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QUALITY ESTIMATION OF EUROPEAN BEECH (*FAGUS SYLVATICA* L.) SEEDS FROM THE EASTERN EUROPE

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INTRODUCTION

The increase in the forest areas is one of the keys to the successful conservation and restoration of biodiversity, maintaining ecological balance and increasing the country's wealth. Beech forests are one of the national treasures of the Republic of Moldova. There are several national reserves in the country, in which forest beech Fagus sylvatica predominates along with such crops as ash *Fraxinus* L., hornbeam *Carpinus* L., sessile and pedunculate oak *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L., linden *Tilia* L., and some other deciduous species trees (maple Acer L., poplar *Populus* L., sallow *Salix* L.) (Postolache and Postolache, 2010; Republic of Moldova. 6th National Report on Biological Diversity, 2019).

To replenish the gene bank of beech, seeds collected in the Republic of Moldova are usually used, as well as seeds of beech growing in the neighboring territory – Ukraine and Romania. In the Republic of Moldova, the main beech plantations (1,441.9 ha from 2,062.8 ha) are concentrated in protected areas: in the scientific reserves "Plaiul Fagului" (d. Ungheni) and "Codrii" (d. Lozova), as well as in protected area "Harjauca – Sipoteni" (d. Calaras), in the landscape reservations "Cabaiesti – Parjolteni" (d. Calaras) and "Cazimir – Milesti" (d. Nisporeni), in forest nature reserves "Cabac" (d. Nisporeni), "Sadova" and "Bogus" (d. Calaras) (Postolache and Postolache, 2010). In Ukraine and Romania, there are significantly more places for collecting beech seeds. In Ukraine, European beech grows mainly in the Carpathian Mountains (Vološčuk, 2014). In Romania, European beech also grows in the Carpathians, but other than that in the Western Romanian mountains, the foothills of the Carpathian and on the Transylvanian plateau. In the form of islands outside of a continuous range it is found in Muntenia, Oltenia, Drobeta-Turnu Severin (Județul Mehedinți), in Dobrudja (Tulcea), etc. (Biriş, 2014).

The physical, physiological and genetic quality of the seeds has a direct impact on their germination and the successful growth of seedlings. The physical quality of seeds is related to characteristics such as their size, color, age, state of seed coat and damages by diseases and pests. Physiological quality characterizes seed maturity, moisture content, vigor and germination capacities. Plant genetic resources allow adaptation to various environmental conditions, including climate change. Taken together, all of the above factors can ultimately significantly affect the seeds germination and seedling viability. In addition, the conditions for drying and storing seeds also significant affect beech germination processes (Bezděčková and Matějka, 2015). Therefore, it is important to know the initial potential of germination for each batch of seeds.

To determine the quality of the harvested seeds, as well as to calculate the sowing rate, high-quality rapid tests are needed to identify the viability of seeds. The method of seed germination requires a long-time interval; therefore, it is most advisable to use tetrazolium tests and treatment with hydrogen peroxide (Tylkowski, 2002; Sharma and Sibi, 2020). The results of tetrazolium test direct correlated with total seed germination (Elisovetcaia et al., 2020).

It is well known that the seed production of beech is rather irregular, with a wide variation over the years - from three to fifteen years (Gavranović et al., 2018). Seed yield is highly dependent on annual climate variability. Also, climate change significantly affects the survival rate of beech forests and the viability of seeds. For these reasons, it becomes necessary, firstly, to control the beech seeds vigor and maintain of their viability for a long time – from two years or more. Secondly, it is required to constantly research for the adaptation of beach from different areas to more warm conditions than near mountains. The pedoclimatic conditions of the Republic of Moldova can provide the best results in obtaining more resistant to hot the young beech forest. The purpose of this work was to evaluate the quality of beech seeds obtained from various beech growing areas, differing in height above sea level, forest stand and trees age.

MATERIALS AND METHOD

Seed collection. Seeds of European beech (*Fagus sylvatica* L.) were collected from different regions of the Republic of Moldova, Romania and

Ukraine in 2020. In total, ten collection places were selected, several for each country (Fig. 1, Table 1). Seed collection places differed in altitude (height above sea level), stand and trees age. The seeds were collected in a pure beech forest (Humosu and Ivano-Frankivsk) and in mixed stands with conifers and broadleaved species such as *Picea abies* (L.) H. Karst.; *Abies alba* Mill., *Carpinus betulus* L.; *Quercus robur* L., *Quercus petraea* (Matt.) Liebl. and others (Table 1).

