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Assessing Phenotypic Diversity and Population-Level Differentiation in *Laurus nobilis* L. Seedlings from the Southwestern Black Sea Region

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ABSTRACT

Bay laurel (*Laurus nobilis* L., Lauraceae) is an economically and ecologically important non-timber forest product widely distributed across Mediterranean and sub-Mediterranean ecosystems. This study assessed phenotypic variation in two-year-old seedlings originating from six natural populations in the Southwestern Black Sea Region. Seedlings were grown under uniform open-field nursery conditions and evaluated using fourteen phenotypic and quality-related traits, including growth parameters, biomass components, and composite seedling quality indices. Univariate and multivariate analyses revealed significant population-level differentiation and supported the emergence of two well-defined distinct phenotypic groups. The first group exhibited higher biomass accumulation, larger root collar diameter, and more balanced quality index values, indicating stronger structural development and superior seedling quality. In contrast, the second group showed lower biomass production and higher slenderness, reflecting a more limited growth pattern. Mantel tests revealed moderate correlations between phenotypic distances and both geographic and environmental distances; however, these relationships were not statistically significant, suggesting that spatial and environmental gradients alone do not fully account for the observed phenotypic structure. Our results highlight the critical influence of population origin in shaping seedling morphology and quality under uniform nursery conditions. These findings provide a scientific basis for provenance-based seed source selection and contribute to sustainable cultivation, breeding, and conservation strategies for bay laurel, with practical implications for horticulture and urban forestry.

Keywords:
bay laurel, nursery performance, phenotypic differentiation, population variability, seedling quality and morphology

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INTRODUCTION

Laurus nobilis L., commonly known as bay laurel, is an evergreen shrub or small tree belonging to the Lauraceae family. It typically reaches heights of 6–12 m and is characterized by leathery, aromatic leaves rich in essential oils. The species is native to the Mediterranean Basin (Akyol et al. 2023), where it forms part of the relict flora of humid forests and contributes to the structural diversity of sclerophyllous woodland ecosystems. Bay laurel occurs naturally across southern Europe, North Africa, and parts of the Middle East, including Portugal, Spain, France, Italy, Greece, Türkiye, Algeria, Morocco, and Libya. It thrives in calcareous soils, prefers sunny exposures, and is well adapted to Mediterranean climatic conditions (Christodoulakis and Fasseas 1990, Özel et al. 2008), although it can tolerate occasional frost in sheltered microhabitats. Depending on local climatic and topographic conditions, bay laurel is found from the sea level up to approximately 1200 m a.s.l. (Davis 1982, Atalay 2002).

Beyond its ecological role in native forest vegetation, where it contributes to slope stabilization, biodiversity support, and habitat provision, *Laurus nobilis* is widely valued for its aromatic properties and ornamental appeal (Marzouki et al. 2009, Yılmaz and Çiftçi 2021, Anzano et al. 2022, Paparella et al. 2022). These characteristics have led to extensive cultivation across the Mediterranean region and beyond. In addition to its importance in forestry, where it enhances the structural diversity and resilience of Mediterranean ecosystems, *Laurus nobilis* holds considerable significance in horticulture, landscape design, and urban forestry. Its dense evergreen foliage, tolerance to pruning, and adaptability to diverse site conditions make it a popular choice for hedges, formal plantings, and mixed shrub compositions in parks, gardens, and urban green spaces. This broad horticultural and urban applicability further underscores the importance of selecting high-quality and well-adapted planting material.

Laurus nobilis exhibits broad ecological plasticity, thriving across diverse soil types and climatic regimes. It is naturally found in both humid riparian zones and dry coastal slopes and tolerates a wide range of soil pH (Ibaoğlu 1998, Özel et al. 2008). Physiological adaptations such as foliar moisture retention and atmospheric water absorption contribute to its drought tolerance. However, seedlings are particularly sensitive to water stress (Rhizopoulou and Mitrakos 1990, Chmit et al. 2014), and juvenile plants display a range of morphological and physiological responses to semi-arid environments (Maatallah et al. 2010, Bernal et al. 2015, Pasha et al. 2019, Ishimwe and Deligöz 2024), including proline and soluble carbohydrate accumulation, modulation of stomatal conductance, and increased photosynthetic pigments levels – traits that collectively enhance drought tolerance.

As in many economically important species (Atar and Turna 2018, Poljak et al. 2018, Tumpa et al. 2021, Atar et al. 2024), genetic and phenotypic variation plays a pivotal role in shaping its ecological resilience and selection potential (Zobel and Talbert 1984). Morphological traits, as phenotypic expressions of the interaction between genetic structure and environmental conditions, are particularly valuable for evaluating seedling quality and predicting field performance (Jaenicke 1999, Grossnickle 2012). To date, most scientific studies on *Laurus nobilis* have focused on essential oil composition, ecology, and molecular-level genetic structure (Marzouki et al. 2009, Maatallah et al. 2010, Rodríguez-Sánchez et al. 2009, Sellami et al. 2011, Bernal et al. 2015, Sırıken et al. 2018, Alessi et al. 2019, Singletary 2021, Yılmaz and Çiftçi 2021, Paparella et al. 2022, Akyol et al. 2023). However, comprehensive studies addressing phenotypic differentiation during the seedling stage remain scarce, even though phenotypic diversity provides important insights into adaptive capacity and evolutionary processes (Nicotra et al. 2010). In this context, parametrically designed morphological studies on *Laurus nobilis* seedlings from diverse geographical origins are therefore needed to support seedling quality assessment, guide breeding efforts, and inform site-specific cultivation strategies (Grossnickle 2012, Ivetić et al. 2016).

The southwestern part of the Black Sea Region represents a distinctive ecological setting for studying *Laurus nobilis*, characterized by a transitional climate between Mediterranean and temperate zones, heterogeneous topography, and diverse forest composition. Türkiye, which encompasses the study area, is the world's leading producer of bay laurel, accounting for approximately 90% of global output. Due to its high export potential and contributions to rural development, the species is recognized as a significant non-timber forest product (Semerci and Çelik 2017). However, natural populations face increasing pressures from uncontrolled harvesting, habitat degradation, and the export of low value-added products (Bilgin et al. 2005, Durgun et al. 2014, Gül and Çelik 2016), raising concerns about long-term sustainability.

