



Ora-pro-nóbis (*Pereskia aculeata* Mill.) propagated by seeds and cuttings under water deficit and biological indicators of soil quality

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ABSTRACT. Ora-pro-nóbis (*Pereskia aculeata* Mill.) is a protein-rich member of the Cactaceae with great potential for cultivation in regions where water can be a limiting factor. The aim of this study was to verify the tolerance of this species to water deficiency and determine how microbial biomass and soil respiration are affected under these conditions. Five treatments were tested, namely control and irrigation suspension for 7, 14, 21, and 28 consecutive days, in plants propagated by seeds and cuttings. The decrease in soil moisture and soil water potential (Ψ_s) after 28 days of water restriction highlighted the severe impact of a lack of water on plants, especially those propagated by cuttings, which showed greater susceptibility to water stress (leaf potential -2.84 MPa). Seedlings, although affected in terms of root development and fresh mass at 28 days (leaf potential of -1.59 MPa), showed advantages in terms of shoot fresh matter and plant diameter, suggesting that propagation by seeds and root development are crucial for water stress resistance. The microbial biomass carbon, in general did not vary among the irrigation suspensions times both in plants propagated by seeds and cuttings, reinforcing the importance of microbial adaptation to stressful conditions.

Keywords: water stress; non-conventional food plants; soil physics; WP4; water potential.

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Introduction

Cacti are characterized by their ability to establish themselves in environments in which a lack of water is a limiting factor. Among the cacti of relevance for human use, ora-pro-nóbis (*Pereskia aculeata* Mill.; OPN) stands out, as a shrubby unconventional food plant (UFP/PANC) with long, evergreen branches. Native to the south, southeast, northeast and central-west regions of Brazil (Powow, 2024), it is particularly common in Minas Gerais, where it is of both ornamental and dietary importance and is used in folk medicine. This cactus, characterized by its true, succulent leaves, has low or no incidence of pests and diseases and is a valuable source of proteins and amino acids for human food.

OPN is resistant and adapts well to a variety of soils, including those that have not necessarily been fertilized (Vega et al., 2020). Many studies on this plant have been carried out for medicinal purposes, as it has properties that reduce the perception and transmission of stimuli that cause pain (Silva Porto et al., 2022) and contains antioxidants (Pinto et al., 2020), a high fiber content, considerable amounts of calcium, magnesium, manganese and zinc, vitamins A and C, and folic acid in the fresh leaves (Takeiti et al., 2009). The protein content is around 25% in dry matter (Amaral et al., 2019; Takeiti et al., 2009; Vega et al., 2020).

In a previous experiment, OPN retained its leaf area and showed no leaf drop when subjected to a water potential of up to -70 kPa (-0.07 MPa) (Queiroz et al., 2015). However, this value is not enough to conclude whether the plant is drought tolerant, as several species show tolerance to greater ranges (-1.4 to -3.2 MPa), including C3 crops, such as bean (*Phaseolus vulgaris*; -0.6 to -1.2 MPa) (Chaves et al., 2003), soybean (*Glycine max*; -0.7 to -1.5 MPa) (Xu et al., 2023) and the C4 maize (*Zea mays*; -0.5 to -1.5 MPa), which is more sensitive to water deficits, especially during the flowering phase (Pedreira et al., 2008). OPN can develop under adverse conditions with unfavorable water potentials, which are the main limiting environmental factors for agriculture in arid and semi-arid regions. Thus, OPN is likely to tolerate even lower water potentials.

Water deficit also significantly affects the soil microbial community, altering both its composition and activity. Water scarcity can reduce microbial biomass, which is essential for organic matter decomposition and nutrient release to plants. During drought, the microbial structure can change, favoring organisms that are more resistant to dehydration, such as certain groups of bacteria and fungi, while more sensitive microorganisms can be reduced or eliminated. In addition, water deficit can influence interactions between plants and microorganisms. For example, a reduction in root exudation due to drought can decrease the beneficial microorganism populations, such as growth-promoting rhizobacteria, which depend on these exuded compounds to survive. These changes can have long-lasting effects on soil fertility and plant health since the recovery of microbial communities after periods of prolonged drought can be slow and incomplete, affecting the soil's ability to support crop growth.

Research results suggest that the response of plants to water stress may be mediated by changes in the structure of the soil microbial community induced by the lower water availability in the soil (Eke et al., 2019; Mehrasa et al., 2022). Some microorganisms, such as fungi and bacteria, have strategies to overcome water stress and are able to tolerate rapid changes in the soil water potential (Buchmann et al., 2020; Gui et al., 2023), favoring and promoting plant growth (Eke et al., 2019). The few studies carried out on the tolerance of OPN to water deficit are inconclusive. Therefore, it is necessary to determine the water use efficiency of Cactaceae and the behavior of the soil microbial biomass under water stress. The aim of this study was to verify how tolerant this species is to water stress when propagated by cuttings and seeds and determine the microbial biomass and respiration under different periods of water restriction.

Material and methods

Experimental project

The work was carried out in a greenhouse at the Federal University of Lavras, Department of Soil Science, located in the municipality of Lavras, Minas Gerais State, Brazil. During the experiment, the humidity was close to 77%, and the minimum and maximum temperatures were 21.8 and 26.2°C, respectively, between February 28 and June 3, 2020. The experiment had a randomized block design with a 2 × 5 factorial scheme: 2 propagation methods (seeds and cuttings), 5 irrigation application treatments (irrigated control throughout the experiment and irrigation suspension for 7, 14, 21, and 28 consecutive days until the end of the experiment) with 4 replications (Figure 1).

