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IMPROVEMENT OF SEED GERMINATION AND SEEDLING RESISTANCE OF BEECH (*FAGUS SYLVATICA*) BY GROWTH REGULATORS

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ABSTRACT

Beech seeds are characterized by a strong exogenous and deep physiological dormancy. They need a long period of stratification, but even through stratification, seed germination is greatly extended in time. The aim of this study was to accelerate the seeds germination and improve the resistance of seedling by application of different growth regulators (gibberellic acid, capsicoside and genistifolioside). The concentration of growth regulators has been modified from 0.1% to 0.001%. The mean daily germination, mean germination time, speed of germination and total germination of seeds were evaluated. The best results of increase in daily germination (up to 18.5%) were obtained under influences of capsicoside and genistifolioside at concentration of 0.001%. Moreover, the time of total seeds germination was reduced by 20-22 days, which allowed for an earlier sowing of germinated seeds. The beneficial effect of treatment with growth regulators was also established when the germinated seeds were transferred to the soil by better adaptation and development of seedlings. The emergence rate and survival rate of beech seedlings increased 2.5-3.3 times. The use of growth regulators affected the morphometric parameters. The leaves from control seedlings were significantly smaller than in the variants with capsicoside and genistifolioside treatment – shorter by 1.7-2.9 cm and narrower by 0.3-1.3 cm. The leaves from seedlings treated with gibberellic acid were 1.5-1.9 times narrower than in the others variants. The relative chlorophyll index determined in the phase of three pairs leaves on seedlings treated with capsicoside and genistifolioside significantly exceeded the control (by 9.2-24.1 g/m^2).

Key words: *Fagus sylvatica, seed germination, seedlings adaptation, capsicoside, genistifolioside.*

INTRODUCTION

The European beech *Fagus sylvatica* L. is one of the main forest-forming trees in the Republic of Moldova. In order to help preserve clean air and ecology, forests should be at least 15% of the entire territory. At the same time, the forest fund in the republic has been rapidly decreasing over the past decades: only 6.7% of forests remain in the southern zone of the Republic of Moldova, 7.2% in the north, and 13% of the entire territory in the center (http://www.moldsilva.gov.md). The main reason for the decline in the forest fund is the rapidly growing deforestation. Another important factor that negatively affects beech forests is climatic changes, which entail an increase in the average annual temperature and a decrease in precipitation (Republic of Moldova Forest Policy Note, 2014). Forest beech is one of the most vulnerable forest-forming species in terms of temperature parameters and is picky about high moisture content in the ambient air (Dolschak *et al.*, 2019). Currently, serious measures are being taken in the Republic of Moldova to preserve forest lands: a draft law on a three-year moratorium on deforestation has been developed (The draft law "On the establishment of a moratorium on timber harvesting", 2020). However, limiting deforestation and natural regeneration of beech forests is not enough. It is necessary to ensure the availability of sufficient quantities of beech seeds for planting in nurseries and for increasing seedling production.

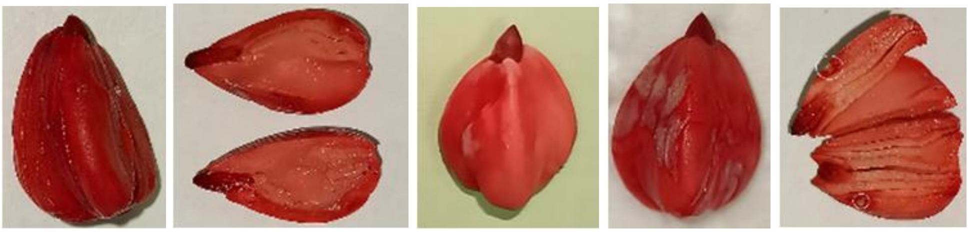
Fagus sylvatica seeds are deeply dormant seeds (Kolařova *et al*., 2010). Most authors agree that in order to successfully interrupt the dormant period, beech seeds need cold stratification (+ 3 - 4 ºC) at a moisture content of 28-30% for at least 12 weeks, which can last up to 17-20 weeks. However, prolonged cold stratification can also lead to a loss of seed viability. Therefore, the problem of reducing the dormancy period of beech seeds is one of the most important tasks at the present stage, which scientists from many European countries are trying to solve (Staszak *et al*., 2019)

The purpose of our research was to study the effect of natural growth bioregulators (genistifolioside and capsicoside) on increasing the germination of European beech seeds and the survival of seedlings in a greenhouse.

