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Biometrics and physiological parameters of sour passion fruit seedlings produced on organic substrates

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Abstract

The organic fruit market has expanded considerably in recent years, but for the establishment of more productive organic orchards, several aspects need to be studied, starting with obtaining good quality seedlings. In this work, different formulations of organic substrates were evaluated to select the one that provides greater vigor to *Passiflora edulis* Sims seedlings. The study was conducted under anti-aphid screen conditions in two regions of Bahia (Recôncavo da Bahia, Chapada Diamantina), using a completely randomized design. The substrates were prepared using different proportions of coconut fiber, vermiculite, bovine manure and bokashi. At 60 days after sowing, germination vigor, gas exchange and growth parameters were evaluated. The data were submitted to principal component analysis and the groups formed were represented by box plots and compared by the Tukey test. Three groups were formed from PCA for the seedlings produced in both regions and the joint analysis, considering the two environments. In Chapada Diamantina, the seedlings produced in group 1, corresponding to 40% of the substrates tested, stood out for their root characteristics. In Recôncavo da Bahia, the seedlings showed low variation between the treatments $G1_C$ and $G2_C$ (80% substrates), compared to the $G3_C$ seedlings. The best performance was observed in the seedlings produced in Chapada Diamantina, but the use of coconut fiber and soil (CfSB), vermiculite and soil (SVB), both with 5% bokashi, produced excellent seedlings in both regions, so it is recommended for the organic production of *P. edulis*.

Keywords: *Passiflora edulis* Sims, principal component analysis, bokashi, bovine manure, photosynthesis.
Abbreviations: E_ emergence; MET_ mean emergence time; PH_ plan height; SD_ stem diameter; LA_ leaf area; APDM_ aerial part dry mass; RDM_ root dry mass; DQI_ Dickson's quality index; TRL_ total root length; MRD_ mean root diameter; RV_ root volume; root length as a function of the root diameter classes RL1_ 0.0 to ≤ 0.5 mm; RL2_ > 0.5 ≤ 1.0 mm; RL3_ > 1.0 mm; A_ photosynthesis; Ci_ internal carbon; E_transpiration; gs_ stomatal conductance; WUE_ water use efficiency; iWUE_ intrinsic water use efficiency; Cf cocou fiber; V_ vermiculite; S_ soil; M_ manure; B_ bokashi; C_ Cruz das Almas; L_ Lençóis. G1L_ group 1 in Lençóis; G2L_ group 2 in Lençóis; G3L_ group 3 in Lençóis; G1C_ group 1 in Cruz das Almas; G2_ group 2 in Cruz das Almas; G3C_ group 3 in Cruz das Almas. G1L, C_ group 1 in Lençóis and Cruz das Almas; G2L, C_ group 2 in Lençóis and Cruz das Almas; G3L, C_ group 3 in Lençóis and Cruz das Almas; PCA_ principal component; T_ air temperature; RH_ relative air humidity.

Introduction

Organic fruit and vegetable production is one of the fastest growing segments in agriculture worldwide (Sediyama et al., 2014; Bezerra et al., 2016; Coelho et al., 2018). The increase in the consumption of organic foods is due, in part, to society's greater awareness of the accumulation of chemical residues in fruits and vegetables grown conventionally, which are the main source of pesticide exposure to most humans (Mie et al., 2017).

The organic production system differs from the conventional system, among other factors, by not using chemical products for the control of pests and diseases and for the fertilization of plants in the field or during seedling production (Coelho et al., 2018; Embrapa, 2018). Due to the complexity of biological interactions in this system, production can be significantly varied, especially during the transition process between conventional and organic systems, which leads to a lower supply of foods in the market and a consequent increase in the price of organic produce (Goetzke et al., 2014; Pawlewicz, 2020). The establishment of a robust organic production system can minimize the variation in fruit production and promote the expansion of orchards, making this market more accessible to consumers (Jesus et al., 2016; Embrapa, 2018). For this, studies are necessary from the initial stages of seedling production to the post-harvest stage of fruits and vegetables.

The production of high-quality seedlings is important mainly for fruiting plants propagated by seeds, such as passion fruit (*Passiflora edulis* Sims), since it is correlated with the higher performance, uniformity and production of plants in the field (Lima Filho et al., 2019; Trazzi et al., 2020). Among the factors that affect the quality of seedlings, the type of substrate, environmental conditions, container volume, seed quality, adequate irrigation and fertilization are among the most important (Furlaneto et al., 2014; Grossnickle and MacDonald, 2018; Marques Honório et al., 2019). It is necessary to identify the formulations that provide the best balance among these factors, which is determined in most studies through biometric, physiological and nutritional parameters of the seedlings.

The substrate is considered the main factor that determines the quality of seedlings, because when well-prepared. it positively influences the vegetative vigor, root initiation and supply of nutrients, thus enhancing the physiological processes and consequent growth of the aerial part. Therefore, special attention should be given to formulations that provide rapid plant growth and good nutrition (Oliveira et al., 2016; Faria et al., 2016).

The use of alternative materials has been studied by various research groups (Oliveira et al., 2016; Faria et al., 2016; Albano et al., 2017), and found to promote the formation of more vigorous seedlings at lower production costs, in addition to providing appropriate use of potentially toxic wastes (Correa et al., 2019; Silva et al., 2019; Lobo et al., 2020). Some works have already analyzed the production of passion fruit seedlings using alternative substrates from different types of plant residues (Farias et al., 2019), such as chicken and cattle manure together with earthworm humus (Ribeiro et al., 2018), and different proportions of bovine manure in soil (Melo et al., 2019). The use of bokashi in the composition is also interesting, since it is a balanced mixture of agro-industrial residues, submitted to fermentation by microorganisms (Lima et al., 2015). However, studies testing this fertilizer for the production of passion fruit seedlings are scarce.

The statistical analyses used for substrate selection in most studies are univariate (Farias et al., 2019; Melo et al., 2019), which makes it difficult to select the best substrate when considering several traits. However, multivariate methods such as principal component analysis and correlation have contributed to the selection of high-quality substrates to produce *Passiflora edulis* Sims (Silva et al., 2021) and *Garcinia mangostana* L. seedlings (Gomes Júnior et al., 2019).

