X-RAY DOSE EFFECTS ON SEEDS GERMINATION AND SEEDLING PERFORMANCE OF EUROPEAN BEECH

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Abstract

The aim of the study was to assess germination dynamics and post-germination adaptation in two populations of European beech (*Fagus sylvatica* L.) seeds collected in autumn 2023 in the 'Plaiul Fagului' State Nature Reserve, Republic of Moldova with contrasting initial viability levels ($PF\text{-}23_{high}$ – 97% and $PF\text{-}23_{low}$ – 57%) under X-ray exposure at doses ranging from 25 to 300 Gy. Germination parameters, including total germination, mean daily germination (MDG), mean germination time (MGT), and germination rate index (GRI), were recorded alongside seedling development under solarium conditions. $PF\text{-}23_{high}$ exhibited a more enhanced response: exposure to 25–125 Gy stimulated earlier and more intense germination (MDG up to 1.55; GRI up to 2.00), with a peak germination of 77.33% at 125 Gy and a 21-day advancement relative to the control. In contrast, $PF\text{-}23_{low}$ showed only moderate stimulation at 25 Gy (germination 65.67%, 13 days earlier than control), and was markedly more sensitive to doses ≥100 Gy, with reduced germination and delayed dynamics (MGT up to 122 days; MDG and GRI consistently lower across treatments). At high doses (200–300 Gy) both populations demonstrated a pronounced decline in germination parameters. Seedling survival under solarium conditions revealed a uniformly negative impact of irradiation: both $PF\text{-}23_{low}$ and $PF\text{-}23_{high}$ showed rapid post-germination mortality, particularly between weeks 6 and 10 at doses ≥50 Gy. Despite the initially higher physiological resilience of $PF\text{-}23_{high}$, neither population exhibited stable adaptation under prolonged post-irradiation stress. These findings may suggest a high level of radiosensitivity in European beech during early ontogenetic development, regardless of initial seed viability.

Keywords: *Fagus sylvatica* seeds, high-viability, low-viability, X-ray irradiation, germination traits

Fagus sylvatica L. (Fagaceae) is one of the key tree species in natural forests of Central Europe, characterized by a moderate lifespan of approximately 300 years and a comparatively late entry into the reproductive phase – at the age of 30-50 years (Packham J.R. *et al*., 2012). The onset of fruiting in European beech varies from 20 to 80 years depending on ecological conditions and growth form – solitary, in stands, or in dense forests (Bonner F.T., Leak W.B., 2008). Stable and abundant fruiting in beech depends on climatic factors and yield cyclicity (Bonner F.T., Leak W.B., 2008; Gavranović Markić A. *et al*., 2024).

Studies conducted across various European regions show that seed yield in *F. sylvatica* is mainly characterized by binary cycles, as well as 3- to 5-year cycles under the influence of various factors (Drobyshev I. *et al*., 2014; Lebourgeois F. *et al*., 2018; Chiavetta U., Marzini S., 2021; Gavranović Markić A. *et al*., 2024). At the same time, the periodicity of mast years varies by region and historical period and may reach 5-7 or even 15-20-year cycles (Piovesan G., Adams J. M., 2001; Drobyshev I. *et al*., 2014; Ascoli D. *et al*., 2017; Brumme R. *et al*., 2021; Hacket-Pain A. *et al*., 2022). Significant changes in mast periodicity are associated with climatic factors such as extreme summer temperatures, drought, and frost during flowering (Nussbaumer A. *et al*., 2020).

Contemporary climate dynamics are increasingly constraining the capacity for natural regeneration of *F.sylvatica* in several parts of its distribution area, particularly in southeastern marginal zones, where elevated heat and drought stress are becoming more prevalent (Bolte A. *et al*., 2016; Muffler L. *et al*., 2021).

Among various approaches to enhancing genetic diversity in plants, radiation-induced mutagenesis remains one of the most reliable and efficient techniques (Ma L. *et al*., 2021). X-rays and gamma radiation are the primary tools in mutation breeding programs, offering promising outcomes for woody species including *F. sylvatica*, which are genetically rich yet have not been subject to intensive selection efforts (Kornienko V.O., Netsvetov M.V., 2016; Holonets R. *et al*., 2021). For example, Holonets et al.)2021) found that gamma irradiation (γ-rays) at a dose of 2 Gy

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significantly increased the germination of *Quercus petraea* acorns to 86.67%. Long-term effects of gamma irradiation on the morphology of *Quercus robur* were also studied (Kornienko V.O., Netsvetov M.V., 2016). Comparable research involving radiation treatments has been extensively carried out on conventional agricultural crops as well as plant species cultivated under *in vitro* conditions (Reznik N. *et al.*, 2021).

