

Phenotypic and Genetic Variation in Medlar Germplasm Based on Morphological, Chemical, and Leaf Traits Using Multivariate Analysis

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Abstract

Medlar (*Mespilus germanica* L.) is an underutilized fruit with notable nutritional benefits; however, limited data on its phenotypic diversity restrict germplasm conservation and breeding efforts. This study characterized the morphological, chemical, color, and leaf traits of 101 naturally growing medlar genotypes from Türkiye's West Black Sea region. Morphometric measurements, chemical analyses, ImageJ for leaf traits, and statistical methods, including ANOVA, principal component analysis (PCA), and heritability estimates (h^2), revealed significant variation across traits. High heritability was observed for fruit length (0.71), volume (0.68), and weight (0.66), indicating strong genetic control. Conversely, seed number (0.02), leaf length (0.07), and titratable acidity (0.05) were predominantly influenced by environmental factors. Fruit weight exhibited broad ranges (7.67–37.70 g); soluble solids content (SSC) 8.53–20.40 °brix; pH 3.72–4.70; and TA 1.08–3.15. Correlations showed a negative relationship between titratable acidity and fruit size. PCA explained 78.9% of the variation, and cluster analysis (CA) identified three distinct genotype groups. These findings provide valuable insights into the genetic diversity of medlar, offering a foundation for targeted breeding and conservation strategies to enhance fruit quality.

Keywords: *Mespilus germanica* L., genetic resource, heritability, agro-morphological traits, principal component analysis

Zusammenfassung

Phänotypische und genetische Variation in Mispel-Genressourcen auf Grundlage morphologischer, chemischer und Blattmerkmale. Die Mispel (*Mespilus germanica* L.) ist eine unzureichend genutzte Obstart mit bemerkenswerten ernährungsphysiologischen Vorteilen. Die begrenzte Verfügbarkeit von Daten zur phänotypischen Diversität beschränkt jedoch die Erhaltung genetischer Ressourcen sowie die züchterischen Bemühungen. Diese Studie charakterisierte morphologische, chemische, farbmétrische und blattmorphologische Merkmale von 101 natürlich vorkommenden Mispel-Genotypen aus der westlichen Schwarzmeerregion der Türkei. Die morphometrischen Messungen, chemischen Analysen,

die bildgestützten Blattanalyse mittels ImageJ und statistischen Methoden, inklusive ANOVA, Hauptkomponentenanalyse (PCA) und Schätzungen der Heritabilität (h^2) zeigten eine signifikante phänotypische Variabilität für sämtliche untersuchten Merkmale. Hohe Heritabilitätswerte wurden für Fruchtlänge (0,71), Fruchtvolumen (0,68) und Fruchtgewicht (0,66) ermittelt, was auf eine überwiegend genetische Kontrolle dieser Merkmale hinweist. Demgegenüber wurden die Samenanzahl (0,02), die Blattlänge (0,07) sowie der titrierbare Säuregehalt (TA; 0,05) hauptsächlich durch Umweltfaktoren beeinflusst. Das Fruchtgewicht unterlag einer großen Variationsbreite zwischen 7,67 und 37,70 g, der Gehalt an löslichen Feststoffen (SSC) zwischen 8,53 und 20,40 °Brix, der pH-Wert zwischen 3,72 und 4,70 sowie der titrierbare Säuregehalt (TA) zwischen 1,08 und 3,15 %. Korrelationsanalysen zeigten einen negativen Zusammenhang zwischen dem titrierbaren Säuregehalt und der Fruchtgröße. Die Hauptkomponentenanalyse erklärte 78,9 % der Gesamtvariation, während die Clusteranalyse drei klar abgegrenzte Genotypengruppen identifizierte. Diese Ergebnisse bieten wertvolle Einblicke in die genetische Diversität der Mispel und liefern eine wissenschaftliche Grundlage für zielgerichtete Züchtungs- und Erhaltungsstrategien um die Fruchtqualität zu verbessern.

Schlüsselwörter: *Mespilus germanica* L., genetische Ressourcen, Heritabilität, agromorphologische Merkmale, Hauptkomponentenanalyse (PCA)

Introduction

The medlar (*Mespilus*), belonging to the Rosaceae family, includes two primary species: *Mespilus germanica* L. (common medlar) and *Mespilus canescens* Phipp. Its cultivation dates back about 3000 years in regions near the Caspian and Black Sea coasts of Türkiye. Despite its historical significance, the medlar was often overlooked in the food industry, and contemporary agricultural interest in it remains limited relative to its potential (Haciseferogullari et al., 2005; Rop et al., 2011; Cevahir and Bostan, 2021; Voaides et al., 2021). Despite being underutilized, medlar fruit has significant nutritional benefits and a distinctive flavor that appeals to diverse preferences. The medlar exhibits notable biological properties, including antioxidant and antimicrobial effects, which are largely attributed to its substantial content of polyphenols, flavonoids, and other antioxidant compounds (Voaides et al., 2021; Cevahir & Bostan, 2021; Stankovic et al., 2022; Popovic-Djordjevic et al., 2023; Drvodelić et al., 2025; Mezhenskyj et al., 2025). These attributes have prompted researchers to promote its inclusion in dietary guidelines and explore its potential role in enhancing food security. Phytochemical analyses have further indicated that medlar tissues are rich

in organic acids, such as malic and citric acids, and volatile constituents, including monoterpenes such as γ -terpinene, which enhance their antioxidant capacity and potential functional value (Tessa et al., 2021; Sadeghinejad et al., 2022). Additionally, various plant parts, including fruits, leaves, and bark, have been traditionally used in folk medicine to treat conditions such as diarrhea and inflammation, underscoring the ethnobotanical relevance and therapeutic potential of this species (Popovic-Djordjevic et al., 2023; Tessa et al., 2021; Yildiz et al., 2023). Typically, the fruit undergoes two color phases: brown when mature and dark-brown when overripe (Bibalani et al., 2012; Voaides et al., 2021). Research has shown that medlar fruit is initially characterized by a hard and acidic texture, which softens over time through a ripening process known as "bletting." This process renders the fruit palatable after exposure to frost or after extended storage (Glew et al., 2003; Lim, 2012; Akçay et al., 2016; Voaides et al., 2021). In Türkiye, medlars are consumed fresh or processed into products such as jams, jellies, candies, sauces and pickles (Bibalani et al. 2012; Lim et al. 2012). According to the Türkiye Statistical Institute, the national medlar production is approximately

