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Effect of Low Gamma Irradiation on the Germination and Morphological Characteristics of Broad Beans (*Vicia faba* L.), Mung Beans (*Vigna radiata* L.), and Peas (*Pisum sativum* L.) Seedlings

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Abstract

Understanding the effects of ionising radiation (IR) on plants has been a major focus of research. Acute high-dose effects are well-documented and understood (mainly through laboratory testing). Lower doses, on the other hand, are less understood, as low dosage research is controversial, and there are only a few studies that use low and ecologically relevant IR levels, particularly those conducted under controlled conditions. The effect of low gamma radiation was investigated in this study using *Vicia faba* L., *Vigna radiata* L., and *Pisum sativum* L. Healthy and viable seeds of these plants were irradiated with varying doses of gamma radiation (Cs^{-137} source) and sown under controlled environmental conditions. The doses/dose rates used were within the scope of the International Commission on Radiological Protection's Derived Consideration Reference Level (DCRL) for these groups of plants (1 - 10 $mGy \cdot d^{-1}$), so this study tested this DCRL. Observations were made on certain germination parameters and growth traits like germination percentage and rate, shoot and root length, seed weight, number of leaves, wet and dry biomass, plant height, leaf chlorophyll content, and leaf area. In the germination phase, the doses employed in this experiment did not affect the seeds' weight, germination percentage, and rate, but there were some interesting effects on the root and shoot length; as all irradiated groups performed better than the control group (particularly the 16.2 mGy and 48.5 mGy dose in *V. radiata* and *P. sativum*, while the 1070 mGy dose had the highest value in *V. faba*). However, the plants were able to compensate for the effects observed in the germination phase and by the end of the experiment, there were no statistically significant effects (at 0.05 p level) in all the morphometric parameters studied; the visible organs appeared normal, and growth rate was normal. This study, therefore, concludes that the DCRL used to protect these groups of plants from the effects of IR (1 - 10 $mGy \cdot d^{-1}$) is appropriate and present regulation appears to be suitable.

Keywords

Ionising Radiation, Morphometric Parameters, Germination Parameters, ICRP DCRLs

1. Introduction

Ionising radiation (IR) is a form of radiation that contains enough energy to displace electrons in an atom's orbit and cause it to become ionised [1]. Examples include swift sub-atomic particles like neutrons, beta particles, etc., as well as the most notable ones which are X rays and gamma rays. Unlike X rays, gamma radiation penetrates easily into the matter and has greater ionization potential because they retain more energy. They can occur naturally as cosmic rays from galaxies and solar radiations, from decaying nuclei of radioactive materials, which are majorly isotopes of uranium, radium, cesium, cobalt, potassium, and lead [2]. Artificial sources however include manmade gamma emitters generally used for industrial and medical purposes (e.g., ^{60}Co or ^{137}Cs), testing of nuclear weapons, uranium mining, fallouts from nuclear plant operations, and nuclear accidents as in the case of Chernobyl (1986) and Fukushima (2011) which are the two most devastating nuclear disasters in history [3] [4].

Living organisms, therefore, may be exposed to the natural background and anthropogenic sources [5], causing disruption in the normal processes and functioning of the cell [6] [7] [8], and due to plants' static lifestyle, they cannot avoid the majority of these environmental stresses. IR can disrupt some regular patterns of development in green plants, as well as other processes that can cause morphological changes in cells, which are generally expressed as morphogenetic traits. However, the effect of these radiations depends on several factors, some of which are related to certain characteristics of plants (e.g., stage of development, physiological conditions, species, varieties), and some are linked to the radiation characteristics such as dose, quality and length of exposure [9], as well as various environmental factors [10] [11].

Presently, the impact of IR on plants is better known at acute high doses because: in the immediate wake of nuclear disasters (e.g., Chernobyl and Fukushima), many field investigations have been conducted; there have also been significant controlled field studies using point sources; so many controlled laboratory experiments have been done using these acute high doses (most of which were used to induce mutation for crop improvement purposes) [12]. In these studies, various plants react differently to varying doses of gamma irradiation, where lower doses (<100 Gray) generally stimulate the growth and development of plants while lethal/inhibitory effects were observed at the higher doses (>200 Gray); as summarised in **Table 1**.

Even though some of these doses categorized as "low" above produced stimulatory effects on plants in most cases, they are rarely encountered in real-life situations, hence, so much less is known about the effect of chronic low doses (especially in controlled conditions). This is a concern because the chronic low dose rates are more environmentally relevant as they are the ones usually encountered in real-world situations like aftermaths of nuclear accidents e.g., Chernobyl (excess of 1 mGy/h [4]), and Fukushima.

One of the few studies investigating the effect of field-relevant dose rates was that of [23]. This is a very recent study where the transgenerational trend in five generations of *Arabidopsis thaliana* (Thale cress) was investigated using soil contaminated with ^{137}Cs at an environmentally relevant dose rate (35 $\mu Gy \cdot h^{-1}$) (0.035 $mGy \cdot h^{-1}$). Some morphological parameters such as leaf area and root length were considered along with some developmental and physiological effects. First, there were no transgenerational trends observed; also, there were no significant differences in the leaf area and root length between treatments and between generations. While there were some effects in the physiology (e.g., there was a significant reduction in the leaves' percentage of methylated DNA in the irradiated group of the first two generations), these weren't things that could negatively impact generations and populations.

