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Original article

## THE INFLUENCE OF DEUTERIUM-DEPLETED WATER LARSEN D100 ON SEED GERMINATION PARAMETERS IN CEREAL, OILSEED, AND LEGUME CROPS

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### *Abstract*

**Background.** The study aimed to investigate the species-specific effects of deuterium-depleted water (Larsen D100, <100 ppm D) on seed germination and early seedling growth parameters of key agricultural crops (barley, wheat, rapeseed, and lentil) under controlled in vitro conditions. Seeds were germinated in Petri dishes on a fabric material moistened with either distilled water (control) or Larsen D100. The experiment was set up in a completely randomized design (CRD) with several biological replications. After 10 days, the following key parameters were assessed: germination rate, seedling length, and fresh biomass. Data were statistically processed using Student's t-test or the Mann-Whitney U-test ( $p < 0.05$ ). Treatment with deuterium-depleted water had a pronounced and species-specific influence. A significant stimulatory effect was recorded in barley: germination increased by 20%, seedling length by 72%, and biomass by 17% compared to the control. Lentil showed a sharp increase in length (222%) and biomass (64%) of seedlings, although germination rate remained unchanged. In contrast, rapeseed exhibited inhibition of biomass accumulation (a 38% reduction), while wheat parameters did not change. Deuterium-depleted water acts as a powerful metabolic modulator, but its effects are strictly species-specific-ranging from significant stimulation to inhibition. The obtained data highlight the potential for its use in pre-sowing seed treatment for crops such as barley and lentil but strongly caution against universal application without mandatory prior species and cultivar testing. The observed effects are associated with isotope-mediated changes in the kinetics of key enzymatic and osmotic processes in the early stages of plant development.

**Purpose.** Of the study was to evaluate the effect of deuterium-depleted water (Larsen D100) on germination efficiency (germination rate) and initial growth vig-

or (length and fresh weight) of seedlings in four economically important species of agricultural crops representing cereals (barley, wheat), oilseeds (rapeseed), and legumes (lentil).

**Materials and methods.** Seeds of spring barley (*Hordeum vulgare* L.), soft wheat (*Triticum aestivum* L.), rapeseed (*Brassica napus* L.), and lentil (*Lens culinaris* Medik) were sterilized and germinated in Petri dishes on filter paper moistened with distilled water (control, pH=5.8, EC<5  $\mu$ S/cm) or deuterium-depleted water (Larsen D100, <100 ppm D). The experiment was set up according to a completely randomized design (CRD) with several biological replications (Petri dishes). Germination was carried out for 10 days at a temperature of  $23\pm 1^\circ\text{C}$ . Germination rate, seedling length, and fresh weight were recorded. Statistical analysis was performed using Statistica 10.0 software employing parametric (Student's t-test) or non-parametric (Mann-Whitney U-test) methods for independent samples at a significance level of  $p<0.05$ .

**Results.** The application of Larsen D100 water led to a complex, species-specific response. For barley, significant stimulation was found for all parameters. Germination rate increased by 20%, seedling length by 72%, and fresh weight by 17%. For lentil, no effect on germination rate (~97%) was characteristic, but a sharp increase in length (222%) and fresh weight (64%) of seedlings was observed. Data analysis for rapeseed showed no significant effect on germination and length, but a substantial reduction (38%) in seedling fresh weight. No statistically significant effect on any of the measured parameters of wheat was found.

**Conclusion.** Deuterium-depleted water Larsen D100 is not a universal biostimulant but represents a powerful species-specific modulator of germination and early growth processes. It significantly enhances the initial growth vigor in barley and lentil but inhibits biomass accumulation in rapeseed, while wheat demonstrates a neutral response. This specificity underscores the critical importance of mandatory preliminary testing on each target crop and cultivar before considering any agro-economic application. The detected effects are likely rooted in isotope-induced modifications of the kinetics of metabolic reactions, energy metabolism, and osmotic processes.

