Bartın Orman Fakültesi Dergisi | Journal of Bartin Faculty of Forestry
2025, 27(2): 142 – 152 | DOI: 10.24011/barofd.1567038

Genetic Variation of *Tilia tomentosa* Moench Populations in Yenice of Karabük

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Article History
Received: 16.10.2024
Accepted: 04.07.2025
Published: 30.08.2025

Research Article

Abstract – Plus trees were selected from five populations (Camiyani, Kavaklı, Karakaya, Kayadibi and Kızılkaya) of silver linden (*Tilia tomentosa*), which has the broadest distribution of linden species in Karabük-Yenice as well as in Türkiye. An index including the number of flowers in an inflorescence, flower rate in the crown, and crown size were used to select plus trees. Seeds were collected from 53 of the 101 selected plus trees. The seeds of each plus tree were sown in the Gökçebey Forest Nursery area using a completely randomized plot design in a common garden test. Root collar diameter (mm) and height (cm) were measured on the seedlings for two consecutive years. No statistical difference was observed between the populations in root collar diameter and height traits in both years. However, the height difference between families (within the population) was statistically significant. For height, individual heritabilities in the first and second years were 0.13 and 0.29, and family mean heritabilities were 0.31 and 0.51, respectively. Phenotypic correlations between root collar diameter and height at the family level were 0.68 in the first year and 0.59 in the second year. This first study on silver linden revealed that seed transfer rules were not required for silver linden populations in Karabük-Yenice and that there was genetic variation among families for height traits. Silver linden, the broadest distribution in Türkiye and Karabuk-Yenice, is crucial for linden flower production and a mixture tree in forests. For sustainable forest management, further research on the species may be conducted.

Keywords – *Silver linden, plus trees, heritability, common garden test, phenotypic correlation*

Karabük-Yenice *Tilia tomentosa* Moench Popülasyonlarının Genetik Varyasyonu

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Makale Tarihçesi
Gönderim: 16.10.2024
Kabul: 04.07.2025
Yayım: 30.08.2025

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Öz – Türkiye'de olduğu gibi Karabük-Yenice'de de en geniş yayılış yapan *Tilia tomentosa*'nın (gümüşi ıhlamur) beş popülasyonunda (Camiyani, Kavaklı, Karakaya, Kayadibi ve Kızılkaya) üstün ağaçlar seçilmiştir. Üstün ağaç seçiminde kuruldaki çiçek sayısı, çiçeklenme oranı ve tepe büyüklüğünü içeren bir indeks kullanılmıştır. Seçilen 101 üstün ağacın 53 adedinden tohum toplanmıştır. Ortak bahçe denemesinde, tesadüf parselleri deneme deseni kullanılarak, her bir üstün ağaç tohumları Gökçebey Orman Fidanlığı alanına ekilmiştir. Yetişen fidanlarda birbirini izleyen 2 yıl kök boğazı çapı (mm) ve boy (cm) ölçülmüştür. Kök boğazı çapı ve boy özelliklerinde, her iki yılda da popülasyonlar arası istatistik farklılık görülmemiştir. Bununla birlikte, aileler arasında (popülasyon içi) boy farklılığı istatistik olarak anlamlı bulunmuştur. Boy için birinci yıl ve ikinci yıl bireysel kalıtım dereceleri 0.13 ve 0.29, aile ortalamaları kalıtım dereceleri aynı sırayla 0.31 ve 0.51 olmuştur. Fidanların kök boğazı çapı ve boyu arasında aile düzeyinde fenotipik korelasyon birinci yıl 0.68 ve ikinci yıl için 0.59 olmuştur. Gümüşi ıhlamurda yapılmış bu ilk çalışma, Karabük-Yenice'de bulunan gümüşi ıhlamur popülasyonları için tohum transfer kurallarına gerek olmadığı, boy özelliğinde ise aileler arasında genetik varyasyon olduğu ortaya çıkmıştır. Türkiye'de ve Karabük-Yenice'de en çok yayılışı olan gümüşi ıhlamur, ıhlamur çiçeği üretimi ve ekolojik açıdan ormanlarda karışım ağacı olarak önemlidir. Sürdürülebilir orman yönetimi için tür üzerinde daha ileri araştırmalar yürütülebilir.

