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Research Article

Optimizing the Technologies of Initiation and Restoring Oak Forests by Applying Physiological Methods

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Abstract

The research goal was to elaborate a method of obtaining pedunculate oak (*Quercus robur* L.) seedlings with a high growth rate and increased resistance to extreme temperatures, thus reducing the costs of plantation care works during the first years after their initiation and the risk of suppression of oak plants due to competing with fast-growing weeds. As a result of the provided research, we elaborated a method of initiation to pedunculate oak forests, including two separate stages. In the first stage, the collected October acorns, after treatment with the biostimulator *Reglalg*, are incubated in specially created laboratory conditions for acorns germination and plantlet growth until May. In the second stage, the five-month seedlings are transferred to the field for cultivation. In the first, second, third, and fourth years of cultivation, the seedlings height reached 52, 95, 120, and 176 centimeters, respectively. During the mentioned periods, plants grown from the sown in the field acorns reached a height of 7, 19, 24, and 31 centimeters, respectively. Taken together, the obtained result gives the possibility to conclude that, during the first four years, the growth rate of the pedunculate oak plants, grown by a newly elaborated method, was at least five times higher. The plant's tolerance to high temperatures was also higher than those of the plants obtained after the ordinary method (by acorns sowing in the fall).

Keywords: *Quercus Robur* L; Oak; Acorn; Leave; Thermotolerance; Seedlings; Growth Rate; Viability; Methods

Introduction

The consequences of predicted climate change on the forest state have attracted large attention after the signing of the Kyoto Protocol (1997). It explicitly states that one way to mitigate the greenhouse effects is to increase the carbon accumulation in forest ecosystems. The consequences of global warming were analyzed at the Ministerial Conferences for the Protection of Forests in Europe [13]. The strategies for preventing and combating the possible consequences were exposed in the "*Adaptation of Forests and People to Climate Change - a Global Assessment*" [17]. The trend of global climate warming is associated with the rise of the minimum temperature at a higher rate than that of the maximum temperature [14], and the need for optimizing existing cultivation technologies has increased.

In new conditions is important to expand forested areas tolerant to extreme temperatures species expected to survive in the new environmental conditions [10] Specialists forecast the evolution of forest vegetation under global warming conditions will lead to the expansion of the areas occupied by oak species throughout Europe [1]. Over time, oak species in the temperate zone have adapted to changing environmental factors, especially climatic ones [16]. Under these conditions, knowing the specificity of regulation plant adaptation processes to the action of extreme temperatures becomes crucial for optimizing the technologies of their cultivation.

Oak species are central to maintaining the state of equilibrium in the natural conditions, promoting the folk traditions, and economic development of the Republic of Moldova and other countries. The maintenance, restoration, and initiation of new oak forests are associated with several problems, the main of which are the following

- During the first four years after acorn germination, oak plants grow very slowly. Therefore, to prevent their replacement by weeds and other fast-growing species, multiple care works are required during this period, associated with financial expenses. Herbaceous vegetation reduces seedlings' growth due to competition. This situation leads to prolongation of the period in which the seedlings are susceptible to weed overwhelm [18]. Both factors must be considered when practicing the direct sowing of oak acorns as a viable alternative to of planting seedlings.

- From the view of the global climate warming trend, the need to choose the species used for afforestation corresponding to the environmental conditions is becoming increasingly evident. Our previous research was specifically toward solving these problems. Concerning them, we mention only some of the previously obtained results. In the fall of 2001, we initiated a plantation of pedunculated oak (*Quercus robur* L.) in the Scientific Reservation *Plaiul Fagului.* In the first four years after the initiation of the plantation, before the oak's plants transitioned to the state of the grove, to protect the suppression of the saplings, 4 - 5 works of elimination of the overwhelming grasses were necessary every year. Currently, the average height of the 22-year-old trees exceeds 18-20 meters. They have 3-4 meters length straight trunks.

Starting from the second year after the initiation of sowing, seedlings obtained from spring-sown acorns manifested a constant tendency to grow faster compared to those obtained from autumn sowing [4] and their average height exceeds the height of the autumn-sowed trees by 60 cm. Spring sowing also has another priority. By applying this method, we reduce the risk of acorn damage by pests and rodents during the winter and early spring [2,9].