MATERIALS AND METHODS

The experiments were carried out in the Laboratory of Natural Bioregulators of the Institute of Genetics, Physiology and Plant Protection, Republic of Moldova during 2020. The seeds of European beech *Fagus sylvatica* L. (Fagaceae) were collected in the autumn of 2019 from beech stand in the Slovak Republic (Tribeč Mountains, Western Carpathians). The beech seeds were dried at ambient temperature and humidity until they reached a moisture content of 8-10% (fresh weight basis) and were stored at the temperature of $+4\pm1^{o}C$ in polyethylene bags, placed in plastic containers.

The viability of seeds was determined by two tests using the 2.3.5-triphenyltetrazolium chloride (2.3.5-TTC) solution (Kerkez *et al.*, 2018) and hydrogen peroxide (HP) solution (Sharma and Sibi, 2020). In each test, 4 replicates were used – in the TTC test 25 seeds, in the HP test – 30 seeds per replicate. In the TTS test, the germination percentage or viability was calculated on the basis of the total number of viable (complete staining of the cotyledons and radicle in bright red color) and conditionally viable (at least 2/3 of the basal part of the cotyledon stained with stained radicle) seeds (Figure 1). In the HP test viability was calculated on the basis of the sum of all germinated seeds – both with a radicle of equal and more than 2 mm (Figure 2).



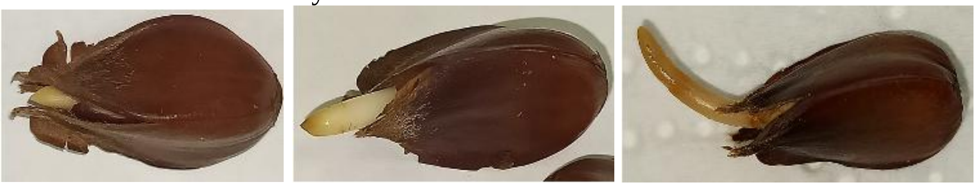
a b c d e

Figure 1. Staining *Fagus sylvatica* seeds in the TTC test: a) completely viable seed with bright red staining; b) completely viable fully stained seed in horizontal cut; c, d) partially stained seeds that may produce either normal or abnormal seedling; e) partially stained seeds in horizontal cut – the circles indicate the infection sites.

a b c

Figure 2. The degree of germination of radicle in *Fagus sylvatica* seeds in the hydrogen peroxide test (a – radicle more than 2 mm; b, c – radicle equal to 2 mm).

The germination test (four replicates of 80 seeds each) were carried out in accordance with the recommendations of the International Seed Testing Association (ISTA, 2006). The germination of *Fagus sylvatica* seeds was stimulated by their treatment with bioregulators - 0.1-0.001% solutions of capsicoside and genistifolioside for 22-24 hours. Genistifolioside (the sum of iridoid glycosides) and capsicoside (the sum of steroid glycosides) were obtained from the aerial part of the *Linaria genistifolia* (L.) Mill and from the seeds of the *Capsicum annuum* L. according to the described techniques (Mascenco *et al*., 2015; Borovskaia *et al.*, 2020). Distilled water was used as a control, and 0.02% solution of gibberellic acid was used as a standard. Seed stratification was carried out at moisture content of 30% and temperature of +4±1 °C until germination. The appearance of radicles was defined as germination (Figure 3). The daily germination counts were made on the seeds until no further germination occurred (for up to 112 days). The following observations: total germination percentage, mean daily germination, germination rate index were made (Al-Ansari and Ksiksi, 2016). Data are presented as the means and standard deviation of four biological replicates. The relationship between particular parameters was examined using Pearson's correlation coefficient analysis.



a b c

Figure 3. The degree of radicle development in different variants of *Fagus sylvatica* seeds treatment for the same period of time: a – control; b – 0.001% capsicoside; c – 0.02% gibberellic acid.

