ANNALS OF THE UNIVERSITY OF CRAIOVA

Series: ✓*Biology*
✓*Horticulture*
✓*Food products processing technology*
✓*Environmental engineering*

Vol. XXXI (LXVII) - 2026

PHENOTYPIC AND INTER-ANNUAL VARIABILITY OF MULBERRY (MORUS SPP.) FRUIT QUALITY IN AN EX-SITU COLLECTION

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Keywords*: germplasm characterization; phenotypic variability; fruit morphometric traits; soluble solids content; vapour pressure deficit.*

ABSTRACT

This paper reports a two-year (2025-2026) phenotypic characterization of an ex-situ mulberry (Morus spp.) germplasm collection at the Research Station for Fruit Growing Băneasa – Moara Domnească experimental base, Romania, comprising two complementary components. First, 17 accessions (one tree per accession, 20 fruits per tree) were evaluated in 2026 for fruit weight, height and diameter; one-way ANOVA indicated significant differences among the sampled accessions for all three traits, with Duncan's multiple range test separating several homogeneous subsets. Second, six of these accessions, sampled identically in 2025 and 2026, were compared between years for fruit weight, height, diameter, soluble solids (Brix) and pH. Fruit weight, height and diameter were significantly higher in 2026 than in 2025 (paired t-tests, $p < 0.05$), and mean Brix was also higher in 2026, although this was not consistent across all six accessions; pH did not differ significantly between years. These inter-annual differences coincided with a lower vapour pressure deficit (VPD) during the fruit-development window in 2026 than in 2025, together with markedly higher rainfall in the same period. Because the trial spanned only two growing seasons, this climatic association is presented as an interpretive hypothesis rather than a statistically tested cause-effect relationship. As each accession in the 2026 characterisation is represented by a single tree, the observed differences describe variability among the sampled accessions rather than a fully replicated test of genotypic effect.

INTRODUCTION

Mulberry (*Morus spp.)* is a fast-growing, multipurpose fruit tree valued for fresh consumption, processing, and its historical role in sericulture, and it is increasingly studied for the nutritional and antioxidant properties of its fruit (Uyak et al. 2024; Farahani et al. 2019; Hashemi & Khadivi 2020; Sümbül 2025; Firouz et al. 2026). Previous studies have documented substantial morphological and pomological diversity among mulberry species and genotypes (Hashemi & Khadivi 2020; Khadivi et al. 2024; Uyak et al. 2024), although most genotype-characterisation studies report results from a single growing season. Environmental and seasonal conditions can substantially modify mulberry fruit characteristics; for example, off-season production has been associated with significant changes in fruit size, weight and soluble solids

compared with normal-season production (Liu et al. 2022). Nevertheless, the influence of climatic variation from one year to another on fruit size and quality, relative to genotypic effects, remains insufficiently understood.

Climatic change is accelerating, and new situations are influencing the life on earth. Flash drought is one concern that widespread over 74 % of global regions (Yuan et al. 2023). These flash droughts pose a serious threat to vegetation ecosystems, water resources, and agriculture. Vapor pressure deficit (VPD) is a key climatological variable that relates to atmospheric dryness, vegetation productivity and terrestrial water and carbon fluxes (Ghausi et al. 2026). Also, VPD has recently been identified as a key climatic driver of fruit growth and quality in several species, acting mainly through its effect on plant water status during fruit cell expansion (Carella et al. 2023).

The present study aimed to characterize the phenotypic and biochemical variability of fruits among genotypes maintained in an ex-situ mulberry collection over two consecutive growing seasons (2025 and 2026). In addition, the climatic conditions of the two seasons were compared using VPD as an indicator of atmospheric evaporative demand, in order to examine its potential association with inter-annual variation in fruit weight and soluble solids content.

MATERIAL AND METHODS

Location

The study was carried out at the Research Station for Fruit Growing Băneasa – Experimental base Moara Domnească (SCDP) located at 44.503986 ^{o}N, 26.246220^{o}E. The experimental site is situated within Vlăsia Plain – a subunit of Romanian Plain. The soil is classified as loamy-clayey in texture, with a slightly acidic pH of 5.27(Olaru et al. 2025).

Climatic conditions

The area is characterised by a temperate continental climate, with warm summers and cold winters. The Sysagria 7049 automated weather station recorded air temperature, relative humidity, precipitation, solar radiation and VPD values at the experimental site throughout 2025 and 2026.