To understand the potential levels of harmful and or deleterious effects of these radiations on plants, international organizations often use the findings from studies on the effects of irradiation on living systems to inform their radiation-related policies, guidelines, regulations, and practices worldwide. The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) suggested a dose rate of 10 mGy/d for plant radioprotection as a benchmark in 1996, although various species (especially different groups of plants) are likely to respond differently to this threshold. To account for this, the International Committee for Radiological Protection (ICRP) developed the use of Reference Animals or Plants (RAPs), which was augmented with Derived Consideration Reference Levels (DCRLs) for each RAP [24] [25]. DCRLs are bands of dose rates where one might start to see deleterious effects for individual RAP. However, there is still limited data from many taxa and this gap has led to the use of extrapolation techniques in making radiation-related recommendations or decisions and therefore is associated with substantial uncertainty [12] [24] [25].

The DCRLs for the environmental protection of each RAP are presented in **Figure 1**. For grass, the ICRP's plant DCRLs are 1 - 10 $mGrayd^{-1}$ (in green), and this provides a reference for herbaceous higher plants.

Due to the gap identified above, this study will be testing/probing the ICRP DCRLs, making use of *Vicia faba* L., *Vigna radiata* L., and *Pisum Sativum* L. as reference plants. Hence the dose/dose rates used for this study fall within the

Table 1. Overview of the effects of acute gamma radiation on the key morphometric parameters in plants.

Plants	Doses	Parameters	Low (5 - 20 Gy)	Medium (20 - 100 Gy)	Mid-High (100 - 200 Gy)	High (>200 Gy)	References
Triticum aestivum L.	100 - 400 Gray	Germination percentage		↔		↔	[13]
		Germination rate		↓		↓	
		Shoot length		↑		↓	
		root and shoot weight		↑		↓	
		root length		↓		↓	
Triticum aestivum L.	600 - 3000 Gray	germination percentage				↓	[14]
		germination rate				↓	
		root length				↓	
Lathyrus chrysanthus B.	50 - 250 Gray	germination percentage		↑		↑	[15]
		seedling height		↑		↓	
		root length		↑		↓	
		fresh weight		↑		↓	
		chlorophyll content				↓	
Glycine max L.	5 - 2560 Gray	germination percentage	↓	↓		↓	[16]
		mean germination time	↑	↑		↑	
		1st day of germination	↔	↑		↑	
		last day of germination	↑	↑		↑	
		seedling height	↓	↓		↓	
		root length	↓	↓		↓	
Abelmoschus esculentus L.	400 - 1000 Gray	seedling survival				↑ - ↓	[17]
		plant height				↑ - ↓	
		number of branches				↑ - ↓	
		number of leaves				↑ - ↓	
		stem diameter				↑ - ↓	
		leaf length				↑ - ↓	
		leaf width				↓	
Vigna radiata L. (TARM1 var.)	50 - 800 Gray	germination percentage		↑		↓	[18]
		shoot length		↓		↓	
		seedling length		↓		↓	
		root length		↔		↔	
Pisum sativum L. (Sahiwal matar var.)	50 - 200 Gray	germination percentage		↓	↓		[19]
		1st day of germination		↑	↑		
		seedling survival		↓	↓		
		plant height		↑	↓		
		root length		↑	↑		
Vicia faba Cv saraziri	25 - 120 Gray	germination percentage		↔	↔		[20]
		germination rate		↓	↓		
		plumule length		↓	↓		
		radicle length		↓	↓		
Helianthus annuus L.	100 - 900 Gray	germination percentage			↑	↓	[21]
		plant height			↑	↓	
		root length			↑	↓	
		dry biomass			↑	↓	

Continued

Capsicum annuum L.	2 - 16 Gray	germination percentage stem length stem diameter leaf area	↑ ↑ ↑ ↑		[7]
Phaseolus vulgaris L.	0.3 - 100 Gray	leaf area leaf weight	↑	↓ ↑	[22]

↔ represents no effect; ↑ represents stimulatory effect; ↓ represents lethal/adverse effect; ↑-↓ represents initial stimulatory effect followed by adverse effect as doses increase. This is particularly common with studies in which their lowest doses are categorised as high.

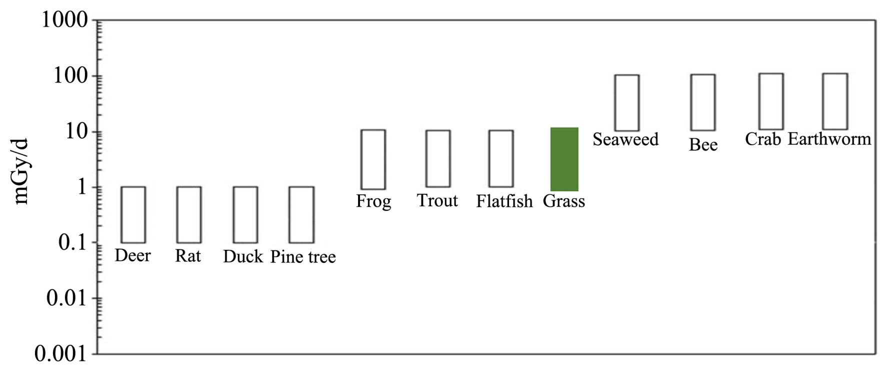

Figure 1. DCRLs for each RAP [24] [25] [26].

