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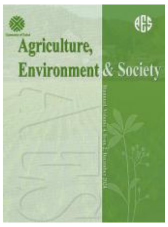

# Assessment of phenotypic diversity among mulberry (*Morus spp.*) genotypes in South Khorasan province

Hadi Zeraatgar *[a], Azam Gideski [b], Maryam Samaie [b], Abbas Mirjalili [c]

[a] Crop and Horticultural Research Department, South Khorasan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Birjand, Iran
[b] Expert of Crop and Horticultural Research Department, South Khorasan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Birjand, Iran
[c] Researcher of Agricultural Research, Education and Extension Organization (AREEO), Emam Khomeini Higher Education Center, Tehran, Iran

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## ABSTRACT

Mulberry (*Morus spp.*) is an economically important plant in Iran. This research was conducted to identify and collect mulberry germplasm to preserve genetic resources over 3 years in South Khorasan province. Accordingly, during different growth stages, visits were made to mulberry cultivation areas to record important characteristics of the genotypes based on the provided descriptors. In total, 31 different mulberry genotypes from 3 species (*M. alba, M. nigra, M. macroura*) from seven regions were identified, and morphological traits were recorded in accordance with the guidelines of UPOV. In quantitative traits, the strongest positive correlations were obtained in shoot number, shoot length, lateral shoot number, leaf blade width, leaf blade length, peduncle length, petiole length, mature fruit length, fruit weight, mature fruit length/width, and sweetness (Brix). In qualitative traits, the strongest positive correlations were obtained in leaf blade texture, leaf blade thickness, leaf blade glossiness of the upper side, and the shape of the apex leaf blade, and the strongest negative correlations were obtained in mature fruit color, shape of the apex leaf blade, and leaf blade shape. The lowest fruit sweetness (Brix) value (15.3) was recorded in genotype M22, whereas the highest value (36.5) was observed in genotype M5. The mean fruit sweetness(Brix) across all genotypes was 24.05. Furthermore, the highest fruit weight (4.26 g) was obtained from genotype M16, while the lowest (1.08 g) was recorded in genotype M2. The average fruit weight among the genotypes was 2.69 g. Cluster analysis using the "Ward" method for farm data classified the studied genotypes into five groups. Apparently, cluster analysis was unable to fully differentiate among the three species. Only the genotypes belonging to *M. nigra* were partially grouped into a single cluster, likely due to the overlap of some of their phenotypic traits. This study revealed high morphological diversity in the mulberry genotypes dispersed in South Khorasan province. 31 distinct selected genotypes were transferred to the collection for the preservation of genetic resources and their use in breeding programs. This study had certain limitations, including potential environmental interference and the lack of molecular validation.

## Highlights

- Most genotypes were spreading but M5/M10 and M31 were semi-upright; M22/M24/M32 and M36 showed high vegetative growth.
- Leaf blade length in M33/M34 genotypes was "long to very long" and in M1/M14 and M29 was very narrow.
- M5/M8 genotypes had cylindrical fruits; M5/M29/M34 had high sweetness, while M6/M22 had low sweetness.
- Strong positive correlation: leaf (thickness with texture, glossiness with apex shape), shoot (thickness with color).
- Strong negative correlation: leaf apex shape with fruit color, leaf shape with fruit color.

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* Corresponding author.
E-mail address: zeraatgarh@yahoo.com

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## 1. Introduction

Mulberry (*Morus spp*.) of the family Moraceae is an economically important plant being used for sericulture, fruit consumption, wood, landscape design, and medicinal purposes (Imran et al., 2010; Rohela et al., 2020). Given that the silk trade has been widespread around the world for centuries, and that this plant has also been valued for its fresh fruit and ornamental use in green spaces, the wide distribution of its germplasm across many countries is not surprising. Today, the plant has a broad distribution across Asia, Europe, Africa, and the Americas. It is believed that most mulberry cultivars originated from regions of China and Japan, particularly in the foothills of the Himalayan mountain range (Singhal et al., 2010). Assessing genetic diversity in plant populations has potential applications in the fields of evolution, breeding, germplasm biology, and conservation. Genetic diversity within populations reflects the intensity of interactions between multiple evolutionary processes such as genetic drift, mutagenesis, and natural selection. Identification and preservation of plant germplasm is essential for distinguishing the genotypes of individuals within a species and determining the extent of diversity among populations of each species.

In China, which is a leading country in the field of silkworm breeding, more than a thousand mulberry cultivars are available for sericulture. These cultivars originate from four main species, namely *M. alba*, *M. multicaulis*, *M. bombycis*, *and M. atropurpurea*. It is believed that the most common species in the world *is M. alba* or *M. indica*. Intense natural selection through open pollination, hybridization, and mutation has led to the creation of thousands of mulberry cultivars, including many polyploids (Singhal et al., 2010). In Brazil, there are about 90 genotypes of the species *M. alba* (De Almeida and Fonseca, 2000).

Vijayan, Saratchandra, et al. (2011) believe that taxonomically, the genus Morus has 68 species, out of which *M. alba, M. indica, M. nigra, M. latifolia,* and *M. multicaulis* are cultivated for silkworm rearing, *M. rubra and M. nigra* for fruit production, and *M. laevigata and M. serrata* for timber production. They have listed the highest number of germplasm in countries such as China (1860 populations), Japan (1375 populations), India (1120 populations), Korea (614 populations), and Vietnam, respectively. Unfortunately, no documented report was found indicating the introduction of mulberry cultivars in Iran. Various types of data are used to analyze the genetic diversity in the collections, of which morphological and horticultural traits are more common for distinguishing the accessions (Höfer et al., 2012). Bootprom et al. (2014) also assessed genetic diversity using horticultural traits (fruit) and total soluble solids in 21 white mulberry cultivars.

Iran is recognized as one of the centers of diversity for various mulberry species (*Morus* spp.), and this crop is cultivated and utilized in many regions of the country (Farrokhi Toolir & Mirjalili, 2023).

According to statistics published by the Ministry of Agriculture Jihad (2025), the total mulberry cultivation area in Iran is approximately 3,652 hectares, with an annual production of 15,689 tons, underscoring the significance of this crop in the country's horticulture sector. The existence of two species of this genus, including white (*Morus alba*) and black (*Morus nigra*) mulberries, has been reported in some parts of Iran (Azizian et al., 2000). The study of morphological traits on mulberry genotypes from 10 provinces of the country showed that leaf margin, petiole length, and inflorescence diameter had a higher variation than the other variables. A positively significant correlation between harvest time and inflorescence shape was observed (Fakhraei Lahiji et al., 2016). Other studies on Iranian mulberry populations revealed that the Gorgan and Shahrood populations had the highest coefficient of variations for morphological, physiological, and biochemical traits. Both fruit size and fruit taste were positively correlated with foliage–related traits (Ebrahimi et al., 2021).

Among the different regions, South Khorasan province is considered one of the most suitable areas for the propagation of this plant species. Characterized by an arid and semi-arid climate, low and irregular rainfall, and various soil limitations, this province provides favorable conditions for the development of diverse plant species adapted to environmental stresses, such as mulberry (Golmohammadi, 2012).

Based on a report by the Ministry of Agriculture Jihad (2025), the mulberry cultivation area in South Khorasan province stands at 178 hectares, with a production of 547 tons. These statistics highlight the significant role of this province in mulberry production within the arid regions of Iran and demonstrate its potential for the sustainable development of this crop under conditions of limited water resources.

Local data show that despite all kinds of environmental stresses, a good yield of native mulberries grown in the climate of South Khorasan province exhibits relatively suitable resistance to high temperature during the growing seasons. This fact justifies their potential exploitation in breeding programs. However, less information is available about the number of varieties and characteristics of these genotypes. Therefore, understanding the diversity among different mulberry genotypes for selecting more parental lines to improve the agronomic traits of existing varieties and identifying superior genotypes is a fundamental step in mulberry breeding programs in this province.

This study does not clearly identify the research gap. Identifying and accessing the primary origins and sources of mulberry and studying the characteristics and features of its different genotypes in Iran can pave the way for achieving a population with more suitable morphological traits and active compounds. Also, studying the genetic structure of mulberry populations, identifying the genetic potential of existing populations in terms of several morphological traits, and identifying genotypes to support complementary breeding plans for this ancient plant are among the goals of this research.

## 2. Materials And Methods

A total of 31 samples of mulberry genotypes were collected from 7 regions located in different parts of South Khorasan province, Iran, in 2022-2024. During 2022–

2024, at different growth stages (before flower buds open, flowering time, active growth period of the tree, and fruit harvest time), areas susceptible to mulberry cultivation in South Khorasan province were visited, and important morphotype characteristics were marked and recorded based on descriptors**.** Since genotype identification and selection were performed based on phenotypic differences, at least one representative tree was selected for each genotype — one that best exhibited all the observable phenotypic distinctions characteristic of that genotype. Subsequently, trait recording and documentation were conducted on this selected tree, following the appropriate characteristic categories and procedures specified in the UPOV Test Guidelines, for example: All observations on leaf characters shall be measured using fully expanded mature leaves in the middle portion (15th leaf) on the longest shoot. All observations on shoot, leaf, and bud characteristics shall be made on the upper (e.g., stipule nature), lower (e.g., mature shoot color and shoot thickness in cm), and 1/3rd portion of the longest shoot. Observations on reproductive characteristics shall be taken during the natural flowering season and fruit ripening period. At least 10 samples shall be used to measure leaf and fruit traits. For the assessment of colour characteristics, the latest Royal Horticultural Society (RHS) colour chart shall be used.

