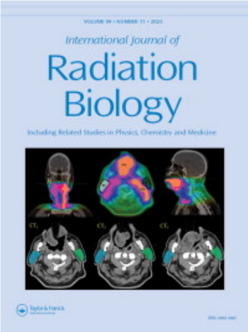

International Journal of Radiation Biology

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/irab20

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To cite this article: Ramazan Beyaz & Jennifer W. MacAdam (2023) X-radiation of *Lotus corniculatus* L. seeds improves germination and initial seedling growth, International Journal of Radiation Biology, 99:11, 1794-1799, DOI: 10.1080/09553002.2023.2204961

To link to this article: https://doi.org/10.1080/09553002.2023.2204961

Published online: 01 May 2023.

INTERNATIONAL JOURNAL OF RADIATION BIOLOGY
2023, VOL. 99, NO. 11, 1794–1799
https://doi.org/10.1080/09553002.2023.2204961

ORIGINAL ARTICLE

X-radiation of *Lotus corniculatus* L. seeds improves germination and initial seedling growth

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ABSTRACT

Purpose: *Lotus corniculatus* L. (bird's foot trefoil, BFT) is a valuable perennial legume forage species due to its high nutritive value, persistence under grazing, and condensed tannin content that improves ruminant production and prevents bloating. However, it is less preferred by farmers compared with other perennial forage legumes such as alfalfa because of slow germination, slow establishment and low seedling vigor. This study was conducted to determine whether X-ray seed priming could improve these deficiencies.
Materials and methods: Seeds of *L. corniculatus* cv. 'AC Langille' were irradiated at 0, 100, and 300 Gy. Non-irradiated and irradiated seeds were sown on Murashige and Skoog/Gamborg medium under in vitro conditions and cultured for 21 days. Germination percentage, mean germination time (MGT), germination rate index, length of shoot and root, fresh and dry weight of shoot and root, dry matter ratios of shoot and root, water content of shoot and root, and seedling vigor index were measured.
Results: The results of this study demonstrated that X-ray seed priming significantly increased the germination percentage of *L. corniculatus*, increased the germination rate and thereby shortened the MGT, and improved seedling growth. However, X-ray pretreatment also decreased seedling shoot and root biomass.
Conclusions: In this study, it is reported for the first time that X-ray seed pretreatment has the potential to address important seedling establishment issues in *L. corniculatus*.

ARTICLE HISTORY
Received 2 November 2022
Revised 23 February 2023
Accepted 21 March 2023

KEYWORDS
Lotus corniculatus L.; X-rays; germination; seedling growth

Introduction

Lotus corniculatus L. (commonly known as bird's foot trefoil, BFT, Fabaceae) is a non-bloating perennial fodder legume that is well-suited to ruminant production on pastures (Hunt et al. 2015). Like alfalfa, *L. corniculatus* is tap-rooted, drought-tolerant, and capable of biological dinitrogen fixation. *L. corniculatus* does not produce bloat when grazed as green, standing forage because it accumulates a condensed tannin (Brummer et al. 2016) that also enhances ruminant production (Waghorn 2008). Due to its high nutritional content and tolerance of unfavorable climatic circumstances, *L. corniculatus* is better-suited for pasture, hay or silage production than white clover (Nikolic et al. 2006). Despite these valuable features, it has slow germination, slow field establishment (McLean and Nowak 1997; Brummer et al. 2016) and low seed vigor (Artola et al. 2003). Compared to red clover or alfalfa, *L. corniculatus* seedling growth is substantially slower, reducing its competition with other plants, especially weeds, in field conditions. The productivity of *L. corniculatus* is primarily constrained by poor seedling emergence and inadequate stand establishment (Farooq et al. 2019). Therefore, increasing the rates of seed germination and seedling establishment would greatly enhance the use of *L. corniculatus* for pasture, hay and silage.

