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MORPHOLOGICAL CHARACTERIZATION AND GENETIC DIVERSITY BASED ON THE MELON ROOT SYSTEM

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Abstract

Studies on plant root system architecture may yield valuable data for genetic improvement programs for developing cultivars that acquire water and nutrients more efficiently. This study used morphological descriptors to characterize the root system and evaluate genetic diversity among 30 melon accessions. A completely randomized experiment was conducted with 30 treatments and 5 replications. The plot consisted of one seedling. After seed germination, five seedlings were fixed in a dried growth medium for 12 days. Then, they were scanned and measured for primary root length and primary root neck diameter. Root hairs were visually evaluated. Finally, basal root angles were measured. The accessions diverged genetically regarding the root system morphological characteristics. The primary root neck diameter contributed the most to the dissimilarity between the accessions (43.06%). The A-50 accession stood out for the highest mean for morphological characteristics. It may represent a reference in genetic improvement programs to develop cultivars that acquire water and nutrients more efficiently.

Keywords: *Cucumis melo*. Genetic variability. Root morphology.

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1. Introduction

Melon (*Cucumis melo* L.) is a vegetable from the Cucurbitaceae family, originating in Central Asia and Africa (Lija and Beevy 2021). It is a commercially relevant species worldwide, characterized by its adaptation to different environments with high temperatures, light, and low relative humidity (Aragão et al. 2019).

In recent years, melon cultivation has gained prominence in the Brazilian agricultural market due to increased cultivated areas, the use of new technologies, and higher production (Nunes et al. 2016). The Brazilian Northeast has a significant share, accounting for more than 98% of national production and exports,

with emphasis on Mossoró-Assú and Vale Baixo Jaguaribe agro-clusters in Rio Grande do Norte and Ceará, respectively (IBGE, 2025).

Besides its high economic value, this species is the most polymorphic of the genus *Cucumis*. It demonstrates wide genetic variability in leaves, flowers, and fruits (Nunes et al. 2016), which motivates studies on diversity. The analysis of genetic diversity is a significant step for improving agronomic species. It allows the identification of divergent genotypes for future crosses (Silveira et al. 2021; Barbosa et al. 2023; Zhang et al. 2023).

The literature on genetic diversity and morpho-agronomic characterization of melons is often linked to yield traits (Aragão et al. 2013; Dantas et al. 2015; Macêdo et al. 2017; Gomes et al. 2021). However, root features are little explored. The root system architecture is also linked to increased productivity in agricultural crops. Root morphology characteristics, such as length, diameter, density, and insertion angle, may directly influence soil spatial exploration and the acquisition of water and nutrients (Lynch et al. 2021). Besides assisting in the fixation of plants in the soil, root hairs are crucial for the uptake of water and poorly mobile nutrients in uneven environments (Vaz-de-Melo et al. 2013; Taiz et al. 2017; Nascimento et al. 2023).

Studies that analyze root system architecture are relevant in providing data that may help genetic improvement programs to develop cultivars with more efficient soil exploitation for water and nutrient acquisition (Lynch et al. 2024). Research was conducted to evaluate root architecture in bean (Ho et al. 2005), soybean (Gonçalves and Lynch, 2014), rice (Uga et al. 2013), corn (Vaz-de-Melo et al. 2013), and tomato (Alaguero et al. 2018) genotypes. However, only a few studies examine genetic diversity through the morphological characteristics of the melon root system (Dias et al. 2004; Fita et al. 2006; Fita et al. 2011).

Therefore, this study characterized the root system and evaluated genetic diversity among 30 melon accessions according to morphological descriptors.

2. Material and Methods

Experiment site

The experiment was conducted in the Plant Production and Technology and Seed Production laboratories of the Jundiaí Agricultural School – Academic Unit Specialized in Agrarian Sciences, in Macaíba, RN, Brazil, from March to June 2021.

Genotypes evaluated

The experiment followed a completely randomized design. It included 30 treatments corresponding to 30 accessions belonging to the melon germplasm collection of the Federal Rural University of the Semi-Arid. The experiment had 5 replications, and the plot comprised one seedling (Table 1).

