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Research Article

Influence of geographical provenance, biostimulatory treatments and their interaction on the seed germination of *Quercus robur* L.

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Abstract

The hereby research aimed to identify the influence of several biostimulants on the germination capacity of pedunculate oak (*Quercus robur* L.), with the goal of enhancing the germination process, which is fundamental for the conservation and development of this ecologically and economically important species. Biological materials were collected from four different geographical provenances in Romania: Prejmer (Brașov), Racășa (Maramureș), Căpâlna (Alba), and Orăștie (Hunedoara). The main phenotypic characteristics of the seeds were analyzed, including length, diameter, and weight. Germination capacity was assessed using different biostimulants, compared with untreated seeds as a control. Results showed significant differences between the investigated provenances regarding morphological traits of seeds, respectively, seed length, diameter, and weight. Additionally, significant differences in germination capacity were noticed among the applied treatments and studied provenances. The treatment with Atonik biostimulant illustrated the best results within all provenances, followed by Nitrozym and Cropmax. The study offers useful information into enhancing the germination capacity of *Q. robur*, highlighting the significance of provenance and stimulation treatments for effective germination, significant for the conservation and sustainable management of this species.

Keywords: biostimulants; conservation; forestry species; germination; seeds

Introduction

The world's growing need for resources resort in conversion of natural forests into farmland, damaging these ecosystems in the process. Logging, habitat fragmentation, and pollution all contribute to this problem

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(Song et al., 2018). Practices that balance human needs with the health of forests are required to ensure these habitats survival for future generations. This means better conservation, more reforestation, and stricter rules about how we use forest resources (Brancalion *et al.*, 2020).

The pedunculate oak (*Quercus robur*), an important species of many European forests, is facing serious threats. Habitat loss, climate change, and diseases are all taking a toll (Muhammad *et al.*, 2023). These trees grow across a large area, from the Mediterranean to northern Europe, including significant stands in Romania (about 130,000 hectares, or 2% of the country's forests). They are valued for their wood, and their acorns are a key food source for wildlife, particularly in autumn when other food is scarce (Deaconu *et al.*, 2023).

Getting these trees to grow well requires tackling environmental challenges. Things like drought, salty soil, extreme temperatures, and nutrient deficiencies can seriously damage oak trees (Morar et al., 2023). One promising approach is to use biostimulants – naturally occurring substances that promote plant growth and resilience. These can boost nutrient uptake, improve root development, and help plants resist stress (Yakhin *et al.*, 2017). Using them is a way to promote sustainable agriculture.

Human activities, like converting forests into farmland and over-logging, are devastating forest ecosystems (Aju *et al.*, 2015; Raw *et al.*, 2024). This highlights the need for new conservation methods. Biostimulants have shown promise in boosting tree health and resistance to stress (Campobenedetto, 2020).

This study looks at how different biostimulants affect *Q. robur* seed germination. Our aim was to find ways to improve germination, which is essential for conserving and managing this important tree species.

Materials and Methods

Biological material

The biological materials were represented by acorns of oak trees collected from different seed source reserves (provenances) from Romania (Figure 1) noted in the National Catalogue of Forest Genetic Resources (Pârnuță *et al.*, 2011).

