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Research article

Effect of Soaking Time and Gamma Radiation on Seed Germination and Seedling Growth of Three Varieties of Chinese Spinach

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Abstract

Pre-soaking seeds prior to radiation exposure is a common technique used in agricultural and biological research to study its effects on seed germination, growth, and overall plant development. This study investigated the impact of varying seed soaking time and radiation exposure on three varieties of Chinese spinach (Type A: red leaf, Type B: long green leaf, and Type C: round green leaf). Seeds were soaked for 0, 12, 24, and 36 h before exposure to 0 and 50 Gy of gamma radiation. Germination percentages were monitored for 72 h, and seedling growth parameters, including height, leaf length, leaf width, and number of leaves, were measured over 28 days. Correlation matrices were generated to analyze the relationships between soaking time, radiation dose, and growth parameters. The results indicated that soaking time and radiation exposure interacted in complex ways to influence plant growth. Type A seeds exhibited increased germination and leaf expansion with longer soaking time under radiation, while Types B and C showed reduced growth in height, leaf length, and width. The findings highlight species-specific responses to radiation and soaking treatments, providing insights into optimizing seed treatment protocols for crop improvement in stress-prone environments. These results contribute to understanding plant resilience mechanisms, offering valuable insights for plant breeders aiming to optimize seed treatment protocols for effective mutation breeding.

Keywords: germination; seedling growth; soaking time; gamma radiation; Chinese spinach

1. Introduction

Pre-soaking seeds prior to radiation exposure is a widely utilized technique in agricultural and biological research that is used to investigate the impact on seed germination, growth, and overall plant development. This method involves immersing seeds in water for varying durations before subjecting them to irradiation, with the aim of modifying their response to radiation stress. By altering the moisture content and physiological state of seeds before

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exposure, pre-soaking has been shown to potentially mitigate the adverse effects of radiation or induce desirable traits such as enhanced germination rates, improve growth parameters, and increase stress tolerance (Myttenaere et al., 1965; Amjad & Anjum, 2002; Al-Bachir et al., 2003; Baek et al., 2003). This approach plays a crucial role in studies exploring the radiation's influence on plant genetics, mutation breeding, and agricultural productivity. It provides valuable insights into optimizing seed treatment protocols for sustainable crop improvement strategies, particularly in environments prone to radiation or other stress factors (Shahab, 2018; Li et al., 2019; Pandit et al., 2021). Understanding the underlying mechanisms of how pre-soaking interacts with radiation exposure (Kiani et al., 2022) contributes to the development of resilient crop varieties capable of thriving in challenging conditions, thereby advancing agricultural innovation and global food security efforts.

Previous study investigated the synergistic effects of gamma radiation and seed hydration on plant germination, growth, and development across multiple species. Research conducted on burdock plants demonstrated that seeds subjected to gamma radiation (10-30 Gy) and subsequently hydrated for 12 h exhibited enhanced germination velocity, with optimal outcomes observed at 30 Gy (Taher et al., 2022). Analogous results were obtained in *Moluccella laevis* L., where hydrated seeds exposed to low-dose gamma radiation (25 Gy) yielded the highest germination percentage (Minisi et al., 2013). The treatments significantly influenced seedling development, with burdock seeds irradiated at 30 Gy displaying superior vegetative and root growth characteristics. In *Moluccella laevis* L., low radiation doses correlated with increased plant height, while higher doses resulted in augmented branch formation and elevated dry weight. Conversely, rice seedlings exposed to higher radiation doses (200 Gy) on pre-hydrated seeds exhibited significantly diminished emergence and growth (da Silva et al., 2011). Morphological alterations were observed in *Moluccella laevis* L. at higher radiation doses (125-175 Gy) on hydrated seeds. Furthermore, the treatments influenced reproductive phenology, with specific doses accelerating flowering and increasing inflorescence production in *Moluccella laevis* L. These findings suggest that the combinatorial approach of gamma radiation and seed hydration presents a promising methodology for enhancing various plant characteristics. However, it is crucial to note that the efficacy of this approach is highly dependent on radiation dosage, plant species, and cultivar specificity.