5,390 tons (TÜİK, 2025). This figure underscores the continued commercial significance of the medlar in this region. Türkiye is also recognized as an important center of origin and diversity for this fruit, hosting rich populations of natural genetic resources (Akbulut et al. 2016; Cevahir and Bostan, 2021). While natural (seed-grown) genotypes are essential resources for breeding programs, prominent commercial varieties in Türkiye include "Istanbul," "Italian," and "Akçakoca 77," and their cultivation is encouraged (Akçay et al., 2016). This diversity in medlar growing, combined with the country's varied agroecological zones, supports medlar cultivation across different climatic conditions and indicates a gradually expanding market for this fruit. Genetic diversity within medlar is essential for breeding initiatives aimed at enhancing fruit quality and improving disease resistance. Phenotypic trait analysis is a vital tool for differentiating cultivars and facilitating targeted breeding. Recent research has emphasized that understanding variation in phenotypic traits is fundamental to breeding programs, as it enables targeted enhancement of specific traits of interest. Numerous studies have documented significant variability in morphometric and quality-related traits among cultivars, underscoring their importance in selection and differentiation (Akbulut et al., 2016; Grygorieva et al., 2018; Tessa et al., 2021). Detailed phenotypic characterization has been demonstrated to aid in the discrimination of genotypes and support breeding efforts aimed at improving commercially relevant attributes, such as fruit size, flavor, and overall quality (Mezhenskyj et al., 2025; Drvodelić et al., 2025).

There is insufficient data on the phenotypic diversity of natural medlar trees in Turkey. This study aimed to characterize and evaluate the morphological variation, chemical properties, and leaf traits of this species growing in its natural habitat. The findings will help conserve local germplasm, identify accessions with desirable traits, and support their integration into the breeding gene pool.

Material and methods

This study was conducted on naturally occurring medlar (*Mespilus germanica* L.) genotypes from the West Black Sea region of Bolu Province, Türkiye. This study encompassed seven distinct locations: Aşağısoku (6 genotypes), Yukarısoku Köy (3 genotypes), Çele (18 genotypes), Yumrukaya (12 genotypes), Değirmenbeli (19 genotypes), Kartalkaya (10 genotypes), and Karacau (33 genotypes), where 101 mature trees were evaluated during the harvest season of the first year (2020). Then, 20 promising genotypes were selected using the "Weighted Rating Method"; their weighted scores out of 100 are presented in Tab. 1. The weighted grading method is employed to enable the concurrent assessment of multiple economically significant traits, including pomological, morphological, agronomic, biochemical, and market-quality characteristics. This method facilitates the identification of superior genotypes based on overall performance. By assigning specific relative coefficients to each criterion, breeders can refine selections for their target markets, making the evaluation process more systematic, objective, and efficient compared to single-trait selection. This approach incorporates various quantitative and qualitative parameters into a composite selection index, considering the relative importance of each trait within specific breeding objectives. These genotypes were further examined in 2021. The fruit characteristics were also assessed. Morphological traits (FWD: Fruit weight; FWT: Fruit width; FL: Fruit length; FV: Fruit volume, FFR: Fruit Flesh ratio; FBW: Fruit Flower branch width; FBD: Fruit Flower branch depth; SN: Seed number; SW: Seed weight; FFH: Fruit Flesh Hardness; FV: Fruit volume), chemical properties (pH, measured with a pH meter (Thermo Science, Orion star A 111) and Titratable acidity (TA)), and leaf traits (LL: Leaf length; L: Leaf width; PL: Petiole length) were evaluated for a minimum of 20 fruits and leaves per tree. The color parameters (L^* , a^* , b^* , chroma, and hue angle) were measured using a hand-type colorimeter (Minolta Co., model CR-

400, Tokyo, Japan). Morphological characteristics, including fruit length, width, height, shape index, and weight, were determined using digital calipers and a precision balance. For the measurement of FV, ten genotypes were randomly selected and placed in a beaker equipped with measurement scales. The beaker was then filled with pure water, and the volumes of the genotypes were subsequently measured. SSC, expressed as degrees Brix (°Bx), indicated in fresh juice using a handheld refractometer (ATC, NTRM01) at 20 °C. TA was determined by titration with NaOH (0.1 N)

at a pH of 8.1 using a titration device, and the results were expressed as the percentage of malic acid. The pH values were recorded using a digital pH meter (Thermo Science, Orion star A 111) by directly immersing the probe in the fruit juice samples. ImageJ (Ver. 1.54d) was used to analyze leaf characteristics. For each selected genotype, 20 leaves were collected at different angles from annual shoots. The leaves were photographed using a camera and saved on a computer, and the images were used to evaluate the various leaf traits.

Tab. 1: The weighted grading table for the Medlar genotypes was based on the evaluated parameters, with minor revisions (Yılmaz, 2015; Közen and Bostan, 2016).

Characters	Reference values and coefficients of classes importance percentage	Score
Fruit weight (g)	≥28.0 =5; 23.0-27.9 =3; ≤22.9 = 1	40
Fruit Flesh/ seed (%)	≥90.0 = 5; 88.6-89.9 = 3; ≤88.5 = 1	25
Tree Yield Status	Very High = 5; High =3; Medium =1	25
SSC (°Bx)	≥9.0 =5; 8.0-8.9 =3; ≤7.9 = 1	10

Statistical Evaluations

Analysis of variance (ANOVA) was used to analyze the data. For each genotype, the mean, standard error, maximum and minimum values, and coefficient of variation (CV) were calculated for every trait. Statistical analyses and graphical representations were performed using the JMP 16 software (SAS Institute Inc., Cary, NC, USA). The relationships and similarities between genotypes based on the evaluated traits were assessed using principal component analysis (PCA). Genotypic variance (σ^2_g), phenotypic variance (σ^2_p), broad-sense heritability (h^2), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), genetic advance (GA), and genetic advance as a percentage of the mean (GAM) were estimated using R Studio version 2025.05.1 Build 513.