DCRL for these groups of plants (1 - 10 $mGy \cdot d^{-1}$). There are various reasons to investigate the appropriateness of this DCRL for herbaceous higher plants. First, there is still a lot of debate over the environmental impacts of radiation, for example, at places like Chernobyl and Fukushima [27], and the creation of RAPs revealed how little is understood about the effects of radiation on plants compared to humans or other animals. This remains true and could be partly due to the difficulties associated with studying radiation effects on plants; for instance, the radiosensitivity of certain above or below-ground organs (e.g., roots, rhizome), difficulty in understanding the interaction between radiation and other stressors/confounding factors [27] [28]. Secondly, many places on the planet have a naturally elevated ambient IR; for instance, prolonged exposure in Ramsar, Iran, has been found to affect plants at dose rates up to 4 $mGy \cdot h^{-1}$ [29]; this is approximately 10 times the world average background [30] and falls within sensitive plants' DCRL. At Fukushima, effects have also been observed at relatively low dosage rates [31], and in an era where decarbonisation is vital for reducing anthropogenic emission of carbon dioxide, global investment in nuclear energy has increased; there are currently 444 nuclear reactors in operation, with 51 more under construction [32]. Therefore, the balance between environmental gains and hazards which these IR standards strive to attain should be scrutinized extensively. Finally, several of the studies used in the development of the DCRLs used field settings with dose rate variations as the foundation for their study. Linking contamination with effect in these kinds of settings is simply one sign of causation as there may be other confounding factors.

Therefore, this research will contribute to the literature on the impacts of low doses of gamma radiation on plants, by providing data on its effect on germination and morphological parameters of *Vicia faba* "Aquadulce Claudia", *Vigna radiata*, and *Pisum sativum* "Terrain". These three plants are legumes, and they play crucial roles in the agricultural and global food systems' long-term viability. They also help to improve soil fertility, safeguard the environment and have great nutritional value [33]-[38]. Therefore, in light of the rising impact of global warming, as well as population increase, both of which are already posing serious threats to global food security, particularly legumes [36] [39] [40] [41] [42], it is very crucial to understand the effect of low gamma doses on these groups of plants. They can also be easily grown and have a two-to-four-month life cycle, making them ideal for this research.

2. Methodology

2.1. Gamma Irradiation

Healthy and viable seeds of *Vicia faba*, *Vigna radiata*, and *Pisum sativum* were obtained from a reliable source. After which, they were submitted for irradiation. Irradiation was performed using a Cesium-137 (^{137}Cs) gamma source at the irradiation facility, at the gardens and grounds, University of Stirling. The seeds were exposed to four treatments (Low, Mid, Mid-High, High), while un-irradiated seeds served as the control. The control seeds, however, were in the same room as the other seeds but were shielded from the radiation source in a separate section (see **Figure 2**). **Table 2** and **Figure 2** show the breakdown of the irradiation process.

The seeds stayed 45 days in the irradiation facility and the seeds were irradiated for 1077.5 hours during that time.

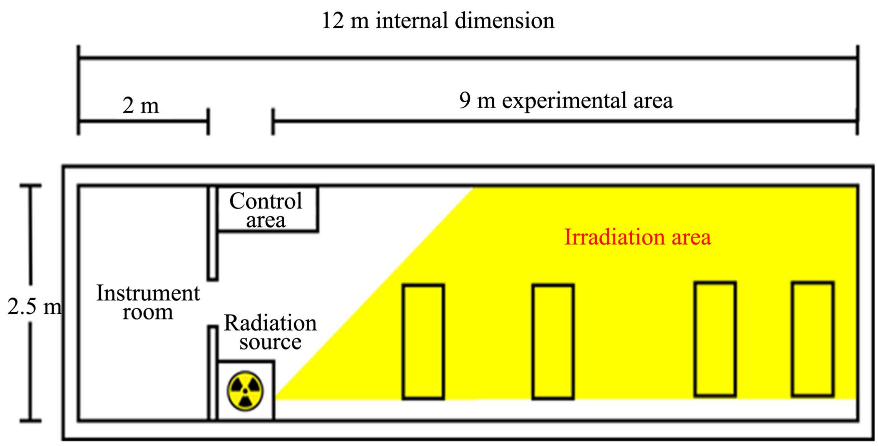

Figure 2. Illustration of the radiation pathway through the irradiation facility from the ^{137}Cs source.

Table 2. Dose rates and accumulated doses used in the experiment.