Planting material

The study was conducted in an ex-situ mulberry (Morus spp.) germplasm collection established in 2021 at a planting distance of 3 × 4 m, comprising 17 accessions grouped by fruit colour class (black, purple, marbled, dirty-white and white). Each accession is represented in the collection by a single tree, trained to either a vase or a palmette crown form depending on the accession. Because each accession corresponds to one tree, the 20 fruits sampled per accession in 2026 are technical replicates (fruit-level subsamples) from that single tree rather than independent biological replicates; consequently, differences among accessions reported below describe variability among the sampled trees of the collection and should be interpreted as a preliminary phenotypic characterisation rather than a fully replicated test of genotypic effect. Six accessions (MOR-102, MOR-104, MOR-105, MOR-106, MOR-206, MOR-210) were sampled under the same protocol in both 2025 and 2026, allowing direct within-accession comparisons across years.

Measurements

Fruit length and diameter were measured individually for each of the 20 fruits per tree using a digital and a manual caliper, and fruit weight with a digital balance.

Fruit were harvested at the visual ripeness stage characteristic of each accession's colour class. After individual measurement, the 20 fruits per tree were crushed together into a single juice sample; soluble solids (Brix) and pH were each read three times from this composite sample, following an approach comparable to Nayab et al. (2020), and averaged to give one Brix and one pH value per tree.

Statistical analysis

All statistical analyses were performed in SPSS v.26. For the six accessions common to both years, fruit weight, height, diameter, Brix and pH were compared between 2025 and 2026 using paired-samples t-tests, with normality of the paired differences confirmed by the Shapiro-Wilk test; the Wilcoxon signed-rank test was run in parallel as a distribution-free cross-check given the small sample size (n = 6), and effect sizes were expressed as Cohen's d for paired samples. As all five traits met the normality assumption ($p > 0.05$), the paired t-test was retained as the primary test. For the 17 accessions sampled in 2026, fruit weight, height and diameter (20 fruits per accession) were compared by one-way ANOVA, with Accession as the fixed factor, followed by Duncan's multiple range test ($\alpha = 0.05$) for post-hoc comparison of accession means; because each accession is represented by a single tree in the collection (see Planting material), accession and tree are confounded in this design, and the ANOVA results should be interpreted as describing variability among the sampled accessions rather than as a fully replicated test of genotypic effect independent of individual-tree or micro-site variation. Pearson correlations among fruit traits were computed at the accession level.

RESULTS AND DISCUSSIONS

The weather station data provided monthly mean values for temperature and precipitation, as shown in Figures 1 and 2.

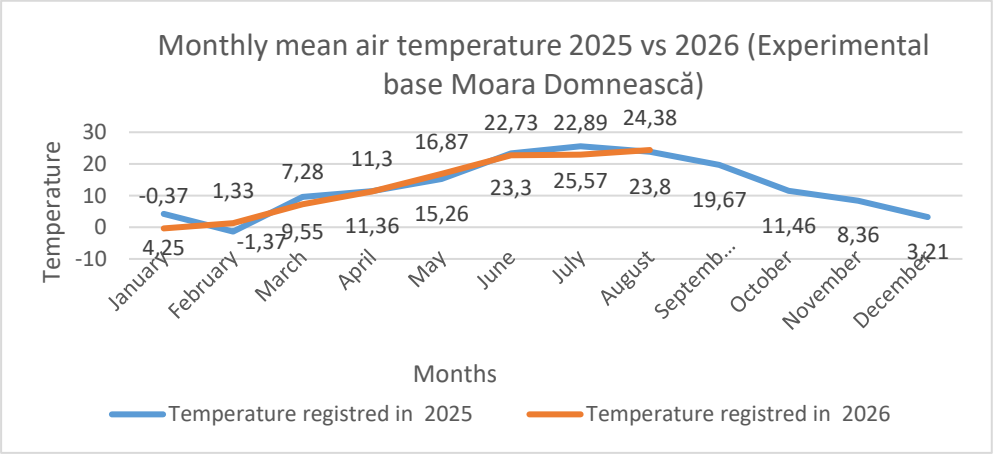


Figure 1. Monthly air temperature registered by Sysagria meteo station

More interesting differences appear in precipitation, with rainy periods occurring during fruit development as shown in Figure 2.

Vapour pressure deficit (VPD) was calculated using the Tetens equation (Murray, 1967): (e_s(T) = 0.6108 \times \exp[17.27T/(T+237.3)]) [kPa], followed by (VPD = e_s \times (1 - RH/100)). Because meteorological data for 2025 were available only

as monthly aggregates, the year-to-year comparison was standardized at the monthly scale. Accordingly, we calculated monthly VPD values for both 2025 and 2026, and summarized precipitation totals over the same time basis to ensure direct comparability between years.

