The Effect of Seed Size on Germination and Seedling Growth in Sweet Chestnut (Castanea sativa Mill.)

Tumpa, Katarina; Vidaković, Antonio; Drvodelić, Damir; Šango, Mario; Idžojtić, Marilena; Perković, Ivan; Poljak, Igor

Source / Izvornik: **Forests (1999-4907) 12 (2021); 858, 11, 2021, 12, 1 - 11**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/10.3390/f12070858

forests

Article

The Effect of Seed Size on Germination and Seedling Growth in Sweet Chestnut (*Castanea sativa* Mill.)

Katarina Tumpa [1], Antonio Vidaković [1], Damir Drvodelić [2], Mario Šango [2], Marilena Idžojtić [1], Ivan Perković [2] and Igor Poljak [1,*]

[1] Institute of Forest Genetics, Dendrology and Botany, Faculty of Forestry and Wood Technology, University of Zagreb, HR-10000 Zagreb, Croatia; ktumpa@sumfak.hr (K.T.); avidakovi@sumfak.hr (A.V.); midzojtic@sumfak.hr (M.I.)

[2] Institute of Ecology and Silviculture, Faculty of Forestry and Wood Technology, University of Zagreb, HR-10000 Zagreb, Croatia; ddrvodelic@inet.hr (D.D.); msango@sumfak.hr (M.Š.); iperkovic@sumfak.hr (I.P.)

* Correspondence: ipoljak@sumfak.hr; Tel.: +385-1-2352547

Abstract: The quantity and quality of seedlings in the nursery has an impact on the success of re-establishment and later growth. High germination rates enable a sufficient number of seedlings, and their quality is assessed using a number of parameters, including seedling height and root collar diameter. These parameters are influenced in some species by seed size, but the correlation between them is species-specific. The model species in this research was sweet chestnut (*Castanea sativa* Mill.), and seeds from 12 populations from two distinct biogeographical regions of Croatia were collected. We examined the influence of seed size on four parameters: germination rate, seedling height, root collar diameter and sturdiness quotient. Seed size has been shown to have a positive influence on both seedling height and root collar diameter, whereas no such correlation was noted for germination rate and sturdiness quotient. Significant differences in nut size and seedling growth parameters were found between the Mediterranean and continental populations, with higher values observed in the coastal Mediterranean populations. We concluded that seed origin and seed size have a significant impact on seedling growth and are important factors to consider when choosing seed material. Further nursery operations should consider seed origin and local environmental conditions when choosing seedlings for reforestation efforts and general forest operations.

Keywords: seed size; germination; seedling height; root collar diameter; sweet chestnut

Citation: Tumpa, K.; Vidaković, A.; Drvodelić, D.; Šango, M.; Idžojtić, M.; Perković, I.; Poljak, I. The Effect of Seed Size on Germination and Seedling Growth in Sweet Chestnut (*Castanea sativa* Mill.). *Forests* **2021**, *12*, 858. https://doi.org/10.3390/f12070858

Academic Editor: Daniel J. Johnson

Received: 27 May 2021
Accepted: 27 June 2021
Published: 29 June 2021

1. Introduction

The nursery sector and seedling production are an integral part of most tree growing programs, providing landholders and communities with high quality planting stock [1,2]. Artificial regeneration requires seedlings that can compete with natural vegetation, tolerate browse damage, and survive adverse conditions [3]. Unlike agricultural crops, seeds from forest trees are often of limited availability, have lower germination rates and are more expensive to come by, making the highest possible germination rate an important factor of production [4]. High germination rates can be aided by pre-sowing germination tests, which can provide nursery operators with an accurate estimation of viable seeds. As a result, the proper sowing rate can be selected to produce the optimum number of seedlings [5]. The quality of seedlings obtained from the nursery will affect the hardening phase of the seedling [6], as well as re-establishment in the field, and has an important role in its subsequent productivity [7,8]. Evaluation of seedling quality is based on the monitoring of growth parameters, such as the morphological attributes of shoot height, root collar diameter and root systems [9]. In addition, sturdiness quotient, a parameter based on non-destructive measurements of root collar diameter and height, can give a more accurate picture of the viability and vigor of the seedling [10]. Together with the assessment of drought resistance, freezing tolerance, mineral nutrient status, root growth potential, and root electrolyte leakage, this evaluation provides a more accurate picture of seedling health and can occur at various stages of nursery production. These parameters and assessments ensure a practical monitoring method and provide initial information on the expression of future growth [11].

Besides being used in forestry, high quality seedlings are the backbone of forest and landscape restoration programs. Failure to produce enough plants of sufficient quality can result in unsuccessful outplanting programs [12]. In recent years, the international community has set the goal of restoring millions of hectares of deforested and degraded lands. Availability of quality nursery seedlings is a crucial component and precursor for meeting this target [12]. Nursery production, especially of broadleaved species, contributes to the sustainability of plantation forestry, promotes community forests, and secures the preservation of endangered species or genotypes.

Some Mediterranean species, like sweet chestnut (*Castanea sativa* Mill., Fagaceae), may have lost their role as a source of subsistence food, but they continue to play a major role in the cultural context and in many agroforestry systems [13]. Sweet chestnut is a noble hardwood species, cultivated for centuries as a nut-producing tree. Besides having edible nuts, the trees are of high value in the production of tannins, honey, and timber [14]. Native across the Mediterranean region and in Asia Minor, sweet chestnut can be found in naturalized stands, managed coppice and grafted fruit orchards [15,16]. Thus, an important aspect of sweet chestnut nursery production is the production of high-quality seedlings with high survival capability for reforestation efforts in forestry, as well as the production of rootstock for grafting cultivated varieties.

The aim of this study was to determine the differences in seed germination and two seedling growth parameters, namely seedling height and root collar diameter, between 12 sweet chestnut populations, originating from two distinct biogeographical regions. The correlation between seed size and growth parameters was examined as well. The results of this research could be significant for future nursery production of sweet chestnut seedlings.

