

# Effect of Elevation on Morphological Characteristics and Germination of Black Alder (*Alnus glutinosa* subsp. *barbata*) Seeds

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## Abstract

*Aim of the study:* This study was undertaken to determine the effect of elevation on the characteristics of cones and seeds of black alder (*Alnus glutinosa* subsp. *barbata*).

*Area of study:* Cones were collected from three different elevations (270 m, 590 m and 860 m) within the native range of the species in Arhavi district of Artvin Province, in October 2017.

*Material and methods:* Morphological characteristic such as diameter and length of cones, width, length and thickness of seeds were measured. In addition, germination percentage and mean germination time parameters were used to evaluate the germination ability of seeds. Germination experiments were carried out with four replications where each replicate contained 50 seeds. Stratification pre-treatment was applied to seeds for 2, 4, 6 and 8 weeks.

*Main results:* Results showed that cone diameter, seed length, seed width and seed thickness were significantly affected by elevation. Differences in germination percentage and average germination time also were significant ( $p<0.05$ ) with respect to elevation. Seeds collected from 270, 590 and 870 m had the 2.71, 2.63 and 2.61 mm, seed lengths, 2.45, 2.49 and 2.08 mm seed width and 0.49, 0.52 and 0.41 mm seed thickness, respectively.

*Research highlights:* Seeds collected from 590 m had the greatest germination percentage (63.7%).

**Keywords:** Black alder, Elevation, Seed, Germination, Cold stratification

## Yükseltinin Sakallı Kızılağaç (*Alnus glutinosa* subsp. *barbata*) Tohumlarının Morfolojik Özelliklerine ve Çimlenmesine Etkisi

### Öz

*Çalışmanın amacı:* Bu çalışma, yükseltinin sakallı kızılğaç (*Alnus glutinosa* subsp. *barbata*) kozalakları ve tohumlarının özellikleri üzerindeki etkisini belirlemek amacıyla yapılmıştır.

*Çalışma alanı:* Sakallı kızılğaç kozalakları, Artvin İli Arhavi ilçesindeki türün doğal yayılış alanı içerisindeki üç farklı yükseltiden (270 m, 590 m ve 860 m) 2017 yılı ekim ayında toplanmıştır.

*Materyal ve yöntem:* Kozalaklarda, kozalak çapı ve uzunluğu, tohumlarda ise tohum genişliği, uzunluğu ve kalınlığı gibi morfolojik özellikler incelenmiştir. Ayrıca tohumların çimlenme kabiliyetini değerlendirmek için çimlenme yüzdesi ve ortalama çimlenme süresi parametreleri kullanılmıştır. Çimlendirme deneyleri, her tekrerde 50 tohum olmak üzere dört tekrerli olarak gerçekleştirilmiştir. Tohumlara 2, 4, 6 ve 8 hafta katlama ön işlemleri uygulanmıştır.

*Temel sonuçlar:* Sonuçlar, kozalak çapı, tohum uzunluğu, tohum genişliği ve tohum kalınlığının yükseklikten önemli ölçüde etkilendiğini göstermiştir. Çimlenme yüzdesi ve ortalama çimlenme süresindeki farklılıklar da yükseltiye göre önemli bulunmuştur ( $p<0.05$ ). 270, 590 ve 870 m'den toplanan tohumlar sırasıyla 2.71 mm, 2.63 mm ve 2.61 mm tohum uzunluğuna, 2.45 mm, 2.49 mm ve 2.08 mm tohum genişliğine ve 0.49 mm, 0.52 mm ve 0.41 mm tohum kalınlığına sahiptir.

*Araştırma vurguları:* 590 m'den toplanan tohumlar en yüksek çimlenme yüzdesine (%63.7) sahiptir.

**Anahtar Kelimeler:** Sakallı kızılğaç, Yükselti, Tohum, Çimlenme, Soğuk katlama



## Introduction

Black alder (*Alnus glutinosa* (L) Gaertn subsp. *barbata*), a subspecies of common alder (*Alnus glutinosa* (L) Gaertn), is found in the eastern Black Sea region of Türkiye (Güner et al., 2012). It grows naturally in Ordu, Giresun, Gumushane, Trabzon, Rize and Artvin provinces and has a significant range in the region only exceeded by oriental spruce, Caucasian fir, Scots pine and oriental beech (Yildirim et al., 2020). It grows on humid slopes, valley bottoms and stream banks at elevations ranging from sea level to 1700 m (Odabaşı et al., 2004; Mamikoğlu 2015).