Treatments	Distance from Source (m)	Dose Rate (mGy/h)	Accumulated Dose (mGy)
Control	-	0.0004	0.431
Low	9	0.015	16.2
Mid	4.5	0.045	48.5
Mid-High	2	0.4	431
High	1	0.9	1070

2.2. Experimental Design

A randomized block design was used, with four treatments (except the broad beans, have three), and each had three replicates. The un-irradiated seeds served as the control. Ten seeds were sown for each experimental group, and sampling followed a random approach. The experiment was done in two phases.

2.2.1. Germination Phase

The experiment followed a completely randomised design with three replicates of 10 seeds from each treatment, and the standard germination test was performed on irradiated seeds according to the International Seed Testing Association methodology [43]. Seeds were immersed for 12 hours in warm water before being uniformly dispersed in 1000 ml plastic dishes on a double layer of tissue paper moistened with 5 ml of distilled water. Seeds were considered germinated when radicle protrusion ≥ 2 mm was observed, and the paper was kept moist by regular watering. After 7 days, measurements were taken, and the following parameters were calculated:

1) Germination percentage using:

Equation (1): Germination percentage [44]

$$\text{Germination\%} = \frac{\text{Total number of normal germinated seeds}}{\text{Total number of seeds}} \times 100\% \quad (1)$$

2) Mean germination rate (MGR) using:

Equation (2): MGR (suggested by [45])

$$\text{MGR} = \frac{N_1T_1 + N_2T_2 + \cdots + N_nT_n}{N_1 + N_2 + \cdots + N_n} \quad (2)$$

where T = Number of days

N = Number of seeds germinated on a given day

3) Shoot and root development: The shoot and root lengths were measured in centimetres using a Westcott TC-385 flexible curve ruler at 14 DAS.

2.2.2. Polytunnel Experiment (Growth Phase)

This was done in one of the polytunnels in the University of Stirling gardens and grounds. Seeds were sown in trays, and a randomised block design with three blocks (A, B and C) was used for the experiment. For each plant in each block, every treatment had 10 seeds sown in inserts (seeds were weighed before sowing), and they were watered regularly following a bottom watering approach to ensure all plants get an equal amount of water and also to prevent fungal infestation. They were monitored closely, and all the seedlings were transplanted into larger pots as required (as seen in Figure 3 and Figure 4).

Sampling was done on the 15th, 30th and 45th DAS for all the parameters. A periodic sampling was adopted to monitor the growth rate of these plants and to see if these plants reacted differently at certain intervals within the growth phase. Six plants were randomly selected within each treatment (two from each block), and the following parameters were studied:

1) A ruler was used to measure the plant's height (in centimeters) from the cotyledon to the apical head.

2) Manual counting was used to record the number of leaves per plant.

3) Leaf area of a mature leaf that has recently fully grown (the fourth leaf from the plant top) was measured using the Image J software (Java version 1.8.0_172).

4) Plant fresh weight (grams) was measured using a Denver XP-300 digital scale.

5) After being cleaned with distilled water and dried in a vented oven at 70˚C for 24 hours, the dry weight (grams) of the plants was also determined.

Figure 3. Experimental set-up 10 DAS.

Figure 4. Experimental set-up 25 DAS.

6) Leaf chlorophyll content was measured from the fourth full mature upper leaf using a SPAD-502 plus digital chlorophyll meter. These readings gotten from the chlorophyll meter served as relative values for chlorophyll content.

2.3. Data Analysis

One-way analysis of variance (ANOVA) was used to analyse data from the germination phase of the experiment. This was followed by Duncan's multiple range post-test, to compare the effect of the different doses on each of the plants. Two-way ANOVA was used for the second (growth) phase, where the doses and blocks served as the factors. Tukey post hoc test was then used to compare the difference of the means of all tested parameters in these plants at the 0.05% level of probability. SPSS software version 27 was used to carry out these analyses.

3. Results

3.1. Effect of Gamma Radiation on Irradiated Seeds' Weight

Upon removal of the seeds from the irradiation facility, wrinkling of the seeds was noticed as doses increased especially with the peas, and this suggested that irradiation might have a direct impact on seed quality. To test this, 10 seeds per treatment (for each replicate) were weighed, and the results are summarized in Table 3.

According to ANOVA, the doses of gamma irradiation used in this experiment had no significant effect on the seeds' weight of these three plants as the p values were all > 0.05 ($p = 0.505$, $p = 0.676$ and $p = 0.110$). Tukey's post hoc test showed that the mean followed by different letters is significantly different at the $p \leq 0.05$ level.

3.2. Effect of Irradiation on Germination Percentage

Data presented in Table 4 revealed that the gamma irradiation doses used did not affect the germination percentage in the three plants.

For the germination percentage, ANOVA showed that there was no statistical difference between the groups ($p = 0.596$, $p = 0.229$ and $p = 0.580$ in *V. faba*, *V. radiata*, and *P. sativum* respectively). Higher doses proved to increase the

Table 3. Effect of gamma irradiation on the weight of irradiated seeds.

