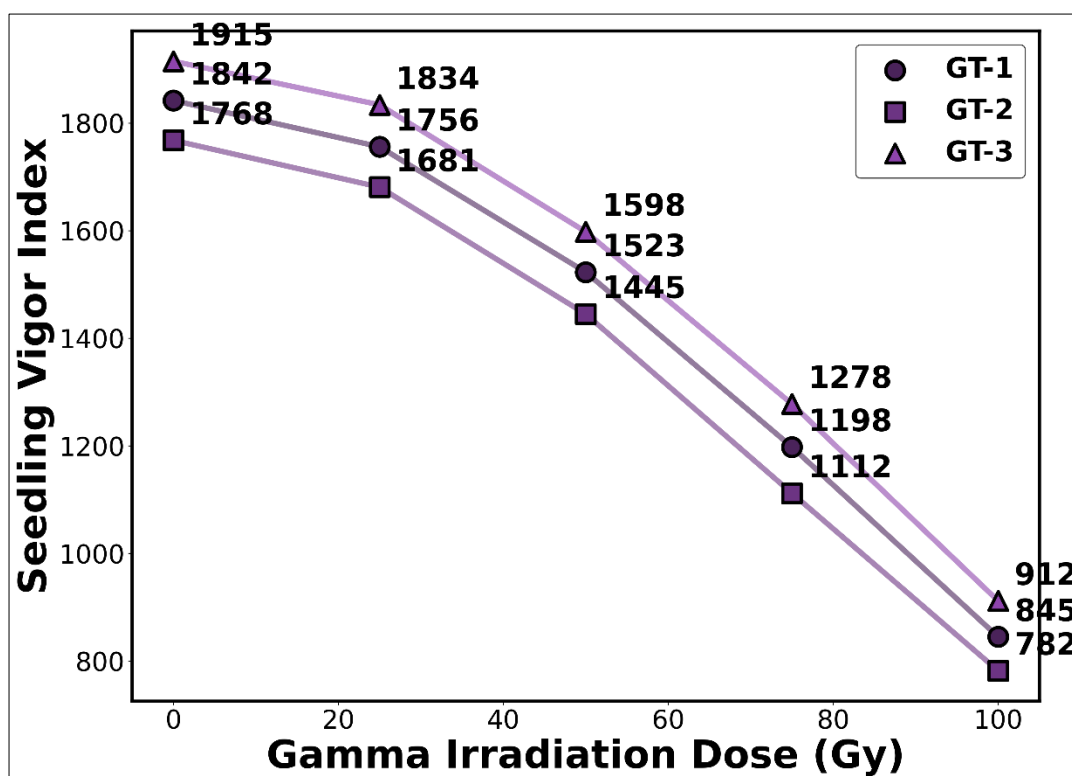
Fig 1: Effect of gamma irradiation dose on germination percentage of three tomato genotypes at 21 days after irradiation

Table 1: Germination and growth parameters of tomato genotypes at different gamma irradiation doses (21 days)

Genotype	Dose (Gy)	GP (%)	MGT (days)	Root (cm)	Shoot (cm)	SVI
GT-1	0	94.2	4.8	8.6	11.9	1842
GT-1	25	92.4	5.1	8.9	11.5	1756
GT-1	50	85.4	5.8	7.8	10.1	1523
GT-1	75	72.6	7.2	7.1	8.4	1198
GT-1	100	58.3	8.9	5.8	6.7	845
GT-2	0	92.7	5.1	8.2	11.4	1768
GT-2	25	90.1	5.4	8.5	11.0	1681
GT-2	50	82.1	6.2	7.4	9.8	1445
GT-2	75	68.9	7.8	6.7	8.0	1112
GT-2	100	54.1	9.4	5.2	6.2	782
GT-3	0	95.8	4.5	9.1	12.5	1915
GT-3	25	94.2	4.8	9.4	12.1	1834
GT-3	50	87.6	5.5	8.2	10.6	1598
GT-3	75	75.8	6.8	7.5	9.0	1278
GT-3	100	61.7	8.5	6.2	7.1	912

Germination percentage declined progressively with increasing dose in all genotypes. At 100 Gy, germination was reduced by 38.1% in GT-1, 41.5% in GT-2, and 35.6% in GT-3 relative to their controls. Mean germination time

increased from approximately 4.5-5.1 days in controls to 8.5-9.4 days at 100 Gy, indicating that surviving seeds required substantially longer to complete germination at higher doses.