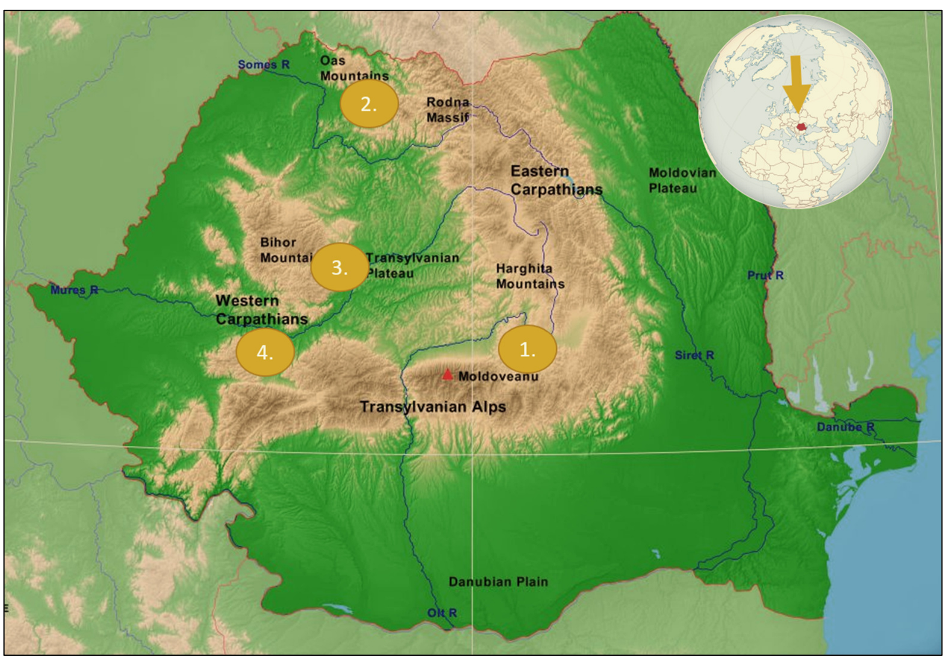


Figure 1. Geographical location of the four *Q. robur* provenances analyzed

The provenances analyzed were selected from four distinct regions, in order to investigate possible variability of reproductive traits of Romanian oaks: P1 - Prejmer (Brașov) – an area known for its specific climate and soil conditions; P2 - Racășa (Maramureș) – offers unique ecological conditions due to the altitude and the influence of the mountain climate; P3 - Câpâlna (Alba) – characterized by a combination of pedoclimatic factors that can influence seed development, and P4 - Orăstie (Hunedoara) – a region with a significantly different soil composition and biodiversity compared to the other locations. By sourcing acorns from these distinct locations, the research aims to assess the influence of genetic variability on germination capacity and seedling development.

Measurements and determinations

The main phenotypic characteristics in regard with the germination evaluation included determination of seed length, seed diameter and seed weight. To accurately determine the phenotypic characteristics of the oak seeds, specialized tools and equipment from the forestry laboratories at ICHAT - UASMV Cluj-Napoca were utilized (Figure 2).

Figure 2. Phenotypic analysis of oak seed characteristics

Germination capacity was assessed using four different biostimulants treatments. The germination capacity of oak seeds from different provenances in Romania was tested according to ISTA recommendations, using four treatments: T1 - Control, T2 - Biostimulator Cropmax, T3 - Biostimulator Atonik and T4 - Biostimulator Nitroyzm. The seeds underwent a preparation process in which they were soaked for 24 hours in water or specific treatment solutions. To determine the germination capacity of acorns from each provenance, the four different treatments were applied, each treatment testing 400 seeds from each location. Treatments include: T1 - Control (Control): the acorns were soaked only in water for 24 hours. T2 - Cropmax: a biostimulator based on amino acids, vitamins and trace elements, known for its role in increasing the efficiency of photosynthesis and resistance to stress, T3 - Atonik: which contains phenolic compounds and is recognized for stimulating physiological processes in plants, including germination and root growth, T4 - Nitroyzm: a biostimulator that influences the development of the root system and stimulates the early growth of seedlings.

The seed preparation process: The acorns were soaked for 24 hours either in water or in the solutions specific to the treatments, to facilitate the absorption of the bioactive substances from the biostimulators. Following this initial phase, they were placed in a germination substrate consisting of 70% soil, 20% perlite, and 10% sand, designed to provide optimal conditions for growth. The germination process was monitored over a 28-day period, with assessments conducted on days 0, 4, 8, 12, 16, 21, and 28 to track the progress of seed sprouting and overall health (Figure 3). This structured timeline allowed for detailed observation of the germination rates and the effects of the various treatments on seed development.

