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Research Article

Gamma Irradiation of *Plukenetia volubilis* L. Seeds Promotes Several Changes during Its Germination and Vegetative Growth

Mike Anderson Corazon-Guivin,[1] **Sofia Rengifo-Del Aguila**,[1] **Angel David Hernández-Amasifuen**,[1] **Víctor Manuel Arévalo-Rojas**,[2] **Ronny Anthony Acosta-Córdova**,[1] **Agustín Cerna-Mendoza**,[1] **Thiago de Araújo Mastrangelo**,[3] **Jorge Damian Valverde-Iparraguirre**,[1] and **Juan Carlos Guerrero-Abad**[4]

[1]*Laboratorio de Biología y Genética Molecular, Universidad Nacional de San Martín, Jr. Amorarca N° 315, Morales, Peru*
[2]*Laboratorio de Biotecnología, Universidad Nacional de Barranca, Avenue Toribio de Luzuriaga N°376, Mz J, Urbanización La Florida, Barranca, Peru*
[3]*Laboratory of Radiobiology and Environment, Center for Nuclear Energy in Agriculture (CENA/USP), Piracicaba, 13416-000, São Paulo, Brazil*
[4]*Instituto de Investigación, Innovación y Desarrollo para el Sector Agrario y Agroindustrial (IIDAA), Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas (UNTRM-A), Calle Higos Urco N° 342, Chachapoyas 01001, Peru*

Correspondence should be addressed to Mike Anderson Corazon-Guivin; macorazong@unsm.edu.pe and Juan Carlos Guerrero-Abad; juan.guerrero@untrm.edu.pe

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Plukenetia volubilis ("sacha inchi") is a perennial plant that produces edible seeds with a remarkable lipid composition that is highly concentrated in polyunsaturated essential fatty acids. Inca nut seeds have potential use for lowering malnutrition, enhancing sustainable food production systems, reforestation, and the pharmaceutical industry. The establishment of genetic variability, through spontaneous mutations or induced mutations, can bring desirable and undesirable agronomic traits. Our research focused on studying the impact of gamma radiation on *P. volubilis* seeds during their germination and vegetative growth. For this purpose, we exposed seeds to different doses of gamma irradiation (0, 500, 550, 600, 650, 700, 750, 800, and 900 Gy) and planted them under *in vitro* and greenhouse conditions following a completely random design. Our findings showed that gamma radiation treatments did not affect the germination of *P. volubilis* seeds but affected its root tip growth. An analysis on morphological and physiological parameters revealed a reduction in seedling size and weight when the irradiation doses were increased. Also, the pattern of plant organ development changed as its gamma irradiation was increased. Finally, our analysis found that median lethal dose (LD_{50}) for *P. volubilis* L. seeds is 618.78 Gy. Our findings can be used as an important reference for plant breeding in this species.

1. Introduction

Plukenetia volubilis (commonly referred to as "maní del Inca," "maní salvaje," "maní de montaña," "sacha inchi," or "inca inchi") is a perennial plant of the Euphorbiaceae family, native to the Amazon region of South America and thriving at altitudes ranging from sea level up to approximately 1,000 m [1]. Inca nut plants maintain continuous production because they start flowering about 5 months after being planted, and they remain in bloom and continue fruiting throughout the growing season. Its edible seeds contain a remarkable lipid composition characterized by very high amounts of the polyunsaturated essential fatty acids such as α-linolenic acid (ω-6) and linoleic acid (ω-3); there fatty acids represent 35.2%–50.8% and 33.4%–41.0% of the total lipid fraction, respectively [2–4]. Due to these reasons and the potential applications for reducing malnutrition, improving sustainable food production systems, aiding reforestation, and supporting the pharmaceutical industry, *P. volubilis* has proven to be a successful addition to agricultural practices in the Peruvian Amazon [2, 5–7].

However, the domestication process of *P. volubilis* is still ongoing. For this reason, the crop is susceptible to pests and diseases such as those caused by *Ralstonia pseudosolanacearum* phylotype I [8] and *Meloidogyne–Fusarium* complex [9], which can cause total wilting and result in significant economic losses [5]. Added to the high requirement of fertilizers for oil production [10], this shows the importance and need to implement a breeding program for this crop. This program should not only provide agronomic benefits but also lead to increased productivity and enhanced fruit characteristics (sensory qualities) [11].

Genetic variability for breeding programs is necessary, and spontaneous mutation or induced by physical or chemical agents are source of genetic variants [12]. In this sense, the induction of point and/or chromosomal mutations using physical and chemical mutagenic agents can be considered a strategic approach to expanding the genetic variability in crops [13]. Physical mutagens such as nonionizing or ionizing radiation often result in large-scale DNA deletion and changes in the chromosomal structure, while chemical mutagens allow for affecting only individual nucleotide pairs [14].

Ionizing radiation is widely recognized as one of the most commonly utilized physical mutagens. The gamma irradiation has been used successfully to generate several positive mutations in crops [15–18], as well as for pathogens reduction and pest control in seeds [19–22]. Gamma rays can promote physiological, morphological, cytological, and biochemical changes in plants by increasing free radicals in cells caused by their ionizing effects [23]. Higher doses inhibit growth and development of plants, while low doses can stimulate them [24]. Experiments to establish the appropriate radiation, known as the median lethal dose (LD_{50}) would be determined by dosimetry assays and it could be used as a reference to generate and recover useful mutations [25–27].

A 2022 study using a chemical mutagenic agent, such as ethyl methanesulfonate (EMS), induced morphological and physiological changes in *P. volubilis* seedlings [28]. However, studies on radiation effects of *P. volubilis* seeds are still unknown. Accordingly, the goal of our research was to study the effect of different doses of gamma radiation (^{60}Co) at the seed level on the growth and development of *P. volubilis* seedlings growth under greenhouse conditions.

