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VARIATION IN TOMATO GENOTYPES RESPONSE TO X-RAY IRRADIATION AND ESTIMATION OF THE EFFECTIVE DOSES

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Abstract: *The study investigates the response of 15 tomato genotypes (cultivated and wild forms) to X-ray irradiation, aiming to determine effective mutagenic doses that result in a 50% reduction in key quantitative parameters: germination rate, radicle length, hypocotyl height, and seedling height. Seeds were exposed to 10 irradiation doses (ranging from 100 to 530 Gy), and the effects were assessed in comparison to a non-irradiated control. Linear, nonlinear, and polynomial regression models were applied, along with dose–response curve analysis, to estimate the effective inhibitory doses (ID_{50}/ID_{30}) for the efficient use of induced mutagenesis in the M_0 and subsequent generations. The results reveal considerable variability among genotypes, including stimulatory (hormetic) effects at lower doses. These findings are essential for optimizing mutagenesis protocols in tomato breeding programs.*

Keywords: mutagenesis, tomato, regression, variability, X-rays.

INTRODUCTION

The induction of mutations using physical agents, such as ionizing radiation (gamma rays, X-rays), is a well-established approach for increasing genetic variability beneficial to plant breeding programs. This method enables the development of novel genotypes exhibiting superior agronomic traits, including stress tolerance, disease resistance, and improved nutritional or technological qualities [1]. However, the success of induced mutagenesis is strongly influenced by a combination of biological, technical, and experimental factors that affect the plant's response to radiation treatments. A key determinant is the proper preparation of seed material. Seed moisture content at the time of irradiation directly affects energy transfer efficiency and consequently influences the frequency and type of induced mutations. Seeds with higher water content are more sensitive to radiation due to the generation of free radicals through water radiolysis,

which can interact with DNA and other cellular structures. Therefore, strict control of seed moisture (typically reduced to 12-14%), according to standardized protocols is essential to ensure the repeatability and comparability of experimental results.

Other critical variables include the radiation dose and the type of radiation source, both of which influence the intensity and specificity of the mutagenic effects. In addition, the physiological stage of the biological material (seeds, seedlings, callus, etc.) and the genetic background of the treated genotypes contribute significantly to variations in radiosensitivity and the plant's capacity to repair radiation-induced damage [2; 3]. Genotypes vary in their response to mutagenic stress, necessitating individualized calibration of radiation doses for each variant under study. This is typically achieved through the determination of parameters such as the median inhibitory dose (ID_{50}). Establishing an effective dose is a critical step to ensure an optimal mutation rate while avoiding lethal damage to plant tissues.

In tomato, the literature reports a wide range of effective doses depending on genotype and the type of radiation or facility used [4; 5; 6]. Moreover, assessment methods such as germination rate, hypocotyl/plant length reduction, and seedling vigor are commonly used to identify promising genotypes for further breeding cycles.

In this context, the primary objective of the present study is to evaluate the differential radiosensitivity of 15 tomato genotypes and to estimate their LD_{50} values based on relevant morphological parameters. By applying mathematical modeling approaches, including both linear and nonlinear regression, we aim to accurately estimate optimal radiation doses and to characterize the dose–response relationships. This contributes to the rational planning of mutagenesis experiments. Therefore, the rigorous integration of these factors into the experimental design is essential for generating reliable, reproducible, and practically applicable results in modern plant improvement programs.

MATERIALS AND METHODS

An essential prerequisite for X-ray treatment is the determination of initial seed moisture content, which in our case was assessed using the gravimetric method, according to [7]. Representative seed samples (three replicates of 500 mg each) were analyzed for three genotypes. The optimal moisture content was standardized to 12–13% [8] through controlled humidification. Seeds were hermetically sealed prior to irradiation to maintain consistent moisture levels.

The evaluation of genotype radiosensitivity and the identification of effective doses were carried out following IAEA protocols [9] and methodologies described by Bado et al. [6]. The irradiation response was tested in vivo across 15 tomato genotypes (including varieties, intra- and interspecific recombinant forms,

and spontaneous variants). A total of 10 X-ray doses ranging from 100 to 530 Gy were applied, with six selected doses (100, 150, 200, 250, 300, and 350 Gy) retained for further experiments based on their preliminary depressive effects. Following irradiation, seeds were placed either in Petri dishes (for germination rate assessment) or directly into soil substrate for seedling development. X-ray treatments were performed using a calibrated Rad Source RS-2400 unit.