Figure 3. Observations of the germination process of oak seeds

Statistical analysis

The statistical analysis of the data was carried out by the method of multiple comparisons with the Duncan test. Significant differences between treatments were tested by ANOVA at the 95% confidence level, followed by Duncan's post hoc test, with significant differences indicated by distinct letters at $P < 0.05$.

Results and Discussion

Results of morphological traits of acorns depending on their provenance

Phenotypic evaluation of acorns, including length, diameter and weight, showed significant difference depending on provenance (Figure 4 A, B, C). The provenance from Alba County had the highest value of acorn length (3.42 cm), statistically significantly superior, while Brașov provenance had the lowest value (2.66 cm). Regarding the diameter of the acorns, Hunedoara (P4) presented significantly higher values compared to the other provenances. These findings suggests that the environmental conditions and genetic factors in the Hunedoara region investigated may contribute to enhanced seed development, which could positively influence germination and seedling vigor. This difference may reflect local adaptations that could influence not only seed size, but also other functional traits associated with germination and seedling survival (Singh *et al.*, 2020). In contrast, the Maramureș provenance (P2) recorded the lowest values for acorn diameter. This may indicate a variety of factors, including local climatic conditions, soil quality, rainfall regime or specific ecological interactions, that could be limiting seed size. These size variations, similar with other studies, suggest that each provenance reflects unique environmental and genetic characteristics, that may influence seed morphology and, by implication, germination capacity and initial seedling development (Roman *et al.*, 2022).