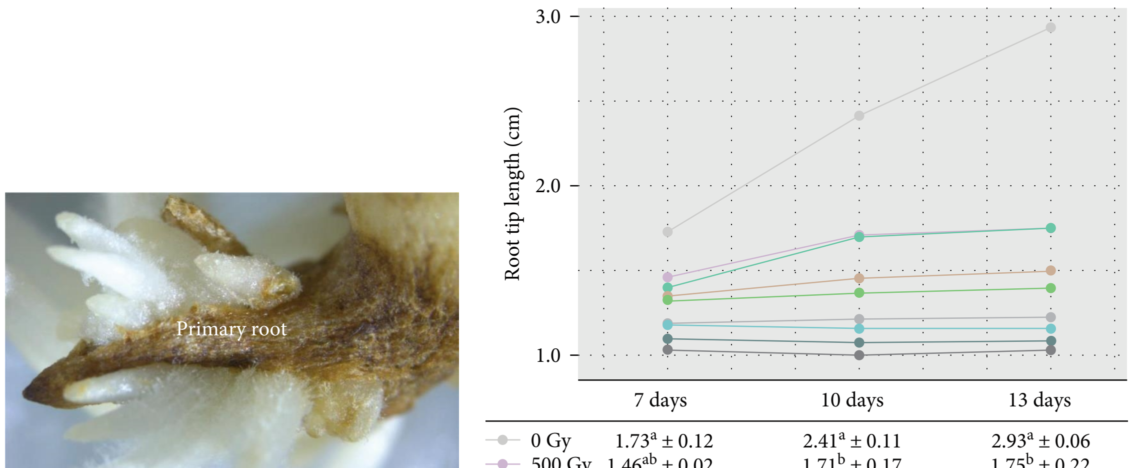
2. Materials and Methods

2.1. Plant Material and Irradiation Seeds. P. volubilis seeds (local ecotype "Shanantina") were harvested and collected in Lamas Province, San Martin, Peru (06°26′47″ S, 076°31′44″ O; 382 m.a.s.l.). Phenotypic characteristics such as color (dark brown), shape (oval), and diameter (1.5–2.0 cm) were considered as traits to select homogeneous seeds for irradiation. Nine treatments with different irradiation doses (0-control, 500, 550, 600, 650, 700, 750, 800, and 900 Gy) were administered in the irradiator type I (^{60}Co) Gammacell 220 Excel, at a dose rate of 1,964.3 Gy/h (Model C-198-GS-401; MDS Nordion International Inc., Canada) located at the Instituto Peruano de Energía Nuclear (IPEN), Lima, Peru. Two sets of 100 seeds were irradiated separately for every gamma doses. To determine the absorbed doses of the gamma source and map the doses, Fricke and alanine dosimeters were employed. To ensure uniform exposure, a dosimeter was irradiated along with the seeds for each dose. Before irradiation, the humidity content of the seeds was determined (10%) following to the handbook instructions of the International Seed Testing Association [29].

2.2. Laboratory Experiment #1: In Vitro Seeds Sowing. One part of the total irradiated seeds were germinated under *in vitro* conditions (laboratory). For each gamma dose, three replicates with 10 seeds per replicate were performed under a completely randomized design. All experiments were repeated for twice in parallel, evaluating a total of 60 seeds per treatment. To obtain healthy and uninfected seedlings, the seeds underwent a surface sterilization process. Initially, the seeds were immersed in a 0.5% sodium hypochlorite solution for a duration of 2 min, followed by a 2-min immersion in 95% ethanol. Subsequently, the seeds were thoroughly rinsed with sterile distilled water, and this step was repeated in two steps. After this, the seeds were placed in humid chamber and incubated at 25 ± 1°C for a period of 5 days to facilitate seed germination. Then, all treatments were transferred to a growth chamber at 28 ± 1°C, illuminated with white fluorescent light (1,800–2,000 Lx) at a photoperiod of 16 hr of light and 8 hr of darkness for a duration of 2 weeks, allowing for optimal growth and development of the seedlings.

2.3. Greenhouse Experiment #2: Ex Vitro Seeds Sowing. The *ex vitro* experiment was conducted in the greenhouse of the Facultad de Ciencias Agrarias, Universidad Nacional de San Martín-Tarapoto from May to August 2018. The temperature ranged between 22.9 and 39.6°C, while the relative humidity was between 38% and 83% during the experiment. Seeds were sown in a vertical position with the hilum oriented downward according to Guerrero-Abad et al. [9]. This orientation aimed to facilitate the direction of the root meristem grown, at a depth of 1.5 cm in plastic bags containing 1 kg substrate (soil : sand : organic material, 1 : 2 : 1) with interday irrigation. For each gamma dose, three replicates were tested, with 20 seeds per replicate, under a completely randomized design. All experiments were independently repeated twice, evaluating a total of 120 seeds per treatment. This approach was adopted to ensure the precision of the study.