In this study, we started from the hypotheses that it would be possible to obtain vigorous and inexpensive seedlings of *P. edulis* using bokashi in the formulation of the substrate; and there would be no substantial variation in the seedlings produced in different regions using the same substrates. Thus, the objectives of this work were to select among different substrate formulations one that provides greater germination, physiological and biometric vigor in *Passiflora edulis* Sims seedlings for an organic production system and to determine possible variation in the quality of seedlings produced in the conditions of two regions in the state of Bahia, Brazil: Recôncavo da Bahia (municipality of Cruz das Almas) and Chapada Diamantina (municipality of Lençóis).

Results

Dispersion of treatments according to the two main components

The first two components (PC1 and PC2) contributed 68.5 and 74.0% of the total variability of the traits evaluated in the conditions of Lençóis and Cruz das Almas, respectively (Fig 1A, C). In the analysis considering the two environments, the characteristics APDM, RL3, LA, RL2, RV, SD, PH, RLT had greatest contribution to PC1, with 71.77%, while WUE, iWUE, A, gs, RL1, TRL, RDM and Ci contributed 78.83% to PC2 (Fig

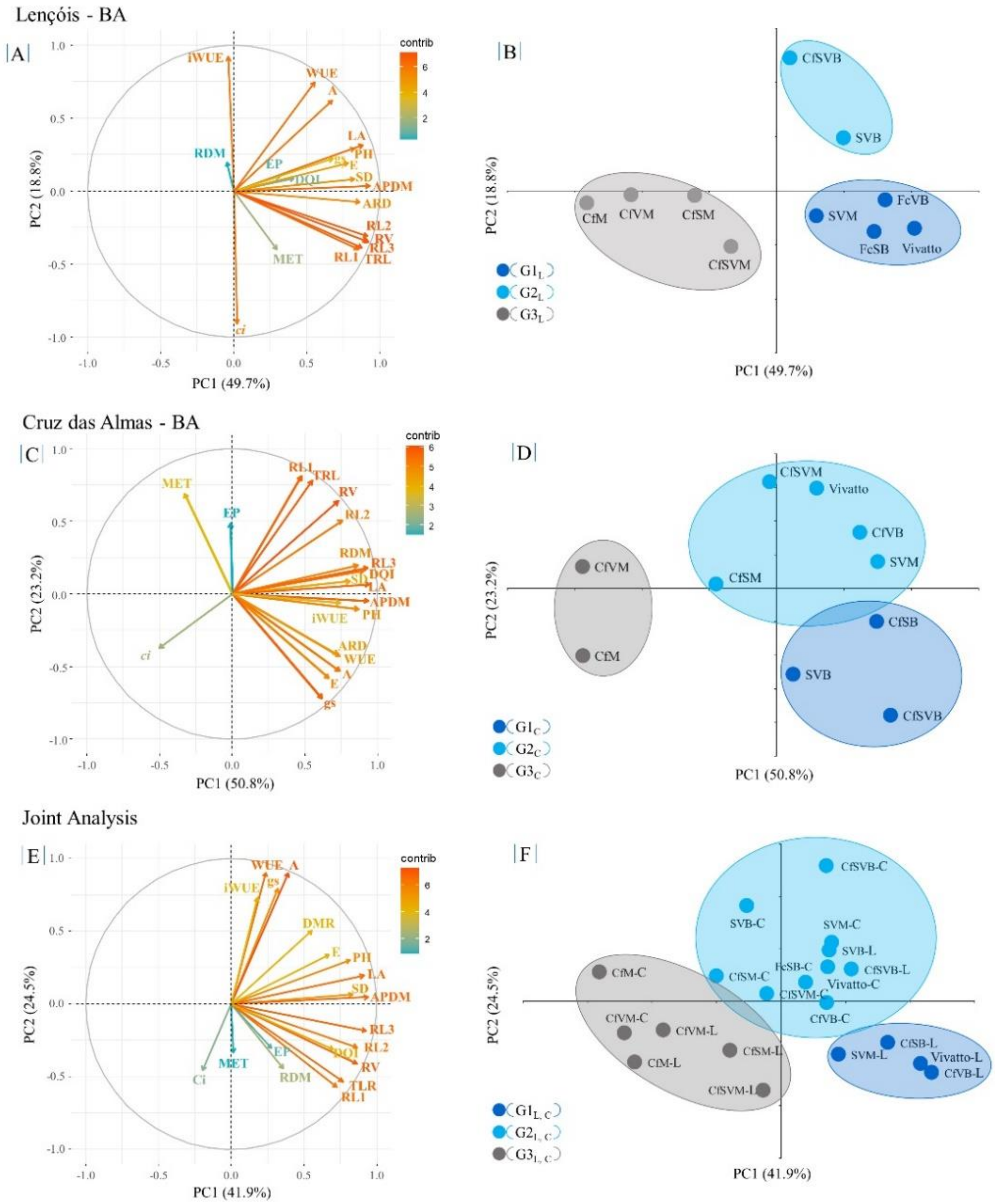

Fig 1. Biometric and physiological characteristics of *Passiflora edulis* seedlings produced in different substrates evaluated at 60 days after sowing in the cities of Lençóis (A-B) and Cruz das Almas (C-D), and joint analysis of the seedlings produced in Lençóis and Cruz das Almas (E-F). A, C and E: circle of correlation for the variables germination vigor, physiological traits of the aerial part and root variables. The arrows represent the direction of the traits, and its gradient colors represent the contribution of each trait to the components, from blue (low) to red (high). B, D and F: scatter plots of points in the first two components for the germination variables, physiological traits, aerial part and root variables. E: emergence; MET: mean emergence time; PH: plan height; SD: stem diameter; LA: leaf area; APDM: aerial part dry mass; RDM: root dry mass; DQI: Dickson's quality index; TRL: total root length; MRD: mean root diameter; RV: root volume; root length as a function of the root diameter classes RL1: 0.0 to ≤ 0.5 mm; RL2: > 0.5 ≤ 1.0 mm; RL3: > 1.0 mm; A: photosynthesis; Ci: internal carbon; E: transpiration; gs: stomatal conductance; WUE: water use efficiency; iWUE: intrinsic water use efficiency. Cf coconut fiber; V: vermiculite; S: soil; M: manure; B: bokashi; C: Cruz das Almas; L: Lençóis. $G1_L$: group 1 in Lençóis; $G2_L$: group 2 in Lençóis; $G3_L$: group 3 in Lençóis; $G1_C$: group 1 in Cruz das Almas; G2: group 2 in Cruz das Almas; $G3_C$: group 3 in Cruz das Almas. $G1_{L, C}$: group 1 in Lençóis and Cruz das Almas; $G2_{L, C}$: group 2 in Lençóis and Cruz das Almas; $G3_{L, C}$: group 3 in Lençóis and Cruz das Almas.