**Keywords:** deuterium-depleted water; seed germination; plant growth; isotope effect

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Научная статья

## **ВЛИЯНИЕ ВОДЫ С ПОНИЖЕННЫМ СОДЕРЖАНИЕМ ДЕЙТРИЯ LARSEN D100 НА ПОКАЗАТЕЛИ ПРОРАСТАНИЯ СЕМЯН ЗЛАКОВЫХ, МАСЛИЧНЫХ И БОБОВЫХ КУЛЬТУР**

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### **Аннотация**

**Обоснование.** Исследование направлено на изучение видоспецифичных эффектов воды с пониженным содержанием дейтерия (Larsen D100, <100 ppm D) на показатели прорастания семян и раннего роста проростков ключевых сельскохозяйственных культур (ячменя, пшеницы, рапса и чечевицы) в контролируемых условиях *in vitro*. Семена проращивали в чашках Петри на тканном материале, увлажненной либо дистиллированной водой (контроль), либо водой Larsen D100. Опыт был заложен в полном рандомизированном дизайне (CRD) с несколькими биологическими повторностями. Через 10 дней оценивали следующие ключевые параметры: всхожесть, длину проростка и сырую биомассу. Данные были статистически обработаны с использованием t-критерия Стьюдента или U-критерия Манна-Уитни ( $p < 0,05$ ). Обработка дейтерий-дефицитной водой оказывала выраженное и видоспецифичное влияние. Достоверный стимулирующий эффект был зарегистрирован у ячменя: всхожесть увеличилась на 20%, длина проростков - на 72%, а биомасса - на 17% по сравнению с контролем. У чечевицы наблюдалось резкое увеличение длины (на 222%) и биомассы (на 64%) проростков, хотя всхожесть осталась неизменной. Напротив, у рапса было выявлено ингибирование накопления биомассы (снижение на 38%), в то время как параметры пшеницы не изменились. Вода с пониженным содержанием дейтерия выступает в роли мощного метаболического модулятора, но ее эффекты строго видоспецифичны - от значительной стимуляции до ингибирования. Полученные данные подчеркивают потенциальную возможность ее использования для предпосевной обработки семян таких культур, как ячмень и чечевица, но настоятельно предупреждают от универсального применения без обязательного предварительного видового и сортового тестирования. Наблюдаемые эффекты связаны с изотоп-опосредованными изменениями кинетики ключевых ферментативных и осмотических процессов на ранних стадиях развития растения.

**Цель.** Цель исследования заключалась в оценке влияния воды с пониженным содержанием дейтерия (Larsen D100) на эффективность прорастания (всхожесть) и энергию начального роста (длину и сырую массу) проростков у четырех экономически важных видов сельскохозяйственных культур, представляющих злаки (ячмень, пшеница), масличные (рапс) и бобовые (чечевица).

**Материалы и методы.** Семена ярового ячменя (*Hordeum vulgare* L.), мягкой пшеницы (*Triticum aestivum* L.), рапса (*Brassica napus* L.) и чечевицы (*Lens culinaris* Medik) проращены в чашках Петри на тканном материале, увлажненной дистиллированной водой (контроль, pH=5,8, ЭП<5 мкСм/см) или водой с пониженным содержанием дейтерия (Larsen D100, <100 ppm D). Опыт был заложен по принципу CRD с несколькими биологическими повторностями (чашки Петри). Проращивание проводили в течение 10 дней при температуре  $23 \pm 1^\circ\text{C}$ . Учитывали всхожесть, длину проростка и сырую массу. Статистический анализ проводили в программе Statistica 10.0 с использованием параметрического (t-критерий Стьюдента) или непараметрического (U-критерий Манна-Уитни) методов для независимых выборок при уровне значимости  $p < 0,05$ .

**Результаты.** Применение воды Larsen D100 привело к сложному, видоспецифичному ответу. Для ячменя выявлена достоверная стимуляция по всем параметрам. Всхожесть увеличилась на 20%, длина проростков - на 72%, сырая масса - на 17%. Для чечевицы характерно отсутствие эффекта на всхожесть (~97%), но резкое увеличение длины (на 222%) и сырой массы (на 64%) проростков. Анализ данных по рапсу показал отсутствие достоверного эффекта на всхожесть и длину, но существенное снижение (на 38%) сырой массы проростков. Статистически значимого влияния на какие-либо из измеряемых параметров пшеницы не выявлено.

**Заключение.** Вода с пониженным содержанием дейтерия Larsen D100 не является универсальным биостимулятором, а представляет собой мощный видоспецифичный модулятор процессов прорастания и раннего роста. Она достоверно усиливает стартовую энергию роста у ячменя и чечевицы, но ингибирует накопление биомассы у рапса, в то время как пшеница демонстрирует нейтральный ответ. Эта специфичность подчеркивает критическую важность обязательного предварительного тестирования на каждой целевой культуре и сорте перед рассмотрением какой-либо агрономической аппликации. Обнаруженные эффекты, вероятно, коренятся в изотоп-индуцированных модификациях кинетики метаболических реакций, энергетического метаболизма и осмотических процессов.