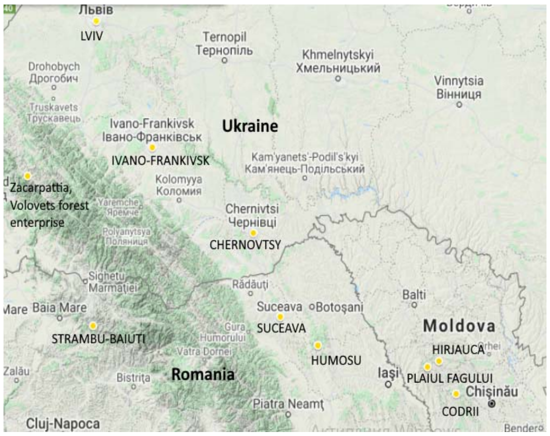

Figure 1. Places of the Republic of Moldova, Ukraine and Romania, where seeds of European beech *Fagus sylvatica* L. were collected in 2020.

Moisture content. The moisture content (W, %) of the seeds calculated on the fresh weight, was determined using two replicates per batch of beech seeds, stripped (cleaned) from pericarp and crushed in a laboratory mill for 10 s (15 g in each replicate), which were then dried 180 min at 105 ± 2 °C (ISTA rules, 2006). Moisture of seeds was determined by formula 1:

$$W = \frac{M-M_1}{M-M_2} \times 100 \quad (1),$$

Where W – moisture, %;
M – weight of the weighing bottle with seeds before drying, g;
M_1 – weight of empty weighting bottle, g;
M_2 – weight of weighing bottle with seeds after drying, g.

After determining the moisture content of beech seeds in all batches they were dried to a water content of 8-10 % and stored sealed in plastic bags, which were placed in plastic boxes or paper bags at a temperature of +4±2 °C.

The viability of seeds was determined by tetrazolium and hydrogen peroxide tests (Gugala, 2002; ISTA, 2003; Procházková and Bezděčková, 2008; Getachew, 2010; Kerkez et al., 2018) and the assessment was made according to the ISTA Rules (ISTA, 2003).

Table 1. Places characteristics of beech seeds collections

Forest stand composition	Altitude, m a.s.l.	Area, ha	Actual age in 2020, years
Republic of Moldova			
Plaiul Fagului – The "Plaiul Fagului" scientific reservation, compartment 26C			
8FS2CB	260	4.1	128
Hirjauca – Calarasi state forest enterprise, Hirjauca forest district, compartment 42A			
8FS2CB	240	4.3	105
Codrii – The "Codrii" scientific reservation, compartment 54I			
5FS1QP1TC2CB1 DS	240	4.1	105
Romania			
Suceava – Suceava forest department, Adincata forest district, compartments 2A, 2C, 3A			
6FS1QP3CB	420	27.6	120
Humosu – "Humosu Old Growth Beech Forest", Iashi forest department, Harlau forest district, compartment 64			
10FS	470	41.2	200
Strambu-Baiut – Maramures forest department, Strambu-Baiut forest district, compartment 63			
6FS4AA	840	26.8	190
Ukraine			
Lviv – Zolochevsky state forest enterprise, Nestyukivske forest district, compartment 38(15)			
10FS+CB+QR	450	15.0	81
Chernovtsy – Berehomet state forest enterprise, Dolishniy Shepit forest district, compartment 22(8)			
5FS4AA1PA	750	5.0	110
Ivano-Frankivsk – State Enterprise "Nadvirnyanske Lisove Gospodarstvo", Nadvirna district, compartment 6(1)			
10FS	575	5.0	110
Zacarpattia – Volovets forest enterprise, Zhdeniievo forest district, compartment 14(60)			
8FS2PA	750	7.3	190

Note: FS – *Fagus sylvatica* L.; CB – *Carpinus betulus* L.; QR – *Quercus robur* L.; PA – *Picea abies* (L.) H. Karst.; AA – *Abies alba* Mill., QP – *Quercus petraea* (Matt.) Liebl.; TC – *Tilia cordata* Mill.; DS – Diverse hardwood forest species

Tetrazolium test (TTC). 100 seeds for four replications of 25 seeds each selected using random sampling techniques were soaked in 50 ml of distilled water for 18-20 hours at temperature 20-25 °C. The pericarp removed and then the seeds were again soaked in distilled water for 6 hours at temperature 20-25 °C. After removing of brown seed coat the seeds were soaked for 10-12 hours in the dark at 25-30 °C in a 1.0% solution of 2,3,5-triphenyltetrazolium chloride (TTC) (ISTA, 2003).