The aim of this study was to conduct a comparative evaluation of phenotypic traits in seedlings originating from six natural populations of *Laurus nobilis* in the southwestern Black Sea Region. Identifying population-level variation in seedling morphology will contribute to understanding intraspecific diversity and determining the most suitable populations and genotypes for local conditions. The study also aims to support sustainable bay laurel cultivation and quality-based seedling selection strategies. We hypothesize that two-year-old seedlings from different populations exhibit significant phenotypic differences and that these differences reflect the geographical origin of each population. Considering that the populations originate from ecologically diverse environments, characterized by differences in climate, altitude, soil, and local microhabitats, we assume that long-term environmental contrasts have contributed to the development of distinct adaptive trait patterns. Because all seedlings were grown under identical nursery conditions, short-term environmental effects and phenotypic plasticity were minimized. Therefore, the differences observed among populations are expected to represent inherent, genetically based variation shaped by adaptation to their native habitats.

MATERIALS AND METHODS

Selection of Populations

This study was conducted using *Laurus nobilis* seedlings grown from seeds collected from six natural populations located in the southwestern part of the Black Sea Region of Türkiye: P1–Kurucaşile, P2–Amasra, P3–İnkumu, P4–Devrek,

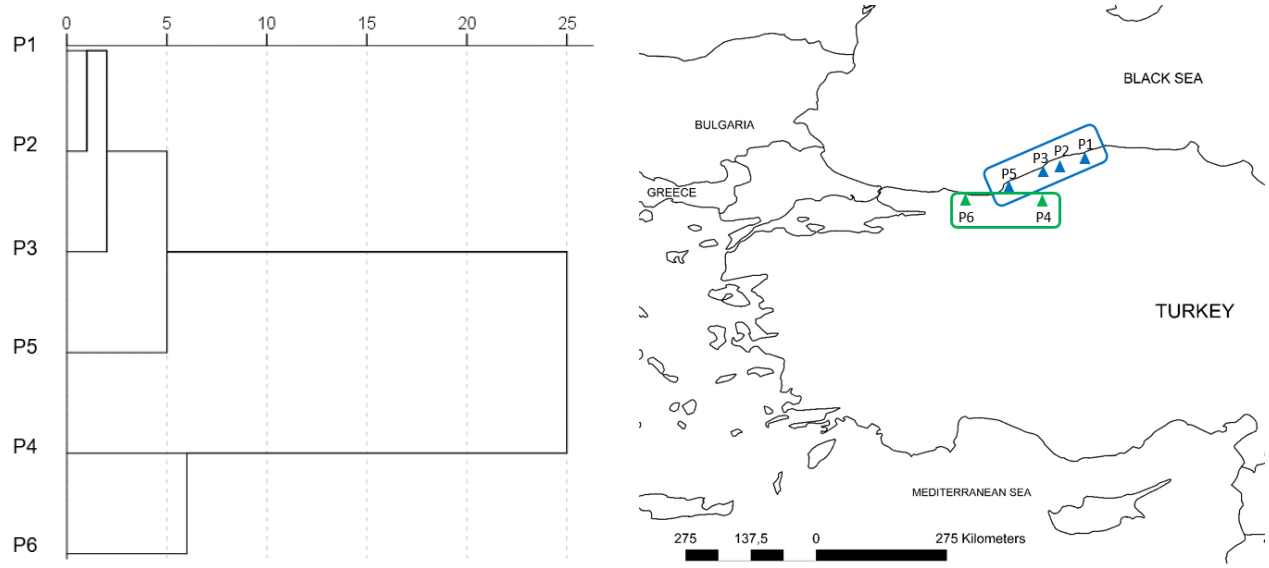

Figure 1 The geographical locations of the studied populations and the results of the hierarchical cluster analysis. Population abbreviations: P1–Kurucaşile; P2–Amasra; P3–İnkumu; P4–Devrek; P5–Ereğli; P6–Akçakoca.

P5–Ereğli, and P6–Akçakoca (Figure 1, Table 1). These populations occur within mixed broadleaf forest ecosystems typically dominated by *Fagus orientalis* Lipsky, *Quercus* spp., *Castanea sativa* Mill., *Carpinus betulus* L., *C. orientalis* Mill., *Tilia platyphyllos* subsp. *corinthiaca* (Bosc ex K.Koch) Pigott, and *Alnus glutinosa* (L.) Gaertn. The dominant soil type across the population sites consists of brown forest soils, typically deep, well-drained, and moderately rich in organic matter, which provide favorable conditions for mixed broadleaf forest development. Climatic data for the period 1970–2000 were obtained from the WorldClim 2 database at ~1 km² spatial resolution (Fick and Hijmans 2017). Mean annual temperature (T) across the sampled populations ranges from 12.3 °C (Akçakoca, P6) to 13.7 °C (Devrek, P4), while mean annual precipitation (P) varies between 743 mm (Kurucaşile, P1) and 1029 mm (Ereğli, P5). These values reflect moderate thermal conditions and a humid climate regime characteristic of the southwestern Black Sea Region, supporting the development of mixed broadleaf forest ecosystems.

Table 1 Geographic origin and climatic conditions of the studied populations.

Population number	Population name	Latitude	Longitude	Altitude (m a.s.l.)	T (°C)	P (mm)
P1	Kurucaşile	41°50'17''	32°42'24''	117	12.9	743
P2	Amasra	41°43'23''	32°17'51''	103	13.1	828
P3	İnkumu	41°40'51''	32°13'49''	65	13.2	859
P4	Devrek	41°18'21''	32°04'39''	225	13.7	881
P5	Ereğli	41°12'47''	31°25'20''	54	13.0	1029
P6	Akçakoca	41°04'54''	31°05'25''	42	12.3	965

T = mean annual temperature; P = total annual rainfall

Seedling Cultivation

To minimize the likelihood of genetic similarity, seeds were collected from 20 spatially separated trees per population, maintaining a minimum distance of 100–150 m between individuals. In total, seeds were obtained from 120 seed-bearing trees. The resulting seedlings were cultivated under open-field nursery conditions at the Gökçebey Forest Nursery, managed by the Zonguldak Regional Directorate of Forestry. According to long-term climate records for Zonguldak province (1939–2024), the site is characterized by an average annual temperature of 13.8 °C and an average total annual precipitation of 1226 mm, providing a temperate-humid environment suitable for seedling development.