Table 1. Classification of accessions used in the experiment.

Accession	Horticultural group	Accession	Horticultural group	Accession	Horticultural group
A-02	*Momordica*	A-25	*Momordica*	A-36	*cantalupensis*
A-07	*cantalupensis*	A-26	*cantalupensis*	A-39	*Momordica*
A-08	*cantalupensis*	A-27	*cantalupensis*	A-41	*cantalupensis*
A-09	*conomon*	A-28	*cantalupensis*	A-42	*Momordica*
A-10	*cantalupensis*	A-29	*cantalupensis*	A-43	*inodorus*
A-11	*conomon*	A-30	*Momordica*	A-44	*cantalupensis*
A-14	*cantalupensis*	A-31	*cantalupensis*	A-45	*cantalupensis*
A-16	*acidulus*	A-33	*Momordica*	A-50	*Momordica*
A-17	*cantalupensis*	A-34	*Momordica*	A-51	*Momordica*
A-22	*Momordica*	A-35	*Momordica*	A-52	*Momordica*

Preparation of seeds and evaluation

A sample of 10 seeds underwent an aseptic process using sodium hypochlorite (0.5%) immersion for 1 minute. Then, it was washed with distilled water. The methodology used in the germination test followed the Rules for Seed Analysis (Brasil, 2009). We used Germitest paper sheets moistened with 2.5 times the mass of the dry substrate. Then, the seeds were uniformly arranged on Germitest paper, wrapped in rolls, and placed in plastic bags labeled for each treatment. The bags were placed vertically in BOD germination chambers under controlled light conditions with 12 hours of light and 12 hours of dark, 80% humidity, and 28°C temperature. They remained in the chambers for 72 hours.

Subsequently, five seedlings with approximately 3 cm of radicle growth were selected per treatment to be fixed in the dried growth medium. Each growth sheet consisted of one Germitest paper sheet folded in half, wrapped in a polyethylene plastic bag, and uniformly perforated with 2-cm-diameter holes for improved aeration.

These dried samples were vertically fixed in a rectangular glass box (aquarium) measuring 30cm wide x 40cm high x 50cm long and containing a distilled water solution up to 14cm high. A wire supported the upper portion of the dried growth medium along the sides of the aquarium, allowing it lower portion to remain immersed in the solution to a height of 5 cm. Regarding capillarity, the solution moistened the entire dried growth medium, which allowed the root system to develop for 12 days. At the end of this period, each germitest sheet containing the root was removed from the polyethylene plastic bag and scanned at 300 dpi resolution with an HP scanner (Vaz-de-Melo et al. 2013).

Variables measured

The length (cm) and neck diameter (mm) of the primary root were measured using a graduated ruler and a digital caliper. Root hairs were visually evaluated using a stereoscope, as per Vieira et al. (2007). A scale from 1 to 10 was employed, where 1 corresponds to the absence of root hair and 2, 3, 4, 5, 6, 7, 8, 9, and 10 corresponds to 20, 30, 40, 50, 60, 70, 80, 90, and 100% of the entire root system with root hairs, respectively. Using “PHOTOSHOP CS6 v13.0” image editing software for Windows, the angles of the basal roots were measured, obtaining the general mean value. The primary root growth axis served as a parameter for measuring the angles.

Statistical analysis

Univariate analyses of variance were performed for all evaluated characteristics, and the means were grouped according to the Scott-Knott test. Subsequently, a multivariate analysis of variance and the Wilks criterion at 5% significance were performed. Cluster analyses were performed using a Euclidean distance matrix among accessions, applying hierarchical clustering with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) implemented via the Pheatmap package in R (Kolde, 2010). The Singh criterion identified the relative contribution of each trait to the genetic divergence between accessions (Cruz et al. 2014).

3. Results

All morpho-agronomic features of the analyzed root yielded significant effects ($p < 0.05$), indicating genetic variability among the evaluated melon accessions (Table 2). The characteristic angle of basal roots exhibited a 13.1% coefficient of variation. The primary root length, the primary root neck diameter, and root hair grades presented coefficient of variations of 46.31%, 22.06%, and 31.64%, respectively (Table 2).