In dependence of the seed staining (after opening the cotyledons fully), the seeds were distributed into three groups: a) the seeds with bright red staining, which are completely viable and give normal seedlings; b) partially stained seeds (with at least two-thirds of the basal part of the cotyledons stained) that may produce either normal or abnormal seedling; c) greyish stained or black seeds indicate the presence of a dead tissue in the seed, they are non-viable (Fig. 2).

Hydrogen peroxide test (HP). ***Soaking.*** 100 seeds for four replications of 30 seeds each selected using random sampling techniques were soaked in

100 ml of 1.0% hydrogen peroxide solution ($H_2 0_2$) at temperature 25 °C for a day. ***Cutting.*** Using a sharp scalpel, the pericarp tip of the seed was cut deep enough to expose but not damage the root tip. The cut was made at tilt angle (not straight across) to reduce radicle damage. After cutting, the seeds were immediately placed in 1.0% $H_2 0_2$ to prevent drying out. ***Incubation.*** 50 ml of 1.0% $H_2 0_2$ were poured into each of four dishes or glasses. Added 30 cut seeds per dish (glass) and incubated in the dark at 25 °C to allow the radicles elongation to occur. ***Assessment.*** After three days of incubation, seeds with "evident" and "slight evident" radicles were counted (Fig. 3). All seeds were returned to fresh 1.0% $H_2 0_2$ 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 Peterson (1983) and all seeds were divided into three classes: evident – radicle > 2 mm long; slight – radicle ≤ 2 mm long and none – no radicle protrusion. Data were expressed as the proportion of "evident" and "slight evident" germinates to the total number of seeds used in the test.

a b c

Figure 2. Distribution of *Fagus sylvatica* seeds into groups of viability depending on the degree of staining using the tetrazolium test (a – the seeds with bright red staining; b – partially stained seeds; c – greyish stained or black seeds and damaged seeds).

a b c

Figure 3. Distribution of beech seeds by classes depending on the degree of radicle germination using hydrogen peroxide test (a, b – radicle protrusion by more than 2 mm, c – radicle protrusion less than or equal to 2 mm).

Statistical analysis. Values were presented as the mean of four replicates ± standard deviation. The data analysis was processed by Excel.

RESULTS AND DISCUSSIONS

The moisture content of beech seeds collected in 2020 from different places of the Republic of Moldova, Romania and Ukraine varied significantly (Table 2). The lowest moisture content was observed in the seed batch collected in Lviv (9.41±0.01 %) and Ivano-Frankivsk (9.51±0.01 %) regions, the highest – seeds collected in Humosu, Romania and Hirjauca, Republic of Moldova, 21.09 and 20.20 %, respectively. The high moisture content was also noted in seed batch from the scientific reservations "Codrii" (17.40 %) and "Plaiul Fagului" (15.90 %), Republic of Moldova.

Storage moisture plays a key role in maintaining high seed viability. Drying seeds leads to a decrease in metabolism and, as a consequence, to a decrease in respiratory activity and preservation of nutrient substances (Gugala, 2002). It is known that for storing beech seeds at +5 °C, the optimum moisture content is 15-16 % (Kruk, 2017). 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 and 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). Thus, the viability of beech seeds obtained from Slovakia (Nitra), dried up to 8-9 %, when stored for a year at a temperature of +4 °C and a relative humidity of up to 50%, remained at a fairly high level – 69.5%. Therefore, before being placed for storage, all batches of seeds obtained by us from different places of the Republic of Moldova, Romania and Ukraine, the moisture content of which exceeded 16%, were dried in the open air at room temperature (+20 °C) until moisture of 8-10 % was reached.

