

EFFECT OF X-RAY RADIATION ON THE VIABILITY OF EUROPEAN BEECH SEEDS

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Summary. *Fagus sylvatica* seeds from the "Plaiul Fagului" Natural Reserve, Republic of Moldova (PF-23) were treated in peeled (without pericarp) and unpeeled (with pericarp) form with X-ray doses from 25 to 425 Gy. No linear dependence of the viability of both peeled and unpeeled seeds on the radiation dose was established using the hydrogen peroxide test (HP). The use of the Indigo-carmin test (IC) made it possible to reveal a linear dependence of the viability of beech seeds (with pericarp) on the irradiation dose of X-rays. X-ray treatment in doses from 50 to 300 Gy reduced seed viability in the range from 39.53 to 48.84% (limit 50%); X-rays treatment above 600 Gy reduced seed viability by 67.44% or more.

Keywords: *Fagus sylvatica*, seeds, viability, X-ray, irradiation.

Introduction

Current climatic conditions may be limiting for natural regeneration of *Fagus sylvatica* in some areas of its distribution, especially at the southeastern edge of its range, where the likelihood of extreme heat and drought increases significantly (Bolte et al., 2016; Muffler et al., 2021). Successful beech regeneration is critical for population maintenance and range expansion. Muffler et al. (2021) previously found that seed germination success of *Fagus sylvatica* increased with increasing air temperature and decreased with decreasing air temperature, while establishment and survival of seedlings were impaired under warmer and drier conditions. The high plasticity of early life history traits found in European beech, regardless of seed origin, may provide short-term establishment (Muffler et al., 2021). However, due to the low survival rate of beech seedlings found in dry and hot conditions, this plasticity may not be enough to ensure beech regeneration in the future. Long-term plant responses to thermal shifts, changes in moisture regimes, insolation, and the spread of pathogens, accompanied by physiological and genetic mutations, can be stretched over several generations (Alberto et al., 2013; Richardson et al. 2014; Oddou Muratorio et al., 2021; Prozherina and Nakvasina, 2022). Spontaneous mutations in nature occur with an extremely low frequency (10^{-6}), which makes the process of selecting useful traits in plants tedious. One of the ways to successfully increase genetic variability is the selection of radiation mutations, which has been used for a number of agricultural and forest plants for over 100 years (Ma et al., 2021). Radiation mutation breeding is usually divided into classical radiation mutation breeding, particle mutation breeding and cosmic radiation mutation breeding. Classical radiation mutation breeding methods mainly involve the use of X-rays and gamma rays. All major forest-forming species, including European beech, have a huge advantage for mutation breeding over agricultural crops, since they are wild species unaffected by breeding and have huge genetic potential.

Currently, mutation breeding studies are carried out with both fruit and forest crops (Kornienko and Netsvetov, 2016; Holonec et al. 2021). Holonec et al. (2021) found that the maximum germination of acorns (86.67%) of *Quercus petraea* (Matt.) Liebl. was induced by gamma irradiation at a dose of 2 Gy, which caused the highest value of germination index (2.02), of germination energy (7.7) and of germination speed (7.24), and also increased the diameter (2.65 cm) and height of seedlings (11.25 cm) compared to the control. Other authors have studied the remote effects of radiation after seed irradiation on the architecture of the aboveground part of the common oak *Quercus robur* L. (Kornienko and Netsvetov, 2016). The scientific literature has described in detail the diverse effects of gamma irradiation on various traditional crop species and

for species cultivated *in vitro* (Reznik et al., 2021, Bliznyuk et al., 2023; Ghanim, 2024). However, research on the effects of beech irradiation is very limited (Lof et al., 2005).

The aim our investigation was to select doses of X-rays irradiation that would cause the death of 25, 50 and 75% of the beech seed population in order to determine which doses to *Fagus sylvatica* seeds could be considered high, moderate and low.

Materials and methods

The objects of the study were *Fagus sylvatica* seeds collected in the fall of 2023 in the "Plaiul Fagului" Natural Reserve, Republic of Moldova (hereinafter referred to as PF-23), the viability of which, determined by hydrogen peroxide method (HP) was 78.0-86.0%. The study was conducted in laboratory conditions in the Laboratory Natural Bioregulators of the Institute of Genetics, Physiology and Plant Protection, Moldova State University, Chisinau, Republic of Moldova.