Based on the Scott-Knott test (5% probability), the primary root neck diameter presented the highest diversity among the accessions, yielding four groups. The primary root length, root hairs, and mean angle formed two groups (Table 3).

Table 2. Summary of the analysis of variance and coefficient of variation (CV%) of the characteristics: primary root length (RL - cm), primary root neck diameter (ND - mm), root hair grades (GRH – grade scale from 0 to 10), and the mean angle between the horizontal axis of seedling growth and basal lateral roots (ANG - degrees).

SV	DF	Mean square			
		RL	ND	GRH	ANG
Treatment	29	54.766	0.795	21.768	397.28
Residue	120	9.605	0.068	3.773	102.81
CV (%)		46.31	22.06	31.64	13.1
F		5.701*	11.669*	5.769*	3.864*

*Significant by the 5% F test; SV: Source of variation; DF: Degrees of freedom.

Table 3. Means of primary root length (RL - cm), primary root neck diameter (ND - mm), root hair grades (GRH – grade scale from 0 to 10), and the mean angle between the vertical axis of seedling growth and basal lateral roots (ANG - degrees).

Access	RL	ND	GRH	ANG
A-02	2.22^{b}	1.40^{b}	7.4^{a}	73.64^{b}
A-07	5.24^{b}	1.32^{b}	8.8^{a}	93.24^{a}
A-08	4.40^{b}	1.12^{c}	5.4^{b}	74.39^{b}
A-09	5.86^{b}	1.16^{c}	6.0^{a}	82.09^{a}
A-10	5.56^{b}	1.18^{c}	7.0^{a}	93.04^{a}
A-11	3.08^{b}	0.66^{d}	2.8^{b}	80.32^{a}
A-14	3.30^{b}	1.12^{c}	8.0^{a}	80.65^{a}
A-16	4.28^{b}	0.62^{d}	3.2^{b}	70.34^{b}
A-17	4.46^{b}	1.54^{b}	8.8^{a}	82.91^{a}
A-22	4.98^{b}	0.74^{d}	7.2^{a}	72.10^{b}
A-25	5.44^{b}	0.84^{d}	7.6^{a}	69.37^{b}
A-26	5.66^{b}	0.92^{d}	8.2^{a}	67.87^{b}
A-27	5.34^{b}	1.12^{c}	7.0^{a}	81.57^{a}
A-28	3.76^{b}	1.04^{c}	8.2^{a}	82.16^{a}
A-29	7.72^{b}	0.82^{d}	7.4^{a}	69.91^{b}
A-30	5.58^{b}	0.68^{d}	3.6^{b}	59.80^{b}
A-31	11.64^{a}	0.90^{d}	2.8^{b}	79.04^{a}
A-33	5.72^{b}	0.62^{d}	6.4^{a}	68.9^{b}
A-34	5.64^{b}	0.70^{d}	6.2^{a}	78.66^{a}
A-35	13.50^{a}	1.16^{c}	3.0^{b}	80.75^{a}
A-36	13.94^{a}	1.78^{a}	2.8^{b}	85.91^{a}
A-39	6.36^{b}	1.94^{a}	6.0^{a}	74.59^{b}
A-41	5.32^{b}	2.06^{a}	8.0^{a}	72.99^{b}
A-42	4.92^{b}	1.24^{c}	9.2^{a}	72.31^{b}
A-43	9.4^{a}	1.20^{c}	4.0^{b}	56.02^{b}
A-44	13.44^{a}	1.34^{b}	5.2^{b}	77.53^{a}
A-45	8.70^{b}	1.80^{a}	5.8^{a}	75.39^{b}
A-50	13.40^{a}	1.52^{b}	9.2^{a}	85.63^{a}
A-51	5.20^{b}	1.50^{b}	4.4^{b}	92.27^{a}
A-52	6.70^{b}	1.42^{b}	4.6^{b}	87.96^{a}
Mean	6.69	1.18	6.14	77.38

Means followed by the same letters in the columns belong to the same statistical group, by the Scott and Knott test at 5% probability.