2.4. Data Collection. For experiment #1 (*in vitro* seeds sowing), germination days and germination percentage during the first 13 days after sowing (DAS) were evaluated, with a seed being considered germinated when it had grown to >2 mm in length. In the experiment #2 (*ex vitro* seeds sowing), emergence days and emergence percentage were evaluated during 30 DAS, considering a plant emerged when it



	7 days	10 days	13 days
0 Gy	$1.73^{a} \pm 0.12$	$2.41^{a} \pm 0.11$	$2.93^{a} \pm 0.06$
500 Gy	$1.46^{ab} \pm 0.02$	$1.71^{b} \pm 0.17$	$1.75^{b} \pm 0.22$
550 Gy	$1.4^{ab} \pm 0.01$	$1.70^{b} \pm 0.09$	$1.75^{b} \pm 0.08$
600 Gy	$1.34^{ab} \pm 0.08$	$1.45^{bc} \pm 0.06$	$1.5^{bc} \pm 0.10$
650 Gy	$1.33^{ab} \pm 0.06$	$1.37^{bc} \pm 0.04$	$1.4^{bc} \pm 0.03$
700 Gy	$1.19^{b} \pm 0.09$	$1.21^{c} \pm 0.08$	$1.22^{c} \pm 0.09$
750 Gy	$1.18^{b} \pm 0.10$	$1.15^{c} \pm 0.03$	$1.15^{c} \pm 0.01$
800 Gy	$1.09^{b} \pm 0.07$	$1.07^{c} \pm 0.01$	$1.08^{c} \pm 0.02$
900 Gy	$1.03^{b} \pm 0.10$	$1.00^{c} \pm 0.02$	$1.03^{c} \pm 0.00$

(a) (b)

FIGURE 1: Gamma irradiation effect on *P. volubilis* seeds during germination and root tip growth. (a) Necrosis on principal root and development of lateral roots. (b) Root tip growth recorded after 7, 10, and 13 days of germination. Values in columns (mean ± SD of 60 germinating seeds) with the same letter are not significantly different (Tukey's test, $p < 0.05$).

had sprouted above the substrate. Additionally, we estimated the percentage of survival according to Rizwan et al. [30]:

$$\text{Survival}(\%) = \frac{\#\ \text{of survival plants}}{\#\ \text{of total irradiated seeds}} \times 100. \quad (1)$$

Other parameters, such as total height, root length, stem diameter, fresh weight, dry weight, number of leaves per plant, leaf area, and morphological alterations (apical meristem, leaf shape, stem fusion, and chlorosis), were registered 30 days after emergence (DAE). Leaf area values were calculated from scanned full leaves using ImageJ software (https://imagej.nih.gov/ij/), and pictures of leaves were taken using an Epson Perfection V370 Scanner.

2.5. Statistical Analysis. The results of the two independent experiments exhibited minimal numerical differences, with no significant statistical differences observed ($p > 0.05$) for each recorded parameter. Consequently, the data from both experiments were merged for further analysis. Normality and homogeneity assessments were performed on the measured variables in this study using the Shapiro–Wilk and Levene's tests, respectively. In cases where the data do not conform to the assumptions, data transformation was employed: an arcsine transformation was utilized for percentages, whereas square root or logarithmic transformations were applied as needed. Subsequently, analysis of variance and comparison of means were performed using Tukey's HSD test ($p < 0.05$). Categorical variables were analyzed using the chi-square test. The results are presented in the original unit of measurement for better interpretation and understanding. A logistic regression between radiation doses and mortality was calculated to estimate the optimum LD_{50} using a generalized linear model. The data were analyzed using R 4.3.0 [31].

3. Results

3.1. Laboratory Experiment #1

3.1.1. Gamma Irradiation of Plukenetia volubilis L. Seeds Does Not Affect Germination but Affects Root Tip Growth. Germination of *P. volubilis* seeds was not affected by any gamma radiation doses (0-control, 500, 550, 600, 650, 700, 750, 800, 900 Gy; see *Supplementary 1*). Similarly, gamma rays did not affect days to germination, which started between 5 and 6 days in all radiation doses (*Supplementary 2*).

As gamma radiation was increased, a significant impact on the growth of the main root of the *P. volubilis* embryos was evident, along with necrosis of the cortical cells and the emergence of new lateral roots (Figure 1(a)). Likewise, a decrease in root tip growth at 7, 10, and 13 days of germination was observed, with significant differences among treatments. Figure 1(b) shows a trend of slowing and eventual stagnation of growth at the dose of 500 Gy, where root size was affected in 40.3% compared to the control plants. The 900 Gy dose increased this reduction to 64.8% at 13 days after germination.

3.2. Greenhouse Experiment #2

3.2.1. The Normal Vegetative Growth and Development of Plukenetia volubilis Are Affected by Gamma Irradiation. There was no significant difference in emergence percentage

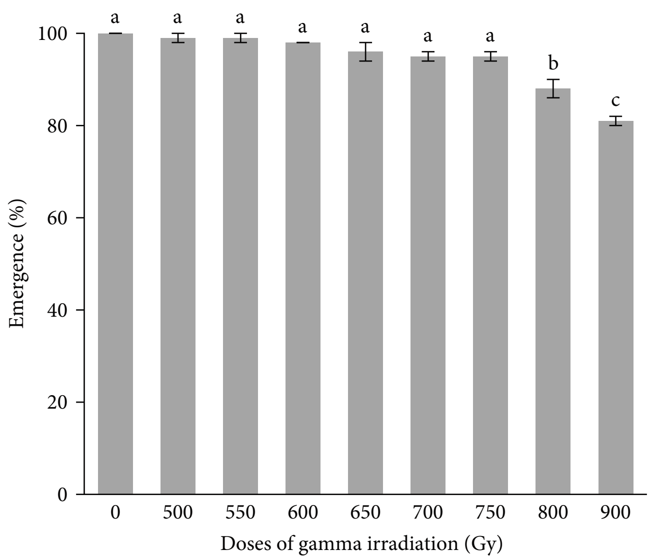


Figure 2: Gamma irradiation effect on *P. volubilis* seeds emergence. Values in columns (mean ± SD of 120 emergence seeds) with the same letter are not significantly different (Tukey's test, $^*p<0.05$).

among treatments lower or equal to 750 Gy. However, the 800 and 900 Gy doses resulted in a significant decrease in emergence percentage (Figure 2). The time of emergence had different patterns between 9 and 21 days for each treatment, showing a trend to extend days for seed emergence above the 500 Gy dose (*Supplementary 3*).