While research has been conducted on the effects of gamma radiation combined with seed soaking in various plant species, there appears to be a gap in the literature regarding similar studies on spinach. This experiment aimed to investigate the relationship between seed moisture content and radiation exposure by varying seed soaking time, and to assess the changes in seed germination and seedling growth parameters such as plant height, leaf length, leaf width, and number of leaves across three varieties of Chinese spinach.

2. Materials and Methods

Three varieties of Chinese spinach—red leaf (Type A), long green leaf (Type B), and round green leaf (Type C)—seeds were used for this study. Each condition involved 500 seeds soaked for 0, 12, 24, and 36 h. Subsequently, seeds from each condition were exposed to doses of 0 and 50 Gy of gamma radiation. Germination percentages were monitored for up to 72 h after germination. One hundred germinated seeds from each condition were randomly planted, with randomized planting positions. Germination percentage and seedling growth parameters were assessed by measuring height, leaf length, leaf width, and number of leaves every 7 days over a period of 28 days.

Correlation matrices were generated for each plant variety, examining the relationships between soaking time, radiation dose, and growth parameters (height, number of leaves, leaf length, and leaf width). Correlation coefficient ranges from -1 to 1, indicating the strength and direction of the linear relationships between variables. The correlation coefficients presented in the heatmap were calculated using the Pearson correlation coefficient formula, which quantifies the linear relationship between two variables X and Y. The formula is:

$$r = \frac{\sum(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum(X_i - \bar{X})^2 \sum(Y_i - \bar{Y})^2}}$$

Where r is the Pearson correlation coefficient, Xi and Y_i are the individual data points, $\bar{X}$ and $\bar{Y}$ are the means of X and Y, and Σ represents the summation over all data points. The calculation involves determining the deviations of each data point from their respective means, multiplying these deviations for corresponding pairs, summing the results, and normalizing by the product of the standard deviations of X and Y.

In the heatmap, this formula was used to compute the correlation coefficients for each pair of variables, such as soaking time, radiation dose, height, number of leaves, leaf length, and leaf width. The resulting coefficients range from -1 to 1, where values close to 1 indicate a strong positive correlation, values close to -1 indicate a strong negative correlation, and values near 0 indicate no linear relationship. The heatmap visualizes these relationships using a gradient of colors, with darker shades representing stronger negative correlations, lighter shades indicating weak or no correlation, and brighter shades signifying stronger positive correlations, revealing how these factors interrelate across plant types (Types A, B, and C).

In the study of radiation effects on seedling growth, plants of Types A, B, and C were subjected to two different conditions: control and radiation. Measurements of plant height, number of leaves, leaf length, and leaf width were recorded at 14, 21, and 28 days. The results are displayed as bar plots showing the mean and standard deviation of the data.

For the relationship between soaking time and radiation, the plants were divided into two main groups based on radiation dosage: 0 Gy and 50 Gy. Each group was further categorized into three types of plants (A, B, and C) and subjected to different soaking times (0, 12, 24, and 36 h). Growth parameters were measured on day 28, and the data were summarized in Table 1 and presented using box plots. Statistical significance was indicated with symbols: ns (not significant), ** ($p < 0.01$), *** ($p < 0.001$), and **** ($p < 0.0001$).

3. Results and Discussion

The germination percentages shown in Figure 1 illustrate the varying responses of different plant types to soaking time and radiation doses. At a soaking time of 0 h, all plant types exhibited a lower germination percentage. As the soaking time increased, the germination percentage decreased for Type B and Type C plants. In contrast, for Type A plants, the germination percentage increased with longer soaking times, showing a significant increase at 36 h of soaking. The findings reveal precise comparisons with previous research. The observed variations in germination percentages across different plant types

Table 1. Summary of growth parameter values for three Chinese spinach varieties exposed to different radiation doses and soaking times on day 28