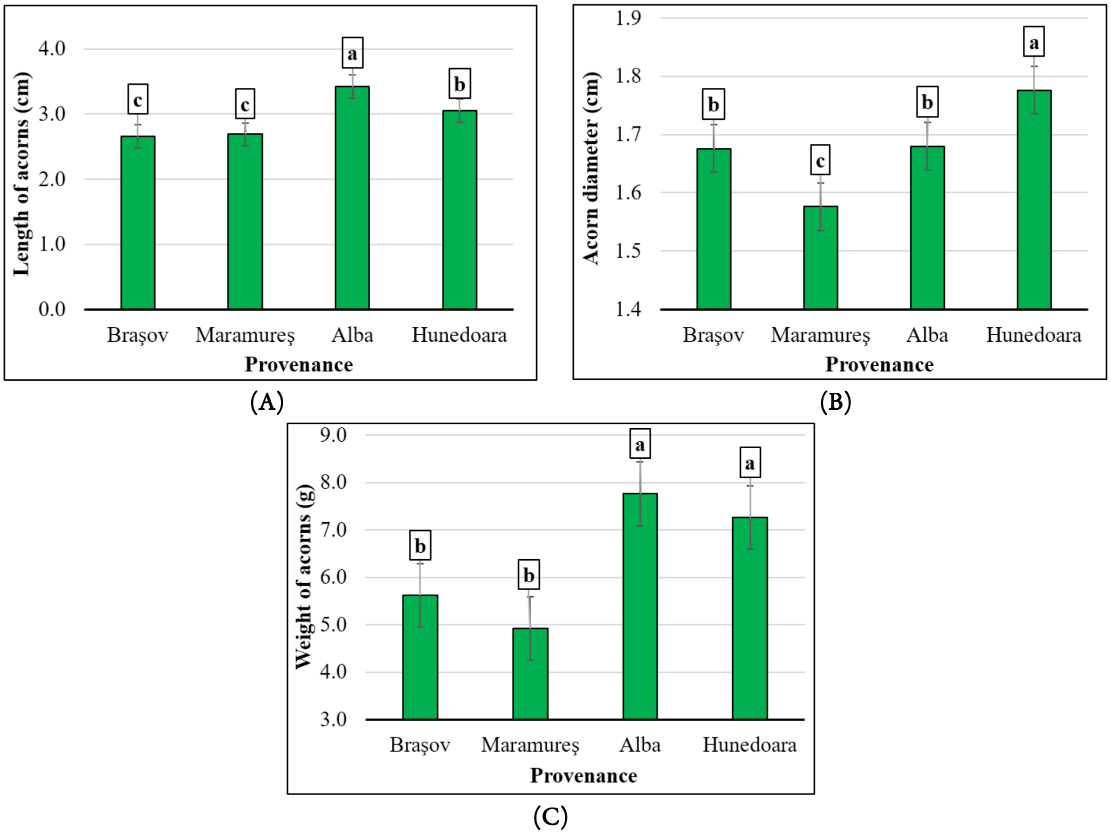


Figure 4. Morphological traits of *Q. robur* acorns according to their provenance: (A) length (cm); (B) diameter; (C) weight. The columns represent mean and error bars as ± SD. Any means followed by the same letter are not significantly different (Duncan test, $P < 0.05$)

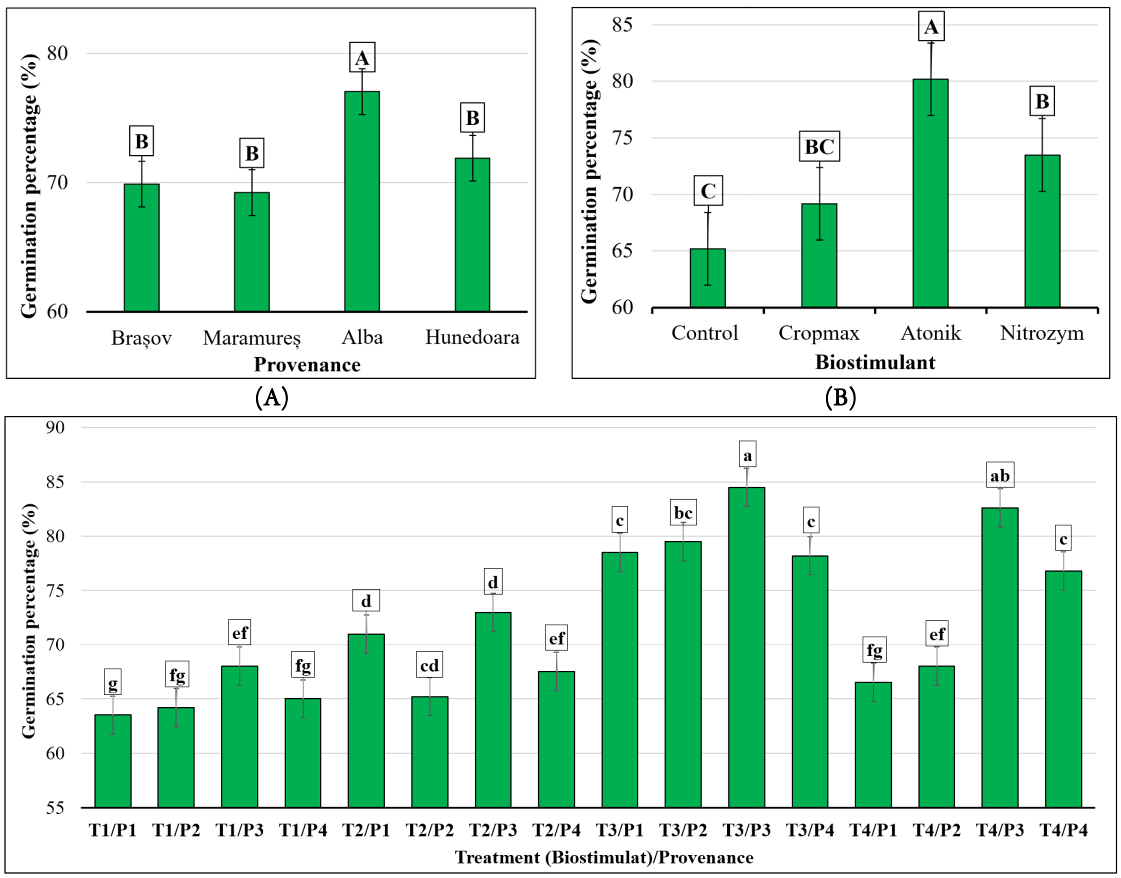
In terms of acorn weight, the highest values were recorded for the provenances P3 (Alba) and P4 (Hunedoara), while the lowest values were found in the provenances P2 (Maramureș) and P1 (Brașov). This variation in acorn weight is significant, as heavier acorns often contain more stored nutrients, which can enhance germination success and seedling establishment. The superior acorn weight observed in the Alba and Hunedoara provenances suggests that these regions may have optimal environmental conditions that may promote quality seed development.