The negative effects of seedling shading were also confirmed in research with downy oak (*Quercus pubescens* Willd). They demonstrated that seedling growth decreases more when the level of seedling shading is higher. For this reason, to avoid the death of seedlings due to competition with other fast-growing plants and to reduce the time required to reach the state of the grove, in the first four years after sowing the acorn, it is necessary not only to minimize the competition with suppressive grasses, but also to avoid shading by trees growing nearby.

Another essential aspect that needs to be considered is maintaining the oak sapling roots intact during transplantation. It has been shown that plants grown by sowing acorns, without transplanting, reach a massive state about two years earlier than those obtained by transplanting seedlings. During the first two years after seedlings transplanting, their growth rate was significantly lower. In common, those affected the efficiency and increased the cost of plantation care works. Thus, the practical results of pedunculated oak forest initiations largely depend on the revision of the applied technologies to the plant species' genetic potential in correlation with the specificity of the environmental conditions. This method of starting new oak forests is more complicated and expensive. The developed seedlings have deep pivoting roots, which assure higher resistance and survival rate in the first year after planting in the natural conditions [19]. Improving cultivating crops in the nursery allows obtaining seedlings of high quality [12], but when afforestation is used the seedlings of oak species grown in the cultivation room and then transplanted; pruning the taproot of seedlings causes a significant slowdown in their growth rate, especially in the first years after transplantation [12].

The mentioned before suggest that although the methods of initiation and restoration of oak forests were developed, their implementation in practice is still associated with the need for expensive care works for new plantations, especially in the first five years after acorn sowing [4].

Another method of initiating oak plantations, based on planting container-grown seedlings over two years, ensures an increased probability of success in new oak forest installation. This method requires at least one supplementary year of seedlings' adaptation to natural conditions. Due to the high costs of growing in containers and then transplanting plants for growing in natural conditions, as well as extending by one year (from five to six years) the age of transition of oak plants the stage in which the trees crowns union are beginning, the large-scale application of this method in forestry is expensive. Considering the mentioned problems, for the initiation of pedunculate oak forests, starting in the 2019 year, we tested the applicability in forestry of some of the methods developed to increase the resistance of wheat plants to extreme temperatures and drought [5]. An additional mode to solve the problems related to the initiation of oak forests involves the enlargement of plants' adaptive capacities to extreme temperatures with the aid of biostimulators [6].

In this paper, we present the results of the initiation of plantation with pedunculate oak by sowing the acorns in autumn (1) and as well by planting in the field during the spring of seedlings obtained after germination of acorns, cultivation of plantlets in artificial conditions during the autumn and winter period (2). Thanks to the application of the new method of initiation oak plantation, we have eliminated the risk of damage caused to acorns by rodents, substantially reduced the work of caring for plantations, and obtained vigorous and fast-growing oak plants.

Materials and Methods

In our studies, we used saplings of English oak (*Quercus robur* L.), grown from acorns harvested in the fall of 2019 from 100-120-year-old trees that grow in the Scientific Reservation *Plaiul Fagului*, Ungheni district of the Republic of Moldova.

Before the initiation of germination, the whole, undamaged, and identical-sized acorns (at least 30 mm in length) were selected. Initially, we sterilized acorns by immersion in twice diluted commercial preparation ACE for 15 minutes; then washed by immersing them in distilled water, thrice for 3 minutes each time. Finally, we divided acorns into control and experimental variants; equal by mass.

In the last decade of November 2019, the acorns from the first variant were sown in the Protection Zone of the Institute of Genetics, Physiology, and Plant Protection in rows, at a distance of 25 cm between plants and 50 cm between rows, at a depth of 6-7 cm.

In the last decade of November 2019, acorns of the experimental variant were immersed in a solution of the biostimulator *Reglalg*, diluted with distilled water in a ratio of 1: 200, and incubated for 24 hours in the refrigerator at a temperature of +4°C. Then, the acorns were sown at a depth of 6–7 cm, in vegetative vessels filled with forest ground. The vessels were placed in a culture room where the temperature was maintained at 15°C at night and 20°C during the day. Between 7:00 a.m. and 11:00 p.m., in addition to window lighting, the vessels were illuminated with 20 Watt lamps by company Philips. The intensity of artificial lighting was 600 - 700 $\mu mol*m^{2}*s^{-1}$. Soil moisture in the vessels was maintained at 78–82%, which corresponded to 70% of the total moisture capacity of the soil. The germination of acorns was assessed on the 30th day after sowing. When oak seedlings reached a height of 5 cm, they were sprayed with the diluted water in a ratio of 1: 200 solutions of the biostimulator *Reglalg*.