Plant Type	Dose (Gy)	Soaking Time (h)	Height (cm)				Number of Leaves				Leaf Length (cm)				Leaf Width (cm)			
			mean	SD	min	max	mean	SD	min	max	mean	SD	min	max	mean	SD	min	max
A	0	0	2.55	0.45	0.6	3.5	6.94	1.10	4	10	2.43	0.32	1.5	3	1.71	0.34	1	2.6
		12	2.50	0.45	0.4	3.4	6.99	1.04	4	9	2.39	0.39	0.6	3.2	1.67	0.34	0.3	2.4
		24	2.39	0.52	0.5	3.4	7.01	1.14	3	10	2.40	0.31	1.2	3.2	1.68	0.29	0.6	2.5
		36	2.14	0.68	0.5	3.2	6.96	1.39	2	9	2.41	0.39	1	3.3	1.67	0.35	0.6	2.5
	50	0	2.41	0.47	0.2	3.1	6.89	1.08	2	9	2.44	0.31	1.5	3.2	1.68	0.30	0.8	2.7
		12	2.26	0.50	0.6	3.3	6.82	0.94	4	9	2.40	0.40	0.6	3.2	1.64	0.34	0.4	2.5
		24	2.32	0.43	1.2	3.2	7.13	0.91	5	9	2.41	0.41	1.2	3.1	1.69	0.31	0.8	2.4
		36	1.95	0.48	0.5	3	6.80	1.26	1	9	2.43	0.35	1.5	3.2	1.68	0.30	0.9	2.6
B	0	0	1.63	0.27	1.1	2.5	5.95	0.22	5	6	7.48	1.01	5.1	10.8	2.17	0.50	1.1	3.5
		12	1.48	0.23	0.9	2.1	5.86	0.35	5	6	7.11	1.04	2.4	9.4	2.00	0.52	0.7	3.3
		24	1.74	0.32	1.1	2.4	5.68	0.47	5	6	7.42	1.15	4.3	9.5	2.31	0.59	1.1	3.7
		36	1.71	0.34	1.1	2.7	5.31	0.58	4	7	7.31	1.36	2.2	11.1	2.44	0.54	0.6	3.8
	50	0	1.55	0.28	1.2	2.5	5.98	0.15	5	6	7.31	0.87	5.3	11.2	2.17	0.46	1.1	3.5
		12	1.51	0.26	0.9	2.4	5.79	0.41	5	6	6.82	1.07	4.3	9.1	1.96	0.48	1	3.1
		24	1.58	0.28	1.1	2.5	4.99	0.41	4	6	5.33	1.13	2.8	9.1	1.49	0.42	0.5	3.5
		36	1.23	0.31	0.7	2.2	4.20	0.97	2	6	3.17	1.46	1.2	8.5	1.33	0.78	0.3	3.1
C	0	0	1.51	0.32	1.1	3.4	4.98	0.15	4	5	4.62	1.07	2.2	7.5	3.14	0.75	1.3	5.5
		12	1.59	0.36	1.1	3	4.92	0.28	4	5	4.59	1.18	2	8	3.13	0.83	1.3	5
		24	1.50	0.35	1.1	2.4	4.94	0.23	4	5	4.76	1.17	1.8	7	3.09	0.74	1.3	4.3
		36	1.38	0.27	0.9	2.4	5.07	0.37	4	6	4.90	1.13	1.8	7.1	3.17	0.84	1.1	4.5
	50	0	1.42	0.17	0.9	1.8	4.96	0.19	4	5	4.59	0.95	2.1	6.4	3.19	0.66	1.8	4.5
		12	1.37	0.23	1	2.8	4.71	0.48	3	5	3.87	1.06	1.8	7.1	2.58	0.70	1.2	5
		24	1.36	0.24	0.9	2.5	4.71	0.54	3	6	3.78	1.22	1.7	7.1	2.41	0.73	1.1	4.8
		36	1.35	0.29	0.9	2.5	4.25	0.61	3	6	2.36	0.89	1.1	5.1	1.69	0.56	0.8	3.5