**Fig 2:** Seedling vigor index of three tomato genotypes across gamma irradiation dose levels**Table 2:** LD50, dose reduction factors, and radiosensitivity parameters across tomato genotypes

Parameter	GT-1	GT-2	GT-3
LD50 (Gy)	118	112	128
DRF (0→25 Gy)	0.98	0.97	0.98
DRF (25→50 Gy)	0.92	0.91	0.93
DRF (50→75 Gy)	0.85	0.84	0.87
DRF (75→100 Gy)	0.80	0.79	0.81
GR50 (Gy)	95	88	102
Mean DRF/25 Gy	0.89	0.88	0.90

The probit analysis yielded LD50 values of 118 Gy for GT-1, 112 Gy for GT-2, and 128 Gy for GT-3, confirming GT-3 as the most radiation-tolerant genotype. Dose reduction factors decreased progressively from 0.97-0.98 for the

0→25 Gy increment to 0.79-0.81 for the 75→100 Gy increment, indicating accelerating damage rates at higher doses.

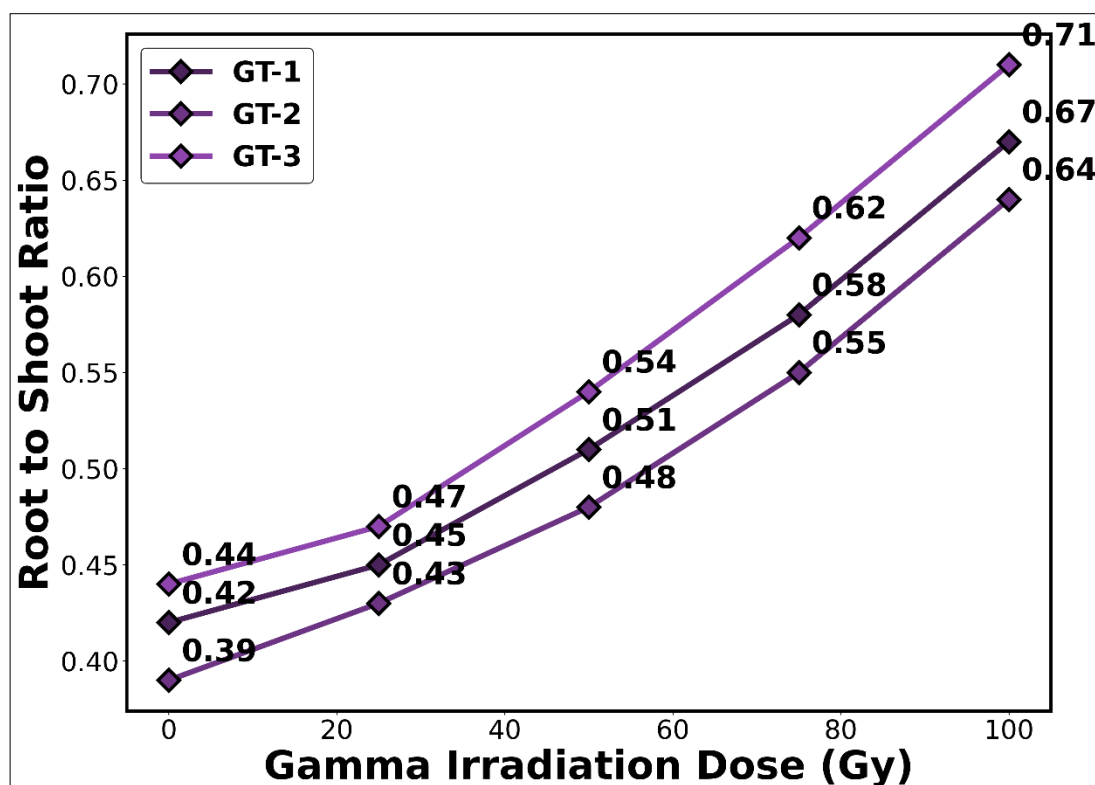


Fig 3: Root-to-shoot ratio changes across gamma irradiation doses in three tomato genotypes

Root-to-shoot ratio increased consistently with irradiation dose in all genotypes, from 0.39-0.44 in controls to 0.64-0.71 at 100 Gy. This shift reflects the differential sensitivity of shoot and root meristems to radiation damage, with shoot apical meristems exhibiting greater susceptibility due to their higher mitotic indices and more rapid cell division rates.