Gamma irradiation showed a clear effect on the size and weight of *P. volubilis* seedlings. A significant reduction ($p \leq 0.05$) in seedling height was evident when comparing the control plants (0 Gy) to the treated plants exposed to gamma radiation doses ranging from 500 to 800 Gy. Although no significant differences in seedling height were observed between intermediate (550 and 600 Gy) and higher (700, 750, and 800 Gy) doses, a decreasing trend was observed (Figure 3(a)). Also, a significant decrease was observed in the root length from the 650 Gy treatment, with no significant differences in root length between the control plants (0 Gy) and plants treated with lower doses (500, 550, and 600 Gy) (Figure 4(b)).

Seedling fresh weight in the 500–800 Gy group exhibited a significant decrease compared to the control treatment, while no significant differences ($p \leq 0.05$) were observed between the intermediate doses (550 and 600 Gy, 650 and 700 Gy, and 750 and 800 Gy), as shown in Figures 3(c) and 3(f). The dry weight of seedlings followed a similar trend to the fresh weight. We observed a significant decrease in the dry weight of seedlings ($p \leq 0.05$) in the 500–800 Gy group compared to the control treatment. In contrast, no significant differences were found among the intermediate doses (500 and 600 Gy, 650 and 700 Gy, and 750 and 800 Gy, Figure 3(d)). Finally, phenotypic observations were made on representative full plants under different treatments of gamma radiation (Figures 4(e) and 4(f)).

Gamma irradiation also affected leaves of seedlings. The number of leaves exhibited a significant difference between the control plants (0 Gy) and doses exceeding 500 Gy ($p \leq 0.05$), but no significant difference was observed between these treated plants (650, 700, and 750 Gy, Figure 4(a)). Similarly, leaf area per seedling was significantly different ($p \leq 0.05$) between the control treatment and the higher doses in all the treatments, observing a significant decrease in leaf area when the irradiation dose increased (Figure 4(b)). Also, the increasing presence of chlorotic leaves was associated with higher doses of gamma irradiation ($p \leq 0.05$), showing an increase in the percentage of chlorotic leaves in treated plants compared to control plants (Figure 4(c)).

Concerning the survival rate, a significant decrease was observed when the control plants (0 Gy) were compared to treated plants with more than 600 Gy (Figure 5(a)). The estimation of the LD_{50} in the gamma-irradiated samples in this study was determined by analyzing the resulting logistic regression of the death rate, as shown in Figure 5(b). In general, a lethal effect was evident at 900 Gy for all the variables described above, and the LD_{50} of the dosimetry assay was 618.78 Gy.

3.2.2. Organ Development Patterns Are Modified by Gamma Irradiation. Negative effects related to loss of apical dominance were significant, with an increase ($p \leq 0.05$) in Gy-treated plants as the dose was increased, with no significant differences in the intermediate doses (550 and 600 Gy and 650 and 700 Gy). The most pronounced negative effects were observed at higher doses (750 and 800 Gy, Figure 6(a)).

The fused shoot rate was significantly different ($p \leq 0.05$) between 0 Gy and doses above 550 up to 800 Gy. It was observed that the 750 Gy treatment presented the highest fused shoot rate (%) when compared to the control plants (Figure 6(c)). Regarding this issue, the observed differences in shoot rate as a function of radiation could be a consequence of the decrease in biomass production in the 500, 600, 650, and 800 Gy treatments described in the previous section. Leaves showed abnormal shapes as a common result of all radiation doses (Figure 6(f)), with smaller, contorted, or absent lateral leaflets. We observed a significant increase in the percentage of abnormal leaves in the treated plants (500–800 Gy treatments) compared to control. However, there was no significant difference among the treatments (Figure 6(e)). Differences in organ development patterns resulting from gamma radiation are shown in Figures 6(b), 6(d), and 6(f).

4. Discussion

In this study, we evaluated the effect of gamma irradiation (^{60}Co) on seed germination and seedlings growth of *P. volubilis*. Previously, a study related to the effect of chemical mutagens in this plant indicated that the use of doses of 3.0% of EMS reduces seedling emergence by up to 50% and produces phenotypic alterations [28]. However, as far as we know, our study is the first to focus on exploring the impact of physical mutagens in *P. volubilis*.

In the *in vitro* experiment, *P. volubilis* seeds treated with different doses of gamma radiation showed no difference in