The dose–response relationship was analyzed using the mean values of measured traits, as well as by the estimation of the DI_{50} (inhibitory dose causing a 50% reduction in the parameter relative to the control). Quantitative indices used to assess the effects of irradiation included germination rate over time, radicle length, hypocotyl length, and total plant height. Measurements were carried out dynamically, starting 72 hours after seed soaking. Germination was assessed *in vivo* using three replicates of 30 seeds per genotype per dose. Seedling measurements were performed 21 days after germination on 10 plants per replicate (3 replicates). Germination was conducted in darkness at approximately 20 °C. Seedlings were grown under controlled laboratory conditions in soil substrate.

Data were analyzed using Excel and Statgraphics Plus software. Linear, nonlinear, and polynomial regression models were applied, along with dose–response curve analysis to estimate effective irradiation doses.

RESULTS AND DISCUSSION

According to the literature data, the optimal doses of ionizing radiation (X-rays and γ-rays) for tomatoes typically range between 200 and 400 Gy. However, these values must be adjusted based on the specific genotype tested. To determine the optimal impact of X-rays, we initially assessed a wide range of doses (100–530 Gy) on key indicators in five genotypes using a limited sample of seedlings and young plants. Based on preliminary lethality observations, we selected six doses between 100 and 350 Gy, where mortality at the seedling stage did not exceed 30%.

In subsequent experiments, the 15 genotypes included in the study exhibited differential responses depending on both the radiation dose and the evaluated trait. In this study, we applied targeted indicators and statistical methods to evaluate their relevance in determining the radiation impact on tomatoes—particularly in assessing the effects of various doses on quantitative traits through the calculation of ID_{50} values.

One of the first measurable indicators was the seed germination rate over time. Starting from 120 hours post-imbibition, we observed a gradual decrease in germination speed with increasing radiation dose, compared to the control. Suppression reached up to 90% at doses of 300–350 Gy. However, beginning at 178–216 hours, the differences in germination rate between irradiated and control groups diminished significantly, with the initial suppressive effect disappearing

entirely. This suggests that irradiation primarily causes a delay in germination rather than a reduction in final germination rate.

Additional indicators, such as radicle length, hypocotyl length, and total seedling length, also showed significant reductions with increasing irradiation dose, varying by genotype (Table 1, 2). A 30–50% decrease in radicle length compared to the control (ID_{50}) was observed across a wide range of doses (150–250 Gy), while a similar decrease in total seedling length (ID ID_{50}) ranged between 150–350 Gy, depending strongly on the genotype. Notably, at the dose 350 Gy, although the final germination rate was not significantly reduced, radicle length decreased by 75–90% and seedling length by 47–100% compared to the control. These findings align with international literature, which emphasizes genotype-dependent variation and radiation type [4, 6].

Further analysis focused primarily on plant height as a function of radiation dose across genotypes, although other indicators could also serve as references for assessing radiation impact. According to our observations, plant height is a more informative and reliable trait for establishing standard impact indices such as ID_{50}. As radiation dose increased, so did the proportion of individuals that remained at the cotyledon stage (BBCH 10), whereas control and some irradiated individuals progressed to BBCH 11–12. Thus, we used two comparative evaluation models: (1) "Plant" model, height was recorded only for individuals that had developed at least one true leaf (BBCH 11 or higher); otherwise, height was recorded as zero, (2)"Cotyledon" model, height was measured for all individuals, regardless of whether they had only cotyledons or true leaves.

Results indicate that data from individuals with true leaves (BBCH 11–13) are more relevant. Higher radiation doses often inhibit further development in seedlings that form only cotyledons, resulting in relatively tall hypocotyls but no progression to later stages. This is confirmed by diagrams showing that including cotyledon-only individuals in the ID_{50} or ID_{30} estimation artificially inflates the doses required to suppress seedling height by 50% or 30%, sometimes beyond 350 Gy – a threshold that does not reflect biological reality, as severe damage to radicle or seedling development occurs at these levels (Figure 1A, Table 3). Moreover, when calculating mean plant height based solely on individuals that had progressed to at least BBCH 11 (i.e., with true leaves), we can more confidently assume a higher likelihood of survival – though this is not guaranteed. From the dose–response diagrams for mean plant height (Figure 1B), we observe that the ID_{50} doses fall within the 250–300 Gy range for over 50% of the genotypes studied.