**Ключевые слова:** вода обедненная дейтерием; прорастание семян; рост растений; изотопный эффект

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## Introduction

Sustainable agricultural development in the face of climate change and growing population pressure requires innovative approaches to increasing the productivity of staple crops [1]. Research on crop growth and adaptation has become a fundamental factor in ensuring global food security. Since the Green Revolution, while the world's population has doubled, the area under grain crops has remained virtually unchanged, while crop yields have tripled. These indicators demonstrate the effectiveness of breeding programs, agronomic practices, and the introduction of innovative technologies [2]. In modern conditions, there is a need to ensure food security for a population close to 10 billion people [3] in the face of climate change, soil degradation, and limited water resources. These challenges require further intensification of scientific research and improvement of agricultural technologies. Seed germination is a critical stage in plant ontogenesis, determining further development and crop formation [4]. Germination energy, germination rate and initial growth vigor directly affect plant density, their competitiveness against weeds and resistance to abiotic stress [5; 6].

Water plays a fundamental role in the germination process, acting not only as a universal solvent and medium for biochemical reactions but also as an active participant in metabolic processes [7]. In recent decades, the biological effects of deuterium-depleted water (DDW) have been actively studied in medicine and microbiology. Existing evidence suggests that deuterium is a natural regulator of cell growth, capable of controlling the balance between mitochondrial oxidation and reduction [8]. Natural deuterium concentrations and a stable D/H ratio are vital conditions for maintaining normal cell growth rates and signaling, as well as for regulating the physiological functions of living systems [9]. In 1993, Somlyai et al. [9] noted the potential effect of DDW in suppressing tumor growth in xenograft-bearing mice. Since then, numerous publications have examined the antitumor effects of DDW both in vitro and in vivo, as well as its potential to prolong the survival of cancer patients and alleviate symptoms [10].

However, in contrast to medical and microbiological studies, the effects of deuterium-deficient water on higher plants, and particularly on the critical stage of seed germination, have been extremely poorly studied. An analysis of the existing literature reveals fragmentary and inconsistent data on the effects of DDW on plants [13]. Existing studies often focus on a single species or a limited set of parameters, preventing generalizable conclusions. A key shortcoming is the lack of comprehensive comparative studies within a single experimental protocol that would encompass representatives of different botanical families and economic groups.

Therefore, the aim of this study was to comprehensively assess the impact of deuterium-reduced water (Larsen D100, <100 ppm D) on germination and early growth parameters of four economically important agricultural crops: barley and wheat (cereals), rapeseed (oilseed rape), and lentils (legumes). Reducing the deuterium concentration in germination water will significantly impact physiological processes at a critical early stage of plant ontogenesis, and this effect will be highly species-specific, conditioned by the metabolic characteristics of each of the studied crops.

**Purpose.** The aim of the study was to evaluate the effect of deuterium-reduced water (Larsen D100) on germination efficiency and initial growth vigor (length and fresh weight) of seedlings in four economically important crop species representing cereals (barley, wheat), oilseeds (rapeseed) and legumes (lentils).

### **Materials and Methods**

Seeds of spring barley (*Hordeum vulgare* L.), common wheat (*Triticum aestivum* L.), rapeseed (*Brassica napus* L.), and lentil (*Lens culinaris* Medik) were visually inspected before the experiment to ensure uniformity and absence of damage. The experiment was designed using the CRD principle. For the control variant, 4 Petri dishes were prepared (one for each of the indicated crops), each containing 25 seeds. For the experimental variant, 12 Petri dishes (biological replicates) were prepared, each containing 25 seeds. Distilled water was used as the control variant; pH = 5.8, electrical conductivity (EC) = <5 µS/cm. The experimental variant consisted of Larsen D100 deuterium-reduced drinking water (produced in Russia; the manufacturer's declared deuterium content is <100 ppm). Sterile Petri dishes (90 mm in diameter) were lined with three sterile cotton pads and moistened with 10 ml of the appropriate aqueous solution. The seeds were laid out with sterile tweezers, avoiding their contact with each other. The closed dishes were placed in a climatic chamber at a constant tem-

perature of  $23 \pm 1$  °C. During the 10-day germination period, the discs were additionally moistened daily with 2 ml aliquots of the respective solutions to maintain humidity. On the 10th day, the following parameters were recorded: seed germination, seedling length (measured from the root collar to the tip of the coleoptile or hypocotyl using a digital caliper), and fresh weight.