Results

Characterization and Phenotypic Diversity

Analysis of variance (ANOVA) identified statistically significant differences ($P\leq0.05$) among the medlar genotypes across all assessed morphological, chemical, and leaf traits. The population demonstrated substantial phenotypic variation, with 17 of the 21 traits exhibiting coefficients of variation (CV) exceeding 10%. Notably, traits such as titratable acidity (TA) and fruit volume (FV) displayed high variability of 73.32% and 34.96%, respectively. The broad-sense heritability (h^2) was highest for fruit length (0.71), followed by fruit volume (0.68) and fruit weight (0.66), indicating that these traits are predominantly governed by genetic factors and are dependent on selection. In contrast, traits such as seed number (h^2 :0.02), leaf length (h^2 :0.07), and titratable acidity (h^2 :0.05) showed low heritability, suggesting a considerable environmental impact on phenotypic expression. Genetic advancement as a percentage of the mean was the most significant for fruit volume (48.65), seed weight (47.01), and fruit weight

(42.39), underscoring the potential for substantial genetic enhancement through direct selection (Tab. 2).

Tab. 2: Descriptive statistics for the characters utilized in the 101 Medlar genotypes

Characters	Abbr.	Unit/code	Minimum	Maximum	Mean	SD	CV	$\sigma^2 g$	$\sigma^2 p$	h^2	GCV	PCV	GA	GAM
L*		code	25.29	68.20	50.37	0.64	13.95	10.82	43.03	0.25	6.53	13.02	3.39	6.74
a*		code	10.62	23.63	16.79	0.24	15.48	1.09	6.85	0.16	6.24	15.59	0.86	5.14
b*		code	13.22	44.04	26.87	0.57	23.15	6.30	30.64	0.20	9.34	20.60	2.34	8.72
Chrome		code	16.25	43.60	30.20	0.55	20.03	9.90	37.12	0.26	10.42	20.17	3.35	11.09
Hue		code	44.80	66.43	55.26	0.49	9.69	9.00	26.18	0.34	5.43	9.26	3.62	6.56
Fruit weight	FWD	g	7.67	37.70	18.51	0.29	31.12	21.89	33.02	0.66	25.28	31.05	7.84	42.39
Fruit width	FWT	mm	24.85	42.72	32.83	0.19	11.31	8.80	13.61	0.64	9.04	11.24	4.91	14.97
Fruit length	FL	mm	20.59	43.66	31.78	0.24	15.15	16.85	23.60	0.71	12.91	15.28	7.14	22.48
Fruit volume	FV	ml	8.00	35.00	17.36	0.30	34.96	24.46	35.55	0.68	28.48	34.34	8.45	48.65
Fruit flesh ratio	FFR	%	68.54	95.60	85.32	0.18	4.21	2.68	12.53	0.21	11.15	24.08	1.56	10.64
Fruit fresh hardness	FFH	Kg cm ⁻²	0.60	4.70	1.89	0.06	36.77	0.14	0.48	0.28	19.91	37.07	0.41	22.02
Flower branch width	FBW	mm	9.60	39.33	17.17	0.16	19.05	5.11	10.70	0.47	13.16	19.05	3.21	18.74
Flower branch depth	FBD	mm	3.58	27.13	9.45	0.18	37.90	4.71	12.66	0.37	22.98	37.67	2.72	28.89
Seed weight	SW	g	0.77	6.94	2.69	0.05	37.07	0.60	0.95	0.62	28.80	36.36	1.26	47.01
Seed number	SN	unit	4.00	7.00	4.99	0.01	3.01	0.00	0.02	0.02	0.49	2.97	0.00	0.16
pH	pH	code	2.00	5.31	4.14	0.05	11.33	0.05	0.15	0.31	5.40	9.63	0.25	6.24
Titrateable acidity	TA	%	0.60	13.00	1.71	0.13	73.32	0.06	1.21	0.05	15.29	64.68	0.12	7.44
SSC	SSC	brix	0.80	28.00	14.74	0.57	38.56	9.59	21.88	0.43	21.00	31.72	4.22	28.64
Leaf width	LW	cm	3.02	9.80	4.05	0.10	22.10	0.07	0.72	0.10	6.79	21.07	0.18	4.51
Leaf length	LL	cm	7.17	13.55	9.91	0.18	16.07	0.18	2.51	0.07	4.38	15.99	0.24	2.47
Petiole length	PL	cm	0.17	1.15	0.56	0.02	32.19	0.003	0.03	0.11	10.95	31.93	0.11	0.04

CV, coefficient of variation; $\sigma^2 g$, genotypic variance; $\sigma^2 p$, phenotypic variance; h^2 , broad-sense heritability; PCV, phenotypic coefficient of variation; GCV, genotypic coefficient of variation; GA, genetic advancement; GAM, genetic advancement as a percentage of the mean.

Color Parameters and Morphometric Characteristics

Significant variations in color characteristics were observed between genotypes, years, and genotype-year interactions. Colorimetric analysis revealed L* values ranging from 41.13 to 59.07, a* values from 14.44 to 18.68, b* values from 21.78 to 32.57, Chr values between 22.09 and 36.45, and hue values between 48.48 and 60.88 (Tab. 3).

