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Physiological and morphometric characteristics of *Pachycereus pringlei (S.Watson) Britton & Rose* seedlings applying organic manures

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Abstract. The establishment of *Pachycereus pringlei* seedlings is a scarce event; soil conditions are one of the factors attributed to its high mortality rate in the early stages of development. The use of organic manures as a substrate helps to improve the structure, porosity, and density of the soil where they live, in addition to providing nutrients that would benefit the roots, increasing the possibility of their establishment during the emergence stage. The objective of this study was to evaluate the effect of different proportions of organic manures as a sown substrate in the emergence and establishment of *Pachycereus pringlei* seedlings, using nine treatments of organic manures in a completely randomized experimental design with four replications. The variables measured were emergence rate (ER) and percentage (EP) and their indices, seedlings' morphometric, and physiological characteristics in the establishment stage. The results showed that the evaluated variables in the emergence and seedling establishment phase indicate the preference for natural substrate and organic manure combinations in proportions of 50 and 30%, respectively. The analysis of variance showed significant differences regarding the morphometric and physiological variables of the stem and root of the evaluated treatments. The emergence and establishment analyses show that cardon seedlings under different organic manure treatments and controlled conditions had excellent survival results compared to the low rates of recruitment and survival reported under natural conditions. Similarly, the implementation of organic manures improved the physiological and morphometric characteristics of the cardon seedlings compared to the control treatment with the natural substrate.

Keywords: *emergence, establishment, cacti, compost, vermicompost*

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Introduction

The Cacti from arid and semi-arid zones where water is limited have unique morphometric, physiological, and anatomical characteristics that allow them to tolerate extreme climatic conditions and complete their life cycle (Nobel *et al.,* 1988; Bravo-Hollis and Scheinvar, 1995). These characteristics for adapting to environments include crassulacean acid metabolism (CAM), thick cuticle, and leaves modified to spines (Granados *et al.,* 1998). Cacti are vulnerable species in the early stages of development (Martínez-Ramos *et al.,* 2016) because of abiotic and biotic factors (Turner, 1990; Valiente-Banuet and Ezcurra, 1991; Mandujano *et al.,* 1997). Cacti seedlings must grow as fast as possible, establish roots for rapid water absorption, and compete for nutrients, light, and space with other plants (Fenner and Thompson, 2005). The most important source of nutrients for plants is organic matter, which in arid regions is limited; therefore, many cacti seedlings do not reach the next stage and die due to a lack of nutrients (Ramirez, 2011).

The early stages of cacti are considered critical, as the seedlings depend on environmental variables for their survival and establishment, such as moisture, light, and nutrients (Sánchez-Soto *et al.,* 2010; Pimienta-Barrios *et al.,* 2012). The emergence is the process where the appearance of the seedling is observed on the soil surface; it is a stage after the seed germination or sprouting of the bud. On the other hand, the seedling establishment is defined as the process by which a plant establishes itself, resists, and survives. It also includes the stages of germination and emergence until the development of true leaves -spines in cacti- (National Research Council, 2002; Dumroese *et al.,* 2012).

The soil represents an important factor in the first stages of plant development, which is why in recent years, the use of organic manures (compost and vermicompost) has increased due to the benefits it has, among which are the increased permeability and retention of moisture in the soil for longer, has a buffer capacity against sudden changes in acidity, and another advantage is the supply of nutrients, which improves the morphometric and physiological characteristics of plants (Bashan *et al*., 2009; Nieto-Garibay *et al.* 2021). Some morphometric and physiological changes that can be evidenced depending on the environment in which these species are found are the presence of a thick cuticle, high osmotic concentrations to extract moisture from very dry soils, great development of the root system, and size of the portion reduced area (González, 2012).

Pachycereus pringlei (S.Watson) Britton & Rose is a species of cactus endemic to the Sonoran Desert, which is a reservoir of nutrients, provides shelter and is a source of food for local fauna (Delgado-Fernández *et al*., 2017). This species has a high germination rate (Suzán-Azpiri and Sosa, 2006; Bacilio *et al*., 2011); however, the establishment of seedlings is a sporadic event due to high mortality rates (Bullock *et al*., 2005), which could be linked to the edaphic conditions of arid environments, such as the low percentage of organic matter (Pérez-Sánchez *et al*., 2015). This study aimed to evaluate the effect of different proportions of organic manures as a sown substrate in the emergence and establishment of *Pachycereus pringlei* seedlings.

Material and Methods

Study site

The experiments of emergence and establishment were carried out in the Center for Biological Research of Northwest México S.C (CIBNOR), west of La Paz city, Baja California Sur (BCS), México located at 24º08'10.03” N and 110º25'35.31” W.

Fruits collection

The ripe cardon fruits were collected between June to July at the Biological Station “Dra. Laura Arriaga-Cabrera” located at 24º 07'41.4” N and 110º 26' 19.0” W (Figure 1a). The seeds were extracted from the fruit through longitudinal dissections and later were dried at room temperature using paper napkins and cotton (Figure 1b).

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a **b**

Figure 1. (a) *Pachycereus pringlei* fruits collected between June and July; (b): Seeds extraction from the fruits and drying at room temperature.

Experimental design

The experiments of the emergence and establishment of seedlings stages were carried out using a completely randomized experimental design where the main study factor was the sowing substrate with nine treatments and four replications of 50 seeds. The treatments consisted in the combination of the substrate in different proportions of organic manure and native soil (Table 1). The organic manures were from agricultural residues and obtained from Rancho Buena Suerte (organic farm) in the town of La Paz, BCS. The physicochemical composition, electrical conductivity (EC), organic matter (OM), apparent density (AD), porosity (Po), and pH of the treatments were analyzed in the soil science laboratory of CIBNOR (Table 1). The substrate's nutrient content (NH_3, NO_3^-, SO_4^{2-}, Ca, P, Mg, and K) were analyzed using a portable photometer (9, Hanna Instruments Inc., Woonsocket, RI, USA).

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Table 1. Physicochemical composition of each treatment (sowing substrates).