Samples of leaves were picked during the summer. The fruits were harvested at horticultural maturity of respective genotypes (starting from early June to mid-August). Samples were collected according to Ebrahimi et al. (2021). All quantitative and qualitative traits of these morphotypes were evaluated and recorded according to the conduct of the test for distinctiveness, uniformity, and stability for mulberry genotypes (IPGRI, 2000; UPOV, 2020)**.** In total, 30 morphological traits, including 16 qualitative and 14 quantitative variables, were characterized. The following traits have been used as a detailed practical guide to identify characteristics that can be used to describe and differentiate mulberry genotypes. Qualitative traits included Tree vigor, Tree Growth Habit, Shoot Thickness, Shoot Color, Leaf Blade Thickness, Shape of Apex Leaf Blade, Shape of Base Leaf Blade, Leaf Blade Shape, Leaf Blade Depth of Sinus, Incisions of Margin, Leaf Blade Texture, Leaf Blade Blistering of Surface, Leaf Blade Color of Upperside, Leaf Blade Glossiness of Upper side, Fruit Shape, Mature Fruit Color. These variables were determined and classified visually. Quantitative traits included Leaf Shoot Number, Lateral Shoot Number, Shoot Length, Internode Length, Leaf Blade Length, Leaf Blade Width, Leaf Blade Length/Width, Petiole Length, Mature Fruit Length, Mature Fruit Width, Mature Fruit length/width, Fruit Weight, Peduncle Length, and Sweetness (Brix) were also examined (Table 1).

**Table 1. List of quantitative and qualitative phenotypic traits of mulberry and their abbreviations**

| Quantitative phenotypic traits | | Qualitative phenotypic traits | |
|---|---|---|---|
| Shoot Number | SN | Tree vigor | TV |
| Lateral Shoot Number | LSN | Tree Growth Habit | TGH |
| Shoot Length | SL | Shoot Thickness | ST |
| Internode Length | IL | Shoot Color | SC |
| Leaf Blade Length | LL | Leaf Blade Thickness | LBT |
| Leaf Blade Width | LW | Shape of Apex Leaf Blade | SALB |
| Leaf Blade Length/Width | LL/LW | Shape of Base Leaf Blade | SBLB |
| Petiole Length | PL | Leaf Blade Shape | LBS |
| Mature Fruit Length | FL | Leaf Blade Depth of Sinus | LBDS |
| Mature Fruit Width | FW | Incisions of Margin | IM |
| Mature Fruit Length/Width | FL/FW | Leaf Blade Texture | LT |
| Fruit Weight | FWe | Leaf Blade Blistering of Surface | LBBS |
| Peduncle Length | PDL | Leaf Blade Color of Upperside | LBC |
| Sweetness(Brix) | S | Leaf Blade Glossiness of Upper Side | LBGU |
| | | Fruit Shape | FS |
| | | Mature Fruit Color | FC |

All measurable data on inflorescences, internodes, and leaves were evaluated by a ruler and a caliper. The weights of fresh and dry fruits and leaves were determined by precision scales.

Sweetness (Brix) was determined using a hand-held refractometer (Atago, Tokyo, Japan) to measure total soluble solids. Principal Component Analysis (PCA) was performed on 14 quantitative traits to explore the patterns of variation and identify the most discriminating traits among the 31 mulberry genotypes. Before analysis, all variables were standardized to have a mean of zero and unit variance to eliminate the effect of different measurement scales. The PCA was conducted using the correlation matrix, and components with eigenvalues greater than one (Kaiser's criterion) were retained. Varimax rotation was applied to enhance the interpretability of the factor loadings. The adequacy of sampling was verified by the Kaiser-Meyer-Olkin (KMO) measure and Bartlett's test of sphericity. All statistical analyses were performed using SPSS Statistics software (version 26). To classify the mulberry genotypes and reveal the phenotypic relationships among them, hierarchical cluster analysis (HCA) was performed. Ward's method was applied as the linkage rule, using squared Euclidean distance as the dissimilarity measure. Ward's method was selected because it minimizes the within-cluster variance and tends to produce distinct and balanced clusters, which is particularly effective for morphological data in plant genetic diversity studies. The optimal number of clusters was determined by cutting the dendrogram at the appropriate distance level to maximize inter-cluster heterogeneity.

## 3. Results And Discussion

Since the identification and data recording of mulberry genotypes were conducted across regions with sometimes differing topographic and climatic conditions, environmental influences could not be controlled. Consequently, the expression of traits is affected by both environmental factors and genotype**.** The highest amount of mulberry (fresh and dried) had been in the regions of Ferdows, Sarayan, and Boshroyeh, and the highest amount of Sericulture Plantations (noghan) had been in the regions of Boshroyeh and Ferdows in South Khorasan province. In general, considering the status of cultivation and exploitation of this important fruit, it can be said that the regions of Boshroyeh, Ferdows, and Sarayan have been the best regions for cultivating and growing mulberries in this province.

Over the course of the experimental years, a total of 31 distinct morphotypes with unique and desirable traits were identified and coded in South Khorasan province (Table 2), and their traits were evaluated and recorded based on Guidelines UPOV (International Union for the Protection of New Varieties of Plants) and IPGRI (International Plant Genetic Resources Institute ) for Mulberry Genotypes evaluated and recorded. (Table S1 in Appendix Section)

**Table 2. Origin and location of 31 studied mulberry genotypes in South Khorasan province**

| No | Code | Type | Genus/Species | Elevation (m) | Latitude (N) | Longitude (E) | Sampling location | Region |
|---|---|---|---|---|---|---|---|---|
| 1 | M1 | Black | M. alba | 1643 | 31° 46′ 180″ | 060° 00′ 694″ | Hamand | Nehbandan |
| 2 | M2 | White | M. alba | 1643 | 31° 46′ 180″ | 060° 00′ 694″ | Hamand | Nehbandan |
| 3 | M3 | White | M. alba | 1643 | 31° 46′ 180″ | 060° 00′ 694″ | Hamand | Nehbandan |
| 4 | M4 | Black | M. nigra | 1643 | 31° 46′ 180″ | 060° 00′ 694″ | Hamand | Nehbandan |
| 5 | M5 | White | M.macroura | 1668 | 31° 46′ 260″ | 060° 00′ 690″ | Hamand | Nehbandan |
| 6 | M6 | Black | M. alba | 1674 | 31° 46′ 232″ | 060° 00′ 596″ | Hamand | Nehbandan |
| 7 | M7 | White | M. alba | 1674 | 31° 46′ 232″ | 060° 00′ 596″ | Hamand | Nehbandan |
| 8 | M8 | Black | M.macroura | 1229 | 31° 33′ 630″ | 060° 06′ 256″ | Khan Sharaf | Nehbandan |
| 9 | M9 | White | M. alba | 1338 | 32° 49′ 817″ | 058° 55′ 476″ | Masoomabad | Khosf |
| 10 | M10 | White | M. alba | 1338 | 32° 49′ 828″ | 058° 55′ 471″ | Masoomabad | Khosf |
| 11 | M14 | White | M. alba | 1642 | 33° 01′ 214″ | 058° 41′ 478″ | Ark | Khosf |
| 12 | M15 | White | M. alba | 1641 | 33° 01′ 230″ | 058° 41′ 480″ | Ark | Khosf |
| 13 | M16 | Black | M. nigra | 1623 | 33° 01′ 210″ | 058° 41′ 503″ | Ark | Khosf |
| 14 | M18 | Black | M. nigra | 1624 | 33° 01′ 212″ | 058° 41′ 497″ | Ark | Khosf |
| 15 | M19 | Black | M. alba | 1391 | 33° 01′ 294″ | 058° 41′ 363″ | Shahabi Qanat | Qaen |
| 16 | M20 | White | M. alba | 1445 | 33° 43′ 499″ | 059° 12′ 625″ | Dastjerd Qanat | Qaen |
| 17 | M21 | Black | M. nigra | 1181 | 33° 49′ 072″ | 057° 15′ 897″ | Raqeh | Boshruyeh |
| 18 | M22 | Black | M. nigra | 1181 | 33° 49′ 072″ | 057° 15′ 897″ | Raqeh | Boshruyeh |
| 19 | M23 | Black | M. alba | 1181 | 33° 49′ 068″ | 057° 15′ 900″ | Raqeh | Boshruyeh |
| 20 | M24 | White | M. alba | 1190 | 33° 52′ 330″ | 057° 13′ 830″ | Jozardan | Boshruyeh |
| 21 | M26 | White | M. alba | 1401 | 33° 51′ 398″ | 057° 25′ 804″ | Sarayan | Sarayan |
| 22 | M27 | Black | M. nigra | 1388 | 33° 51′ 281″ | 058° 29′ 147″ | Sarayan | Sarayan |
| 23 | M28 | White | M. alba | 1425 | 33° 51′ 167″ | 058° 30′ 311″ | Sarayan | Sarayan |
| 24 | M29 | White | M. alba | 1863 | 34° 00′ 967″ | 058° 28′ 304″ | Masabi | Sarayan |
| 25 | M30 | White | M. alba | 1863 | 34° 00′ 955″ | 058° 28′ 326″ | Masabi | Sarayan |
| 26 | M31 | White | M. alba | 912 | 33° 48′ 142″ | 059° 58′ 342″ | Mirabad | Zirkooh |
| 27 | M32 | Black | M. alba | 912 | 33° 48′ 142″ | 059° 58′ 342″ | Mirabad | Zirkooh |
| 28 | M33 | White | M. alba | 912 | 33° 48′ 142″ | 059° 58′ 342″ | Mirabad | Zirkooh |
| 29 | M34 | White | M. alba | 912 | 33° 48′ 142″ | 059° 58′ 342″ | Mirabad | Zirkooh |
| 30 | M35 | White | M. alba | 2032 | 32° 38′ 646″ | 059° 30′ 841″ | Shavakand | Sarbisheh |
| 31 | M36 | White | M. alba | 2032 | 32° 38′ 646″ | 059° 30′ 841″ | Shavakand | Sarbisheh |

### 3.1. Descriptive statistics

**Tree/growth Habit** (Upright, Semi-upright, Spreadding, Drooping, Weeping)**:** Most genotypes were “Spreading” and “Drooping”, and only the genotypes M5, M10, and M31 were “Semi-upright”.

