







## Morphological characterization and genetic divergence among melon accessions<sup>1</sup>

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### ABSTRACT

Melon, a member of the Cucurbitaceae family, is cultivated in over 100 countries and is a crop of great global importance. The significant morphological variability, encompassing attributes such as size, shape, color, and texture of the rind and flesh, alongside aroma and flavor, indicates the extensive phenotypic diversity the crop manifests. This diversity represents a crucial resource for breeding programs aimed at the species' improvement. Such variation enables breeders to select superior individuals for subsequent crosses, to develop cultivars adapted to diverse environmental and management conditions. The objective of this study was to estimate the genetic divergence between melon accessions for morphological traits. Twenty-four accessions and one commercial cultivar were evaluated. The generalized Mahalanobis distance was used as a measure of dissimilarity. Then, the clustering analysis of the accessions was performed using the Tocher optimization method and the hierarchical agglomerative clustering method (UPGMA). Both the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) hierarchical method and the Tocher optimization agglomerative method yielded the formation of three distinct groups, exhibiting a congruence of 96% between them. It was found that the longitudinal diameter, transverse diameter, and length-width/fruit ratio were the characteristics that presented the highest percentage of contribution to divergence between the accessions evaluated.

**Keywords:** *Cucumis melo* L., dissimilarity, multivariate analysis.

## INTRODUCTION

Melon (*Cucumis melo* L.) is a widely cultivated Cucurbitaceae crop, grown in over 100 countries. The vast diversity of horticultural groups and high consumer demand have driven the selection and cultivation of hundreds of cultivars, adapted to various climates and consumer preferences.<sup>(1)</sup>

Based on DNA and morphological analyses, recent studies suggest that Asia, particularly India and Pakistan, is the primary center of melon diversification.<sup>(2,3)</sup> This hypothesis is supported by the presence of wild melon relatives in India and Australia, along with the extensive diversity of cultivated melons in India and East Asia.<sup>(3)</sup>

Global melon production reached over 28 million tons in 2022, with China accounting for almost half of this total. Brazil is the eighth largest producer. In 2023, Brazil produced 862,387 tons, with the Northeast region contributing 98%. Within the Northeast, Rio Grande do Norte is the leading producer, followed by Bahia, Ceará, and Pernambuco. Melon cultivation is thus a significant source of food and income for farmers worldwide.<sup>(4,5)</sup>

The wide range of variations in melon characteristics, such as size, shape, color and texture of the peel and pulp, aroma, and flavor, indicates the great phenotypic diversity manifested by the crop, which is a reflection of the genotypic diversity, being a vital resource for breeding programs of the species.<sup>(2,3,6)</sup>

It is established that the comprehension of the genetic basis of diversity is of paramount importance for the development of varieties exhibiting desirable traits, such as enhanced disease resistance, tolerance to abiotic stresses, and superior fruit quality.<sup>(7)</sup> Currently, 451 melon cultivars are registered in the National Cultivar Registry.<sup>(8)</sup>

The study of genetic divergence among melon accessions has broad applications. In conservation, it aids in identifying populations with high genetic diversity to ensure species preservation and adaptability. In breeding, it facilitates the selection of parents for crosses to exploit heterosis and accelerate the development of cultivars with desirable traits.<sup>(3,9,10)</sup> In evolutionary studies, it elucidates the phylogenetic relationships among melon groups, providing insights into domestication events and dispersal patterns. In conclusion, the analysis of genetic divergence is essential for conservation, breeding, and evolutionary studies of melon.<sup>(9,11)</sup>

Genetic diversity can be inferred through various meth-

ods, including analyzing morphological, physiological, and molecular traits. Dissimilarity measures, such as Mahalanobis or Euclidean distances, can be quantified using multivariate analyses like principal component analysis, canonical variate analysis, and hierarchical clustering.<sup>(12,13)</sup>

Dissimilarity measures can be used in clustering and graphical dispersion analyses to identify homogeneous subgroups among accessions. Hierarchical methods such as maximum linkage, complete linkage, UPGMA, and optimization methods such as Tocher's and modified Tocher's methods are commonly employed in these analyses.<sup>(13,14)</sup>

The primary aim of this study was to assess genetic divergence among melon accessions using morphological traits, to provide insights for the selection of genotypes in breeding programs.

## MATERIAL AND METHODS

The experiment was conducted in a greenhouse located in the Phytotechny Area of the Agronomy Department at the Universidade Federal Rural de Pernambuco, during October 2023. Twenty-four melon accessions, sourced from the Melon Active Germplasm Bank (BAG) of Embrapa Vegetables (Brasília-DF), were evaluated, along with the commercial cultivar Eldorado 300, which exhibits oval-shaped fruits with yellow rind coloration and light green flesh. This cultivar possesses a firm rind, rendering it suitable for long-distance transportation (Table 1).