Anahtar Kelimeler – *Gümüşi ıhlamur, üstün ağaç, kalıtım derecesi, ortak bahçe testi, fenotipik korelasyon*

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1. Introduction

The *Tilia* (linden) genus includes 23 forest tree species that are whole insect-pollinated and mainly found in the temperate zone in the northern hemisphere (Pigott, 2012). Among the four linden taxa naturally distributed in Türkiye (*Tilia cordata* Miller subsp. *cordata*, *Tilia dasystyla* Steven subsp. *caucasica* (V.Engl.) Pigott, *Tilia platyphyllos* Scopoli and *Tilia tomentosa* Moench), the most striking taxon, with its intensely scented flowers and population density in its natural distribution areas is the *Tilia tomentosa*. The most important feature that distinguishes it from other species is the presence of silvery-white star hairs that completely cover the lower surface of its leaves like felt (Oral, 2022). Usually, lindens are subject to flower production and they are also crucial in forestry in terms of being as a mixture species and as a wood source (Jensen, 2003; Radoglou et al., 2009; Alan, 2022).

Silver linden is the most widely distributed linden taxon in Türkiye and is found in the European-Siberian phytogeographic region, along the Black Sea Region of Northwest Anatolia, to the east of Karabük, and is also found as isolated populations in İzmir, Balıkesir, Aydın and Amanos (Demir, 2003; Ebcin, 2022; Güngöroğlu, 2022). As in Türkiye in general, silver linden is the most widely distributed and utilzed (for flower collecting and honey production) linden species in Yenice-Karabük (Canlı, 2021). In this context, "Yenice Linden Honey" was granted a Geographical Indication (Türk Patent, 2023).

Usually, flower collecting has faced some problems such as tall trees, difficulties in flower collecting due to tall trees, short collecting period (20-30 days), time-consuming (12 times going to the forest in a period), and damaging linden trees because of cutting branches to collect flower (Alan et al., 2018; Ateş, 2021). On the other hand, some procedures can increase flower quality. If the linden flower can be collected within four days of flower blooming and from the south aspect, the linden flower can be of higher quality and preferable for commercial purposes (Uslu, 2004; Korkusuz and Dirik, 2011; Alan et al., 2018; Alan, 2022). Considering flower collecting, global markets and the pharmaceutical industry demand high-quality and highly active ingredient products: "standard products." Unfortunately, it is impossible to obtain a standard product by collecting natural plants, but selection and breeding can be used to develop genotypes that produce standard product (Bayram et al., 2010; Alan et al., 2018). In this context, regarding linden flower collecting, Alan et al. (2018) proposed the domestication of silver linden in Yenice-Karabük. Domestication includes the following procedures: the plus tree selection, growing seedlings from the seeds of each plus tree, and establishing a linden flower orchard with these seedlings. In this direction, the best 20 of all plus trees were selected with an index. A linden flower orchard was planned to be established with the number of seedlings (about 30) of each plus tree of the best 20-plus trees using the two-year-old seedlings.