2. Materials and Methods

2.1. Experimental Material

The nuts used in this research were collected from 12 native sweet chestnut populations in Croatia (Table S1). Populations Poreč, Učka, Cres and Buje represented the Mediterranean biogeographical region, and populations Krndija, Psunj, Bilogora, Moslavačka gora, Medvednica, Žumberak, Samoborsko gorje and Zrinska gora the temperate, continental biogeographical region. In each population, ten trees were selected for sampling, each at least 50 m apart to minimize the possibility of half-sib progeny being mixed. Finally, 200 nuts were collected from each individual tree. Both central and side nuts from burrs were collected. Nuts were collected in October 2019 and were immediately prepared for stratification to prevent desiccation.

Seed preparation and stratification were performed according to ISTA (International Seed Testing Association) rules [17]. The float/sink test was performed to remove empty or damaged nuts. Other visibly damaged nuts were also discarded. Healthy nuts were counted and weighed individually and for total fresh mass (SM) to the nearest 0.01 g. To prepare the stratification medium, distilled water and quartz sand (granules 0.1–0.4) were mixed, with the water having 37% of the total volume of sand. The stratification medium and nuts were mixed in a ratio of approximately 2:1, and this mixture was placed in perforated plastic freezer bags. The bags were labelled and placed in a refrigerator at 4 °C. Plastic bags were checked on a weekly basis for mold and germinated seeds. Moldy, rotten nuts were removed to prevent the spread of decay, whereas germinated seeds were pushed deeper into the stratification medium to prevent drying out. To preserve the moisture within the stratification medium, distilled water was sprayed over the medium every seven to fourteen days, whenever it was noticed that the sand medium was drying out. Stratification ended in March, and germinated seeds were transplanted into the nursery.

2.2. Experimental Design and Nursery Production

Raised beds were prepared for the planting of the germinated seeds. Each of the 12 populations, represented by 10 trees each, were sown in three repetitions. The nursery beds were relatively uniform and the environmental conditions identical. Within each bed, six rows, approximately 15 cm deep and spaced 25 cm apart, were dug out, and seeds were planted with their radicule facing downward. Each tree was clearly marked with wooden stakes tagged with metal plates and the corresponding tree code. Each seed had ample growing space, being spaced 5 cm apart. Beds were covered with agrotextile (frost cloth) until mid-April. Throughout the growing season, young plants were regularly checked, watered during the summer, and the beds were weeded. The seedlings were healthy, and no fertilization or anti-disease treatments were applied.

2.3. Germination and Seedling Data Collection

Seed germination, i.e., plumule emergence was recorded for each tree on a weekly basis. The percentage of germinated seedlings was calculated as the number of seedlings with emerged plumule in the total number of sown seeds (emerged plumule/all sown seeds $\times$ 100). Seeds that germinated during the stratification were included within the germinated seeds, since germination during stratification was uniform.

All seedlings were measured in early December 2020. Seedling height (SH) was measured with a measuring tape to the nearest 0.1 cm, from root collar up to the apical bud. Root collar diameter (RCD) was measured with a digital caliper to the nearest 0.01 cm. Sturdiness quotient was calculated as the ratio of the height of the seedling to the root collar diameter [18,19].

2.4. Data Analyses

All statistical analyses were performed using the software packages STATISTICA version 13 [20] and R v.3.4.3 [21]. For all the studied variables (seed mass, germination, seedling height, root collar diameter and sturdiness quotient), standard descriptive statistical parameters were calculated: arithmetic mean ($\overline{X}$), standard deviation (SD) and coefficient of variation (CV). Correlations between variables were analyzed using Pearson's correlation coefficient. To assess the possibility of conducting multivariate statistical analyses and parametric tests, the symmetry, unimodality and homoscedasticity of the data were verified [22]. Assumptions of normality were checked using the Shapiro–Wilk test, and the assumption of homogeneity of variance was checked using Levene's test. Statistically significant differences between the studied sweet chestnut populations were tested using analysis of variance (ANOVA). To explore the variation in individual studied traits, differences among population means were tested for each trait (Y) using the model Y = P + T(P) + e, where P was the population effect, T(P) was the tree effect nested within the population, and e was the error. For each analysis, statistically significant differences between means were identified using the Tukey–Kramer multiple comparison test, at $p \leq 0.05$. Multivariate statistical methods were used to identify the population differentiation. Principal component analysis based on five studied traits was performed. The biplot was constructed by two principal components showing the analyzed individuals and traits. In addition, cluster analysis was used to identify population differentiation. The conducted cluster analysis resulted in a hierarchical tree, where the unweighted pair-group method with arithmetic mean (UPGMA) was used to join the clusters, and the Euclidean distance was used to define the distance between the studied objects. Prior to ANOVA and multivariate statistical analysis, values of the germination percentages were square root arcsine transformed. In addition, the input data in multivariate statistical methods were previously standardized, i.e., standardization of characters to zero mean, and unit standard deviation was performed prior to each multivariate analysis.

3. Results and Discussion

Statistically significant differences between the studied populations were found for germination rates (F = 3.317, $p < 0.001$), sturdiness quotient (F = 3.334, $p < 0.001$), and the three measured parameters: seed mass (F = 6.824, $p < 0.001$), height of the seedling (F = 16.658, $p < 0.001$), and diameter of the root collar (F = 6.118, $p < 0.001$). Similarly, statistically significant differences among nested trees within populations were confirmed for all of the studied traits. In addition, most of the population pairs from different biogeographical regions had significant pairwise values (Table 1).

Table 1. Arithmetic means ($\overline{X}$), standard deviations (SD) and coefficients of variation (CV) for seed mass (SM), germination rates, seedling height (SH), root collar diameter (RCD) and sturdiness quotient (SQ) in 12 sweet chestnut populations. Population ID: BU—Buje; CR—Cres; PO—Poreč; UC—Učka; BI—Bilogora; KR—Krndija; ME—Medvednica; MG—Moslavačka gora; PS—Psunj; SG—Samoborsko gorje; ZG—Zrinska gora; ZU—Žumberak. Means marked with different letters were significantly different according to the Tukey–Kramer multiple comparison test, at $p \leq 0.05$ level.