Tab. 3: The variability of fruit color between genotypes

Genotypes	L*	a*	b*	Chr	Hue
DB008	51.38±1.57bd	16.14±0.67ae	26.35±3.03ad	30.87±2.88ae	57.47±2.45ae
KK001	58.30±2.56ab	18.34±1.53ac	32.43±1.15a	36.45±1.56a	57.70±0.85ae
KK004	50.05±2.35cd	14.60±1.02de	24.96±3.53bd	22.09±0.46g	54.73±2.67ch
KK005	52.97±2.54ad	14.63±0.78de	21.78±2.37d	26.33±2.30dg	55.34±2.11bg
KK006	48.74±3.21cd	18.48±1.42ab	29.01±5.67ac	29.55±3.14bf	53.72±3.40dh
KK007	50.63±3.58cd	14.44±0.34e	23.10±2.82cd	27.11±2.17cg	50.97±2.27gi
KK008	59.07±2.70a	18.05±1.19ac	32.57±0.65a	36.11±1.04a	57.55±0.74ae
KK009	49.12±1.63cd	18.05±0.62ac	24.86±0.88bd	30.91±0.65ae	53.67±1.60di
KK010	49.00±1.46cd	18.03±0.59ac	26.15±1.48ad	31.84±1.25ad	56.68±1.49af
S001	52.34±1.80ad	14.47±1.05e	30.04±1.14ab	31.07±2.12ae	60.35±0.86ab
S002	53.31±1.74ac	16.84±0.63ae	29.33±1.62ac	32.91±1.80ac	60.88±1.38a
S003	46.08±4.03ce	16.95±1.12ae	30.54±1.53ab	32.78±2.68ac	59.77±1.43ac
S004	50.82±1.82cd	17.90±0.53ac	27.43±1.22ad	32.92±0.70ac	58.46±1.19ad
S005	41.13±4.14e	16.73±0.77ae	25.20±0.58bd	23.94±3.12fg	50.11±1.71hi
S006	52.46±1.60ad	18.68±1.36a	29.59±1.45ac	33.66±1.19ab	54.93±2.28ch
YK011	48.34±3.42cde	15.76±1.09be	23.00±3.89cd	28.06±3.79bf	53.62±2.96di
YK012	45.91±3.61de	15.64±1.00ce	22.20±3.31d	25.31±2.40efg	48.48±0.72i
YS001	50.48±2.16cd	17.10±1.04ae	27.13±1.59ad	29.25±1.71bf	51.63±1.74fi
YS002	47.22±1.01cde	17.33±1.01ad	25.08±1.18bd	30.96±2.21ae	52.77±0.77ei
YS003	50.05±1.51cd	17.65±0.69ac	26.56±1.30ad	31.89±1.34ad	56.30±1.06af
Genotypes	***	***	***	***	***
Years	***	ns	***	ns	**
Genotypes X Years	***	***	***	***	***

Considerable variation in morphological traits was observed among genotypes, with significant differences in the fruit dimensions. Specifically, the FL ranged from 24.75 mm to 37.79 mm. The fruit volume ranged from 9.00 to 25.11. whereas the FWT varied from 27.26 mm to 37.27 mm. FWD exhibited a broad range from

10.66 g to 26.60 g, with an average of 18.48 g and a CV of 31.12%. The FW ranged from 8.95 to 24.85 g. FFR ranged from 80.04% to 88.07%. The FFH was between 1.18 and 2.97 Kg cm⁻². FBW ranged from 13.28 mm to 20.52 mm, and the FBD demonstrated high variability (CV = 37.90%), ranging from 6.36 mm to 13.80 mm (Tab. 4).

Tab. 4: The variability of morphological characters between genotypes

Genotypes	FL	FV	FWT	FWD	FFR	FFH	FBW	FBD
DB008	30.61±0.63f	16,74±0,31d	30.96±0.48gi	15.89±0.61ef	86.50±0.51bd	2.33±0.23ac	16.94±0.49ef	7.37±0.49gh
KK001	28.73±0.63gh	13,79±0,94fg	29.48±0.69j	13.62±0.81hi	83.93±0.51hi	2.55±0.21ab	13.28±0.51i	6.76±0.35h
KK004	29.72±0.59fg	12,42±0,73g	29.82±0.38ij	14.31±0.53fi	84.86±0.51gi	1.43±0.12gh	13.70±0.34i	7.34±0.30gh
KK005	29.74±0.48fg	14,63±0,96e	29.40±0.36j	15.12±0.94eh	85.16±0.51eh	1.58±0.13eh	13.75±0.27hi	7.64±0.22gh
KK006	24.75±0.29i	10,95±0,24hi	27.26±0.34k	10.66±0.36j	85.81±0.51cg	1.40±0.09gh	13.35±0.37i	7.87±0.30fh
KK007	27.54±0.88h	11,00±0,40hi	29.94±0.60ij	13.78±0.78gi	83.88±0.52hi	2.13±0.11bf	15.26±0.53gh	7.49±0.28gh
KK008	28.17±0.48gh	15,89±0,66df	32.23±0.55fg	16.26±0.64ef	83.66±0.51i	2.28±0.22bd	16.36±0.58fg	7.59±0.33gh
KK009	24.93±0.47i	9,00±0,24i	30.20±0.43hj	12.69±0.49ij	85.93±0.52cg	1.57±0.18eh	16.27±0.29fg	7.99±0.21eh
KK010	25.25±0.59i	11,05±0,24hi	30.63±0.31hj	12.99±0.41hi	87.01±0.51ac	1.63±0.22dh	16.33±0.39fg	7.68±0.23gh
S001	33.95±0.50de	15,75±1,34df	33.60±0.43ef	21.26±0.66d	86.42±0.51be	2.12±0.25bf	18.41±0.42ce	13.80±1.44a
S002	32.38±0.44e	17,63±0,59d	31.45±0.30g	16.61±0.35e	85.12±0.51fh	1.82±0.11ch	18.97±0.71bcd	7.98±0.32eh
S003	33.72±0.74de	23,42±1,14a	34.53±0.87d	20.73±1.25d	84.32±0.51hi	2.26±0.09be	19.71±1.15ac	8.59±0.35dg
S004	35.63±0.68bc	22,20±0,52b	36.44±0.52a	23.83±1.05bc	86.32±0.51bf	2.05±0.23bg	19.34±0.46ad	11.00±0.40bc
S005	37.79±0.67a	25,11±1,21a	37.01±0.75a	26.60±0.80a	86.66±0.51bd	1.57±0.25eh	20.07±0.52ab	10.21±0.53cd
S006	35.77±0.68bc	24,32±1,41a	37.27±0.63a	24.54±1.19ab	80.04±0.51j	1.60±0.32eh	20.52±0.75a	9.60±0.39cdef
YK011	35.07±0.48bc	23,47±0,85a	35.35±0.36b	22.13±0.48cd	83.89±0.51hi	1.52±0.15fh	18.13±0.36de	9.76±0.19cde
YK012	34.50±0.42cd	20,21±0,14c	34.85±0.37c	21.24±0.41d	85.16±0.51dh	1.95±0.39bg	19.18±0.40ad	12.69±0.93ab
YS001	36.54±0.68ab	17,05±1,16d	35.24±0.67b	21.95±1.01cd	86.06±0.51cg	2.97±0.56a	18.68±0.67bd	12.25±0.84ab
YS002	33.94±0.57de	20,11±1,01c	34.14±0.56d	21.55±1.18d	87.71±0.51ab	1.86±0.25bh	17.04±0.49ef	12.75±0.93ab
YS003	36.38±0.67ab	22,95±0,59a	36.22±0.41ac	23.86±0.63bc	88.07±0.51a	1.18±0.14h	17.94±0.32de	12.43±1.43ab
Genotypes	***	***	***	***	***	***	***	***
Years	ns	***	***	**	***	ns	ns	***
Genotypes X	***	***	***	***	***	***	***	***