These results align with findings from other studies in the field, which have also reported significant variations in seed morphological traits among different provenances. Similar research has demonstrated that environmental factors, such as soil composition and climate, can markedly influence seed characteristics, including weight and size (Fenner, 1992; Kozlowski, 2002; Gomes *et al.*, 2023; Gonçalves and Fonseca, 2023; Ferreira and Vieira, 2024). This variability between provenances can have a direct impact on the success of the germination process and subsequent plant development, which can be valuable in reforestation and ecological conservation programs.

Seed germination depending on provenances, biostimulant treatments and their interaction

Geographic provenances, treatments applied to seeds with biostimulators, as well as their interactions significantly influenced the germination capacity of seeds (Figure 5 A, B, C). Analyzing the unilateral influence of geographical provenances on seed germination capacity (Figure 5 A), regardless of the biostimulatory treatments applied to the seeds, the Alba provenance was recorded with the highest seed germination capacity, presenting significant differences compared to the other provenances.

Examining the singular impact of biostimulator treatments on seed germination capacity (Figure 5 B), irrespective of geographical provenance, the biostimulant Atonik (T3) produced the highest germination rate (approximately 80.0%), demonstrating its efficacy in improving seed germination performance. These results suggest that Atonik may improve seed performance by activating physiological processes that support rapid and vigorous seedling germination (Djanaguiraman *et al.*, 2005). Nitrozym (T4) followed, but to a lesser extent than Atonik, with about 73% germination rate, suggesting that it also positively impacts germination and highlighting the benefits of using biostimulants in promoting seed germination. In contrast, the Cropmax assured the lowest germination capacity among the biostimulants used, with no significant difference from the untreated control (T1). However, these results are in line with other studies that have shown that biostimulants can positively influence seed germination by increasing enzyme activity and water absorption, thus facilitating the initiation of the germination process (Calvo *et al.*, 2014).



(C)

Figure 5. Mean germination percentage of *Q. robur* seeds (%), according to their provenances – P (A); biostimulant treatments – T (B); the interaction between biostimulator treatments and provenances – T/P (C). The columns represent mean and error bars as ± SD. Any means followed by the same letter are not significantly different (Duncan test, $P < 0.05$)

Analysis of interactions between biostimulator treatments and provenances highlights notable differences between the combinations of experimental factors tested (Figure 5 C). For Alba (P3 provenance), Atonik (T3) showed the best germination with a percentage over 80%, suggesting its strong efficacy in promoting seed germination. This result showed that Atonik is an effective stimulant for oak seed germination, probably due to its ability to activate essential biochemical processes in mature seeds (Xu *et al.* 2016). In contrast, the control group (T1) showed the lowest germination rate at 68%, highlighting once again the advantages of using biostimulants. In Hunedoara (P4 provenance), Atonik (T3) also led the results with a germination percentage of 78.2%, while the control group (T4) recorded the lowest rate at 65.0%. In Maramureș (P2 provenance), Atonik and Nitrozym treatments showed the best results, followed by Cropmax, while the untreated seeds had the lowest germination percentage. These findings underscore the importance of selecting appropriate treatments to improve germination success in acorns from this provenance.