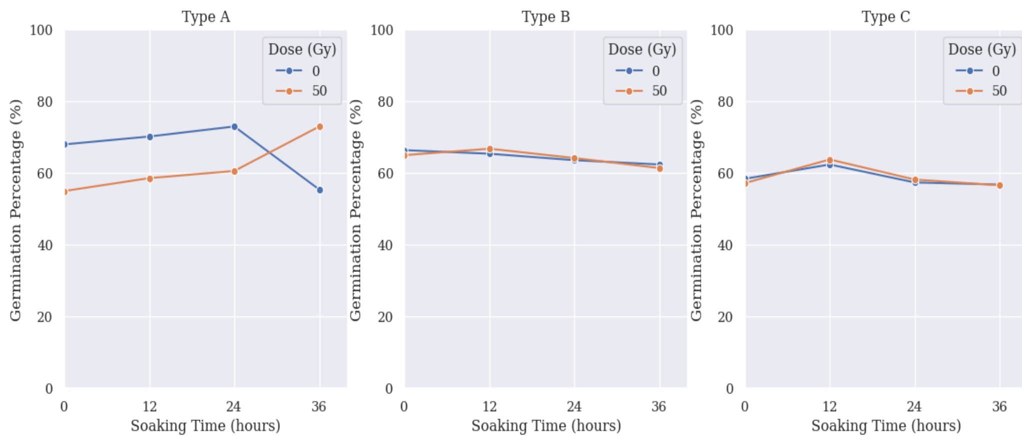

Figure 1. The germination percentage of three types of Chinese spinach as function of soaking time with subgroup based on radiation doses (0 and 50 Gy)

under varying soaking times and radiation doses partially corroborate earlier studies. Specifically, our results align with Minisi et al.(2013), who noted negative responses of gamma radiation on *Moluccella laevis* L. seed germination. While our study demonstrates decreased germination percentage for plant types B and C with increased soaking time. Taher et al. (2022) similarly reported reduced seed germination percentages when burdock seeds were subjected to soaking and gamma radiation. Similar effects were reported by da Silva et al. (2011), where rice seedlings showed decreased emergence under elevated gamma radiation doses.

The correlation matrix heat map (Figure 2) revealed relationships among variables. It indicated that height, number of leaves, leaf length, and width exhibited negative correlations with radiation dose and soaking time. Conversely, these variables demonstrated positive correlations with each other. Type A demonstrated stronger correlations between height and other growth parameters, which may suggest a more direct influence of these parameters on overall plant height. In contrast, Types B and C exhibited more moderate correlations, indicating potential variations in growth patterns or genetic factors influencing these relationships. The number of leaves showed stronger correlations with leaf length and width in Types B and C compared to Type A, suggesting that leaf growth in B and C was more coordinated or synchronized. This may reflect inherent genetic differences or varying responses to environmental conditions between these plant types. The very high correlation between leaf length and width across all types indicated a fundamental relationship in leaf development, regardless of plant type. This suggests that these two dimensions are tightly regulated and grow in tandem. The weak correlations of soaking time and radiation dose with growth parameters across all types highlighted the complexity of these factors impacting plant growth. These results suggest that the effects of soaking time and radiation dose are not straightforward and may involve interactions with other variables such as an increase in free radical content by increasing dose (Kiani et al., 2022).

From Figure 3, the data indicates that radiation affected the growth parameters of plants differently depending on the plant type. Overall, all types showed growth in height, number of leaves, leaf length, and leaf width over time under both control and radiation