Seedling emergence studies were carried out in a greenhouse conditions, namely seasonal fluctuation of temperature, natural ventilation and drip irrigation. A mixture of white peat with a pH of 5.5 and soil 1:1 was used as a substrate. Seeds were sown as they were germinated.

The assessment of the total proportion of emergent seedlings and development of plants continued until the appearance of the first and second pairs of true leaves. During the growing season two foliar treatments with aqueous solutions of bioregulators (0.1-0.001%) were applied to the seedlings (in the growth phase of the second pair of true leaves and one month after the first treatment). Seedlings treatment with water and gibberellic acid solution (0.02%) was used as control and standard, respectively.

Relative chlorophyll content was quantified as the chlorophyll content index (CCI), measured for the first and second pair of true leaves with the FIELD SCOUT CM1000TM Chlorophyll Meter (Spectrum Technologies, Inc., USA). For each leaf, ten measurements on different locations were taken, avoiding the mid-vein (Van Wittenberghe, 2012).

RESULTS AND DISCUSSION

The viability of seeds of *Fagus sylvatica* L., determined by TTC and PH tests, was on average 69.50% and 61.68%, respectively. Mathematical analysis of the data showed a high degree of correlation between the viability established by the TTC and total germination of seeds (Pearson correlation coefficient was equal 0.9892).

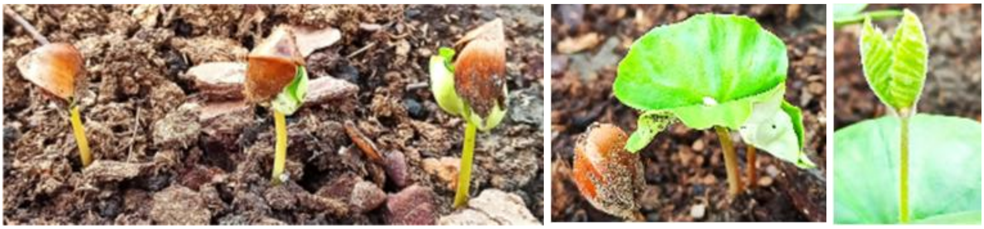
The highest rates of seed germination were obtained by treating seeds with solutions of capsicoside and genistifolioside at a concentration of 0.001%. Treatment with bioregulators at higher concentrations, despite the positive effect – a decrease in the proportion of infected seeds, still leads to a decrease in seed germination compared to a concentration of 0.001%. It was noted that pretreatment of beech seeds with 0.001% capsicoside and genistifolioside contributed to a significant increase in daily seed germination (up to 18.5%) in laboratory conditions compared to the control and standard (Table 1). 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), stimulation of daily seed germination from 0.54 (control) and 0.57 (gibberellic acid) up to 0.64 and 0.66 seeds (capsicoside and genistifolioside, respectively) leads to a significant reduction in the total period of seed germination by 20-22 days (in comparison with gibberellic acid and control). This significant reduction in germination time is very important for planting. Moreover, treatment with growth regulators depending on the duration of the stratification period, increases seed germination by 13-56% (of the total number of germinated seeds) in comparison with gibberellic acid and control.