Propagation methods

To produce cuttings, the terminal portions of the branches were collected during the vegetative period of the mother plant, cleaned and then cut to form conical cuttings 15 cm in length and 5 mm in diameter. To prevent the tissues from dehydrating, they were kept in a glass of water until they were inserted into tubes (one cutting per tube) with one-third covered by a substrate and kept in a greenhouse. The seeds were placed in Petri dishes containing moistened and sterilized absorbent cotton and filter paper. Seed germination was monitored for 15 days in an incubator at 28°C until the main root emerged. After germination, the seeds were transplanted into tubes (one germinated seed per tube). The surface of the propagating material was disinfected using 70% ethanol for 1 min., followed by treatment with 1% sodium hypochlorite for 3 min., and then rinsed 6 times with distilled water.

The substrate used to produce the seedlings from seeds and cuttings was a mixture of washed sand passed through a 2 mm sieve and vermiculite, in a 1:1 ratio, packed in 280 cm³ tubes. Seedlings and cuttings were grown in the tubes for 45 days and then transferred to permanent growing containers with a capacity of 3.45 L.

To maintain substrate moisture and meet the nutritional needs of the seedlings under ideal temperature and water conditions, irrigation during acclimatization was carried out every 3 days with 40 mL of complete nutrient solution (Hoagland & Arnon, 1950), with the following composition of stock solutions added to 4 L of water: 4 mL of 236.16 g L⁻¹ CaN₂O₆·4H₂O; 1 mL of 115.03 g L⁻¹ NH₄ H₂PO₄; 6 mL of 101.11 g L⁻¹ KNO₃; 2 mL of 246.9 g L⁻¹ MgSO₄·7H₂O; 1 mL of 10 g L⁻¹ FeCl₃; and 1 mL of micronutrients (2.86 mg L⁻¹ H₃BO₃; 2.03 mg L⁻¹ MnSO₄·4H₂O; 0.22 mg L⁻¹ ZnSO₄·7H₂O; 0.08 mg L⁻¹ CuSO₄·5H₂O; and 0.09 mg L⁻¹ Na₂MoO₄·H₂O).

Experimental design

The substrate used in the final containers was a typical Latossolo Vermelho distroférrico (LVdf) (Curi et al., 2020) with a very clayey texture, collected on the campus of the Federal University of Lavras. The water supply

in the experiment after the acclimatization period was by capillarity and supplied from the base of the pot. Irrigation was suspended for 7, 14, 21, and 28 days after the 15-day acclimatization period, with the experiment ending 43 days after the plants were transplanted (Figure 1).

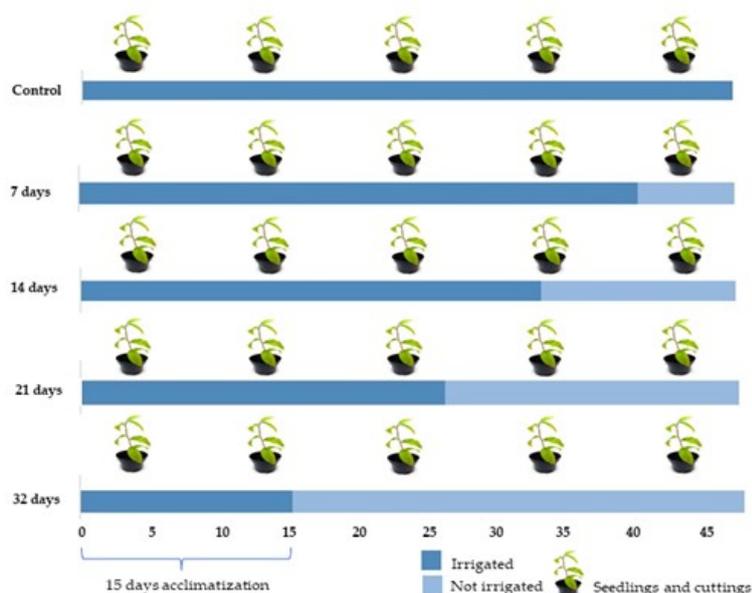


Figure 1. Distribution of treatments according to water availability. The x axis indicates the time (in days) when the experiment was carried out. The y axis indicates the treatments (in days) in which irrigation was suspended for seeds and cuttings. Image at 15 days of acclimatization.

Evaluation parameters

Plant height (PH) (cm) was measured with a graduated ruler, considering the distance between the ground and the apex of the plant. Stem diameter (SD) was measured with a digital caliper at the beginning, after 15 days and at the end of the experiment (Figure 2a). At the end of the experimental period, the following biometric traits were quantified: total number of leaves, fresh mass of the shoots and roots (SFM and RFM) (g), dry mass of the shoots and roots (SDM and RDM) (g) after drying in a forced air circulation oven for 3 days at 65°C.