**Shoot/number** (Few, Few to medium, Medium, Medium to many, Many)**:** Most genotypes were “Medium”. Genotypes M22, M24, M32, and M36 were “Many” and only genotypes M21 and M35 were “Few”**.**

**Shoot/number of lateral shoots** (Absent or few, Medium, Many)**:** Most genotypes were “Medium”. Genotypes M22, M24, M32 and M36 were “Many” and Genotypes M21, M23, M28, M29 and M35 were “Absent or few”.

**Shoot/length** (Short, Short to Medium, Medium, Medium to Long, Long)**:** Shoot length in most genotypes varied from “Short” to “Medium”.

**Shoot/thickness** (Thin, Medium, Thick)**:** Most genotypes were “Medium” and only genotypes M6, M8, and M14 were “Small” in shoot thickness.

**Shoot/color** (Light grey, Greyish brown, Greenish brown, Yellowish brown, Reddish brown, Medium brown, Dark brown)**:** Most genotypes were varied from “Greyish brown” to “Yellowish brown” and only genotype M8 was “Light grey”.

**Shoot/length of internode** (Short, Medium, Long)**:** Most genotypes varied from “Short” to “Medium” and only genotype M2 was “Long”.

**Leaf blade/length** (Very short, Very short to short, Short, Short to medium, Medium, Medium to long, Long, Long to very long, Very long)**:** genotype M14 was “Very short to short”, and genotypes M33 and M34 were “Long to very long”, and the other genotypes varied between these groups.

**Leaf blade/width** (Very narrow, Very narrow to narrow, Narrow, Narrow to medium, Medium, Medium to broad, Broad, Broad to very broad, Very broad)**:** Only genotypes M1, M14, and M29 were "Very narrow" and genotypes M22, M33, and M34 were" broad" and the other genotypes varied between these groups..

**Leaf blade/(length/width)** (Low, Medium, High)**:** Most genotypes were "Medium". genotypes M10, M15 and M31 were "Low" and genotypes M1, M6, M8 and M29 were "High" .

**Leaf blade/thickness** (Thin, Medium, Thick)**:** Most genotypes were "Medium". Genotypes M2, M9, M14 and M29 were "Thin" , and genotypes M5, M7, M8, M21, M22 and M33 were "Thick".

**Leaf blade/shape of apex** (Caudate, Acuminate, Acute, Obtuse, Retuse)**:** Most genotypes were between "Acuminate" and "Obtuse".

**Leaf blade/shape of base** (Cuneate, Truncate, Retuse, Cordate)**:** Most genotypes were "Cordate" and a few were "Truncate" and "Retuse".

**Leaf blade/shape** (Triangular, Cordate, Ovate, Circular, Pentagonal)**:** Most genotypes varied from "Triangular" to "Ovate". Only genotype M4 was "Circular", and two genotypes, M2 and M9, were "Pentagonal".

**Leaf blade/symmetry** (Absent, Present)**:** The leaves of all genotypes were symmetrical.

**Leaf blade/depth of sinus** (Absent or very shallow, Medium, Deep)**:** The depth of sinus of the leaf blade in all of the genotypes was "Absent or very shallow" and only genotype M2 was "Deep".

**Leaf blade/incisions of margin** (Repand, Crenate, Dentate, Serrulate, Biserrate, Serrate, Aristate)**:** The shape of the margin of the leaf blade in most genotypes was "Crenate". Genotypes M1 and M7 were "Dentate", genotypes M3 and M4 were "Aristate", genotypes M6 and M8 were "Serrulate", genotypes M29 and M30 were "Biserrate", genotypes M16 and M18 were "Serrate", and only genotype M5 was "Repand".

**Leaf blade/texture** (Smooth, Medium, Rough)**:** In most genotypes, leaf texture was "Medium". Genotypes M9 and M29 were "Smooth", and the remaining genotypes were "Rough".

**Leaf blade/blistering of surface** (Absent or weak, Medium, Strong)**:** Most genotypes were"Absent or Weak". Genotypes M4, M16, and M33 were "Medium", and genotype M6 was "Strong".

**Leaf blade/color of upper** (Yellow, Yellowish green, Light green, Medium green, Dark green)**:** The leaf surface color of most genotypes varied from "Light green" to "Dark green", and no genotype was "Yellowish green".

**Leaf blade/glossiness of upper side** (Absent or very weak, Weak, Medium, Strong)**:** The glossiness of the upper leaf surface varied between "Medium" and "Strong" in most genotypes.

**Leaf/presence of stipules** (Absent, Present)**:** In all genotypes studied, the leaves lacked stipules ( "Absent").

**Petiole/length** (Absent or very short, Very short to short, Short, Short to medium, Medium, Medium to long, Long, Long to very long, Very long)**:** Genotypes M4, M16, M21, and M22 were "Short" and only genotypes M1 and M30 were "Very long". The remaining genotypes were between these groups.

**Infructescence/length** (short, short to medium, medium, medium to long, long)**:** The Infructescence length of most genotypes varied from "Short" to "Medium", and only genotypes M5 and M8 were "Long", and genotype M15 was "Medium to long".

**Infructescence/width** (Narrow, Medium, Broad)**:** Most genotypes varied from "Medium" to "Broad".

**Infructescence/(ratio length/width)** (Low, Medium, High)**:** Most genotypes varied from "Low" to "Medium" in this trait, and genotypes M4, M5, and M8 were 'High".

**Infructescence/weight** (Low, Medium, High)**:** Genotypes M10, M16, and M36 had the highest, and genotypes M2, M21, and M34 had the lowest Infructescence weight, and the remaining genotypes were between these two groups**.**

**Infructescence/shape** (Globose, Ellipsoid, Cylindric)**:** Most genotypes were "Ellipsoid". Genotypes M10 and M21 were "Globose" and genotypes M5, M8, and M34 were "Cylindrical" shape.

**Infructescence/color** (White, Yellowish white, Pink, Reddish purple, Light purple, Dark purple, Black purple)**:** The Infructescence color in most genotypes was "Yellowish white". Only 3 genotypes, M7, M14, and M24, were "White". Genotypes M1, M6, M19, M23, and M32 were "Black" or "Dark purple". Genotypes M20 and M29 were "Light purple". Genotypes M4, M16, M18, M21, M22 and M27 were "Reddish purple"( M. nigra).

**Infructescence/length of peduncle** (Short, Short to medium, Medium, Medium to long, Long)**:** Most genotypes were "Short" to "Medium" in length of peduncle. The lowest length of peduncle was related to genotypes M27 and M21, and the highest length of peduncle was related to genotypes M5, M15, and M30.

**Infructescence/sweetness(Brix)** (Low, Low to medium, Medium, Medium to high, High)**:** Genotypes M6 and M22 had the Lowest Infructescence sweetness, and genotypes M5, M29, and M34 had the Highest Infructescence sweetness. Coefficient of Variation (CV) was the highest value for leaf shape (CV= 52.36%), and Leaf blade texture (CV= 40.86%). Minimum values of CV were observed in Leaf Blade width (CV= 12.01%), and Internode length (CV= 12.21%) (Table 3). The CV and mean are the most commonly used statistical indices (Ebrahimi et al., 2021).

In previous studies on mulberry genotypes, CVs ranged from 12.78 to 84.09 % (Krishna et al., 2018), 0 to 203.19% (Farahani et al., 2019), and 25.9 to 91.4 (Ebrahimi et al., 2021). Fruit weight, fruit width, and leaf length were reported as 84.04, 20.25, and 25.55%, respectively, by Ebrahimi et al. (2021). The main reason for the observed high CV in qualitative traits refers to the type of sampling and its statistical analysis (Bakhshalizadeh et al., 2012).

Mean of fresh fruit weight was (2.56 g), and fruit length (28.9cm) (Table 3). The highest mean of fresh fruit weight (3.75 g) and fruit length (2.12 cm) were reported in Kermanshah genotypes by Fakhraei et al. (2016). Our results showed that South Khorasan genotypes have enough marketability in terms of fresh weight and fruit length to be introduced to the markets.