The primary root length (RL) ranged from 2.22 (A-02) to 13.94 cm (A-36) (Table 3). Accessions A-31, A-35, A-36, A-43, A-44, and A-50, clustered in the first group, stood out for presenting larger primary roots, while 80% of accessions had smaller primary roots than 8.70 cm, without significant differences.

The root neck diameter (ND) means values ranged from 0.62 to 2.06 mm (Table 3), joining the accessions into four groups. Accessions A-36, A-39, A-41, and A-45 formed the group with the largest neck diameter. Accession A-41 stood out for the largest primary root diameter. The second group consisted of 23% of accessions, ranging from 1.32 to 1.54 mm. The third group comprised 30% of accessions. Finally, the last group included 33% of accessions with the lowest mean estimates.

The mean root hair (GRH) grades ranged from 2.8 to 9.2 (Table 3), forming two groups. The means of 63% of accessions in the first group were above 5.8. Accessions A-42 and A-50 stood out for yielding the highest root hair density values, with a mean of 9.2.

The means of accessions for the characteristic basal root angle (ANG) varied from 56.02 to 93.24° (Table 3). The first group was formed by accessions with higher angles between the basal and primary roots. Accession A-07 stood out with a higher mean (93.24°). Thus, the accessions in this class present more superficial basal roots. Approximately 47% of accessions were placed in the second group, with means lower than 75.39°.

The UPGMA cluster analysis revealed five groups formed among accessions (Figure 1). The first group corresponded to A-50. This accession stood out for yielding higher means in all analyzed characteristics. The second group, consisting of accessions A-36, A-44, A-31, and A-35, presented higher values for primary root length, primary root neck diameter, and basal root angle, and lower values for root hair grades.

The third group was formed with accessions A-11, A-16, A-30, and A-43 (Figure 1). This group presented lower mean values for primary root length, primary root neck diameter, root hair grade, and basal root angle. Accessions A-41, A-39, and A-45 were allocated to the fourth group. They demonstrated higher mean values for primary root neck diameter and root hair grade, and lower values for primary root length and basal root angle.