The substrate moisture, substrate water potential (Ψ_s) and leaf water potential (Ψ_f) were determined every 4 days. Leaf samples and 2 g substrate subsamples were collected from the treatment pots with the greatest suspension of irrigation, between 5:00 and 5:30 in the morning, to measure Ψ_f and Ψ_s using a WP4-T device (Figures 2b). To quantify Ψ_f , one leaf from the lower third of the sample was collected, placed in a plastic bag wrapped in laminated paper and taken to the laboratory, where 1.2-cm diameter disks were prepared and evaluated using the WP4-T. The substrate samples were collected at the same depth (approximately 2.5 cm) so that the gravitational potential could be considered the same for all measurements (Reichardt & Timm, 2004). Calibration was carried out with 5 continuous evaluations of a KCl solution (0.5 mol L⁻¹) of known

potential ($\Psi = -2.19$ MPa) according to the supplier (Decagon Devices, 2003) when the equipment was used for the first time during the day. The normalized difference vegetation index (NDVI) was assessed using a handheld sensor (Greenseeker, Trimble-Inc.) at the end of the experiment (Figure 2c).

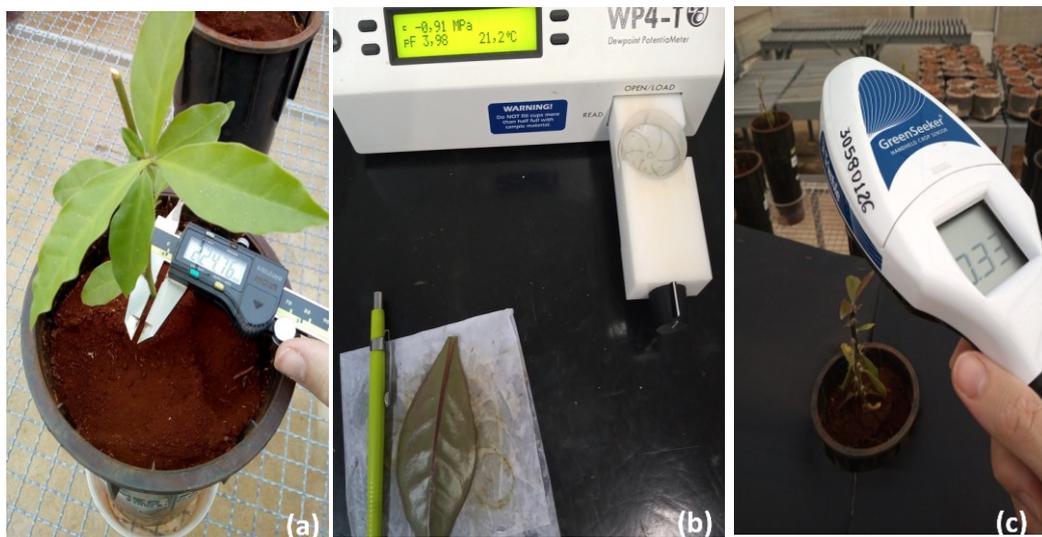


Figure 2. Stem diameter (SD) measured with a digital caliper (a), soil (Ψ_s) and leaf (Ψ_f) water potential (b) measured using a WP4-T device, and normalized difference vegetation index (NDVI) determined using the Greenseeker sensor (c).

Microbial biomass carbon (C_{mic}) (Vance et al., 1987) and basal microbial respiration of CO_2 (Alef & Nannipieri, 1995) were estimated during the incubation process following the protocol. Based on the C_{mic} and respiration data from the samples, we calculated their metabolic quotient (qCO_2) (Anderson & Domsch, 1993).

Images of the roots, distributed evenly and without overlapping in polyethylene plastic trays with a contrasting background and of known size, were collected using a semi-professional digital camera at chest height. After scanning, the images were corrected, aligned and analyzed using SafiraR® software (Stonway, Brazil) to estimate the following root traits: root length (cm), root area (mm^2), and root volume (mm^3).

Statistical analysis

The mean tests of the data, including the biological tests in triplicate, those measured with the WP4-T and NDVI were performed at a 5% probability by the Scott–Knott test using Sisvar version 5.7 (Ferreira, 2019) and Pearson correlation tests. Principal component analysis (PCA) was performed on this dataset in R version 1.3.1073 (R Core Team, 2018). Correlations were performed using the Spearman test with the R package ‘multtest’, and heat maps were generated using the package ‘corrplot’.

Results

Soil moisture showed a significant reduction in the first 12 days of water restriction for both propagation methods, and it was accompanied by a decrease in Ψ_s values, indicating the progressive depletion of available water in the substrate (Figure 3). These substrate moisture values ranged from 33.51% (seeds) and 43.06% (cuttings) in the constantly irrigated control to 6.43% (seeds) and 5.43% (cuttings) at 28 days.

In the treatments with irrigation suspension for 21 and 28 days, there was a significant difference in Ψ_s in the treatments propagated by seeds, reaching average values of -49.63 and -70.09 MPa, respectively. There were statistical differences between 7 and 14 days for cuttings and between 14 and 21 days for seeds (Table 1). The Ψ_f of cuttings was similar at 21 and 28 days, differing significantly from the values recorded in the previous stages (control, 7 and 14 days) and cuttings have significant variation in relation to seeds under the same conditions. In comparison, cuttings showed a significantly lower Ψ_f than plants propagated by seeds in 21 and 28 days. After irrigation suspension for 21 and 28 days, the Ψ_f of the cuttings showed significantly more negative values than seeds. The average NDVI values differed only in the treatments in which irrigation was suspended for 28 days in both seeds and cuttings, reaching values close to 0.1. There was no difference between the treatments for seeds and cuttings after 14 days of irrigation suspension.