Results obtained through multivariate analysis

Employing Principal Component Analysis (PCA) for dimensionality reduction, the first principal component (PC1) accounted for 50.2% of the total variance in the original dataset. Thus, PC1 emerged as the most significant principal component in PCA, succeeded by PC2, which accounted for 35.6% of the total variance in the original dataset. The PCA plot displayed the four provenances in distinct quadrants. The provenances of Alba and Brașov were situated at a considerable distance from one another on one diagonal, while Maramureș and Hunedoara were positioned oppositely on the other diagonal (Figure 6).

The primary morphological elements of the acorns were situated in proximity inside the first quadrant, with germination being somewhat distanced and positioned in the second quadrant. In addition, the traits of the acorns were situated in the same quadrant as temperature, while germination was positioned in the quadrant associated with precipitation.

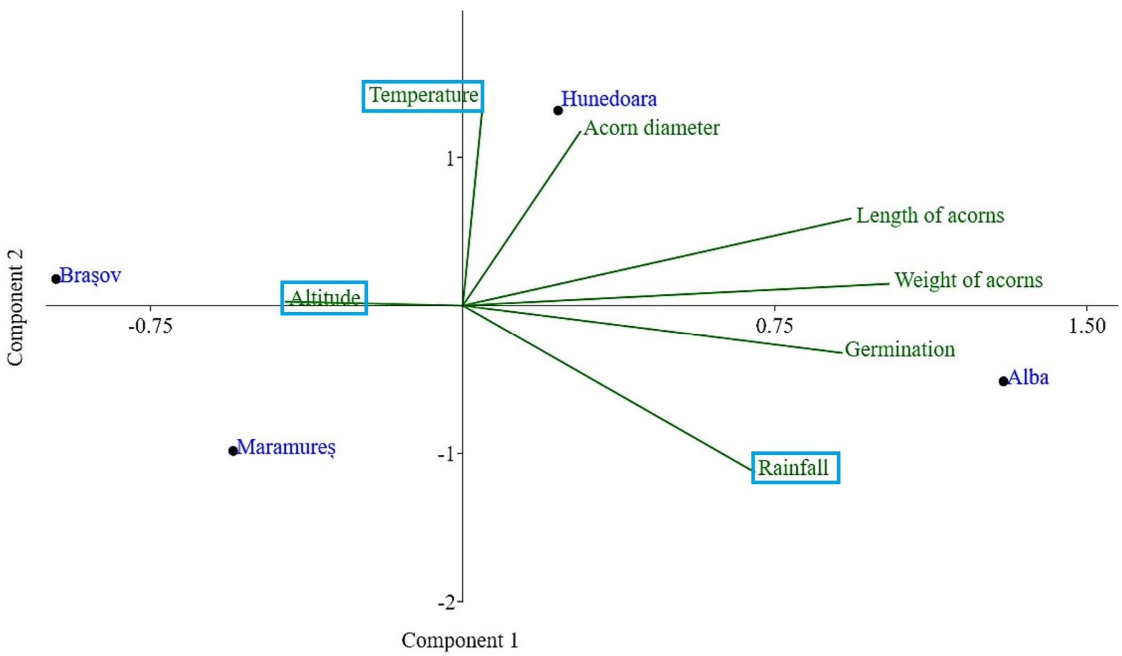


Figure 6. Principal component analysis (PCA) based on morphological traits of *Q. robur* acorns and seed germination, four geographical provenances, and their ecological conditions (altitude, temperature, and rainfall). The first axis explains (PC1) 50.2% of the total variance, while the second (PC2) explains 35.6%

The outcomes of the hierarchical UPGMA analysis (Figure 7) demonstrate that the Alba provenance occupies a separate subcluster inside the provenance dendrogram, but Maramureș and Brașov are in proximity, constituting a pair. The horizontal dendrogram contains two primary subclusters. In the uppermost subcluster, acorn diameter correlates with temperature, which is further associated with site elevation. In the lower subcluster, acorn length and weight have a greater correlation with germination, while precipitation is positioned on a separate branch, further detaching from these variables.