White et al. (2007) stated that two empirical methods were available for indicating environmental and genetic outcomes: common garden tests and molecular genetic studies. Regarding the common garden test, they underlined that seeds were collected from trees in stands (populations) distributed in many diverse environments, and these seeds were planted in randomized, replicated experiments in single or multiple field tests to understand adaptive properties. Schwinning et al. (2022) also indicated that the common garden test could vary from one-to-many gardens and from a few populations characterizing noticeable ecotypes to numerous populations representing whole metapopulation, and it could be indoors (in a greenhouse) or outdoors. Studies exist on common garden tests related to forest trees, such as Isik (1986); Kaya and Isik (1997) for *Pinus brutia* Ten; Rehfeldt and Gallo (2001) for *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus ponderosa* Laws., Jensen and Hansen (2008) for *Quercus petraea* (Matt.) Liebl and *Quercus robur* L., Alan and Ezen (2018) for *Liquidambar orientalis* Mill. and Dixit and Kolb (2020) for *Pinus ponderosa* var. *scopulorum* Lawson. On the other hand, some researchers also used common garden tests related to climate change, adaptation and phenotypic plasticity in recent times (Cooper et al., 2018; Sáenz-Romero et al., 2019; Salk, 2020; Matyas, 2021; Schwinning et al., 2022). No studies on the common garden test have been found on *Tilia tomentosa* in Türkiye.

Mixed forests play a crucial role in terms of stability, production potential and adaptation to climate change. They mitigate the negative impacts of changing environmental conditions better than monoculture. At the same time, mixed stands can be more productive (depending on tree species composition and environmental conditions) than monocultures during climate change (Vacek et al., 2021). Lime species are admixture trees

with both broad leaves and conifers trees. *Tilia tomentosa* can also grow in steppe with *Qurecus pupescens* Willd., *Fraxinus ornus* L. etc. (Radoglou et al., 2009). On the other hand, between 2014 and 2023, the annual average production of linden flowers and leaves was 68 tons in Türkiye (OGM, 2024), indicating the economic importance of linden species. Tuttu et al. (2017) also reported that linden flower production in Turkey was conducted in Adapazarı, Amasya, Balıkesir, Bolu, Bursa, Çanakkale, Isparta, İstanbul, Kastamonu and Zonguldak forestry regional directorates, and Türkiye's annual average production in 27 years between 1989 and 2015 was about 22 tones.

The linden species are economically important as non-wood products and crucial for admixture species in mixed forests. In this context, the study aimed to reveal genetic differences between populations and families (within populations) and interpret forest management using the first common garden test of silver linden in Türkiye.

2. Material and Method

A total of 101 plus trees had been selected from five populations before this study was conducted in Yenice-Karabük. However, seeds were collected from 53 of 101 plus trees in four of the four populations primarily due to flower harvest before the seed collection, illegal cutting or difficulty reaching the tree to collect seeds (Table 1). Plus tree selection included 13 traits, some of which were geographic, like altitude and latitude, and some of them were biological characteristics like height, number of flowers in an inflorescence, crown size, flowering rate in the crown, stem form, and age. However, the number of flowers in an inflorescence, crown size, and flowering rate in the crown were used for a selection index to select the best plus trees. All 53 plus trees were used for a common garden test in the Gökçebey Nursery in Zonguldak.

Table 1
Populations, selected plus trees, seed collected plus trees

Populations	Altitude[a] (m)	Latitude N Longitude E	Mean annual precipitation[b]	Mean annual temperature[b]	Mean stand age[b]	Selected plus trees	Seed collected plus trees
Camiyanı	281	41° 24' 90" 32° 89' 29"	650	14	48	22	12
Karakaya	397	41° 25' 33" 32° 30' 49"	650	14	49	20	9
Kızılkaya	353	41° 25' 00" 32° 29' 96"	650	14	48	28	15
Kavaklı	296	41° 24' 30" 32° 29' 54"	650	14	48	27	17
Kayadibi	88	41° 28' 73" 32° 31' 60"	650	14	50	4	0
Total						101	53

[a]Average altitude and age of plus trees in the population (stand) [b]Marchi et al. (2020)