Treatments	EC	OM	PO	DA	pH	NO_3^-	NH_3	SO_4^{2-}	Mg	P	Ca	K
	dS m^{-1}	%	%	G cm^{-3}		mg L^{-1}						
NS100 (control)	0.4	0.4	19.25	1.51	7.45	ND	0.006	0.1	0.5	0.008	0.9	0.1
C100	30.6	39.6	22.26	0.41	8.33	2.17	0.04	16.7	0.3	0.44	156.7	11.0
V100	15.2	20.8	10.19	0.73	8.16	1.17	0.12	16.7	21.7	0.60	176.7	96.7
C30NS70	6.8	14.6	1.89	1.05	8.42	0.67	0.36	13.3	5.0	0.40	166.7	8.7
C70NS30	21.3	30.1	17.36	0.54	8.79	1.33	0.13	20.0	6.7	0.27	116.7	10.5
C50NS50	10.6	16.7	4.91	0.87	8.51	0.83	0.08	15.0	13.3	0.47	110.0	9.5
V30NS70	5.2	3.1	15.85	1.42	8.50	1.00	0.43	18.3	5.0	0.43	200.0	11.7
V70NS30	8.7	18.6	3.02	1.08	8.45	1.17	0.14	15.0	6.7	0.50	160.0	8.7
V50NS50	9.1	6.3	9.96	1.24	8.38	0.50	0.10	15.0	5.0	0.57	166.7	10.5

NS100: 100% Native Soil. C100: 100% Compost. V100: 100% Vermicompost. C30NS70: Compost 30%-Native Soil 70%. C70NS30: Compost 70%-Native Soil 30%. C50NS50: Compost 50%-Native Soil 50%. V30NS70: Vermicompost 30%-Native Soil 70%. V70NS30: Vermicompost 70%-Native Soil 30%. V50NS50: Vermicompost 50%-Native Soil 50%. EC: electrical conductivity. OM: Organic matter. PO: Porosity. AD: Apparent density. ND: Not detected. $(n = 3)$.

Seedlings emergence stage

The seeds were sown in trays of 200 cavities divided previously into four sections of 50 cavities, each representing one replication of 50 seeds. The trays were covered with plastic micro-tunnels to prevent rapid evaporation and placed inside a 2.5 × 2.5 m anti-aphid mesh. The treatments were irrigated every 4 to 7 days with water from the desalination plant of the experimental field. The emergence was considered when seedlings began to sprout 2 mm above the substrate and were recorded daily until 33 days after sowing.

Emergence Indices

Through the emergence indices, the favorable performance of seedling emergence was determined. Table 2 describes the emergence indices calculated using the "GerminaR" package in RStudio (Lozano-Isla *et al*., 2018).

Table 2. Description of emergence indices.

Emergence Indices	Units	Formulas	Reference
Emergence Percentage (EP)	%	$EP = \frac{n_i}{N}\, x\, 100$ Where: n_i = Seedlings emerged on the i^{th} day N = Total number of seedlings in the experiment	Maguire (1962)
Speed of emergence (SE)	%	$SE = \sum_{i=1}^{k} \frac{n_i}{n_i X_i}\, x\, 100$ Where: n_i = Seedlings emerged on the i^{th} day X_i = Number of days since seed sowing k = The last day of emergence evaluation	Ranal and Santana (2006)
Emergence Index (EI) or Mean Emergence Time	time	$EI = \sum_{i=1}^{k} \frac{n_i t_i}{n_i} = \frac{1}{MER}$ Where: n_i = Seedlings emerged on the i^{th} day t_i = Time from the start of the experiment to the i^{th} day of observation k = The last day of emergence evaluation MER = Mean emergence rate	Ranal and Santana (2006)
Emergency Rate Index (ERI)	time	$ERI = \frac{\sum_{i=1}^{k} n_i}{MER}$ Where: n_i = Seedlings emerged on the i^{th} day k = The last day of emergence evaluation MER = Mean emergence rate	Bilbro and Wanjura (1982)

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Mean Emergence Rate (MER)	time^{-1}	$MER = \frac{1}{EI}$ Where: EI = Emergence Index	Ranal and Santana (2006)

Seedlings establishment stage

The seedlings' establishment stage was evaluated at the end of the emergence stage and until 153 days after sowing. The physiological and morphometric measurements of survived seedlings were evaluated individually in the plant physiology laboratory of CIBNOR, selecting three seedlings per replication and treatment. The treatments were irrigated every 10 days with water from the desalination plant of the experimental field during this phase.

Survival curve

The seedlings' establishment was performed using Kaplan-Meir survival curves (Kaplan and Meier, 1958) and compared using the Mantel-Haenszel log-rank analysis (Mantel and Haenszel, 1959). Survival curves were evaluated (Figure 2) from the day 33 when an asymptote was observed in the emergence of seedlings, and 5 treatments that registered at least 1 dead individual were included. The treatments C30NS70, C70NS30, V30NS70, and V70NS30 were discarded from the analysis because they did not register the deaths of individuals during the evaluated period.

$$S(t_i) = \prod_{t_i \leq t} \frac{n_i - d_i}{n_i}$$

Where, n_i is the number of living individuals before the time t_i, d_i is the number of events at the time t_i, and t_i is the inter-occurrence time of the i^{th} event in the i^{th} research unit.

Morphometric variables (stems and roots)

The spine length, the primary root length, diameter, and stem length were measured using a digital vernier (Cole-Parmer, General model 143). The number of spines was determined for each seedling.

The dry and fresh weight of stems and roots were determined using an analytical balance (Mettler Toledo, model AG204). The stems and roots were placed in paper bags and placed in a laminar flow oven (Sheb-Lab, model FX-5, series 1000203) for 24 hours at 70°C until entirely dehydrated to obtain the dry weight.

The root area was determined using an area meter (LICOR, model LI-1300) scanning the entire root. Previously, the roots were rinsed with topwater for 5 min to remove the excess substrate and stored in the refrigerator until scanning. The values for the root area variable are expressed in centimeters squared (cm2).