Population ID	Seed Mass (g)		Germination (%)		Root Collar D. (mm)		Seedling Height (cm)		Sturdiness Quotient	
	$\overline{X} \pm$ SD	CV (%)	$\overline{X} \pm$ SD	CV (%)	$\overline{X} \pm$ SD	CV (%)	$\overline{X} \pm$ SD	CV (%)	$\overline{X} \pm$ SD	CV (%)
BU	6.0 ± 1.6 ab	25.7	57 ± 16.6 ab	29.4	5.2 ± 1.2 a	23.5	24.0 ± 4.5 ab	18.5	4.7 ± 0.8 abc	16.9
CR	10.5 ± 2.4 d	22.6	67 ± 12.8 abc	19.0	8.4 ± 1.1 bc	12.8	32.3 ± 4.2 cd	13.1	3.9 ± 0.3 abc	8.1
PO	7.8 ± 1.1 ac	14.4	58 ± 12.9 abc	22.2	9.5 ± 1.9 c	19.9	38.8 ± 6.2 de	15.9	4.2 ± 1.1 abc	24.9
UC	9.4 ± 2.8 cd	30.2	69 ± 17.0 abc	24.8	8.5 ± 1.9 bc	22.3	40.5 ± 5.6 e	13.8	4.9 ± 1.0 bc	20.7
BI	5.0 ± 1.5 b	31.0	60 ± 15.3 abc	25.3	7.3 ± 1.1 abc	14.9	25.5 ± 4.4 abc	17.4	3.5 ± 0.6 a	15.8
KR	7.0 ± 1.1 abc	16.3	80 ± 12.1 c	15.2	5.9 ± 1.5 a	25.0	29.0 ± 4.0 bc	13.8	5.1 ± 1.0 c	19.6
ME	6.7 ± 2.3 abc	34.0	63 ± 17.6 abc	27.9	6.6 ± 1.5 ab	23.0	26.5 ± 5.0 abc	18.9	4.1 ± 0.5 abc	11.0
MG	7.0 ± 2.2 abc	30.8	62 ± 17.0 abc	27.5	6.5 ± 1.4 ab	21.7	26.5 ± 6.1 abc	23.0	4.1 ± 0.5 abc	12.1
PS	7.0 ± 0.7 abc	9.8	70 ± 12.0 abc	17.1	7.0 ± 1.9 ab	26.7	26.2 ± 4.8 abc	18.3	4.0 ± 1.1 abc	27.1
SG	6.7 ± 1.4 ab	21.2	77 ± 10.6 bc	13.7	5.9 ± 2.0 a	33.9	21.4 ± 3.7 a	17.1	3.9 ± 1.2 abc	29.6
ZG	6.2 ± 1.4 ab	21.9	53 ± 17.5 a	32.9	6.4 ± 1.3 ab	21.0	23.6 ± 2.1 ab	9.1	3.8 ± 0.5 ab	12.4
ZU	7.8 ± 2.0 ac	25.5	55 ± 13.9 a	25.3	6.2 ± 2.0 ab	33.0	23.6 ± 4.4 ab	18.8	4.0 ± 0.9 abc	21.6

The average seed mass of three out of four Mediterranean populations had the highest values: Cres (SM = 10.5 ± 2.4 g), Učka (SM = 9.4 ± 2.8 g) and Poreč (SM = 7.8 ± 1.1 g). Seeds from Bilogora and Buje populations had the lowest values: 5.0 ± 1.5 g and 6.0 ± 1.6 g, respectively (Table 1). Previously published data by Idžojtić et al. [23] showed similar values, with Mediterranean populations having the heaviest nuts. Some populations, like Moslavačka gora, showed notable differences in seed mass results from this research compared with the results from 2009 [23]. Seasonal variation and the specifics of the environmental conditions of each population are most likely to be the cause of this variability over the years [24–27]. In addition, Idžojtić et al. [23] did not measure the central fruit inside the chestnut burrs, which differs morphologically and is likely to influence the average seed mass values.

Chestnut seeds are the least dormant and easiest to germinate of all tree species, rotting easily and requiring high moisture content in order to stay viable [28]. Therefore, stratification is needed to not only overcome dormancy but to keep the seeds viable as well. The ideal temperature for stratification is in the range of +3 to +5 °C [28]. Reported average germination rates of chestnut seeds are within the range of 65% to 87% [29,30]. Germination rates obtained in this study were comparable at 53% to 80% (Table 1). Mediterranean populations had somewhat lower germination rates on average (57%–69%), compared with continental populations (53%–80%). It is reported that seed germination is influenced by both genetic as well as environmental factors [31]. All 12 populations had already started to germinate during stratification, and seed emergence occurred simultaneously. In addition, it is important to note that germination during stratification did not negatively affect the sowing and development of seedlings. The same results were previously reported by Tilki and Alptekin [32].

Seedlings from the three Mediterranean populations (Cres, Učka and Poreč) were higher on average and had a larger root collar diameter than the continental populations (Table 1). The tallest seedlings, on average, were measured in the population Učka (SH = 40.5 ± 5.6 cm), and the seedlings with the largest root collar diameter on average were measured in the population Poreč (RCD = 9.5 ± 1.9 mm). Samoborsko gorje was the population with the shortest seedlings (SH = 21.4 ± 3.7 cm), and population Buje had the lowest average value for the root collar diameter (RCD = 5.2 ± 1.2 mm).

Sturdiness quotient values ranged from 3.5 ± 0.6 (Bilogora population) to 5.1 ± 1.0 (Krndija population). The ideal value for a seedling to be considered sturdy is less than six [19], and our results are within the recommended values. This proves that seedlings are vigorous and robust, making them more likely to survive out-planting, especially on windy or dry sites [18].