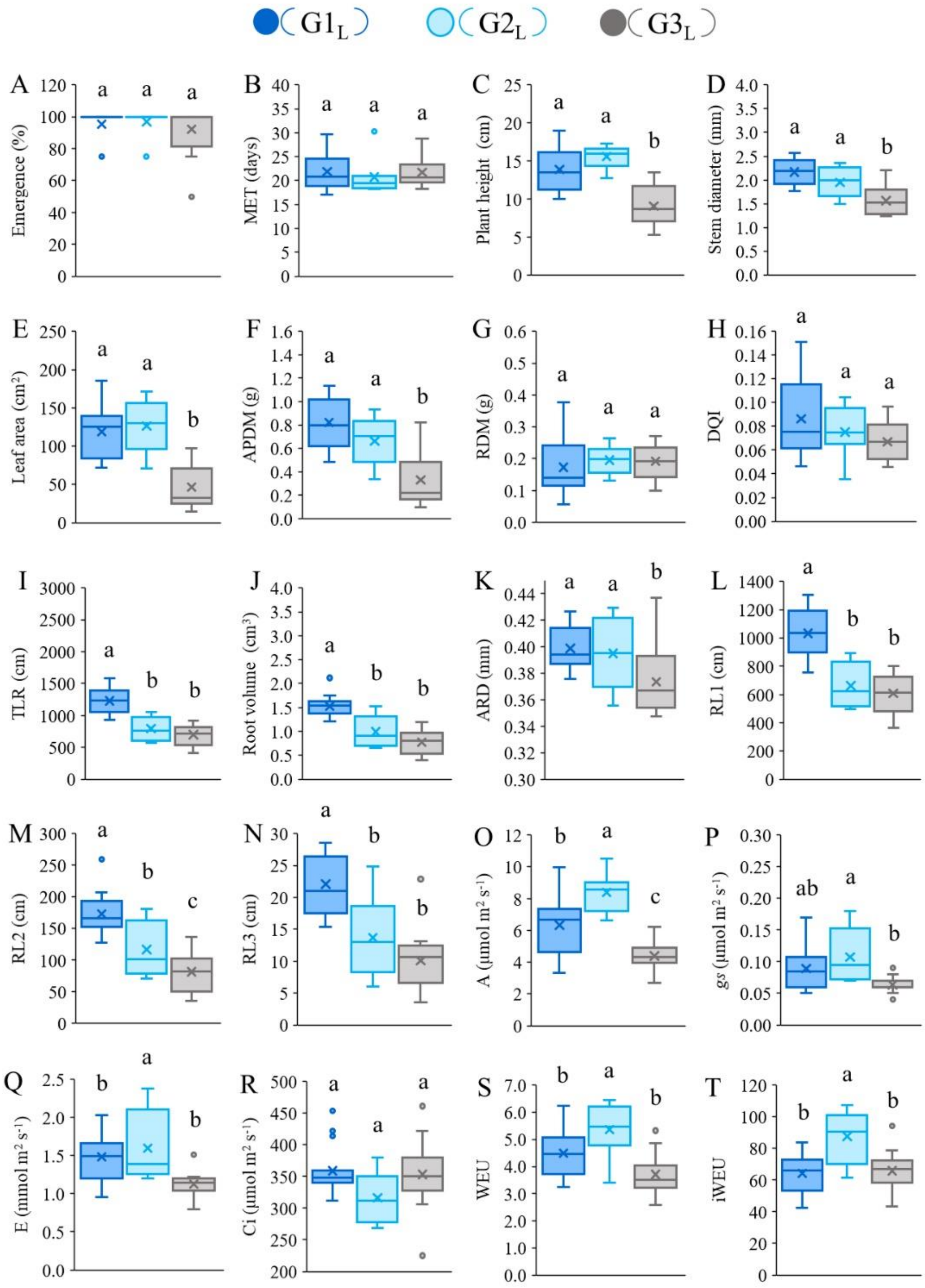

Fig 2. Germination vigor, areal part, root and physiological characteristics of *Passiflora edulis* plants produced in different organic substrates evaluated at 60 days after sowing in Lençóis. A-T: box plots comparing the means of each group for each variable using the Tukey test ($p \leq 0.05$). E: emergence; MET: mean emergence time; PH: plant height; SD: stem diameter; LA: leaf area; APDM: aerial part dry mass; RDM: root dry mass; DQI: Dickson's quality index; TRL: total root length; MRD: mean root diameter; RV: root volume; root length as a function of the root diameter classes RL1: 0.0 to ≤ 0.5 mm; RL2: > 0.5 ≤ 1.0 mm; RL3: > 1.0 ≤ 1.5 mm; A: photosynthesis; Ci: internal carbon; E: transpiration; gs: stomatal conductance; WUE: water use efficiency; iWUE: intrinsic water use efficiency. $G1_L$: group 1 in Lençóis; $G2_L$: group 2 in Lençóis; $G3_L$: group 3 in Lençóis.

1E).

The seedlings produced in Lençóis in the different substrates formed three groups based on the PCA, with better performance being verified for the substrates of Group 1 ($G1_L$), whose composition was soil, coconut fiber, vermiculite, bokashi and manure, in addition to the control treatment. $G1_L$ was influenced mainly by the aerial part, roots and physiological characteristics such as stomatal conductance (gs) and transpiration (E). Group 2 ($G2_L$), formed by two treatments, which had in common the bokashi component in the formulation, was more influenced by the physiological characteristics such as the water use efficiency (WUE) and photosynthesis (A). Group 3 ($G3_L$), formed by four treatments, which had in common the manure component in the formulation, presented a lower result in relation to the other substrates (Fig 1A-B).