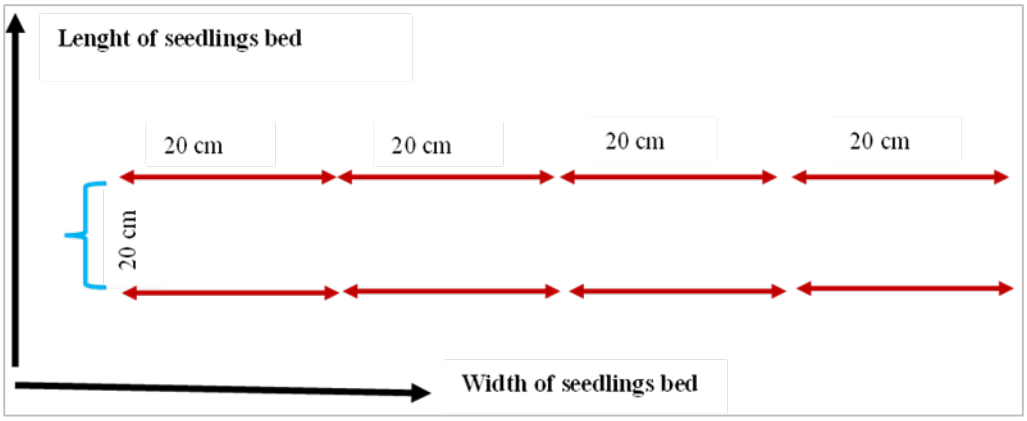


Figure 1. Sowing seed plan for seedling (test design)

The collected seeds from plus trees were sown the next day in seedbeds of Gökçebey Nursery with a completely randomized design with eight replications in the common garden test. In the design, seeds were sown in September 2022 using 20 cm between lines and inter-sown points for equal space of seedlings (Figure 1). However, the seedling number and spacing were unbalanced due to unbalanced germination[2]. Therefore, all germinated seedlings were transported to plastic bags in April 2023 to provide homogeneity, keeping the test design (completely randomized). Plastic bags with a width of 17 cm and a length of 35 cm were used, and the material in the plastic bags was comprised of a mixture of forest soil, peat, and perlite. All seedling heights (cm) and root collar diameters (mm) were measured for two consecutive years, June 2023 and 2024 (Figure 2). Therefore, the seedling's ages were accepted as one- and two-year-old for 2023 and 2024, respectively.



Figure 2. Height and root collar diameter measurement on tagged seedlings in plastic bag

The data collected from the common garden test were checked for normal distribution. As data on both traits were not normally distributed, they were transformed by taking the square root. Subsequently, data were

[2] Nursery staff have suggested to sow in seedling bed for more germination

evaluated using the following mixed model and assumed population as fixed and family as random variables. Data was analyzed using the software SAS ver. 9.0 (SAS Institute Inc, 2002).

$$y_{ij} = \mu + P_i + F_{j(i)} + e_{ij} \quad (2.1)$$

Where, y_{ijk} is k^{th}, in the j^{th} family of i^{th} population, μ is general mean, P_i is i^{th} population, i= 1, 2, ... 4, $F_{j(i)}$ is j^{th} family within i^{th} population, j= 1, 2, …. 53, and e_{ij} is error.

Individual and family mean heritability were estimated following equations.

$$h_i^2 = \frac{4\sigma_f^2}{\sigma_f^2 + \sigma_e^2} \quad (2.2)$$

$$h_f^2 = \frac{\sigma_f^2}{\sigma_f^2 + (\sigma_e^2/n)} \quad (2.3)$$

$$Cv_g = \frac{\sqrt{4\sigma_f^2}}{\bar{X}} \quad (2.4)$$

Where, σ_f^2 is family variance, σ_e^2 is error variance, n is the harmonic mean (13.486) of seedlings in the common garden, Cv_g is the additive genetic coefficient of variations and $\bar{X}$ is the general mean of the trait. Families were assumed to be the whole half-sib, so the coefficient was accepted as 4 in Equation 2 and 4. Estimating standard errors of heritabilities in Equation 2 and 3 were used in Dickerson's method suggested by Dieters et al. (1995).

The phenotypic correlations (Pearson) of family and individual levels were also estimated between height and root collar diameter to evaluate the relationship between traits.