Physiological variables

Relative water content (RWC). The RWC was calculated using the formula proposed by Yamasaki and Dillenburg (1999):

$$RWC = \frac{F_w - T_w}{D_w - T_w} x\, 100$$

Where, F_w is the fresh weight, D_w is the dry weight, and T_w is the turgent weight. The spines of the stems were removed and subsequently weighed to obtain the fresh weight (F_w). To determine the turgid weight (T_w), stems were placed in distilled water inside a closed Petri dish for 24 hours. After that, the sample of the stems was placed in a laminar flow oven (Sheb-Lab, model FX-5, series 1000203) at 70°C for 24 hours to obtain the dry weight (D_w). The weights of the plant material were obtained using an analytical balance (Mettler Toledo, model AG 204). *Osmotic potential (Ψo).* The measurements of osmotic potential were made according to the method proposed by Moghaieb *et al*. (2004). The seedlings were placed in vials and subsequently placed in an ultra-freezer at -20 °C. The vials were thawed and centrifuged at 1200 rpm × g for 20 min at 4°C to extract the cells from the sap. The osmotic potential was determined with a vapor pressure osmometer (Wescor, VAPRO model). The data were transformed to MPa using the formula proposed by Van't Hoff (Salisbury and Ross, 1992), which states the following:

$$\Psi o = -C\, x\, R\, x\, T$$

Where, C is the molarity of the solution (mol of the solute kg^{-1} H_2O), R is the universal gas constant (0.0831 kg Mpa mol^{-1} K^{-1}), and T is the absolute temperature (K).

Water potential (Ψw). The measurements of water potential were made at the critical time (approximately 12:00 h), considering the interval in which the highest temperatures of the day occur. The water potential was determined using a dew point potentiometer (Dewpoint PotentiaMeter, model WP4-T). Three readings of each treatment were made, using complete samples of cardon seedlings at the end of the experiment.

Turgor pressure (Ψt). The turgor pressure was calculated considering the difference between the water potential (Ψw) and the osmotic potential (Ψo) (Moghaieb *et al*., 2004).

$$\Psi t = \Psi w - \Psi o$$

Chlorophyll a, b, and total. A hollow steel punch was used to obtain the fresh tissue discs, to which 80% acetone was added, and it was left to stand for 48 hours in a test tube at room temperature and in the dark. Subsequently, the absorbance was read in a UV/Visible spectrophotometer (HACH, model DR 3900). Chlorophyll contents were calculated with the equation proposed by Strain and Svec (1996):

$$Chl\ a\ (mg\ mL^{-1}) = 11.64\ x\ (A663) - 2.16\ x\ (A645)$$
$$Chl\ b\ (mg\ mL^{-1}) = 20.97\ x\ (A645) - 3.94\ x\ (A663)$$

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Where, $A663$ and $A645$ represent the absorbance values read at a wavelength of 663 nm and 645 nm, respectively. Additionally, total chlorophyll was measured indirectly, through the sum of chlorophylls a and b, to obtain a prediction model of total chlorophyll values.

Statistical analyses

The normality and homoscedasticity of variances of the data were verified, and subsequently, a one-way ANOVA test was performed. The mean differences among treatments of each variable were detected with a mean difference test (Tukey HSD, $p \leq 0.05$). A nonparametric Kruskal-Wallis test was performed when the data did not meet the statistical assumptions, followed by Dunn's post-hoc test for group comparisons. Statistical analysis was performed with the free access software RStudio. The significance levels for each treatment were later identified using the "Agricolae" and "MultcompView" packages (De Mendiburu, 2014; Graves *et al*. 2019).

Results and discussion

Seedlings emergence stage

Emergence Indices

The results showed statistically significant differences ($p<0.05$) in the emergence indices evaluated for each treatment in this study (Table 3). Regarding the percentage of emergence (EP), the highest values were obtained for the treatments V30NS70, C30NS70, V50NS50, and C50NS50. On the other hand, the 100% treatments with organic manures registered low values of the emergence percentage (EP) and slow emergence speed (SE). Treatments NS100 and C30NS70, which showed the first seedlings emerged from the substrate, registered the highest emergence speed (SE) values. Concerning the emergence index (EI), which estimates the time it takes for the seedling to emerge, the highest values were recorded for the V100 and C100 treatments because the mean total seedling emergence time for these treatments was slower than the other treatments. The treatments with an organic substrate, including the control (NS100), that was watered every 4 to 7 days, showed more than 19 % of the seedlings' emergence. These results are similar to those reported by Puente (2004), who described that in the control treatment with only saturation irrigation, cardon seeds had a high germination percentage. Marchiol *et al*. (1999) subjected different types of substrate treatments with compost to seeds from herbaceous plants and found that the lowest germination percentage was recorded for the 100 % compost treatment. These results are similar to the treatments of C100 and V100 of this study, which registered less than 34 % of emerged seedlings. In addition, there was a delay in the emergence phase, evidenced by the speed of emergence (SE) and the emergence index (EI), which could be associated with the combination of high levels of electrical conductivity and potassium, which affects germination in some cactus (Teixeira *et al.*, 2004; Sarria-Perea, 2010; Freire *et al*., 2018).

The emergence percentage increased by more than 25% for the V30NS70 treatment concerning control group. Organic manures in proportions of 50 and 30 % for C30NS70, V50NS50, and C50NS50 also presented a high percentage (>58%) of seedlings emerged on day 33. Likewise, the seedling emergence variables showed an important variation concerning the C100 and V100 treatments, which indicates the preference for the combination of natural substrate and organic manure in proportions of 50% and 30 %, which is corroborated with the emergence indices (Table 3).

The NS100 and C30NS70 treatments recorded the highest values of emergence speed (SE) and emergence rate index (ERI) since on days 1 and 2 the first seedlings began to sprout from the substrate. The average speed of emergence for *P. pringlei* in the treatments with organic manures evaluated was 8 days. Regarding the above, Godínez-Alvarez and Valiente-Banuet (1998) mention that the seeds of cacti species germinate quickly in the first weeks when they have moisture, for example, some species of *Opuntia* take to germinate an average of 13 days, but under controlled conditions, they germinate between 5 and 6 days (Gonzalez-Cortés *et al*., 2018).