The first two components from the principal component analysis explained 38.21% and 28.02% of the total variation, respectively (Table 2). The first principal component (PC1) was highly negatively correlated with seedling height and root collar diameter, and moderately negatively with fruit size. The second principal component (PC2) was strongly negatively correlated with the sturdiness quotient, and moderately positively with the root collar diameter. From the biplot on Figure 1, it is quite evident that the coastal Mediterranean populations are associated with larger seeds and seedlings, and the continental populations with smaller seeds and seedlings. However, population Buje was somewhat specific, characterized by unexpected values of seed size and seedling growth. Although biogeographically related to the rest of the Mediterranean populations, population Buje was characterized by smaller nuts and smaller seedlings, and it showed similarity and linkage to the populations from the continental region (Figure 2). This curious fact can be attributed to previously published results by Poljak et al. [33]. The mentioned authors [33] revealed that the Mediterranean population Buje exhibits clear genetic differentiation from other Croatian coastal populations. A similar effect of genetic variability on seedling growth was noted for several other species [34–37].

Table 2. Pearson's correlation coefficients between five seeds and seedling traits and scores of the first three principal components.

Trait	PC—Principal Component		
	PC1	PC2	PC3
Seed Mass	−0.587506	−0.354325	0.183710
Gremination	−0.257149	−0.259451	−0.930168
Root Collar Diameter	−0.855226	0.481539	0.015402
Seedling Height	−0.869198	−0.278959	0.154842
Sturdiness Quotient	0.111337	−0.947850	0.148190
Eigenvalue	1.91	1.40	0.95
% of Variance	38.21	28.02	18.90

In this research, we found no evidence of a decisive influence of seed weight on germination performance, with only extremely low correlations being observed between these two parameters (Table 3). Although the data for sweet chestnut is limited, the connection between seed size, germination rate and survival has been well-studied in another Fagaceae genus, i.e., oak (*Quercus* L.). Our results are in accordance with Alptekin and Tilki [38] and Garcia-De La Cruz et al. [39], who concluded that seed size did not affect the acorn germination rate or seedling emergence of the four analyzed oak species. On the other hand, Purohit et al. [40] and Pandey et al. [41] described a positive relationship between seed size and germination rate in oaks. In addition, they concluded that the impact of seed size on germination rate was primarily due to the increased water content in larger seeds. Similar positive correlations between seed mass and germination rate were documented in other plant species [42–46]. These different results, even within the same genus, can possibly be explained as a species-specific reaction to the environmental conditions, variation in maternal origin, differences in provenance and intraspecific variation within a larger range of a species [39,47]. However, storage and proper stratification prior to sowing can also influence the germination and subsequent development of seedlings [32]. This was confirmed by Hatzig et al. [31]. Their results indicated that genetic determinants for the manifestation of seed dormancy may be a decisive factor influencing the inheritance of germination performance in plant species.

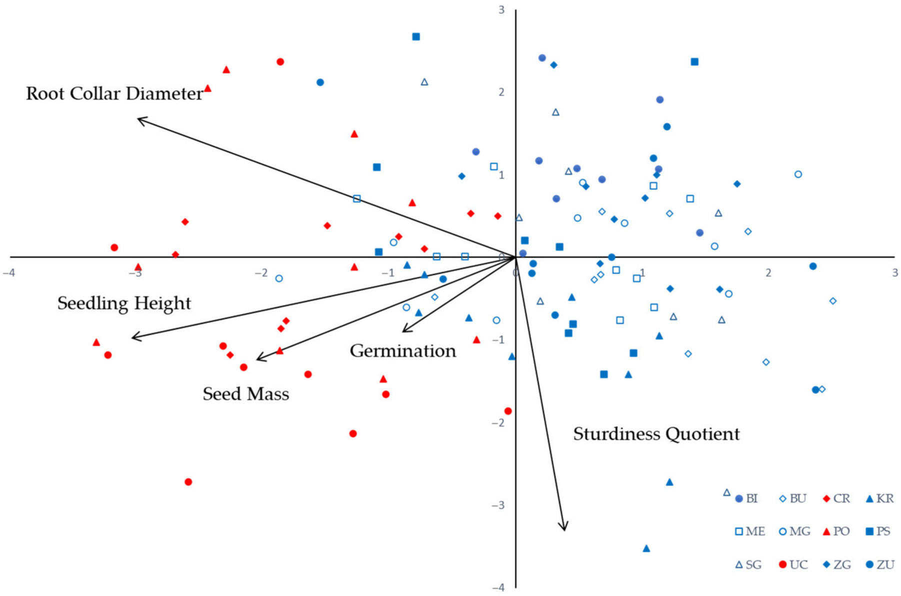

Figure 1. Biplot of principal component analysis based on seed mass, gemination and seedling characteristics in the 12 studied sweet chestnut populations. Population ID: BU—Buje; CR—Cres; PO—Poreč; UC—Učka; BI—Bilogora; KR—Krndija; ME—Medvednica; MG—Moslavačka gora; PS—Psunj; SG—Samoborsko gorje; ZG—Zrinska gora; ZU—Žumberak.

Similar to the correlation between seed size and germination rate, varying results exist for the correlation between seed mass and seedling height and seed mass and root collar diameter. In our research, a low-to-moderate positive correlation between seed mass and seedling height, as well as seed mass and root collar diameter, has been observed (Table 3). These correlations for sweet chestnut were found to be positive by Soylu and Serdar [48] and Cicek and Tilki [49], as well as by Pinchot et al. [3] and Shepard et al. [50] for Chinese and American chestnut species. Purohit et al. [40] reported the same results for two important central Himalayan oak species, *Q. semecarpifolia* Sm. and *Q. oblongata* D. Don. They concluded that seedlings grown from larger acorns generally had significantly longer stems and root collar diameters, as well as a greater number of leaves and total leaf area. Furthermore, Jarvis [51], Ke and Werger [52], Seiwa [53], Gómez [54], Navarro et al. [55], González-Rodríquez et al. [56], and Pesendorfer [57] found out that the acorn mass of various oak species was positively correlated with seedling height growth parameters and survival. In general, the fact that larger seeds produced significantly more vigorous seedlings than smaller ones is well-documented in various tree [54,58,59] and crop [60–62] species and varieties. In contrast, Clark and Schlarbaum [63] revealed that neither acorn size nor mass could be used reliably to predict seedling survival or the morphological indicators of seedling quality. However, they hypothesized that the results from their study were affected by an unusually long growing season and advanced fertilization regimes at the nursery, which may have negated acorn size/mass effects on seedling growth.