X-ray treatment. PF-23 seeds were treated according to the relevant protocol (Protocol for X-ray mutagenesis of plant material: seed, 2010; FAO/IAEA, 2018) with an RS-2400-Q Rad Source Technologies ionizer-radiator, at different X-ray doses. The seeds (100 seeds in each variant) were packed into labeled paper bags, which were placed in the middle of the containers and covered with grain to minimize air space. After irradiation with different doses, the viability of seeds was determined – in the first experiment, using the HP test; and in the second experiment, using the indigo-carmin test (IC).

Hydrogen peroxide test (HP). Each variant contained 100 seeds, in four replications. Seeds were selected using random sampling techniques and soaked in 100 ml of 1.0% hydrogen peroxide solution (H_2O_2) at temperature 25 °C for a day. The pericarp tip of the seed was cut deep enough to expose but not damage the root tip. After cutting, the seeds were immediately placed in 100 ml of 1.0% H_2O_2 to prevent drying out and incubated in the dark at 25 °C to allow the radicles elongation to occur. After three and six days of incubation, the seeds were returned to fresh 1.0% H_2O_2 solution. On the 7th day, all seeds were evaluated and the test was completed. The grouping of seeds into classes through the hydrogen peroxide test was carried out according to Kerkez et al. (2018) and all seeds were divided into three classes: evident – radicle > 2 mm long; slight – radicle \leq 2 mm long and none – no radicle protrusion (Fig. 1). Data were expressed as the proportion of "evident" and "slight evident" germinates to the total number of seeds used in the test (Kerkez et al., 2018; Sharma and Sibi, 2020; Elisovetcaia et al., 2021).

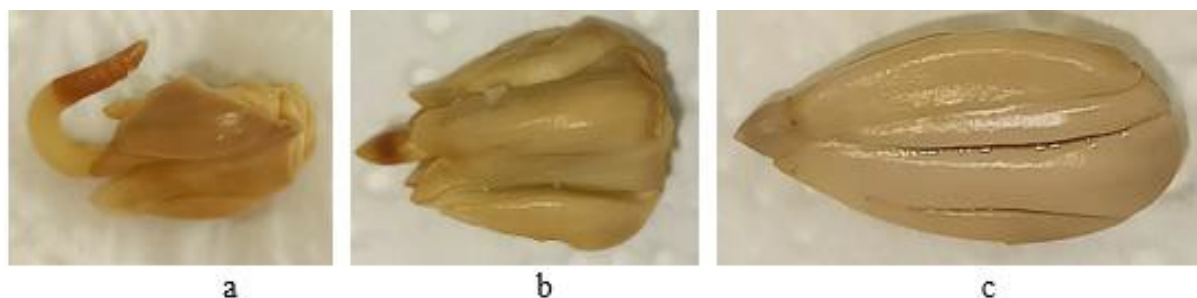


Figure 1. The degree of germination of radicle in *Fagus sylvatica* seeds in the HP test (a – completely viable seed with radicle more than 2 mm; b – conditionally viable seeds with radicle equal to 2 mm; c – ungerminated non-viable seeds) (photo Dina Elisovetcaia)

Indigo-carmin test (IC). Each variant contains 4 replicates of 25 seeds each. Duration of testing: 2 days, temperature +30 °C (Gugala, 2002). The beech seeds (100 seeds for four replications of 25 seeds each selected using random sampling techniques) are pre-soaked in Petri dishes in 50 ml distilled water for 18-24 hours at temperature 30 °C, after which the pericarp and brown seed coat is carefully removed. Staining is carried out with a 0.5% aqueous solution of

indigo carmine (disodium salt of indigo-5,5'-disulfonic acid) for 2 hours in the thermostat. After the staining time has passed, the seeds are thoroughly washed with water. IC test stains dead seed tissues dark blue or gray-blue (Fig. 2, C-E). Living seed tissue remains white or light blue (Fig. 2, A-B). A light blue border around the radicle is allowed (Fig. 2 A).

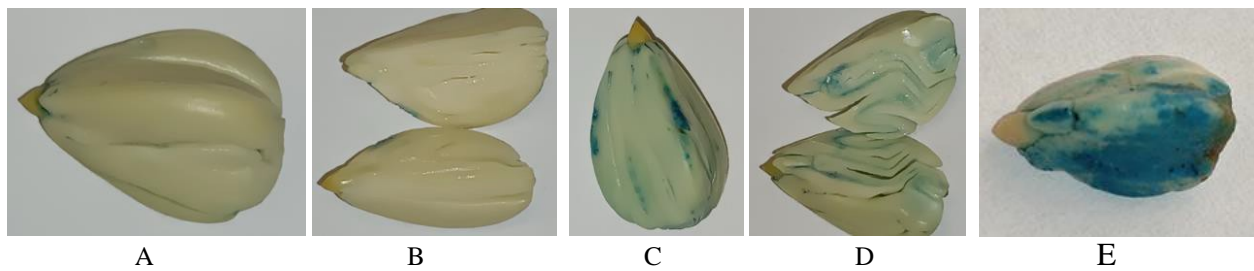


Figure 2. Distribution of beech seeds in groups according to the degree of fabric staining: A-B – viable seeds and C-E non-viable seeds (photo Dina Elisovetcaia)