Table 1. Effects of pretreatment on seed germination, adaptation and survival of *Fagus sylvatica* seedlings

Variant	In laboratory conditions			In greenhouse conditions	
	Mean daily germination	Total germination, %	Germination rate index	Adapted seedlings, %	Survival rate of seedling, %
Control	0.54	84.51	1.23	12.77	10.64
Gibberellic acid, 0.02%	0.57	76.12	0.89	16.33	12.24
Capsicoside, 0.001%	0.64	87.01	1.18	32.79	31.15
Genistifolioside, 0.001%	0.66	87.34	1.39	19.64	35.29

During seed stratification, it was noted that treatment with gibberellic acid 0.02% often led to lengthening and thinning of the radicle, and also promoted the germination of infected seeds (Figure 3c), which died after sowing.

The beneficial effect of treatment with capsicoside and genistifolioside 0.1-0.001% was also established during the adaptation of germinated seeds sown in a greenhouse (Figure 4). The highest rates of seedling survival were obtained from seeds and seedlings treated with solutions of capsicoside and genistifolioside at a concentration of 0.001%. The seedling emergence rate was 31.15 and 35.29%, which 2.5-2.9 times higher than in the variant with gibberellic acid, and 3.0-3.3 times higher than in the control (Table 1). In the hot summer period, when the temperature in the greenhouse sometimes reached to 48 ºC, the number of seedlings decreased in all variants. However, the proportion of seedlings that survived after foliar treatment with bioregulators capsicoside and genistifolioside 0.001 % was on average 2.7-3.0 times higher than in the variants with gibberellic acid and control (Table 1). It was revealed that the treatment with gibberellic acid leads to an increase in growth. Thus, it was found that the seeds treated with 0.001% capsicoside and genistifolioside become more resistant during the periods of adaptation to sowing and growth in suboptimal conditions. In addition, the seedlings obtained in variants with bioregulators were distinguished by bright color of leaves, strong stem and rapid development. Thus, the presented results show the advantage of processing by these natural growth regulators, which turned out to be much more effective than gibberellic acid.



a b c

Figure 4. Germination and development of *Fagus sylvatica* in a greenhouse, treatment with 0.001% genistifolioside (a – seedling emergence, b – unfolding of the cotyledons, c – development of first pair of true leaves).

It was found that the use of bioregulators and a growth stimulator of gibberellic acid affects the morphometric parameters of beech seedlings, in particular, the leaf shape (Figure 5). Thus, in the control, the leaves were significantly smaller than in the variants with bioregulators treatment – shorter by 1.7-2.9 cm and narrower by 0.3-1.3 cm. On average, the length and width of leaves were 3.9 ± 1.5 x 2.9 ± 1.3 cm in the control, 5.1 ± 0.7 x 3.5 ± 0.4 cm and 5.3 ± 0.9 x 3.8 ± 0.9 cm in the capsicoside and genistifolioside, correspondingly.

a b c d

Figure 5. Leaf shape in different variants of treatment: a, b - gibberellic acid (seedling and leaf in autumn); c, d –capsicoside (seedling and leaf in autumn).

The gibberellic acid treatment not only promoted seed germination, but also accelerated the growth of beech seedlings by stretching internodes (Figure 5a). Moreover, gibberellic acid led to a significant change in the leaf shape, the leaves were 1.5-1.9 times narrower than the others variants (Figure 5b). The ratio of length-to-width increased 1.5 times and reached 2.3±0.6 cm instead of 1.5±0.2 cm (control and treatment with bioregulators).

Relative chlorophyll index in the phase of three pairs of true leaves from seedlings in the variants capsicoside and genistifolioside 0.001% was 144.9±3.5 and 145.6±3.7 g/m^2 respectively, that significantly exceeded the control (135.7±3.2 g/m^2) and the variant with gibberellic acid (121.5±2.9 g/m^2).

CONCLUSION

The pre-germination treatment of European beech seeds with natural growth regulators capsicoside and genistifolioside in concentration of 0.001% significantly increased the mean daily germination, total germination of seeds, adaptation and survival of *Fagus sylvatica* seedlings.

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