Ionizing radiation, including both X-rays and γ-rays, has been actively explored in seed science for its potential to stimulate germination processes in seeds characterized by low physiological quality (Akshatha S. *et al.*, 2013; Holonets R. *et al.*, 2021; Flores-López L.Y. *et al.*, 2022; Beyaz R., Macadam J.W., 2023). When applied at low intensities (10–100 Gy), such irradiation can trigger a cascade of beneficial responses, including enhanced metabolic activity, increased permeability of the seed coat, activation of antioxidant defenses, and initiation of DNA repair, ultimately resulting in improved and accelerated germination (Akshatha S. *et al.*, 2013; Beyaz R., Macadam J.W., 2023). Such effects have been observed in the seeds of certain oak species (*Q.robur* and *Q. petraea*), which belong to the Fagaceae family and share close physiological and ecological traits with *F. sylvatica* (Kornienko V.O., Netsvetov M.V., 2016; Holonets R. *et al.*, 2021). Recent studies conducted on two genotypes of okra (*Hibiscus esculentus* L.) showed that low doses of X-ray irradiation (up to 5 Gy) stimulated seed germination, seedling growth, and antioxidant activity, while higher doses caused oxidative stress and suppressed plant development (Rezk A.A., *et al.*, 2019). Studies on *Rhynchostylis retusa* orchid seeds have shown that low doses of X-ray irradiation (up to 4 rad) enhance seed germination and seedling morphological development, including shoot, root, and leaf growth (Paul M. *et al.*, 2021). These findings highlight the potential of X-rays specifically for stimulating physiological processes and improving germination, which is particularly important for species with low seed viability.

Nevertheless, specific data concerning the response of *F. sylvatica* seeds to X-ray irradiation are scarce in the current literature. This gap is especially important in the context of poor mast years, when beech seeds often display reduced viability due to incomplete physiological development, embryonic abnormalities, or unfavorable storage conditions. Under these conditions, conventional pre-sowing treatments such as stratification and scarification frequently prove ineffective in improving germination outcomes (Ratajczak E. *et al.*, 2015; Małecka A. *et al.*, 2021).

As a result, applying low-dose X-ray irradiation may serve as a valuable alternative for improving germination rates in physiologically weak or dormant beech seeds, particularly under circumstances of limited high-quality seed stock. This strategy holds both practical relevance for seedling production and forest regeneration initiatives, and scientific value for exploring the radiotolerance of tree seeds.

The aim of this study is to evaluate how varying doses of X-ray exposure influence the germination performance of *Fagus sylvatica* seeds with differing levels of viability, and to assess the potential applicability of this method for enhancing seedling adaptation during early stages of development.

MATERIAL AND METHOD

The experiments were carried out in the laboratory Natural Bioregulators, in solarium of the Institute of Genetics, Physiology and Plant Protection, Moldova State University during 2024-2025. The seeds of *F. sylvatica* collected in autumn 2023 from the "Plaiul Fagului" Nature Reserve, Republic of Moldova (hereafter referred to as population PF-23) with different initial viability levels (high – 97%, PF-23_{high}, and low – 57%, PF-23_{low}) were used in the study.

The viability of seeds was determined by test using the 2.3.5-triphenyltetrazolium chloride (TTC) solution (França-Neto J.B., Krzyzanowski F.C., 2019).

X-ray irradiation of beech seeds were carried out following the Protocol for X-ray mutagenesis of plant material using an RS-2400 X-ray device, with doses ranging from 25 to 300 Gy (Protocol for X-ray mutagenesis of plant material: seeds, 2010; FAO/IAEA, 2018; Reznik N. *et al.,* 2021; Chanim A.M.A., 2024). Each variant of irradiation consisted of three replicates of 100 seeds each. The control variant included seeds that were not exposed to irradiation. After X-ray irradiation, the seeds were germinated during cold stratification at +4±1°C. Monitoring of seed germination was carried out each 7 days for the period of 150 days of stratification. The total germination percentage (TGP), mean daily germination (MDG), mean germination time (MGT) and germination rate index (GRI) were calculated using formulas described by F. Al-Ansari and T. Ksiksi (2016).