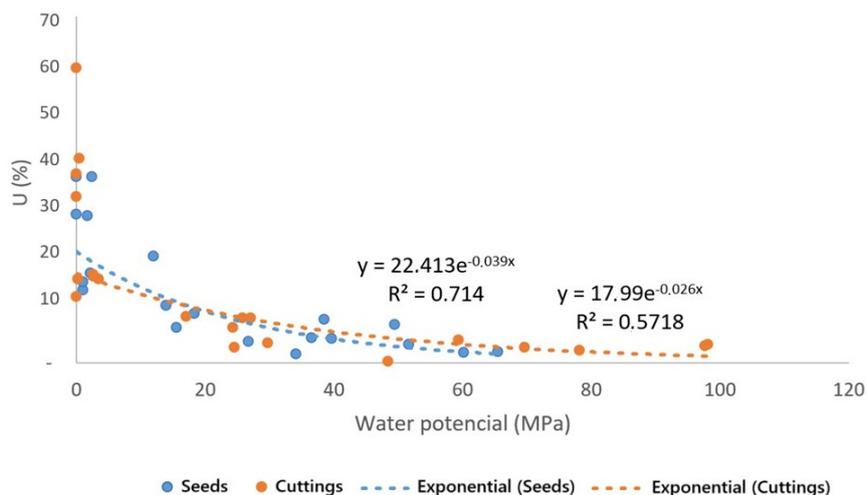


Figure 3. Distribution of substrate moisture (U%) and water potential over time for seeds and cuttings. A higher R² (0–1) indicates greater explanatory power and improved fit of the exponential decay model to the samples.

Table 1. Average values of soil water potential (Ψ_s), leaf water potential (Ψ_f) and NDVI of *Pereskia aculeata* propagated by C = Cuttings and S = Seeds in different periods under irrigation suspension.

Irrigation Suspension Period (days)	Ψ_s (kPa)				Ψ_f (MPa)				NDVI			
	S		C		S		C		S		C	
Control	-0.69	Aa	-0.37	Aa	-2.25	Ba	-1.91	Aa	0.37	Aa	0.26	Ab
7	-12.92	Aa	-11.40	Aa	-0.75	Aa	-1.49	Aa	0.41	Aa	0.25	Ab
14	-16.54	Aa	-54.51	Bb	-0.60	Aa	-1.44	Aa	0.29	Aa	0.21	Aa
21	-49.63	Ba	-48.27	Ba	-1.72	Ba	-2.91	Bb	0.27	Aa	0.24	Aa
28	-70.09	Ca	-88.67	Cb	-1.59	Ba	-2.84	Bb	0.10	Ba	0.12	Ba

Averages followed by the same capital letter indicate statistical differences between treatments within the propagation medium and lower case between propagation methods, using the Scott–Knott (1974) test at a 5% level of significance.

There were significant differences in the SFM of seedlings after 28 days of irrigation suspension with lower values compared to the other treatments (Table 2). No statistical differences among treatments were observed for plants obtained from cuttings. Compared to the cuttings, the values were higher for the plants that originated from seeds and were only similar after 28 days of irrigation suspension. The difference in root dry matter (RDM) was only evident when comparing the propagation methods after 7 days of water suspension.

Table 2. Values of shoot fresh mass (SFM) and shoot dry mass (SDM) and of root fresh mass (RFM) and root dry mass (RDM) of *Pereskia aculeata* propagated by C = Cuttings and S = Seeds in different periods under irrigation suspension.

Irrigation Suspension Period (days)	SFM (g plant) ⁻¹		SDM (g plant) ⁻¹		RFM (g plant) ⁻¹		RDM (g plant) ⁻¹									
	S	C	S	C	S	C	S	C								
Control	11.80	Aa	5.88	Ab	2.23	Ba	1.49	Aa	20.90	Aa	23.59	Aa	4.87	Aa	4.38	Aa
7 days	13.33	Aa	6.22	Ab	2.71	Aa	1.53	Ab	22.22	Aa	19.47	Aa	5.85	Aa	4.00	Ab
14 days	9.80	Aa	5.01	Ab	2.09	Ba	1.30	Aa	19.65	Aa	18.05	Aa	4.71	Aa	3.87	Aa
21 days	10.54	Aa	6.21	Ab	3.10	Aa	1.67	Ab	21.90	Aa	19.37	Aa	5.79	Aa	4.24	Aa
28 days	5.16	Ba	3.72	Aa	1.57	Ba	1.13	Aa	14.22	Ba	18.12	Aa	3.46	Aa	3.75	Aa

Averages followed by the same capital letter indicate statistical differences between treatments within the propagation method and lowercase letters between propagation methods, using the Scott–Knott (1974) test at a 5% level of significance.

C_{mic} (Figure 4a) showed differences between the propagation methods only in the control (cuttings) and at 14 days (seeds). For the root surface area and volume, no differences were observed after irrigation suspension treatments for each of the propagation methods, except between seeds and cuttings after 0, 7, and 21 days of irrigation suspension (Figure 4d and e). The PH (Figure 4g) differed only between propagation methods for the control, 14 and 28 days treatments.