Black alder requires high humidity and full sunlight at maturity even though it can withstand low light levels during early stages of growth (Kaliniewicz et al., 2018). It grows fast during the initial years but growth rate decreases with increasing age and can reach 30 m in height (Carus, 1994). Seed production begins at 20 and 30 years of age in stands with full and normal canopy closure, respectively. Seed year occurs every two to three years though some seeds are produced in years when seeds are low in quantity (Kaliniewicz et al., 2018).

Alders have nodules in their roots. The symbiotic bacterium within these nodules, *Frankia alni*, (Figueiredo et al., 2010; Orczewska et al., 2012) enriches poor soils by fixing atmospheric nitrogen (Diagne et al., 2013; Stokdyk & Herrman, 2014). Therefore, alders are used in restoration of mining sites, reforestation of disturbed forest areas, afforestation of sites low in organic nutrients (Karthikeyan et al., 2013; Bissonnette et al., 2014; Diagne et al., 2015) and as biofertilizer in industrial plantations to support the growth of economically valuable tree species (Güner et al., 2018). At the same time, if the growing conditions are suitable for alder, it is among the species preferred by the local people in agroforestry.

The production of alder seedlings has become important due to its increase use and potential use in a wide range of industries. The

most important factor affecting seedling production is seed quality. Therefore, it is important to obtain seeds with high genetic diversity and quality primarily for seedling production. Alder seeds lose their vigor if they're not collected shortly after maturation and not stored at low temperatures (Baskin & Baskin, 2014). The vigor loss resulting in low seed quality in alders can be minimized by monitoring the maturation stages of seeds. Elevation is another important factor affecting the seed maturation process as temperature has an inverse relationship with elevation. The length of the growing period decreases with increasing elevation, which subsequently affects flowering, seed formation and maturation processes in trees.

Since alder trees are plants with high moisture demands, changes in the amount of precipitation with the changing elevation within the optimum distribution limits may cause the seed characteristics to differ. In this study, it was aimed to determine whether the characteristics of black alder cones and seeds change according to elevation differences.

## Materials and Methods

Black alder seeds used in the study were collected from natural alder forests, which are not protected as a seed source within Arhavi district of Artvin province in October 2017 (Figure 1, Table 1). The areas remain within the boundaries of Artvin Forest Regional Directorate Arhavi Forest Management District. Care was taken to ensure that trees from which seeds were collected had the same diameter and height same characteristics and equal settlement areas as much as possible. Cones were collected from trees that are as far apart as possible and have no neighborly boundaries to minimize relatedness of the mother-trees. At least 150 cones (50 from each tree) were collected from 3 trees at each elevation. Measurements to determine seed and cone characteristics, pre-treatment and germination experiments were conducted at the Seed and Afforestation Laboratory of Artvin Çoruh University.