**Table 1.** Melon accessions were used in the genetic divergence study

Code	Accesses	Code	Accesses
1	CNPH 15-1064	14	CNPH 11-1059
2	CNPH 11-1063	15	CNPH 16-1086
3	CNPH 11-1072	16	CNPH 11-1075
4	CNPH 11-1070	17	CNPH 11-1074
5	CNPH 11-1067	18	CNPH 17-0913
6	CNPH 11-1066	19	CNPH 00-881
7	CNPH 17-001	20	CNPH 13-962
8	CNPH 09-002	21	CNPH 11-961
9	CNPH 11-069	22	CNPH 11-939
10	CNPH 11-1054	23	CNPH 11-930
11	CNPH 08-1051	24	CNPH 13-963
12	CNPH 15-1061	25	Eldorado 300
13	CNPH 11-1058		

Sowing was conducted in 162-cell polypropylene trays utilizing a commercial pine bark-based substrate. The trays were moistened, stacked, and covered to ensure photoperiodic exclusion for 72 hours. Subsequently, seedlings were maintained for eight days in a greenhouse under a subirrigation hydroponic system. Following this period, when the seedlings had developed four true leaves, they were transplanted into 5.5-liter capacity pots containing washed sand. Plant water requirements were met via a drip hydroponic system, controlled by a digital timer and adjusted by fluctuations in climatic conditions. Mineral nutrition was supplied through a nutrient solution.

Fruits were obtained through successive self-crosses within each accession, beginning with the pollination of plant 1 by plant 2 and so on. Female and male flowers were protected with gelatin capsules during the pre-anthesis stage. On the following day, pollen was transferred to the female flower, and the flower was then protected to prevent contamination with pollen from other accessions.

Harvesting commenced 63 days after sowing, with five fruits collected per accession. The fruits were identified and transported to the Phytopathology Laboratory, where physical evaluations were conducted. The following traits were assessed: fruit mass (FM, kg) using a balance; longitudinal diameter (LD, cm) and transverse diameter (TD, cm) using a tape measure; longitudinal internal cavity (LIC, cm) and transverse internal cavity (TIC, cm) using a tape measure; peel thickness (PET, mm) and pulp thickness (PUT, mm) using a digital caliper; calyx length (CA, mm) and corolla length (CO, mm) using a digital caliper; pulp firmness (FIR, N) using a penetrometer; soluble solids content (SS, °Brix) using a portable refractometer; and fruit shape index (FSI, dimensionless).

Data standardization was performed, followed by the calculation of the Mahalanobis generalized distance matrix ( $D^2$ ). Subsequently, cluster analysis was conducted using Tocher's optimization method and the hierarchical clustering method (UPGMA).

The relative importance of each trait in predicting genetic diversity was also studied, based on the contribution of the components of  $D^2$  to the total observed dissimilarity.<sup>(15)</sup>

The cophenetic correlation coefficient was estimated to test the efficiency of the hierarchical clustering method. Data were obtained from 1,000 simulations and analyzed using a t-test. The cutting point ( $P_c$ ) of the dendrogram generated by the UPGMA method was defined according to a previously established criterion,<sup>(16)</sup> following the formula

$P_c = m + kdp$ , where  $m$  is the mean of the distance values of the fusion levels corresponding to the stages;  $k = 1.25$ ;<sup>(17)</sup> and  $dp$  is the standard deviation.

Statistical analyses were conducted using a completely randomized design (CRD), although the nature of the experiment did not fully meet all the assumptions required by the method. Due to the nature of the experiment, which required frequent crosses, complete randomization was not feasible. However, the design was considered adequate for processing this fraction of the data.

Data analysis was performed using the GENES software.<sup>(18)</sup>

## RESULTS AND DISCUSSION

Significant differences were found among accessions for all evaluated traits, according to the F-test ( $p < 0.05$ ) (Table 2). This result demonstrates the existence of phenotypic variability and highlights the need for clustering techniques, such as Tocher's and UPGMA, to identify the most divergent genotypes based on Mahalanobis generalized distance ( $D^2$ ).

Singh's method<sup>(15)</sup> assumes that the most important traits express greater variability. Using this method, it is possible to verify the relative importance of the analyzed traits in genetic dissimilarity. In this case, longitudinal diameter (29,8%), transverse diameter (18,3%), and fruit shape index (11,8%) were the traits that contributed the most to the divergence among the evaluated accessions, explaining 59,9% of the total genetic dissimilarity (Table 3). Analogous results were observed in a previous study,<sup>(19)</sup> who, upon evaluating 38 distinct melon accessions, identified the longitudinal diameter as the characteristic exhibiting the greatest contribution to variability. It has been pointed out that the most important traits for estimating diversity among genotypes are those that exhibit the greatest variability.<sup>(13)</sup>

Based on the evaluated morphological variables, a dissimilarity matrix of the 25 melon genotypes was generated. The estimated dissimilarity coefficients ranged from 0.00 to 512.0, indicating the existence of genetic diversity among the materials used. The dissimilarity measures based on Mahalanobis generalized distance ( $D^2$ ) among genotypes ranged from 3.99 to 512.01, with a mean of 104.10. The greatest distance was recorded between accessions 16 and 24, and the smallest distance between accessions 8 and 22 (Figure 1).