Seed priming is an age-old technique, but is still widely used today. It is a relatively easy to apply, low-cost and effective method that can improve crop performance (Farooq et al. 2019). Seed priming improves the performance of seeds by making them sprout quickly and evenly, improving seedling establishment. This creates good physiological conditions that help many crops sprout faster and healthier. Rapid seed germination and stand establishment are crucial factors affecting crop productivity (Khan et al. 2022). Target seeds are altered physiologically during germination by priming, through osmotic changes, membrane restructuring, and delaying electrolyte seepage (Mansour et al. 2019; Srivastava et al. 2021). Priming may also promote protein formation, repairs to cellular bio-membranes, increase antioxidant enzymes, and aid DNA repair. It also increases ATP availability (Hussain et al. 2019; Mansour et al. 2019; Sen and Puthur 2020). Seed priming can be conducted using conventional (hydro-priming, chemical priming, bio-priming, nutrient priming, priming with plant growth regulator, priming with plant extract, and osmo-priming) or advanced methods (seed priming through

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nanoparticles or through physical agents such as exposure to a magnetic field, UV radiation, gamma radiation, X-radiation, or microwaves) (Khan et al. 2022). Plant growth and development can be affected by physical mutagens like gamma rays and X-rays, both high-frequency ionizing radiation that can cause cytological, genetic, biochemical, and physiological alterations in cells and tissues (Dada et al. 2022). Ionizing radiation is a useful tool in agricultural research (IR). In Sievert units (Sv), the absorbed dosage of IRs can also be expressed, with 1 Sv dose equaling 1 J of radiation energy absorbed for every kilogram of organ or tissue weight; the rate of dosage (rate of energy deposition, expressed as Gy h^{-1}) (Araújo et al. 2016).

Gamma-rays have greater energy than X-rays and are continuously emitted from the nucleus of radioactive source materials, while X-rays can be generated on demand from an X-ray tube. Although the two forms of ionizing radiation may have similar effects when used to prime seeds, more is known about the effect of gamma irradiation than X-radiation on seed germination and seedling growth (Araújo et al. 2016; Beyaz et al. 2016; Deshmukh et al. 2018). Hydropriming (Artola et al. 2003) and osmopriming (Aydınoğlu 2019) have been used to stimulate germination in *L. corniculatus*; however, no study has been conducted on the effect of X-rays on germination and seedling traits of *L. corniculatus*, despite the slow germination, slow establishment and low seedling vigor in this crop. A 2016 study (Beyaz et al. 2016) of the lagure *Lathyrus chryanthus* demonstrated that seed germination and seedling growth were improved by exposure to 150 Gy gamma radiation while exposure of seeds of the perennial legume, sainfoin (*Onobrychis viciifolia*) to 400, 500, and 600 Gy gamma radiation showed elevated chlorophyll concentration at all levels of irradiation. The objective of this study was to determine if germination, establishment and seed vigor index (VI) of *L. corniculatus* could be similarly enhanced using X-rays, a more readily available form of ionizing radiation. This study is the first to examine the general impact of X-radiation on seed germination and seedling growth of *L. corniculatus*.

Materials and methods

Plant material and X-radiation

In this study, seeds of the BFT cv. 'AC Langille' (Papadopoulos et al. 1997) were used as plant material. Seeds were obtained from Power Seeds Inc. (Lindsay, Canada). Seeds were irradiated with 100 and 300 Gy in an RS-2000 X-rays generator (Rad Source Technologies, Buford, GA). The dose rate of the device was 0.495 Gy/h. Seeds were placed in a 100 mm diameter polystyrene petri dish in a single layer, and the petri dish was placed in the center of the aluminum shelf which was inserted at the highest level in the chamber. Seeds were irradiated for 12 min. 7 s (100 Gy) or 36 min 21 s (300 Gy).

Plant tissue culture conditions

The growth medium, standard Murashige and Skoog/Gamborg (Plant Media, Dublin, OH) (Gamborg et al. 1968), contained 3% sucrose (Research Product International, Mount Prospect, IL) and 7% agar (Plant Media, Dublin, OH). Before autoclaving at 121 °C, 7.25 psia for 20 minutes, the pH of the medium was corrected to 5.7 with 1 M NaOH or HCl. The non-irradiated (0 Gy control) and irradiated (100 and 300 Gy) *L. corniculatus* seeds were surface sterilized in 50% commercial bleach (Clorox, Oakland, CA, containing 8.25% sodium hypochlorite) in which one drop of Tween-20 (Acros Organics, Geel, Belgium) was added for 20 minutes and then rinsed three times with distilled water. Sterilized non-irradiated and irradiated seeds were sown on 2.5 cm of medium in 7.62- × 7.62- × 10.16-cm magenta boxes (BioWorld, Visalia, CA). Germination of seeds and subsequent seedling development were carried out at 25 ± 1 °C under white fluorescent lamps at an intensity of 30 μmol m^{-2} s^{-1} (PAR) in a photoperiod of 16 h light and 8 h dark.