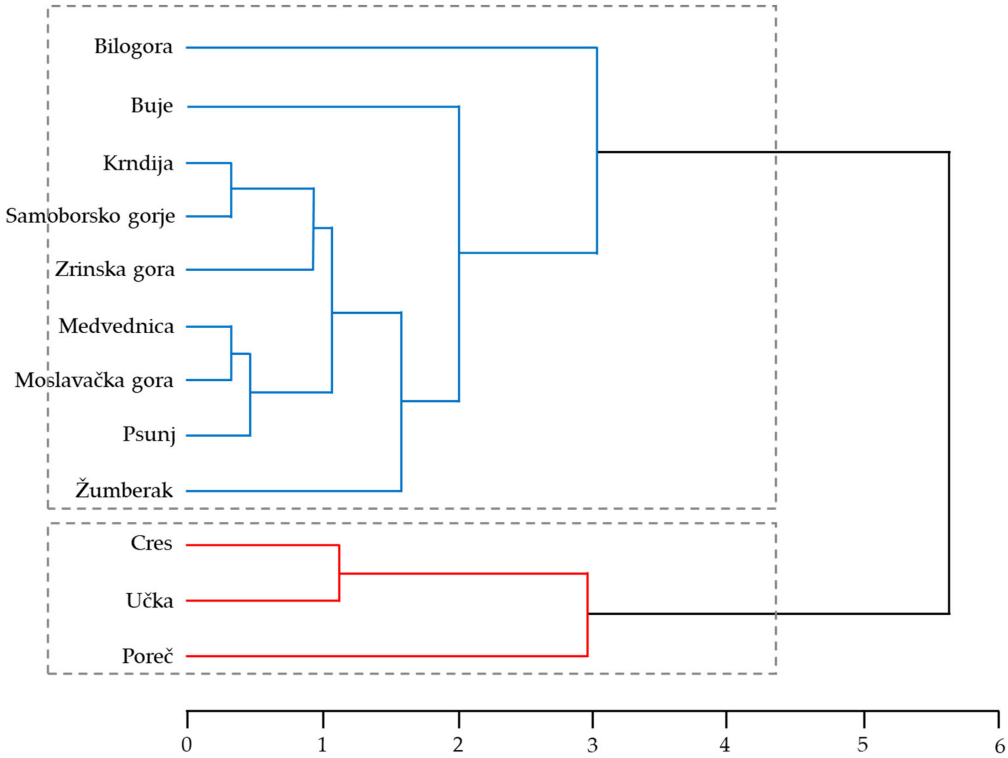

Figure 2. Tree diagram of the 12 sweet chestnut populations studied. The unweighted pair-group method with arithmetic mean (UPGMA) was used to join the clusters, and the Euclidean distance was used to define the distance between the studied populations.

Table 3. Pearson's correlation coefficient among the seed mass, germination and seedling characteristics of the 12 sweet chestnut populations studied. Statistically significant values are marked in bold. Levels of significance: *** significant at $p < 0.001$, ** significant at $0.001 < p < 0.01$, * significant at $0.01 < p < 0.05$, ns non-significant values ($p > 0.05$).

Trait	SM	G	RCD	SH	SQ
Seed Mass (SM)		ns	*	**	ns
Gremination (G)	0.097		ns	ns	ns
Root Collar Diameter (RCD)	0.295	0.075		***	***
Seedling Height (SH)	0.381	0.138	0.657		*
Sturdiness Quotient (SQ)	0.125	0.070	−0.502	0.272	

As already mentioned, seed size can influence the survival rate of seedlings, since larger seeds provide young plants with a number of advantages in dealing with environmental stressors [46]. For instance, several authors noted increased drought tolerance in seedlings grown from larger seeds [41]. Thus, our results are somewhat consistent with these studies and suggest that larger nuts and larger seedlings from Mediterranean populations may be a result of the major selective force acting on adaptation to drought. Large-seeded trees produce more robust seedlings that are more likely to survive drought in the Mediterranean climate.

These findings may be applicable in the production of rootstock for cultivated sweet chestnut varieties in these areas. Taller seedlings and larger root collar diameters from Mediterranean populations make them the best choice for vegetative propagation and use in agroforestry. Rootstocks have considerable effects on the performance of the grafted scion and subsequent growth, making the production of suitable rootstock a priority in nurseries. In order to obtain rootstock of native chestnut genotypes, high seed germination rates and seedling emergence, as well as vigorous growth and drought resistance, are desirable parameters [64]. Considering that all populations have shown a desirable sturdiness quotient (values < 6), seedlings from all 12 populations could be used in out-planting programs and rootstock production. The biogeographical provenance of seeds, however, must be taken into account in order to provide optimal suitability for newly produced seedlings in the targeted location of out-planting or orchards.

4. Conclusions and Practical Implications

Differences in seed and seedling size can be attributed to intraspecific variability and the development of ecotypes. The Mediterranean populations in this research have been shown to have larger seeds, and their seedlings were characterized by more vigorous growth. These populations are genetically and environmentally specific and thus exhibit specific growth parameters. In addition, dry environmental conditions in their natural habitat could also influence the selection of larger seeds, as this provides increased drought tolerance.