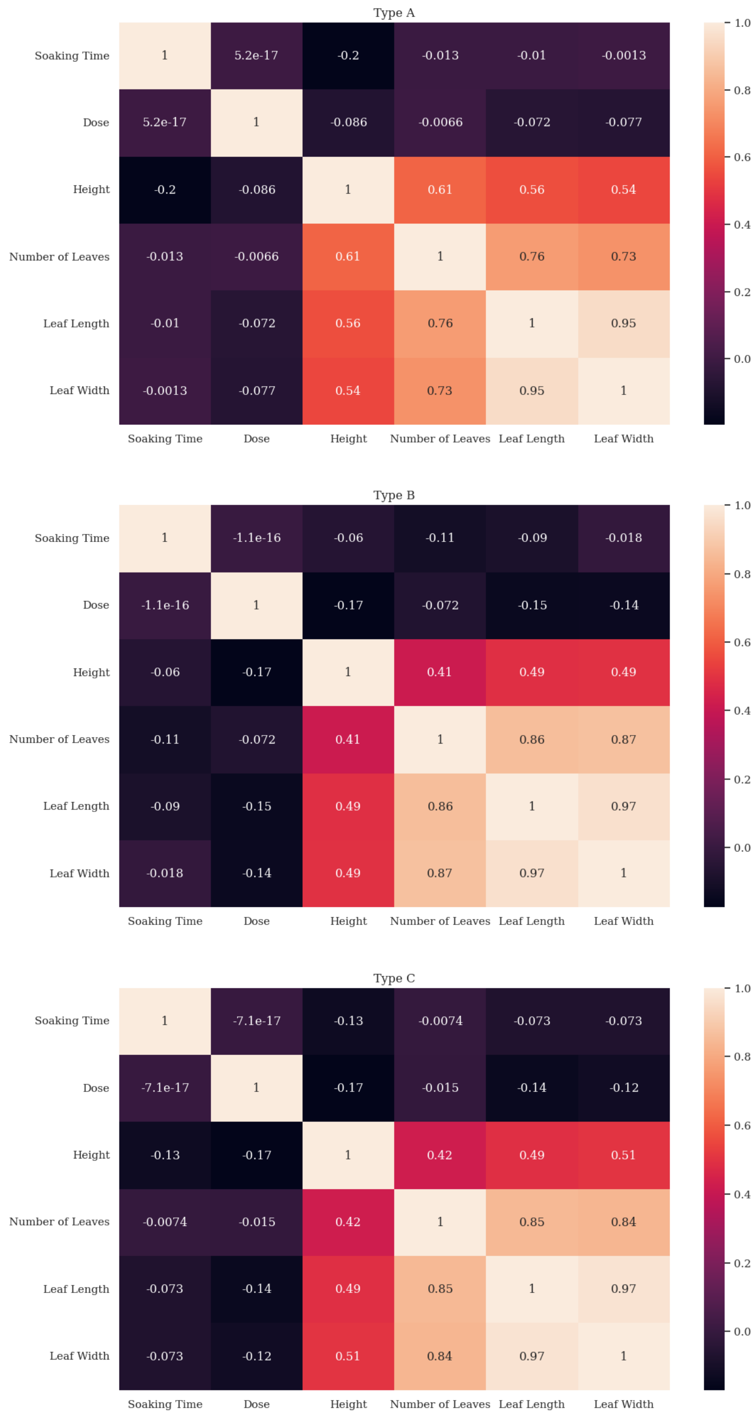

Figure 2. Correlation matrix heatmap of soaking time, radiation dose, height, number of leaves, leaf length and leaf width of three types of Chinese spinach