The average genetic dissimilarity between each pair

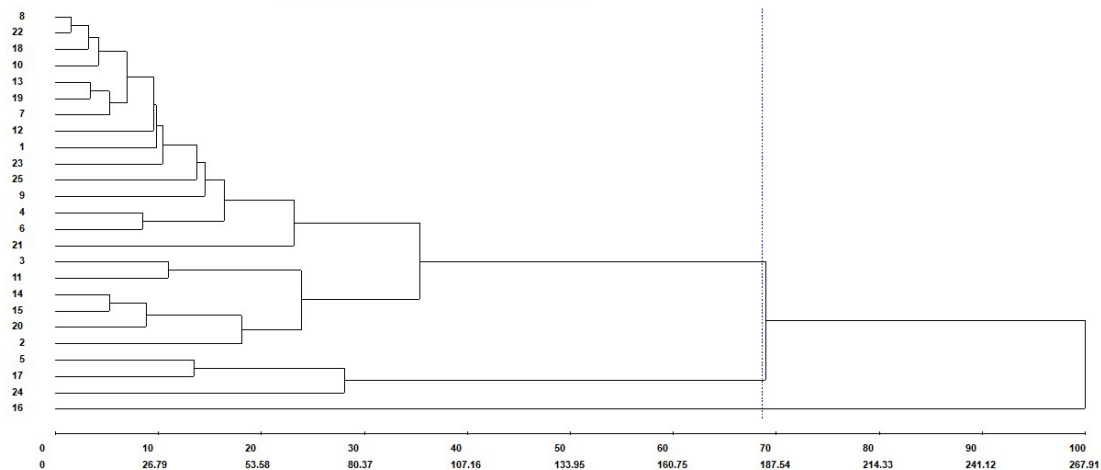
**Table 2.** Means of 12 traits evaluated in 25 melon accessions at UFRPE, Recife, PE, 2023

Accesses	FM	LD	TD	FSI	LIC	TIC	PET	PUT	FIR	SS	CA	CO
1	1223	17.5	13	1.3	7	11.5	1.4	29.1	16.4	8.5	6.3	20.5
2	315.6	10.3	8.2	1.2	6	7.4	1.6	11.7	21.8	7.7	4.8	13
3	570.4	16.9	7.9	2.1	3.9	13.1	1.2	17.2	51.4	4.4	5.7	10.2
4	2381	22.7	15.8	1.4	10.3	18.3	1.8	24.4	22.2	3.5	6.2	17
5	2068.6	32.5	12.9	2.5	6.9	25.6	1.6	29.3	33.2	4.4	6.2	13.9
6	2566.4	21.4	16.8	1.2	7.9	15.2	1.8	39.7	23.9	5	6.5	13.9
7	1469.2	16.3	13.2	1.3	5.9	10.9	1	25.1	21.3	6.1	6.3	16.3
8	1347	14.7	13.7	1	6.3	9.7	1.7	32.6	16.6	7.1	6.1	13.9
9	1546.2	15	14.3	1	6.4	9.9	1.2	37.3	29	6.7	4.1	6.9
10	1800.4	15.7	15.2	1	6.7	10.2	1.2	39.8	27.8	8	5.3	12.7
11	785.2	11	10	1.1	4.7	6.9	0.5	26.2	65.5	3.6	5.9	12.5
12	1065.1	15.2	11.2	1.3	6.9	11.2	0.8	22.5	38.2	4.2	5.9	18.9
13	1533.6	17.2	13.3	1.2	7.1	11.8	0.8	29	23.2	7.9	6.3	17
14	49.4	5.24	3.9	1.3	2.8	4	0.8	5.6	41.6	5.9	5	10.7
15	163	8.26	5.5	1.4	4.8	7.2	0.4	3.4	45.5	5.2	5	11.8
16	192.8	7.72	6.5	1.1	3.9	5.7	4.9	1.3	93.2	4.3	5.2	11.4
17	1821.8	37.6	10.1	3.7	5.2	31.4	1.6	24.3	37.6	2.7	7.1	18.6
18	1612.9	14.8	14.6	1	6.8	9.3	1.7	40.5	15.4	5.3	6	13.5
19	986	12.9	12.6	1	6.9	7.8	0.6	25.8	16.7	9.5	5.9	13
20	147.2	7.84	5.7	1.3	3.7	5.9	0.3	9.25	31.6	5.5	6.6	16.8
21	551.2	15.9	9.1	1.7	5.4	11.6	1.2	15.3	17.3	5.1	6.4	24.8
22	1475.6	15.7	13.4	1.2	6.9	11	1.7	29.4	21.1	6.1	5.7	12.6
23	1190.8	17.4	11.8	1.4	5.9	9.5	1.9	28	17.3	4.8	6.7	15.1
24	1247.4	36.6	8.5	4.2	5.6	32.5	1.5	13.6	21	3.8	6.4	11.6
25	1514.6	19.1	14	1.3	7.7	14.1	0.3	27.2	23.8	10.7	6	14.7
CV (%)	27.4	12.2	10.6	15.3	16.7	16.4	32.5	22.6	31.7	20.1	8	14.8
SM <sub>treatment</sub>	2567091.4**	350.6**	62.7**	3.6**	13.6**	290.3**	4.2**	641.2**	1931.4**	17.0**	2.3**	85.5**
Media	1184.9	17	11.2	1.58	6.1	12.5	1.3	22.6	30.9	5.8	5.9	14.5