Doses (mGy)	Weight(g) (Mean ± S.D)		
	V. faba	*P. sativum*	*V. radiata*
Control	$12.3^{a} \pm 0.5$	$1.92^{a} \pm 0.07$	$0.69^{a} \pm 0.02$
16.2	$12.9^{a} \pm 1.5$	$2.04^{ab} \pm 0.72$	$0.65^{a} \pm 0.02$
48.5	$11.6^{a} \pm 0.6$	$1.96^{ab} \pm 0.04$	$0.66^{a} \pm 0.02$
431	-	$1.99^{ab} \pm 0.23$	$0.67^{a} \pm 0.05$
1070	$12.0^{a} \pm 1.2$	$2.00^{ab} \pm 0.23$	$0.64^{a} \pm 0.08$

Table 4. Effect of gamma irradiation on the germination percentage and mean germination rate of *V. faba*, *P. sativum* and *V. radiata*.

DOSES (mGy)	*V. faba*		*P. sativum*		*V. radiata*	
	Germination percent (%)	MGR (days)	Germination percent (%)	MGR (days)	Germination percent (%)	MGR (days)
Control	93.3[a]	4.05[a]	76.7[a]	4.06[ab]	100[a]	4[a]
16.2	93.3[a]	4.64[ab]	90[a]	4.20[b]	100[a]	4[a]
48.5	100[a]	4.1[a]	80[a]	4.05[ab]	96.7[a]	4[a]
431	-	-	76.7[a]	4.12[ab]	100[a]	4[a]
1070	100[a]	4.03[a]	80[a]	4.04[a]	96.7[a]	4[a]

germination rate of *V. faba* and *P. sativum*, especially the 1070 mGy dose. *V. radiata* however, showed no response to any of the doses.

3.3. Effect of Irradiation on Root and Shoot Development

Figure 5 presents the effect of the irradiation doses used in this study on the root and shoot length at 14DAS. The figure reveals that all the irradiated groups performed better than the control group in all cases. Secondly, the lower doses (16.2 mGy and 48.5 mGy) performed better on the root and shoot development of *V. radiata* and *P. sativum*, with the 16.2 mGy dose having the longest roots in both plants, while the 48.5 mGy dose had the longest shoots in both plants. In *V. faba* however, 1070 mGy dose gave a stimulatory effect on both root and shoot development. ANOVA showed that there is a significant difference between the doses and root length of all the three plants (with p values < 0.05). Similarly, for the shoot development, ANOVA showed a significant difference between the doses and shoot length (all p values < 0.05). Duncan's posthoc test also showed a significant difference between the groups (doses) in all cases.

3.4. Polytunnel Experiment (Growth Phase)

As stated earlier, this phase of the experiment was set up in blocks (A, B and C), and for all the parameters measured, there were no significant differences across the blocks, according to two-way ANOVA.

Effect of Gamma Irradiation on the Morphometric Parameters

Figure 6 presents the effects of gamma radiation doses used in this experiment on the various morphometric parameters of the plants, such as plant height, number of leaves, leaf chlorophyll content, leaf area, fresh and dry weight at 45 DAS. There was no correlation between the doses and the plants' height in all three plants.

For the number of leaves, *V. radiate* showed no correlation between the doses and the number of leaves, while *P. sativum* and *V. faba* showed a low positive correlation between these two variables; however, ANOVA showed that these changes within the treatments were not significant (p = 0.992 and p = 0.960 in *V. faba*, and *P. sativum* respectively).