Statistical analysis was performed using Statistica 10.0 software (StatSoft, USA). Separate analyses were performed for each plant species. The normality of distribution of quantitative parameters (seedling length and weight) was tested using the Shapiro-Wilk test. Comparisons of quantitative parameters between the control and experimental groups were performed using the Student's t-test for independent samples (under the conditions of normal distribution and homogeneity of variances) or the Mann-Whitney U-test. Data are presented as the arithmetic mean and standard deviation ( $M \pm SD$ ). Differences were considered statistically significant at  $p < 0.05$ .

## Results

In the course of the experiment, the effect of an aqueous solution with a reduced deuterium content (Larsen D100) on key parameters of seed germination of four agricultural crops was assessed (Figs. 1-4; Table 1). The results demonstrate a species-specific response to the applied treatment.

Table 1.

**The effect of water with reduced deuterium content (Larsen D100)  
on seed germination parameters**

Culture	Group	Germination rate, % (average)	Seedling length, cm (mean $\pm$ SD)	Seedling fresh weight, g (mean $\pm$ SD)	p-value (length)	p-value (mass)
<i>Hordeum vulgare</i> L.	Control	64	$3.6 \pm 2.0$	$0.133 \pm 0.033$	<0.001	0.004
	Test (D100)	84	$6.2 \pm 2.4$	$0.156 \pm 0.030$		
Lens culinaris Medik	Control	96	$0.9 \pm 0.5$	$0.058 \pm 0.015$	<0.001	<0.001
	Test (D100)	97	$2.9 \pm 1.5$	$0.095 \pm 0.020$		
Brassica napus L.	Control	92	$1.3 \pm 0.6$	$0.016 \pm 0.009$	0.25	0.004
	Test (D100)	84	$1.4 \pm 0.7$	$0.010 \pm 0.001$		
Triticum aestivum L.	Control	56	$5.0 \pm 1.7$	$0.107 \pm 0.018$	0.51	0.20
	Test (D100)	69	$5.5 \pm 3.3$	$0.098 \pm 0.035$		

Treatment with Larsen D100 water did not have a statistically significant effect on the germination of *Lens culinaris Medik* seeds, which remained at an exceptionally high level in both groups: 96% in the control and 97% in the experimental group ( $p = 0.74$ ). However, a reliable stimulating effect on growth characteristics was observed. The average length of seedlings in the experimental group was more than three times higher than in the control group (2.9 cm versus 0.89 cm;  $p < 0.001$ ). Similar positive dynamics were observed for seedling biomass: their fresh weight in the experimental group was significantly higher (0.095 g versus 0.058 g in the control;  $p < 0.001$ ).

In *Brassica napus L.* seeds, the treatment showed an inhibitory effect on biomass accumulation. Despite a statistically insignificant difference in germination (92% in the control and 84% in the experiment;  $p = 0.32$ ) and seedling length (1.27 cm and 1.44 cm, respectively;  $p \approx 0.25-0.31$ ), the fresh weight of seedlings in the experimental group was significantly lower than in the control (0.010 g versus 0.016 g;  $p < 0.01$ ).

In *Triticum aestivum L.* seedlings, no statistically significant effect of experimental water was found on any of the studied parameters. Germination rates (56% in the control and 69% in the test;  $p = 0.22$ ), average length (5.0 cm and 5.5 cm), and fresh weight (0.107 g and 0.098 g) of seedlings did not differ significantly between groups ( $p > 0.19$  for all comparisons).

The greatest stimulating effect of treatment with Larsen D100 water was recorded in *Hordeum vulgare L.* The treatment significantly increased seed germination by 20% compared to the control (84% versus 64%;  $p = 0.003$ ). A significant increase in seedling length (6.2 cm in the experiment versus 3.6 cm in the control;  $p < 0.01$ ) and their fresh weight (0.156 g versus 0.133 g;  $p < 0.01$ ) was also observed.

