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UALITY CHANGES DURING STORAGE OF BEECH SEEDS COLLECTED FROM DIFFERENT PLACES

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Summary: In this study, the changes in quality of *Fagus sylvatica* seeds collected in 2020 from different places of the Republic of Moldova (Plaiul Fagului, Hîrjauca) and Ukraine (Zakarpattia) on the third year of storage were evaluated. Beech seeds quality maintenance depended on the values of moisture, initial viability and seeds germination. The seeds from the Plaiul Fagului with a higher initial germination 76.7-84.0% and lower moisture content (8.69-9.24%) slightly reduced their quality during storage, by 1.0-2.3%.The germination indices such as germination rate, mean daily germination, average seed germination time, total time of seed germination, were improved by treatment of stored seeds with the natural bioregulator, genistifolioside. Treatment with genistifolioside also contributed to the elongation of seed roots by 17-24% in all variants.

Keywords: *Fagus sylvatica, seed moisture, viability, germination, bioregulator genistifolioside.*

Introduction. European beech forests (*Fagus sylvatica*) are one of the national treasures of the Republic of Moldova. At present, special attention is paid to the restoration and maintenance of beech forests in the republic. For successful artificial reforestation of beech, it is desirable that the planting material be genetically diverse for better adaptation and survival of the new population in changing conditions. The annual collection of beech seeds is problematic, since the most productive years occur once every 3-7 years, sometimes much less often – once every 15-20 years (Gavranović et al., 2018; Övergaard et al., 2010). In this regard, the creation of a seed stock, the optimal storage of beech seeds and the use of bioregulators to increase the viability of seeds is very relevant.

The aim was to study changes in viability and germination of beech seeds after storage and the effect of the bioregulator genistifolioside on increasing germination indices during stratification.

Materials and methods. The experiments were carried out at the Laboratory of Natural Bioregulators of the Institute of Genetics, Physiology and Plant Protection, Moldova State University. The objects of research were the seeds of *Fagus sylvatica*, collected in the autumn of 2020 in three places: The Scientific Reserve "Plaiul Fagului" and the Hîrjauca forest, Republic of Moldova (RM) and in the Zakarpattia forest, Ukraine (UA). Seeds from October 2020 were stored at the temperature of +4±1 ^{o}C and a relative humidity to the air of 45±5% in plastic bags. Seeds were manually separated from impurities and obviously empty and infested seeds before storage. The study was carried out on the 3rd year of storage of seeds.

Seed weight and seed traits. The mass of seeds was determined by random counting – 100 seeds in 3 repetitions. Each repetition was weighed separately and the weight was recorded in grams (g). Random samples of 40 seeds per each collection (population from different places) were taken for morphometric analysis. Seed length and width were measured by digital calliper with a precision of 0.01 mm. Standard deviations of the average weight of 100 seeds and of the average length and width of 40 seeds were calculated using Microsoft Excel.

Seed moisture was measured using the RADWAG moisture analyser and classic oven drying method (ISTA, 1985; Rao et al., 2006). For each determination, 3 grams of seeds were used. Three determinations were made for each lot of seeds (places). Moisture was determined using the RADWAG moisture analyser by gradual drying with a halogen lamp at a temperature of 160 °C to a constant weight (if the change in weight does not exceed 1 mg in 10 sec). The classic method consisted in drying the crushed seeds in a drying oven at a temperature of +103±2 °C for 17 hours.

Seed viability was determined by two methods: using a 1.0% hydrogen peroxide (HP) solution and 0.5% solution of the 2.3.5-triphenyltetrazolium chloride (TTC). Each variant consisted of four repetitions of 25 seeds each. In the TTC test, the total viability percentage was calculated based on the number of viable and conditionally viable seeds, and in the HP test, it was calculated from the sum of all germinated seeds with roots equal to or greater than 2 mm (Elisovetcaia et al., 2021).

Seed stratification. The beech seeds selected from three places, after determining the viability, were subjected to stratification. Each variant contained 4 repetitions, 50 seeds each. The seeds were soaked in water (control) and a 0.01% solution of natural growth regulator (bioregulator) genistifolioside (GL) for 24 hours, and then were subjected to stratification at the temperature of $+4\pm1^{o}C$ in coconut substrate (pH=5.5). Seed germination was monitored two times a week. Germination defined as the appearance of the first roots (Fig. 1).

In the process of stratification of *F. sylvatica* seeds, the following indices of germination were determined: germination rate, mean germination time, mean daily germination, coefficient of speed germination (Bezděčková et al., 2013).

Figure 1. Monitoring the germination of *Fagus sylvatica* seeds during stratification.