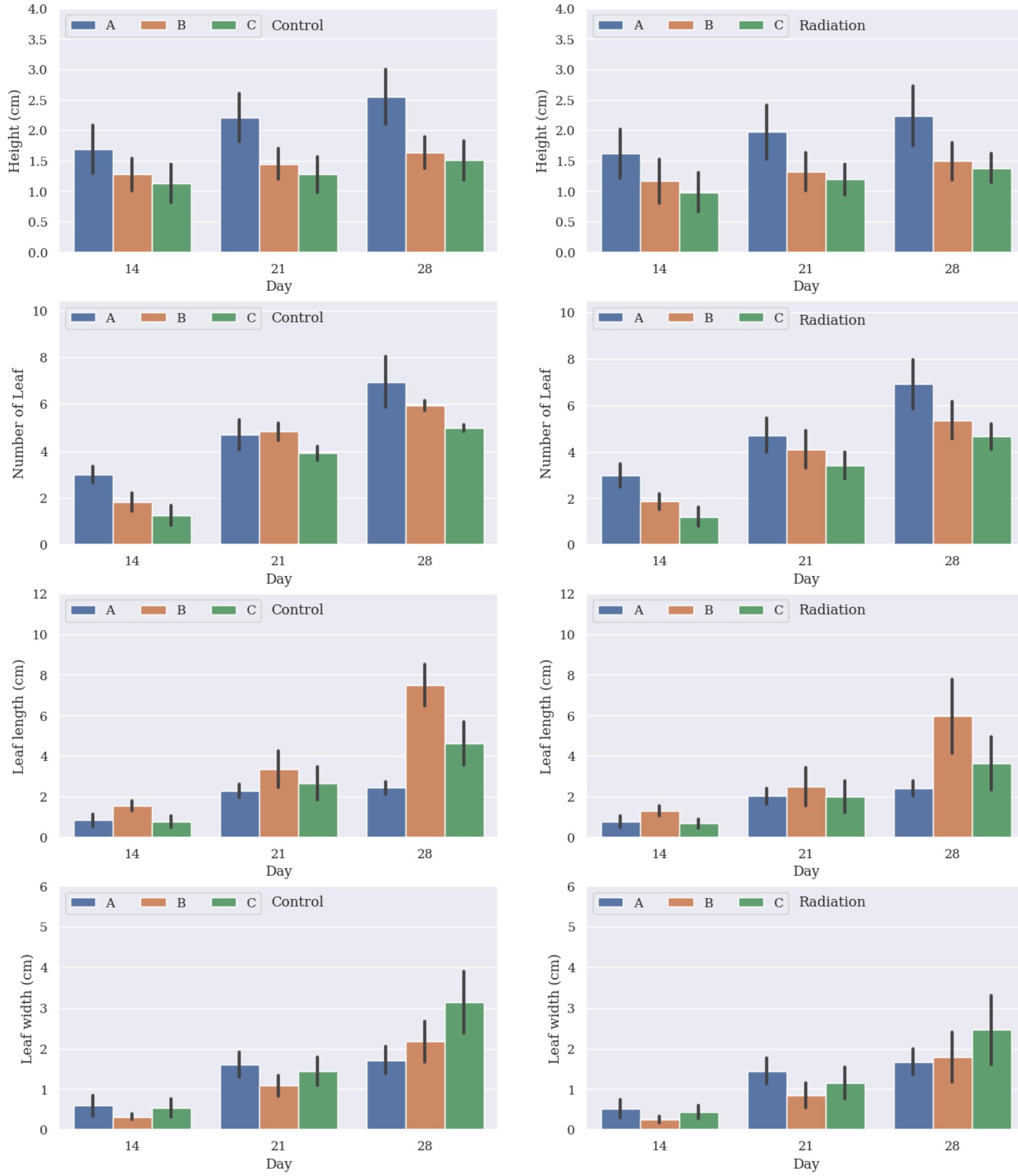

Figure 3. Seedling growth parameters of three types of Chinese spinach (height, number of leaves, leaf length, and leaf width) as a function of time after planting, displayed in columns with non-radiated (Control) on the left and radiated (Radiation) on the right.

conditions. However, plants under radiation tended to exhibit slightly reduced growth compared to the control group, particularly in leaf length and width. The radiation condition appeared to slightly inhibit the growth of Types B and C compared to the control group by the end of the 28-day period. Type A under radiation showed a higher number of leaves compared to the control group by day 28, suggesting a potential compensatory response to radiation stress. Both parameters showed increased value over time, with plants under control conditions generally displaying greater growth compared to those under radiation.