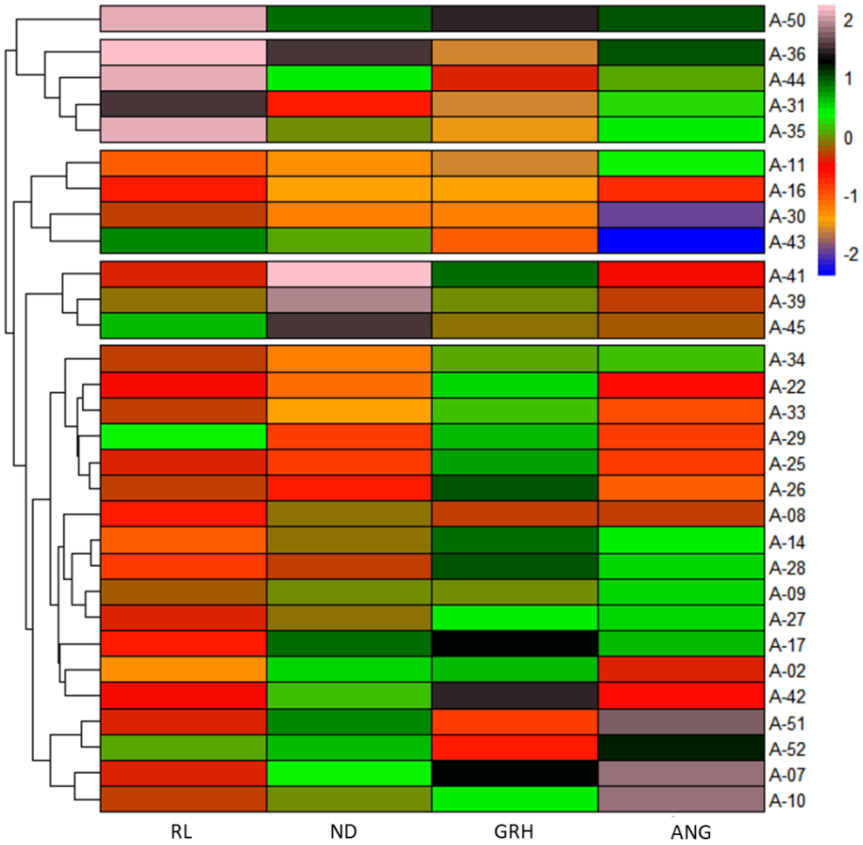

Figure 1. Dendrogram generated using the UPGMA method from the Euclidean distance matrix among 30 melon accessions for root morphology characteristics. RL: primary root length (cm); ND: Primary root neck diameter (mm); GRH: Root hairs grade (grade scale from 0 to 10); ANG: Mean angle between the horizontal axis of seedling growth and basal lateral roots (degrees). Cophenetic correlation coefficient (r = 0.80**).

Finally, accessions A-34, A-22, A-33, A-29, A-25, A-26, A-08, A-14, A-28, A-09, A-27, A -17, A-02, A-42, A-51, A-52, A-07, and A-10 constituted the fifth group (Figure 1). This group yielded higher means for root hair grades and lower values for primary root length. Accessions A-51, A-52, A-07, and A-10 stood out for presenting higher mean values for basal root angle and primary root neck diameter than the other accessions in the group.

A cophenetic correlation coefficient higher than 0.7 suggests a good fit of the clustering method (Figure 1). The groups formed using this method are reliable, as the estimated cophenetic correlation coefficient in this study exceeded the reference value (r = 0.80**).

The root morphology characteristics used in this study allowed the visualization of species phenotypic plasticity. The primary root neck diameter contributed the most to genetic divergence (43.06%) (Table 4). Breeders should use this information as a parameter for selecting cultivars with higher water and nutrient acquisition efficiency. Singh's contribution demonstrates the characteristic root hair grades throughout the root system (21.42%) and primary root length (20.42%). The angle between basal and primary roots contributed the least to the divergence (15.08%).

Table 4. Contribution of root morpho-agronomic traits to genetic divergence among melon accessions.

Characteristic	S_j (%)
Primary root length	20.42
Primary root neck diameter	43.06
Root hair grades	21.42
Angle	15.08
Total	100.00

S_J (%): Relative contribution of variables (Singh 1981).

4. Discussion

The root is a key plant organ. It is responsible for fixing and supporting the plant in the soil, uptaking and conducting water, and acquiring mineral elements. It is also a source of starch storage. A vigorous and healthy root system offers benefits to the plant, such as the interaction with valuable soil microorganisms and tolerance to environmental stresses (Fita et al. 2006; Lynch et al. 2021).

The primary root length findings of the present study corroborate those of Fita et al. (2011). They found melon root architecture ranging from 4.6 to 14.6 cm. These authors verified that the accessions from the groups *Momordica*, *flexuosus*, *Dudaim*, and *inodorus* yielded the highest means, while those from the groups *agrestis* and *conomon* presented the lowest means. The A-36 accession from the cantalupensis group and the A-50 accession from the Momordica group presented the highest means for most analyzed variables. Melon has a shallow, highly branched root system, and approximately 80% of melon roots are concentrated in a layer up to 20cm deep. In this sense, melon accessions with a deeper root system can explore a larger soil volume in search of water and nutrients (Nunes et al. 2016).

Accessions A-36, A-39, A-41, and A-45 exhibited larger root neck diameters, and A-41 stood out for producing the highest value. The root diameter and its ability to penetrate compacted soils are strongly correlated. Roots with larger diameters can exploit a higher soil volume (Lynch, Chimungu, and Brown, 2014). Root diameter is a significant morphological feature. It has the potential to increase plant adaptation to water stress (Yuguda et al. 2020; Zhuo et al. 2020). Neck diameter is also a vital trait in selecting compatible rootstocks (Silva et al. 2025).