Table 3. Emergence indices of cardon seedlings under organic manure treatments.

Treatments	EP	SE	EI	ERI	MER
	%	%time^{-1}	time^{-1}	time^{-1}	time
NS100 (control)	57.5 ± 10.6 ^{abc}	12.839 ± 0.73 ^{a}	7.806 ± 0.42 ^{c}	3.683 ± 0.731 ^{ab}	0.1283 ± 0.0073 ^{a}
C100	19 ± 2.58 ^{d}	6.129 ± 0.29 ^{c}	13.315 ± 0.78 ^{a}	0.581 ± 0.064 ^{c}	0.0612 ± 0.0029 ^{c}
V100	33 ± 6.83 ^{cd}	5.876 ± 0.29 ^{c}	17.050 ± 0.83 ^{a}	0.968 ± 0.192 ^{c}	0.0587 ± 0.0029 ^{c}
C30NS70	69.5 ± 12.4 ^{ab}	10.799 ± 1.26 ^{ab}	9.347 ± 0.99 ^{bc}	3.718 ± 0.429 ^{ab}	0.1079 ± 0.0126 ^{ab}
C70NS30	54 ± 16.6 ^{abc}	8.144 ± 0.99 ^{bc}	12.415 ± 1.48 ^{ab}	2.175 ± 0.707 ^{bc}	0.0814 ± 0.0099 ^{bc}
C50NS50	59 ± 9.31 ^{abc}	9.874 ± 2.29 ^{ab}	10.618 ± 2.84 ^{bc}	2.778 ± 0.467 ^{ab}	0.0987 ± 0.0229 ^{ab}
V30NS70	83 ± 22.5 ^{a}	10.429 ± 2.17 ^{ab}	9.993 ± 2.60 ^{bc}	4.153 ± 1.810 ^{a}	0.0104 ± 0.0217 ^{ab}
V70NS30	47 ± 4.76 ^{bcd}	9.094 ± 1.22 ^{bc}	11.148 ± 1.51 ^{ab}	2.108 ± 0.380 ^{bc}	0.0909 ± 0.0122 ^{bc}
V50NS50	66 ± 14 ^{ab}	10.453 ± 1.22 ^{ab}	9.666 ± 1.13 ^{bc}	3.414 ± 0.613 ^{ab}	0.1045 ± 0.0229 ^{ab}

NS100 (control): 100% Native Soil. C100: 100% Compost. V100: 100% Vermicompost. C30NS70: Compost 30%-Native Soil 70%. C70NS30: Compost 70%-Native Soil 30%. C50NS50: Compost 50%-Native Soil 50%. V30NS70: Vermicompost 30%-Native Soil 70%. V70NS30: Vermicompost 70%-Native Soil 30%. V50NS50: Vermicompost 50%-Native Soil 50%. EP: Emergence percentage. SE: Speed of emergence. EI: Emergence Index. ERI: Emergency rate index. MER: Mean emergence rate. Means ± standard deviation followed by the same letter in the same column are not significantly different. The significance level was set at $p \leq 0.05$. (n=4).

Seedlings establishment stage

Survival curve

No significant differences were found in seedling survivalduring the seedling establishment stage between treatments of organic manures and control (LR=7.02, g.l.=4, $p>0.05$). The establishment showed that cardon seedlings had high survival, with 1 to 2 deaths recorded after 153 days (Figure 2), compared to the low rates of recruitment and survival recorded under natural conditions (Bullock *et al*., 2005). These results are related to the availability of nutrients and the retention of humidity as benefits of the use of organic manures. It is noteworthy that the survival success of seedlings, and especially cacti, can be determined by the amount of water stored in the tissues during the first stage of growth, which is favored by water retention by vermicompost and compost.

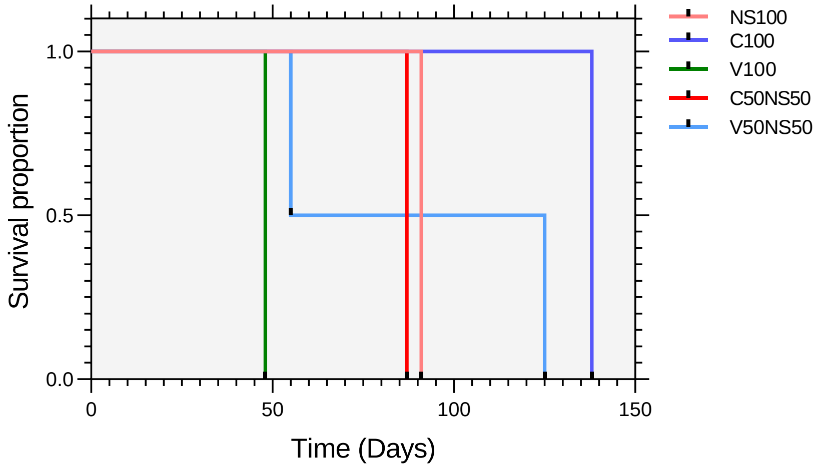

Figure 2. Survival of cardon seedlings under organic manure treatments.

The arid and semi-arid zones are characterized by environments where water resources are limited, and temperatures are extreme. These zones have soils with low nutrient availability and atmospheric decompensation due to water loss by evapotranspiration (Vásquez-Méndez *et al*., 2011; Montaño *et al*., 2016). These characteristics cause cacti seedlings vulnerable in the early stages of life, with slow growth rates (Martínez-Ramos *et al*., 2016).