The statistical data, such as mean, standard deviation, minimum, and maximum growth parameters from the 28-day period, are shown in Table 1. The box plot in Figure 4 indicates p-values for analyzing the differences between soaking time and growth parameters under radiation conditions. The results indicated that radiation exposure (50 Gy) combined with prolonged soaking times significantly affected plant growth parameters. For all plant types, height was generally reduced at longer soaking times under radiation, suggesting a detrimental effect on vertical growth, which was partially consistent with da Silva et al. (2011) and Taher et al. (2022), who observed reduced height in plant seedlings that were exposed to higher doses of gamma radiation. Leaf length and width decreased only in Types B and C. Conversely, the leaf length and width of Type A showed significant increases at 24 and 36 h of soaking under radiation, indicating that while vertical growth may be inhibited, leaf expansion might be enhanced under these conditions, potentially due to hormesis effect, a non-linear dose-dependent response where low doses provide beneficial outcomes while high doses cause inhibition (Amjad & Anjum, 2002). These results indicated that soaking time played a major role in inhibiting seedling height under radiation in all plant types and enhanced leaf length and width only in Type A, suggesting a variation in response among different plant types. The results align with Kaini et al. (2022), indicating that moisture significantly influenced the production of reactive oxygen species (ROS). Gamma radiation exposure in plants triggers ROS production through water radiolysis by splitting water molecules into hydroxyl radicals and other reactive species that damage cellular components. High moisture content significantly amplifies this effect as observed in our study. This mechanism explains why soaking time strongly influenced seedling inhibition across all plant types. The differential response among plant types, particularly enhanced leaf dimensions in Type A, likely originates from variations in antioxidant capacity, membrane composition, growth regulator balance and DNA repair mechanisms. At low doses, ROS can stimulate growth through hormetic effects by modulating cell expansion genes and enhancing metabolic processes. Consequently, plant breeders should consider reducing the irradiation dosage for seeds with high moisture levels to prevent excessive inhibition of plant growth.

4. Conclusions

The germination of seeds was influenced by both radiation dosage and soaking time. Type B and C seeds were most sensitive to radiation, exhibiting significant reductions in germination as soaking time increases. In contrast, Type A seeds appeared more resilient, maintaining consistent germination rates regardless of radiation exposure, with minimal differences observed between radiation dosages. Radiation exposure had a measurable impact on plant growth, with variations observed among different plant types. While Type A plant showed higher germination percentage and continued growth in leaf number under radiation due to hormesis, where low-dose radiation triggered beneficial physiological responses, other types exhibited reduced growth in height, leaf length, and width, and were