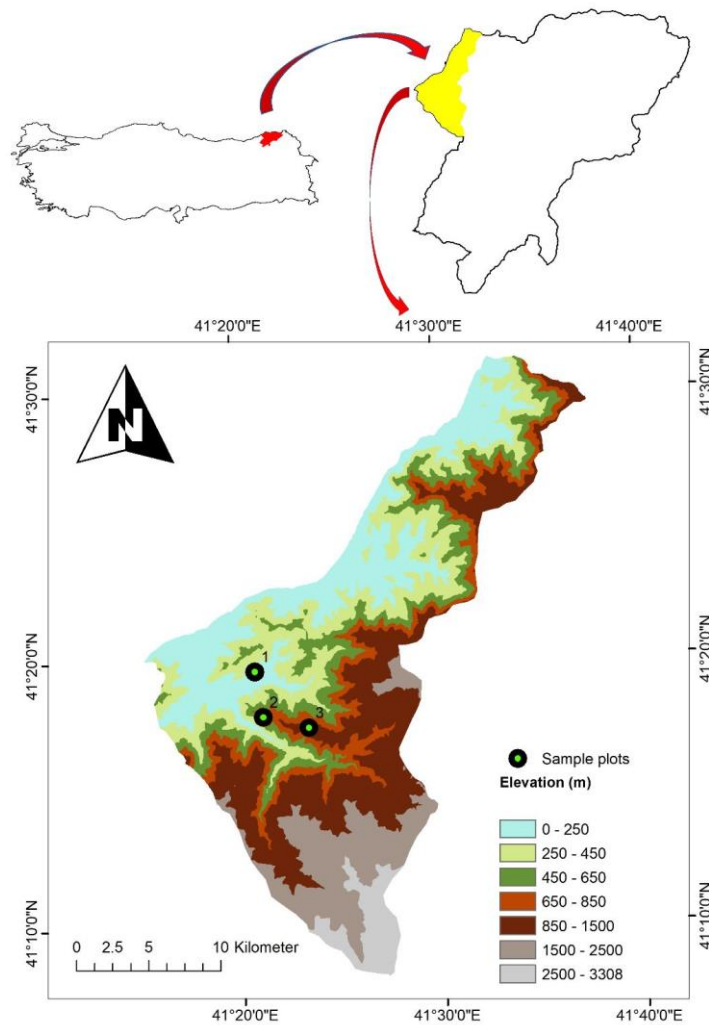


Figure 1. Cone collection sites.

Table 1. Site characteristics.

No	Compartment	Elevation (m)	UTM Coordinates	
			x	y
1 (bottom)	72	270	696476	4577669
2 (middle)	133	590	697077	4574514
3 (top)	213	860	700252	4573792

Cone diameter and length were measured before cones lost moisture and opened. Cone diameters were determined by average of two perpendicular measurements from the middle part of the cones. Cones were spread on a dry surface in a single layer at room temperature in laboratory settings after measurements were completed and seeds were separated from cones. Seeds were kept at room temperature for 10 days before measurement and pre-treatment. In the calculation of seed weights (1000 pcs), the average weight

calculation method was used in 8 x 100 seeds (ISTA, 1993). The sound seed rates were detected by cutting 100 seeds taken from seed weight calculated samples. Seed length, width and thickness were measured with an electronic caliper (Standard Gage 00534021) with 0.01 mm precision.

Stratification pre-treatment was done by placing the seeds between moist sand layers in plastic storage containers. Cheesecloth bags were used to prevent seeds from mixing with sand. Seed stratification was conducted in a

refrigerator at  $3\pm 1$  °C. Stratification pre-treatment periods were conducted at two, four, six and eight weeks. The seeds in the refrigerator were used as a control.

Germination experiments were carried out with four replications. Each replicate contained 50 seeds resulting in 200 seeds total.

Filtrate paper was spread into plastic petri dishes to prepare the germination medium. The germination experiment was conducted at 22 °C in an oven in a dark medium. Seeds which root grows at least 3 mm have considered germinated. Germination tests were continued up 28 days.

Germinating seeds were counted on the 3<sup>rd</sup>, 7<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> days from the beginning of germination experiment and periodically at weekly intervals thereafter until germination ended. Germination percentage and average germination time were determined using the following equation 1-2:

$$GP = \frac{\sum n}{N} \times 10 \quad (1)$$

where GP is germination percent, n is number of seeds that were germinated and N is total number of sown seeds.

$$AGT = \frac{\sum(t_i \cdot n_i)}{\sum n_i} \quad (2)$$

where AGT is average germination time,  $n_i$  is number of seeds that germinated over  $t_i$  days and  $t_i$  is the period from the beginning of

germination experiment until germination check (day).

The effect of elevation on seed properties, cone characteristics, seed germination percentage and average germination time were determined by analysis of variance (ANOVA) and the Duncan test with 0.05 significant level. Pearson correlation analysis was performed to determine the relationship between elevation and the parameters examined. Statistical analyses were carried out using SPSS (Version 19, 2019) statistical package program.

### Results and Discussion

Analyses revealed that cone length was not affected by elevation whereas cone diameter varied in relation to elevation. The widest diameter (10.80 mm) was measured on the cones collected at 860 m elevation (Table 2). Yilmaz (2020) reported that the greatest cone diameter and length on cones native to Bahce were collected at 460 m for Turkish alder (*Alnus orientalis*). Yilmaz and Yilmaz (2021) measured the greatest length (21.54 mm) and diameter (12.33 mm) in cones collected from Türkoğlu and the smallest diameter in cones collected from Karaisali for common alder (*Alnus glutinosa*). The elevation of these sites are 1110 m and 697 m, respectively. There were seven sites whose elevation ranged from 100 m to 675 m in the study conducted by Yilmaz (2020). In this study, cones were collected from different elevations within the same site.