Morphometric variables of the stems

The analysis of variance showed significant differences ($p<0.05$) with respect to the variables evaluated of the stem between treatments of organic manures and control (Figure 3). It is observed that the treatment of NS100 (control) registers the thinnest and smallest stems, unlike the treatments with organic manures, which retain a higher humidity of the substrate. It is observed that the largest seedlings were registered for the treatment of C30NS70, C50NS50, C70NS30, V30NS70, V50NS50 and V70NS30. Even though the C100 and V100 treatments registered low emergence percentages, the few seedlings that emerged were larger than the NS100 treatment (control). The morphometric variables of the stem showed that the combination of organic manures and native substrate allowed rapid growth of the stems, due to the contribution of nutrients and moisture retention by the organic substrate (Domínguez, 2004; Doan *et al*., 2015; Nieto-Garibay *et al*., 2021). The smallest seedlings were recorded the treatments with extreme values of electrical conductivity and organic matter, the NS100 treatment had the lowest values of these parameters, while the V100 and C100 treatments were the opposite (Figure 3). According to González and Chueca (2010), Celaya-Michel and Castellanos-Villegas, (2011), the high saline content of soils can affect the development of the seedlings leading to water deficit; this can be a consequence of the use of pure proportions of organic manures which might hamper the growth in seedlings. The proportions combined with organic manures showed better seedling development than to the control treatment (NS100) (Figure 3). The above is supported by Uddin *et al*., 2012, who evidenced a positive effect application of organic manure with soil in leguminous seedlings.

Likewise, it is observed that there were significant differences ($p<0.5$) in the fresh weight of the seedlings between treatments. Graphs 3a and 3b show a directly proportional relationship between the stems' size and the fresh weight of each treatment. The number of spines also showed statistically significant differences ($p<0.5$) between the control (NS100) and organic manure treatments. The combined treatments of organic manures showed a more substantial number of spines than the control group (NS100) and the pure treatments with organic manures (V100 y C100). Likewise, while counting the spines, it was possible to observe that the combined treatments

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had thicker spines while the control treatment had thinner spines. The cardon seedlings exhibited morphometric characteristics like those registered for other species of the Pachycereeae tribe, where it was observed as they grew that the first areoles had tiny spines and whitish trichomes (Loza-Cornejo and Terrazas, 2011).

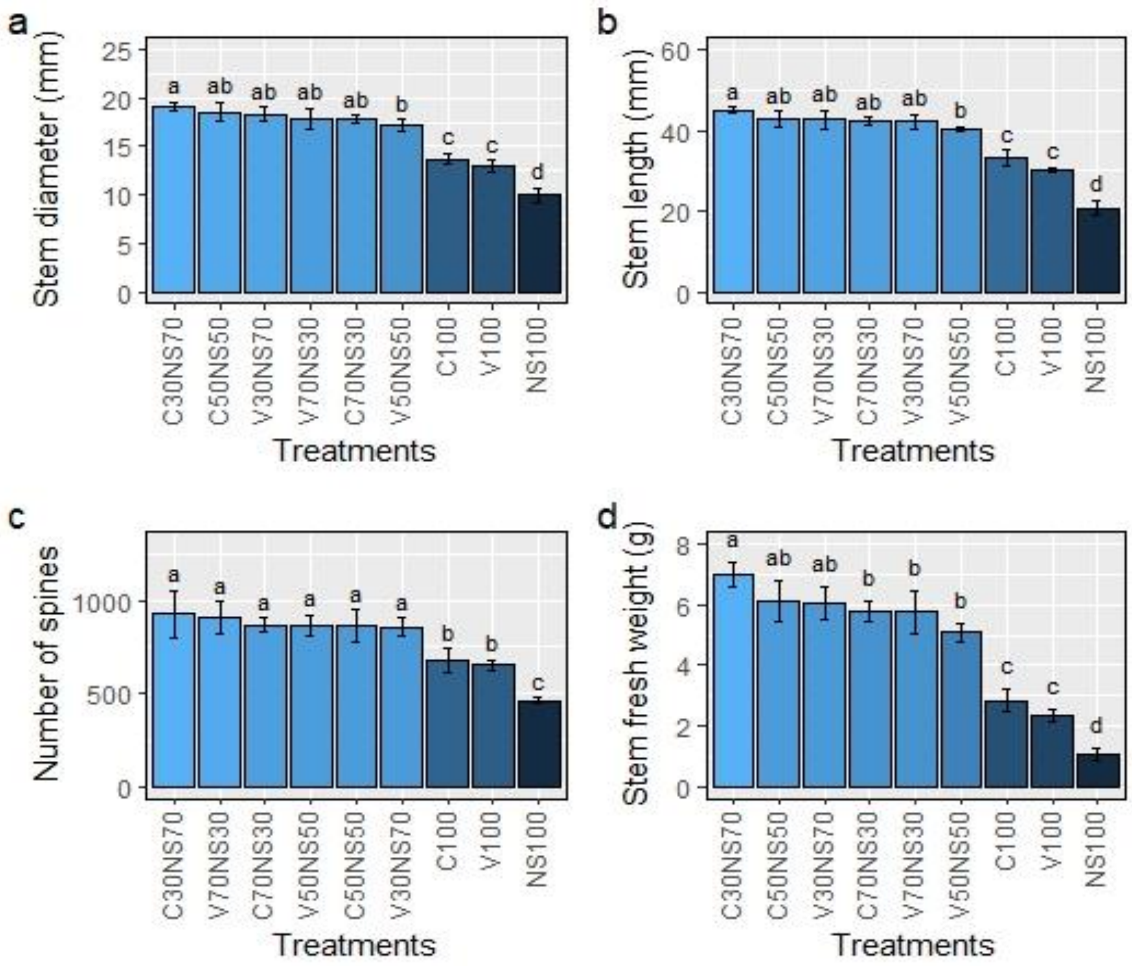

Figure 3. Average values (±SD) of stem morphometric variables of cardon seedlings under organic manure treatments, (a) Stem diameter; (b) Stem length; (c) Number of spines; (d) Stem fresh weight. The bars followed by the same letter are not significantly different (Tukey HSD *p*≤0.05). The vertical lines at the top of the bars represent the standard deviation.

Morphometric variables of the roots

There are statistically significant differences (p<0.5) in the evaluated variables of the root between treatments of organic manures and control (Figure 4). The same trend is observed with the treatments' seedlings of C30NS70, C50NS50, C70NS30, V30NS70, V50NS50, and V70NS30, which acquired seedlings of greater diameter and length showing higher root area values. Similarly, the same pattern is repeated for the average length of the tap root. The treatments of C100, V100, and NS100 showed little root growth, which may be related to the scarcity and ratio of organic manure used for these treatments as indicated previously. Loza-Cornejo *et al*. (2003) mention that the success of the establishment of cacti depends on the root system responsible for the absorption of water and nutrients from the soil. There is a relationship between the area of the root and the aerial part of the seedling since the treatments of NS100, V100, and C100 reported the thinnest and smallest stems. That is, as the plant has more root hairs, the size of the plant should be due to the greater absorption of water and nutrients (Niklas *et al*., 2000, 2002).