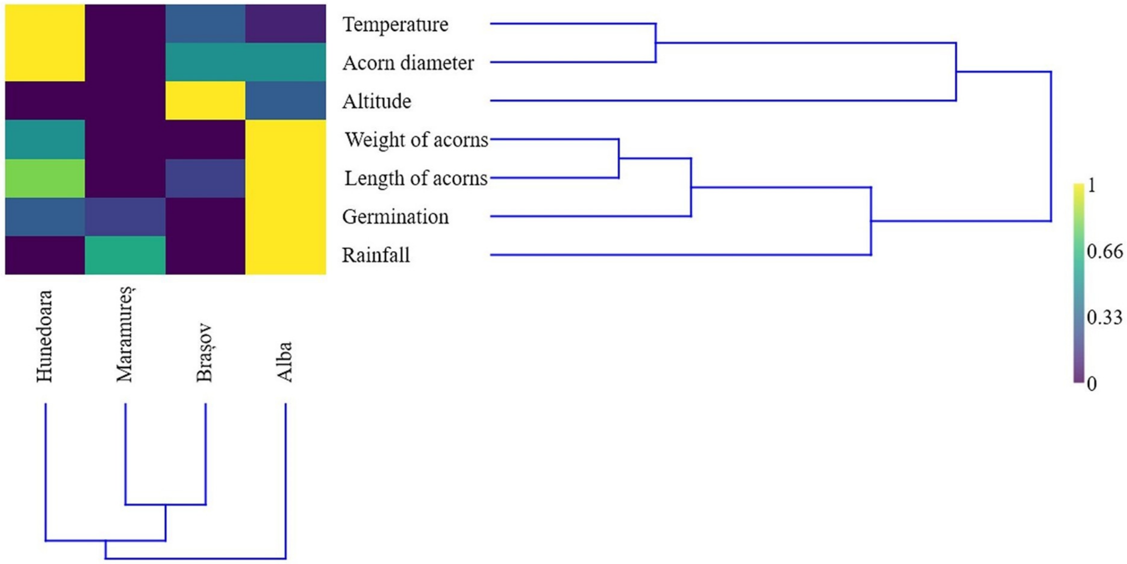


Figure 7. Hierarchical clustering - unweighted pair group method with arithmetic mean (UPGMA) algorithm, and Euclidean distance matrix dendrograms of the four *Q. robur* provenances and features of acorns, seed germination, and key ecological variables from provenance stands of acorns

The calculated values of the Pearson correlation coefficients between the analyzed parameters (Table 1) have both positive and negative values, the latter indicating inversely proportional relationships between the variables. However, the correlations were only significant in one pair of traits: the length of the acorns and their weight (positive and quite predictable close connection). Values close to the 5% threshold of significance were also recorded between the acorn weight and germination, or acorn diameter and temperature in the area of origin of the provenances. Negative 'r' values between acorn features and altitude, albeit not significant, suggest that acorn size and weight decrease as the altitude of the seed plantation location increases. Additionally, non-significant negative correlations between altitude and seed germination were noted.

The usefulness of identifying possibilities for increasing seed germination in forest trees

Seed length, diameter, and weight were determined as essential phenotypic traits in this study. Seed length serves as an indicator of resource storage potential, while seed diameter reflects initial growth potential. Additionally, seed weight acts as a measure of nutrient content, which significantly influences the germination success of the seeds. Together, these characteristics provide valuable insights into the viability and growth prospects of the tree seeds (Morar *et al.*, 2023).