Statistical analysis. Values of the seeds viability were presented as the mean of four replicates. The length of the roots was calculated for each variant for all germinated seeds. Pearson coefficient was calculated using the Excel program. The obtained data were analysed by Tukey's HSD test (HSD – Honestly Significant Difference) at level $p\leq0.05$ with Statgraphics Plus 5.0.

Results and discussion. Analysis of the obtained data showed that beech seeds from different places differed significantly in their parameters – length, seed width and weight (Table 1). The largest mean value of a seed length and width was in Plaiul Fagului population, while the lowest mean value was in Zakarpattia population. Mathematical analysis showed that the seed width of the Plaiul Fagului population differs significantly from the seed width of the Hîrjauca and Zakarpattia populations ($p\leq0.05$). There were no significant differences between the width of seeds from Hîrjauca and Zakarpattia populations. A direct correlation was found between the size of the seeds (length and width) and their weight – larger seeds had more weight (Table 1).

Table 1. Weight and moisture of *Fagus sylvatica* seeds from different places ~~(populations)~~ of the 3rd year of storage

Variants (places)	Seed traits 3rd year storage		100 seeds weight 3rd year storage, g	Seed moisture before storage (RADWAG analyser), %	Seed moisture 3rd year storage,%	
	length, mm	width, mm			RADWAG analyser	Classic method
Plaiul Fagului (RM)	17.29±1.23	9.48±1.27	25.58±0.48	7.50±0.03	8.72±0.10	7.34±1.69
Hîrjauca (RM)	16.35±1.61	8.04±1.06	23.62±0.31	9.05±0.04	8.78±0.20	8.17±0.49
Zakarpattia (UA)	15.39±1.11	7.64±0.91	19.33±0.47	9.56±0.02	9.16±0.79	8.78±0.20
+/- limits (Tukey HSD mean)	0.59	0.60	0.52	-	-	
Pearson correlation coefficient	-	-	-	-	0.7822	

Our data are in good agreement with those previously obtained by other authors. For example, Gavranovich et al. (2018) established that there is a direct relationship between the weight of seeds and their size. Correlation analysis showed a positive and statistically significant correlation between beech seeds sample mass and seed width (R^2 =0.5875); no significant correlation was found between seed weight and seed length (Gavranovich et al., 2018). In our studies, a direct correlation was found both between the width of the seed and their weight, and between the length of the seed and their weight (Table 1).

The yield of beech seeds is influenced by many factors, such as environmental conditions (abiotic and biotic), altitude and micro-site (Overgaard, 2010; Gavranović et al., 2018). There are also studies proving that the meteorological conditions of previous years significantly affect the yield of beech seeds (Öwergaard et al., 2007; Piovesan and Adams, 2001; Drobyshev et al., 2010). Therefore, the place of growth, namely soil, climatic and other conditions certainly affect the size and weight of the collected seeds. This was partly confirmed by our studies – the seeds collected in one year, but in different places, differed significantly both in weight and in the length and width of the seed (Table 1).

The seeds were dried before storage to a moisture content of approximately 8-10% (Table 1). Compared to 2020, in the 3rd year of storage, the moisture content of the seeds has changed and differs depending on the origin. In the batch of seeds from Plaiul Fagului (RM), the moisture index increased by an average of 1.22%, while in populations Hîrjauca (RM) and Zakarpattia (UA) it decreased by 0.27-0.40%. However, despite this, the moisture content of the Plaiul Fagului (RM) seeds remained the lowest. The moisture values of seeds from the same places determined by two different methods (RADWAG moisture analyser and classic method) had no significant difference and correlated well with each other. Pearson's correlation coefficient is 0.7822 (Table 1).

It well known that for storing beech seeds at +5 °C, the optimum moisture content is 15-16 % (Hong 1996). These parameters allow maintaining the maximum germination of beech seeds for at least one to two years. However, some researchers recommend drying beech seeds to a moisture content of 8-9 % and storing them at +3-4 °C (Ratajczak & Pukacka 2005), which significantly increases both the storage period (up to 3-4 years) and seed germination. In this case, a 45 % humidity regime is preferred over 75 %. These data have been confirmed by our earlier studies (Elisovetcaia et al. 2020, Elisovetcaia et al., 2021). The present study also confirms that the lower the moisture contents of stored seeds, the higher their viability during storage (Table 1, 2). The seeds from the Plaiul Fagului with a higher initial germination 76.7-84.0% and lower moisture content (8.69-9.24%) slightly reduced their quality during storage, by 1.0-2.3%. At the same time, seeds with an initial germination below 70% and higher moisture content of 9.16-9.56% more significantly reduced their quality in the third year of storage (Table 2). Data analysis showed that there is a high degree of correlation between the two methods for determining the viability of seeds – TTC and HP. Pearson's correlation coefficients were 0.9955 for 2020 and 0.9838 for 2023 (Table 2).