Box plots of the three groups formed from PCA of the seedlings produced in Lençóis revealed that the information corroborated in general the PCA data, since $G1_L$ stood out in the aerial part traits, such as plant height, stem diameter, leaf area and aerial part dry mass; and root traits, such as total root length, root volume, average root diameter and root length in the 0 to 0.5 mm diameter classes, with 0.5 to 1.0 mm and 1.0 to 1.5 mm being higher by 35%, 28%, 61%, 59%, 60%, 7.7%, 8%, 57%, 47% and 49%, respectively, in relation to the seedlings produced in the $G3_L$ substrates, which contained 5% manure (Fig 2C-F, I-N). The traits emergence percentage (E), mean emergence time (MET), root dry mass (RDM) and Dickson quality index (DQI) did not differ among groups for seedlings produced in Lençóis (Fig 2A- B, G-H).

Regarding the physiological characteristics of the seedlings formed in Lençóis, higher values were found for the seedlings produced in the $G2_L$ substrate, with emphasis on photosynthesis, transpiration, water use efficiency and intrinsic efficiency in the use of water, which differed from the seedlings produced in the $G1_L$ and $G3_L$ substrates (Fig 2O, Q, S, T). The $G2_L$ substrates had in common the use of soil, vermiculite and bokashi in the formulation (Table 1, Supplementary Table 1).

For the seedlings produced in Recôncavo da Bahia (Cruz das Almas), three groups were also formed based on principal component analysis. Group 1 ($G1_C$) contained plants grown in the substrates that showed the best performance. However, for most traits, the response was similar to the substrates in $G2_C$ (Fig 1C-D, Fig 3). $G3_C$, formed by the treatments of coconut fiber with vermiculite in the proportion 1:1 with 5% bovine manure (CfVM) and coconut fiber with 5% bovine manure (CfM), had a worse result, not being recommended for the production of passion fruit seedlings in the conditions of Recôncavo da Bahia, with strong contributions from the mean emergence time (MET) and internal carbon (Ci) (Fig 1C-D).

Considering the PCA, for the seedlings produced in Cruz das Almas and Lençóis the first two components contributed 66.4% of the total variation (Fig 1E). The dispersion of treatments in the first two components formed three groups, with emphasis on $G1_{L,\ C}$ with seedlings formed only in Lençóis, while $G2_{\ L,\ C}$ was formed by seedlings produced in both locations (Fig 1E). Group 3 ($G3_{L,\ C}$) contained the six lowest performing substrates. In this group, the substrates had in common the use of 5% bovine manure and had an inferior result for both places (Fig 1F).

The performance of seedlings produced in Cruz das Almas in $G1_C$ and $G2_C$ substrates was similar for most characters, with the exception of the average emergence time, total root length and root length in the diameter class between 0 and 0.5 mm (RL1), which were 17%, 33% and 37% higher for seedlings formed in $G2_C$ substrates (Fig 3B, I, L). The aerial part dry mass and the average root diameter were 26 and 9.0% higher in the substrates of $G1_C$ compared to $G2_C$ (Fig 3F, K). The seedlings produced in the $G3_C$ substrates showed lower results for most of the traits (Fig 4A-T), having in common the presence of coconut fiber and manure in the formulation (Table 1).

Fig 4 shows that the seedlings produced in Lençóis had the highest averages, except for photosynthesis (Fig 4O), stomatal conductance (Fig 4Q) and water use efficiency (Fig 4S), which were 78%; 73% and 71% higher in seedlings produced in Cruz das Almas. The characters emergence percentage, mean time of emergence, plant height, stem diameter, leaf area, aerial part dry mass and mean root diameter were similar for both sites (Fig 4A-F, J, P, R, T).

Pearson's correlation analysis, represented in the form of a network (Fig 5), showed a strong correlation for both locations between the chemical characteristics of the substrates for Ca, Na and P; and the physiological characteristics stomatal conductance (gs), transpiration (E), photosynthesis (A) and water use efficiency (WUE), as well as the biometrics LR2, LR1, APDM, LR3, RV, TRL and MRD. In Lençóis, there were strong positive correlations between transpiration and height (r = 0.93) stomatal conductance and height (r = 0.79), photosynthesis and height (r = 0.73), in addition to a strong relationship between leaf dry mass and the total root length (r = 0.67) and the sum of bases (r = 0.54) of the substrates (Fig 5A). The correlations in Cruz das Almas demonstrated a strong relationship between the QDI and the root characters, stem diameter and aerial part dry mass. Regarding the physiological traits, strong positive correlations were observed between photosynthesis and seedling height (r = 0.76), photosynthesis and total root length (r = 0.67), and