Significant differences between some morphological characteristics, including SW between 1.50 and 4.77 g and SN between 4.90 and 5.00 pieces, were identified. Notable variations in chemical properties were observed among genotypes. Specifically, pH ranged from 3.72 to 4.70,

TA varied from 1.08 to 3.15 %, and SSC ranged from 8.53 to 20.40 Brix. Investigated leaf characteristics: LW ranged from 3.47 to 5.82 cm, LL ranged from 8.41 to 12.16 cm, and PL ranged from 0.38 to 0.81 cm (Tab. 4).

Tab. 5: The variability of morphological, chemical and leaf traits between genotypes

Genotypes	SW	SN	pH	TA	SSC	LW	LL	PL
DB008	2.10±0.06ij	5.00±0.00b	4.23±0.04ae	1.80±0.09ab	13.60±1.17bg	4.39±0.41b	8.82±0.72de	0.76±0.04ab
KK001	2.11±0.10ij	5.00±0.00b	4.25±0.16ad	2.00±0.43ab	9.63±1.85g	5.82±1.39a	8.85±0.68de	0.64±0.12ad
KK004	2.16±0.09hi	5.00±0.00b	4.15±0.06ae	1.44±0.12b	17.80±2.40ac	3.96±0.43b	9.45±0.39be	0.60±0.10ae
KK005	2.16±0.06hi	5.00±0.00b	3.96±0.07ce	2.34±0.21ab	20.40±4.26a	3.99±0.25b	8.41±0.41e	0.69±0.09abc
KK006	1.50±0.08k	4.90±0.07c	3.74±0.38de	1.54±0.08b	10.20±0.49g	4.06±0.27b	9.27±0.64ce	0.62±0.05ad
KK007	2.28±0.14hi	5.00±0.00b	4.27±0.17ad	1.88±0.13ab	18.60±3.72ab	3.57±0.05b	10.33±0.73ae	0.67±0.08ad
KK008	2.70±0.18fg	5.00±0.00b	3.97±0.07cde	1.64±0.16ab	17.40±1.81ad	4.09±0.28b	9.99±0.80ae	0.62±0.06ad
KK009	1.72±0.09jk	5.00±0.00b	3.76±0.17de	1.72±0.14ab	11.80±0.20dg	3.73±0.16b	8.72±0.58de	0.59±0.09ae
KK010	1.68±0.08k	5.00±0.00b	3.76±0.14de	1.78±0.18ab	10.40±0.51g	3.54±0.16b	9.18±0.93ce	0.55±0.05be
S001	2.81±0.12efg	5.00±0.00b	4.46±0.19ac	1.34±0.14b	12.20±0.97cg	3.93±0.21b	10.49±0.42ae	0.65±0.07ad
S002	2.49±0.14gi	5.00±0.00b	4.58±0.10ab	1.67±0.32b	17.17±1.92ad	4.67±0.30ab	10.77±1.05ad	0.81±0.17a
S003	3.29±0.21bd	5.00±0.00b	4.70±0.25a	1.38±0.20b	17.50±1.89ae	4.04±0.29b	10.18±0.45ae	0.38±0.07e
S004	3.26±0.20bd	5.00±0.00b	4.21±0.13ae	1.30±0.11b	17.60±1.75ad	3.88±0.26b	10.13±0.92ae	0.44±0.07de
S005	3.59±0.24b	5.00±0.00b	4.40±0.14ac	1.24±0.26b	17.60±2.50ad	4.05±0.42b	10.24±0.70ae	0.52±0.10ce
S006	4.77±0.17a	5.00±0.00b	4.09±0.19bce	1.08±0.09b	11.40±0.24eg	4.07±0.38b	10.14±1.26ae	0.43±0.05de
YK011	3.54±0.11bc	5.10±0.10a	4.21±0.09ae	1.68±0.11ab	11.00±0.84fg	3.78±0.29b	10.98±0.82ac	0.46±0.05ce
YK012	3.16±0.10ce	5.00±0.00b	3.72±0.37e	3.15±1.98a	8.53±1.60g	4.13±0.30b	9.55±0.41bce	0.54±0.12be
YS001	3.02±0.16df	4.95±0.05bc	4.05±0.15ce	2.00±0.21ab	19.40±2.82ab	4.32±0.32b	12.16±0.55a	0.43±0.13de
YS002	2.53±0.21h	5.00±0.00b	4.29±0.22ad	1.08±0.06b	19.00±2.04ab	3.47±0.15b	11.31±0.65ab	0.49±0.06ce
YS003	2.84±0.18eg	4.90±0.07c	4.19±0.21ae	1.72±0.19ab	16.80±2.75af	3.54±0.31b	10.73±1.00ad	0.51±0.08ce
Genotypes	***	ns	***	ns	***	**	ns	**
Years	***	**	***	ns	***	**	ns	ns
Genotypes	***	ns	ns	ns	***	*	ns	ns

Correlations among the characters

Positive and negative correlations were observed between the traits (Fig. 1). L^* was positively correlated with b^* ($r = 0.54$), chroma (Chr; $r = 0.61$), and hue ($r = 0.52$) values. Chroma was positively correlated with a^* ($r = 0.67$) and b^* ($r = 0.77$) values for the color parameters. Hue was positively correlated with b^* ($r = 0.65$) and chroma ($r = 0.64$) values. Conversely, TA was negatively correlated with FBW ($r = -0.77$), FV ($r = -0.75$), FWD ($r = -0.77$), FWT ($r = -0.76$), and SW ($r = -0.77$). Leaf length was negatively correlated with chroma ($r = -0.75$) and FBW ($r = -0.74$),

respectively. Seed weight was positively correlated with FBD ($r = 0.46$), FBW ($r = 0.70$), FFR ($r = 0.46$), FL ($r = 0.81$), FV ($r = 0.85$), FWD ($r = 0.86$), FWT ($r = 0.88$), and LL ($r = 0.60$). Leaf length was positively correlated with FBD ($r = 0.71$), FBW ($r = 0.58$), FL ($r = 0.70$), FV ($r = 0.55$), FWD ($r = 0.68$), and FWT ($r = 0.65$). Leaf width was positively correlated with b ($r = 0.48$), FFH ($r = 0.61$), and L^* ($r = 0.53$). Additionally, pH was positively correlated with fruit length FL ($r = 0.52$) and FV ($r = 0.50$).