In the present study, we aimed to apply a variety of statistical techniques and methods to highlight the relevance of each factor – genotype, radiation dose, evaluation index, assessment matrix, and both statistical and empirical approaches – in determining the impact of radiation on plants. Specifically, our objective was to identify effective mutagenic doses that induce minimal adverse effects on plant

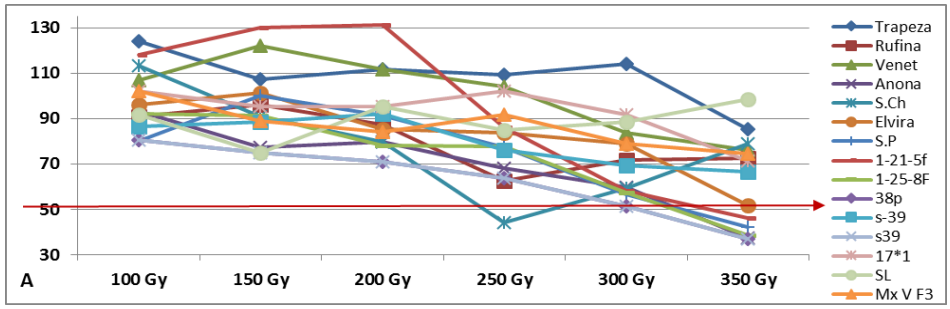
vitality, as well as to determine the ID_{50} (inhibitory dose for 50% effect).

By employing dedicated statistical methods, we were able to assess the radiation impact either at the level of individual genotypes, genotype groups, or specific irradiation doses. When analyzing the mean radiation impact expressed as ID across 15 genotypes, a clear decrease in ID values was observed with increasing doses (Table 1). For instance, a dose of 100 Gy led to a 3.8% reduction in average plant height (ID_{50}) compared to the control, while a 300 Gy dose resulted in a 47.1% reduction. Furthermore, analysis using Multiple Range Tests (a component of dispersion analysis) confirmed that different radiation doses produced significantly distinct effects on the suppression index (ID) compared to the control. Statistically significant differences were recorded for each of the doses ranging from 250 to 350 Gy (Table 1). A similar trend is also observed in the average plant height (expressed in cm) across 15 genotypes exposed to six radiation dose levels ranging from 0 to 350 Gy.

Table 1. Multiple range tests for plant height and percent values relative to the control (ID), according to radiation dose (100–350 Gy), summarized across 15 genotypes. Method: 95.0% LSD

X ray dose, Gy	Genotypes number	LS Mean	Homogeneous Groups	X ray dose, Gy	Total evaluation	LS Mean	Homogeneous Groups
ID – trait value, % of control				Plant height, cm, LS Sigma - 0.09			
350	15	42.9	C	350	311	3.64	F
300	15	56.7	CB	300	311	4.72	E
250	15	72.0	B	250	311	5.23	D
200	15	90.0	A	200	311	6.05	C
100	15	96.2	A	150	311	6.50	B
150	15	100.1	A	100	311	6.58	AB
		LS Sigma 5.9		0	311	6.75	A

It is also worth noting that repeated experiments revealed a hormesis effect in some genotypes, typically at doses between 100 and 150 Gy, with certain exceptions. This phenomenon is illustrated in Figure 1.



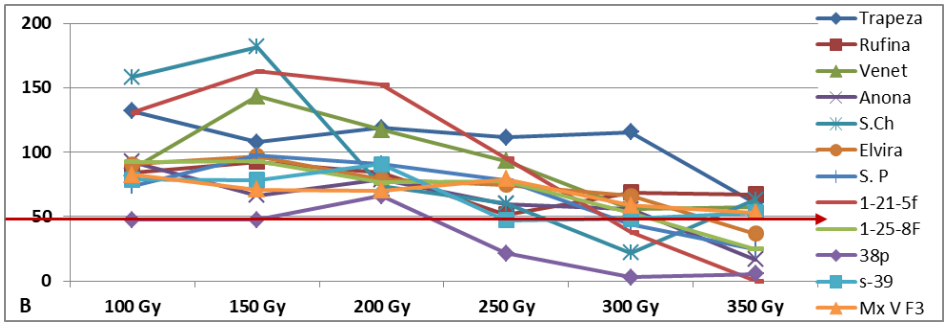


Figure 1. Variation in the trait *Plant height* as predicted by Model 1 (B – 15 genotypes) and Model 2 (A – 12 genotypes), in response to increasing X-ray radiation dose, expressed as a percentage of the control value.

The divergence in response becomes apparent starting at 200 Gy, with the height of plants irradiated at 350 Gy showing a reduction of approximately 46% compared to the control (Table 1). However, the response of plants to ionizing radiation varied significantly depending on the genotype. The application of Multiple Range Tests revealed the classification of seven statistically homogeneous groups with respect to the overall effect of radiation doses (0–350 Gy) on plant height. The differences between group means were consistent and statistically significant (Table 2).