3. Result and Discussion

The number of individuals per family (plus tree) varied between 4 and 32 due to some germination barriers of silver linden. In most linden species, the collected seeds should be planted in moist soil or sandy environments without delay. After sowing, the seeds should be kept outdoors during the winter. However, if they are not to be sowed, they should be kept at a temperature of 0-5ºC. The high number of unfertilized seeds also reduces the germination percentage of linden seeds (Pigot, 2012; Ebcin, 2022). Collected seeds were sown the next day in the study to obtain a high germination rate because it has been suggested that to grow a sufficient number of seedlings from silver linden seeds and to prevent the seeds from aging, it is appropriate to collect them when the pericarp turns from green to greenish-yellow, the seed coat turns tan (light brown), the endosperm becomes semi-solid, and the embryo is predominantly green, and be sowed immediately without any processing (Ebcin, 2022). In this context, our study's germination rate might have been low due to unsuitable seed or pericarp colour. As mentioned above, color determination could be needed experience, and collecting time according to color could increase the germination rate and, by the way, the number of seedlings.

The overall mean of seedlings for height was 23.85 and 36.64 cm at the first and second age, and the root collar diameter was 6.37 and 8.14 mm, respectively. Alan and Ezen (2017) found 40.58 and 89.93 cm in height and 11.72 and 33.41 mm in root collar diameter at the second and third age, respectively, for *Liquidambar orientalis*. On the other hand, the mean of each silver linden population, both height and root collar diameter, was close to the general mean of populations. However, differences between minimum and maximum values

at individual levels showed considerable height and root collar diameter variability in each population. Standard errors of the mean for each population were acceptable, especially the Kavaklı population (Table 2).

Table 2
Basic statistics for height and root collar diameter for populations

Seedling ages	Population	Height						Root collar diameter				
		N	Mean	Std dev.	Std error	Min	Max	Mean	Std dev.	Std error	Min	Max
One-year-old	Camiyani	89	24.61	11.61	1.23	4.00	58.00	6.54	2.62	0.28	1.47	13.70
	Kavakli	325	23.22	9.43	0.52	4.00	59.00	6.03	2.10	0.12	1.27	13.00
	Karakaya	181	26.13	11.30	0.84	6.00	57.50	6.71	2.27	0.17	2.46	14.78
	Kızılkaya	157	21.68	10.11	0.81	4.00	60.00	6.20	2.45	0.20	1.75	13.68
	Total/Mean	752	23.91					6.37				
Two-year-old	Camiyani	79	38.98	13.39	1.51	9.00	63.40	8.04	2.34	0.26	3.15	13.42
	Kavakli	311	35.53	13.12	0.74	10.00	72.30	7.99	2.39	0.13	1.44	15.92
	Karakaya	176	37.91	14.59	1.10	10.00	70.00	8.61	2.59	0.20	3.06	17.42
	Kızılkaya	143	34.15	12.05	1.01	9.70	67.00	7.92	2.77	0.23	2.53	16.75
	Total/Mean	709	36.64					8.14				

Significant differences were observed between populations for height and root collar diameter but families (within populations) for height in the first year; however, there were only significant differences between families for height in the second year (Table 3). A multiple comparison test (Tukey) was given in Table 4 due to the statistical differentiation of the population for both traits at first age. The groups were intertwined, and no clear separation occurred between populations.

According to White et al. (2007), a short-term common garden test could be conducted to study the natural geographic variation and develop seed transfer guidelines. In this direction, seeds could be obtained from different provenances and grown for a short time (a few months to a few years) in growth rooms, greenhouses, nurseries, or other artificial environments. Subsequently, seedling traits could be revealed the variation across different geographic regions. In this context, whole populations could be used everywhere in Yenice due to no differences in population in the second age and at the limit value or close to the limit value of significance in first age (Table 3 and Table 4). However, *Liquidambar orientalis* differed from the silver linden in Yenice Town (Alan and Ezen, 2018). Population differences in *L. orientalis* were statistically significant in seedlings' second and third ages. Significance in *L. orientalis* populations might be sourced from broader distribution and more extensive altitude ranges (5 to 1100 m) than from silver lindens. Elevation differences, such as Isik (1986) and Dixit and Kolb (2020), could reflect genetic differences. In addition, *L. orientalis* is distributed in Mugla and Denizli provinces, while silver linden populations are only distributed in Yenice Town of Karabük Province.