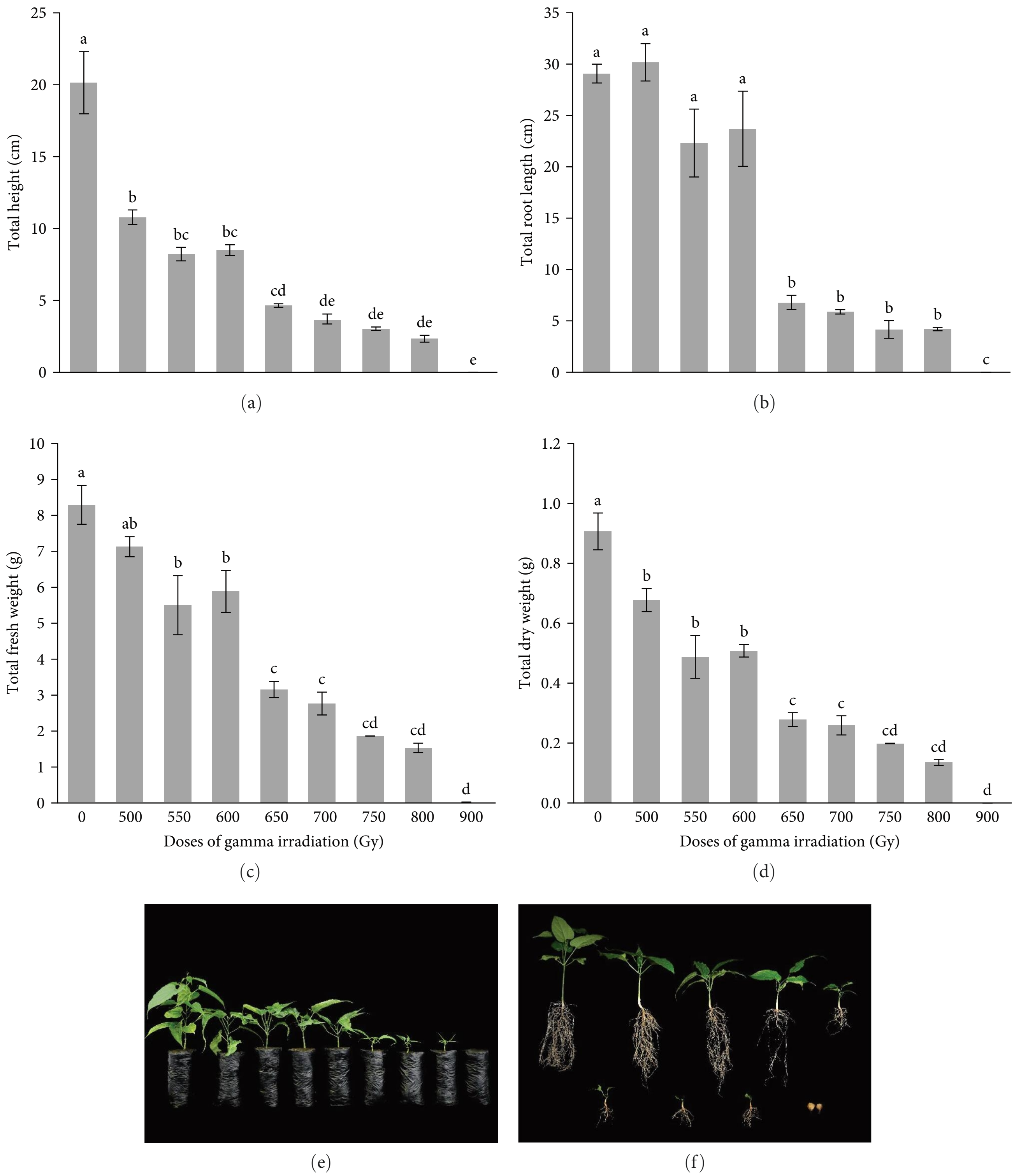


Figure 3: Effect of gamma irradiation on size and weight of *P. volubilis* seedlings. (a) Effect on total height. (b) Effect on total root length. (c) Effect on total fresh weight. (d) Effect on total dry weight. (e) and (f) Illustration of plant morphology. Values in columns (mean ± SD of 120 seedlings) with the same letter are not significantly different (Tukey's test, $p < 0.05$).

germination percentage compared to the control (all had values close to 100%, *Supplementary 1*). Nonetheless, a significant decrease in root tip growth was observed from the 500 Gy dose, becoming particularly noticeable from day 10 of germination (Figure 1). In contrast to our results, several studies show that germination is affected by increasing irradiation dose. Songsri et al. [32] showed that germination percentages in *Jatropha curcas* reduced from 82.5% with 0 Gy (control) to 4.0% at 100 Gy, while irradiations higher than 600 Gy resulted in 0%. Similarly, Zhang et al. [33]