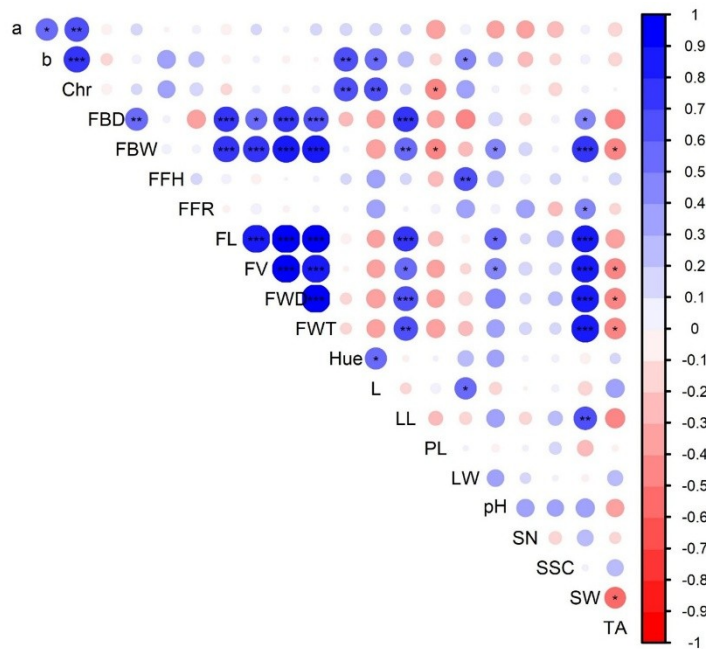


Fig. 1: Pearson’s correlations of morphological, chemical, and leaf traits of the medlar genotypes.

Principal component analysis (PCA)

PCA was employed to elucidate the relationships among genotypes and examine the correlations between phenotypic traits. The analysis revealed that the first five components accounted for 78.89% of the observed variability (Fig. 2). Specifically, PC1, PC2, and PC3 explained 34.65%, 18.59%, and 10.80% of the variability, respectively, accounting for 64.05%. PC1, identified as the most significant component, exhibited positive correlations with FBD, FBW, FL, FV, FWD, FWT, SW, and TA. Furthermore, the variables with the highest loadings on PC2 were a*, b*, and Chr values, as well as FFH, FFR, Hue, L*, and LW. PC3 was positively correlated with PL, LW, pH, and SN.

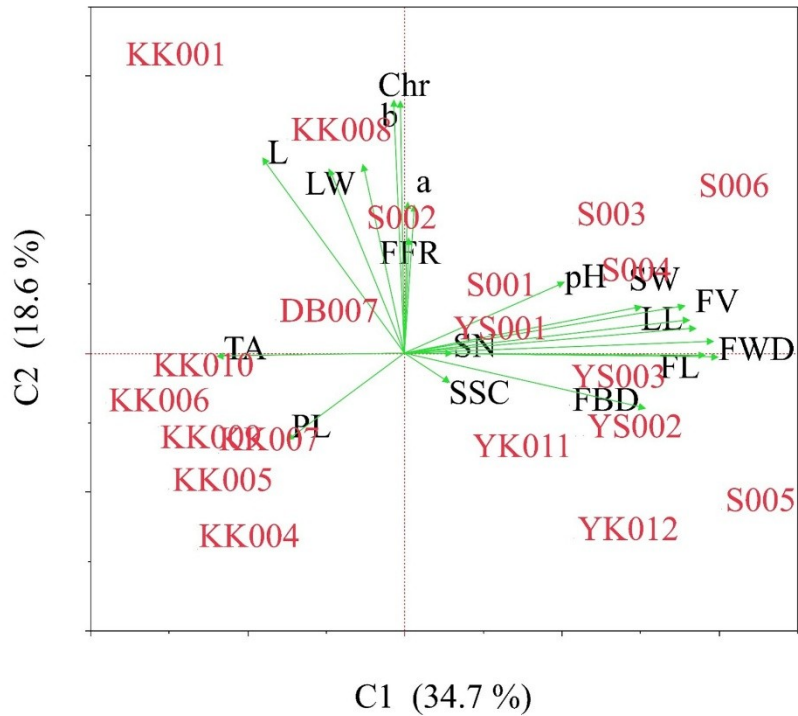


Fig. 2: Biplot of the principal component analysis based on morphological, chemical, and leaf traits of promising medlar genotypes.

Cluster analyses (Heat map)

CA is a widely used multivariate technique that categorizes accessions into groups based on their similarities. A dendrogram generated through cluster analysis divided the populations into three primary groups. The first cluster (I) comprised four genotypes, whereas the second cluster (II) was further divided into two subclusters, each containing three genotypes. Cluster (III) was divided into two subclusters: the first included six genotypes,

and the second contained four genotypes. The identified clusters and subclusters were consistent with those derived from heat map clustering. Cluster I was distinguished by elevated values of color characteristics (L^* , a^* , b^* , Chr, and Hue) as well as FFH, FFR, LW, and SN. Cluster II exhibited higher values for PL and TA, whereas Cluster III was characterized by FBD, FBW, FL, FV, FWD, FWT, LL, pH, and SSC (Fig. 3).