Root length of the germinated beech seeds was measured using a VOREL digital caliper with a resolution 0.1mm and an accuracy of ±0.2 mm.

Beech seedling emergence studies were carried out by planting in substrate under solarium conditions (unheated greenhouse) with regulated humidity. The experiment was conducted in a randomized design with three replicates per variant.

Statistical analysis. The results were analyzed using Statgraphics Plus 5.0 software, applying standard statistical procedures including one-way ANOVA and Multiple Range Tests at a significance level of $p \leq 0.05$ (Tukey's HSD — Honestly Significant Difference and LSD – Least Significant Difference).

RESULTS AND DISCUSSIONS

A comparative analysis of seed germination in European beech (*F. sylvatica*) from populations with low (PF-23_{low}) and high (PF-23_{high}) initial viability revealed that the total germination percentage of control seeds on the 150th day of stratification differed insignificantly between the two populations and averaged 63.33% and 67.33%, respectively, for PF-23_{low} and PF-23_{high}. Although germination in both populations started at approximately the same time (on the 63rd day of stratification), their germination dynamics differed markedly. By the 80th day of stratification, germination in the PF-23_{low} population was observed only in the 25 and 75 Gy treatments (0.33–0.67%). In contrast, in the PF-23_{high} population, germination occurred in the control and in all irradiated treatments (0.33–2.33%), except at the highest dose of 300 Gy (*figure 1*).

In PF-23_{high}, germination progressed at a faster rate compared to PF-23_{low} – more than 20% of seeds had germinated by the 105th day, particularly in the 25 and 50 Gy doses (28.0–30.0%). The majority of seeds in PF-23_{high} germinated by the 120th day, which was 15 days earlier than in PF-23_{low}, where peak germination occurred only by the 135th day. This indicates higher metabolic activity and a faster response to stimulatory exposure in the more viable PF-23_{high} population.

Irradiation with the lowest dose of 25 Gy had a stimulatory effect on both populations, though to different extents: an increase of 6.67% in PF-23_{high} and 2.34% in PF-23_{low} compared to the control. With increasing doses (50–100 Gy), the proportion of germinated seeds in PF-23_{low} decreased compared to both the control and the 25 Gy treatment (down to 56.33–59.67%), while in PF-23_{high} a high germination level was maintained (71.33–72.67%) (*figure 1*).

At 125 Gy, a marked divergence was observed: PF-23_{low} showed suppression (46.33%), whereas PF-23_{high} exhibited the strongest stimulatory effect (77.33%). At high irradiation doses (200–300 Gy), PF-23_{low} displayed pronounced inhibition of germination, while PF-23_{high} retained high viability (58.67–59.00%) (*figure 1*). It was found that in PF-23_{high}, irradiation at 25–125 Gy resulted in earlier germination compared to the control, while in PF-23_{low} only the 25 Gy dose provided a moderately higher germination rate than the control (*figure 1*).

Statistical analysis (Multiple Range Tests, $p \leq 0.05$, Tukey's HSD and LSD) revealed that although the mean germination values in the 50, 75, and 100 Gy treatments for PF-23_{low} were lower than those in the control and the 25 Gy treatment, these differences were not statistically significant ($p \leq 0.05$) (*figure 2*). Similarly, no statistically significant differences were found between the 25–125 Gy treatments and the control in the PF-23_{high} population (*figure 2*).

At the same time, for PF-23_{low}, germination in the high-dose treatments (200–300 Gy) was significantly lower than in the control, whereas in PF-23_{high}, germination in these variants was at the level of the control and significantly lower than at 25, 100, and 125 Gy (*figure 2*).

Statistical analysis confirmed that for PF-23_{low}, only the control and the 25 Gy treatment provided significantly higher germination compared to the 200 and 300 Gy treatments (*figure 2*).

An analysis of Mean Daily Germination (MDG), Mean Germination Time (MGT), and Germination Rate Index (GRI) supported the differences in seed germination between populations of different initial viability: PF-23_{high} showed higher MDG and GRI values and shorter MGT across all treatments (*table 1*).

In PF-23_{high}, irradiation at 25 Gy produced a slight stimulatory effect, with minor increases in MDG and GRI and a slight reduction in MGT. However, starting from 50 Gy, there was a gradual decline in MDG and GRI along with increased MGT, indicating the onset of stress response. At 200 and 300 Gy, values dropped sharply: MDG decreased to 0.50–0.70, GRI to 0.63–0.84, and MGT reached its maximum, indicating a strong inhibitory effect of high doses (*table 1*).