Besides assisting in the fixation of plants in the soil, root hairs are crucial for the uptake of water and poorly mobile nutrients (Taiz et al. 2017). In this research, accessions A-42 and A-50 from the Momordica group stood out for presenting the highest root hair density. Studies report a positive correlation between root hair density and phosphorus acquisition (Miguel et al. 2015; NIU et al. 2013). Bayuelo-Jiménez et al. (2012) also showed that increased root hair density and length in primary and seminal roots of maize genotypes were stimulated by soil phosphorus deficiency. Jungk (2001) found that root hairs are genetically controlled. Therefore, genotypes with higher length and root hair density values may be used in genetic improvement programs (Lynch 2022).

The arrangement of angles between basal and primary roots is the source of variation in plant root architecture, even within the same species. Studies suggest that basal root angulation determines whether roots will dig deep in the soil or remain in surface layers (Vieira et al. 2007; Fita et al. 2011). Plants with a superficial root system, i.e. basal roots with larger angles than the primary root, acquire immobile nutrients more efficiently, while roots with smaller angles are more efficient in absorbing water (Ho et al. 2005; Lynch, 2019; Lynch et al. 2024).

Root architecture directly influences the uptake of water and mineral nutrients with low mobility, which are mainly in the soil surface layers, especially phosphorus (Gonçalves and Lynch, 2014; Li et al. 2022). The dynamic modulation of root architecture over time by genotype-environment interactions determines root plasticity responses and allows plants to efficiently adapt to environmental constraints (Koevoets et al. 2016).

Fita et al. (2011) evaluated root architecture diversity among 12 melon accessions from different groups. They found that the different architectural arrangements of the root system directly influence phosphorus use efficiency. Among the accessions studied by Fita et al. (2011), the most efficient belonged to the horticultural groups *conomon* and *Momordica*, respectively. Low nutrient availability in the soil and water stress may restrict global agricultural production. Currently, plant genetic improvement programs are researching the selection of promising genotypes in water and nutrient acquisition through basal root angulations in soybeans (Gonçalves and Lynch, 2014), beans (Ho et al. 2005), rice (Uga et al. 2013) and melon (Li et al. 2022).

In non-irrigated crops, roots with smaller angles are crucial to absorbing water in deeper soil layers (Paez-Garcia et al. 2015). Melon crops are predominantly drip-irrigated, so employing genotypes with shallow basal roots may yield higher water uptake efficiency by exploiting surface soil layers (Pereira et al. 2021).

Our findings are highly relevant, as they provide new information on melon breeding programs. This research advances our knowledge of melon root phenotyping and presents practical implications for melon breeding programs in Northeastern Brazil. The findings contribute to the existing body of knowledge by showing the development of the melon root system in different genotypes and its architecture. This information benefits breeders, as they can consider it in the selection process.

These findings allow future studies to explore melon breeding programs that aim to produce varieties with a more robust root system, capable of exploring a higher soil volume to uptake water and nutrients. Another approach is to obtain a rootstock. We plan to extend our root phenotyping studies to additional melon accessions and commercial cultivars and test their performance against nutrient deficiency and water stress. Additionally, the employed methods and approaches may be applied to other areas, making this research a valuable biotechnology tool to understand the genetic mechanisms controlling root development. Validating the performance of accession A-50 under abiotic stresses, such as drought or low nutrient availability, is vital to confirm its agronomic potential. Its superior root morphology traits suggest higher efficiency in water and nutrient uptake under limiting conditions, but this advantage must be experimentally verified. Comparing A-50 with a commercial cultivar or a contrasting genotype would help contextualize its performance and determine whether it surpasses widely used materials. Thus, controlled experiments under abiotic stress would provide robust evidence to support the use of A-50 in breeding programs aimed at improving resource-use efficiency.

5. Conclusions

Accessions differ genetically according to root system morphology characteristics. The primary root neck diameter contributed the most to the dissimilarity between the studied melon accessions. Accession A-50 stood out for presenting the highest means for the evaluated morphological characteristics. It may be used as a source of alleles in genetic improvement programs.

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