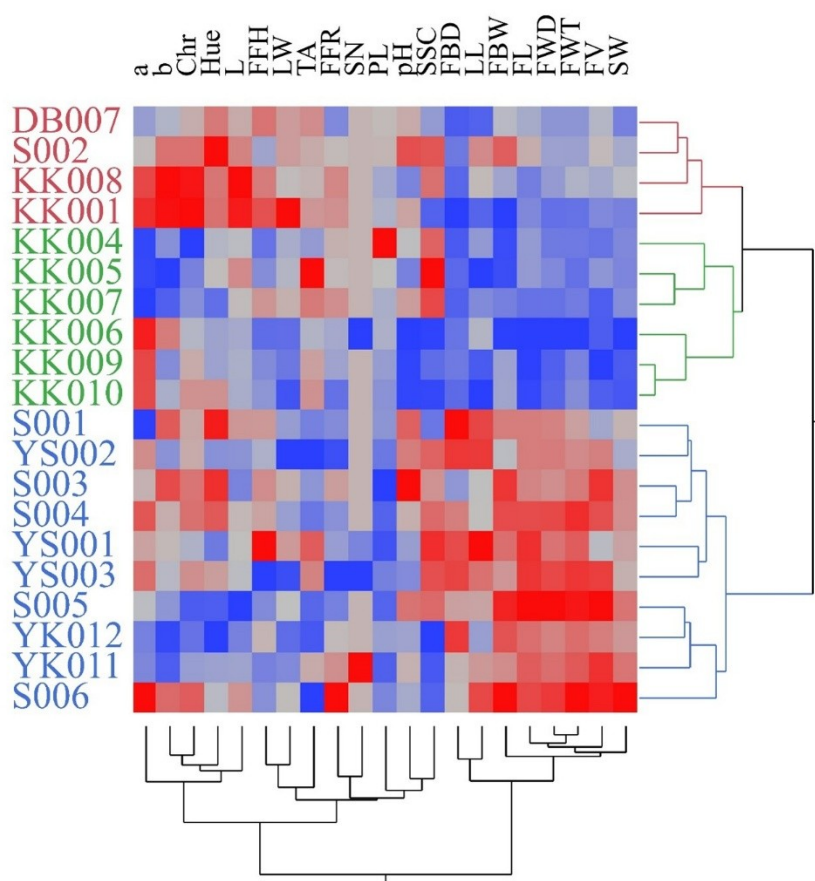


Fig. 3: Heat map and cluster analysis of promising medlar genotypes.

Discussion

The systematic characterization of germplasm is a crucial prerequisite for effective crop improvement and the management of genetic resources (Bezu et al. 2019; Zargar et al. 2021). In fruit, evaluating phenotypic diversity based on morphological, biochemical, and quality traits provides essential information for breeding programs, cultivar development, and

conservation strategies (Güler et al., 2021; Muradoğlu and Kayakeser, 2022; Dong et al., 2023). The obtained data showed statistically significant differences ($P \leq 0.05$) among the medlar genotypes for all measured morphological, chemical, and leaf traits, indicating considerable phenotypic variability within the gathering. In germplasm characterization studies, significant findings across multiple

trait categories are generally seen as indicators of exploitable genetic diversity suitable for classification, selection, and breeding (Sadeghinejad et al., 2022; Solaiman et al., 2014). The medlar genotypes showed significant phenotypic diversity, with 17 out of 21 traits exhibiting CVs over 10%, and very high CVs for titratable acidity (73.32%) and fruit volume (34.96%). In germplasm diversity assessments, CV is commonly used to quantify trait dispersion and identify characteristics with discrimination among accessions, with fruit size and quality traits contributing most to overall variation (Grygorieva et al., 2018; Cosmulescu et al., 2020; Muradoğlu et al., 2019; Mezhen-skyj et al., 2025). Previously studied levels of variability in fruit-related traits across different species have been observed, often with CVs exceeding 30%, indicating valuable genetic and phenotypic diversity useful for breeding and improvement programs were reported (Tian et al., 2024; Tunç et al., 2025).

Broad-sense heritability (h^2) estimates further elucidated the genetic architecture of the evaluated traits. Fruit length (0.71), volume (0.68), and weight (0.66) had the highest heritability values, suggesting a strong genetic influence on these morphometric characteristics. This finding aligns with those of previous studies on other fruit crops, in which size-related traits typically exhibit moderate to high heritability (Cheng et al., 2011; Chanda et al., 2020). Conversely, seed number (0.02), leaf length (0.07), and titratable acidity (0.05) displayed low heritability, indicating a more substantial environmental influence and potentially stronger genotype $\times$ environment interactions than with the other traits. The GAM was notably high for fruit volume (48.65%), seed weight (47.01%), and fruit weight (42.39%), which are generally considered advantageous for effective selection in breeding programs (Gurung et al., 2020; Ali et al., 2025). According to quantitative genetic principles, traits with high heritability and significant genetic gains are effective targets for direct phenotypic selection. However, studies on diversity emphasize the importance of phenotypic variability and multivariate structural parameters, with the stability of genetic inference depending

on the experimental design and variance divisions (Zargar et al., 2021; Bezu et al., 2019; Dong et al., 2023). Therefore, while the h^2 and GAM patterns observed in this medlar dataset support established selection rationale, the results ultimately depend on environmental and experimental contexts.

Colorimetric analysis revealed significant variations in fruit appearance parameters, with L values ranging from 41.13 to 59.07, indicating notable diversity in fruit lightness. The observed ranges for a, b*, chroma (Chr), and hue further demonstrated measurable differences among the genotypes, underscoring the substantial contribution of color traits to phenotypic variation. In horticultural germplasm studies, fruit appearance and quality attributes, particularly color measurements, are widely employed as market- and preference-related descriptors to distinguish and classify accessions, especially when evaluated alongside fruit size and texture (Ayaz et al. 2008; Muradoglu and Balta 2010; Rop et al. 2011; Gruz et al. 2011). Therefore, the wide variation across multiple color dimensions in this studied data supports the general differentiation of the ripening stage.