In contrast, the PF-23_{high} population exhibited a positive response to irradiation at 25–125 Gy: MDG and GRI increased progressively, peaking at 125 Gy (1.55 and 2.00, respectively), while MGT remained in the range of 112.95–117.67 days – significantly lower than in PF-23_{low}, including its control (121.91). At high doses (200–300 Gy), PF-23_{high} values also declined, but remained higher than those of PF-23_{low} at comparable doses and were similar to the control values of the less viable population (*table 1*). This indicates higher resistance to X-ray irradiation and an enhanced capacity to compensate for radiation-induced stress in PF-23_{high}.

Overall, the results confirm that physiological responses to irradiation were strongly dependent on the initial viability of seeds. While PF-23_{low} demonstrated pronounced sensitivity and reduced germination rates even at moderate doses, PF-23_{high} exhibited stable adaptive responses, including accelerated germination and enhanced intensity under stimulatory exposures.

Thus, the reaction to irradiation directly depended on the initial viability of seeds. The PF-23_{high} population maintained a consistently high germination rate across the entire dose range up to 125 Gy and showed particular sensitivity to the 125 Gy dose (peak stimulatory effect). Even at 300 Gy, germination remained high (>50%). PF-23_{low} exhibited only a slight increase in germination at 25 Gy and, starting from 100 Gy, seed germination declined relative to the control. At doses ≥125 Gy, germination of beech seeds in PF-23_{low} was significantly lower than in PF-23_{high}.

The PF-23_{high} population demonstrated greater tolerance to X-ray irradiation during stratification. In contrast, PF-23_{low} showed high sensitivity to doses ≥100 Gy and no statistically significant positive response to low irradiation doses.

An analysis of beech seedling survival under solarium conditions showed that irradiation had a pronounced negative effect on seedling adaptation regardless of the initial viability of the population. Both populations – PF-23_{low} and PF-23_{high} exhibited a sharp decline in the number of viable seedlings within 3–6 weeks post-germination (*figure 3 a, b*).

At early stages (1–2 weeks post-germination), seedling survival was relatively high in the 25 Gy and control treatments, especially in PF-23_{high}. However, this effect proved temporary. By the 6th and especially the 10th week, almost all seedlings derived from seeds irradiated at 50 Gy and above had died. Even in the control, there was a noticeable reduction in the number of viable seedlings, particularly in the PF-23_{low} population, indicating generally low resistance (*figure 3 a, b*).

Therefore, irradiation adversely affected not only initial seed germination but also further adaptation and survival of beech plants under solarium conditions. Neither population showed a stable adaptive response to increasing doses, suggesting high sensitivity of beech to X-ray irradiation during the early stages of ontogenesis.

Our findings on the radiosensitivity of European beech during early ontogenetic stages are consistent with several studies on the effects of ionizing radiation on seed germination in other species. For example, Hong M.J., et al. (2018) reported that low doses of gamma radiation up to 10 Gy stimulate germination and seedling growth in wheat, resembling the hormetic responses we observed in *F. sylvatica* under X-ray exposure (i.e., enhanced germination in the PF-23 high population at 25–125 Gy) (Hong M.J. *et al.*, 2018).

Similarly, recent studies on European beech seeds from the same nature reserve (Plaiul Fagului State Nature Reserve, Republic of Moldova) indicate that X-ray doses ranging from 50 to 300 Gy reduce viability by approximately 40–50%, supporting our conclusions regarding the significant population decline in PF-23low under higher doses (Elisovetcaia D. *et al.*, 2024).