In the second decade of May 2020, the seedlings of the experimental variant were transplanted into open ground, in parallel with the rows of the control variant. When planting, damage to the main root of the seedlings was rigorously avoided. Immediately after planting and after the next two months (in the second ten days of July), the control plants were sprayed with water, and the experimental plants were sprayed with the biostimulator *Reglalg*, diluted with water in a ratio of 1: 400.

The growth dynamics and physiological state of control and experimental plants were assessed throughout the entire growing season, in 2020, 2021, 2022, and 2023. During the year, we provided phenological observations and plantation care works. In October of each year, we measured the height of trees with the ruler to the accuracy of ± 1 cm and the diameter of the stem - using the caliper (accuracy ± 0.1 mm). We characterized the morphological parameters and physiological state of plants obtained after direct acorns sowing and those planted with seedlings grown in autumn-winter by the appreciation of total and annual growth in height and diameter of trees at the trunk level [4] and the chlorophyll index using the SPAD apparatus (USA) (Diagnostics, 1988). During the growing season, in at least 20 leaves from 10 plants, the chlorophyll content, using a chlorophyll meter (CM-1000 Spectrum Brands Holdings, Inc, (USA)), as well as the quantum yield of chlorophyll [7], using a PAM-2100 fluorimeter, Walz, Germany, were determined.

The intensity of electrolyte leakage from segments of oak plant leaves was determined using a conductivity meter N5721 (ELWRO, Poland), using the method [15]. The relative value of electrolyte leakage (RVEL) was calculated using the formula

$RVEL = + (\mu_t - \mu_{25}) / (\mu_{100} - \mu_{25})$, where

μ_t - electrical conductivity of the solution at the time t of incubation at 25°C of the sample, previously subjected to heat shock;

μ_{25} - electrical conductivity of the solution with the control sample (preliminarily not subjected to heat shock) at the time t of incubation at 25°C;

μ_{100} - electrical conductivity of the solution after 120 minutes of incubation at 25°C of the sample, previously subjected to heat shock with 100°C, for 10 minutes.

The results obtained were processed statistically, calculating the average value of each type of measurement, the standard deviation of the average, as well as the level of reliability of the difference between the averages [3].

Results and Discussions

Figure 1 shows the images of the pedunculate oak plants from the control and the experimental variant in each of the first four years of plant growth. The analysis of these images suggests that during every year, the experimental plants' growth rate was higher than those of the control one, due to which the difference between their heights increased with increasing plants' age.

The quantitative results regarding the height and diameter of the oak plants at the end of the active vegetation period determined each year (in the third decade of November) are presented in Table 1. Comparing the growth parameters of the plants from

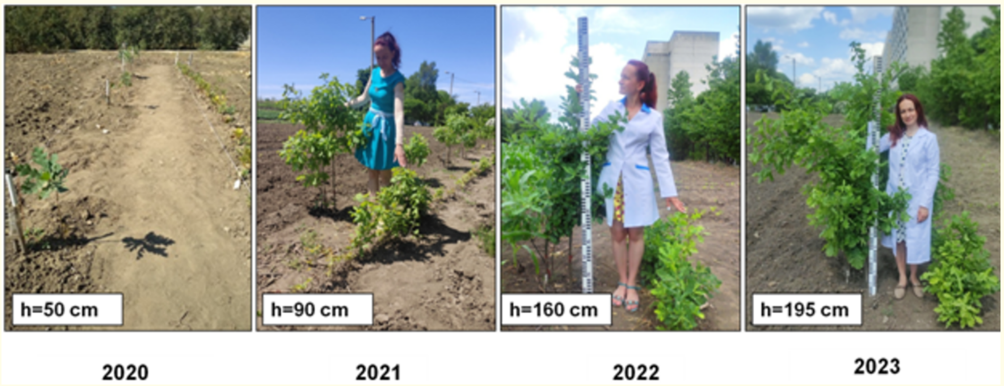

Figure 1: Images of oak seedlings in the first, second, third, and fourth years of growth (2020, 2021, 2022, and 2023) obtained from acorns sown in the field in autumn (left-hand on the photo) and from the seedlings obtained and cultivated from November 2019 until Mai 2020 in artificially created conditions (right-hand on the photo), then transplanted in the field.