**Table 3. The descriptive analysis of 30 qualitative and quantitative variables.**

| Qualitative variables | Minimum | Maximum | Mean | Std deviation | CV% |
|---|---|---|---|---|---|
| Tree vigor | 3.00 | 7.00 | 5.53 | 1.59 | 28.75 |
| Tree growth habit | 3.00 | 7.00 | 4.60 | 1.88 | 37.32 |
| Shoot thickness | 3.00 | 5.00 | 4.81 | 1.14 | 23.53 |
| Shoot color | 3.00 | 7.00 | 4.80 | 2.21 | 37.25 |
| Leaf blade thickness | 3.00 | 7.00 | 4.33 | 1.44 | 33.25 |
| Shape of apex leaf blade | 3.00 | 7.00 | 5.31 | 1.36 | 25.12 |
| Shape of the base leaf blade | 3.00 | 7.00 | 5.13 | 1.59 | 30.99 |
| Leaf blade shape | 1.00 | 9.00 | 4.73 | 2.81 | 52.36 |
| Leaf blade depth of the sinus | 3.00 | 7.00 | 4.6 | 1.35 | 29.34 |
| Incisions of the margin | 3.00 | 7.00 | 4.6 | 1.12 | 24.34 |
| Leaf blade texture | 3.00 | 7.00 | 4.6 | 1.88 | 40.86 |
| Leaf blade blistering of the surface | 3.00 | 7.00 | 3.4 | 1.73 | 24.31 |
| Leaf blade color on the upper side | 3.00 | 7.00 | 5.2 | 1.85 | 35.57 |
| Leaf blade glossiness of the upper side | 2.00 | 8.00 | 6.2 | 1.25 | 22.41 |
| Fruit shape | 1.00 | 3.00 | 2.1 | 1.75 | 19.91 |
| Mature fruit color | 1.00 | 9.00 | 8.4 | 1.43 | 24.96 |
| **Quantitative variables** | **Minimum** | **Maximum** | **Mean** | **Std. Deviation** | **CV%** |
| Shoot Number | 1.00 | 7.00 | 4.80 | 1.35 | 20.45 |
| Lateral Shoot Number | 3.00 | 7.00 | 4.87 | 1.75 | 18.54 |
| Shoot Length | 15.0 | 50.0 | 32.58 | 0.90 | 16.33 |
| Internode length | 5.00 | 17.0 | 8.67 | 0.56 | 12.21 |
| Leaf Blade length | 5.70 | 13.16 | 9.18 | 2.31 | 19.21 |
| Leaf Blade width | 4.09 | 10.96 | 7.37 | 1.14 | 12.01 |
| Leaf Blade Length/Width | 0.85 | 1.81 | 1.25 | 0.42 | 17.52 |
| Petiole length | 0.50 | 30.7 | 7.52 | 0.80 | 14.67 |
| Mature Fruit length | 15.0 | 89.1 | 28.9 | 0.40 | 29.32 |
| Mature Fruit width | 10.7 | 17.57 | 14.3 | 0.33 | 20.24 |
| Mature Fruit Length/Width | 1.07 | 7.62 | 2.07 | 0.45 | 19.91 |
| Fruit weight | 1.08 | 6.9 | 2.65 | 0.35 | 13.67 |
| Peduncle Length | 0.50 | 14.3 | 5.81 | 0.09 | 13.63 |
| Sweetness (Brix) | 15.3 | 36.3 | 24.05 | 1.35 | 13.37 |

## 3.2. Relationships between Variables

The results of the correlation analysis of quantitative traits in mulberry genotypes (Table 4) showed that significant relationships exist between vegetative growth traits and several fruit characteristics. Shoot number (SN) and lateral shoot number (LSN) exhibited a very strong and positive correlation, indicating a high level of coordination between overall vegetative vigor and lateral branching, which is likely influenced by shared genetic control. In addition, shoot length (SL) showed a significant positive correlation with both shoot number and lateral shoot number, suggesting that genotypes with greater longitudinal growth tend to produce more branches. These findings reflect a structural association between longitudinal and lateral growth of shoots in mulberry. This aligns with previous observations reporting that increased shoot proliferation and branching are associated with enhanced plant productivity in mulberry. In that study, total shoot length and plant height, as well as leaf area, showed significant positive associations with leaf yield, highlighting the contribution of vegetative growth to yield traits (Singhvi et al., 2000).

**Table 4. Correlation matrix of 14 variables of quantitative phenotypic traits using Pearson's method in 31 mulberry genotypes.**

| | SN | LSN | SL | IL | LL | LW | LL/LW | PL | FL | FW | FL/ FW | Fwe | PDL | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SN** | 1 | | | | | | | | | | | | | |
| **LSN** | 0.96$^{**}$ | 1 | | | | | | | | | | | | |
| **SL** | 0.50$^{**}$ | 0.54$^{**}$ | 1 | | | | | | | | | | | |
| **IL** | 0.17 | 0.14 | 0.03 | 1 | | | | | | | | | | |
| **LL** | 0.05 | 0.12 | 0.28 | 0.02 | 1 | | | | | | | | | |
| **LW** | 0.17 | 0.24 | 0.19 | 0.12 | 065$^{**}$ | 1 | | | | | | | | |
| **LL/LW** | -0.07 | -0.12 | -0.11 | 0.08 | 0.24 | -0.37$^{*}$ | 1 | | | | | | | |
| **PL** | 0.07 | 0.11 | 0.26 | -0.09 | 0.39$^{*}$ | 0.35 | -0.01 | 1 | | | | | | |
| **FL** | 0.01 | 0.02 | 0.28 | -0.12 | 0.19 | 0.08 | -0.03 | 0.44 | 1 | | | | | |
| **FW** | 0.04 | 0.05 | 0.24 | -0.39$^{*}$ | 0.23 | 0.29 | -0.33 | -0.06 | 0.08 | 1 | | | | |
| **FL/ FW** | 0.12 | 0.14 | -0.06 | 0.10 | 0.06 | -0.08 | 0.14 | 0.15 | 0.54$^{**}$ | -0.44$^{*}$ | 1 | | | |
| **Fwe** | -0.04 | -0.07 | 0.23 | -0.21 | 0.11 | 0.10 | -0.27 | 0.20 | 0.65$^{**}$ | 0.44$^{*}$ | 0.24 | 1 | | |
| **PDL** | -0.08 | -0.13 | 0.05 | -0.04 | -0.07 | 0.09 | -0.05 | 0.57$^{**}$ | 0.24 | -0.20 | 0.11 | -0.03 | 1 | |
| **S** | -0.15 | -0.16 | -0.09 | -0.24 | -0.13 | 0.17 | -0.31 | 0.44 $^{*}$ | 0.18 | -0.20 | -0.02 | 0.002 | 0.53$^{**}$ | 1 |

Shoot Number (SN), Lateral Shoot Number (LSN), Shoot Length (SL), Internode Length (IL), Leaf Blade Length (LL), Leaf Blade Width(LW), Leaf Blade Length/Width (LL/LW),)Petiole Length (PL), Mature Fruit Length (FL), Mature Fruit Width (FW), Mature Fruit length/width (FL/ FW), Fruit weight (FWe), Peduncle Length (PDL), Sweetness(Brix)(S).

* and ** Correlations significant at P< 0.05 and P< 0.01, respectively.

Among leaf traits, leaf length (LL) was positively and significantly correlated with leaf width (LW), indicating that genotypes with longer leaves generally develop wider leaves as well, reflecting coordinated growth across leaf dimensions. Conversely, the leaf length-to-width ratio (LL/LW) showed a significant negative correlation with leaf width, suggesting that as leaf width increases, the ratio decreases, and the leaf shape shifts from elongated to broader. The positive correlation found between leaf length (LL) and leaf width (LW) in our data is consistent with morphological studies in mulberry that report proportional growth of leaf dimensions among genotypes (Gharibi et al., 2023).

Fruit-related traits also demonstrated important correlation patterns. Fruit length (FL) had a significant positive correlation with fruit weight (FWe), highlighting its value as a reliable indicator for predicting fruit weight in mulberry genotypes. The fruit length-to-width ratio (FL/FW) showed a significant negative correlation with fruit width (FW), and fruit width showed a significant positive correlation with fruit weight; thus, fruits with greater width tend to be less elongated and generally heavier. Regarding fruit quality, a significant positive correlation was observed between fruit petiole (PL) and sweetness(Brix) (S), indicating that fruits with longer petioles are typically sweeter. Fruit peduncle length (PDL) also showed significant positive correlations with fruit petiole and sweetness(Brix), which may reflect the role of the petiole and peduncle in facilitating efficient translocation of photosynthates to the fruit, thereby improving fruit quality.

Regarding fruit traits, the significant positive correlation between fruit length (FL) and fruit weight (FWe) observed in our study is in agreement with findings by Nonthakod et al. (2019), who reported that among 71 mulberry hybrids, fruit length and width had a very high positive correlation with fruit weight (r = 0.91) (Nonthakod et al., 2019). This suggests that fruit length and overall size parameters are reliable predictors of fruit biomass in mulberry — a useful insight for selection in breeding programs. Our observed negative correlation between fruit length-to-width ratio (FL/FW) and fruit width and positive correlation between fruit width and fruit weight indicate that heavier fruits tend to be less elongated and more plump. This pattern aligns with morphological variation reported in mulberry genotypes, where fruit size and shape vary significantly, and weight often associates with larger diameter/width rather than high elongation (Gharibi et al., 2023).

Furthermore, the positive association between fruit width and sweetness(Brix) (or soluble solids) in our data mirrors the reported trend that thicker mulberry fruits tend to accumulate more soluble solids and bioactive compounds. For instance, biochemical characterization of a wide set of mulberry genotypes demonstrated substantial variation in fruit weight, size, soluble solids content, and antioxidant compounds, implying that fruit morphological traits may co-vary with quality traits (Uyak et al., 2024).