Table. 2. Quality of *Fagus sylvatica* seeds from different locations

Variants	100 seeds weight, g	Seed moisture, %
Republic of Moldova		
Plaiul Fagului	30.20±1.87	15.90±0.03
Hirjauca	31.46±1.05	20.30±0.04
Codrii	23.57±0.34	17.40±0.03
Romania		
Suceava	25.50±0.50	10.65±0.02
Humosu	33.44±0.54	21.09±0.05
Strambu-Baiut	25.29±0.11	11.13±0.02
Ukraine		
Lviv	26.85±0.37	9.41±0.01
Chernovtsy	24.03±0.26	9.80±0.02
Ivano-Frankivsk	26.93±0.22	9.51±0.01
Zakarpattia	18.31±0.03	9.58±0.02

Based on the results of visual examination, it was revealed that the highest proportion of infected seeds (visually determined by the mycelium of the fungus on the surface of the pericarp) was observed in the batch collected in the "Humosu Old Growth Beech Forest" (Romania). We have found that seeds are most commonly damaged by fungi from the genus *Penicillium*, as well as by some other imperfect fungi. It is possible that the increased moisture content of the seeds (21.09 %) contributed to the accumulation and spread of infection during transportation and storage. In all batches of seeds from Ukraine, a significant proportion of empty or substantially immature ones was noted, a particularly high percentage was in the batch of seeds from Ivano-Frankivsk – more than 50 %. The highest damage by pests was observed in seeds from Lviv (up to 30 %). The smallest and lightest seeds were found in consignments from Ucraine – Zacarpattia (Volovets forest enterprise, Zhdeniievo forest district), the largest – from Romania – Humosu, and Republic of Moldova – Hirjauca and "Plaiul Fagului" (Table 2).

As a result of the experiments, it was found that the percentage of viability of seeds collected in the fall of 2020 and obtained by us from different places of the Republic of Moldova, Romania and Ukraine varied significantly in the range of 66.0-92.0 % for tetrazolium test and in the range of 50.7-76.7 % for hydrogen peroxide test (Table 3).

Table. 3. Viability of *Fagus sylvatica* seeds from different locations

Variants	Total number of viable (and conditionally viable) seeds, %	
	tetrazolium test	hydrogen peroxide test
Republic of Moldova		
Plaiul Fagului	84.0	73.4
Hirjauca	68.0	61.7
Codrii	88.0	68.3
Romania		
Suceava	92.0	76.7
Humosu	76.0	65.0
Strambu-Baiut	84.0	63.8
Ukraine		
Lviv	78.0	56.7
Chernovtsy	80.0	72.6
Ivano-Frankivsk	66.0	63.0
Zakarpattia	70.0	50.7
$LSD_{0.05}$	*8.2*	*14.8*

The tetrazolium test indirectly determines the respiratory activity of the cells that make up the seed tissues. Respiratory tissue can be found in the embryo of the seed, in cotyledons, root, and some other tissues of the seed (França-Neto and Krzyzanowski, 2019). Under the action of certain enzymes, the tetrazolium salt (TTC) in living tissues is reduced to form triphenylformazan (or formazan) – a stable, non-diffusible red substance. The decrease in TTC with the formation of formazan indicates that respiratory activity occurs in the mitochondria of the cells of the seed tissue, which are considered alive (França-Neto and Krzyzanowski, 2019). Thus, the red color of the seed tissue is a positive sign of viability by indirectly determining the respiratory activity at the cellular level. Non-viable seed tissues do not react with TTS and therefore do not stain.

Analysis of the data revealed no direct relationship between the proportion of viable seeds and their weight. The absence of a direct dependence on the weight of seeds was confirmed by us when determining the viability by both the tetrazolium and hydrogen peroxide methods (Pearson's coefficient was – 0.1078 and 0.2871 consequently). However, we found a moderate correlation between the age of beech trees and seed weight – Pearson's coefficient reached 0.3142. At the same time, a fairly close correlation was revealed between tetrazolium and hydrogen peroxide methods – the Pearson coefficient attain the value 0.6859. A direct relationship was also established between the increase in the proportion of empty seeds in the batch and the decrease in the Pearson coefficient: for batches of seeds from the Republic of Moldova and Romania, Pearson reached 0.8022 and 0.8209, respectively, and for Romania – only 0.4155. Mathematical analysis of the data of the tetrazolium test revealed a significant difference between the variants ($LSD_{0.05}$ = 8.2, $p \leq 0.05$). The highest results in terms of viability were shown by seeds from Suceava, "Plaiul Fagului", "Codrii" and Strambu-Baiut. When analyzing the data of the hydrogen peroxide test ($LSD_{0.05}$ = 14.8, $p \leq 0.05$), the highest level of viability was noted in the variants of a batch of seeds from Suceava, "Plaiul Fagului", Chernovtsy, "Codrii", Humosu, Ivano-Frankivsk and Strambu-Baiut.

We have established an inverse relationship between the weight of seeds and the height above sea level of growth of beech trees (-0.5258). The higher the plants grow, the lower the weight of the harvested seeds.