\*\* Significant by the F-test at a 5% probability level. FM=fruit mass; LD= longitudinal diameter; TD=transverse diameter; FSI=fruit shape index; LIC=longitudinal internal cavity; TIC=transverse internal cavity; PET=peel thickness; PUT=pulp thickness; FIR=pulp firmness; SS=soluble solids content and CA= calyx length and CO=corolla length.

**Table 3.** The relative contribution of quantitative traits to genetic divergence among 25 melon accessions, using Singh's method, UFRPE, Recife, PE, 2023

Characters	Relative contribution (%)
Longitudinal diameter (cm)	29.8
transverse diameter (cm)	18.3
Fruit shape index	11.8
Peel thickness (mm)	7.4
Pulp firmness (N)	7.3
Corolla length (mm)	6.0
Pulp thickness (mm)	6.0
Longitudinal internal cavity (cm)	4.4
Soluble solids content (°Brix)	4.3
Calyx length (mm)	2.3
Transverse internal cavity (cm)	2.0
Fruit mass (g)	0,4



**Figure 1.** Dendrogram obtained by the UPGMA clustering method, using Mahalanobis distance ( $D^2$ ) resulting from the analysis of 25 melon accessions. UFRPE, Recife, PE, 2023.

**Table 4.** Grouping by the Tocher method, using Mahalanobis distance ( $D^2$ ) resulting from the analysis of 25 melon accessions. UFRPE, Recife, PE, 2023

GROUP	ACCESSES
1	8, 22, 18, 10, 7, 19, 12, 1, 23, 6, 9, 25, 4, 2, 21, 3, 11, 20, 15, 5, 14
2	17 e 24
3	16

of genotypes was used to construct a dendrogram using the UPGMA hierarchical method. A significant cut-off of 70%<sup>(16)</sup> allowed the formation of three similarity groups (Figure 1). With this cut-off, three distinct groups were formed: group 1 consisted of 21 accessions (84% of the evaluated accessions), including the commercial hybrid Eldorado 300 (Figure 1). Group 2 consisted of three accessions (5, 17, and 24), and group 3 was represented only by accession 16, showing divergence from the others by forming an exclusive group, which could be explored in breeding programs. This distribution indicates that, regarding the evaluated traits, most genotypes showed high levels of similarity.<sup>(20)</sup>

The cophenetic correlation coefficient ( $r$ ) was 0.84, confirming a good fit between the graphical representation of the distances and the original matrix. According to Rohlf,<sup>(21)</sup> the fit of the cophenetic correlation coefficient is considered good when it is equal to or greater than 0.70. In this case, the higher the ( $r$ ), the lower the distortion of the clustering, indicating a good fit between the matrix and the resulting dendrogram.<sup>(12)</sup>

The grouping of genotypes by the UPGMA method showed partial similarity to the Tocher method when forming groups among the most divergent genotypes (Table 4).

The similarity between the different clustering techniques can be observed by the fact that the genotypes belonging to groups 2 and 3 of Tocher were, for the most part, the same as those of the UPGMA clustering. Accession 5 was the sole accession to be grouped distinctly between the two analytical methods.

In general, multivariate analysis methods (UPGMA and Tocher), used to estimate genetic dissimilarity, have shown to be similar and efficient, constituting important tools for finding good parents.<sup>(22)</sup>

## CONCLUSION

Genetic divergence was observed among the melon accessions, with partial congruence (96%) between the two clustering methods employed. The characteristics exhibiting the greatest influence on this divergence were fruit longitudinal diameter, fruit transverse diameter, and fruit shape.

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## DATA AVAILABILITY

We declare that all data are contained within the manuscript.

## AUTHOR CONTRIBUTIONS

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