the experimental variant with those of the plants from the control one, we note that at the end of the fourth year of vegetation, the average height of the seedlings from the experimental and the control variants reached 176 and 31 cm, respectively; stem diameter of the plants from these variants reached 31 and 6 mm, respectively. In this way, in the first four years of cultivation, the average growth speed in height and diameter of the experimental variant seedlings was 5.7 and 5.2 times higher than that of the seedlings from the control variant. From a practical point of view, it is essential that the annual increase in the height and diameter of the seedlings from the experimental variant, in each cultivation year, exceeded that of the seedlings from the control variant by 3.5 -8.0 times. From this, it follows that the modification of the method of initiation of germination and growth of seedlings carried out during their preparation period to be transplanted in field conditions had beneficial effects on the growth rate of plants obtained during the four years of realization of research. It is interesting to note that starting from the second year of growing the plants in field conditions (year 2021), the ratio between the annual growth in height, or diameter, of the plants from the experimental variant compared to that characteristic for the plants from the control variant has stabilized, varying between 5 and 8. Also, in all variants, the ratio between the annual growth in height, or diameter, compared to the respective values of the saplings at the beginning of the reference year stabilized, the values varying between 1.20 and 1.29. From this, it follows that starting from the second year of cultivation; plants have installed processes that ensure the same relative growth in diameter and height. At the same time, during the initiation of this state, the plants from the experimental variant, being taller and having a larger diameter of the stem, according to the absolute value of the growth speed, continuously exceeded the characteristic values for the plants from the control variant.

Year	Trunk height. cm		Trunk diameter. mm	
	Classic method	Laboratory method	Classic method	Laboratory method
2020	7 ± 1.32	52 ± 3.23	1 ± 0.01	7 ± 1.32
2021	19 ± 1.05	95 ± 2.92	4 ± 0.03	19 ± 1.05
2022	24 ± 2.12	120 ± 4.12	5 ± 0.03	24 ± 2.12
2023	31 ± 1.98	176 ± 3.98	6 ± 0.02	31 ± 1.98

Table 1: Dynamics of pedunculate oak (*Quercus robur* L.) seedlings growth in the first 4 years of the growing season on the experimental field of the Institute of Genetics. Physiology and Plant Protection. Academy of Sciences of the Republic of Moldova.

The data presented in table 1 demonstrate that the growth rate obtained according to the newly proposed method of oak saplings exceeds by more than seven times that characteristic of the saplings from the control group. Although in the following years, the relative growth compared to the height at the beginning of the respective year in seedlings from both batches became practically identical, the ratio between the absolute values of the annual growth in these variants in seedlings at the age of 2 and 3 years, remained identically to the one calculated in the first year. Due to this, according to the absolute values, the differences between the height of the seedlings from the experimental variant and the control one increased progressively in each year of cultivation. Considering the mentioned data, we decided to determine if the faster growth of the seedlings in the experimental variant, according to the law of *Bergonie and Tribondo* [11], led to a decrease in their resistance to the action of high temperatures. Figure 2 shows the data on the influence of the HS with 50°C on the leakage of electrolytes from the leaf segments taken for analysis from the third year of growing oak saplings in the summer of 2022. From the figure, we notice that already in the first 5 minutes after the exposure of the leaf segments to the HS with 50°C, during 30 minutes, about 50% of the electrolytes were drained in solution. The prolongation of the period of leaf segment incubation after exposure to HS resulted in a slow increase in the rate of electrolytes leaching from the segments.