Table 3
Variance analyses for height and root collar diameter

Seedling ages	Variance source	DF	Height			Root collar diameter		
			Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
One-year-old	Population	3	2.621	3.88	0.014	0.668	3.00	0.039
	Family (Population)	49	1.605	1.47	0.021	0.217	1.01	0.457
	Error	697	1.099			0.214		
Two-year-old	Population	3	2.666	1.24	0.302	0.429	1.82	0.151
	Family (Population)	48	2.344	1.85	0.0006	0.243	1.19	0.178
	Error	657	1.265			0.203		

Table 4
Multiple comparison of populations in one year old seedling

Height		Root collar diameter	
Populations	Mean	Poulations	Mean
Karakaya	26.13 a*	Karakaya	6.71 a*
Camiyanı	24.61 ab	Camiyanı	6.54 a
Kavaklı	23.22 ab	Kızılkaya	6.20 ab
Kızılkaya	21.68 b	Kavaklı	6.03 ab

*Different letters show different groups

Besides the molecular study, Lobo et al. (2018) assessed the bud burst of all trees in the clonal test for 2004 and 2006 using a score from one to six situated on the showing level of bud burst from fully closed winter buds (1) to fully opened buds named as leaves (6) in *Tilia cordata* Mill. Lobo et al. (2018) used nine populations and 91 families and stated that matched geographic divisions and corresponding differences between populations tended to be related. Unlike *Tilia tomentosa* in Yenice, the population and geographical distance of *Tilia cordata* are further than those in Yenice. On the other hand, the study of Lobo et al. (2018) included highly fragmented *Tilia cordata* populations, which were expected to be very diverse from each other.

Tilia tomentosa populations were divided into only two groups and sourced comparison tests in only the first year (Table 4). In contrast to *T. tomentosa*, populations of *Liquidambar orientalis* were split into three groups for root collar diameter and height in the second and third years (Alan and Ezen 2018). Besides populations, families in *L. orientalis* were also significantly different in height and root collar diameter at the second and third age. In *T. tomentosa*, significant family differences were only in height traits for two consecutive years. Both populations and family differences between species might be sourced beacuse *L. orientalis* comprised nine populations, and *T. tomentosa* comprised four. Another reason may be that the entire *T. tomentosa* population in Yenice might already be genetically similar. Advanced research is needed to understand the reasons for this.

Individual (0.13±0.09) and family mean (0.31±0.22) heritability for height was low for the first age but acceptable (0.29±0.12 and 0.51±0.21) for the second age in silver linden. Alan and Ezen (2017) also found low heritabilities in the second age (0.07±0.02 and 0.31±0.11) and third age (0.08±0.03 and 0.34±0.10) for root collar diameter but higher heritabilities for height in the second age (0.39±0.05 and 0.72±0.10) and third age (0.45±0.05and 0.75±0.10), respectively in *Liquidambar orientalis*. It might be sourced that nine populations and 223 families were used in *L. orientalis*, while four populations and 53 families were used in

Tilia tomentosa. Lower standard errors in *L. orientalis* than in *T. tomentosa* might be sourced to more population and family numbers in *L. orientalis* than in *T. tomentosa*. In addition, the *L. orientalis* experimental design included 25 blocks (replication), while *T. tomentosa* included less replication. Regarding heritabilities, Baliuckas et al. (2005) studied a common garden experiment with open-pollinated families from six and four populations of *Prunus padus* L. and *Sorbus aucuparia* L., respectively. They found 0.35±0.10 in age three for *P. padus* and 0.45±0.20 and 0.39±0.17 individual heritabilities in age two and three, respectively, for *S. aucaparia* for height. Individual heritabilities for both species were found to be higher than silver linden. Higher individual heritability of *P. padus and S. aucaparia* than *T. tomentosa* may be sourced from larger species distributions in Sweden. That is, *S.aucaparia* covers between 55° and 62°, and *P. padus* between 58° and 62° N latitudes, while *T. tomentosa* in Yenice covers only 41° 24' and 41° 28' N latitudes. Therefore, a more extensive distribution might be attributed to higher heritability due to more considerable geography and family differences.