Seed size positively correlated with both seedling height and root collar diameter. In contrast, we found no evidence of the decisive influence of seed mass on germination rate and sturdiness quotient. Larger seeds give young seedlings a head start in early development by providing them with enough nutrients to spur growth. This aids early competition in the natural setting and increases the likelihood of this trait further surviving in the population. In contrast, the ideal conditions of stratification and later nursery treatments provided equal germination conditions for all seeds, regardless of their size. This could explain the lack of influence of seed mass on germination. By providing optimum and uniform germination conditions, nurseries can maximize the production of seedlings.

Since chestnut seed and seedling quality is affected by seed origin and environment, choosing a proper seed source is an important factor in seedling production. Seed origin must be observed when selecting out-planting materials, especially when the conditions are unfavorable, e.g., drought stress. Properly selected seeds and seedlings ensure the success of reforestation and provide the best chance for the establishment of young plants. Choosing the right seed source for rootstock production is also of paramount importance in nursery operations, as it increases the viability of the grafted plant and provides a genetically more diverse rootstock, which is more likely to cope with the changing environment and possible new pests.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12070858/s1, Table S1: Studied populations.

Author Contributions: Conceptualization, K.T., D.D., M.I. and I.P. (Igor Poljak); Sample collection, K.T., A.V., I.P. (Ivan Perković), M.I. and I.P. (Igor Poljak); Experiment, K.T., M.Š. and A.V.; Statistical analysis, I.P. (Igor Poljak); Writing–Original Draft Preparation, K.T. and I.P. (Igor Poljak); Writing–Review & Editing, A.V., D.D., M.I. and I.P. (Ivan Perković). All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Croatian Science Foundation (grant number IP-2018-01-1295) and University of Zagreb financial support.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Gregorio, N.O.; Herbohn, J.L.; Harrison, S.R. The potential role of nurseries in improving access to high quality planting stock and promoting appropriate silvicultural systems to improve the productivity of smallholder tree farms in Leyte, Philippines. In *ACIAR Smallholder Forestry Project–Redevelopment of a Timber Industry Following Extensive Land Clearing: Proceedings from the End-of-Project Workshop*; Harrison, S.R., Herbohn, J.L., Suh, J., Mangaoang, E., Vanclay, J., Eds.; ACIAR Smallholder Forestry Project: Ormoc City, Phillipines, 2004; pp. 269–278.
2. Allen, K.S.; Harper, R.W.; Bayer, A.; Brazee, N.J. A review of nursery production systems and their influence on urban tree survival. *Urban For. Urban Green.* **2017**, *21*, 183–191. [CrossRef]
3. Pinchot, C.C.; Clark, S.L.; Schlarbaum, S.E.; Saxton, A.M.; Sung, S.S.; Hebard, F.V. Effects of temporal dynamics, nut weight and nut size on growth of American chestnut, Chinese chestnut and backcross generations in a commercial nursery. *Forests* **2015**, *6*, 1537–1556. [CrossRef]
4. Prasetyo, L.B.; Mansur, I.; Djamhuri, E. Nursery establishment and management. In *Survey on Silvicultural Techniques and Plantation Promoting Policies in Indonesia*; Ando, K., Ed.; FORDA (Forestry Research and Development Agency) and JICA (Japan International Cooperation Agency): Ibaraki, Japan, 2003; pp. 51–68.
5. Willan, R.L. Seed testing. In *A Guide to Forest Seed Handling with Special Reference to Tropics*; FAO: Rome, Italy, 1985.
6. Bae Park, B.; Han, S.H.; Hernandez, J.O.; An, J.Y.; Nyam-Osor, B.; Jung, M.H.; Sang-Hoon Lee, P.; Lee, S.I. The use of deep container and heterogeneous substrate as potentially effective nursery practice to produce good quality nodal seedlings of *Populus sibirica* Tausch. *Forests* **2021**, *12*, 418. [CrossRef]
7. Clark, S.L.; Schweitzer, C.J.; Schlarbaum, S.E.; Dimov, L.D.; Hebard, F.V. Nursery quality and first-year response of American chestnut (*Castanea dentata*) seedlings planted in the Southeastern United States. *TPN* **2010**, *53*, 13–21.
8. Bhardwaj, R.L. Effect of growing media on seed germination and seedling growth of papaya cv. 'Red lady'. *Afr. J. Plant Sci.* **2014**, *8*, 178–184.
9. Davis, A.S.; Jacobs, D.F. Quantifying root system quality of nursery seedlings and relationship to outplanting performance. *New For.* **2005**, *30*, 295–311. [CrossRef]
10. Ivetić, V.; Davorija, Z.; Vilotić, D. Relationship between morphological and physiological attributes of hop hornbeam seedlings. *Glas. Sumar. Fak.* **2013**, *108*, 39. [CrossRef]
11. Grossnickle, S.C.; MacDonald, J.E. Why seedlings grow: Influence of plant attributes. *New For.* **2017**, *49*, 1–34. [CrossRef]
12. Haase, D.; Davis, A.S. Developing and supporting quality nursery facilities and staff are necessary to meet global forest and landscape restoration needs. *Reforesta* **2017**, *4*, 69–93. [CrossRef]
13. Bounous, G. Revival of chestnut culture in Mediterranean countries: Factors to improve the quality of productions. *Adv. Hortic. Sci.* **2006**, *20*, 7–15.
14. Fernández-López, J.; Alía, R. *Technical Guidelines for Genetic Conservation and Use for Chestnut (Castanea sativa Mill.)*; EUFORGEN International Plant Genetic Resources Institute: Rome, Italy, 2003.
15. Mattioni, C.; Cherubini, M.; Micheli, E.; Villani, F.; Bucci, G. Role of domestication in shaping *Castanea sativa* genetic variation in Europe. *Tree Genet. Genomes* **2008**, *4*, 563–574. [CrossRef]
16. Mattioni, C.; Ranzino, L.; Cherubini, M.; Leonardi, L.; la Mantia, T.; Castellana, S.; Villani, F.; Simeone, M.C. Monuments unveiled: Genetic characterization of large old chestnut (*Castanea sativa* Mill.) trees using comparative nuclear and chloroplast DNA analysis. *Forests* **2020**, *11*, 1118. [CrossRef]
17. ISTA (International Seed Testing Association). *Chapter 5: The Germination Test, International Rules for Seed Testing*, 1st ed.; The International Seed Testing Association (ISTA): Bassersdorf, Switzerland, 2006.
18. Takoutsing, B.; Tchoundjeu, Z.; Degrande, A.; Asaah, E.; Gyau, A.; Nkeumoe, F.; Tsobeng, A. Assessing the quality of seedlings in small-scale nurseries in the highlands of Cameroon: The use of growth characteristics and quality thresholds as indicators. *Small-Scale For.* **2014**, *13*, 65–77. [CrossRef]
19. Gebretsadik, W. Assessing the role of quality thresholds on early performance of tree seedlings planted on degraded highlands. *For. Res.* **2018**, *7*, 220.
20. *Statistica (Data Analysis Software System)*, Version 13; TIBCO Software Inc.: Palo Alto, CA, USA, 2018. Available online: http://www.statsoft.com.
21. R Core Team. *R: A Language and Environment for Statistical Computing, v.3.4.3*; R Foundation for Statistical Computing: Vienna, Austria, 2020.
22. Sokal, R.R.; Rohlf, F.J. *Biometry: The Principles and Practice of Statistics in Biological Research*, 4th ed.; W.H. Freeman and Co.: New York, NY, USA, 2012; p. 937.
23. Idžojtić, M.; Zebec, M.; Poljak, I.; Medak, J. Variation of sweet chestnut (*Castanea sativa* Mill.) populations in Croatia according to the morphology of fruits. *Sauteria* **2009**, *18*, 323–333.
24. Parsons, P.A.; Allard, R.W. Seasonal variation in lima bean seed size: An example of genotypic-environmental interaction. *Heredity* **1960**, *14*, 115–123. [CrossRef]
25. Crochemore, M.L.; Huyghe, C.; Papineau, J.; Julier, B. Intra-plant variability in seed size and seed quality in *Lupinus albus* L. *Agronomie* **1994**, *14*, 5–13. [CrossRef]
26. Parciak, W. Environmental variation in seed number, size and dispersal of fleshy-fruited plant. *Ecology* **2002**, *83*, 780–793. [CrossRef]