In general, the viability of *Fagus sylvatica* seeds from the three studied places of Plaiul Fagului and Hîrjauca (RM), and Zakarpattia (UA) for the third year of storage did not reveal a significant decrease in seed viability compared to their initial values ($LSD_{0.05}$= 6.8, p<0.05= 4,66E-08), except for Hîrjauca seeds.

Table 2. Viability of *Fagus sylvatica* seeds tested before and at the 3rd year of storage

Variants (places)	Viability of seeds, %			
	Before storage (2020)		3rd years storage (2023)	
	TTC	HP	TTC	HP
Plaiul Fagului (RM)	84.0	76.7	81.7	75.7
Hîrjauca (RM)	68.0	67.8	61.7	49.7
Zakarpattia (UA)	70.0	68.0	72.0	59.0
Pearson correlation coefficient	0.9955		0.9838	
$LSD_{0.05}$	6.8			

Following the viability determinations, the beech seeds selected from three localities were subjected to stratification at a temperature of +4±1°C. The first germinated seeds were identified on the 34th day of cold stratification, and at 104th day the monitoring of seed germination was stopped. The highest rates of seed germination during cold stratification were obtained in samples of seeds from Plaiul Fagului and Hîrjauca treated with solution of genistifolioside (Table 3). It was noted that pre-germination treatment of these beech seeds with genistifolioside contributed to a significant increase in the germination rate of seeds by 6.0-12.5%, mean daily germination by 0.1-0.3, average seed germination time by 2.1-15.5 days, daily seed germination (up to 12.5-37.5%) compared to the control (Table 3).

Considering that the germination time of a large batch (1000 and more) of beech seeds is rather long and amounts to 90-140 days (Bonner and Leak, 2008; Elisovetcaia et al., 2021), stimulation of daily seed germination from 0.8 (control) up to 0.9-1.1 seeds (genistifolioside) leads to a significant reduction in the total seed germination time by 11.5-15.6 days for seeds from Plaiul Fagului and Hîrjauca in comparison with control.

Table 3. Germination indices of *Fagus sylvatica* seeds during 104 days of cold stratification

Variants (places)	Variant	Germination rate, %	Mean daily germina-tion	Mean germi-nation time, days	Coefficient of speed germination
Plaiul	Control	41.5±4.7	0.8	58.1	1.7

Fagului	GL 0.01%	47.5±5.7	0.9	56.0	2.0
Hîrjauca	Control	42.0±4.6	0.8	56.9	1.3
	GL 0.01%	54.5±5.1	1.1	72.4	1.8
Zakarpattia	Control	48.5±5.4	0.9	44.0	2.3
	GL 0.01%	43.0±4.9	0.8	42.8	1.9

The seeds from Zakarpattia treated by bioregulator showed no significant lower germination rate compared to the control, at average 5.5% (Table 3). These results can be explained by the fact that the seeds from Zakarpattia initially had more parameters that are vulnerable: lighter weight, higher humidity and low initial viability (Table 1, 2). Therefore, the additional load in the form of a biostimulator genistifolioside led to some negative impact.

The treatment of seeds with the bioregulator genistifolioside also contributed to an increase in the length of roots in germinated seeds (by 17-24%) in comparison with the control in all studied variants (Table 4).

Table 4. Effect of the bioregulator genistifolioside on the length of roots of *Fagus sylvatica* seeds during stratification

Variants (places)	The length of the roots, cm					
	Control			Genistifolioside (GL) 0,01%		
	Max	Min	Average	Max	Min	Average
Plaiul Fagului	3.1	0.5	1.28	3.6	0.3	1.09
Hîrjauca	2.0	0.5	1.12	3.7	0.2	1.21
Zakarpattia	2.5	0.4	1.03	3.7	0.2	1.21

Conclusions. Our results show that the decrease in the viability of seeds of *F. sylvatica* of various origins during storage at a temperature of +4 ± 2°C significantly depended both on the moisture content of the seeds before storage and on the quality of the seeds themselves. The higher the seed viability before storage and the lower the moisture content (7.50%), the better the seed quality was preserved. The bioregulator genistifolioside (0.01%) significantly affects the germination parameters of beech seeds during stratification – increases germination rate, mean daily germination and speed germination, and reduces the mean and total germination time. Seeds of poor quality may experience an increased load when treated with a bioregulator, therefore, the bioregulator genistifolioside should be used differentially to increase the germination of seeds of various qualities.

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