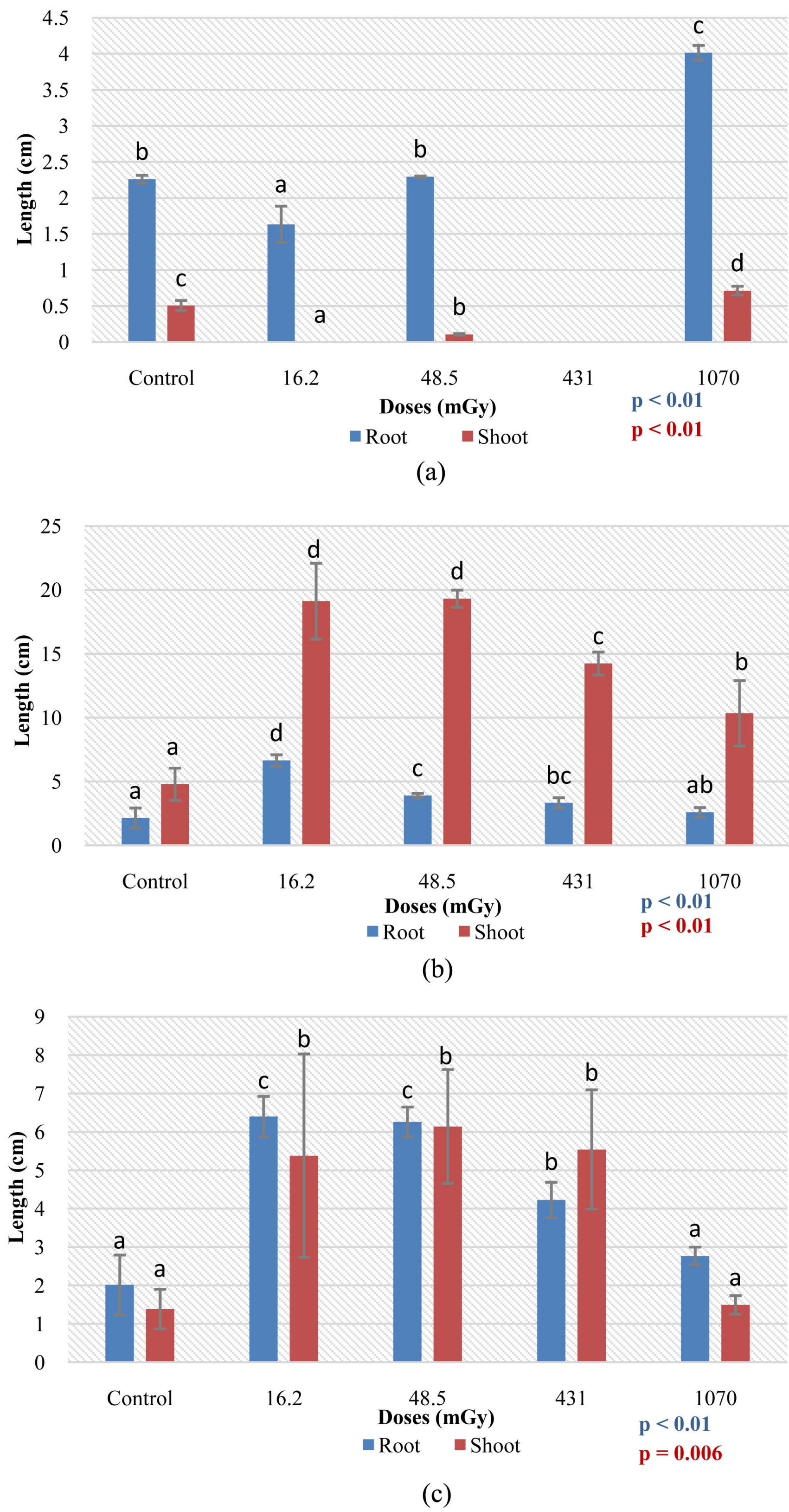

Figure 5. Effect of gamma radiation on root and shoot length at 14DAS; (a) *V. faba* (b) *V. radiata* (c) *P. sativum*. Each bar represents the average value (with error bars showing the standard deviation from the mean).