Results and discussions

It was necessary to select not only the X-ray doses, but also a method for determining the viability of seeds that would most objectively reflect the real picture. Initially, the experiment was conducted on the PF-23 seeds with and without pericarp with X-ray doses from 25 to 425 Gy. Viability was determined by the HP method (which had proven itself well previously) by germinating the seeds in a 1% hydrogen peroxide solution (Table 1). It was found that the HP test does not provide a linear dependence of the decrease in viability according to X-ray data (Fig. 3). Moreover, even at the maximum dose of 425 Gy, the decrease in the viability of beech seeds did not exceed 37.18% (Fig. 3). The absence of a linear dependence of the viability of beech seeds on the dose was obvious both for seeds with and without pericarp. We believe that one of the reasons for such sinusoidal fluctuations in viability is the high heterogeneity of seeds, i.e. the resistance of individual seeds to irradiation, which results in a nonlinear relationship. Another reason for the sinusoidal fluctuation may be that the surviving roots of irradiated seeds can germinate significantly even with significant damage to the cotyledons in the HP test. At the same time, such sinusoidal of the results suggests the induction of mutations in irradiated seeds.

In order to obtain an objective picture of the death of living seed tissue during irradiation, we conducted an experiment to study the effect of different X-ray doses on seeds with pericarp using the IC test. As a result, it was calculated that X-rays from 25 to 35 Gy reduce seed viability by 25%; X-rays from 50 to 300 Gy reduced seed viability in the range from 39.53 to 48.84% (limit 50%); X-rays above 600 Gy reduced seed viability by 67.44% or more (limit 75%) (Fig. 4).

Table 1
Viability of *Fagus sylvatica* seeds from the "Plaiul Fagului" Natural Reserve, Republic of Moldova (PF-23) in laboratory conditions under the influence of X-ray doses, HP test, 2024

Variants (X-ray doses, Gy)	Viability (HP test), %	
	without pericarp	with pericarp
Control	78.00	78.00
25	76.00	51.00
125	68.00	65.00
225	50.00	54.38
325	52.13	51.00
425	57.81	49.00

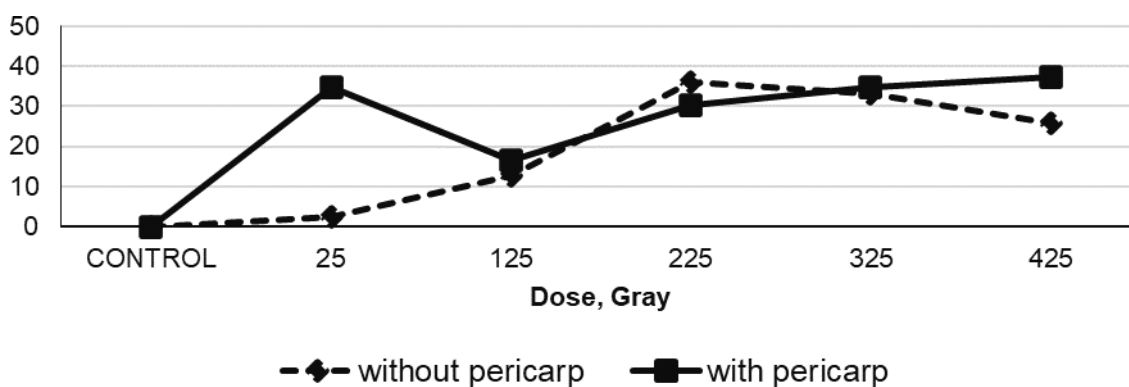


Figure 3. Decrease in the viability of *Fagus sylvatica* seeds from the "Plaiul Fagului" Natural Reserve, Republic of Moldova (PF-23) in laboratory conditions under the influence of X-ray doses, HP test, 2024