Table 2. Parameters of cones coming from different elevations and their statistical comparison

Elevation (m)	Variable	N	F value	Mean	Minimum	Maximum	Standard error
270	Cone length (mm)	50	0.44	16.47	11.63	21.26	1.42
590		50		16.62	14.46	21.26	1.33
860		50		16.73	13.71	22.85	1.43
270	Cone diameter (mm)	50	22.64*	10.09 <sup>b</sup>	8.82	11.76	0.68
590		50		9.95 <sup>b</sup>	8.32	11.06	0.55
860		50		10.80 <sup>a</sup>	8.45	13.25	0.78

\* Indicates significance at 95% ( $p < 0.05$ )

The weights (1000 pcs) of seeds collected from 270 m, 590 m and 860 m elevations were determined as 0.94 g, 1.17 g and 0.80 g, respectively, while the sound seed rates were detected as 74%, 90% and 69%.

Black alder seed lengths, seed widths and seed thickness varied with elevation ( $p < 0.05$ ).

The greatest values for seed length and width were found in seeds collected at 270 m elevation while the greatest seed thickness was reported in seeds collected at 590 m elevation (Table 3). The difference observed between elevations can be attributed to the fact that elevation affects environment

conditions for growth, which subsequently determines seed shape and size (Baskin & Baskin, 2014).

Seed length, width and thickness of seeds with the greatest cone diameter collected at 860 m elevation were smaller compared to the seeds collected at other elevations. Yilmaz and Yilmaz (2021) determined that common alder seed dimensions varied with populations and seed length, seed width and seed thickness

with values ranging between 2.93-2.42 mm, 2.60-2.05 mm and 0.80-0.65 mm, respectively. The longest and widest seed was reported from the Düziçi population at 164 m elevation and the thickest seed was found in the Kozan population at 747 m elevation (Yilmaz & Yilmaz, 2021). The differences observed in seed characteristics among populations in their study was observed among elevations as well in this study.

Table 3. Parameters of seeds coming from different elevations and their statistical comparison.

Elevation (m)	Seed characteristic	F value	Mean	Minimum	Maximum	Standard deviation
270	Seed length (mm)	6.73*	2.71 <sup>a</sup>	2.11	3.22	0.19
590			2.63 <sup>b</sup>	2.26	3.03	0.16
860			2.61 <sup>b</sup>	1.42	3.00	0.22
270	Seed width (mm)	76.38*	2.45 <sup>a</sup>	1.62	3.07	0.32
590			2.49 <sup>a</sup>	1.88	3.15	0.27
860			2.08 <sup>b</sup>	1.65	2.69	0.19
270	Seed thickness (mm)	72.11*	0.49 <sup>b</sup>	0.40	0.64	0.05
590			0.52 <sup>a</sup>	0.39	0.65	0.07
860			0.41 <sup>c</sup>	0.10	0.57	0.07

\* Indicates significance at 95% (p < 0.05)

Untreated (control) seeds had low percentage of germination, indicating that cold stratification treatment stops germination inhibition in this species. Black alder seeds pretreated with cold stratification had greater germination percentage compared to the control seeds (Figure 2). The duration of cold stratification treatment had no significant effect on either germination percentage or

average germination time. Yilmaz (2020) reported that cold stratification treatment duration had no significant effect on germination response in Turkish alder seeds, similar to the findings of this study. However, Yilmaz and Yilmaz (2021) found that cold stratification treatment for 8 weeks had a significant effect on germination response in common alder seeds.

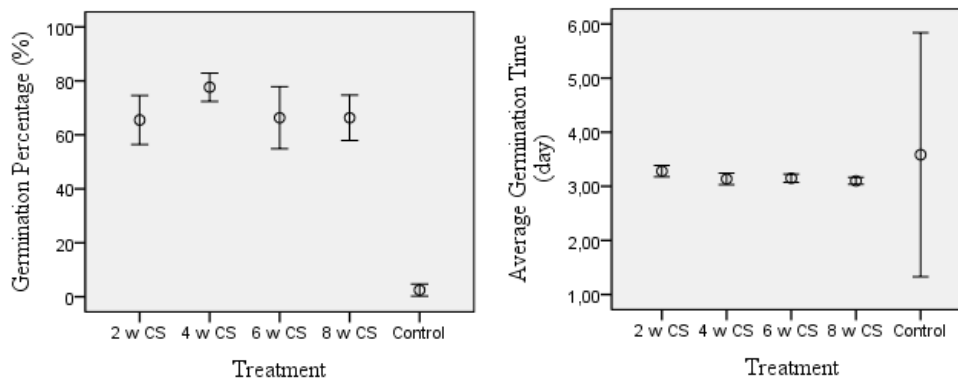


Figure 2. Germination parameters of seeds after different pre-treatment (w: week, CS: Cold Stratification)