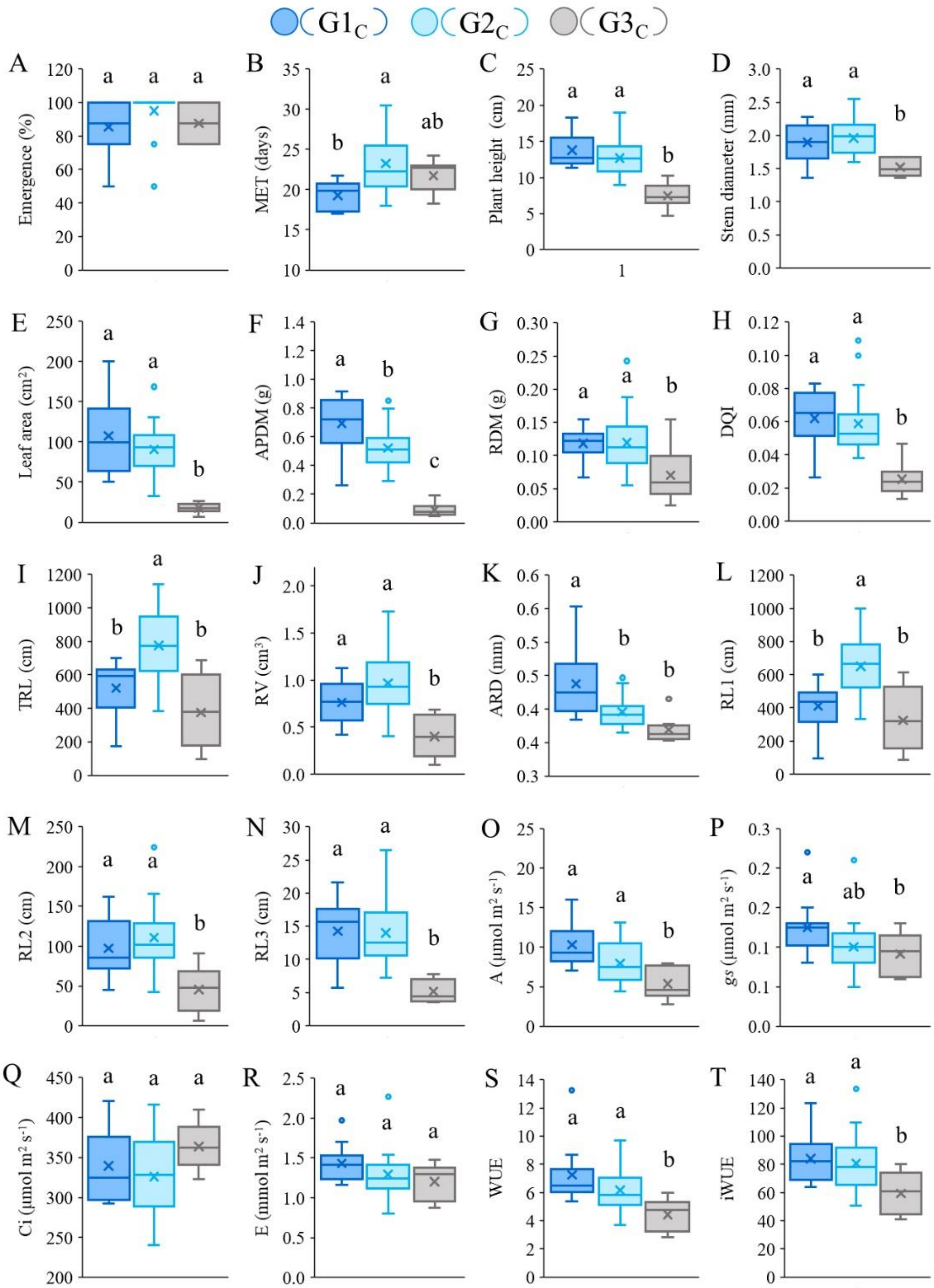

Fig 3. Germination vigor, aerial part, root and physiological characteristics of *Passiflora edulis* plants produced in different organic substrates evaluated at 60 days after sowing in Cruz das Almas. A-T: box plots comparing the means of each group for each variable using the Tukey test ($p \leq 0.05$). E: emergence; MET: mean emergence time; PH: plant height; SD: stem diameter; LA: leaf area; APDM: aerial part dry mass; RDM: root dry mass; DQI: Dickson's quality index; TRL: total root length; MRD: mean root diameter; RV: root volume; root length as a function of the root diameter classes RL1: 0.0 to ≤ 0.5 mm; RL2: > 0.5 ≤ 1.0 mm; RL3: > 1.0 ≤ 1.5 mm; A: photosynthesis; Ci: internal carbon; E: transpiration; gs: stomatal conductance; WUE: water use efficiency; iWUE: intrinsic water use efficiency. $G1_C$: group 1 in Cruz das Almas; $G2_C$: group 2 in Cruz das Almas; $G3_C$: group 3 in Cruz das Almas.

photosynthesis and root volume (r = 0.69), and strong negative correlation between pH and efficiency of intrinsic water use (r = -0.88) (Fig 5B).

Discussion

For the production of organic seedlings, it is necessary for the substrates to have adequate physical, chemical and biological characteristics, since these positively influence the vegetative vigor, root initiation and the supply of nutrients to enhance the physiological processes and consequent growth of the aerial part, and thus the quality of seedlings (Nadai et al., 2015; Oliveira et al., 2016; Faria et al., 2016).

For the conditions of Lençóis and Cruz das Almas, the $G1_{L,\ C}$ substrates, formed by coconut fiber, soil and vermiculite in different proportions, with bokashi in the concentration of 5% in common, stood out in relation to the others, being recommended for the production of *P. edulis* seedlings (Table 1, Fig 1). In addition to promoting favorable performance of seedlings, the substrates of this group had low cost, providing greater potential for use in the production of seedlings.

Bokashi is a natural fertilizer widely used in organic agriculture to supply nutrients to plants. It improves the physical, chemical and biological characteristics of the soil, thus contributing to the formation of seedlings with a more developed root system, positively influencing the establishment and productivity of plants in the field (Van Bruggen, 2016). Additionally, it can be formulated with various materials available in farms, such as manure, wheat bran, castor cake and rock powder (Jaramillo-López et al., 2015). The use of bokashi has shown promising results both for the production of seedlings and fertilization in the field (Jaramillo-López et al., 2015; Embrapa, 2018). Its use is thus an alternative to reduce costs in relation to commercial substrates for passion fruit production.

Despite the similarity in the composition of the substrates that stood out in the production of seedlings in both locations, the seedlings produced in Lençóis performed better in seven of the 20 traits evaluated, while in Cruz das Almas the seedlings showed higher averages in only three physiological variables (Fig 4). This result of the physiological variables may be related to the environmental conditions at the time of reading, which favored the seedlings produced in Cruz das Almas, thus indicating the influence of the environment on the physiological responses and the consequent quality of the seedlings, since the substrate, container and irrigation were the same in both locations.

The better performance of seedlings produced in Lençóis might also be associated with the origin of the genotype used in the test, which was from Brumado, Bahia, close to Chapada Diamantina, with environmental conditions similar to those to which the genotype is adapted. In addition, during the study period, the average values of maximum (36.2 °C), average (23.5 °C) and minimum (16.2 °C) temperature in Lençóis were 1.1, 1.0 and 3.1 °C lower than the average values recorded in Cruz das Almas, respectively, which may have favored greater growth and mass accumulation (Supplementary Fig 1).