Finally, the coordinated variation among vegetative traits (shoot and leaf parameters) and reproductive traits (fruit morphology and quality) observed in this study reinforces the idea that in mulberry, vegetative vigor provides the structural and physiological basis for fruit development and yield efficiency. Similar conclusions have been drawn in studies evaluating genetic variability and trait associations in mulberry under temperate conditions, which suggested that traits like fruit length, fruit width, and shoot thickness are among key yield-attributing traits (Nagoo et al., 2024). The correlation analysis of qualitative morphological traits among mulberry genotypes (Table 5) showed that most traits were mutually independent; however, several important trait pairs exhibited statistically significant relationships. These associations reflect coordinated variation between vegetative and reproductive traits, which may arise from shared genetic background, developmental linkages, and long-term farmer selection during domestication. Similar results indicating partial independence with a limited number of stable correlations have been reported in previous mulberry diversity and germplasm evaluation studies (Vijayan et al., 2011; Maji et al., 2015; Ebrahimi et al., 2017)

**Table 5. Correlation matrix of 16 variables of qualitative phenotypic traits using Spearman's method in 31 mulberry genotypes.**

| | TV | TGH | ST | SC | LBT | SALB | SBLB | LBS | LBDS | IM | LT | LBBS | LBC | LBGU | FS | FC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **TV** | 1 | | | | | | | | | | | | | | | |
| **TGH** | -0.06 | 1 | | | | | | | | | | | | | | |
| **ST** | 0.08 | 0.01 | 1 | | | | | | | | | | | | | |
| **SC** | 0.08 | 0.04 | $0.35^{*}$ | 1 | | | | | | | | | | | | |
| **LBT** | 0.21 | 0.023 | 0.04 | -0.09 | 1 | | | | | | | | | | | |
| **SALB** | 0.27 | -0.21 | 0.12 | 0.07 | -0.007 | 1 | | | | | | | | | | |
| **SBLB** | -0.10 | 0.05 | 0.29 | 0.30 | -0.10 | 0.01 | 1 | | | | | | | | | |
| **LBS** | 1 | -0.15 | 0.33 | 0.02 | -0.09 | 0.22 | 0.18 | 1 | | | | | | | | |
| **LBDS** | 0.09 | -0.11 | 0.05 | 0.01 | -0.32 | -0.21 | -0.18 | 0.31 | 1 | | | | | | | |
| **IM** | -0.005 | 0.26 | -0.17 | -0.22 | -0.11 | -0.32 | -0.08 | -0.30 | -0.10 | 1 | | | | | | |
| **LT** | 0.03 | 0.03 | -0.08 | -0.002 | $0.64^{**}$ | -0.30 | 0.06 | 0.003 | -0.07 | 0.16 | 1 | | | | | |
| **LBBS** | -0.01 | -0.07 | -0.23 | -0.27 | 0.08 | -0.11 | 0.01 | -0.03 | -0.07 | 0.39 | 0.21 | 1 | | | | |
| **LBC** | 0.22 | $0.40^{*}$ | -0.09 | -0.23 | 0.19 | 0.06 | 0.22 | -0.07 | -0.04 | 0.14 | 0.15 | 0.20 | 1 | | | |
| **LBGU** | 0.322 | -0.20 | -0.15 | -0.15 | 0.07 | $0.58^{**}$ | -0.22 | 0.09 | -0.25 | -0.008 | -0.26 | -0.25 | 0 | 1 | | |
| **FS** | 0.10 | -0.02 | -0.16 | $-0.43^{*}$ | 0.22 | 0 | -0.35 | 0.17 | -0.04 | 0.16 | 0.07 | 0.11 | 0.14 | 0.27 | 1 | |
| **FC** | -0.05 | 0.03 | -0.12 | -0.10 | 0.09 | $-0.52^{**}$ | -0.18 | $-0.50^{**}$ | -0.11 | 0.27 | 0.08 | 0.20 | -0.09 | -0.32 | -0.07 | 1 |

Tree vigor (TV), Tree Growth Habit (TGH), Shoot Thickness (ST), Shoot Color (SC), Leaf Blade Thickness (LBT), Shape Of Apex Leaf Blade (SALB), Shape Of Base Leaf Blade (SBLB), Leaf Blade Shape (LBS), Leaf Blade Depth Of Sinus (LBDS), Incisions of Margin (IM), Leaf Blade Texture (LT), Leaf Blade Blistering of Surface (LBBS), Leaf Blade Color of Upperside (LBC), Leaf Blade Glossiness Of Upper side (LBGU), Fruit Shape (FS), Mature Fruit Color (FC).

* and ** Correlations significant at P< 0.05 and P< 0.01, respectively.

A strong and positive correlation was observed between leaf blade thickness (LBT) and total leaf thickness (LT), indicating a close structural dependence between these two traits. Since overall leaf thickness is mainly determined by the thickness of mesophyll tissues forming the blade, this association is biologically expected. Thicker leaves are generally associated with greater photosynthetic capacity, improved water storage, and enhanced tolerance to environmental stresses.

Ebrahimi et al. (2017) also reported strong consistency among thickness-related leaf traits in mulberry accessions, emphasizing their importance in environmental adaptation. Similarly, Vijayan et al. (2011) highlighted the major contribution of leaf anatomical traits to ecological stability and productivity in mulberry. Therefore, the observed correlation confirms the reliability of leaf thickness-related traits as useful indicators for selecting stress-tolerant genotypes.

The strong positive correlation between the shape of apex leaf blade (SALB) and leaf blade glossiness of upper side (LBGU) suggests a close association between external leaf morphology and epidermal surface characteristics. Leaf glossiness is largely governed by cuticular wax deposition, epidermal microstructure, and surface cell arrangement, whereas apex shape reflects genetic regulation of leaf development.

Azad et al. (2014) and Maji et al. (2015) identified both leaf shape and surface appearance as stable and highly discriminative characters for mulberry cultivar identification. The observed relationship indicates that certain apex shapes tend to develop simultaneously with specific epidermal surface properties, which can influence light reflectance, transpiration rate, and resistance to abiotic stresses such as drought and high radiation.

A strong negative correlation was detected between leaf apex shape and fruit color (FC), indicating that particular vegetative morphologies are commonly associated with distinct fruit pigmentation patterns. Although these traits belong to different plant organs, such relationships may reflect a shared genetic background, linkage between controlling loci, or the outcome of long-term traditional selection of specific trait combinations. Previous studies on mulberry diversity have consistently shown that genotypes differentiated based on leaf morphology often exhibit clear differences in fruit traits, especially fruit color (Maji et al., 2015; Ebrahimi et al., 2017).

This association is of great practical value because it indicates that fruit color may be indirectly predicted from leaf characteristics at early developmental stages when fruits are not yet formed.

The negative correlation between overall leaf blade shape (LBS) and fruit color further confirms the coordinated variation between vegetative and reproductive traits in mulberry. This finding suggests that specific leaf shapes tend to be consistently associated with particular fruit color classes.

Several researchers have emphasized that leaf blade shape is one of the most informative morphological descriptors in mulberry and plays a key role in genotype discrimination (Vijayan et al., 2011; Azad et al., 2014).

Its association with fruit color highlights the usefulness of vegetative traits as indirect selection tools in mulberry breeding programs aimed at improving both leaf yield and fruit quality.

The significant positive correlation between shoot thickness (ST) and shoot color (SC) indicates that thicker shoots usually exhibit more intense pigmentation. Thicker shoots represent greater vegetative vigor and often accumulate higher levels of phenolic compounds and pigments, which results in darker shoot coloration.

Maji et al. (2015) reported that shoot pigmentation in mulberry is frequently associated with shoot robustness and general plant vigor. Similarly, Azad et al. (2014) showed that shoot color is a genetically stable trait that often covaries with growth-related characters. Therefore, this relationship suggests that shoot color may serve as an indirect visual indicator of vegetative strength in mulberry genotypes.

The positive correlation between tree growth habit (TGH) and leaf blade color of upperside (LBC) indicates that canopy architecture influences leaf pigmentation. Trees with different growth habits (erect versus spreading) differ in light interception patterns within the canopy, which directly affects chlorophyll accumulation and leaf color intensity.

Vijayan, Tikader, et al. (2011) demonstrated that canopy structure has a significant influence on physiological and morphological leaf traits in mulberry, particularly chlorophyll content and photosynthetic performance.

Ebrahimi et al. (2017) also reported a close relationship between growth habit and leaf color. Thus, the observed correlation can be interpreted as the combined effect of genetic growth pattern and microclimatic conditions within the tree canopy.

The significant negative correlation between shoot color and fruit shape (FS) indicates that specific shoot pigmentation patterns tend to be associated with particular fruit morphology classes. Although shoot color and fruit shape are developmentally independent traits, their association likely reflects long-term selection and stabilization of genotype groups within different agro-ecological regions.

Azad et al. (2014) and Maji et al. (2015) reported that mulberry genotypes classified based on shoot and leaf characters often show marked differences in fruit shape and size. Therefore, this relationship further supports the concept of coordinated variation among vegetative and fruit traits in mulberry.

The dendrogram was constructed using Ward's linkage method and Euclidean distance, with a cut-off at 10.5, resulting in five distinct clusters (Fig 1). These clusters reflect groups of mulberry genotypes with similar and differentiable morphological and fruit traits (Table 6).