Cold stratification speeds up the morphological and physiological development of seed embryos and results in increased germination percentage (Baskin and Baskin, 1991). The stimulation of seed

germination percentage by cold stratification pre-treatment in this study, similar to the results reported by Yilmaz and Yilmaz (2021) for common alder seeds, indicates the resting requirement of black alder seeds. The

identified resting requirement in black alder seeds can be described as a physiological barrier to germination (Yilmaz, 2020). Results suggest that cold stratification pre-treatment can be used to remove this barrier in black alder seeds.

The greatest germination percentages were observed in the seeds subjected to cold

stratification pre-treatment for four weeks across the three different elevations. For seeds subjected to cold stratification pre-treatment for four weeks, 75%, 85.5% and 72.5% germination was recorded at 270 m, 590 m and 860 m, respectively (Table 4).

Table 4. ANOVA results for germination percentage.

Treatment	Elevation (m)								
	270			590			860		
	F value	Mean	Std. Deviation	F value	Mean	Std. Deviation	F value	Mean	Std. Deviation
2 w CS		71.5 <sup>a</sup>	9.00		71.5 <sup>b</sup>	5.00		53.5 <sup>b</sup>	18.86
4 w CS		75.0 <sup>a</sup>	6.00		85.5 <sup>a</sup>	6.61		72.5 <sup>a</sup>	6.61
6 w CS	68.21**	75.0 <sup>a</sup>	3.46	124.35**	80.0 <sup>ab</sup>	9.93	24.66**	44.0 <sup>b</sup>	8.49
8 w CS		63.0 <sup>a</sup>	12.38		80.5 <sup>ab</sup>	5.74		55.5 <sup>b</sup>	4.43
Control		1.5 <sup>b</sup>	3.00		1.0 <sup>c</sup>	1.15		5.0 <sup>c</sup>	4.76
Total	23.60*	57.2 <sup>b</sup>	29.710		63.7 <sup>a</sup>	32.980		46.1 <sup>c</sup>	24.789

\* Indicates significance at 95% ( $p < 0.05$ ); Letters denote significant differences ( $p < 0.05$ ) within a line; \*\* Indicates significance at 95% ( $p < 0.05$ ); Letters denote significant differences ( $p < 0.05$ ) within a column; w: week, CS: cold stratification

The germination percentage of seeds collected at 590 m elevation (63.7%) was greater than seeds collected at 270 m and 860 m (57.2% ve 46.1%) ( $p < 0.05$ ) (Table 4). Even though a decrease in germination percentage with respect to elevation generally has been reported in studies conducted with different species (Salehani et al., 2013; Güney et al., 2014), studies reporting increases in germination percentage with increasing elevation also exist (Bhatt & Ram, 2007). The differences in germination percentages of seeds collected at different elevations probably is related to the percentage of sound seeds, collection year, variation in factors affecting plant development, seed production and germination including temperature, soil moisture, wind speed, soil structure and radiation (Baskin & Baskin, 2014; Tajbakhsh & Ghiyasi, 2008).

The greatest germination percentage (55.3%) for common alder was reported in the Andirin population at an elevation of 1121 m while the Kozan population at the lowest elevation of 164 m found 33% of germination percentage (Yilmaz & Yilmaz, 2021). Yilmaz (2020) reported the greatest germination percentage of 54.5% for Turkish alder in Akseki population at 600 m elevation. Yilmaz (2020) found germination percentages of 33.3% and 24.8% for the highest elevation

Osmaniye (765 m) and the lowest elevation Bucak (190 m) populations, respectively in the same study. Although Yilmaz (2020) did not take into account the elevation differences in his study, it can be said that the results of the study and the findings we obtained are similar.

Kaliniewicz et al. (2018) report the effect of physical parameters of black alder seeds on germination and a concurrent increase in germination potential with increase in seed length, diameter and thickness. The presence of lower germination percentages in seeds with the greatest length and width values collected at 270 m elevation compared to the seeds collected at 590 m elevation in the current study may be attributed to the effect of elevation rather than seed characteristics.