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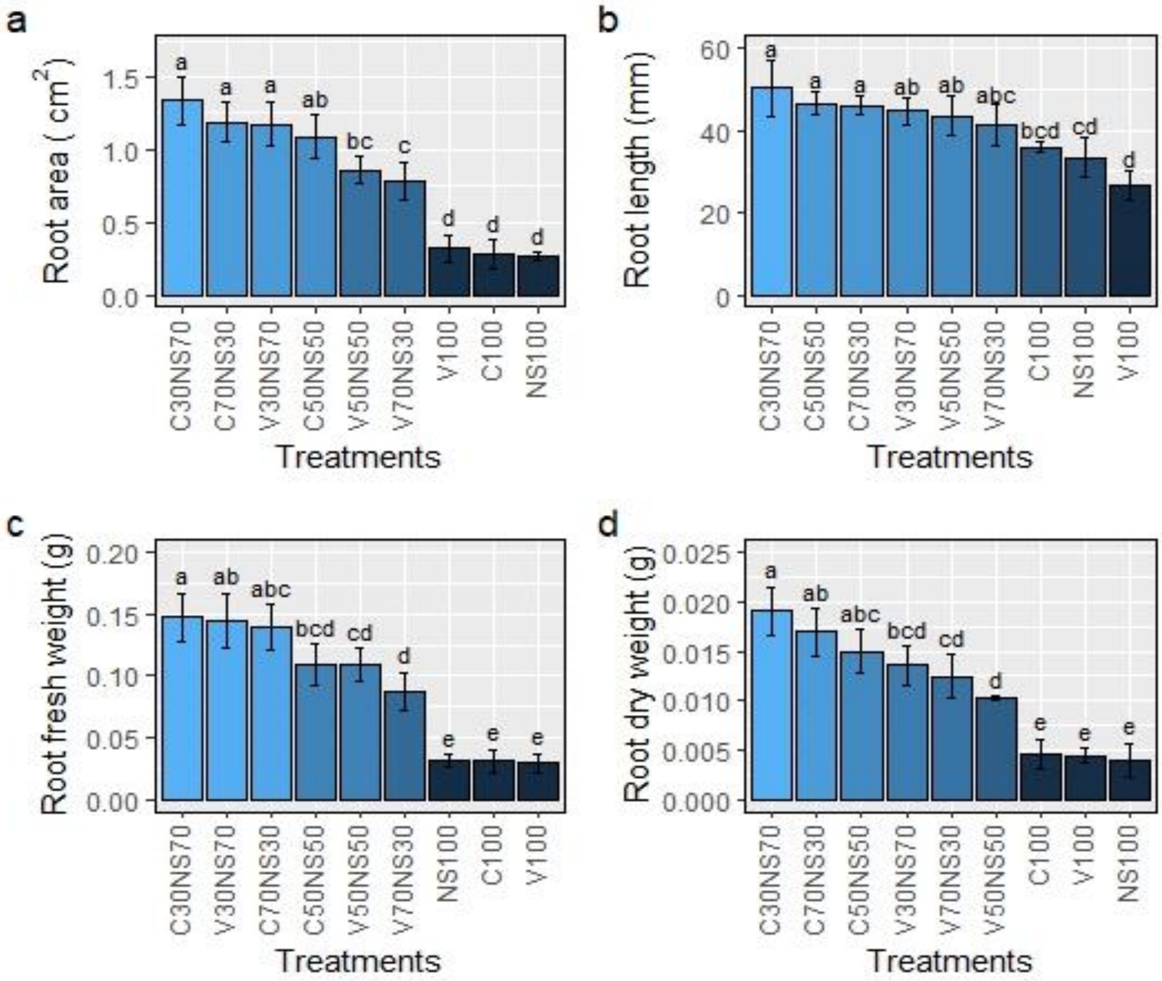

Figure 4. Average values (±SD) of root morphometric variables of cardon seedlings under organic manure treatments, (a) Root area; (b) Root length; (c) Root fresh weight; (d) Root dry weight. Significance levels were given by the Tukey test. The bars with different letters indicate significant differences at $p<0.05$, and the vertical lines at the top of the bars represent the standard deviation.

Physiological variables

Relative water content

In arid zones, the ability of a plant to maintain its water status as the soil water potential decreases constitutes an adaptation to water deficit, which is reflected in the relative water content (RWC) (Ortiz *et al*., 2003). It can be seen in Figure 5a, that the seedlings of the treatments do not present significant differences concerning the relative water content. Considering the importance of water in plants and the adaptations that cacti have developed to use water efficiently and to be able to survive in adverse conditions, it can be considered essential to know the water status of CAM plants. Even though the seedlings of the V100, V50NS50, and C100 treatments register shallow values of water potential (Figure 5c), it can be observed that the RWC was greater than 80% for these treatments. Cacti tissues have large vacuoles that occupy more than 90 % of the cell volume, which improves their water storage capacity (Pérez-Sánchez *et al*., 2015). This ability could explain why most treatments' RWC values are not as marked, and according to Flexas and Medrano (2002), the seedlings evaluated from 9 treatments showed moderate (70-85%) to slight (85-95%) stress.

Osmotic potential

The variable osmotic potential or osmotic pressure is expressed in megapascals (MPa) and is the concentration of osmotically active solutes (soluble sugars, ions, and amino acids) in the vacuole (Taiz and Zeiger, 2006; Salmon *et al*., 2020), which indicates the water status of the plant (Azcón-Bieto and Talón, 2013). The results showed that osmotic potential was affected in the C100, V100, and NS100 treatments (Figure 5b), registering the most negative values and smallest seedlings concerning the treatments with organic manures in proportions of 50 and 30 %. The above indicates that the stems under these treatments have dehydrated cells due to the low concentration of malate in the vacuoles (Azcón-Bieto y Talón, 2013; Delgado-Sánchez *et al*., 2017). However, cacti can adjust osmotically to tolerate dry environments (Goldstein *et al*., 1991; Delgado-Sánchez *et al*., 2013), and this can be compared in the present study, where the measured values of osmotic potential at the level of the lowest ones do not exceed < -1.2 MPa even though during the establishment phase it was irrigated every 10 days.