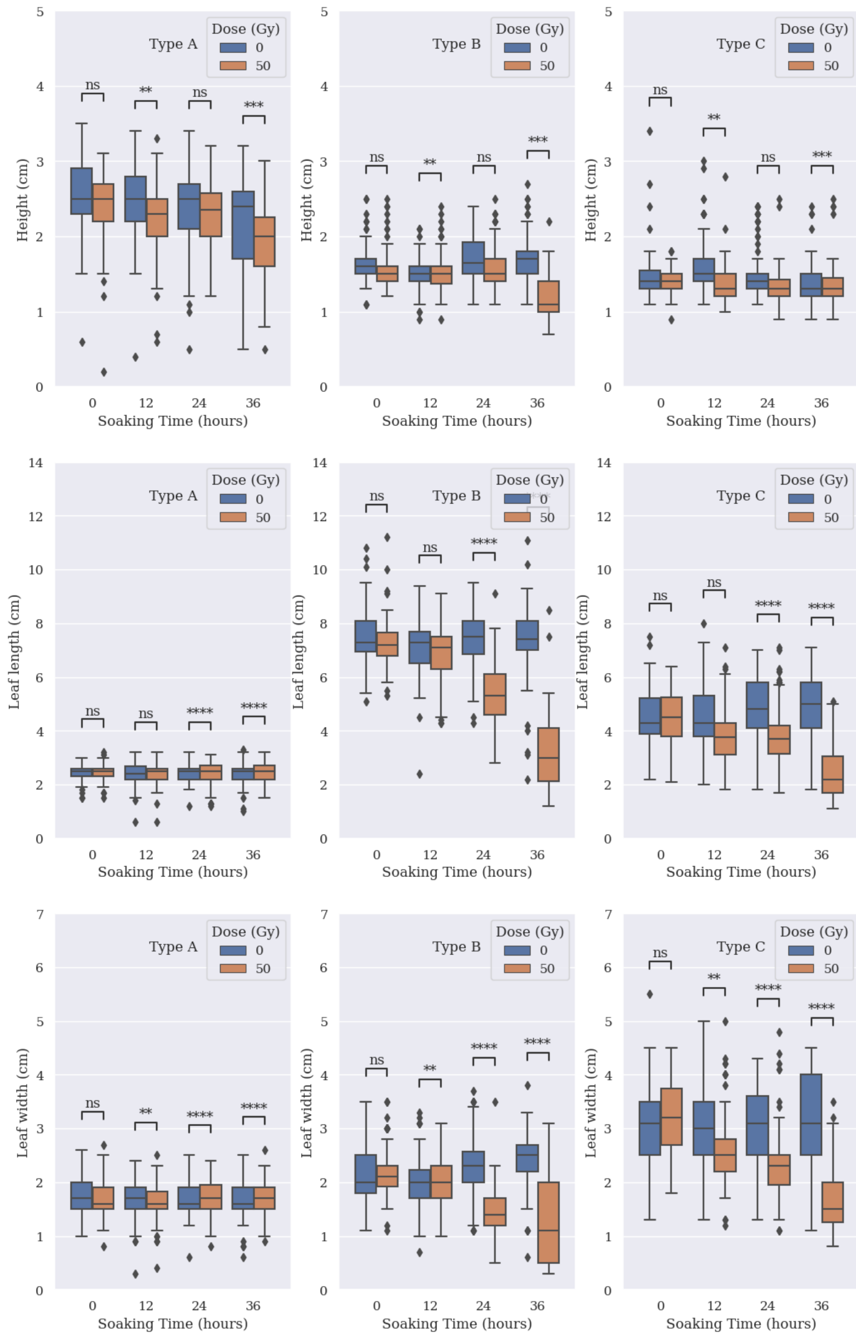

Figure 4. Seedling growth parameters of Chinese spinach (height, leaf length, and leaf width) as a function of soaking time, with subgroups based on radiation dose, separated by columns for the three types of spinach. The data include statistical analysis indicating p-values. (ns: 5.00e-02 < p ≤ 1.00e+00 *: 1.00e-02 < p ≤ 5.00e-02
: 1.00e-03 < p ≤ 1.00e-02" *: 1.00e-04 < p <= 1.00e-03 ****: p <= 1.00e-04)

probably more sensitive to radiation stress beyond their hormetic threshold (Agathokleous et al., 2019; Volkova et al., 2022). These findings suggest a differential response to radiation among plant types, highlighting the importance of considering species-specific responses in studies of environmental stressors. Gamma radiation exposure at 50 Gy coupled with increased soaking times significantly influenced plant growth parameters. Height, leaf length and width were generally reduced for Types B and C, while leaf length and width of Type A increased, particularly at 24 and 36 h soaking times. These findings suggest a complex interaction between radiation exposure, water absorption, and plant varieties that needs further investigation to fully understand the underlying mechanisms and explore potential applications in agricultural practices. From the results, it may be concluded that the radiation dose serves as a primary determinant of the stress level experienced by plants, directly influencing physiological and growth responses. However, soaking time plays a critical modulatory role, either enhancing tolerance in more resilient plant types or amplifying sensitivity in susceptible ones. This interaction highlights the dynamic nature of soaking time, which appears to exert a more substantial and variable influence on plant growth when combined with radiation exposure, underscoring its importance as a key factor in determining plant responses under such environmental stress conditions.

In conclusion, the results clearly demonstrate the inhibitory and enhancing effects of radiation and soaking time on the growth of three varieties of Chinese spinach—Type A (red leaf), Type B (long green leaf), and Type C (round green leaf)—with relevance for optimizing agricultural practices in environments subject to these conditions, particularly in plant mutation breeding with gamma radiation.

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6. Authors' Contributions

Kittikhun Prakrajang was primarily responsible for the experimental design, data analysis, and preparation of the manuscript. Sureeporn Sarapirom contributed significantly to the design of the experiment, participated in data analysis, and performed the critical review and revision of the manuscript. Thasapong Saibunpang and Rapeepan Luejai were involved in conducting the experiments, data collection, and contributed to the analysis of the experimental data.

7. Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper. This includes financial, personal, or professional relationships that could be seen as influencing the research. The work was conducted independently, and no organization or individual with a potential interest in the outcome influenced the study design, data collection, analysis, or writing of the manuscript.

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