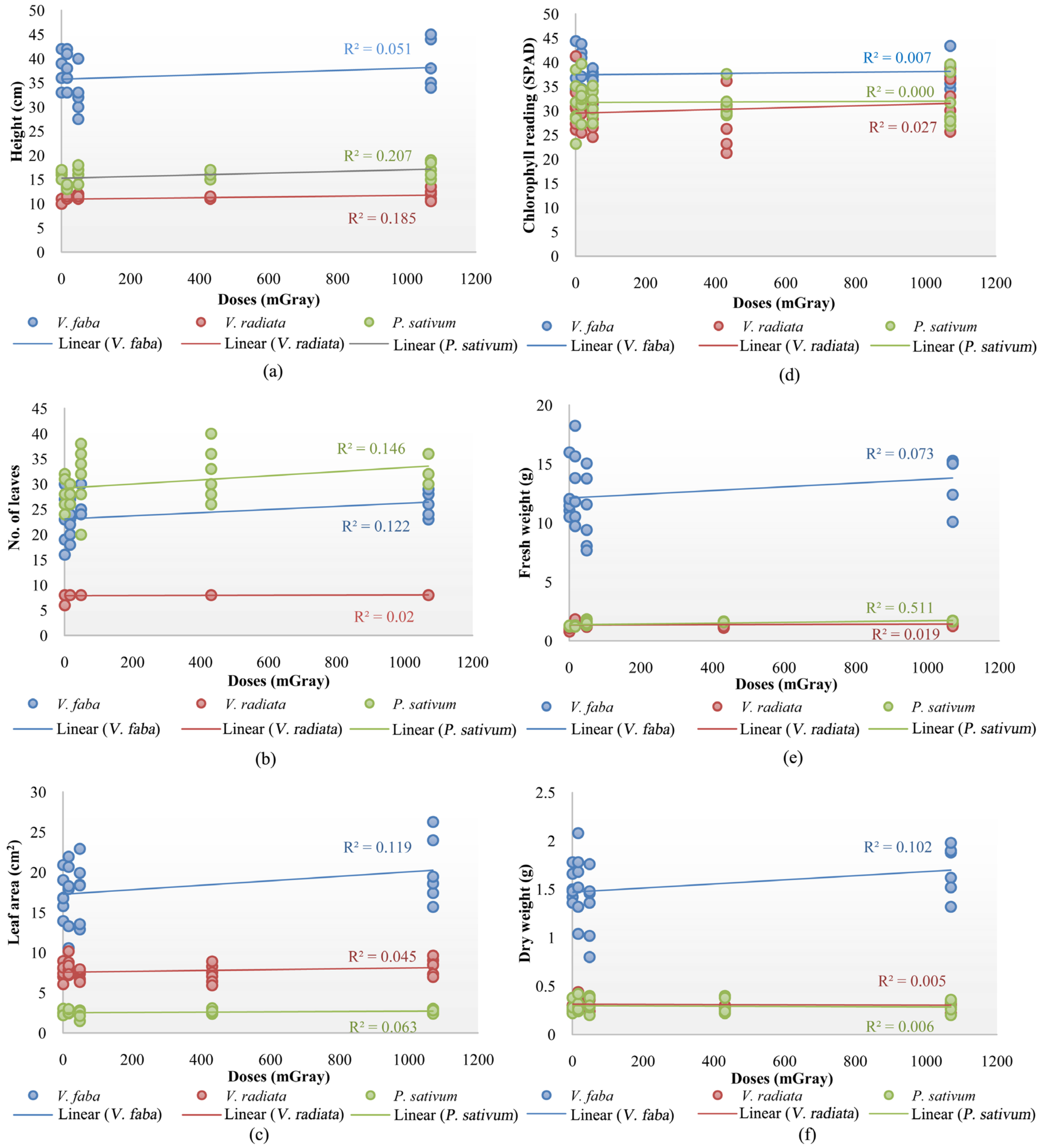

Figure 6. Effect of gamma radiation on the three plants (a) Height, (b) Number of leaves, (c) Leaf area, (d) Leaf chlorophyll, (e) Fresh weight and (f) Dry weight at 45 DAS.

For the leaf area, *P. sativum* and *V. radiata* showed no correlation to the doses, while a low positive correlation was observed between the doses and leaf area in *V. faba*. ANOVA, however showed that these changes were not statistically significant ($p = 0.530$).

The three plants showed no correlation between the leaf chlorophyl content and the doses. For the fresh weight, *P. sativum* and *V. radiata* showed no correlation between the fresh weight and the doses, while *V. faba* showed a low positive correlation. However, ANOVA showed that these changes were not statistically significant. A similar trend was observed for the dry weight with *V. radiata* and *P. sativum* both showing no correlation between the dry weight and the doses while *V. faba* showed a low positive correlation between the two variables.

4. Discussion

This study found some interesting effects of irradiating the seeds of these three plants, especially in the germination phase where the lower doses tend to stimulate the majority of the parameters considered. In the second phase, however, irradiation had no significant effect on all the parameters studied in these three plants. Also, there is a considerable level of variability in these data (as portrayed by the error bars in the bar charts and R^2 values in the scatter plots). These might be the consequence of several environmental factors, such as the amount of water that each plant receives. Even though we adopted a bottom watering technique in this experiment, it is still difficult to ensure that each individual gets exactly the same quantity of water; also, even under controlled conditions, certain traits/features in plants can be quite variable. However, the substantial sample size used in this study, and randomised block design were used to account for any significant variation. It's also worth noting that a correlation or link between doses and parameters doesn't always imply causality, especially if it only explains a small portion of the observed variance [46] [47]. These results, therefore, need to be interpreted with caution.

4.1. Effect of Gamma Radiation on Irradiated Seeds' Weight

Contrary to expectation, **Table 3** showed that the gamma doses used for this study had no significant effect on the weight of the seeds of the three plants. This finding is contrary to that of [48] in their work on four varieties of *Vicia faba*. A possible explanation for this could be as a result of the doses used in that study which are quite high (0 - 50,000 mGray); another possible explanation for this could be ascribed to the different variety of *V. faba* used in this present study as it has been established in literature that different varieties/genotypes/accessions of plants react differently to gamma irradiation [48] [49] [50]. Reference [48] also noticed a similar reaction in the four varieties used in the study. Reference [51] however noticed a significant decline in the weight of 100 seeds of *Arachis hypogaea* in all the three accessions used for their study, their finding is also not in harmony with the result of this study, and this could be attributed to the high doses used (0 - 600,000 mGray), and they are intended for crop improvement.

4.2. Effect of Gamma Irradiation on Germination Percentage and Rate

This experiment revealed that the gamma doses used had no significant effect on the germination percentage and rate of the three plants. This is contrary to most of the studies in the literature as there are many studies (majority of which are acute high doses) where lower doses (5 - 50 Gy) gave a stimulatory effect, followed by a substantial drop in germination percentage with increasing doses as seen in **Table 1**; [16] [18] [19]. In most cases, lethal effects are usually observed at the higher doses (>250 Gy), and this has been ascribed to a variety of factors, including an increase in membrane permeability, a decrease in α-amylase activity, a disruption in the meristematic tissues of the seeds, and in oily seeds, a reduction in lipase activity [52] [53]. Since the doses used within the present study are very low compared to these studies where acute high doses were used, this could be a possible explanation for this result. However, this finding is consistent with [20], where no significant effect was observed when *V. faba* seeds were exposed to 0 to 120,000 mGray gamma doses at intervals. A similar result was also reported in [54] in a study on *P. sativum.* Bringing it down to the context of this present study (doses within ICRP's DCRLs), a dose rate of 40 μGy/h (0.04 mGy/h) was recorded in [23], and there was no long-term trend in the germination percentage of *A. thaliana* as there was no statistical difference in almost all the generations studied. This finding is in harmony with the result of this present study.