27. Zas, R.; Sampedro, L. Heritability of seed weight in Maritime pine, a relevant trait in the transmission of environmental maternal effects. *Heredity* **2015**, *114*, 116–124. [CrossRef] [PubMed]
28. Gosling, P. *Raising Trees and Shrubs from Seed*; Forestry Commission Practice Guide, Forestry Commission: Edinburgh, UK, 2007; pp. 1–28.
29. Young, J.A.; Young, C.G. *Seeds of Woody Plants in North America*; Dioscorides Press: Portland, OR, USA, 1992.
30. Benedetti, S.; Gonzalez, M.; Garcia, E.; Quiroz, I. An analysis of the physical and germination parameters of the sweet chestnut (*Castanea sativa*). *Cien. Inv. Agric.* **2012**, *39*, 185–192. [CrossRef]
31. Donohue, K.; Dorn, L.; Griffith, C.; Kim, E.; Aguilera, A.; Polisetty, C.R.; Schmitt, J. Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. *Evolution* **2005**, *59*, 740–757. [CrossRef]
32. Tilki, F.; Alptekin, C.U. Germination and seedling growth of *Quercus vulcanica*: Effects of stratification, desiccation, radicle pruning, and season of sowing. *New For.* **2006**, *32*, 243–251. [CrossRef]
33. Poljak, I.; Idžojtić, M.; Šatović, Z.; Ježić, M.; Ćurković-Perica, M.; Simovski, B.; Acevski, J.; Liber, Z. Genetic diversity of the sweet chestnut (*Castanea sativa* Mill.) in Central Europe and the western part of the Balkan Peninsula and evidence of marron genotype introgression into wild populations. *Tree Genet. Genomes* **2017**, *13*, 18. [CrossRef]
34. Fredrick, C.; Muthuri, C.; Ngamau, K.; Sinclair, F. Provenance variation in seed morphological characteristics, germination and early seedling growth of *Faidherbia albida*. *JHSF* **2015**, *7*, 127–140.
35. Calvo, L.; Hernández, V.; Valbuena, L.; Taboada, A. Provenance and seed mass determine seed tolerance to high temperatures associated to forest fires in *Pinus pinaster*. *Ann. For. Sci.* **2016**, *73*, 381–391. [CrossRef]
36. Santelices Moya, R.; Espinoza Meza, S.; Magni Díaz, C.; Cabrera Ariza, A.; Donoso Calderón, S.; Peña-Rojas, K. Variability in seed germination and seedling growth at the intra- and inter- provenance levels of *Nothofagus glauca* (*Lophozonia glauca*), an endemic species of Central Chile. *N. Z. J. For. Sci.* **2017**, *47*, 10. [CrossRef]
37. Doffou Akaffou, S.; Kouassi Kouame, A.; Boh Gore, N.B.; Yao Abessika, G.; Kouassi, H.K.; Hamon, P.; Sabatier, S.; Duminil, J. Effect of the seeds provenance and treatment on the germination rate and plants growth of four forest trees species of Côte d'Ivoire. *J. For. Res.* **2021**, *32*, 161–169. [CrossRef]
38. Alptekin, C.; Tilki, F. Effects of stratification and pericarp removal on germination of *Quercus libani* acorns. *Silva Balc.* **2002**, *2*, 21–28.
39. Garcia-De La Cruz, Y.; Lopez-Barrera, F.; Ramos-Prado, J.M. Germination and seedling emergence of four endangered oak species. *Madera Bosques* **2016**, *22*, 77–87. [CrossRef]
40. Purohit, V.K.; Tamta, S.; Nandi, S.K.; Rikhari, H.C.; Palni, L.M.S. Does acorn weight influence germination and subsequent seedling growth of central Himalayan oaks? J. *Trop. For. Sci.* **2003**, *15*, 483–492.
41. Pandey, R.; Bagali, K.; Bargali, S.S. Does seed size affect water stress tolerance in *Quercus leucotrichophora* A. Camus at germination and early seedling growth stage? *Biodiver. Int. J.* **2017**, *1*, 24–30.
42. Winn, A.A. Ecological and evolutionary consequences of seed mass in *Prunella vulgaris*. *Ecology* **1988**, *69*, 1537–1544. [CrossRef]
43. Vera, M.L. Effects of altitude and seed mass on germination and seedling survival of heathland plants in north Spain. *Plant Ecol.* **1997**, *133*, 101–106. [CrossRef]
44. Kaliniewicz, Z.; Tylek, P. Influence of scarification on the germination capacity of acorns harvested from uneven-aged stands of pedunculated oak (*Quercus robur* L.). *Forests* **2018**, *9*, 100. [CrossRef]
45. Mao, P.; Guo, L.; Gao, Y.; Qi, L.; Cao, B. Effects of seed size and sand burial on germination and early growth of seedlings for coastal *Pinus thunbergii* Parl. in the Northern Shandong Peninsula, China. *Forests* **2019**, *10*, 281. [CrossRef]
46. Martínez González, I.; Sanchez-Velazquez, L.R.; Ruiz-Guerra, B.; del Rosario Pineda-Lopez, M.; Velazquez-Rosas, N. The role of seed size in the emergence and survival of seedlings in contrasting environments: The case of *Ceiba aesculifolia*. *New For.* **2020**, *52*, 493–507. [CrossRef]
47. Agboola, D.A. The effect of seed size on germination and seedling growth of three tropical tree species. *J. Trop. For. Sci.* **1996**, *9*, 44–51.
48. Soylu, A.; Serdar, U. Rootstock selection on chestnut (*Castanea sativa* Mill.) in the middle of Black Sea region in Turkey. *Acta Hortic.* **2000**, *538*, 483–487. [CrossRef]
49. Cicek, E.; Tilki, F. Seed size effects on germination, survival and seedling growth of *Castanea sativa* Mill. *J. Biol. Sci.* **2007**, *7*, 438–441. [CrossRef]
50. Shepard, E.; Miller, D.D.; Miller, G.; Miller, D. Effect of weight on emergence and seedling vigor of Chinese chestnut. *Hortic. Sci.* **1989**, *24*, 516–519.
51. Jarvis, P.G. The effects of acorn size and provenance on the growth of seedlings of sessile oaks. *QJF* **1963**, *57*, 11–19.
52. Ke, G.; Werger, M.J.A. Different responses to shade of evergreen and deciduous oak seedlings and the effect of acorn size. *Acta Oecol.* **1999**, *20*, 579–586. [CrossRef]
53. Seiwa, K. Effect of seed size and emergence time on tree seedling establishment; importance of developmental constraints. *Oecologia* **2000**, *123*, 208–215. [CrossRef] [PubMed]
54. Gómez, J.M. Bigger is not always better: Conflicting selective pressures on seed size in *Quercus ilex*. *Evolution* **2004**, *58*, 71–80. [CrossRef] [PubMed]
55. Navarro, F.B.; Jimenez, M.M.; Ripoll, M.A.; Ondono, E.; Gallego, E.; Simon, E. Direct sowing of holm oak acorns: Effects of acorn size and soil treatment. *Ann. For. Sci.* **2006**, *63*, 961–967. [CrossRef]