After five minutes of exposure to HS at 50°C, during 30 minutes, the rate of electrolytes that leaked from the leaf segments of the control variant was 10% higher than that from the experimental variant. After exposure to HS, the value of the difference in electrolyte leakage from the leaf segments of control and experimental variants, with increasing the duration of incubation slowly decreased, reaching 4% after 120 minutes of incubation. These data demonstrate that the plant leaves of the experimental variant were more resistant to HS than those of the control variant. As expected, increasing the duration of exposure to HS from 30 minutes to 60 minutes caused an increase in the level of damage. During the first 5 minutes of incubation after exposure to HS, about 75% of the electrolytes leached from the segments. After exposure to this dose of HS, an extension of the incubation duration larger than 10 minutes resulted in a slow increase in the electrolyte leakage rate. Although at each incubation period, the rate of electrolytes that leaked from the leaf segments of the experimental variant showed a tendency to be higher compared to that characteristic of the control variant, the differences between the two variants demonstrate a tendency to decrease. This was waited since, in line with theory, the differences between the resistances of biological systems to stress factors are most pronounced after their exposure to doses that suppress function by about 50%. Namely, this level of suppression of the ability to maintain electrolytes by the segments of oak leaves was manifested after exposure to 50°C, for 30 minutes.

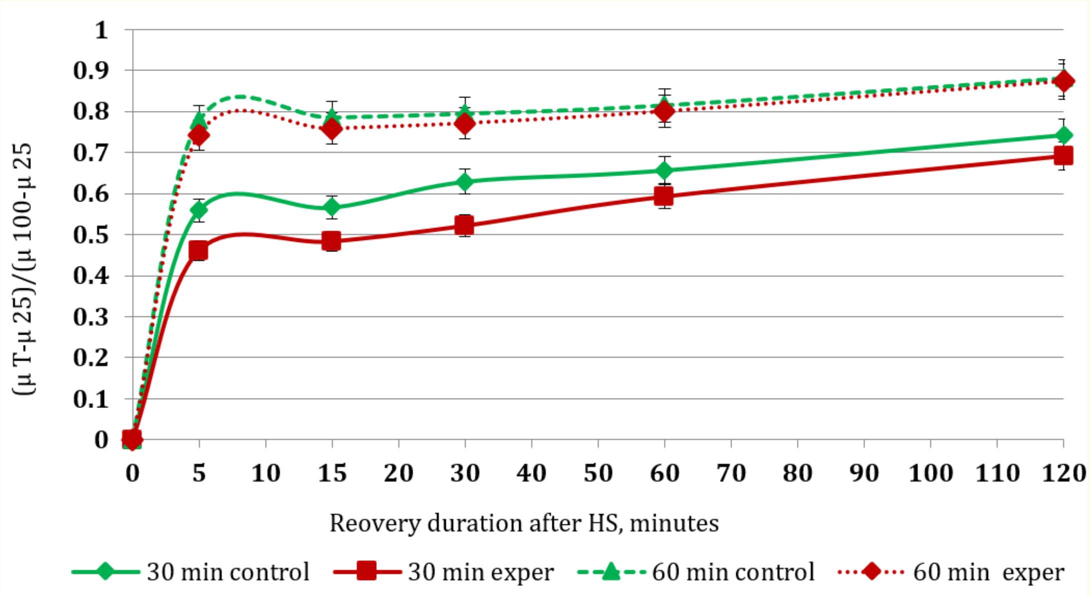

Figure 2: The influence of heat shock caused by incubation at a temperature of 49°C for 30, or 60 minutes, of oak leaf segments on the relative values dynamic of electrolyte leakage from leaf segments of control and experimental oak seedlings collected in June 2021 (The second year after sowing/planting), in the control and experimental variants.

Another applied method to compare the resistance of oak seedlings from the experimental variant with those from the control one is based on the influence of HS on the activity of leaf chloroplasts photosystem II (PS II), taken in the second year of growth of oak seedlings. The obtained data are presented in Figure 3. Analyzing the obtained results, we note that because intact leaves were exposed to HS after their exposure, the gradual decrease in the relative activity of PS II reached the minimum value 24 hours after exposure, subsequently being associated with the increase of this value. It should be noted that the relative activity of PS II at the minimum point (at 24 h of incubation) and the rate of subsequent recovery, as expected, were higher in leaves exposed to ST at 50°C compared to those exposed at 52°C. At the same time, we mention the higher value at the minimum point and the faster restoration in the later period of the relative activity of PS II in the leaves taken from the seedlings of the experimental variant. According to the results presented in Figure 2, the minimum relative value of PS II activity in the leaves of the experimental variant, exposed to HS for 10 minutes with a temperature of 50°C, was higher than that of the leaves of the control variant. In the analogical way, the kinetics of restoring the relative value of PS II activity was faster in them. In common, the data presented in Figures 2 and 3 suggest that the leaves of the experimental variant, compared to those of the control one, the recovery of damage caused by HS faster and more effectively.