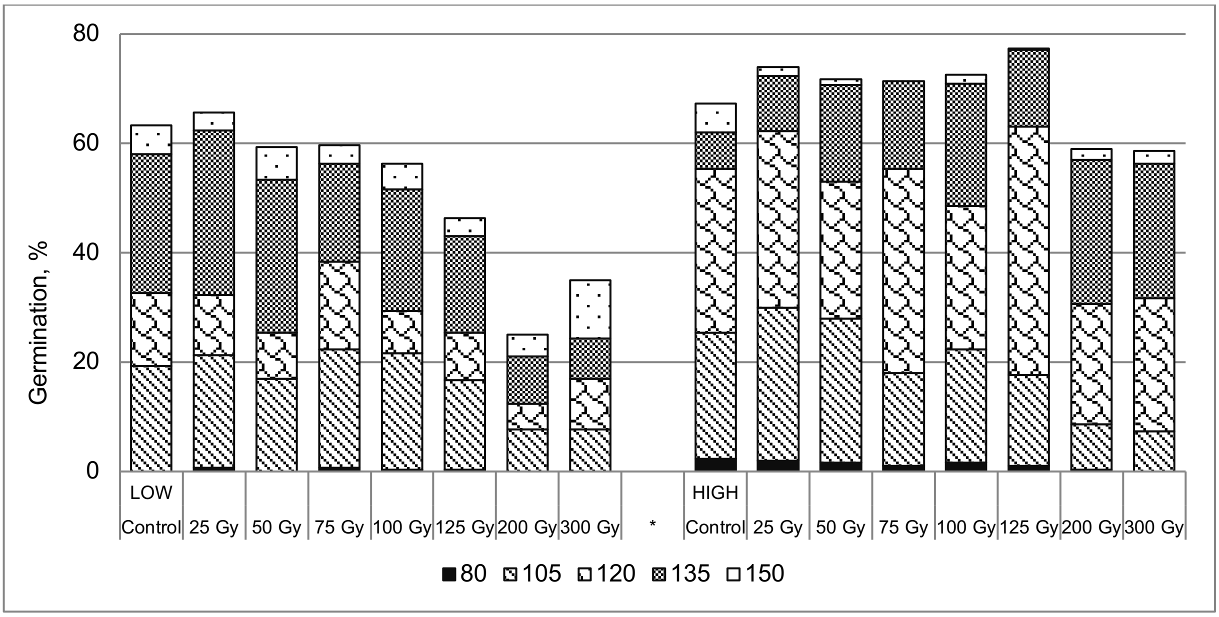


Figure 1 **Germination dynamics of beech seeds from the Plaiul Fagului-23 populations with low and high initial viability exposed to different X-ray irradiation doses during stratification under controlled laboratory conditions**

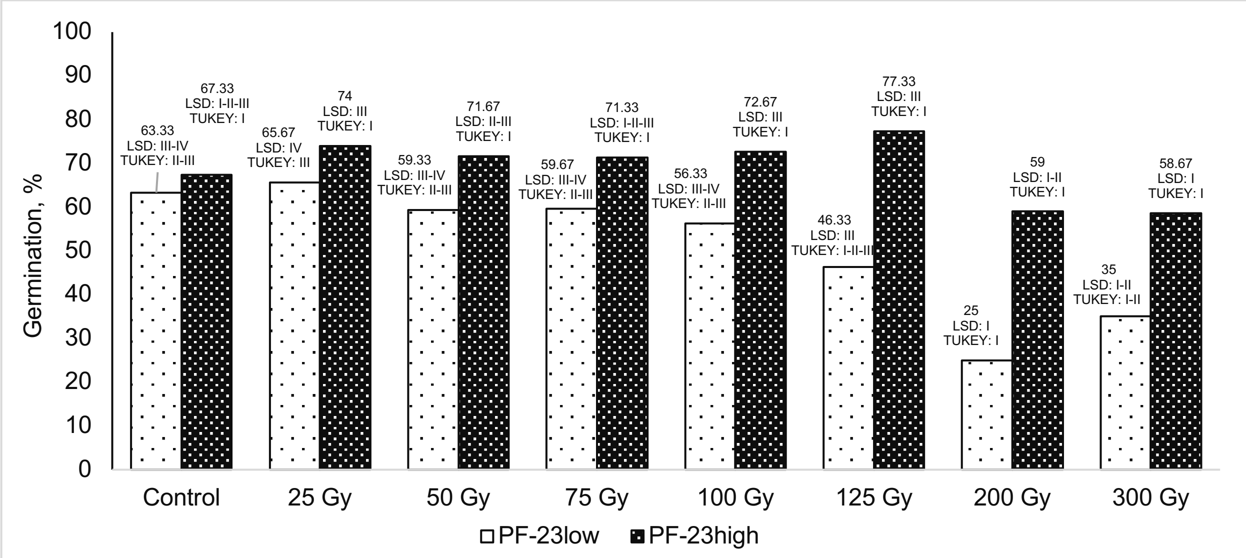
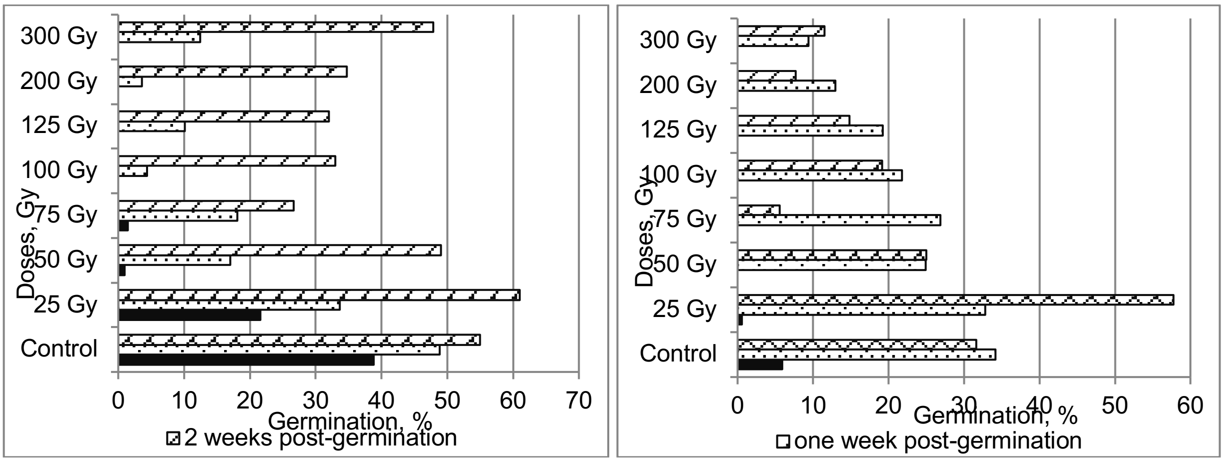