56. González-Rodríquez, V.; Villar, R.; Navarro-Cerrillo, R.M. Maternal influences on seed mass effect and initial seedling growth in four *Quercus* species. *Acta Oecol.* **2011**, *37*, 1–9. [CrossRef]
57. Pesendorfer, M.B. The effect of Seed Size Variation in *Quercus pacifica* on Seedling Establishment and Growth. General Technical Report PSW-GTR-251. In Proceedings of the 7th California Oak Symposium: Managing Oak Woodlands in a Dynamic World, Visalia, CA, USA, 3–6 November 2014.
58. Bonfil, C. The effects of seed size, cotyledon reserves and herbivory on seedling survival and growth in *Quercus rugosa* and *Q. laurina* (Fagaceae). *Am. J. Bot.* **1998**, *85*, 79–87. [CrossRef] [PubMed]
59. Sage, R.D.; Koenig, W.D.; McLaughlin, B.C. Fitness consequences of seed size in the valley oak *Quercus lobata* Née (Fagaceae). *Ann. For. Sci.* **2011**, *68*, 477–484. [CrossRef]
60. Burris, J.S.; Edje, O.T.; Wahab, A.H. Effects of seed size on seedling performance in soybeans: II. Seedling growth and photosynthesis and field performance. *Crop Sci.* **1973**, *13*, 207–210. [CrossRef]
61. Adebisi, M.A.; Kehinde, T.O.; Salau, A.W.; Okesola, L.A.; Porbeni, J.B.O.; Esuruoso, A.O.; Oyekale, K.O. Influence of different seed size fractions on seed germination, seedling emergence and seed yield characters in tropical soybean (*Glycine max* L. Merrill). *Int. J. Agric. Res.* **2013**, *8*, 26–33. [CrossRef]
62. Snider, J.L.; Collins, G.D.; Whitaker, J.; Chapman, K.D.; Horn, P. The impact of seed size and chemical composition on seedling vigor, yield and fiber quality of cotton in five production environments. *Field Crop Res.* **2016**, *193*, 186–195. [CrossRef]
63. Clark, S.L.; Schlarbaum, S.E. Effects of acorn size and mass on seedling quality of northern red oak (*Quercus rubra*). *New For.* **2018**, *49*, 571–583. [CrossRef]
64. Bounous, G.; Beccaro, G. Chestnut culture: Directions for establishing new orchards. *Nucis-Newsletter* **2002**, *11*, 30–34.