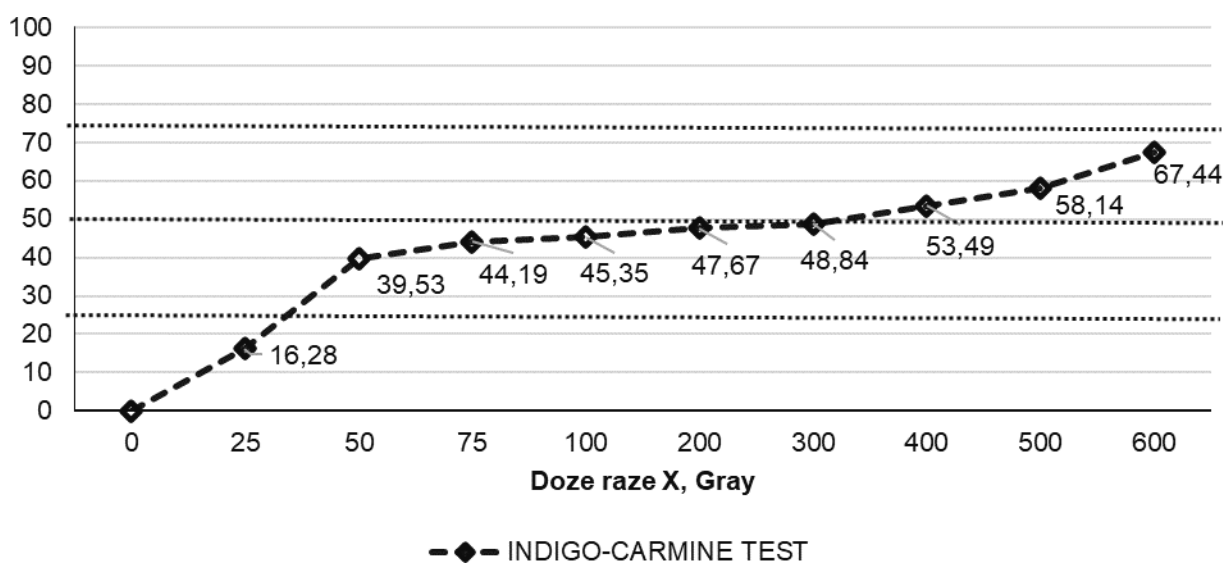


Figure 4. Decrease in the viability of *Fagus sylvatica* seeds from the "Plaiul Fagului" Natural Reserve, Republic of Moldova (PF-23) in laboratory conditions under the influence of X-ray doses, IC test, 2024

Statistical analysis of the data showed a linear relationship between the viability of seeds and X-ray irradiation doses when determining the IC test (Table 2). It was noted that there was no significant difference between seed irradiation with a dose of 75 g and 100-200 Gy. Also, no significant difference was found between doses of 100-200 Gy and a dose of 300 Gy (Table 2). Thus, in the specified range from 75 to 300 Gy, mutations with varying degrees of resistance can be obtained, with an insignificant decrease in viability in a small range.

Thus, for further study of the effect of radiation on beech seeds during stratification, a dose limit of X-ray radiation from 50 to 300 Gy was chosen.

Table 2

Distribution of variants in groups according to the confidence levels in dependencies on the viability of *Fagus sylvatica* seeds from the "Plaiul Fagului" Natural Reserve, Republic of Moldova (PF-23) under the influence of X-ray doses, IC test, 2024

No.	Variants (X-ray doses, Gy)	Viability, %	Distribution of variants into groups depending on the confidence levels																							
			$\alpha = 0.05$ +/- limits 3.06847								$\alpha = 0.01$ +/- limits 3.61242								$\alpha = 0.001$ +/- limits 3.87456							
			1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
1.	Control	86.00							X								X								X	
2.	25	72.00						X								X								X		
3.	50	52.00					X							X								X				
4.	75	48.00				X							X								X					
5.	100	47.00			X	X						X	X							X	X					
6.	200	45.00			X	X						X	X							X	X					
7.	300	44.00			X							X								X						
8.	400	40.00		X							X								X							
9.	500	36.00		X						X									X							
10.	600	28.00	X						X									X								

Conclusions

The use of two tests – hydrogen peroxide (HP) and indigo-carmin (IC) showed that the IC test is better suited for identifying the viability of beech seeds exposed to X-ray irradiation, as it allows for identifying a linear dependence and prevents distortion of the results. The HP test can produce distortions, since even heavily damaged seeds can produce significant root growth when stimulated by peroxide. The dose range between 50 and 300 Gy is of interest for studying the effect on beech seed germination. In this range, seed viability decreases within 39.53-48.84%, which leaves more than half of the seeds alive, and at the same time provides opportunities for obtaining plants with useful mutations.

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