Seed collection was carried out during the first week of November 2022, coinciding with the natural maturation period of *Laurus nobilis*. Following harvest, seeds were manually separated from their pericarps (fruit flesh) and prepared for sowing. Sowing was carried out in open-field nursery conditions using polyethylene bags (15 × 28 cm), arranged according to a randomized plot experimental design. The potting substrate consisted of a uniform mixture of brown forest soil, peat, sand, and bark material in equal proportions (1:1:1:1). Throughout the two-year cultivation period, standard nursery practices, including irrigation, shading, weeding, and general maintenance, were applied consistently to ensure optimal seedling development.

Measurements of Seedling Traits

Phenotypic assessments were performed on a total of 1200 seedlings at the 2+0 age class. Root collar diameter (RCD) was measured using a digital caliper with a precision of

±0.01 mm, while seedling height (SH), root length (RL), and current-year shoot length (CYSL) were recorded using a measuring tape with an accuracy of ±0.1 cm. CYSL was defined as the shoot elongation that occurred during the second growing season of the 2+0 seedlings and was measured at the end of this growing period. Leaf length (LL) and leaf width (LW) were determined by analyzing digital images of a representative leaf from each seedling, and measurements calculated in millimeters using ImageJ software. Fresh biomass parameters, including fresh shoot weight (FSW), fresh root weight (FRW), and total fresh seedling biomass (FSB), were measured using an analytical balance with a precision of ±0.001 g. For dry weight measurements, the samples were oven-dried at 105 °C for 24 hours. Subsequently, dry shoot weight (DSW), dry root weight (DRW), and total dry seedling biomass (DSB) were determined using the same precision balance.

Based on the collected phenotypic data, the sturdiness quotient (SQ) was calculated for each seedling as the ratio of seedling height (SH, cm) to root collar diameter (RCD, cm), according to the following formula:

$$SQ = SH / RCD$$

This index serves as a key morphological criterion reflecting seedling robustness and structural stability (Thompson 1985, Aldhous 1994, Jaenicke 1999). According to SQ values, seedlings were categorized into three quality classes: high-quality (SQ < 50), moderate-quality (50 ≤ SQ ≤ 60), and low-quality (SQ > 60) (Yahyaoğlu and Genç 2007).

In addition, the Dickson quality index (DQI) was calculated for each seedling using the formula:

$$DQI = DSB / (SQ + DSW/DRW)$$

Originally proposed by Dickson et al. (1960), this composite index is widely used to evaluate the overall quality and field performance potential of forest tree seedlings. Values approaching or exceeding 1 are generally considered indicative of high seedling quality (Mañas 2009).

Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) and R software (version 4.x). Prior to statistical evaluation, all phenotypic variables were examined for their suitability for parametric analyses. Data distribution was initially assessed using the Kolmogorov–Smirnov test as a diagnostic procedure. Although strict normality is not a prerequisite for analysis of variance (ANOVA), particularly in studies with sufficiently large and balanced sample sizes, this test was applied to provide an initial evaluation of distributional properties. In addition, homogeneity of variances was evaluated using Levene's test, and the results indicated that this assumption was satisfied for all examined traits ($p > 0.05$). Therefore, no data transformation was applied, and parametric statistical procedures were considered appropriate.

Differences in phenotypic traits among populations were evaluated using one-way analysis of variance (ANOVA). When statistically significant differences were detected ($p < 0.05$), Duncan's multiple range test was applied to identify homogeneous population groups. In addition, Pearson correlation analysis was performed to assess the strength and direction of linear relationships among phenotypic traits and to examine potential redundancy among variables. Although some traits showed moderate correlations, all variables were retained in subsequent analyses, as they were considered biologically meaningful and did not exhibit excessive multicollinearity that could compromise model stability.

Prior to multivariate analyses, all phenotypic variables were standardized using z-score transformation to eliminate scale effects among traits measured in different units. Patterns of phenotypic variation among populations were explored using hierarchical cluster analysis (HCA) and principal component analysis (PCA), both performed on the standardized dataset in R software. Hierarchical clustering was conducted using Ward's minimum variance method, while PCA was employed to reduce data dimensionality and to evaluate relationships among phenotypic traits and populations.

To further assess and confirm population differentiation revealed by the exploratory multivariate analyses, discriminant analysis (DA) was applied. DA was performed using the pooled within-groups covariance matrix, corresponding to Mahalanobis distances, which is appropriate for continuous multivariate phenotypic data. This method maximizes between-group variation relative to within-group variation while accounting for internal group structure. The statistical significance of the canonical discriminant functions was evaluated using Wilks' lambda, with associated chi-square statistics and degrees of freedom. The contribution of individual phenotypic traits to group discrimination was examined using the structure matrix, representing pooled within-group correlations between the discriminating variables and the standardized canonical discriminant function. The robustness and predictive performance of the discriminant model were assessed using a leave-one-out cross-validation procedure.

Mantel tests were performed to assess the relationship between multitrait phenotypic differentiation among populations and their geographic and environmental distances. Phenotypic dissimilarity was quantified using Euclidean distance matrices calculated from population scores on the first two principal components derived from all phenotypic traits. Environmental dissimilarity was computed as Euclidean distances between population means for the first two principal components summarizing the environmental variables (elevation, mean annual temperature, and mean annual precipitation). Geographic distances were calculated as great-circle distances (km) between population coordinates and subsequently log-transformed [ln(km)] to reduce skewness. The significance of correlations between distance matrices was evaluated using Mantel tests with Pearson correlation coefficients and 9,999 permutations. This approach is widely used to test isolation-by-distance and isolation-by-environment patterns by quantifying the association between multivariate dissimilarity matrices. Two simple Mantel tests were conducted: (1) between phenotypic and geographic distances (isolation by distance, IBD), and (2) between phenotypic and environmental distances (isolation by environmental distance, IBE).