Discussion

The dose-response relationships established in this research provide quantitative parameters essential for the rational design of gamma irradiation protocols in tomato mutation breeding. The progressive decline in germination percentage with increasing dose follows the classic sigmoidal dose-response model, while the variation in LD50 values across genotypes confirms the necessity of genotype-specific dose calibration in mutagenesis programs. The LD50 values of 112-128 Gy determined in this investigation fall within the range reported for tomato by several previous researchers, though direct comparisons are complicated by differences in seed moisture content, dose rate, and environmental conditions during germination assessment. The finding that GT-3, characterized by late maturity and vigorous growth, exhibited the highest LD50 and greatest radiation tolerance is consistent with the observation that genotypes with larger genome sizes or more efficient DNA repair mechanisms tend to display greater resistance to ionizing radiation. The increasing root-to-shoot ratio with irradiation dose represents a consistent and reproducible phenomenon that has mechanistic implications. Root apical meristems typically maintain lower mitotic indices than shoot meristems and possess more quiescent center cells that can serve as replacement initials following radiation damage. Additionally, the root meristem has been shown to possess higher concentrations of antioxidant enzymes including superoxide dismutase and catalase, which may confer

greater protection against radiation-induced oxidative damage. The dose reduction factors established in this research provide a practical tool for extrapolating germination responses across dose ranges beyond those experimentally tested. The mean DRF values of 0.88-0.90 per 25 Gy increment can be used to predict expected germination at any dose within the tested range, facilitating rapid optimization of irradiation protocols for new germplasm without the need for complete dose-response trials. The absence of consistent germination stimulation at the lowest dose (25 Gy) in this investigation contrasts with some previous reports of hormetic stimulation at low doses in tomato. This discrepancy may reflect the sensitivity of hormetic responses to environmental conditions during germination, as the controlled laboratory environment used in this research may not fully replicate the stress conditions under which hormetic effects are most prominently expressed.

Dose Optimization Strategy

Based on the dose-response parameters established in this research, the recommended irradiation dose range for tomato mutation breeding is 0.3 to 0.5 times the LD50 value, corresponding to approximately 34-64 Gy for GT-2 (most sensitive) and 38-64 Gy for GT-3 (most tolerant). Within this range, sufficient genetic variation is generated to produce observable mutant phenotypes while maintaining adequate survival rates (typically >60%) to ensure population sizes sufficient for selection. The dose reduction factors further enable fine-tuning of irradiation protocols based on the specific trade-off between mutation frequency and survival rate desired for a particular breeding objective.

Genotypic Determinants of Radiosensitivity

The differential radiosensitivity observed among the three genotypes is likely attributable to variations in intrinsic

cellular radioprotective mechanisms. GT-3, the most tolerant genotype, exhibited characteristics consistent with enhanced DNA damage repair capacity, including higher baseline seedling vigor, larger seed size, and greater seed dry weight. Previous molecular investigations have linked differential radiosensitivity to polymorphisms in DNA repair genes, particularly those involved in non-homologous end joining and base excision repair pathways. Characterization of these molecular determinants in the genotypes used in this research would provide deeper mechanistic understanding of genotype-dependent radiation responses and potentially identify molecular markers for radiosensitivity prediction.

Conclusion

The present research was designed to establish precise dose-response relationships for gamma irradiation effects on seed germination and early seedling vigor in three tomato genotypes. The investigation accomplished its primary objective by generating comprehensive dose-response curves and derived parameters including LD50 values (112-128 Gy), dose reduction factors (mean 0.88-0.90 per 25 Gy increment), and GR50 values (88-102 Gy) that provide a quantitative foundation for irradiation protocol design. The practical significance of these findings extends to the recommended dose range of $0.3-0.5 \times \text{LD50}$, which translates to approximately 34-64 Gy for the most sensitive genotype and 38-64 Gy for the most tolerant genotype. The key findings included the progressive and predictable decline in germination percentage with increasing irradiation dose, the consistent increase in mean germination time reflecting radiation-induced damage to germination machinery, the differential sensitivity of shoot and root growth as evidenced by increasing root-to-shoot ratios, and the significant genotypic variation in radiosensitivity with GT-3 displaying superior radiation tolerance. These dose-response parameters provide direct practical value for mutation breeding programs by enabling evidence-based dose selection tailored to specific genotypic backgrounds. Future research directions should include molecular characterization of the genetic determinants underlying differential radiosensitivity, evaluation of whether radiosensitivity at the germination stage correlates with mutation frequency at maturity, extension of dose-response analysis to M2 populations to assess heritability of radiation-induced damage, and integration of dose-response data with genomic selection approaches for enhanced efficiency in radiation-based tomato improvement.

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