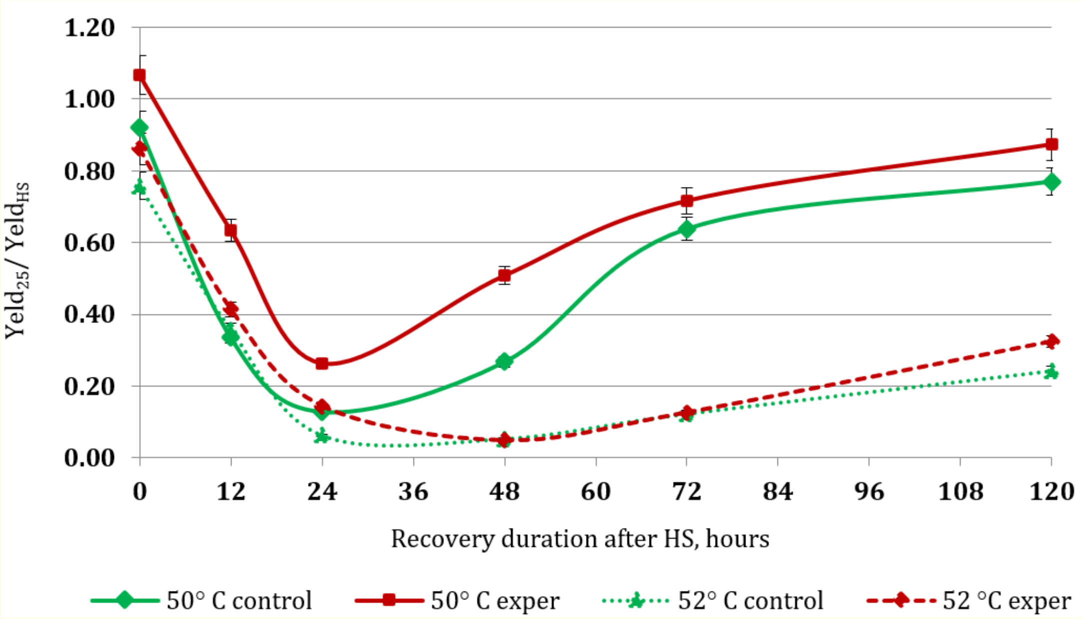

Figure 3: The influence of heat shock, applied to leaves, collected from oak seedlings in June 2021 (the second year after sowing/planting), by incubation at a temperature of 50°C and 52°C for 10 minutes on the relative values dynamic of PS II activity of leaves in control and experimental variants.

Under the influence of stress factors, plants are induced changes in genome expression, which are directed to repair the provoked damages and to increase their resistance [8]. Depending on the exposure dose and genotype initial resistance, the final effects of stress factors influence are different. After exposure to a relatively small or medium dose of stress factor, the plant resistance increases; reparation, recovery, and restoration processes prevail. As a rule, following the mentioned changes, the resistance of biological systems to the action of stress factors becomes higher, a phenomenon confirmed in the experiments that provided for the fractionation of the dose of exposure to different stress factors [4]. At the same time, after exposure of the plants to doses of the stress factor, exceeding the tolerance level of the genotype, the processes of amplifying damage dominate, which leads to the death of the plants [8]. The data presented in Figures 2 and 3 demonstrate this legitimacy. Exposure to higher doses of HS caused a decrease in the speed of restoration or even a loss of the ability of leaves to restore the capacity to maintain electrolytes or the activity of PS II [7].