Germination and morphological observations

When the growing radicle lengthened to 2 mm, the seed was considered germinated. For 14 days, the proportion of seeds that germinated was recorded every 24 hours (ISTA 2003). Mean germination time (MGT) was calculated according to Ellis and Roberts (1980). MGT $= \sum Dn/\sum D$, where n is the number of freshly germinated seeds on day D, and D is the number of days since the start of the experiment. The percentage of seeds that germinated after being exposed to X-rays was estimated using the equation:

$$\text{Germination percentage (GP)} = (\text{number of germinating seeds/total number of seeds}) \times 100$$

(Al-Khayri et al. 2012)

Morphological observations including shoot and root length (cm) and fresh and dry weight (g) were made on developed seedlings 21 days after the start of the experiment (Figure 1). Dry weights were calculated after samples were dried in an oven (VWR Scientific Inc., Logan Township, NJ) at 70 °C for 48 hours (Beyaz et al. 2011). Water content (WC), dry matter (DM, %) and VI were calculated according to the following formulas, respectively:

$$\text{Water content (WC)} = (\text{fresh weight} - \text{dry weight})/\text{fresh weight} \times 100$$

(Zheng et al. 2008)

$$\text{Dry matter (DM)} = (\text{dry weight/fresh weight}) \times 100$$

(Breś et al. 2022)

$$\text{Vigor index (VI)} = (\text{average root length} + \text{average hypocotyl length}) \times \text{germination percentage(GP)}$$

(Abdul-Baki and Anderson 1973)

Germination rate was expressed as the germination rate index (GRI) according to Maguire (1962):

$$GRI = \frac{\Sigma \text{ no. of germinated seeds}}{\Sigma \text{ no. of days}}$$

Statistical analysis

The study was carried out as a completely randomized design with three replications where each magenta box was considered a replication and 20 seeds were planted in each magenta box. For each treatment, a one-way ANOVA was done using the SPSS statistical program (Version 22) (SPSS Inc., Chicago, IL). The means were compared using Duncan's multiple range test at $p < .01$. Before statistical analysis, the data in percentages were transformed using arcsine transformation (Snedecor and Cochran 1967). Each treatment's standard deviation was also calculated.

Results

Effects of X-radiation on germination

Germination percentage, GRI (speed of germination), MGT, and seedling VI were all significantly ($p < .01$) affected by X-ray irradiation (Table 1). Germination percentage was 53.3%, 91.6% and 93.0% under control, 100 Gy treatment and 300 Gy treatments, respectively. As compared to the control, germination percentage was increased by 71.85% under 100 Gy, and 74.48% under 300 Gy treatments. The maximum GRI (27.67%) was measured in the 300 Gy treatment, which was followed by the 23.93% GRI in 100 Gy treatment, and 9.72% in control. The GRI was increased by 146.19% under 100 Gy treatment, and 184.46% under 300 Gy treatment, in comparison with control. On the other hand, the MGT decreased with increasing X-radiation. The lowest value was recorded for the 300 Gy treatment, as 4.61 days. Mean germination time was reduced by 6.99% and 9.96% under 100 Gy and 300 Gy treatments, respectively, as compared with control. For the seedling VI, the values obtained from the 100 Gy treatment, 300 Gy treatment and control were 1329.35, 1209.00 and 530.57, respectively. X-ray treatments increased the seedling VI by 150.58% and 127.86% under 100 Gy and 300 Gy treatments, respectively.

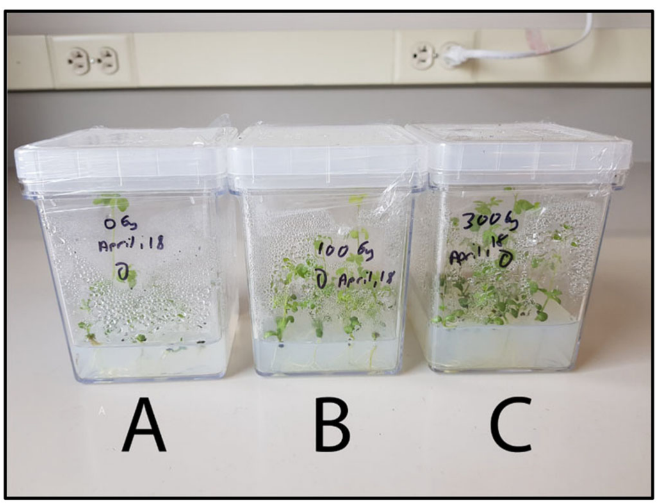

Figure 1. Effect of different doses (A: 0 Gy-control; B: 100 Gy; C: 300 Gy) of X-radiation on *L. corniculatus* seed germination and seedling growth. The photo was taken 21 days after the start of the experiment.