RESULTS

Population Variability – Descriptive Statistics and Variance Analysis

Significant phenotypic variation was observed among seedlings derived from different *Laurus nobilis* populations. Analysis of variance (ANOVA) revealed that all examined traits exhibited statistically significant differences among the populations ($p < 0.05$). Table 2 presents the mean values, coefficients of variation (CV%), results of Duncan's multiple range test, and levels of statistical significance for twelve phenotypic traits.

The highest mean seedling height was recorded in the P2 (Amasra) and P3 (İnkumu) populations, whereas the P4 (Devrek) population had the shortest seedlings. Similarly, the P3 (İnkumu) population exhibited the greatest root collar diameter among all groups.

With respect to leaf traits (LL and LW), the P2 (Amasra) and P3 (İnkumu) populations exhibited higher values, whereas P4 (Devrek), P5 (Ereğli) and P6 (Akçakoca) recorded relatively lower measurements. In particular, the P4 (Devrek) and P6 (Akçakoca) populations stood out due to their lower biomass values. Regarding root morphology, the longest root lengths (RL) were observed in the P1 (Kurucaşile) and P2 (Amasra) populations, while the shortest roots were measured in P6 (Akçakoca). In terms of the sturdiness quotient (SQ), the lowest values were recorded in the P3 (İnkumu) and P1 (Kurucaşile) populations, whereas the highest SQ values were found in P4 (Devrek), P5 (Ereğli) and P6 (Akçakoca). In terms of the Dickson quality index (DQI), the highest value was observed in P4 (Devrek), 2.13, while the remaining populations ranged from 0.95 in P2 (Amasra) to 1.50 in P3 (İnkumu).

Table 2 Mean values, coefficients of variation, Duncan's test groupings (superscript letters), and significance levels (ANOVA p-values: *** significant at $p < 0.001$; ** significant at $0.001 < p < 0.01$; * significant at $0.01 < p < 0.05$) for studied phenotypic traits and populations. Population abbreviations: P1–Kurucaşile; P2–Amasra; P3–İnkumu; P4–Devrek; P5–Ereğli; P6–Akçakoca. Trait acronyms: seedling height (SH), root collar diameter (RCD), leaf length (LL), leaf width (LW), fresh and dry shoot weight (FSW, DSW), fresh and dry root weight (FRW, DRW), fresh and dry total seedling biomass (FSB, DSB), root length (RL), current-year shoot length (CYSL), sturdiness quotient (SQ), and Dickson quality index (DQI).

Seedling trait	Statistical parameter	Population						Overall mean	p-value
		P1	P2	P3	P4	P5	P6		
SH (cm)	Mean	21.4^{b}	25.0^{a}	23.5^{a}	19.6^{c}	23.4^{a}	20.5^{bc}	22.2	***
	CV (%)	13.0	6.53	7.73	6.07	8.47	8.94	12.0	
RCD (cm)	Mean	0.26^{b}	0.26^{b}	0.34^{a}	0.18^{c}	0.23^{bc}	0.20^{bc}	0.25	***
	CV (%)	26.9	19.9	28.4	23,3	29.3	40.8	34.8	
LW (cm)	Mean	2.86^{b}	4.04^{a}	3.86^{a}	1.80^{d}	2.22^{c}	2.11^{c}	2.82	***
	CV (%)	8.60	6.82	5.35	8.69	23.5	13.1	32.62	
LL (cm)	Mean	8.39^{b}	9.26^{a}	8.77^{ab}	5.33^{d}	6.59^{c}	6.88^{c}	7.54	***
	CV (%)	6.78	4.05	2.58	7.91	24.1	5.72	20.8	
FSB (g)	Mean	3.43^{a}	3.25^{ab}	3.46^{a}	2.53^{c}	3.10^{b}	2.58^{c}	3.06	***
	CV (%)	11.6	10.9	8.02	7.04	10.6	13.5	15.9	
DSB (g)	Mean	2.33^{a}	2.46^{a}	2.49^{a}	1.85^{b}	2.47^{a}	1.96^{b}	2.26	***
	CV (%)	11.9	15.1	8.10	7.22	18.2	25.1	18.6	
FSW (g)	Mean	1.72^{b}	2.03^{a}	1.68^{b}	1.71^{b}	1.53^{b}	1.03^{c}	1.62	***
	CV (%)	12.8	23.8	23.5	16.4	16.6	14.3	26.5	
FRW (g)	Mean	1.70^{ab}	1.31^{cd}	1.83^{a}	0.87^{e}	1.47^{bc}	1.14^{d}	1.38	***
	CV (%)	15.3	24.8	27.6	20.7	16.3	16.3	31.7	
DSW (g)	Mean	1.25^{a}	1.18^{a}	1.25^{a}	1.23^{a}	1.04^{a}	0.78^{b}	1.12	**
	CV (%)	20.1	14.6	30.1	19.2	23.8	30.0	26.8	
DRW (g)	Mean	1.16^{a}	1.29^{a}	1.10^{a}	0.63^{b}	1.17^{a}	0.77^{b}	1.02	***
	CV (%)	9.75	16.7	35.2	23.7	21.0	22.8	31.9	
RL (cm)	Mean	15.1^{a}	15.4^{a}	13.8^{a}	9.38^{bc}	10.7^{b}	8.93^{c}	12.2	***
	CV (%)	10.6	13.0	15.9	17.7	21.1	17.3	26.3	
CYSL (cm)	Mean	3.96^{ab}	3.99^{ab}	4.68^{a}	3.22^{b}	4.06^{ab}	3.11^{b}	3.84	*
	CV (%)	28.5	25.5	27.8	28.0	23.3	29.2	29.6	
SQ	Mean	87.5^{ab}	99.8^{ab}	74.4^{b}	116^{a}	112^{a}	121^{a}	102	*
	CV (%)	29.1	22.1	30.1	32.9	37.8	45.3	37.9	
DQI	Mean	1.11^{a}	0.95^{a}	1.50^{a}	2.13^{b}	1.00^{a}	1.12^{a}	1.30	**
	CV (%)	18.5	8.46	82.6	38.0	51.5	49.7	59.6	

Examination of the coefficient of variation (CV%) values revealed considerable levels of variation among populations for several phenotypic traits. When considering all seedlings collectively, high CV values were observed for RCD, LW, FSW, FRW, DSW, DRW, RL, CYSL, SQ, and DQI, indicating a broader range of individual variation and a wider distribution of values within populations for these traits. Additionally, Duncan's multiple range test identified distinct groupings among populations for many traits, with most traits forming three or more statistically homogeneous groups. This result suggests clear phenotypic differentiation among the populations.