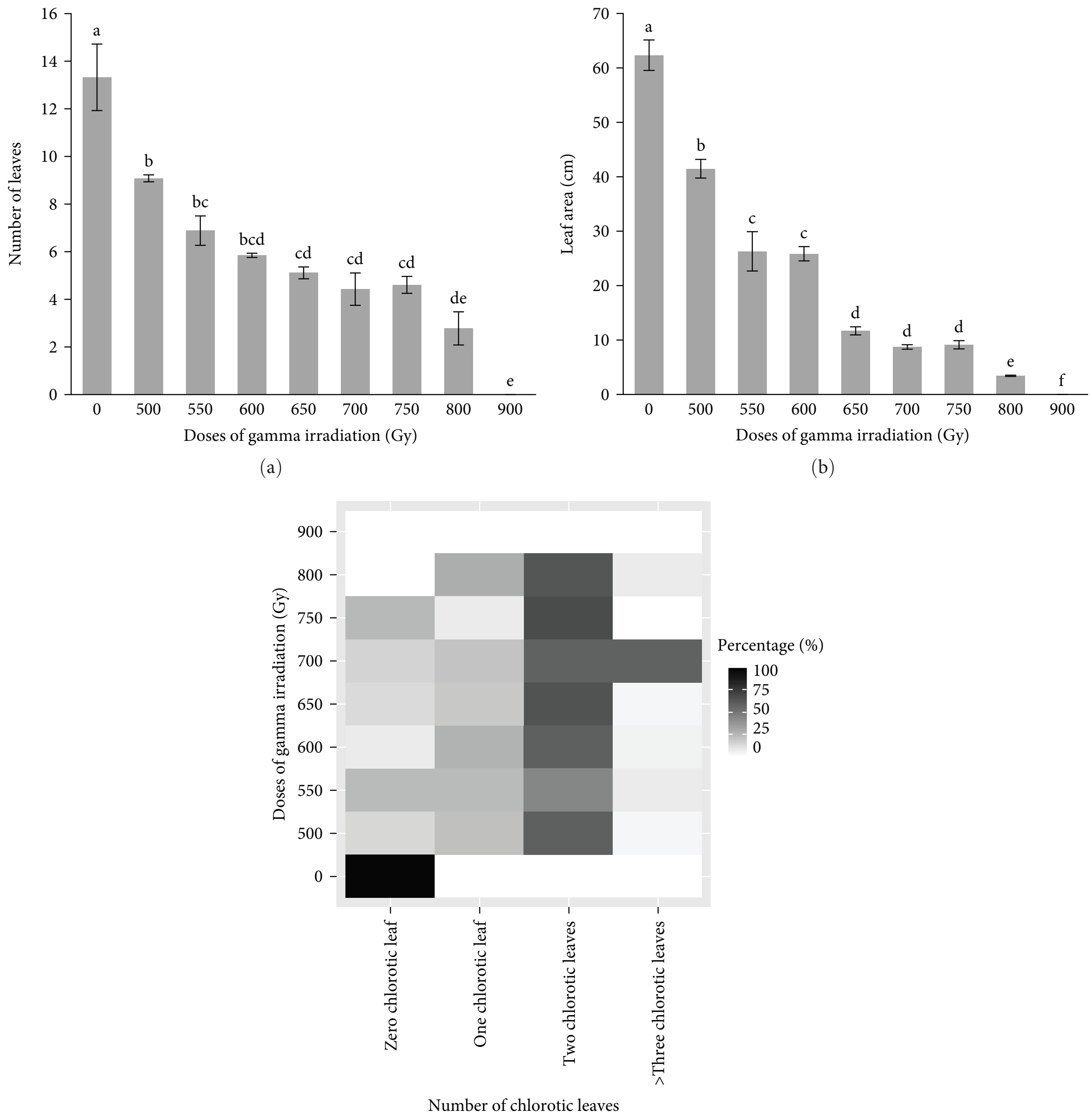


Figure 4: Effect of gamma irradiation in leaves of *P. volubilis* seedlings. (a) Number of leaves. (b) Leaf area. (c) Effect on chlorosis leaves rate. Values in columns (mean ± SD of 120 seedlings) with the same letter are not significantly different (Tukey's test, $p < 0.05$).

reported that the application of radiation doses higher than 600 Gy results in the total decrease in wheat seed germination percentage (0%). Generally, the decrease in germination percentage with high amounts of radiation is related to a delay in nutrient transport to the embryo, a decrease in respiration rate, and a decrease in the number of internal regulators [34]. However, in our case, the seed coat thickness of *P. volubilis* could have provided the seed with a certain level of protection against radiation in the first stages of development [35]. It is possible that this protective effect is also present in the emergence of the seedlings, as a significant decrease in emergence percentage was only noticed at doses of 800 and 900 Gy (Figure 2).

Seeds that germinate even at high doses of radiation, frequently, exhibit short, thickened, and poorly defined roots, leading to less vigorous plants. In our case, the results showed a significant decrease in root tip length. According to Sudhakaran [36], this phenomenon can be caused by a

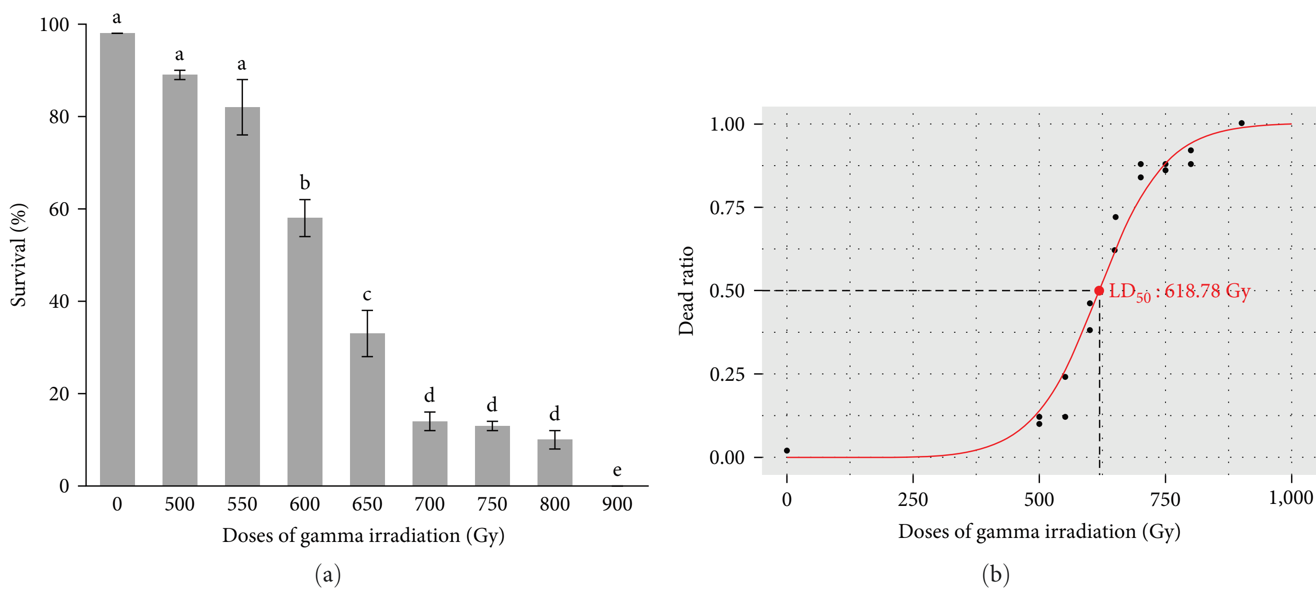


FIGURE 5: Effect of gamma irradiation effects on survival and mortality of *P. volubilis* seedlings. (a) Survival rate. (b) Median lethal dose (LD_{50}) determination. Values in columns (mean ± SD of 120 germinating seeds) with the same letter are not significantly different (Tukey's test, $p < 0.05$).