Effects of X-ray irradiation on seedling growth

The X-radiation had an impact on seedling growth parameters as well (Tables 1 and 2). Shoot length ($p < .05$), root length ($p < .01$), and root to shoot ratio ($p < .01$) were significantly influenced by the X-rays treatments (Table 1). Due to increased X-rays treatments, shoot length decreased (except 100 Gy treatment), while root length and root to shoot ratio increased. The highest shoot and root lengths were recorded for the 100 Gy treatment, at 5.61 cm and 8.88 cm, respectively. Root-to-shoot was recorded as 1.22, 1.57 and 1.86 under the control, 100 Gy and 300 Gy treatments, respectively. When the control and 300-Gy X-ray treatments were compared, it was determined that there was an increase of only 2.58% in the root and an increase of 46.18% in the root. However, the root to shoot ratio was increased by 28.68% for the 100 Gy treatment, and 52.45% for the 300 Gy treatment, in comparison with the control. The fresh weights of both the shoot and root were significantly ($p < .01$) decreased by the X-ray treatments (Table 2). The lowest shoot and root fresh weight values (0.0000072 g per plant and 0.0000074 g per plant, respectively) were recorded for the 300 Gy treatment. As compared to control, shoot fresh weight was reduced by 3.48% under 100 Gy, and 16.27% under 300 Gy X-rays treatments. The root fresh weight was reduced by 14.28% under 100 Gy treatment, and 29.52% under 300 Gy treatment, in comparison with control. Dry weight of shoots and roots were also significantly ($p < .01$) influenced by the X-rays treatments (Table 2). Shoot and root dry weight values were decreased by increasing X-radiation doses. The highest values

Table 1. The effect of different doses of X-radiation on germination percentage (GP), germination rate index (GRI-speed of germination), mean germination time (MGT), seedling vigor index (SVI), shoot length (SL), root length (RL), and root/shoot ratio (R/S ratio) of 21-day-old *L. corniculatus* seedling.

X-rays doses	GP (%)	GRI (%)	MGT (days)	SVI	SL (cm)	RL (cm)	R/S ratio (%)
0 Gy	$53.3^b \pm 1.66$	$9.72^c \pm 3.01$	$5.12^a \pm 0.14$	$530.57^b \pm 136.02$	$4.64^b \pm 0.30$	$5.76^b \pm 0.90$	$1.22^b \pm 0.11$
100 Gy	$91.6^a \pm 3.33$	$23.93^b \pm 0.54$	$4.72^b \pm 0.05$	$1329.35^a \pm 137.91$	$5.61^a \pm 0.38$	$8.88^a \pm 0.97$	$1.57^a \pm 0.06$
300 Gy	$93.0^a \pm 1.82$	$27.67^a \pm 0.41$	$4.61^b \pm 0.04$	$1209.00^a \pm 23.17$	$4.52^b \pm 0.09$	$8.42^a \pm 0.98$	$1.86^a \pm 0.082$
Summary of one-way-ANOVA							
X-rays	**	**	**	**	*	**	**

Means are the average of three replicates with a ± standard deviation of the mean. Different letters in the same column show significant differences at $p < .05$. *significant at $p < .05$, **$p < .01$ and ns: non-significant.

Table 2. The effect of different doses of X-radiation on shoot fresh weight (SWF), root fresh weight (RWF), shoot dry weight (SDW), root dry weight (RDW), shoot dry matter (SDM), root dry matter (RDM), shoot water content (SWC), and root water content (RWC) of 21-day-old *L. corniculatus* seedling.

X-rays doses	SFW (g per plant)	RWF (g per plant)	SDW (g per plant)	RDW (g per plant)	SDM (%)	RDM (%)	SWC (%)	RWC (%)
0 Gy	$0.000086^{a} \pm 0.000$	$0.000105^{a} \pm 0.002$	$0.0000081^{a} \pm 0.0001$	$0.0000065^{a} \pm 0.0001$	$9.35^{a} \pm 0.20$	6.16 ± 0.06	90.64 ± 0.20	93.83 ± 0.06
100 Gy	$0.000083^{a} \pm 0.000$	$0.000090^{ab} \pm 0.004$	$0.0000070^{b} \pm 0.0001$	$0.0000055^{ab} \pm 0.0004$	$8.36^{b} \pm 0.07$	6.15 ± 0.80	91.63 ± 0.07	93.84 ± 0.80
300 Gy	$0.000072^{b} \pm 0.001$	$0.000074^{b} \pm 0.007$	$0.0000066^{c} \pm 0.0005$	$0.0000048^{b} \pm 0.0006$	$9.12^{a} \pm 0.62$	6.46 ± 0.24	90.87 ± 0.62	93.53 ± 0.24
Summary of one-way-ANOVA								
X-rays	**	**	**	*	**	ns	ns	ns

Means are the average of three replicates with a ± standard deviation of the mean. Different letters in the same column show significant differences at $p < .05$. *significant at $p < .05$, ** $p < .01$ and ns: non-significant.