Hierarchical clustering showed that *M. alba* genotypes were mainly located in larger clusters (1 and 2), while *M. nigra* and *M. macroura* genotypes formed smaller and more distinct clusters, indicating significant phenotypic diversity in the South Khorasan mulberry population. Although the cluster analysis was unable to fully differentiate all species, with some genotypes grouped in mixed clusters (clusters 2, 3, and 4), it successfully distinguished *M. alba* (Cluster 1) and *M. nigra* (Cluster 5) based on phenotypic traits. But, the lack of absolute segregation of genotypes based on their species, even in these two clusters, shows that selected morphological descriptors are not efficient due to the lack of necessary precision and overlapping of some vegetative and reproductive traits in different species, and must use molecular markers.

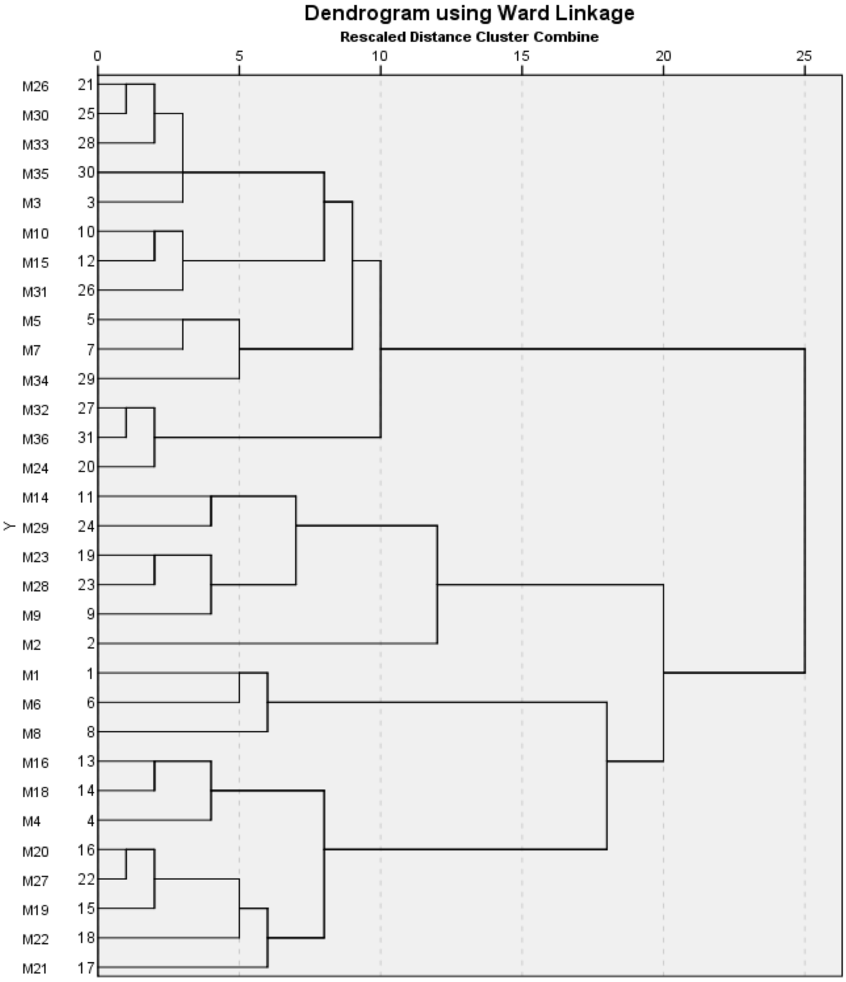

**Figure 1. Cluster analysis dendrogram of South Khorasan mulberry genotypes based on squared Euclidean distance and Ward**

**Table 6. Clusters of South Khorasan Mulberry Genotypes Based on Morphological Traits**

| Cluster | Genotypes | Species (Genus/Species) | Tree Vigor (TV) | Soot Number (SN) | Fruit Sweetness (S) | Fruit weight (FWe) | Fruit Size (FW/FL) | Fruit length (FL) |
|---|---|---|---|---|---|---|---|---|
| 1 | M26, M30, M33, M35, M3, M10, M15, M31, M5, M7, M34, M32, M36, M24 | M. alba,<br>M. macroura | Strong | Medium to many | Medium to high | Medium to high | Medium to Broad, Ellipsoid / Cylindric | Medium to long |
| 2 | M14, M29, M23, M28, M9 | M. alba | Medium / Strong | Few to medium | Medium | Medium to high | Medium | Short to medium |
| 3 | M2 | M. alba | Strong | Medium to many | Medium | low | Medium, Ellipsoid | short |
| 4 | M1, M6, M8 | M. alba,<br>M. macroura | Strong | Many | Medium | Medium to high | Medium, Ellipsoid / Cylindric | long |
| 5 | M16, M18, M4, M20, M27, M19, M22, M21 | M. nigra,<br>M. alba | Medium / Strong | Few/ medium/ many | Few to medium | Medium to high | Medium to Broad, Ellipsoid / Globose | medium |

Key morphological traits, including tree vigor, shoot number as well as fruit weight, size, and sweetness, were clearly associated with the observed cluster structure. These findings provide valuable guidance for targeted breeding programs and selection of superior genotypes, facilitating both improvement and genetic studies in mulberry. The considerable phenotypic diversity observed among the studied genotypes and species has been influenced by the varying environmental and climatic conditions and different management in sites. The existence of differences in latitude (from 912 to 2032 m) and differences in climatic conditions can justify some morphological changes observed in different mulberry species and genotypes. The primary objective of this study was to assess phenotypic diversity under natural growing conditions; therefore, environmental factors were not experimentally controlled through common garden trials. However, the clustering pattern did not follow geographical origin or altitudinal gradients, and partial species separation was observed even within overlapping elevation ranges, suggesting that genetic differentiation also contributed to the observed diversity.

### 3.3. Principal Component Analysis (PCA)

PCA, as a flexible approach by reduction of numerous correlated variables to a few main factors, could be successfully utilized to comprehend the patterned variation in a set of variables. In view of the diversity of mulberry accessions under study, the analysis into main components was carried out to determine the contribution and effect rate of each trait under study on the present diversity. As a criterion to extract the main principal components, eigenvalues greater than one were taken into account. In this study, the KMO criterion for sampling adequacy was 0.62 and Bartlett's test of sphericity was significant ($p < 0.001$), indicating that the data were suitable for PCA. Principal component analysis extracted five components with eigenvalues greater than one, which together accounted for 85.79% of the total phenotypic variance (Table 7). Standard Principal Component Analysis (PCA), being fundamentally a variance-based technique, necessitates numerical data measured on continuous scales. The inclusion of qualitative (categorical) variables would require arbitrary numerical coding, potentially imposing artificial ordinal structures and distorting the underlying relationships among variables. Accordingly, PCA and biplot analysis were performed on 14 quantitative phenotypic traits to identify the traits contributing most significantly to the total phenotypic variance.

The PCA results were largely consistent with the hierarchical cluster analysis, confirming the robustness of the morphological differentiation observed among mulberry genotypes. The first five principal components explaining 85.79% of the total variance indicate that the selected 14 quantitative traits effectively capture the major sources of morphological variation in the studied germplasm (Table 7, 8). The strong association of fruit-related traits (FL, FL/FW, FWe) with PC1 suggests that fruit characteristics are the primary drivers of morphological diversity in this collection, which aligns with findings of Ebrahimi et al. (2017) who reported that fruit traits explained the largest proportion of variation in Iranian mulberry germplasm. Similarly, Gharibi et al. (2023) identified fruit weight and dimensions as key discriminating traits in South Khorasan mulberry genotypes.

**Table 7. Eigenvalues, percentage of variance, and cumulative variance for the first five principal components from PCA of 14 quantitative morphological traits in 31 mulberry genotypes**

| **Item** | **PC axis** | | | | | |
|---|---|---|---|---|---|---|
| **Eigenvalue** | 4.32 | 3.09 | 2.08 | 1.50 | 1.02 | 0.75 |
| **variance** | 30.86 | 22.05 | 14.84 | 10.74 | 7.30 | 5.36 |
| **Cumulative** | 30.86 | 52.91 | 67.75 | 78.49 | 85.79 | 91.15 |

| **Eigenvector** | | | | | |
|---|---|---|---|---|---|
| **Variable*** | **PC1** | **PC2** | **PC3** | **PC4** | **PC5** |
| **SN** | -0.08 | **0.82** | -0.06 | 0.04 | 0.02 |
| **LSN** | -0.05 | **0.85** | -0.03 | 0.01 | 0.03 |
| **SL** | 0.12 | 0.37 | -0.35 | 0.44 | 0.31 |
| **IL** | -0.09 | 0.22 | 0.32 | **0.61** | 0.11 |
| **LL** | 0.21 | 0.28 | **0.78** | -0.02 | 0.08 |
| **LW** | 0.13 | 0.17 | **0.88** | 0.04 | 0.06 |
| **LL/LW** | 0.04 | 0.06 | **-0.63** | -0.13 | 0.04 |
| **PL** | 0.19 | 0.13 | 0.06 | -0.05 | **0.78** |
| **FL** | **0.91** | 0.04 | 0.11 | -0.04 | 0.07 |
| **FW** | -0.01 | -0.05 | 0.21 | **0.83** | -0.11 |
| **FL/FW** | **0.92** | 0.02 | -0.04 | -0.14 | 0.08 |
| **FWe** | 0.54 | -0.08 | 0.23 | **0.63** | -0.05 |
| **PDL** | 0.23 | 0.06 | -0.04 | -0.06 | **0.71** |
| **S** | 0.44 | -0.14 | 0.26 | -0.11 | 0.46 |

Note: Values in bold indicate factor loadings greater than 0.60, representing the strongest contributions to each principal component