Table 2. Multiple Range Tests for plant height affected by X-Ray exposure, (*LS Sigma* - 0,13)

Genotype	8	5	10	9	12	7	1	2	4	11	3	6
Count	168	168	168	168	168	168	168	168	168	168	168	168
LS Mean, cm	2,64	2,87	3,11	3,92	4,42	4,48	4,84	4,96	5,13	5,34	5,93	6,65
Groups	H	GH	G	F	E	E	D	D	CD	C	B	A

Note: Genotypes code: 1 - Trapeza, 2 - Rufina, 3 - Venеț, 4 - Anona, 5 - *Solanum cheesmaniae* (S.Ch), 6 - Elvira, 7 - *S. pimpinellifolium* (S.P), 8 - 1-215f, 9 - 1-218f, 10 - 38p, 11 - S-39, 12 – F3 Mary Gratefully x Venet

The relationship between variables is effectively illustrated through regression analysis, which allows us to investigate how a dependent variable (e.g., growth suppression) changes in response to one or more independent variables (e.g., radiation dose). Moreover, regression models can support the prediction of trends and the estimation of effect magnitude.

Using linear, nonlinear, or polynomial regression equations generated in

Excel or other data analysis software, it is possible to estimate the dose required to achieve a 50% or 30% suppression of a trait's value (ID_{50}, ID_{30}), either for individual genotypes or across multiple genotypes within a given experiment. In this study, 12 genotypes were subjected to six irradiation doses ranging from 100 to 350 Gy, based on 90 observations (mean values of trait suppression). The results revealed a strong negative correlation between radiation dose and the evaluated index (R=-0,6) (Table 3).

Table 3. Summary of ID_{50} index for plant height based on different regression types and trait evaluation modes (Mode 1/2)

Traits		Type of regression				
		Linear simple Y=a+b*X		Polynomial Y=a+b*X-c*X²		Nonlinear Y=d / (1+(X/e) ^b)
		Model 1	Model 2	Model 1	Model 2	Model 1
Plante height,	ID50	328 Gy	422 Gy	321 Gy	388 Gy	316 Gy
		R^2=37	R^2 =36	R^2=39	R^2=38	R^2=40
		R=-0,6	R=-0,6			
Hypocotyl height,	ID50	418 Gy		392 Gy		
		R^2=47		R^2=48		
		R=-0,7				

Individual genotype evaluation			
Genotype	Equation	R^2	ID50, Gy
Trapeza	y = -0.2041x + 124.49	R^2 = 0.87	364
Venet	y = -0.2238x + 121.19	R^2 = 0.72	318
S.P	y = -0.2476x + 117.94	R^2 = 0.93	274

Returning to earlier discussions on how plant height should be measured, we compared results using two models: Model 1, which included only plants that reached at least BBCH stage 11, and Model 2, which included also seedlings measured at the hypocotyl stage. The ID_{50} values derived from these two models – both under linear and polynomial regression – differed significantly. However, when comparing the modes' predictive accuracy to actual observed responses, Model 1 yielded more consistent results, with estimated ID_{50} values of 321 Gy (linear regression) and 328 Gy (polynomial regression), compared to Model 2 with ID_{50} values of 422 and 388 Gy, respectively. In parallel, a third nonlinear regression mode, commonly applied in mutagenesis and pharmacological studies, was also used. The ID_{50} value derived from this method was likewise 316 Gy, closely aligning with the other estimates. This suggests that applying any of these doses would likely result in a 50% reduction in trait value compared to the control. An alternative and recommended approach would be also to estimate ID values for each

genotype individually, based on irradiation dose. These ID values showed wide variation across genotypes, but these were not always consistent with the biological manifestations, as reflected also in the distribution graphs presented in Figure 1.

A key metric for assessing the quality of model fit in regression analysis is the coefficient of determination (R^2). The closer R^2 is to 1, the better the model explains the variance in the dependent variable. In our analyses, R^2 values ranged between 0.36 and 0.40, indicating that, depending on the model used, approximately 40% of the total variability in the observed data is explained (Table 3). Although the level of correlation observed in our case is moderate, it can be considered acceptable under complex experimental conditions or in the presence of high biological variability. However, when evaluating individual genotypes, the coefficient of determination (R^2) shows relatively high values, suggesting a greater accuracy of the resulting equation in capturing the variability of the trait.

CONCLUSIONS

The study highlights the importance of comprehensive assessment in determining radiosensitivity, taking into account both individual genotype responses and aggregated data across the entire genotype set. The application of regression models – linear, polynomial, and nonlinear and the analysis of dose–response curves facilitates the identification of optimal doses for mutation induction without compromising seedling viability. These findings are essential for the effective design of mutagenesis strategies.

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