Correlation Analysis

The correlation matrix (Figure 2) revealed three strongly positive relationships ($r > 0.70$) and two strongly negative relationships ($r < -0.70$) among the measured traits. Strong positive correlations were observed between leaf width and leaf length (LW–LL; $r = 0.80$), fresh seedling biomass and dry seedling biomass (FSB–DSB; $r = 0.71$), and fresh shoot weight and dry shoot weight (FSW–DSW; $r = 0.72$). In contrast, strong negative correlations were detected between sturdiness quotient and root collar diameter (SQ–RCD; $r = -0.88$) and between Dickson quality index and dry root weight (DQI–DRW; $r = -0.72$). Overall, the strongest positive associations were mainly concentrated among biomass-related traits and leaf size traits, indicating coordinated variation in growth and allocation patterns.

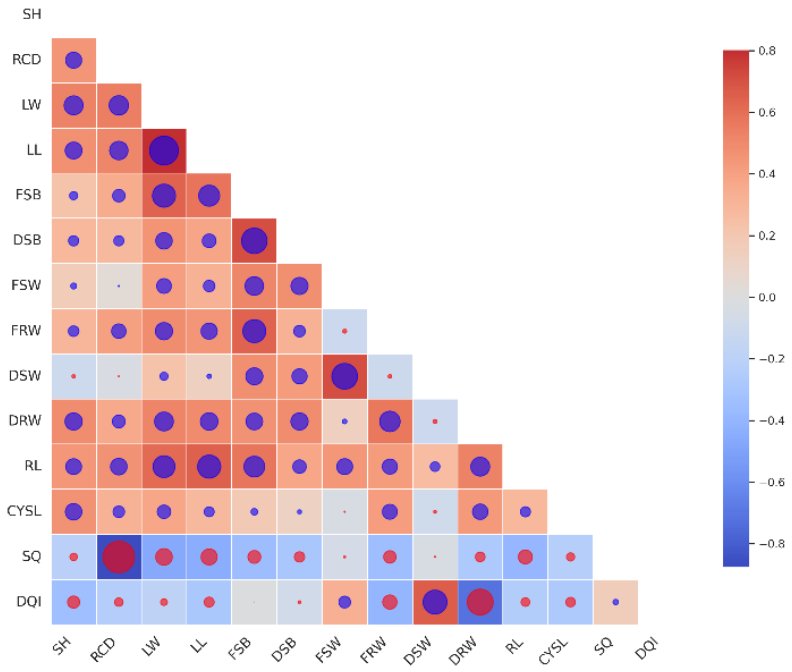

Figure 2 Pearson correlation matrix heatmap showing relationships among studied phenotypic traits. Trait acronyms: seedling height (SH), root collar diameter (RCD), leaf length (LL), leaf width (LW), fresh and dry shoot weight (FSW, DSW), fresh and dry root weight (FRW, DRW), fresh and dry total seedling biomass (FSB, DSB), root length (RL), current-year shoot length (CYSL), sturdiness quotient (SQ), and Dickson quality index (DQI).

Population Structure

Hierarchical Cluster Analysis

The dendrogram resulting from the hierarchical cluster analysis is presented in Figure 1. The analysis revealed that the populations clustered into two main groups based on their phenotypic similarities. The first group initially comprised the P3 (İnkumu) and P1 (Kurucaşile) populations, followed sequentially by the inclusion of P2 (Amasra) and P5 (Ereğli). In contrast, the P4 (Devrek) and P6 (Akçakoca) populations formed a second group, joining the cluster at a higher dissimilarity level.

Principal Component Analysis (PCA)

Table 3 presents the factor loadings and explained variance from the principal component analysis (PCA) conducted on 14 phenotypic traits of *Laurus nobilis* populations. The first four principal components had eigenvalues greater than 1 and together they explained 84.8% of the total variation. The first component (PC1) showed high loadings for FSB, DSB, FSW, DSW, DRW, FRW, CYSL, and DQI, representing a dimension associated with biomass accumulation and overall seedling quality. The second component (PC2) was strongly associated with RCD, LL, and SQ, reflecting traits related to seedling form and structural sturdiness. The third component (PC3) was loaded by SH and RL, while the fourth component (PC4) included only LW. This distribution indicates that different groups of traits are clustered within distinct components, highlighting the multidimensional nature of phenotypic diversity.

Figure 3 presents a two-dimensional biplot displaying the distribution of individuals from the studied populations within the PCA space, along with the vector orientations of phenotypic traits. The populations were clearly separated into two main groups on the plot: the first group included P1 (Kurucaşile), P2 (Amasra), P3 (İnkumu), and P5 (Ereğli), while the second group consisted of P4 (Devrek) and P6 (Akçakoca).

Table 3 The results of the principal component analysis showing Pearson correlations between the measured traits and the principal components. Only statistically meaningful loadings (|loading| ≥ 0.50) are presented to enhance interpretability. Eigenvalues and the proportion of variance explained by each of the first four principal components are also reported. Trait acronyms: seedling height (SH), root collar diameter (RCD), leaf length (LL), leaf width (LW), fresh and dry shoot weight (FSW, DSW), fresh and dry root weight (FRW, DRW), fresh and dry total seedling biomass (FSB, DSB), root length (RL), current-year shoot length (CYSL), sturdiness quotient (SQ), and Dickson quality index (DQI).