Pre-treatment and elevation had no significant effect on average germination time ( $p > 0.05$ ). However, in Table 5, it seems that cold stratification unifies germination rate and there is a high variability of average germination time compare to pre-treated seeds. Results show that average germination time of black alder seeds is quite short (3.10-5.17 days). Similarly, Yilmaz and Yilmaz (2021) reported an average germination time of common alder seeds of 2.9-5.2 days and Yilmaz (2020) reported 4.55-5.78 days for Turkish alder seeds.

Table 5. ANOVA results of average germination time.

Treatment	Elevation (m)								
	270			590			860		
	F value	Mean	Std. Deviation	F value	Mean	Std. Deviation	F value	Mean	Std. Deviation
2 w CS		3.39	0.09		3.11	0.10		3.34 <sup>a</sup>	0.15
4 w CS		3.24	0.14		3.06	0.13		3.10 <sup>a</sup>	0.20
6 w CS	2.03	3.15	0.17	0.21	3.19	0.07	5.67*	3.11 <sup>a</sup>	0.13
8 w CS		3.13	0.10		3.07	0.08		3.10 <sup>a</sup>	0.13
Control		1.33	2.67		4.25	5.06		5.17 <sup>b</sup>	1.67
Total	1.12	2.85	1.32		3.34	2.07		3.56	1.07

\* Indicates significance at 95% ( $p < 0.05$ ); Letters denote significant differences ( $p < 0.05$ ) within a column; w: week; CS: cold stratification.

Pearson correlation analysis results show a positive relationship between height and cone diameter. And also, there is a negative relationship between elevation and seed length, seed width and thickness at the 0.01

significance level (Table 6). In table 6, it is seen that the relations between altitude and pretreatment, germination percentage and average germination time are not significant

Table 6. Pearson correlation analysis explaining the relationship between elevation and parameters examined

	Cone length	Cone Diameter	Seed length	Seed width	Seed thickness	GP	AGT
Pearson Correlation	0.077	0.375**	-0.199**	-0.459**	-0.412**	-0.144	0.192
Sig. (2-tailed)	0.351	0	0.001	0	0	0.273	0.142
N	150	150	300	300	300	60	60

\*\* Correlation is significant at the 0.01 level (2-tailed).

Selahani et al. (2013) reported decreased average germination time with increase in elevation. The shortening of growing period with increasing elevation may cause the seeds of plants found at these elevations to genetically start germination later and end germination earlier. Consequently, a shorter germination period at higher elevations compared to lower elevations is an expected phenomenon.

### Conclusions

In the study carried out to determine whether the cone and seed characteristics of the black alder species change with the increase in elevation, the characteristics, germination percentages and average germination times of the cones collected from three different elevations in alder stands within the boundaries of Artvin Regional Directorate of Forestry Arhavi Forest Management Directorate were investigated. As a result of the study, it was revealed that in terms of cone characteristics, cone length is not affected by elevation, and cone diameter

varies according to elevation. In terms of the germination characteristics examined, it was concluded that the germination percentage was affected by the elevation, but the average germination time was not affected. On the other hand, it was determined that the cold stratification pretreatments applied to eliminate the superficial physiological germination barrier in Alder seeds provided higher germination than the control group seeds, and the highest germination rate in all three elevations occurred in the seeds that were treated with cold stratification pretreatment for four weeks. While the average germination time did not change according to the elevation and the pretreatment applied, the results revealed that the average germination time of black alder seeds was quite short. According to the results, it can be said that the characteristics of the seeds collected from 590 m elevation, which is considered in the study, are better. Although the study was based only on elevation differences within a single population, it can be said that the study should

cover a wider time period in order for the results to be considered binding because the observed trends are a combination of many interrelated genetic, environmental and weather factors. In order to reach more definite conclusions on this subject, it may be suggested to repeat the studies for several years by increasing the number of seeds collected trees, the number of seed collection areas and the number of elevation steps in both black alder and other alder species.

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### Ethics Committee Approval

N/A

### Peer-review

Externally peer-reviewed.

### Author Contributions

Conceptualization: S.G.; Investigation: A.G., S.G.; Material and Methodology: A.G.; Supervision: S.G.; Visualization: A.G.; Writing-Original Draft: A.G., S.G.; Writing-review & Editing: A.G., S.G.; Other: All authors have read and agreed to the published version of manuscript.

### Conflict of Interest

The author has no conflicts of interest to declare.

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