Water potential

The results show that the V100, C100 and NS100 treatments, where the irrigation water evaporated faster, recorded the most negative values of water potential and smaller seedlings compared to the other treatments. According to the Hsiao (1973) classification of water potential levels, the seedlings subjected to the treatment C30NS70 registered mild stress, moderate stress under C70NS30, C50NS50, V30NS70, and V70NS30 and severe stress with V50NS50, C100, V100, and NS100 (Figure 5c). Agentel *et al*. (2006) mentioned that the RWC and water potential influence the water relationships of plants. Likewise, the water deficit not only occurs when there is little water available in the environment, but also due to low temperatures and high soil salinity (Moreno, 2009). As it was explained in previous points, the treatments that registered severe stress have high levels of electrical conductivity, which cause the water potential of the seedlings more negative. However, cacti have developed adaptations and strategies that allow them to survive in these limiting conditions. Nilsen and Orcutt (2000) mention that xerophytic plants adapt to arid environments and can tolerate water potentials more negative than -4 MPa, which coincides with the results of this study related to the water potential and survival.

Turgor pressure

The variance analysis of the turgor pressure variable showed statistically significant differences ($p<0.5$) between the organic manures and control treatments (Figure 5d). The most negative turgor pressure treatment was recorded for V100. The turgor pressure suggests that the V100, NS100, V50NS50, and C100 treatments have less rigid cell walls, which would be related to the high content of EC in the pure proportions of organic manures that causes dehydration in the cells (Nobel, 2006; Schuch and Kelly, 2008). Furthermore, the control treatment without organic substrate was also affected by the turgor loss due to the irrigation water's rapid volatilization.

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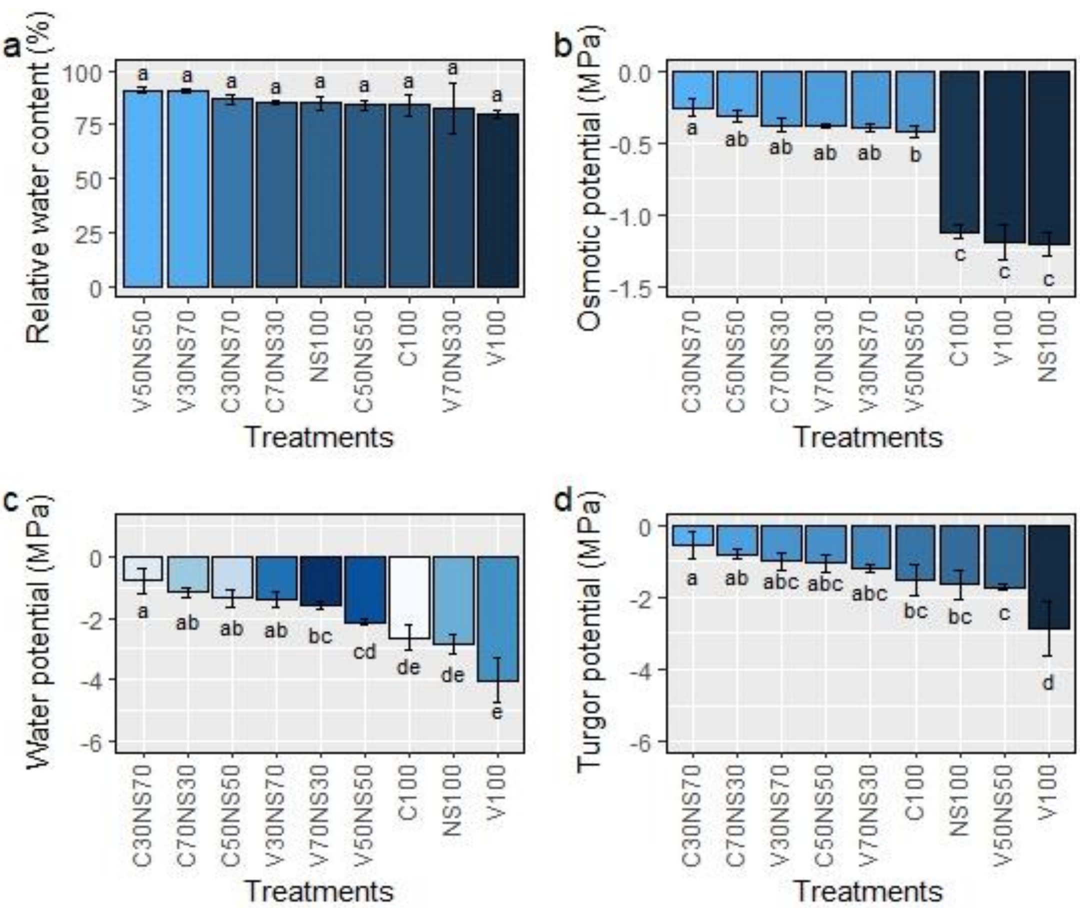

Figure 5. Average values (±SD) of physiological variables of cardon seedlings under organic manure treatments, (a) Relative water content; (b) Osmotic potential; (c) Water potential; (d) Turgor pressure. Significance levels were given by the Tukey and Dunn test. Different letters above the bars indicate significant differences at $p<0.05$, and the vertical lines at the top of the bars represent the standard deviation.