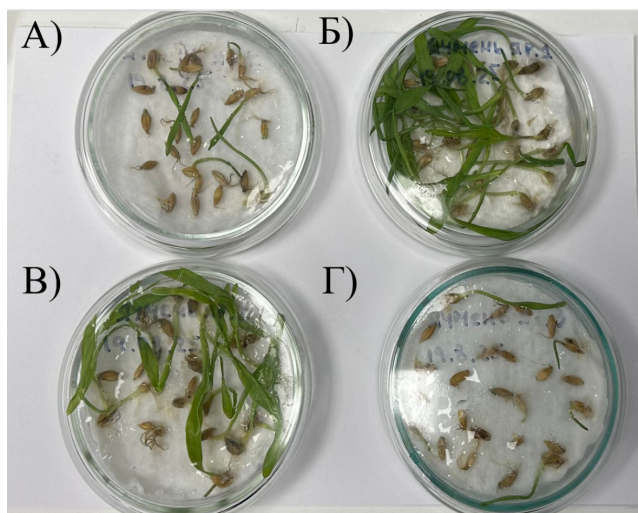
Thus, water with a reduced deuterium content had a pronounced multidirectional effect on the germination and initial growth of the studied crops: from significant stimulation in *Hordeum vulgare L.* and *Lens culinaris Medik* to inhibition of biomass accumulation in *Brassica napus L.* The studied factor did not have a significant effect on *Triticum aestivum L.*

## Conclusion

The study demonstrates complex and distinctly species-specific effects of deuterium-reduced water (Larsen D100) on the physiological processes of seed germination and initial seedling growth. The data obtained do not support a universal stimulating effect, but rather indicate the activation of multidirectional metabolic pathways in different species, requiring in-depth interdisciplinary anal-

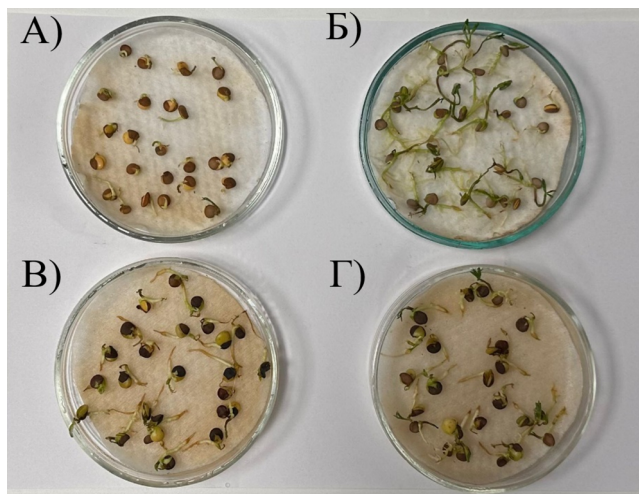
ysis. Statistical analysis of the data confirmed a significant interaction between the factors “crop type” and “treatment type” ( $p < 0.001$  for seedling length and weight), highlighting the complexity and non-linearity of the observed effects.

The most significant positive dynamics were observed in *Hordeum vulgare* L. (Fig. 1), which affect both germination energy (germination) and initial growth vigor (seedling length and weight), suggests that deuterium-deficient water acts as a metabolic modulator. The primary hypothesis explaining this effect is the influence of the isotopic composition of water on the kinetics of biochemical reactions. Reducing the concentration of deuterium, which has twice the mass of protium, increases the rate and efficiency of enzymatic processes, particularly those associated with the hydrolysis of endosperm storage substances in cereals.

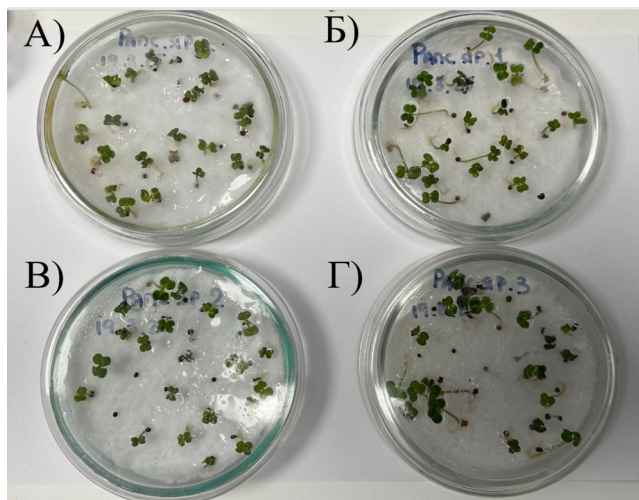


**Fig. 1.** Appearance of *Hordeum vulgare* L. seedlings 10 days after germination in distilled water (A) and in Larsen D100 water (B, C, D)

In *Lens culinaris* Medik, stimulation was observed exclusively in morphometric parameters, not in germination. This may indicate that the biochemical pathways responsible for overcoming seed dormancy and initiating cell division (which determine germination) are less sensitive to isotopic composition than the processes of subsequent cell elongation and differentiation (which determine growth). The effect is related to the influence on aquaporin function and the osmotic gradient, facilitating cell hydration and subsequent elongation.