**Table 8. Interpretation of principal components based on traits with highest factor loadings**

| **Principal Component** | **Proposed Name** | **Traits with Highest Loadings** | **% of Variance** |
|---|---|---|---|
| **PC1** | Fruit Characteristics | FL (0.91), FL/FW (0.92), FWe (0.54), S (0.44) | 30.86 |
| **PC2** | Shoot Traits | SN (0.82), LSN (0.85) | 22.05 |
| **PC3** | Leaf Morphology | LL (0.78), LW (0.88), LL/LW (-0.63) | 14.84 |
| **PC4** | Fruit Dimensions | FW (0.83), FWe (0.63), IL (0.61) | 10.74 |
| **PC5** | Peduncle/Petiole Traits | PL (0.78), PDL (0.71), S (0.46) | 7.30 |

The biplot of the first two principal components (PC1 vs. PC2), which together explained 52.91% of the total variance, provided a clear visualization of the relationships among genotypes and traits (Fig 2). The direction and length of the vectors indicated that fruit-related traits, including fruit length (FL), fruit length-to-width ratio (FL/FW), fruit weight (FWe), and sweetness (S), were the main contributors to PC1, while shoot-related traits, including shoot number (SN) and lateral shoot number (LSN), were the primary contributors to PC2. Genotypes positioned on the right side of the biplot (e.g., M5, M8, and M15) exhibited higher values for fruit-related traits, indicating superior fruit size and quality. In contrast, genotypes located in the upper region (e.g., M22, M24, M32, and M36) showed higher scores for shoot traits, reflecting greater vegetative vigor. The *M. nigra* genotypes (M4, M16, M18, M21, M22, and M27) clustered separately from most *M. alba* genotypes, confirming significant morphological differentiation between species. These genotypes were characterized by broader leaves, as indicated by their proximity to the leaf width (LW) vector. The acute angles between fruit-related vectors (FL, FL/FW, FWe) indicated strong positive correlations among these traits, suggesting that selection for increased fruit length would concurrently improve fruit weight and shape. The near-perpendicular angle between fruit and shoot characteristics suggested that these trait groups are largely independent, allowing simultaneous selection for both fruit quality and vegetative vigor in breeding programs. The clear separation of *M. nigra* genotypes from *M. alba* in the PCA biplot corroborates the taxonomic distinction between these species and supports the utility of morphological markers for species identification. This finding is consistent with Vijayan et al. (2011), who reported significant morphological divergence between *M. alba* and *M. nigra* accessions.

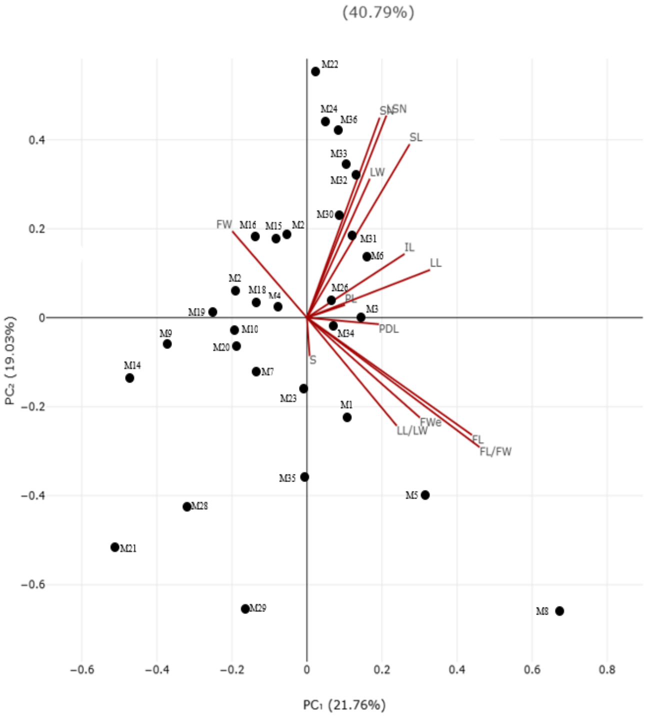

**Figure 2. Biplot between PC1 and PC2 showing the distribution of mulberry genotypes based on quantitative variables.**

## 4. Conclusions

This study indicated that the selected morphological traits (such as fruit weight, size, and sweetness, as well as tree vigor) are not sufficient on their own to fully differentiate the species due to overlapping characteristics. Therefore, the use of molecular (genetic) markers is necessary for a more precise distinction. Furthermore, the observed diversity is influenced by varying environmental and climatic factors, different management practices across various sites, and genetic differentiation. This study shows that although most traits in mulberry vary independently, a limited number of key vegetative traits are strongly correlated with fruit traits. The identification of five distinct groups of traits (fruit traits, branch traits, leaf morphology, fruit dimensions, and peduncle/petiole traits) provides valuable insights for targeted breeding programs. For example, genotypes with high scores on PC1 (e.g., M5, M8, M15) could be selected for improved fruit size and quality, while genotypes with high scores on PC2 (e.g., M22, M24, M32, M36) may be valuable for vegetative vigor and leaf production. Fruit and branch traits indicate that these trait groups are largely independent, allowing for simultaneous selection for fruit quality and vegetative vigor in breeding programs.

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# Appendix Section

**Table S1. Evaluated traits of South Khorasan mulberry genotypes based on UPOV and IPGRI guidelines**

| Code | Genus/Species | TV | TGH | SN | LSN | SL | ST | SC |
|---|---|---|---|---|---|---|---|---|
| M1 | M. alba | Strong | Spreading | Medium | Medium | Short to Medium | Medium | Greenish brown |
| M2 | M. alba | Strong | Spreading | Medium | Medium | Short to Medium | Medium | Greenish brown |
| M3 | M. alba | Strong | Drooping | Medium | Medium | Short to Medium | Medium | Greenish brown |
| M4 | M. nigra | Strong | Spreading | Medium | Medium | Short to Medium | Medium | Greenish brown |
| M5 | M. macroura | Strong | Semi-upright | Medium | Medium | Short to Medium | Medium | Greenish brown |
| M6 | M. alba | Strong | Spreading | Medium | Medium | Medium | Thin | Greyish brown |
| M7 | M. alba | Strong | Spreading | Medium | Medium | Short | Medium | Greyish brown |
| M8 | M. macroura | Strong | Drooping | Medium | Medium | Short to Medium | Thin | Light gery |
| M9 | M. alba | Strong | Spreading | Medium | Medium | Short | Medium | Greenish brown |
| M10 | M. alba | Strong | Semi-upright | Medium | Medium | Short to Medium | Medium | Greenish brown |
| M14 | M .alba | Medium | Spreading | Medium | Medium | Short to Medium | Thin | Greenish brown |
| M15 | M. alba | Strong | Spreading | Medium | Medium | Medium | Medium | Greenish brown |
| M16 | M. Nigra | Medium | Drooping | Medium | Medium | Medium | Medium | Greenish brown |
| M18 | M. Nigra | Strong | Spreading | Medium | Medium | Medium | Medium | Greenish brown |
| M19 | M. alba | Strong | Spreading | Medium | Medium | Short to Medium | Medium | Greenish brown |
| M20 | M. alba | Strong | Drooping | Medium | Medium | Short to Medium | Medium | Greyish brown |
| M21 | M. Nigra | Medium | Spreading | Few | Absent or Few | Short | Medium | Yellowish brown |
| M22 | M. Nigra | Strong | Drooping | many | Many | Short to Medium | Medium | Yellowish brown |
| M23 | M. alba | Medium | Spreading | Few to Medium | Absent or Few | Short to Medium | Medium | Greenish brown |
| M24 | M. alba | Strong | Drooping | many | Many | Medium | Medium | Greenish brown |
| M26 | M. alba | Strong | Drooping | Medium | Medium | Medium | Medium | Yellowish brown |
| M27 | M. Nigra | Strong | Drooping | Medium | Medium | Short to Medium | Medium | Greenish brown |
| M28 | M. alba | Medium | Drooping | Few to Medium | Absent or Few | Short | Medium | Greyish brown |
| M29 | M. alba | Medium | Drooping | Few to Medium | Absent or Few | Short | Medium | Greenish brown |
| M30 | M. alba | Strong | Drooping | Medium | Medium | Medium | Medium | Greenish brown |
| M31 | M. alba | Strong | Semi-upright | Medium | Medium | Short to Medium | Medium | Yellowish brown |
| M32 | M. alba | Strong | Spreading | many | Many | Medium | Medium | Greenish brown |
| M33 | M. alba | Strong | Spreading | Medium | Medium | Medium | Medium | Greyish brown |
| M34 | M. alba | Medium | Spreading | Medium | Medium | Short to Medium | Medium | Greyish brown |
| M35 | M. alba | Strong | Drooping | Few | Absent or Few | Short to Medium | Medium | Yellowish brown |
| M36 | M. alba | Strong | Drooping | many | Many | Medium | Medium | Yellowish brown |