Studied trait	PC–Principal component			
	PC1	PC2	PC3	PC4
SH			0.806	
RCD		0.935		
LW				0.872
LL		0.698		
FSB	0.732			
DSB	0.718			
FSW	0.884			
FRW	-0.767			
DSW	0.895			
DRW	-0.682			
RL			-0.596	
CYSL	-0.798			
SQ		-0.826		
DQI	0.964			
Eigenvalue	5.51	3.25	1.7	1.6
Variation (%)	41.2%	19.6%	12.6%	11.4%

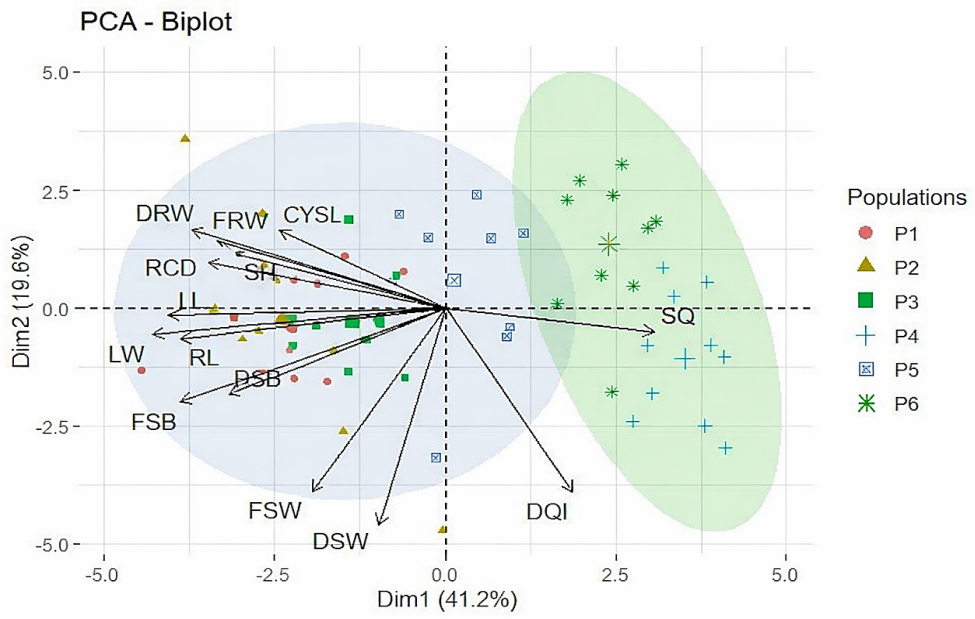

Figure 3 The results of the principal component analysis (PCA) based on 14 seedling phenotypic traits in the studied *Laurus nobilis* populations. Population abbreviations: P1–Kurucaşile; P2–Amasra; P3–İnkumu; P4–Devrek; P5–Ereğli; P6–Akçakoca. Trait acronyms: seedling height (SH), root collar diameter (RCD), leaf length (LL), leaf width (LW), fresh and dry shoot weight (FSW, DSW), fresh and dry root weight (FRW, DRW), fresh and dry total seedling biomass (FSB, DSB), root length (RL), current-year shoot length (CYSL), sturdiness quotient (SQ), and Dickson quality index (DQI).

Discriminant Analysis

Discriminant analysis revealed a clear and statistically significant phenotypic separation between the two predefined geographic groups. The overall discriminant model was highly significant, as indicated by Wilks' lambda (0.286), with a corresponding chi-square value of 63.911 (df = 14, $p < 0.001$), confirming marked phenotypic differentiation between the two groups.

Among the traits examined, several variables emerged as the most influential in distinguishing the two geographic groups. Fresh shoot weight (FSW) was the strongest discriminator, followed by root length (RL), leaf width (LW), and

dry shoot weight (DSW). Together, these traits contributed most prominently to the phenotypic separation observed between the groups.

The discriminant model demonstrated high classification performance. Based on the original classification results, 96.7% of individuals were correctly assigned to their respective geographic groups. Leave-one-out cross-validation yielded an overall classification accuracy of 83.3%, with cross-validated accuracies of 82.5% for Group 1 and 85.0% for Group 2, indicating strong model robustness and generalizability.

The histogram of canonical discriminant scores further illustrates the effectiveness of the discriminant model (Figure 4). Individuals belonging to the first group were predominantly distributed along positive canonical values, whereas those assigned to the second group were concentrated in the negative region of the discriminant axis, with minimal overlap between the distributions. This clear separation visually confirms the strong phenotypic differentiation detected by the statistical analysis and supports the high classification accuracy obtained in both original and cross-validated models.

Mantel Test

Mantel tests were used to evaluate the relationship between phenotypic variation and both geographic and environmental distances among *Laurus nobilis* populations (Figure 5). The tests comparing phenotypic and geographic distances showed a moderate positive correlation (r = 0.4371, *p* = 0.103), indicating a tendency for geographically closer populations to exhibit greater phenotypic similarity. Although the correlation was not statistically significant, the pattern is consistent with a weak signal of isolation by distance (IBD), where spatial separation contributes to phenotypic

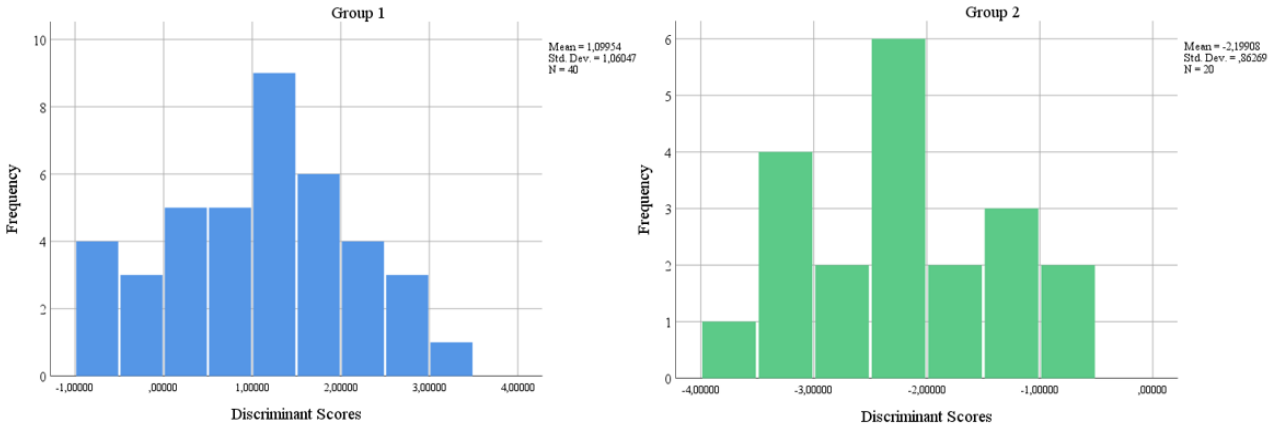

Figure 4 Frequency distribution of individual canonical discriminant scores (Function 1) for the two predefined geographic groups of *Laurus nobilis*. The clear separation between score distributions with minimal overlap indicates strong phenotypic differentiation and supports the robustness of the discriminant model.