(0.0000081 g per plant and 0.0000065 g per plant, respectively) were recorded in the control group for both shoot and root. Dry weight of shoot showed the 13.58% reduction under 100 Gy and 18.51% under 300 Gy treatments. However, the dry weight of roots was less than controls by 15.38% under 100 Gy and 26.15% under 300 Gy treatments. While X-ray treatments caused a statistically significant ($p < .01$) decrease in the percentage of shoot DM, there was no significant difference in the percentage of root DM among treatments (Table 2). There was a decrease in shoot DM ratio due to increasing X-rays doses. However, there was a slight increase (4.87%) in root DM when comparing control and 300 Gy treatment. There was no statistical significance between the means of all treatments (control, 100 Gy and 300 Gy) in terms of shoot and root WC (Table 2).

Discussion

The wavelengths of X-rays are between 0.01 and 10 nm in the electromagnetic spectrum (Kotwaliwale et al. 2014), and their effects on biological things are not as well-understood as the effects of gamma rays. The best soft X-rays for agricultural research are those with energy between 0.12 and 12 keV because of their limited penetration potential (Kotwaliwale et al. 2014). The understanding of X-rays' role as a seed energizing agent or their stimulating impacts on germination needs to be expanded (Araújo et al. 2016). To the best of our understanding, very few publications after the 1960s dealt with the effects of X-radiation on seed performance (Einset and Collins 2015; Pérez-Torres et al. 2015). In the current study of X-radiation seed priming of *L. corniculatus* seeds, both 100 and 300 Gy treatments improved the rates of seed germination and seedling growth. One or both radiation levels positively affected all measured germination and growth parameters with the exception of shoot and root DM accumulation.

Gamma radiation can have a positive effect on germination due to enzyme activators that support the germination process and physiological changes (Dada et al. 2022). According to Abdel-Hady et al. (2008), ionizing radiation's stimulatory effect on seed germination may be due to the activation of RNA or protein synthesis. In addition, according to Kovacs and Keresztes (2002), ionizing radiation can contribute to germination by softening or weakening the seed coat. A softened seed coat may have improved permeability that enhances water and hormone uptake from the medium, which results in high tissue metabolic activity (Beyaz et al. 2016). The current study's germination results were consistent with earlier reports from Thapa (2004), Marcu et al. (2013), and Oladosu et al. (2016) who reported positive effects of ionizing radiation on seed germination in various plants. In addition, Qi et al. (2015) reported that the germination rate was noticeably stimulated by seed irradiation at total doses less than 100 Gy in *Arabidopsis thaliana*. Contrary to these research findings, Al-Khayri et al. (2012) reported that every level of X-radiation from 0.05 to 15 Gy progressively reduced germination in the date palm plant, while Dada et al. (2022) found that levels of both gamma and X-radiation above 100 Gy consistently reduced or inhibited germination and subsequent seedling development of three cultivars of *Coffea arabica*. It has been shown that the biological effects of ionizing radiation are highly contingent on intensity, dose rate, and exposure duration (Araújo et al. 2016).

The early stage of germination requires activation of RNA and protein synthesis that in turn can boost seedling vigor (Dada et al. 2022). Our findings were parallel to that of Dada et al. (2022) who reported that X-ray irradiation pretreatment (50 and 100 Gy) increased seedling VI of coffee varieties. Artola et al. (2003) reported that seedlings of *L. corniculatus* had low seed vigor and this resulted in poor stand establishment of crops. The results of the current study showed that the seedling VI of *L. corniculatus* increased by X-radiation of both 100 and 300 Gy, which is a promising finding suggesting that the establishment of *L. corniculatus* can be improved using X-irradiation. According to Iglesias-Andreu et al. (2012), gamma doses less than 1 kGy (1000 Gy) are regarded as low doses. Low-dose gamma application has demonstrated that seedling development and germination rate in cucumber and okra are positively impacted (Jaipo et al. 2019). Low-dose ionizing radiation stimulates the production of nucleic acids, proteins, and enzymes in seedlings that have been exposed to it (Abdel-Hady et al. 2008).