Variations in seedling quality as a function of the environment have also been reported for *Passiflora alata* (Pio et al., 2004) and for other species not belonging to the *Passiflora* genus, such as *Copernicia hospita* Martius (Oliveira et al., 2009) and *Bauhinia forficata* Link (Ronchi et al., 2016), indicating the relationship between environment and substrate regarding seedling quality.

The seedlings produced in the substrates with bovine manure in the formulation in general performed worse (Fig 1-3). However, several studies have reported a beneficial effect of this waste on the formulation of substrates to grow *P. edulis* (Moreira et al., 2013; Cavalcante et al., 2016), possibly associated with the concentration reported by these authors, which ranged from 25 to 75% in the formulations, while in our study the concentration was 5% (Table 1). The use of coconut fiber was shown to be promising for the formation of *P. edulis* seedlings, since it facilitated the development of roots throughout the substrate, providing an increase in the uptake of water and nutrients.

From the correlation analysis it was possible to identify a relationship between root and aerial part regarding physiological, biomedical, nutritional and physiological traits (Fig 5). The correlation estimates allow predicting the behavior of a certain characteristic when the selection is made based on another one (Jesus et al., 2021). Photosynthesis (A) is a very important variable, because the quality of the seedlings is linked to the photosynthetic capacity of the species in relation to the growing conditions and the level of photoassimilates (Santos et al., 2019; Lima et al., 2020). The plant height and leaf dry mass are important aspects to determine the quality of seedlings, because they are related to the performance of the plant in absorbing nutrients and producing photoassimilates, which will later be partitioned in terms of root system and aerial part, and thus affect the ability to adapt in the field after transplantation (Silva et al., 2019).

Strong correlations between the cation exchange capacity and intrinsic water use efficiency (r = 0.63) were recorded in Cruz das Almas. This same correlation in Lençóis was r = 0.19, indicating that the correlations may vary depending on the environmental conditions (Fig 5). Although there are often consistent patterns between the characteristics evaluated, these correlations can be

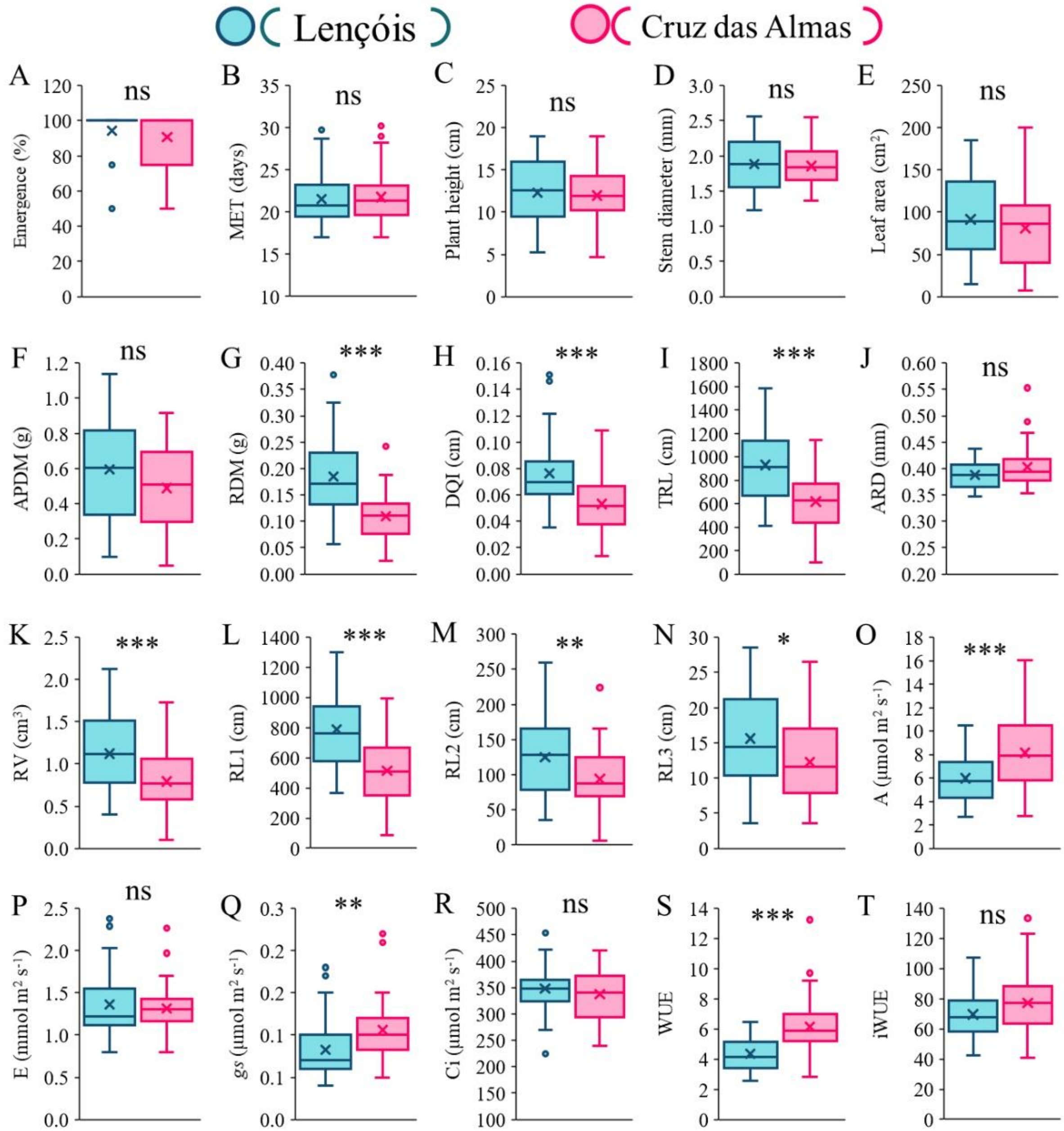

Fig 4. Comparison between the characteristics of germination vigor, aerial part, root and physiological characteristics of *Passiflora edulis* seedlings submitted to the substrates evaluated at 60 days after sowing in Lençóis and Cruz das Almas. A-T: box plots comparing the averages of each location for each variable. ns, *, ** and *** indicate non-significant, significant variation at 5%, 1% and <1%, respectively by the F-test. E: emergence; MET: mean emergence time; PH: plant height; SD: stem diameter; LA: leaf area; APDM: aerial part dry mass; RDM: root dry mass; DQI: Dickson's quality index; TRL: total root length; MRD: mean root diameter; RV: root volume; root length as a function of the root diameter classes RL1: 0.0 to ≤ 0.5 mm; RL2: > 0.5 ≤ 1.0 mm; RL3: > 1.0 mm; A: photosynthesis; Ci: internal carbon; E: transpiration; gs: stomatal conductance; WUE: water use efficiency; iWUE: intrinsic water use efficiency.

absent in different environments (Sgrò and Hoffmann, 2004), due to the interaction of the genotype with the environment, which can change the correlations between the characters (Sgrò and Hoffmann, 2004; Sikkink et al., 2017) and influence the genetic interactions between traits, as well as the genetic variance in the same trait (Sgrò and Hoffmann, 2004).