The additive genetic coefficient of variations was 16% and 19% for first and second age in height, respectively. Cornelius (1994) reviewed the additive genetic coefficient of variations for forest trees and silver linden is higher than that of forest trees in height (%11). Houle (1992) underlined that the populations studied have a good potential for adaptation under changing environmental conditions when a trait has a high additive genetic coefficient. Pliura and Baliuckas (2007) stated that one of the best adaptive traits was bud flushing for *Fraxinus excelsior* L. due to the high additive genetic coefficient. Therefore, height is a better indicator than root collar diameter in terms of adaptation in silver linden. In addition, silver linden might have the potential to adapt to climate change and tree breeding for height due to its high additive genetic coefficient, considering the need for additional research.

A phenotypic correlation of 0.77 at the family level and 0.68 at the individual level was found between height and root collar diameter at first age. It was 0.59 and 0.55 at the second age, respectively. The correlation at the family level was higher than at the individual level, while both correlations at the second age were lower than correlations at the first age. Moreover, the correlations were low or moderate.

According to Berend et al. (2019), common garden tests involve inside (greenhouse or chamber) or outside (nursery, field) plantings of species or populations that have been collected from multiple distinct geographic locations and grown together under standard conditions. The objective of these experiments is to investigate how heritable traits and phenotypic plasticity contribute to population reactions to climate conditions and how natural selection forms these traits to provide the best possible results for population endurance (Cooper et al., 2018; Dixit and Kolb, 2020; Salk, 2020; Matyas, 2021; Schwinning et al., 2022). In this context, research that started with the aim of domestication led to the establishment of the first common garden test in silver linden in Karabük-Yenice, and the first results were achieved. These results can be considered an essential step in investigating the genetic structure of silver linden Yenice and the whole of Türkiye.

4. Conclusion

Tilia species contribute to the forest ecosystem as a good mixture of trees and are valuable in wood production. However, non-wood production, such as flower collection and honey production, deprioritizes the contribution of linden trees in terms of stand mixture and wood production function. With the domestication of non-wood output to increase flower production, silver linden's contribution to the forest ecosystem and use of wood production can be increased. Common garden tests to reveal genetic structure are essential regarding the non-wood utilization of linden, its evaluation for the forest ecosystem, and wood production. The findings show a part difference between populations in height and root collar diameter, and there is a difference between families (within populations) in height trait for silver linden in Karabük-Yenice. However, there is no difference between families for root collar diameter. Although studies are necessary for older ages, it might be accepted that there was no need for seed transfer rules for forestry practices in silver linden in Karabük-Yenice. Heritabilities on height traits were compatible with forest trees. The additive genetic coefficient of variations

in height trait shows the evolvability of silver linden. On the other hand, a phenotypic correlation between height and root collar was low or moderate at the family and individual levels. Continuing these studies may enable more sustainable management of silver linden for non-wood products and use in forest ecosystems such as mixed trees and wood production.

Acknowledgement

This was a Research Project which was supported by the Scientific Research Projects Coordination Unit of Karabuk University. The project Number is KBÜBAP-17-KP-388. Support of the Scientific Research Projects Coordination Unit is gratefully acknowledged.

Zonguldak Forest Regional Directorate supported selecting plus trees, collecting seed from plus trees, and growing seedlings in Gökçebey Nursery Directorate. Many thanks to the Zonguldak Forest Regional Directorate for this valuable support.

Author Contributions

Murat Alan: Prepared draft, collected and analyzed data.

Rumeysa Kumral: Contributed to draft, collected and cured data.

Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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