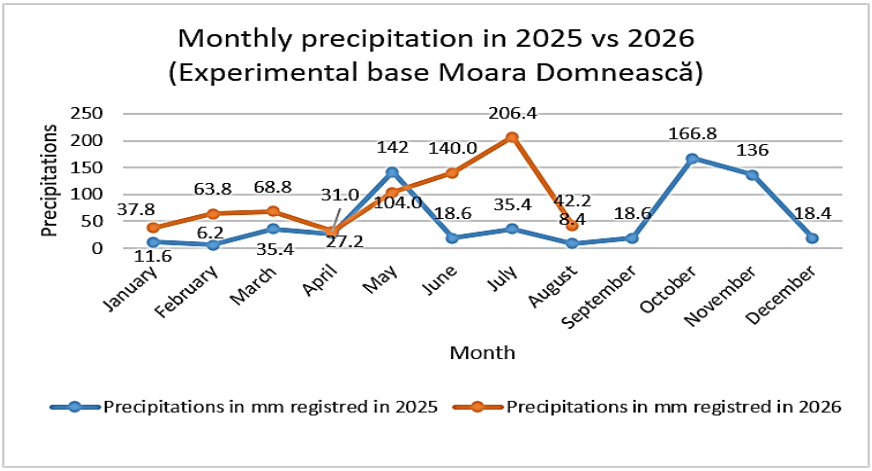
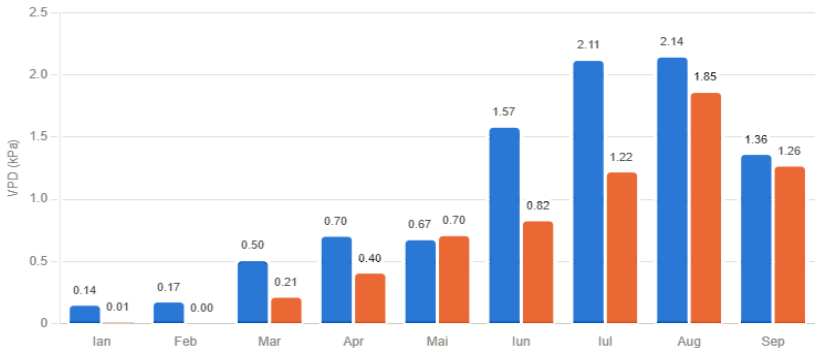


Figure 2. Monthly precipitation in 2025 and 2026 at the Moara Domneasca experimental station, with the mulberry growing season (April-September) and dormant period highlighted.

For the period of active fruit development, VPD and precipitation were additionally summarized for the fruit-set-to-harvest interval of each year (Table 1), corresponding to the period during which the sampled fruits developed. We used daily VPD values available for 2026 only to provide a higher-resolution description of within-season atmospheric conditions and did not use them for direct comparison with 2025. Figure 3 presents the calculated VPD values and corresponding precipitation data.



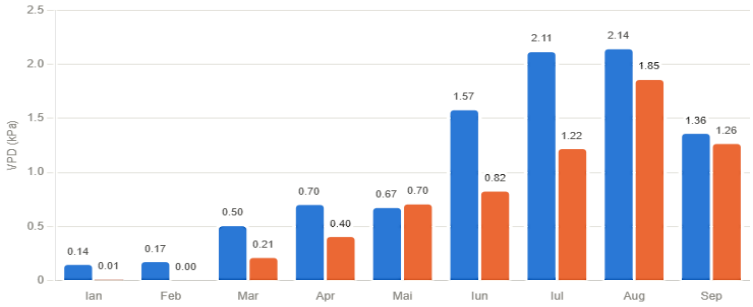


Figure 3. Monthly mean VPD in 2025 and 2026 at the Moara Domneasca Experimental Base

For all the genotypes were noted the phenological stages and are shown in Table 1.

Table 1.