There were no statistical differences for qCO₂ or RDM in any of the treatments, for seeds or cuttings, or between propagation methods (Figure 4c and f). The diameter (Figure 4h) differed for all treatments when comparing propagation methods, with the highest values found for seeds. Leaf dry mass was higher at 7 and 21 days in plants propagated by seeds, and these treatments were also higher than in plants propagated by cuttings (Figure 4i).

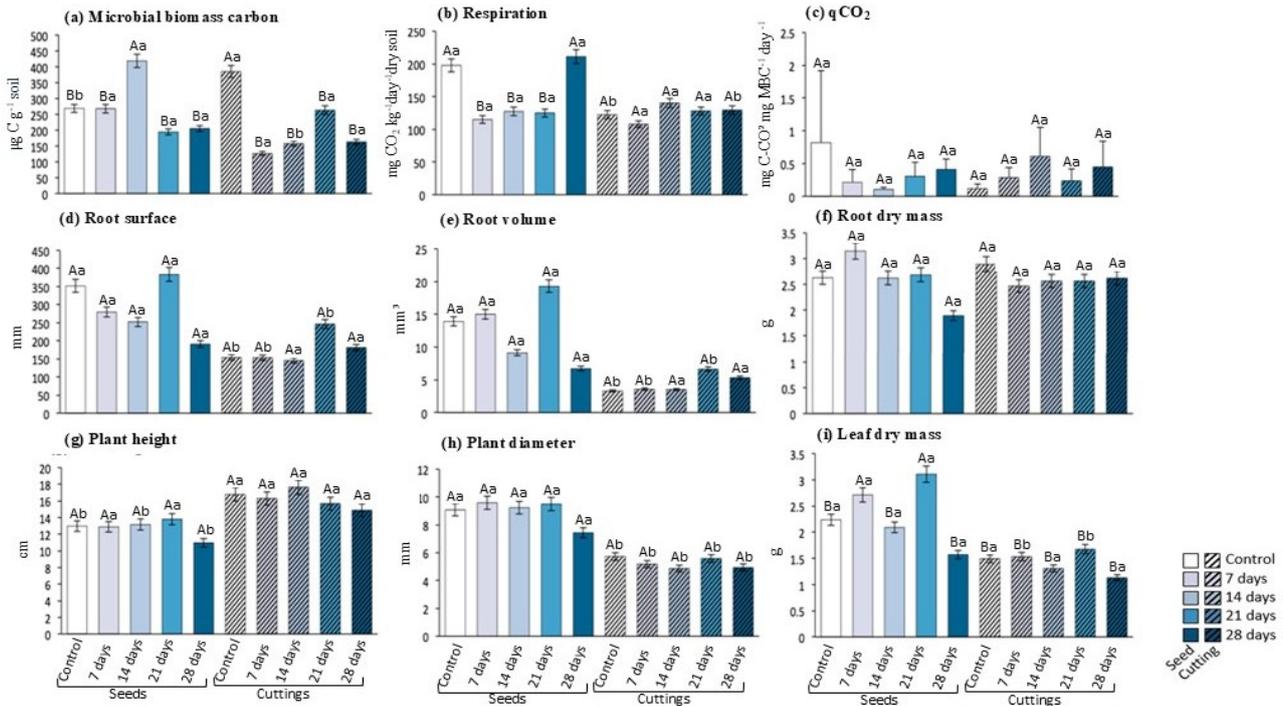


Figure 4. Parameters assessed throughout the experiment for *Pereskia aculeata* plants (seeds and cuttings). Uppercase letters indicate statistical differences between irrigation suspension period within propagation method and lowercase letters between propagation methods.

PCA between the plant traits evaluated during the experiment explained 84.2% of the total variance (PC1, 71.6% and PC2, 12.6%) for seeds and 98.6% for cuttings (PC1, 96.6% and PC2, 2.0%) (Figure 5). Seed-derived plants exhibited positive correlations with plant diameter, leaf mass (fresh and dry weight), NDVI (greenseeker) and C_{mic} , as indicated by their grouping in the lower and upper left quadrants. Ψ_s showed an expected positive correlation with root parameters and Plant Height. However, the root-related parameters exhibited a slightly closer correlation with some of the treatments propagated by cuttings. With regard to the cuttings, the variables were concentrated in the lower left quadrant and showed a negative correlation with qCO_2 . The cutting treatments were less similar to the seed treatments, with the exception of the 28-day irrigation suspension, which were similar for both. In addition, root volume was positively correlated with respiration and plant diameter, which did not show significant changes over time for cuttings.