**Table S1 (continued). Evaluated traits of South Khorasan mulberry genotypes based on UPOV and IPGRI guidelines**

| Code | IL | LL | LW | LL/LW | LBT | LBA | LBB | LS |
|---|---|---|---|---|---|---|---|---|
| M1 | Short | Medium to long | very narrow | High | Medium | Acute | truncate | Triangular |
| M2 | Long | Medium | narrow to medium | Medium | Thin | Acuminate | Retuse | Pentagonal |
| M3 | Medium | long | medium | Medium | Medium | Acute | Cordate | Ovate |
| M4 | Short | Medium to long | narrow | Medium | Medium | Acuminate | Cordate | Circular |
| M5 | Short | Medium | narrow | Medium | Thick | Obtuse | truncate | Ovate |
| M6 | Medium | long | narrow | High | Medium | Acute | truncate | Triangular |
| M7 | Short | Medium to long | narrow to medium | Medium | Thick | Obtuse | truncate | Ovate |
| M8 | Medium | long | narrow to medium | High | Thick | Acuminate | truncate | Triangular |
| M9 | Short | short | narrow | Medium | Thin | Obtuse | Cordate | Pentagonal |
| M10 | Short | short | narrow to medium | low | Medium | Obtuse | Cordate | Cordate |
| M14 | Medium | Very short to short | very narrow | Medium | Thin | Acute | Cordate | Cordate |
| M15 | Short | short | narrow to medium | low | Medium | Obtuse | Cordate | Ovate |
| M16 | Short | Medium to long | narrow | Medium | Medium | Acuminate | Cordate | Cordate |
| M18 | Short | Medium to long | narrow | Medium | Medium | Acuminate | Cordate | Triangular |
| M19 | Short | Medium | narrow | Medium | Medium | Acuminate | Retuse | Triangular |
| M20 | Short | short | narrow | Medium | Medium | Acuminate | Cordate | Cordate |
| M21 | Short | Short to Medium | narrow | Medium | Thick | Acuminate | Cordate | Cordate |
| M22 | Medium | long | broad | Medium | Thick | Acuminate | Cordate | Cordate |
| M23 | Medium | Short to Medium | narrow | Medium | Medium | Acute | Cordate | Ovate |
| M24 | Medium | Short to Medium | narrow | Medium | Medium | Acute | Cordate | Ovate |
| M26 | Short | Medium to long | narrow to medium | Medium | Medium | Obtuse | Cordate | Cordate |
| M27 | Short | Medium to long | narrow to medium | Medium | Thick | Acuminate | Cordate | Cordate |
| M28 | Short | Medium | narrow | Medium | Medium | Acute | Retuse | Ovate |
| M29 | Short | short | very narrow | High | Thin | Acuminate | Retuse | Triangular |
| M30 | Short | long | medium | Medium | Medium | Obtuse | Cordate | Triangular |
| M31 | Medium | Medium to long | medium to broad | low | Medium | Acute | Cordate | Cordate |
| M32 | Short | Medium | narrow | Medium | Medium | Obtuse | Cordate | Cordate |
| M33 | Short | long to very long | broad | Medium | Thick | Obtuse | Cordate | Cordate |
| M34 | Short | long to very long | broad | Medium | Medium | Acute | Cordate | Cordate |
| M35 | Short | long | medium | Medium | Medium | Obtuse | truncate | Cordate |
| M36 | Short | Medium | narrow to medium | Medium | Medium | Obtuse | Cordate | Cordate |

**Table S1 (continued). Evaluated traits of South Khorasan mulberry genotypes based on UPOV and IPGRI guidelines**

| Code | LBDS | IM | LT | LBS | LBC | LBGU | PL | FL |
|---|---|---|---|---|---|---|---|---|
| M1 | Absent or very shallow | Denate | Medium | Absent or weak | Light green | Medium | very long | medium |
| M2 | deep | cernate | Medium | Absent or weak | Medium green | Weak | medium | short |
| M3 | Absent or very shallow | Aristate | Medium | Absent or weak | Dark green | Strong | medium to long | medium |
| M4 | Absent or very shallow | Aristate | Rough | Medium | Medium green | Weak | Short | short to medium |
| M5 | Absent or very shallow | repand | Medium | Absent or weak | Medium green | Strong | long to very long | long |
| M6 | Absent or very shallow | serrulate | Medium | Strong | Medium green | Medium | medium | medium |
| M7 | Absent or very shallow | Denate | Rough | Absent or weak | Medium green | Strong | medium | medium |
| M8 | Absent or very shallow | serrulate | Rough | Absent or weak | Dark green | Strong | medium | long |
| M9 | Absent or very shallow | cernate | Smooth | Absent or weak | Light green | Strong | Short to medium | short to medium |
| M10 | Absent or very shallow | cernate | Rough | Absent or weak | Medium green | Medium | medium | medium |
| M14 | Absent or very shallow | cernate | Medium | Absent or weak | Medium green | Medium | Short to medium | short |
| M15 | Absent or very shallow | cernate | Medium | Absent or weak | Light green | Strong | medium | medium to long |
| M16 | Absent or very shallow | Serrate | Rough | Medium | Dark green | Weak | Short | short to medium |
| M18 | Absent or very shallow | Serrate | Medium | Absent or weak | Medium green | Strong | Short to medium | medium |
| M19 | Absent or very shallow | cernate | Medium | Absent or weak | Medium green | Medium | Short to medium | short |
| M20 | Absent or very shallow | cernate | Medium | Absent or weak | Dark green | Weak | Short to medium | medium |
| M21 | Absent or very shallow | cernate | Rough | Absent or weak | Light green | Weak | Short | short |
| M22 | Absent or very shallow | cernate | Rough | Absent or weak | Medium green | Weak | Short | short |
| M23 | Absent or very shallow | cernate | Medium | Absent or weak | Medium green | Medium | medium | medium |
| M24 | Absent or very shallow | cernate | Medium | Absent or weak | Dark green | Medium | Short to medium | short to medium |
| M26 | Absent or very shallow | cernate | Medium | Absent or weak | Medium green | Medium | medium | medium |
| M27 | Absent or very shallow | cernate | Rough | Absent or weak | Medium green | Weak | Short to medium | short to medium |
| M28 | Absent or very shallow | cernate | Medium | Absent or weak | Medium green | Medium | Short to medium | medium |
| M29 | Absent or very shallow | Biserrate | Smooth | Absent or weak | Light green | Weak | Short to medium | short |
| M30 | Absent or very shallow | Biserrate | Medium | Absent or weak | Dark green | Medium | very long | medium |
| M31 | Absent or very shallow | cernate | Medium | Absent or weak | Medium green | Medium | long to very long | medium |
| M32 | Absent or very shallow | cernate | Medium | Absent or weak | Dark green | Medium | long | short to medium |
| M33 | Absent or very shallow | cernate | Medium | Medium | Dark green | Medium | long to very long | medium |
| M34 | Absent or very shallow | cernate | Medium | Absent or weak | Medium green | Medium | long | short to medium |
| M35 | Absent or very shallow | cernate | Medium | Absent or weak | Medium green | Strong | long | short to medium |
| M36 | Absent or very shallow | cernate | Medium | Absent or weak | Dark green | Medium | long | medium |

**Table S1 (continued). Evaluated traits of South Khorasan mulberry genotypes based on UPOV and IPGRI guidelines**

| Code | FWe | FL/FW | Fw | FS | FC | PDL | S |
|---|---|---|---|---|---|---|---|
| M1 | medium | medium | medium | Ellipsoid | black purple | medium to long | low to medium |
| M2 | low | low | medium | Ellipsoid | yellowish white | medium | medium |
| M3 | medium | medium | medium | Ellipsoid | yellowish white | medium to long | medium to high |
| M4 | high | high | Broad | Ellipsoid | reddish purple | short | low to medium |
| M5 | medium | high | medium | cylindric | yellowish white | long | high |
| M6 | medium | medium | medium | Ellipsoid | black purple | medium to long | low |
| M7 | medium | medium | medium | Ellipsoid | white | medium | medium |
| M8 | high | high | medium | cylindric | dark purple | short | low to medium |
| M9 | medium | medium | medium | Ellipsoid | yellowish white | medium to long | medium |
| M10 | high | low | Broad | globose | yellowish white | medium | medium to high |
| M14 | low | low | medium | Ellipsoid | white | medium | medium to high |
| M15 | high | medium | Broad | Ellipsoid | yellowish white | long | medium to high |
| M16 | high | low | Broad | Ellipsoid | reddish purple | short | low to medium |
| M18 | medium | low | Broad | Ellipsoid | reddish purple | short | low to medium |
| M19 | low | low | medium | Ellipsoid | black purple | medium | low to medium |
| M20 | medium | medium | medium | Ellipsoid | light purple | short to medium | medium |
| M21 | low | low | medium | globose | reddish purple | short | low to medium |
| M22 | low | low | Broad | Ellipsoid | reddish purple | short | low |
| M23 | high | medium | medium | Ellipsoid | black purple | medium to long | low to medium |
| M24 | low | medium | medium | Ellipsoid | white | medium to long | low to medium |
| M26 | medium | low | Broad | Ellipsoid | yellowish white | medium to long | medium to high |
| M27 | medium | low | Broad | Ellipsoid | reddish purple | short | medium |
| M28 | high | low | Broad | Ellipsoid | yellowish white | medium to long | medium |
| M29 | low | low | medium | Ellipsoid | light purple | medium to long | high |
| M30 | medium | low | Broad | Ellipsoid | yellowish white | long | medium to high |
| M31 | high | medium | medium | Ellipsoid | yellowish white | medium | medium to high |
| M32 | medium | medium | medium | Ellipsoid | dark purple | medium to long | medium |
| M33 | high | low | Broad | Ellipsoid | yellowish white | medium to long | medium to high |
| M34 | low | medium | medium | cylindric | yellowish white | medium to long | high |
| M35 | medium | low | Broad | Ellipsoid | yellowish white | medium to long | medium |
| M36 | high | low | Broad | Ellipsoid | yellowish white | short to medium | medium |