Phenological stages noted for genotypes in 2025-2026

Phenological stage	**2025**	**2026**
Bud swelling	10.04	15.04
Early inflorescence, closed	24.04	24.04
Open flowering	~29.04	~05.05
Fruit set	~05.05	≤15.05
Harvest	12.06	09–11.06
Fruit set-to-harvest duration	~38 days	~27 days

Fruit weight in 2026 ranged from 1.89 g (MOR-106) to 3.55 g (MOR-306) across the 17 genotypes (Table 2), with coefficients of variation between 16 % and 34 %, comparable to the ranges reported to other mulberry germplasm collections, for example 1.8 g to 5.84 g in Turkish black mulberry accessions (Uyak et al.2024) or 2.00-5.13 g in Morus alba genotypes from northeastern Turkey, where the first three principal components explained 54.05% of total phenotypic variation (Sümbül 2025).

Table2.

Mean fruit weight, height and diameter of the 17 mulberry genotypes evaluated in 2026

Accession	**Weight (g)**	**CV W (%)**	**Height (mm)**	**CV H (%)**	**Diameter (mm)**	**CV D (%)**
MOR-102	1.99	31.6	19.85	10.9	12.61	9.6
MOR-104	1.96	29.3	20.24	12.7	12.13	8.6
MOR-105	2.28	26.9	24.11	16.5	14.87	31.6
MOR-106	1.89	19.5	20.55	9.0	12.53	8.6
MOR-203	2.63	33.3	25.10	13.5	13.34	8.4
MOR-204	2.17	33.5	20.60	12.3	12.86	15.8
MOR-205	2.42	22.4	22.06	11.1	12.62	8.4
MOR-206	2.30	18.5	20.26	10.3	12.86	10.8

MOR-208	3.06	25.9	22.87	14.9	14.58	6.8
MOR-210	2.53	19.7	21.15	8.6	13.83	11.7
MOR-306	3.55	17.8	25.10	13.7	13.36	8.3
MOR-306B	3.49	23.5	24.07	10.8	15.41	15.9
MOR-308	2.42	32.6	21.84	12.1	13.02	12.0
MOR-309	2.11	31.6	22.48	18.1	12.34	12.2
MOR-310	3.30	23.2	27.03	11.9	14.18	6.6
MOR-407	3.27	25.4	24.92	14.8	14.00	7.9
MOR-412	2.76	16.3	23.24	16.0	13.50	6.3

One-way ANOVA indicated highly significant differences among the sampled accessions for all three morphometric traits: weight $F(16,323) = 13.72$, $p < 0.001$; height $F(16,323) = 10.00$, $p < 0.001$; diameter $F(16,323) = 5.75$, $p < 0.001$. As each accession is represented by a single tree in the collection (see Planting material), these results describe substantial phenotypic variability among the sampled accessions rather than a fully replicated test of genotypic effect independent of individual-tree variation; they are reported here as a preliminary characterisation to guide future multi-tree replication.

Duncan's multiple range test (Figure 5) separated MOR-306 and MOR-306B, the heaviest genotypes, from most other accessions, while MOR-106, MOR-104 and MOR-102 formed the lightest, overlapping subset; a broadly similar pattern was observed for height and diameter, with MOR-310 tallest and MOR-306B widest.

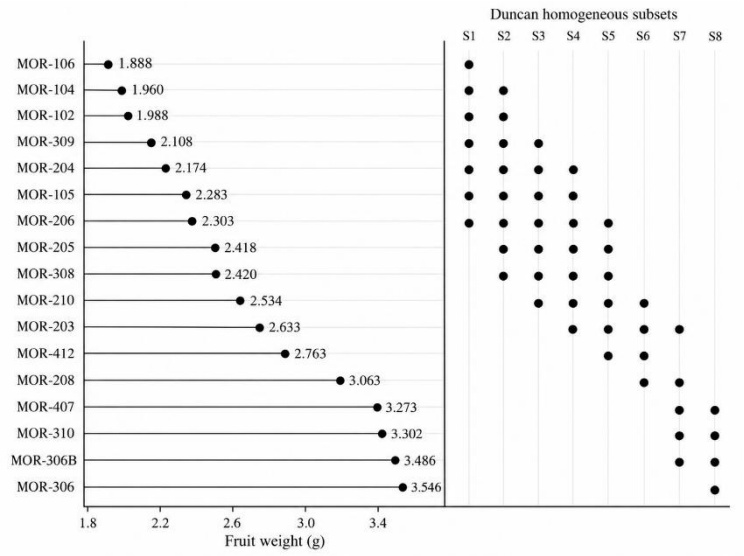


Figure 5. Duncan's homogeneous subsets for weight

All six genotypes sampled in both years showed markedly higher fruit weight in 2026 than in 2025 (Table 3), together with higher soluble solids content (Brix) in five of the six genotypes. This combination indicates that the higher fruit mass in 2026 was not accompanied by a proportional dilution of soluble solids.