The data obtained show that the tetrazolium test for determining the viability of seeds is quite accurate and most fully characterizes the state of both the cotyledons and the radicle. The disadvantages of the method are the energy consumption for cleaning seeds from pericarp and coat, as well as the need for an expensive reagent. The method using hydrogen peroxide has the advantage that the 1% hydrogen peroxide used is readily available and inexpensive, and there is no need to clean the seeds. Despite the fact that the method takes longer (up to 7-8 days), already on the third day one can judge the quality of the seeds. Also, the size of the radicle seedling most accurately characterizes the strength of the seeds. The advantages include the possibility of sowing germinated seeds and obtaining seedlings. Also in the literature, many studies are devoted to the disinfecting properties of hydrogen peroxide (Szopinska, 2014). It should be noted that the hydrogen peroxide test practically does not characterize the state of the cotyledons, which, for example, can be significantly damaged by infection. Certainly, the degree of seed viability is well characterized by the size of the germinated radicle, as well as the time required for the seed to germinate. However, it was noted that the germination of radicle still does not guarantee good development of the seed after sowing. If the cotyledons have little reserve substances or they are damaged by infection, then, as our experiments have shown, such a plant can die prematurely even at the stage of epicotyl elongation and opening of a pair of true leaves.

At the same time, the hydrogen peroxide method is quite simple to implement, does not require expensive reagents, and is not time consuming. Hydrogen peroxide test results are easy to interpret even for novice researchers. So, to carry out the HP test, only 8 days are required, and preliminary results can be obtained already on the 4th day of the experiment (on the third day of germination), when more than 80% of all viable seeds in the experiment germinate. Data analysis showed that in our experiments on the 3rd day germinated from 82.2 to 99.7% of all viable seeds, on average, the difference in seed germination between the 3rd and 7th days was 7%. The advantage of the hydrogen peroxide method is also the preservation of the viability of the seeds, the possibility of sowing them into the soil immediately after the test. Peroxide treatment (with 1.0% hydrogen peroxide solution) also promotes accelerated germination of beech seeds, which can be used to reduce the time required for seed germination.

CONCLUSION

This analysis of seed viability using tetrazolium and hydrogen peroxide test, covering batches collected in ten locations with different altitude, type of stand and age of beech trees, showed that the degree of viability is insignificantly influenced by both the trees age and the seeds weight. The seeds weight is in an inverse dependence on altitude of European beech growing. The revealed correlation between the data of tetrazolium and hydrogen peroxide tests allows it possible to recommend the latter as an express analysis for assessing the viability of beech seeds. Obtained results of seed viability from different places will be used to study their dynamics during seeds storage, as well as to comparatively analysis of the beech seedlings adaptation under the conditions of the Republic of Moldova in order to select the most optimal seeds for creating a new beech gene pool.

ABSTRACT

European beech *Fagus sylvatica* in the Republic of Moldova is located on the eastern border of its range. Therefore, the ongoing climatic changes may negatively affect its populations and ecology. The search for genetic material that is resistant to high temperatures and drought increases the chances of beech seedlings surviving in new climatic conditions. One of the most important characteristics of plant seed quality is the determination of their viability. The aim this study was to assess the quality of beech seeds obtained from various beech growing areas, differing in height above sea level, age of trees and forest stand. The tetrazolium and hydrogen peroxide tests were used as methods for quick and effective evaluation of viability of *Fagus sylvatica* seeds. The direct correlation between both methods by Pearson's coefficient (0.6859) was established. The highest percentage of viable seeds harvested in 2020 was in batches from Suceava (Romania), “Plaiul Fagului” and “Codrii” (Republic of Moldova). The rate of viable seeds from the named locations ranged from 68.3 to 76.7% for hydrogen peroxide test and from 84.0 to 92.0% for tetrazolium test. The smallest proportion of viable seeds among those examined was found in batches from Lviv, Ivano-Frankivsk and Zakarpattia (Ukraine) and Hirjauca, (Republic of Moldova); 50.7-63.0% and 66.0-78.0%, for hydrogen peroxide and tetrazolium tests, respectively. It should be noted that there was no direct relationship between the percentage of viable seeds in both the tetrazolium and hydrogen peroxide tests and their weight (Pearson's coefficients were -0.1078 and 0.2871). At the same time, it was found that the high content of empty and infected seeds was detected in the batches of beech seeds collected from Ukraine.

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