New environments can induce positive correlations between traits because the expression of new genes will break negative correlations (Sgrò and Hoffmann, 2004).

The multivariate investigation through principal component analysis and the correlation network proved to be efficient for the selection of substrate, since superior performance was observed of the seedlings

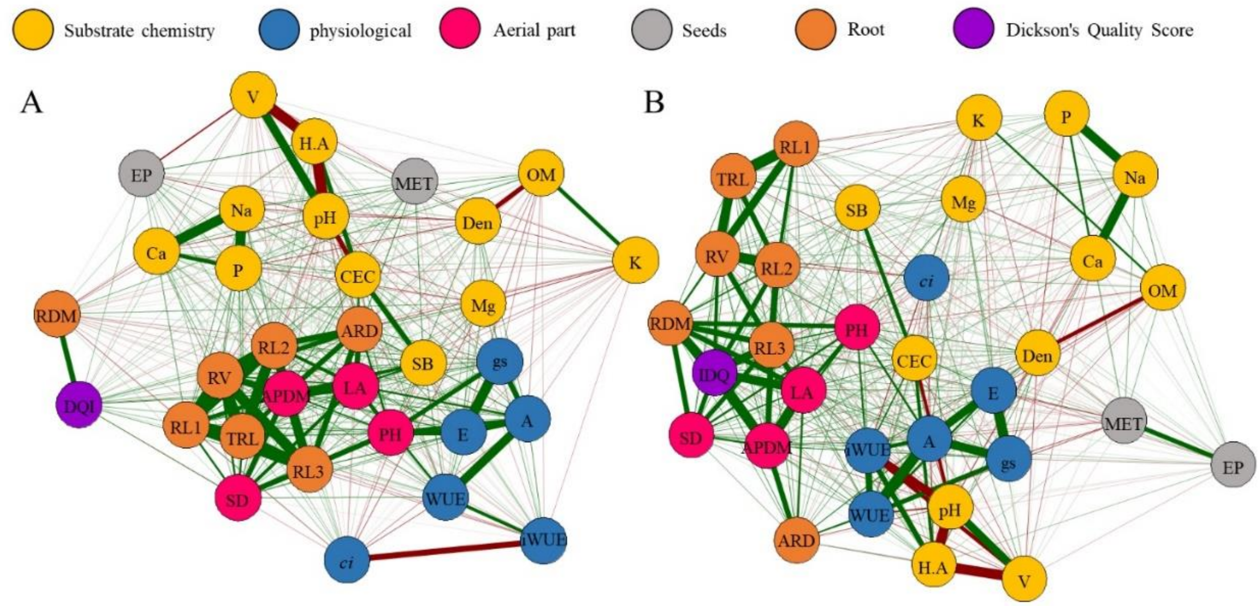

Fig 5. Correlation networks of chemical characteristics of the substrates, germination vigor, and physiological, aerial part and root traits *Passiflora edulis* seedlings formed in different substrates and evaluated at 60 days after sowing in Lençóis (A) and Cruz das Almas (B). The red and green lines represent positive and negative correlations, respectively. The width of the line is proportional to the intensity of the correlation. E: emergence; MET: mean emergence time; PH: plant height; SD: stem diameter; LA: leaf area; APDM: aerial part dry mass; RDM: root dry mass; DQI: Dickson's quality index; TRL: total root length; MRD: mean root diameter; RV: root volume; root length as a function of the root diameter classes RL1: 0.0 to ≤ 0.5 mm; RL2: > 0.5 ≤ 1.0 mm; RL3: > 1.0 mm; A: photosynthesis; Ci: internal carbon; E: transpiration; gs: stomatal conductance; WUE: water use efficiency; iWUE: intrinsic water use efficiency.

produced in the substrates of $G1_{L, C}$ for most of the characters (Figs 2, 3). Promising results using PCA for the selection of substrate for the production of *Garcinia mangostana* L. seedlings were also reported by Gomes Júnior et al. (2019), and for *Passiflora edulis* by Silva et al. (2021). This approach is efficient mainly when seeking to select substrates among many formulations and to indicate which characters are most important in the selection process of vigorous seedlings. Here we identified that characteristics of aerial part and root had high contribution to the first two components, in both locations, while the characters of seeds presented low contribution.

The results obtained in this study indicated that the mixture of coconut fiber and soil in the proportion 1:1 with 5% bokashi was the most efficient, because this formulation presented promising results for the seedlings produced in the two environments. Future studies evaluating the performance of seedlings formed in this substrate under field conditions should be conducted to confirm the findings of the seedling quality in the field.

Materials and methods

Location of the experiments

The experiments were conducted under an anti-aphid screen with transparent plastic film cover at the experimental farm of the Embrapa Cassava and Fruits research unit (*Embrapa Mandioca e Fruticultura*), located in Cruz das Almas, Bahia (BA) (12°39'25” S, 39°07'27” W, 222 m) and the company Bioenergia Orgânico, located in Lençóis, BA (12°32'54.1" S, 41°20'26.4" W, 457 m), with distance apart of 302 km. During the experiment, relative humidity (%) and temperature (°C) (minimum, average and maximum) were monitored daily at 10 min reading intervals (Supplementary Fig 1).