Table 3

Fruit weight, size and quality traits of six mulberry genotypes common to the 2025 and 2026 growing seasons

Code	W 2025 (g)	W 2026 (g)	H 2025 (mm)	H 2026 (mm)	D 2025 (mm)	D 2026 (mm)	Brix 2025	Brix 2026	pH 2025	pH 2026
MOR-102	1.02	1.99	16.67	19.85	10.57	12.61	14.3	20.8	5.96	5.72
MOR-104	0.96	1.96	14.91	20.24	10.22	12.13	14.3	20.0	5.85	5.85
MOR-105	0.95	2.28	14.74	24.11	10.68	14.87	14.2	17.4	5.87	5.95
MOR-106	1.24	1.89	17.91	20.55	11.00	12.53	17.3	19.0	6.16	5.92
MOR-206	1.49	2.30	19.62	20.26	11.99	12.86	16.1	15.9	4.86	5.21
MOR-210	1.67	2.53	19.78	21.15	12.26	13.83	11.8	16.2	5.44	5.23

A simultaneous increase in fruit weight and soluble solids is biologically plausible in mulberry, as both traits may increase during fruit maturation (Nayab et al. 2020)

Despite the shorter estimated fruit-development period in 2026, fruit mass was greater, suggesting that development duration alone cannot explain the observed inter-annual differences. Differences in atmospheric and/or soil water availability represent plausible contributing factors, a hypothesis that warrants confirmation with continuous soil and plant water-status measurements in future seasons.

Paired-samples t-tests confirmed that the increase in fruit weight, height and Brix from 2025 to 2026 was statistically significant despite the small sample size (n = 6), with large to very large effect sizes (Table 4).

Table 4.

Paired-samples comparison (2025 vs 2026) for the six common genotypes

Trait	Mean 2025	Mean 2026	Paired t-test (p)	Wilcoxon (p)	Cohen's d
Fruit weight (g)	1.22	2.16	0.0002	0.031	4.08
Height (mm)	17.27	21.03	0.035	0.031	1.18
Diameter (mm)	11.12	13.14	0.0074	0.031	1.77
Brix (%)	14.7	18.2	0.018	0.063	1.41
pH	5.69	5.65	0.671	0.750	-0.18

No significant difference was found for pH (p= 0.671). For Brix, the paired t-test was significant (p = 0.018) but the Wilcoxon signed-rank test was not (p = 0.063); given this discrepancy and the small sample size (n = 6), the Brix difference between years is reported with caution rather than as a fully robust result. The magnitude of the weight increase suggests that genotypes with smaller 2025 fruit responded proportionally more to the favorable 2026 conditions. This paired inter-annual design (Figure 5), absent from the single-season studies cited above, is a distinct methodological contribution of the present study.

CONCLUSIONS

The 2026 growing season produced significantly heavier, taller and wider mulberry fruit than 2025 across all six directly compared accessions, and mean Brix was also higher in 2026, although this increase was not consistent across all six

accessions. These inter-annual differences coincided with a lower vapour pressure deficit during the fruit-development window in 2026 than in 2025, together with markedly higher rainfall over the same period; because the study spans only two growing seasons, this climatic association is presented as an interpretive hypothesis for future multi-year testing rather than a demonstrated cause-effect relationship. Flowering was delayed by approximately seven days in 2026 relative to 2025, and the fruit-development window was correspondingly shorter, yet fruit size was still greater, indicating that development duration alone does not explain the observed inter-annual difference. Among the 17 accessions evaluated in 2026, MOR-306 produced the heaviest fruit (3.55 g) and MOR-106 the lightest (1.89 g); as each accession is represented by a single tree in the collection, these results indicate substantial phenotypic variability among the sampled accessions, comparable to other mulberry germplasm collections reported in the literature, and should be regarded as a preliminary characterisation pending multi-tree replication. These findings support the value of multi-year phenotypic evaluation, combined with climatic monitoring, for the characterisation of mulberry genetic resources.

ACKNOWLEDGMENT

This research work was carried out with the support of the Thematic Plan on the Implementation of the 'A.S.A.S. Strategy on Research—Development—Innovation in Fruit Growing' for the period 2021–2027, research topic: 1.1.4. Dissemination of scientific results through technology transfer actions, using the infrastructure of the Research Station for Fruit Growing Băneasa (SCDP Băneasa). The authors thank all team members, the administrative staff, and the director for their support in conducting this research.

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