Figure 2 **Significance of differences in total germination percentage of beech seeds from the Plaiul Fagului-23 population with low and high initial viability exposed to different irradiation doses assessed using Multiple Range Tests (LSD and Tukey HSD)**

Table 1

Germination indices of beech seeds from the Plaiul Fagului-23 population with low and high initial viability exposed to different doses on the 150th day of stratification

Dose of irradiation	$PF\text{-}23_{low}$			$PF\text{-}23_{high}$		
	Mean daily germination (MDG)	Mean germination time (MGT)	Germination rate index (GRI)	Mean daily germination (MDG)	Mean germination time (MGT)	Germination rate index (GRI)
Control	1.27	121.91	1.60	1.35	114.19	1.80
25 Gy	1.31	120.90	1.68	1.48	112.95	1.99
50 Gy	1.19	123.80	1.48	1.43	115.48	1.89
75 Gy	1.19	118.38	1.56	1.43	116.14	1.86
100 Gy	1.13	119.66	1.46	1.45	117.67	1.88
125 Gy	0.93	120.00	1.19	1.55	116.95	2.00
200 Gy	0.50	123.37	0.63	1.18	122.93	1.46
300 Gy	0.70	127.68	0.84	1.17	124.26	1.43



a b

Figure 3 **The effect of X-ray radiation doses on the germination dynamics and plant adaptation of Plaiul Fagului-23 populations with low (a) and high (b) initial viability under solarium conditions**

Overall, the comparisons indicate that low to moderate irradiation doses may lead to a slight stimulatory effect, particularly in populations with higher initial viability. However, this effect remains limited and does not persist under higher or prolonged exposures. These observations contribute to the general knowledge of radiosensitivity in beech seeds during early developmental stages and can serve as a basis for further studies on stress resilience and adaptive potential.

CONCLUSIONS

The study demonstrated that the physiological response of European beech (*Fagus sylvatica* L.) seeds and seedlings to X-ray irradiation is strongly influenced by the initial viability of the seed population. The high-viability population (PF-23_{high}) showed enhanced germination performance and greater tolerance to irradiation up to 125 Gy, including earlier and more uniform germination. In contrast, the low-viability population (PF-23_{low}) exhibited lower total germination percentage and was more sensitive to increasing doses, especially ≥100 Gy. Despite these differences, both populations experienced severe post-germination seedling mortality under solarium conditions, particularly at doses of 50 Gy and above. These findings may suggest a generally high radiosensitivity of European beech during early ontogenetic development, with limited adaptive capacity under combined radiation and environmental stress.

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