Chlorophyll a, b, and total

The content of chlorophyll a, b and total did not show significant differences between treatments (Figure 6); however, the highest average values of total chlorophyll were recorded for the C70NS30, V30NS70, and V100 treatments this pattern was recorded in the same way for chlorophyll a and chlorophyll b. The treatments with lower values of chlorophyll a, b, and total were recorded for C100 and NS100 (control). The total chlorophyll content with the organic manure treatments increased in 29 a 40 ug cm^{-2}. In studies using organic manures, there has been an increase in chlorophyll content which is directly related to good nutritional status (Degli-Esposti *et al*., 2011; Flores *et al*., 2022), while in substrates where the salt content is high, the presence of photosynthetic pigments decreases due to the inhibition of specific enzymes responsible for chlorophyll synthesis (Franco-Salazar and Véliz, 2008; Naseer *et al*., 2022). These results are similar to those reported by Meléndez *et al*. (2006) and Trujillo *et al*. (2010) who mention that the chlorophyll content decreases in soils with low moisture gradients and increases in soils with high moisture gradients. On the other hand, stress due to water deficit can also produce photoxidation and degradation of photosynthetic pigments, such as chlorophyll (Flexas and Medrano, 2002; Anjum *et al*., 2011).

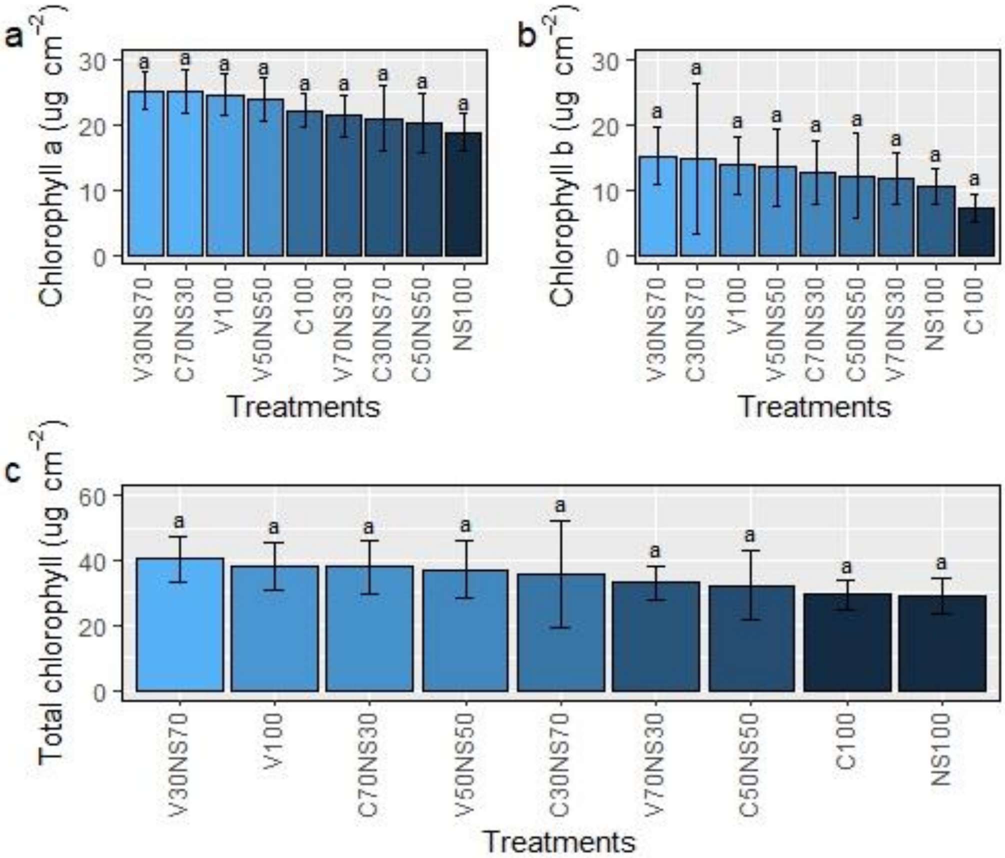

Figure 6. Average values (±SD) of physiological variables of cardon seedlings under organic manure treatments, (a) Chlorophyll a; (b) Chlorophyll b; (c) Total chlorophyll content. Significance levels were given by the Tukey and Dunn test. Different letters above the bars indicate significant differences at $p<0.05$, and the vertical lines at the top of the bars represent the standard deviation.

Conclusions

The results reinforce the information about the benefits by the organic manures of *P. pringlei* during the emergency and establishment phase. Incorporating organic manures in proportions of 50 and 30 % recorded higher values in different emergency indexes and increased the emergence of cardon seedlings. However, the pure treatments with organic manures decreased the emergence of cardon seedlings due to the combination of high levels of electrical conductivity and potassium content.

The establishment of seedlings was high in all treatments. Nevertheless, the seedlings under treatments with proportions of 30, 50 and 70 % of organic manures recorded higher values in the morphometrics variables of the stem and root, which is attributed to the retention of moisture and the supply of nutrients by organic manures, which are essential factors in the first stage of their life cycle. The physiological variables corroborated that cardon seedlings can tolerate negative water potentials of up to -4MPa; it was also possible to observe that the pure proportions of organic manures registered low levels of osmotic potential. The total chlorophyll content was not affected by using different substrates; however, there was a tendency to favor organic manure treatments.

Considering the importance of water in plants and the adaptations that cacti have developed to make this resource more efficient and to survive in adverse conditions, it is essential to know the water status of CAM plants and strategies to ensure establishment in the early stages of life.

Organic manures are a hopeful tool to increase the emergence and establishment of seedlings of *Pachycereus pringlei* (S.Watson) Britton & Rose and improve their morphological and physiological characteristics. In the same way, these results could have important implications for planning reforestation practices for cacti in danger of extinction.

Ethics statement

Not applicable

Consent for publication

Not applicable

Availability of supporting data

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

Conceptualization, J.S.., A.N.G. and A.M.N.; methodology, J.S., A.N.G., T.T., A.M.N., E.T.D. and B.M.A.; formal analysis, J.S. and E.T.D.; investigation, J.S.; resources, A.N.G.; data curation, J.S. and E.T.D.; writing—original draft preparation, J.S. and A.N.G.; writing—review and editing, J.S., A.N.G., T.T. and B.M.A.; visualization, A.N.G.; supervision, A.N.G.; project administration, A.N.G.; funding acquisition, A.N.G.

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