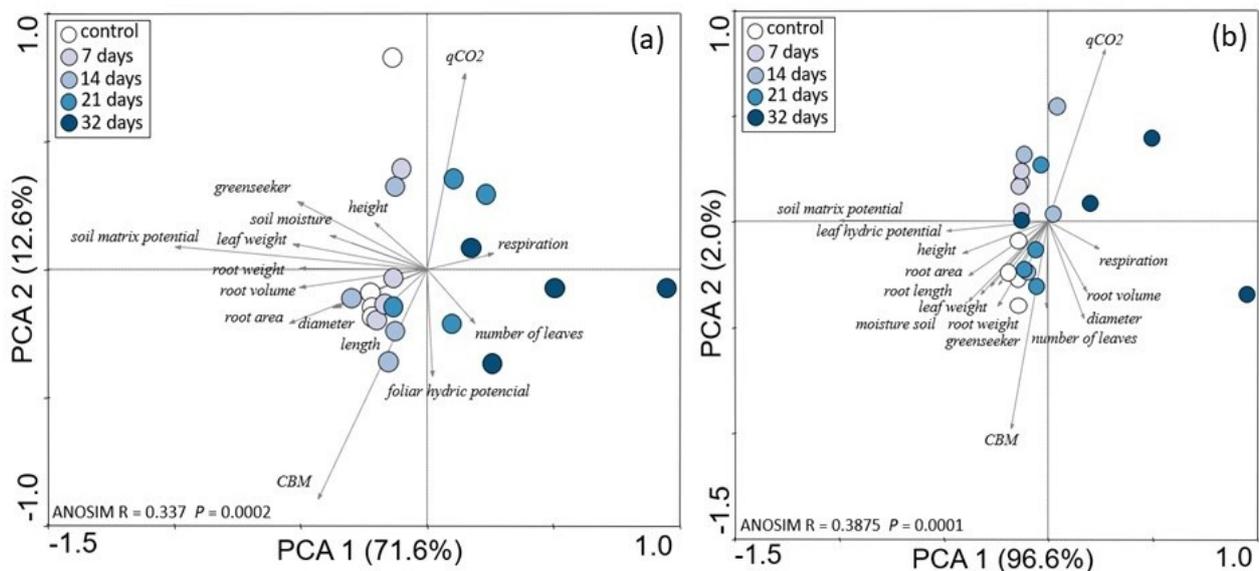


Figure 5. Principal component analysis (PCA) showing the significant variation ($p < 0.05$) in treatments in relation to the analyzed variables, with vectors illustrating the sign and intensity of the relationships between treatments and variables for plants from seeds (a) and cuttings (b).

The positive correlation observed between plant diameter, leaf mass (fresh and dry weight), NDVI (greenseeker) and C_{mic} in seed-propagated plants indicates that these variables are interdependent and demonstrate greater vigor in seed-propagated plants. The most robust root architecture was greater in the treatments subjected to water suspension for 7 and 21 days in the seed-propagated plants, which contributed to greater SFM and SDM, standing out compared to those propagated by cuttings.

These results, together with the Spearman correlation matrix (Figure 6), enabled us to better visualize the relationships between these variables for each propagation method. For plants propagated by seeds, Ψ_f showed a positive correlation with the NDVI, i.e., the greater the volume of water, the greater the green content of the plant. NDVI also showed a positive correlation with SFM and SDM. The qCO_2 was negatively correlated with the RDM and seedling diameter. There was a negative correlation between leaf potential and root volume and area (seeds), i.e., the higher the Ψ_f , the smaller the root volume. Root length directly influenced the plant in relation to SFM and RFM, plant diameter, root area and volume and moisture, showing a positive correlation.

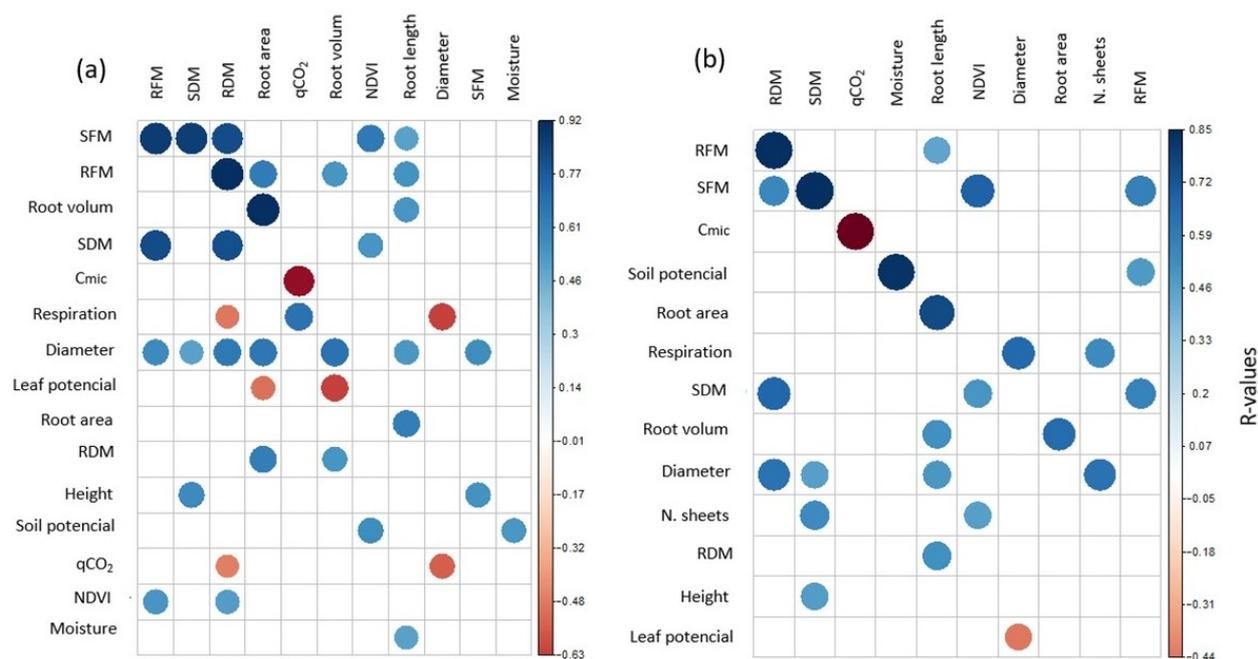


Figure 6. Heat maps of Spearman's rank correlation coefficients between parameters measured for seeds (a) and cuttings (b). The color gradient and the size of the circle are proportional to the weight of the correlation. Positive correlations are shown in blue, and negative correlations are shown in red. Only significant correlations are shown ($p < 0.05$).