Plant material and experimental design

Passiflora edulis Sims seeds (BGP418) from the Passion Fruit Germplasm Bank of Embrapa Mandioca e Fruticultura were used. The seeds were extracted from ripe fruits obtained from controlled pollination, washed in running water, and dried at room temperature for one week until their use in the tests. The experimental design was completely randomized, consisting of 10 treatments (Tables 1 and 2) distributed in four replications of four plants per plot.

Preparation of substrates

The substrates were formulated from a mixture of different proportions of coconut fiber (Golden Mix, Amafibra, Artur Nogueira, SP), soil (dystrophic red oxisol, alic, with clayey texture), (Embrapa, 1999),

Table 1. Proportion of substrates and percentage of organic fertilizer used to produce *Passiflora edulis* Sims seedlings.

Treatment.	Coconut fiber (%)	Soil (%)	Vermiculite (%)	Bovine manure (%)	Bokashi (%)	Density (kg m^{-3})
CfSB	47.5	47.5	0.0	0.0	5.0	629
SVB	0.0	47.5	47.5	0.0	5.0	804
CfVB	47.5	0.0	47.5	0.0	5.0	364
CfSVB	47.5	47.5	47.5	0.0	5.0	640
CfE	47.5	0.0	0.0	5.0	0.0	269
CfSM	47.5	47.5	0.0	5.0	0.0	551
SVM	0.0	47.5	47.5	5.0	0.0	763
CfVM	47.5	0.0	47.5	5.0	0.0	330
CfSVM	47.5	47.5	47.5	5.0	0.0	600
Vivatto (control)						288

Cf coconut fiber; S: soil; V: vermiculite; M: manure; B: bokashi.

vermiculite (Brasil Minérios, Santa Genoveva, Goiânia, GO), bovine manure and bokashi (Embrapa, 2018), totaling 10 different substrates in terms of ingredients and proportions (Tables 1 and Supplementary Table 1). The commercial substrate Vivatto Plus® (Vivatto, Technes, Guatapará, SP) was used as a control treatment, distinct from the other substrates tested in terms of formulation and composition and not recommended for organic agriculture because it is an artificial input.

The substrates were mixed and kept at rest for ten days and then distributed in polyethylene tubes with dimensions of 12.0 x 1.4 cm and volume of 280 cm^3. The seeds were sown at a depth of 1.0 cm and irrigation was applied daily in the morning.

Biometric and physiological characteristics

The variables evaluated in both locations were the seedling emergence percentage (EP) at 45 days after sowing (DAS) and the mean emergence time (MET). Seedlings were considered emerged when they had free and normal cotyledons (Brasil, 2009).

The biometric variables evaluated at 60 DAS in both locations were plant height (PH) in cm, measured using a tape measure from the soil level to the apical meristem; stem diameter (SD) in mm, measured using a pachymeter at 3.0 cm from the soil level; total leaf area (LA) in cm^2 (all leaves of the plant were photographed and the area was estimated using ImageJ®); and the Dickson quality index (DQI), based on the formula proposed by Dickson et al. (1960):

$$\mathrm{DQI} = \mathrm{TDM}/\left[\left(\frac{\mathrm{PH}}{\mathrm{SD}}\right) + \left(\frac{\mathrm{APDM}}{\mathrm{RDM}}\right)\right]$$

where, TDM = total dry mass (g); PH = plant height (cm); SD = stem diameter (mm); APDM = aerial part dry mass (g); and RDM = root dry matter (g).

The roots were placed in 30% alcohol and refrigerated until the process of digitalizing the images, carried out by means of a scanner using the software WinRizho 2013d, with resolution of 400 dpi, to obtain the following variables: total root length (TRL) in cm; average root diameter (ARD) in mm; total root volume (RV) in cm^3; and total root length as a function of the following root diameter classes: RL1: 0.0 to ≤ 0.5 mm; RL2: > 0.5 ≤ 1.0 mm; and RL3: > 1.0 mm diameter. The aerial part dry mass (APDM) and root dry matter (RDM) were obtained after drying in an oven with forced air circulation at 65 °C until constant weight.

The physiological variables evaluated at 60 DAS in fully expanded leaves previously selected for both experiments were: internal carbon (Ci); transpiration (E); stomatal conductance (gs); photosynthesis (A); water use efficiency (WUE); and intrinsic water use efficiency (iWUE). Readings were performed using a portable LCpro-SD IRGA meter (ADC BioScientific Limited, UK) at 1000 µm photons m^{-2} s^{-1} of photosynthetically active radiation (PAR). The evaluation was carried out between 9:00 and 11:00 a.m., after stabilization of the physiological characteristics.

Statistical analysis

The data were submitted to principal component analysis (PCA) for each evaluation site and a third analysis with data from both sites, in which the treatments were plotted in relation to the two main components (PC1 and PC2), which explained the greatest part of the variability of the data.

The analysis was also carried out in order to identify which characteristics most contributed to the discrimination of groups. The number of groups was defined based on the dispersion of treatments indicated by the first two components (PC1 and PC2). From the groups, box plots were generated for each characteristic and the data were subjected to analysis of variance and the means, when significant, were differentiated by the Tukey test (p ≤ 0.05) for the data for each region and compared by the F-test of ANOVA in the comparison between the seedlings produced in Lençóis and Cruz das Almas. To compare the variables analyzed with the chemical attributes of the soil, Pearson's correlation was performed and the data were plotted in a correlation network graph. All analyses were performed using the software R V4.0.1 (R Development Core Team, 2020).

Conclusion

There was significant variation between the traits analyzed depending on the place where the seedlings were produced, with the best performance being in Chapada Diamantina. The mixtures of coconut fiber and soil (CfSB), in the proportion 1:1, and soil plus vermiculite (SVB), both with 5% bokashi, are recommended for the production of *P. edulis* seedlings under the conditions in Chapada Diamantina and Recôncavo da Bahia, due to the high vigor of seedlings in the two evaluated environments.

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Authors' Contributions

ONJ, MOCF and LKSL, conception and design; LNS, ISS, SRS, LKSL, ONJ and MACF, acquisition of data; OND, LKSL and LNS, analysis and interpretation of data; LNS, ISS, SRS, LKSL and ONJ, drafting the article; and LKSL, ONJ and MACF, critical review of important intellectual content.

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