divergence. A similar pattern was observed for the relationship between phenotypic and environmental distances, which also showed a moderate positive correlation (r = 0.449, *p* = 0.092). While not statistically significant, this result suggests a potential influence of isolation by environment (IBE), implying that environmentally similar sites may promote convergence or reduced divergence in phenotypic traits. Taken together, these findings indicate that both spatial and environmental gradients may shape phenotypic differentiation in the studied species, with environmental variation showing a slightly stronger, though still statistically unsupported, association with phenotypic patterns.

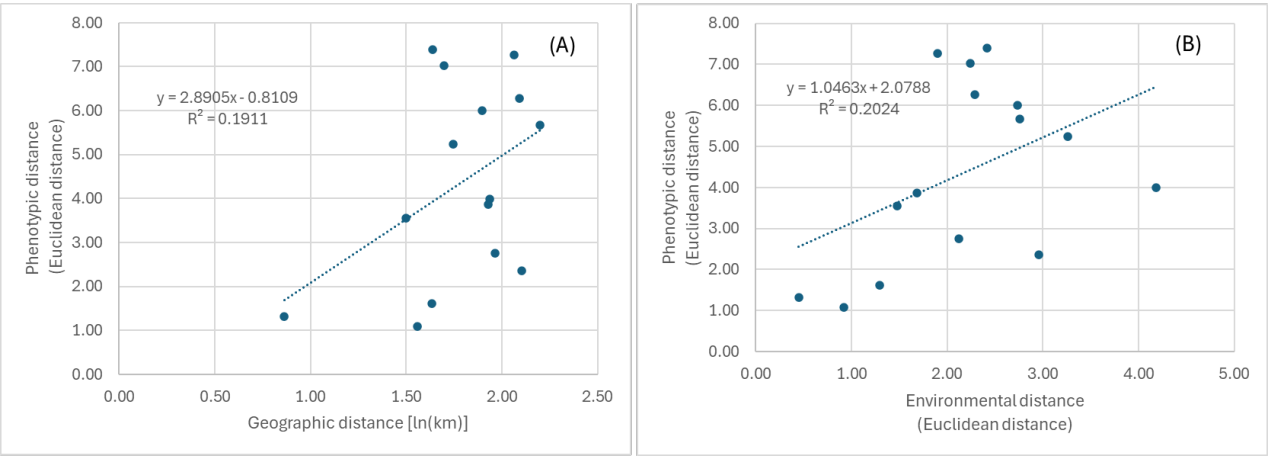

Figure 5 Isolation by distance (IBD) and isolation by environmental distance (IBE) in *Laurus nobilis* populations. Scatter plots of simple Mantel tests showing the relationships between (A) geographic and phenotypic distances (r = 0.4371, p = 0.103, R2 = 0.1911); and (B) environmental and phenotypic distances (r = 0.449, p = 0.092, R2 = 0.2024).

DISCUSSION

The present study revealed pronounced phenotypic variation among *Laurus nobilis* seedlings derived from six natural populations in the Black Sea Region of Türkiye. All evaluated traits, including seedling height, root collar diameter, leaf dimensions, root and shoot biomass (fresh and dry), as well as quality indices such as the sturdiness quotient and Dickson quality index, differed significantly among populations. This variability is in line with earlier reports describing the species' broad ecological amplitude and high phenotypic plasticity across Mediterranean and sub-Mediterranean environments (Alessi et al. 2021, Paparella et al. 2022). Similar population-specific differentiation has been reported for *Laurus nobilis* in previous genetic studies (Yılmaz and Çiftçi 2021), suggesting that such phenotypic contrasts may reflect underlying genotypic divergence shaped by local environmental conditions.

Analysis of the coefficient of variation (CV) revealed substantial intra-population variability across several phenotypic traits. High CV values for traits related to root collar diameter, biomass components, and seedling quality indices indicate a wide range of phenotypic expressions among individuals within populations. This pronounced intra-population variability suggests heterogeneity in growth performance and phenotype development, which may influence population-level responses to local environmental conditions, consistent with previous observations reported for *Laurus nobilis* under contrasting environmental stresses (Maatallah et al. 2010, Bernal et al. 2015).

Multivariate analyses provided an integrated view of population differentiation in *Laurus nobilis*. Comparable integrative approaches, which combine exploratory and confirmatory multivariate methods, have been widely applied to investigate population structure and phenotypic divergence in Mediterranean and sub-Mediterranean taxa. Such approaches have been used in studies of *Ceratonia siliqua* L. (Bolarić et al. 2021), *Castanea sativa* Mill. (Ertan 2007, Tumpa et al. 2021), *Pistacia terebinthus* L. and *P. lentiscus* L. (Temunović et al. 2024), *Pyrus spinosa* Forssk. (Vidaković et al. 2021, 2025a, 2025b), as well as conifer species within the genera *Abies* Mill. (Sękiewicz et al. 2013) and *Juniperus* L. (Klimko et al. 2007, Mazur et al. 2018, Vidaković et al. 2024b).