The beneficial effects of biostimulators and other biologically active substances on resistance, viability, and plant productivity, are manifested at a maximum level under stress conditions, which do not substantially exceed their tolerance level [6]. The data presented in Figures 2 and 3 demonstrate that the beneficial effect of the biostimulator *Reglalg* on the oak leaves' resistance was manifested only in the case of exposure to doses of HS, which did not substantially exceed their thermotolerance Figure 2 and 3. Because of this, the beneficial effect of the biostimulator *Reglalg* on the productivity of different genotypes varies widely and differently depending on the genotype and the conditions of the year [6]. During the daylight hours, the photosynthetic activity of the experimental plant leaves was higher than that of the control variant plant one. This confirms the greater vigor of the oak saplings from the experimental variant compared to those from the control one. As a rule, in the spring the plants of the experimental variant earlier emerged from the dormant state and entered this state later in the fall, compared to the plants of the control variant.

In general, the presented results are consistent with the vision that in ontogenesis the plants adapt to external factors, consecutively acquiring the capacity to survive the seasonal changes in external conditions. Thanks to this, each time plat is the creature of dynamic interaction between genotypes, life history, and environment. Thus, at any stage of ontogenesis, plants' state is determined by their previous "*life history*." The specificity of the environmental conditions alteration during ontogenesis induces in plants a network of specific processes to be considered by cultivators for obtaining the best practical results. Although each parameter characterizes the influence of stressors on the different life processes, for their theoretical assessment and rational application in practice, with perspective is the systemic approach [8]. According to this approach, to obtain the desired results, the most reasonable for the plant and less expensive for the grower is the initiation of non-specific processes of adaptation to stress factors at the first stages of plant ontogenesis thanks to the application biostimulators separately, or in combination with moderate doses of stress factors [6,8].

To reduce the negative effects of stress factors, to which under natural conditions oak saplings, with a high probability, will be exposed, we induced their initiation in advance, thanks to the treatment of the acorn with the biostimulator *Reglalg* and obtained seedlings growth of the in artificially created conditions. Thanks to this, the vigor and viability of the obtained seedlings increased. After their transfer to open field conditions, the adaptation processes, already initiated during the period of germination and growth, in natural conditions developed faster and more completely; Thanks to this, the damage caused by the stress factors, to which the plants were subjected during the vegetation, decreased. This ensured the reduction of the consumption of energy and substances allocated by the plant to repair damages and adapt to stress factors. It is also necessary to mention that under the influence of the biostimulator *Reglal*g, the growth rate of the root system [6] is activated. Thanks to this, the roots penetrate faster and deeper into the moister layers of the soil, which contributes to avoiding the negative effects of drought on the experimental plants. Finally, the mentioned ensured the increase of the growth rate and the adaptive capacity of the oak saplings against thermal stress and drought during the entire vegetation period.

The high growth rate and increased viability of plants, obtained according to the described earlier methods, make it possible to reduce the costs associated with initiating and preserving the new oak plantations. Thanks to this, the costs associated with plant care are reduced, and the risks of eliminating oak seedlings due to competition with other fast-growing plant species are reduced. The presented in this article results can be considered as a supplementary argument in favor of the application of biostimulators, optimization of acorn germination, and seedling growth conditions to accelerate growth and increase the resistance to stress factors of oak plants.

Conclusion

- From the acorn of *Quercus robur* L. treated with the biostimulator Reglalg, under artificially created conditions, saplings with the following essential characteristics are obtained for the initiation of a new pedunculated oak plantation:
  - During embryo germination and seedling growth, it uses the endosperm substances more fully and effectively for germination, growth, and initiation of non-specific adaptation processes to abiotic stress factors;
  - During ontogenesis, under the influence of stress factors, in plants obtained according to the newly developed method, adaptation processes are initiated more quickly, thanks to which the energy cost allocated by them for the recovery of damages and adaptation to stress conditions is minimized;
- Due to the increase in vigor and adaptability, the growth rate of the seedlings in the first four years after the germination of the acorn exceeds an average of five times that characteristic for seedlings obtained by traditional methods.
- Due to the high growth rate and increased viability of obtained plants, implementing the proposed method makes it possible to reduce the costs associated with initiating and preserving the new oak plantation and the risks of eliminating oak seedlings due to competition with other fast-growing plant species.

- The presented supports the perspectives of biostimulators application for optimization the oak acorn germination, and to accelerate seedlings growth and increase the resistance of oak plants to stress factors.

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