Seedling traits of *L. corniculatus* were also affected by the X-radiation pretreatments, resulting in greater shoot length at 100 Gy and an increase in root length at both levels. Similarly, Al-Khayri et al. (2012) reported an increased root length with X-ray treatments in date palm. Moreover, positive effect of different dose levels of ionizing radiation on plant growth was observed in other different plant including wheat (Melki and Marouani 2010), chickpea (Melki and Sallami 2008), lettuce (Marcu et al. 2013), tomato (Wiendl et al. 2013) and in cucumber and okra (Jaipo et al. 2019). The increase in plant growth is explained by the fact that ionizing radiation speeds up cell division while inhibiting the repair process (Ulukapi 2021). In reaction to acute radiation, plant metabolism is shifted to immediate repair of the damage, activation of pro-survival mechanisms and possibly the inhibition of cell division/cell differentiation (Araújo et al. 2016).

On the other hand, shoot and root fresh and dry weight, which are important parameters, decreased due to X-ray treatments, while there was no consistent decrease in either shoot or root DM percentages. Al-Khayri and Al-Enezi (2012) noted that there was a strong correlation between X-rays doses and proline accumulation in palm (*Phoenix dactylifera* L.), and as a result of this relationship, fresh weight and WC of palm seedlings increased. The accumulation of proline or other osmotica could explain why shoot and root percent WC (SWC and RWC) were not affected by increased growth rate. Similarly, Ulukapi (2021) noted that shoot and root fresh weight decreased in the common bean with increasing gamma ray doses (from 10 Gy to 40 Gy). In addition, Beyaz et al. (2016) stated that seedling fresh weight and seedling DM content of *Lathyrus chrysanthus* Boiss. were both decreased by increasing gamma radiation from 100 Gy to 250 Gy compared with 50 Gy. According to Borzouei et al. (2010), the radiation dose affects how much the root and shoot dry weights are reduced. Low-dose ionizing radiation applied to seeds has been shown to promote germination, plant development, and the production of pigments used in photosynthetic processes (Kovacs and Keresztes 2002; De Micco et al. 2014; Macovei et al. 2014). However, the same studies emphasized the negative effects of high radiation doses or extended exposure in living things (Araújo et al. 2016). In the current study, the X-ray doses applied were high enough to have a negative effect on the biomass increase of *L. corniculatus*. This appears to be the simple result of increasing the rate of elongation more than the rate of photosynthesis. The question that would need to be addressed is whether this results in a competitive advantage for *L. corniculatus* seedlings in the field when faced with competition from weeds for light, nutrients and water.

Conclusions

As a result of this research, it has been demonstrated that promising results can be obtained from the priming of *L. corniculatus* seeds using X-ray irradiation. However, while the doses applied in the study increased the rate of germination and seedling development, shoot and root fresh and dry weight were decreased. A better understanding of the plant's physio-biochemical and molecular response to X-radiation on potentially beneficial effects, such as seed coat permeability, protein synthesis, and stimulation of responses to the transient generation of reactive oxygen species is needed.

Acknowledgements

The authors thank Prof. Dr. Jennifer W. MacAdam, Utah State University (Utah-Logan-USA), for generously using her laboratory in the conduct of the research.

Author contributions

RB and JWM: conceived the research plans and designed the experiments; RB: performed the experiments; RB and JWM: wrote the article.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

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Jennifer W. MacAdam's graduate training was at the University of Missouri-Columbia (UM-C) in agronomy with an emphasis on the physiology of forage plants; her graduate research focused on the anatomy and physiology of grass leaf development. Following her PhD in 1988, she taught the Crop Physiology course at UM-C for one semester and gained postdoctoral experience in animal science and basic plant biochemistry and physiology. In 1991, she accepted a Forage research and teaching position at Utah State University. She has taught the foraging class at Utah State University and developed an undergraduate course, The Structure and Function of Plants, for which she also published a text. She has served as the chair of the Forage and Grazinglands Division of the Crop Science Society of America, as a technical editor of the Forage and Grazinglands journal, and as an associate editor for both the Crop Science and Agronomy Journal. She is currently an editor of the Journal of Agricultural Science, Cambridge, and is on the editorial boards of the MDPI journals Agronomy and Grasses, as well as the Wiley journal Grassland Research. She is a fellow of the American Society of Agronomy and the Crop Science Society of America.

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