A negative correlation between Ψ_f and plant diameter was observed in cuttings, unlike in seeds, which showed no variation. The results also showed that the number of leaves, SFM and SDM were positively correlated with the NDVI. Under both propagation methods, RFM and RDM were positively correlated with SDM.

Discussion

The data presented indicate a significant reduction in soil moisture after 21 days of water restriction for both propagation methods, accompanied by a decrease in the soil water potential (Ψ_s), reflecting the progressive depletion of available water in the soil. The literature corroborates these findings, suggesting that the reduction in soil moisture during periods of water stress is directly related to the decrease in Ψ_s , which negatively impacts the water availability for plants and, consequently, their growth and development (Ali et al., 2022; Bhattacharya, 2021; Pamungkas et al., 2022). Studies have shown that a drop in water potential and soil moisture leads to a series of physiological responses in plants, including stomatal closure, reduced photosynthesis and limited growth (Herrera et al., 2022; Sousa Leite et al., 2023). Therefore, under conditions of water restriction, plants propagated by seeds and cuttings tend to exhibit different responses, as observed in the Ψ_f values of -0.6 and -1.44 MPa, respectively, after 14 days of irrigation suspension and Ψ_s of -16.54 and -54.51 kPa, respectively.

The Ψ_s values suggest that stress is much lower than that found in savannah soils (-1.22 ± 0.21 MPa) and the Brazilian cerrado or forests (-0.64 ± 0.45 MPa) (Fu et al., 2022). Given that soil texture is the main determinant of Ψ_s , the higher clay content in Latosol soil may have contributed to greater water retention at depth. The greater capillarity, water adsorption capacity and microbial diversity may have mitigated the water stress in the substrate used in the experimentation (Krishna et al., 2022; Mehra et al., 2022; Zia et al., 2021) as observed after 28 days due to its good aggregation and porosity.

The progressive reduction in Ψ_s in the treatments propagated by seeds and cuttings reached average values of -70.09 and -88.67 kPa, respectively, after 28 days of irrigation suspension, indicating a severe depletion of available water in the soil. The literature suggests that under conditions of prolonged water deficit, the soil tends to retain less and less water available to the plants, resulting in increasingly negative Ψ_s values (Bhatt et al., 2024). These values are critical, since such low water potentials can compromise the plants' ability to extract water from the soil, especially in advanced stages of water shortage (Tatar et al., 2023).

The statistical differences observed between 14 and 21 days for seeds and from 7 and 14 days for cuttings suggest that seed-propagated plants have a slightly greater capacity to maintain Ψ_s at less negative values in the early stages of water stress. This may be related to the early development of the root system of seed-propagated plants, making them more efficient in absorbing water from the soil than cuttings. As water stress progresses, internal reserves (and the growing medium) are depleted, leading to a sharp decrease in Ψ_s (Zhua et al., 2024). This rapid decrease indicates that cuttings are more vulnerable to water stress in the early stages, affecting their establishment and long-term development. Ψ_f reflects the balance between water loss via evapotranspiration and soil water availability (Ψ_s).

Notably, soil moisture remained satisfactory for approximately 4 days after the end of irrigation under greenhouse conditions, with an average ambient temperature of $18.9 \pm 1.7^\circ\text{C}$. Only after 21 days did Ψ_f show variation (for both seeds and cuttings), which is very interesting for the crop since the leaves are consumable. In an experiment with OPN, there were no differences in leaf potential in plants originating from cuttings (Queiroz et al., 2015), while in our experiment, Ψ_f varied statistically between 14 and 21 days after water suspension. This difference was also observed by NDVI measurements, which differed under water restriction at 28 days. OPN also presented Ψ_f values much lower than those found for other crops, such as corn (*Zea mays*), in which values of -0.75 MPa have been observed (Veroneze-Júnior et al., 2019). For beans (*Phaseolus lunatus* L.), values of -0.8 (Nascimento et al., 2019) and for OPN -0.07 MPa (Queiroz et al., 2015) have been observed.

The mean NDVI only differed in the treatments after 28 days of irrigation suspension, which reached values close to 0.1, indicating a significant decrease in photosynthetic activity and amount of chlorophyll in the plants. A low NDVI is commonly associated with plants under severe stress, where chlorophyll loss and leaf senescence are accentuated.

The data presented show that water stress impacts SFM of plants propagated by seeds, especially under prolonged periods of water restriction. Conversely, RDM did not vary both in seedlings and cuttings. Besides, among the treatments root volume was in general higher in plants propagated by seeds than cuttings. These observations are consistent with the literature on the response of plants to water stress, which highlights variations in plant biomass and composition depending on the propagation method and stress duration (Abbas et al., 2023; Atzori & Caparrotta, 2023; Rosso et al., 2023). Compared to cuttings, plants propagated from seeds had a higher SFM and root volume, except after 28 days of water restriction, when the values were similar. This behavior can be explained by the difference in the initial development between the two propagation methods. Plants propagated from seeds tend to develop a more robust root system (Larson et al., 2020; Ranjan et al., 2022), which may provide greater access to water resources in the early stages of development, resulting in a higher SFM under normal or moderate water stress conditions.