**Fig. 2.** Appearance of *Lens culinaris* Medik sprouts 10 days after germination in distilled water (A) and in Larsen D100 water (B, C, D)



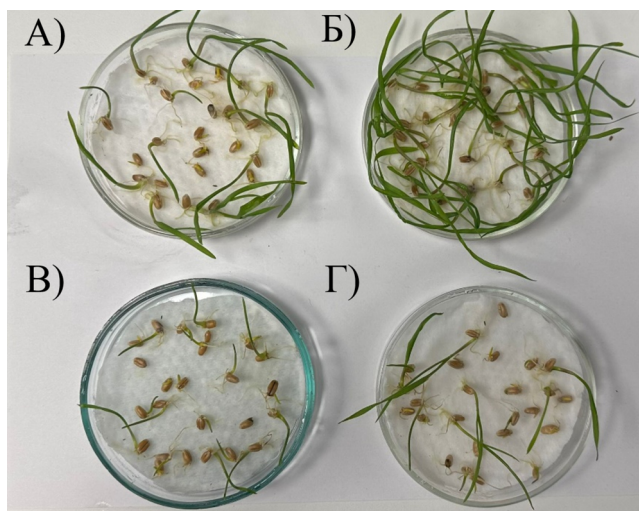
**Fig. 3.** Appearance of *Brassica napus* L. sprouts 10 days after germination in distilled water (A) and in Larsen D100 water (B, C, D)

The inhibitory effect on biomass accumulation in *Brassica napus* L. is of particular interest. For this crop, the reduction in deuterium levels disrupted the



delicate balance of isotope effects in key metabolic cycles, such as lipid biosynthesis, which is crucial for oilseed crops. An alternative explanation is the induction of mild oxidative stress, forcing the plant to expend energy resources to compensate, ultimately reducing growth productivity in the early stages.

The neutral response of *Triticum aestivum* L. highlights the role of the genetic background and selection history of the species, which led to the fixation of alleles that ensure high metabolic stability regardless of slight variations in the isotopic composition of the water.



**Fig. 4.** Appearance of *Triticum aestivum* L. seedlings 10 days after germination in distilled water (A) and in Larsen D100 water (B, C, D)

Sensitivity to deuterium correlates with the species' initial metabolic activity, genome size, cell division rate, and seed biochemical composition (protein, carbohydrate, and lipid ratios). Cereals (*Hordeum vulgare* L., *Triticum aestivum* L.) and legumes (*Lens culinaris* Medik) exhibit different responses, which may be due to differences in the mechanisms for mobilizing reserve nutrients. Evolutionary adaptation of species to specific ecological niches with different isotopic compositions of natural waters could also contribute to the observed specificity.

The results of the multivariate analysis confirmed that the effect of Larsen D100 water treatment significantly depends on the crop species ( $p < 0.001$  for all main parameters). This underscores the need for a species-specific approach when assessing such effects. However, it should be noted that statistical analy-

sis based on individual seeds (rather than average values across replicates) can lead to potentially false-positive results due to intra-group correlation. Future studies plan to increase the number of biological replicates (Petri dishes) for each condition and conduct analyses based on average values, which will enhance the reliability of the conclusions.

The obtained results open up prospects for developing new pre-sowing seed treatment techniques for crops such as *Hordeum vulgare* L. and *Lens culinaris* Medik, aimed at improving field germination and initial development. However, the pronounced negative effect on *Brassica napus* L. is a serious warning against the general use of this technology without preliminary species-specific and varietal testing.

The main limitation of this study is the lack of direct instrumental measurements of the final deuterium concentration in the working solutions, which prevents a definitive attribution of the observed effects to this factor. Furthermore, the experiment was conducted under controlled in vitro laboratory conditions, which can mitigate the complex soil-microbial interactions occurring in natural agrocenoses.

To gain a deeper understanding of the mechanisms underlying the observed effects, it is necessary to monitor deuterium content (using isotope mass spectrometry) not only in water but also in seedling biomass to assess its biochemical composition. Gene expression associated with the metabolism of reserve nutrients, stress response, and cell division must be assessed. Validation of the obtained results in the field will assess not only initial growth but also final plant productivity. Screening of different varieties within each species will be necessary to identify the most responsive genotypes.

Thus, deuterium-reduced water represents a powerful tool for controlling the germination process, but its use requires a highly differentiated approach and a deep understanding of the underlying physiological mechanisms.

**Conflict of interest information.** The authors declare that they have no conflict of interest.

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