Principal component analysis and hierarchical clustering consistently identified two major population groups, with Devrek and Akçakoca forming a distinct cluster separate from Kurucaşile, Amasra, İnkumu, and Ereğli. The Devrek–Akçakoca group was generally characterized by comparatively lower biomass accumulation and less favorable composite quality metrics, reflecting a more slender growth pattern and reduced structural robustness. In contrast, populations within the second group displayed higher biomass-related traits, greater root collar diameter, and more balanced quality index values, indicating stronger structural development and potentially superior seedling quality. Discriminant analysis confirmed the separation of two morphologically distinct geographic groups suggested by PCA and cluster analysis, with a high classification accuracy. The successful validation of PCA- and HCA-derived groupings using CDA has also been reported in *Ceratonia siliqua* and *Salix eleagnos* Scop., where CDA effectively resolved population-level differentiation and strengthened the interpretation of multivariate patterns (Tumpa et al. 2022, Poljak et al. 2024). The CDA structure matrix further showed that fresh shoot weight, root length, leaf width, and dry shoot weight were the primary traits contributing to group discrimination, emphasizing the role of biomass allocation and root-related characteristics in shaping population-level phenotypic differentiation. These findings emphasize the utility of DA as a confirmatory tool in studies of population structure based on phenotypic traits.

The recent molecular studies provide a relevant comparative framework, demonstrating that *Laurus nobilis* populations exhibit pronounced genetic structuring at regional and broader geographic scales. Using ISSR and SCoT markers, Yılmaz and Çiftçi (2021) identified two main genetic groups within Turkish *Laurus nobilis* germplasm, largely corresponding to geographic regions, while nuclear microsatellite analyses across the Mediterranean basin revealed a marked east–west differentiation, with Turkish populations forming a distinct eastern gene pool (Marzouki et al. 2009). At a broader evolutionary scale, plastid DNA-based phylogeographical analyses indicate that these regional lineages have been shaped by long-term range fragmentation and refugial persistence since the Neogene (Rodríguez-Sánchez et al. 2009). Accordingly, the multivariate trait syndromes identified in the present study may be interpreted as phenotypic-level expressions of provenance-related differentiation rather than isolated phenotypic responses. Nevertheless, as the present study relies on phenotypic data, these patterns should be regarded as indirect indicators of underlying genetic structure, and future studies integrating molecular markers with phenotypic traits will be required to explicitly test genotype–phenotype associations in *Laurus nobilis*.

The Mantel test results indicate that neither geographic nor environmental distance alone provides strong explanatory power for the observed phenotypic differentiation among *Laurus nobilis* populations. Although moderate positive trends were detected, these relationships were not statistically supported, suggesting that isolation by distance is not a dominant structuring mechanism within the study area. Similar patterns have been reported in *Zanthoxylum armatum* DC., *Salix triandra* L. and *Phlomis olivieri* Benth., where geographic separation alone did not sufficiently account for phenotypic or genetic divergence (Zare Hoseini et al. 2022, Tumpa et al. 2022, Yan et al. 2023). A comparable tendency was observed for environmental distances, implying that environmental heterogeneity may contribute to phenotypic variation, but without clear statistical support in the present dataset. Previous studies on *Mentha longifolia* (L.) Huds., *Melissa officinalis* L., *Eucommia ulmoides* Oliv. and *Juniperus sabina* var. *balkanensis* R.P.Adams et Tashev have demonstrated that environmental gradients, particularly elevation and climatic variables, can interact with spatial factors to shape variation patterns (Devi et al. 2022, Koohdar and Sheidai 2022, Gong et al. 2023, Poljak et al. 2025). However, in our case, neither geographic nor environmental gradients alone appear sufficient to explain the detected population-level variation. Overall, these findings indicate that phenotypic differentiation in *Laurus nobilis* within the Southwestern Black Sea Region is likely shaped by multiple

interacting processes, including local adaptation and potential gene flow, rather than by clear isolation-by-distance or isolation-by-environment patterns acting independently.

Practical Implications

Several traits evaluated in this study are directly linked to seedling quality and have practical relevance for nursery production and field performance. Root collar diameter, biomass allocation, sturdiness quotient, and Dickson quality index are widely recognized as integrative indicators of mechanical stability, physiological balance, and stress tolerance (Ritchie 1984, Thompson 1985, Grossnickle 2018). Population-level differences in these indices, observed under uniform nursery conditions, indicate that seed source selection plays a critical role in determining seedling quality.

From an applied perspective, populations such as Amasra and İnkumu appear particularly suitable for producing high-quality planting stock, as reflected by higher root collar diameter and more favorable DQI values. In contrast, populations characterized by elevated SQ values, such as Devrek and Akçakoca, may produce more slender seedlings that are potentially more susceptible to mechanical damage and post-planting stress (Haase 2008). Nevertheless, the presence of high-quality individuals within these populations suggests opportunities for selective propagation and breeding, as emphasized in previous studies (Özel et al. 2010, Güney et al. 2013, Atar 2022).

In the context of ecological restoration and afforestation, the observed variation in seedling quality traits has important implications. Seedlings with balanced biomass allocation and higher DQI values are generally better equipped to cope with heterogeneous and stressful site conditions, particularly in degraded landscapes. Moreover, populations exhibiting higher phenotypic variability may possess greater adaptive capacity under variable climatic regimes. Given the ecological and economic importance of *Laurus nobilis* and its sensitivity to water availability (Pasha et al. 2019, Ishimwe and Deligöz 2024), the integration of population-based phenotypic and quality traits into nursery and restoration planning can enhance establishment success and long-term ecosystem resilience.

CONCLUSION

This study demonstrated significant phenotypic differentiation among *Laurus nobilis* seedlings derived from six natural populations in the Black Sea Region of Türkiye. The observed variation in key traits, including seedling height, root collar diameter, root and shoot biomass, leaf dimensions, and seedling quality indices (sturdiness quotient and Dickson quality index), indicates substantial population-level variability under uniform nursery conditions, suggesting a strong influence of population origin on seedling development.

The integration of multivariate statistical approaches, including principal component analysis and hierarchical cluster analysis, further confirmed the differentiation of populations into distinct phenotypic groups. Together, these results provide a robust framework for identifying high-performing seed sources, which is essential for the sustainable cultivation, genetic conservation, and restoration of *Laurus nobilis* in both natural and managed landscapes.

Given the species' wide ecological distribution and economic importance as a non-timber forest product, prioritizing populations with superior phenotypic performance and seedling quality traits is crucial for afforestation, cultivation, and domestication programs. Future studies integrating phenotypic data with physiological and molecular markers are recommended to more explicitly elucidate the genetic basis of population-level variation and to support provenance-based management strategies for *Laurus nobilis*.

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