The differences observed in SFM between propagation methods indicate that the impact of water shortage may be more pronounced in cuttings but this difference decreases with prolonged stress, reflecting a convergent response between the two propagation methods. However, under severe and prolonged water stress, the differences between seeds and cuttings become less pronounced, possibly due to the exhaustion of available water resources, affecting both propagation methods equally (Makena et al., 2023). The greater plant diameter and greater number of leaves in plants propagated by seeds suggest that these plants have a superior vegetative growth capacity, possibly due to a more efficient root system (root volume) that supplies more water and nutrients to the aerial parts of the plant.

C_{mic} reflects the soil's ability to sustain essential biological processes (Silva et al., 2021). This parameter is influenced by the type of plant, root exudates and nutrient availability. In this experiment, there was, in general, no significant difference between the propagation methods.

The higher microbial respiration in seeds after 28 days of irrigation suspension can be attributed to the increase in root exudates and organic matter, providing a substrate for microorganisms. Although water stress normally reduces microbial biomass, it may stimulate an adaptive response in microorganisms under seed-propagated plants, resulting in higher respiration (Lopes et al., 2021).

There were no significant differences in root surface or volume between the treatments. However, differences were observed between seeds and cuttings after 0, 7, and 21 days of irrigation suspension. Plant water requirements are specific to each species, and, in this study, they were influenced by genetic morphophysiological characteristics (Pinto et al., 2020; Silva Porto et al., 2022). Plants propagated by seeds generally develop more vigorous roots, which gives them an advantage in water and nutrient absorption, especially under water stress conditions. However, in this experiment, they may have been limited by the size of the pot. This difference in root development may be critical for plant survival under drought conditions (Ranjan et al., 2022), as a more robust root system can access water in deeper soil layers.

The distinction between plants propagated by seeds and cuttings suggests that the former present greater initial vigor, root development and distinct physiological responses (Mbogue et al., 2021). The formation of specific groups indicates that the different methods result in unique biological profiles that impact plant performance under stress conditions. The negative correlation between Ψ_s and root parameters highlights the complex interaction between water availability, physiology and growth. Under water shortage, plants have difficulty maintaining water potential, leading to adaptations that, although aiding survival, reduce root growth and height. With a decrease in Ψ_s , growth is compromised (Álvarez et al., 2020). Cuttings with greater structural strength show less intense responses to water stress, reflected in more negative values for Ψ_f and Ψ_s , compared to plants propagated by seeds. Wilting and reduced growth were observed under both propagation methods only after 28 days of water restriction.

The association between root parameters and some cutting-propagated treatments suggests that although cuttings may have a less robust initial development compared to seeds, they still exhibit significant acclimation to water restriction. This acclimation may include resource allocation to root development under adverse conditions (Karlova et al., 2021), enabling cuttings to maintain water uptake at adequate levels to survive prolonged water deficit. The negative correlation between Ψ_s and root growth underscores the importance of Ψ_s as a critical factor for plant development under water stress.

The positive correlation of NDVI with SFM and RDM reinforces the idea that vegetative vigor, as measured by NDVI, is associated with greater biomass accumulation in both shoots and roots. This is supported by the literature, which suggests that plants with a higher chlorophyll content (higher NDVI) tend to have a greater photosynthetic efficiency, which translates into greater biomass accumulation (Farias et al., 2023; Marín et al., 2020). This suggests a reduction in chlorophyll levels in the leaves, which is indicative of a substantial metabolic impact due to water deficiency. However, other Cactaceae species demonstrate high survival rates during the initial 6 months of water deprivation, with some species, such as *A. tetragonus* and *M. curvispinus*, persisting for up to 8 months (Silva et al., 2023). Prolonged water stress can lead to plasmolysis, which is characterized by the detachment of the plasma membrane from the cell wall. This phenomenon is governed by the relationships established between the properties of the stems and leaves of *Pereskia* spp. (Edwards, 2006).

The negative correlations of qCO_2 , root dry weight and plant diameter indicate that higher microbial respiratory activity is associated with lower growth efficiency. Under conditions of greater stress, there is greater respiratory demand and reduced carbon allocation to structural growth. The increase in qCO_2 may be related to organic matter decomposition or greater physiological stress, thereby compromising growth efficiency (Agumas et al., 2021; Qadeer et al., 2024). The positive correlation between Ψ_s and NDVI in seed-propagated plants highlights the importance of water availability for vegetative vigor, reflecting that better water conditions promote greater biomass and photosynthetic activity, especially in the early stages of development.

Conclusion

The results demonstrate that plants derived from seeds exhibit superior performance in root volume development, stem diameter, and leaf dry mass and shoot fresh matter when compared to those propagated from cuttings. Moreover, water stress significantly impacted the fresh weight of seed-derived plants,

particularly under 28 days of water restriction, whereas plants from cuttings displayed a more stable and less variable response over time. Microbial biomass carbon remained unaffected by water shortage, likely due to microbial resilience and physiological adaptation. In contrast, basal microbial respiration (CO₂ emission) was significantly higher in plants propagated by seeds, both under continuous irrigation and prolonged irrigation suspension. This indicates that the microenvironment surrounding the seed provided sufficient conditions to sustain microbial activity, even under reduced water availability.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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