reduction of the mitotic index of the root tip cells of seedlings treated with high doses of gamma radiation. In addition, we observed necrosis in cortical cells of the primary root and subsequent development and death of lateral roots (Figure 1). The reason why the seeds cannot germinate or survive for more than a few days following gamma irradiation may be related to metabolic abnormalities in the seeds [37].

Ionizing radiation is known to have diverse effects on plant's morphology, anatomy, biochemistry, and physiology depending on the dosage [38]. In our greenhouse assays, we found a significant decrease in plant height in all radiation treatments (Figure 3). These findings concur with those of Kaur et al. [39] and Albokari et al. [40], who found a reduction in plant height under doses from 20 to 100 Gy and 50 to 500 Gy in *Calendula* and wheat, respectively. However, they are contrary to Shah et al. [41], who reported 64.1% and 51.85% increment in plant height with 300 and 500 Gy in *Crotalaria saltiana*. On the other hand, Di Pane et al. [42] reported that the roots of wheat and triticale cultivar seedlings were more affected by gamma radiation than the aerial portion. This affirmation contrasts with our results, which showed that the aerial sections of *P. volubilis* were more sensitive to gamma radiation than the roots, because the significant differences in root length between the control plants and treated plants were only noticeable at 650 Gy or higher doses.

Decrease in plant height caused by irradiation can be attributed to various factors. Kiong et al. [34] suggested that the sensitivity of plants becomes greater after irradiation at high doses, which could lead to a decrease in the level of endogenous growth hormones, such as cytokinins, due to decomposition or inadequate synthesis. Similary, Walther [43] and Jan et al. [44] have also indicated that the decrease in the plant height might be related to change in plant hormones, primarily auxins, and DNA biogenesis. The changes could adversely affect the process of cell division and cell elongation as a consequence of the mutagenic treatment. However, it's worth noting that despite these observations, a phenomenon known as hormesis has been described, involving the stimulation of various biological processes, including accelerated germination and increasing shoot and root development, in response to low doses of radiation [45].

The decrease in plant biomass following radiation exposure has been documented in various cultivated species, including corn [46], whip beans [47], rice [48], and wheat [49]. The reduction in growth attributed to radiation stress could be a result of decreased mitotic activity in meristematic tissues and a decline in moisture content [50]. In our experiment, the trend in dry weight of seedlings mirrored that of fresh weight in both shoots and roots (Figure 3). Majeed et al. [21] reported similar findings in *Lepidium sativum* and noted that both fresh and dry weights increased at the lowest radiation exposure (0 Gy) and decreased as the dose increased (until to 800 Gy). Similarly, Kon et al. [47] observed a tendency for the shoot dry weight of *Vigna unguiculata* to decreased after exposure to high doses (up to 800 Gy) of gamma radiation.

In terms of leaf characteristics, our results demonstrated a significant reduction in leaf area and number, along with an elevated proportion of chlorotic leaves, attributed to varying radiation doses (Figure 4). Asare et al. [51] reported similar results, showing a decrease in the size and number of okra leaves as gamma radiation dosage increased up to 1,000 Gy (except in 400 Gy). Additionally, Wi et al. [52] observed that subjecting pumpkin plants to a high dose of gamma radiation (1 kGy) could disrupt the equilibrium of plant development regulators, leading to leaf curling and yellowing. This