The wide range of fruit dimensions and weight-related characteristics indicates substantial morphological diversity within the genotypes. Fruit length ranged from 24.75 to 37.79 mm, and fruit weight ranged from 10.66 to 26.60 g, reflecting strong differentiation in size-related attributes with potential relevance for diverse market applications. The morphological characteristics of medlar fruits show significant differences depending on the variety, and fruit diameters range from 1.5 to 3 cm, and weights range from 10 to 80 g, as reported by Grygorieva et al. (2018) and Mezhen-skyj et al. (2025). Similar results have been observed by different researchers in medlar fruit germplasm, where fruit size and weight are the most quantitative traits and major contributors to overall phenotypic variation and multivariate genotype (Akbulut et al. 2016; Suluşoğlu and Unver 2016; Khadivi et al. 2019). Variations in fruit fresh ratio, fruit flesh firmness, and seed weight

importance of morphometric and structural traits in understanding diversity patterns. These parameters are critical for commercial evaluation in both fresh and processed markets and are routinely emphasized in germplasm characterization studies of fruit species (Akbulut et al., 2016; Uzun and Bostan, 2019). Overall, the pronounced variability in fruit size, volume, weight, and firmness shows that these traits may be particularly useful for genotype differentiation and selection in breeding programs.

In gene source evaluations, chemical traits such as SSC and acidity-related variables are routinely assessed because of their direct relationship with consumer preference, taste perception, and technological quality (Zargar et al., 2021; Dong et al., 2023; Cheng et al., 2011). Significant variations were observed in primary chemical quality attributes. TA ranged from 1.08% to 3.15%, pH from 3.72 to 4.70, and SSC from 8.53 to 20.40, consistent with the findings of Haciseferoğlu et al. (2005), Cevahir and Bostan (2021), and Tessa et al. (2021), Šebek et al., (2019); Cevahir & Bostan (2021); Mezhenjskyj et al. (2025), indicating considerable diversity in the sweetness-acidity balance and potential processing suitability. The relationship between ripening stage and chemical composition underscores the critical importance of precise harvesting methods because acidity levels tend to decline markedly during fruit softening and maturation.

Low broad-sense heritability was found in leaf characteristics for LL (0.07), LW (0.10), and PL (0.11), whereas fruit length showed high heritability (0.71). The leaf length (0.07) and leaf width (0.10) were extremely low. The leaf length (LL) of the examined population ranged from 7.17 to 13.55 cm, whereas the leaf width (LW) ranged from 3.02–9.80 cm. In similar studies, genotypes exhibited narrower ranges, with lengths between 8.8 and 12.18 cm and widths between 3.5 and 5.60 cm in Romania (Cosmulescu et al., 2020). Healthy mature plants had shorter lengths (6.5–10.0 cm) and much narrower widths (2.9–3.5 cm)

in Kocaeli, Türkiye (Sulusoglu-Durul and Unver, 2019), and accessions showed significantly smaller dimensions, with lengths ranging from 3.45 to 7.42 cm, in Iran (Khavidi et al., 2019). This suggests that these vegetative traits are heavily influenced by environmental factors, such as localized rainfall, humidity, and soil composition, rather than by genetic control alone.

Strong positive correlations were observed among the color parameters (L, a, b*, chroma, and hue) and between fruit length, width, weight, and volume, as well as between these traits and seed weight, indicating a coordinated relationship among these traits. Negative correlations between TA and several size-related traits (fruit width, volume, weight, and seed weight) suggest potential trade-offs between fruit size and acidity. In fruit research, the relationships between biochemical attributes (e.g. sugars and acids) and morphometric traits are routinely examined to understand quality–yield interactions and to inform balanced breeding strategies (Cheng et al., 2011; Dong et al., 2023). Additionally, the positive correlations between leaf length and fruit size suggest potential physiological connections between vegetative vigor and fruit quality. From a breeding perspective, these correlation patterns highlight that selecting larger fruits could also influence seed and vegetative size.

The application of multivariate statistical methods, especially principal component analysis (PCA) and cluster analysis, has become a standard approach in germplasm evaluation across different crop species (Gülsoy et al. 2023; Kirs et al. 2024). In this dataset, the first five principal components explained 78.89% of the total phenotypic variation, with PC1–PC3 accounting for 64.05%. This level of explained variance reflects the characteristics of the germplasm, with PC1 mainly linked to fruit- and seed-size-related traits, indicating that morphometric variation is the primary axis of differentiation among genotypes. PC2 is mainly characterized by color and texture traits, representing a secondary, yet biologically meaningful,

fruit quality gradient. PC3 appears to capture vegetative and chemical traits (such as pH, leaf size, and seed number), adding another dimension to the observed variability. Similar results with principal components are often seen in germplasm studies of various fruit crops, including apples, apricots, rosehips, and mangoes (Bezu et al., 2019; Kirs et al., 2024; Muradoglu and Kayakeser, 2022; Güler et al., 2021). Hierarchical clustering grouped the genotypes into three main clusters, each with distinct trait profiles. This pattern is frequently observed in studies on the morphological diversity of fruit germplasm (Barracosa et al., 2007; Chanda et al., 2020). The agreement between the dendrogram and heat map patterns bolsters confidence in the identified groups, consistent with multivariate methods that combine clustering with ordering techniques like PCA to enhance interpretability and structural understanding. Cluster I was mainly characterized by higher color parameters and fruit quality traits, Cluster II by leaf and acidity-related traits, and Cluster III by fruit size and chemical quality parameters (such as SSC and pH). The existence of these distinct groups suggests that the collection contains phenotypically structured and complementary trait combinations.

Conclusions

Comprehensive phenotypic analysis of medlar genotypes has demonstrated significant genetic variation in agro-morphological, chemical, and quality attributes. Notable results from the ANOVA, high coefficients of variation, and distinct multivariate models indicate that the genotypes have valuable diversity for breeding and genetic materials. Estimates of heritability and genetic predictions have identified fruit size traits as the primary targets for selection, whereas correlation analysis has elucidated important trait relationships to guide breeding strategies. Principal Component Analysis (PCA) and cluster analysis provide a foundation for understanding population structure and optimizing utilization. Collectively, these findings have enhanced our understanding of phenotypic diversity and supported evidence-based medlar improvement programs. Moreover, the significant genetic variation and distinct population structures uncovered here provide the essential groundwork for genomic-assisted breeding. Moving forward, these phenotypic benchmarks will enable the integration of molecular markers to refine selection accuracy for complex chemical and quality attributes.

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