Seed germination is marked by the development of vegetative organs that enable the new plant to sustain its growth. The process is deemed complete once the radicle emerges through the seed coat. The germination process involves a series of complex biochemical and physiological changes that facilitate the embryo activation and support the development of a new plant (Bewley *et al.*, 2013). In a narrower sense, germination refers to the physiological process in which a seed transitions from a dormant state to an active one, ultimately leading to the formation of a new plant (Baskin and Baskin, 2014). Seed germination is a highly complex process involving numerous biological factors that affect the embryo within the seed (Fenner and Thompson, 2005). In this sense, germination is not a unitary process, but involves a series of interdependent steps, each influenced by internal (genetic and physiological) and external (climatic and edaphic) factors. The current study looked at how these factors affected the ability of seeds from four different areas in Romania to germinate. It showed how the seeds from these areas had different phenotypes and responded differently to different biostimulant treatments.

Table 1. Pearson correlation* between the analyzed characteristics. '*P*' values are presented on the upper right diagonal, and 'r' values (phenotypic correlations) on the lower left diagonal

Correlated trait	Acorn weight	Acorn length	Acorn diameter	Altitude	Temperature	Rainfall	Germination
Acorn weight	–	0.049	0.574	0.723	0.884	0.431	0.059
Acorn length	0.951	–	0.314	0.777	0.596	0.714	0.211
Acorn diameter	0.426	0.686	–	0.874	0.095	0.500	0.906
Altitude	-0.277	-0.223	-0.042	–	0.804	0.683	0.695
Temperature	0.116	0.404	0.905	-0.196	–	0.306	0.788
Rainfall	0.569	0.286	-0.500	-0.317	-0.694	–	0.187
Germination	0.941	0.789	0.094	-0.305	-0.212	0.813	–

*The correlation coefficient is considered statistically significant when the *P*-value is less than 5% ($P<0.05$)

Our results underline the effectiveness of Atonik in promoting the germination of oak seeds, regardless of provenance, suggesting that this biostimulant could be an optimal option for use in silvicultural and reforestation practices. In contrast, the results for Cropmax were variable, showing that its effect may be less pronounced or dependent on the ecological conditions specific to each provenance. This research adds information to the understanding of how biostimulants can be used to improve oak seed germination and highlights the need for further studies to fully assess interactions between provenance' genetics and biostimulant treatments, to optimize reforestation success in different regions. The results of this work emphasize the importance of seed source and environmental factors specific to each region in determining essential phenotypic characteristics of oak seeds, which influence germination and initial seedling development. Identifying these variations contributes to a better understanding of oak seed adaptability, which is essential in reforestation and conservation practices that require careful selection of provenances to optimize initial success and long-term sustainability.

Conclusions

This study findings demonstrate a significant impact of biostimulant treatments on *Quercus robur* seed germination across different geographic origins. Atonik consistently yielded superior germination rates, particularly in seeds from Alba and Hunedoara, suggesting its potential as a valuable tool for oak seedling production. In contrast, the control group showed significantly lower germination, highlighting the substantial benefits of using biostimulants. These results highlight the critical importance of selecting appropriate biostimulants to optimize germination success, a crucial factor in successful reforestation and conservation efforts. Effective reforestation requires careful consideration of both genetic factors (seed provenance) and the local environmental conditions to ensure long-term sustainability. This study contributes valuable knowledge by emphasizing the need to combine effective biostimulants with judicious seed provenance selection for sustainable and healthy forest regeneration.

Authors' Contributions

Conceptualization, I.D.A., I.M.M.; Methodology, I.M.M. and F.A.R.; Software, I.M.M.; Formal analysis, I.M.M., C.D., and A.M.T.; Investigation, I.M.M., I.D.A. and A.M.T.; Resources, I.M.M.; Data curation, I.D.A. and C.D.; Supervision, M.B. and F.I.; Validation, I.D.A. and C.D.; Writing—original draft preparation, I.D.A. and F.A.R..; Writing—review and editing, I.M.M. and C.D. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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