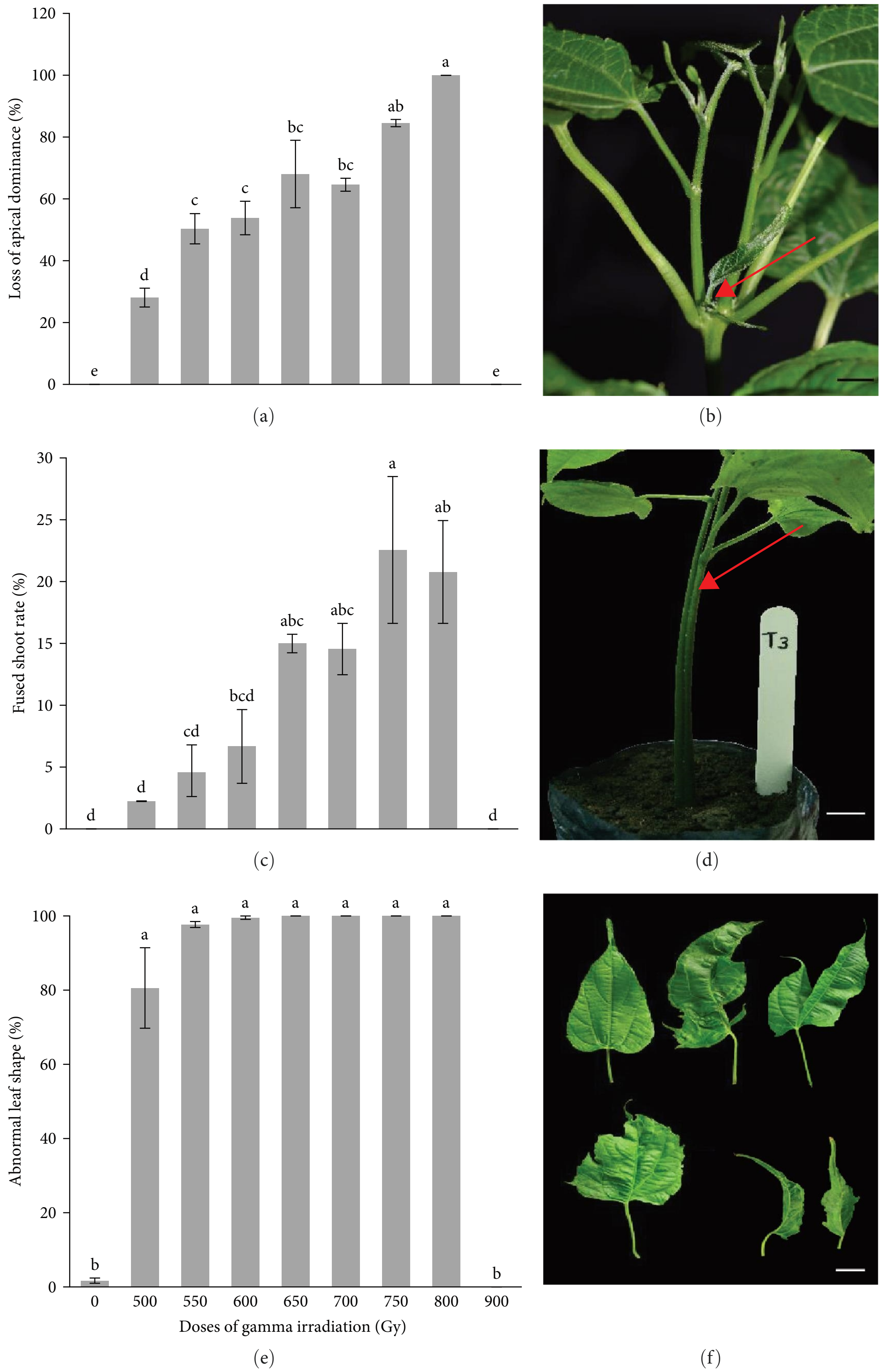


Figure 6: Changes in normal development patterns of different organs of *P. volubilis* seedlings induced by gamma irradiation. (a) Loss of apical dominance. (b) Plant with axillary shoot activation. (c) Fused shoot rate (%). (d) Plant with two fused stems (arrow). (e) Abnormal leaf shape (%). (f) Different abnormal leaf shapes. Values in columns (mean ± SD of 120 seedlings) with the same letter are not significantly different (Tukey's test, $p < 0.05$).

impact on leaf parameters could have significant implications for plant physiology, potentially leading to suppressed plant development and decreased yield. Primarily, this could be attributed to potential disruptions in photosynthetic pigments, potentially resulting in a diminished photosynthetic capacity [53, 54].

Significant changes in organ development were observed, including the loss of apical dominance, an increase in fused shoots, and a more abnormal leaf shape, after radiation exposure (Figure 6). In this context, Majeed et al. [55] and Wang et al. [56] suggested that these growth abnormalities could be attributed to the ionization of water within cells and the subsequent formation of reactive oxygen species and free radicals. These reactive entities can interact with other cellular molecules, potentially leading to detrimental functional and structural alterations. According to Nurmans et al. [57], gamma radiation can also induce abnormal development of leaflets, flowers, and pollen grains.

In general, high doses of gamma radiation can lead to a decrease in the survival rate in various crops, including wheat [49], rice [48, 58], maize [37], and among others [44]. Our findings suggest a correlation between increased radiation dose and a reduction in the percentage of survival. This decline can be linked to high genomic instability resulting from DNA damage, which impacts the G2/M phase of somatic cell division [59]. Furthermore, utilizing the survival percentage, we determined that the LD_{50} for *P. volubilis* seed was 618.8 Gy (Figure 5). This value was higher than that reported for *Manihot esculenta* (27.5 Gy) [60], two varieties of *Vigna subterranea* (160 and 148 Gy) [61], *Capsicum annuum* (178 Gy) [62], and *Oryza sativa* MR219 (450 Gy) [63]. However, it was lower than the value reported for *Echinochloa* spp. (800 Gy) [64]. Finally, the dosage of 900 Gy in this investigation demonstrated a lethal effect on *P. volubilis* seeds. Regarding this, it is important to note that different plant species vary widely in their sensitivity to gamma radiation, a trait that can be influenced by genetic factors among others [65, 66].

5. Conclusions

The study revealed that gamma radiation has a dose-dependent impact on the development and morphology of *P. volubilis* seedlings. Exposure to gamma radiation at dosages between 500 and 900 Gy resulted in inhibitory effects on seed emergence, size, weight, and seedling survival, as well as significant changes in organ development patterns. Furthermore, the LD_{50} was established at 618.78 Gy. These findings contribute to our understanding of the effects of gamma radiation on *P. volubilis*, a plant with significant potential for various applications, including nutrition, agriculture, and pharmaceuticals. The observed changes in seedling growth and development suggest that gamma irradiation can be used as a tool to induce genetic variability in *P. volubilis*, which could be valuable for future breeding programs aimed at improving agronomic traits and productivity in this species. Further research is needed to explore the specific genetic changes induced by gamma radiation and their potential applications in *P. volubilis* cultivation and breeding.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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Supplementary Materials

Supplementary 1. Germination percentage after exposure to different gamma radiation doses.

Supplementary 2. Germination percentage in *P. volubilis* seeds treated with different doses of gamma radiation.

Supplementary 3. Emergence percentage in *P. volubilis* seeds treated with different doses of gamma radiation.

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