4.3. Effect of Gamma Irradiation on Root and Shoot Development

Figure 4 clearly showed that in *Vigna radiata* and *Pisum sativum*, all irradiated groups enhanced root and shoot length as they outperformed the control group. In both plants, the lower doses (16.2 mGy and 48.5 mGy) gave the best results for root and shoot length respectively at 7 and 14 DAS. This finding is consistent with that of [20] in a study on *V. faba* Cv. Saraziri where stimulatory effects were recorded at lower doses (25 Gy) for both root and shoot length. Reference [19] also recorded a similar trend to the one observed in the present study in their work on *Pisum sativum*, where all irradiated groups (except the highest-200 Gy) had a stimulatory effect on the root length. In most of these studies cited in the literature review (**Table 1**), it is very common to see the higher doses (>50 Gy) having lethal effects on the root and shoot with values most times lower than the control [13] [16] [55]. These changes in root length have been reported to be linked to ROS changes in roots after high acute irradiation [56].

In *V. faba* however, the high dose (1070 mGy) had the longest root and shoot. This finding is reasonable since 1070 mGy still falls within the range of DCRL for this plant and is still very low compared to these high acute doses that are commonly encountered in the literature, and this could serve as the explanation for why this dose had the highest value, as well as why all the irradiated groups performed better than the control in *Pisum sativum* and *Vigna radiata*. However, as previously argued by [57] and [58], attributing these effects to radiation may not be the best conclusion.

4.4. Effect of Gamma Irradiation on the Morphometric Parameters

The experiment did not detect any notable/significant effect of the gamma doses used in this study on all the morphometric parameters measured in the three plants. The number of leaves, dry weight, plant height, leaf chlorophyll content, leaf area, and wet weight all showed a normal reaction to the doses. This is contrary to the majority of the study reviewed in the literature review (**Table 1**) [13] [15] [16] [18] [19] [21]. This result may be explained by the fact that the doses used in this experiment are very low, and falls within the ICRP DCRLs for these group of plants (1 - 10 $mGy \cdot d^{-1}$), as compared to these acute high dose studies (with environmentally unrealistic dose rates). Also from literature, it is very rare to find effects of IR within this threshold [12]. For example, a study on *A. thaliana* recorded leaf area readings that were normally distributed, and while statistically significant mean values were found across the treatments, the changes were minor [23]. This finding corroborates that of the present study where a haphazard trend was recorded between the doses, and in cases where differences were observed, they were of small value and were not statistically significant. In a controlled experiment using *Lemna minor*, no influence on physiological, developmental, or morphological characteristics of the plant at dose rates of 0.08 mGy/h to 4.95 mGy/h [59]. Although the higher range of the dose used in their study is quite higher than the one used in the present study, a similar result was obtained. However, a study of crested hair grass (*Koeleria gracilis*) that had inhabited contaminated soil with a dose rate of 4 - 265 mGy/Year (0.00046 - 0.030 mGy/h) recorded cytogenetic effects in the higher doses, but there were no morphological changes [60]. This also accords with our findings, but since this study didn't focus on the genetics aspect, little is known about the cytogenetic effects of the doses used in the present study on *V. faba*, *V. radiata*, and *P. sativum*, and this is an important issue for future research.

There are other several studies where mutation and genetic effects of IR were recorded in doses within the scope of those used in this study, for example; aberrant cells in *Avena sativa*, *Hordeum vulgare* and *Triticum sativum* at doses range of 5.32 - 47.8 mGy [61]; chromosome fragments in *Phragmites australis* (0.01 - 9.30 mGy) [62]; variation in allele number in *Typha latifolia* (0.13 - 7.52 mGy) [63]. However, the majority of these effects are subcellular and have shown no apparent morphological trend after exposure to IR at these doses, and in rare cases where plants exposed to ecologically relevant dose rates of IR experience cytogenetic or physiological effects, there is no substantial body of evidence of effects at higher levels of biological organisation (individual, reproductive, transgenerational, population and community) [12].

5. Conclusions

Because of the prevalence of data from acute high doses in the literature, as well as the scarcity of data from plants exposed to these low levels in controlled environments, it is necessary to conduct more research on the effects of low, environmentally realistic IR doses on plants. The plants in this experiment (*V. faba*, *V. radiata*, and *P. sativum*) were able to compensate for the effect observed in the germination phase, and by the end of the experiment, there were no statistically significant effects in all the morphometric parameters studied; the visible organs appeared normal, and growth rate was normal. We, therefore, conclude that the data presented here increases confidence in the ICRP's DCRLs by giving evidence from controlled environmental conditions and contextualising effects observed in field research.

Also, the effects on plant populations (in cases where there are) at DCRL levels appear to be quite low, as evidenced by the literature, and there are considerable advantages to radiation usage. Therefore, according to our findings, we believe that the DCRLs used to protect plants against the effects of IR are adequate and present regulation appears to be suitable.

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Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this paper.

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