

## Use of physiological parameters to assess seedlings quality of *Eugenia dysenterica* DC. grown in different substrates

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### Abstract

The aim of this study was to evaluate the quality and photosynthetic metabolism of *Eugenia dysenterica* DC. seedlings grown in different substrates. The seed were sown in the following substrates: MecPlant® (MP), rice husks (RH), subsoil (SB), fine vermiculite (FV), coarse sand (CS), tanned cattle manure (CM), decomposed corn silage (CS), and soil collected from around parent plants (SN). The volume-based substrates were formulated as follows: MP+RH (7:3), SB+FV+RH (1:2:2), SB+FV+RH (1:1:1), SB+FV+CS (1:3:6) SB+CS+CM (2:2:1), and SN. After 127 days, the seedlings were evaluated according to their emergence and vigor, biometric characteristics, leaf area (cm<sup>2</sup>), Dickson Quality Index (DQI), stomata conductance (mol m<sup>-2</sup> s<sup>-1</sup>), transpiration rate (mmol m<sup>-2</sup> s<sup>-1</sup>), photosynthesis (μmol m<sup>-2</sup> s<sup>-1</sup>) and leaf mineral nutrient levels. Overall, the SB+CS+CM substrate resulted in higher values for all of the characteristics analyzed, except for the leaf nutrient levels. However, in total, this substrate also resulted in high content of minerals in plant. Likewise, the SB+FV+CS substrate showed the second higher content of nutrients. Based on the results of this study, we concluded that tanned cattle manure and decomposed corn silage resulted in the best *Eugenia dysenterica* seedling quality.

**Keywords:** Cerrado; mineral nutrition; native fruit; physiological analysis; seedling production.

**Abbreviations:** *A*<sub>net</sub> CO<sub>2</sub> assimilation; *Ci/Ca* Ratio of internal to external; CM\_Tanned cattle manure; CO<sub>2</sub> concentration; CS\_Coarse sand; CS\_Decomposed corn silage; *E*<sub>v</sub> Transpiration; ETR\_ Electron transport rate; *F<sub>v</sub>/F<sub>m</sub>* Maximum photochemical efficiency; FV\_Fine vermiculite; *g<sub>s</sub>* Stomatal conductance; MP\_MecPlant; φPSII\_Effective quantum yield of PSII; RMC<sub>L</sub> Relative leaf moisture content; RMC<sub>S</sub> Relative substrate moisture content (dry basis); RH\_Rice husks; SB\_Subsoil, ; SN\_Soil collected from around parent plants.

### Introduction

The Brazilian Savanna (Cerrado) is the second largest Brazilian biome which covers some 2 million km<sup>2</sup> of Central Brazil representing about 22% of the land surface of the country. This Biome also possesses great social importance for fruit production. Fruits are consumed fresh or processed as juice, ice cream, jam and liqueurs. Native fruit species are unique in this biome because they exhibit high content of sugars, vitamins, proteins, and mineral salts (Almeida et al., 1987). One such species is *Eugenia dysenterica* DC., known as “cagaita” which belongs to the family Myrtaceae. This plant is widely used by popular medicine. Their leaf is used to treat diarrhea and diabetes (Lima et al., 2010 and 2011). The cagaita fruit has a distinctive taste and pleasant flavor. In addition, the cagaita fruit has nutraceutical and pharmacological potential (Lima et al. 2010). Knowledge of methods of conservation and propagation of native tree species, such as substrate, may assure the quality of seedlings and is important to reduce biodiversity loss. However, few

studies have examined development of native fruits in Brazilian Savanna species, especially *Eugenia dysenterica* DC. Native fruits species are mainly exploited an extractive form and is necessary to develop appropriate techniques essential to produce high-quality seedlings (Sousa et al., 2007). A high-quality seedling exhibits the attributes necessary for survival and development after being transplanted into the field (Duryea, 1985). Morphological and physiological characteristics are used to determine seedling quality. The morphological characteristics include the phenotypic traits of the seedlings, and the physiological characteristics include the internal factors that determine the visual appearance of the plants. Additionally, substrate quality is important to support plant growth during the rooting, nutrient and water supply (Ferraz et al., 2005) and affects the physiological and metabolic activities of plants. The substrate should have the ability to retain sufficient water, have a porous structure to allow a good aeration,

which might interfere on the growth of plants. The slow decomposing substrates with high cationexchange capacity and free of phytopathogens and other plant seeds are available for purchase at a reasonable price (Dantas et al., 2009). Thus, various mixtures of substrates can be suggested, by mixing two or more components that have different characteristics, when mixed together can provide a substrate with characteristics that are suitable for growing vigorous seedlings (Araújo Neto et al., 2009). However, the good substrate that will result in high-quality seedlings varies with the plant species to be propagated. A few studies exist on the quality of fruit tree seedlings. To increase the information on the seedling production and quality of native Cerrado species, the objective of this study was to characterize the growth, quality, mineral nutrition, and physiology metabolism of *Eugenia dysenterica* DC. seedlings grown in different substrates.

## Result

### *Effect of substrate chemical analysis on quality and growth of seedlings E. dysenterica*

The amount of organic matter ( $61.95 \text{ dag kg}^{-1}$ ) was higher in the MP+RH (7:3) substrate compared with the other substrates. The high organic matter (OM) content is important to reduce the solid soil compounds, which increased nutrient availability in the soil solution. In addition, the MP+RH substrate had the highest P, K, Fe, Cu, and B levels. However, this substrate had the lowest percent base saturation (Fig 1; Table 1).

The quality of the seedlings was affected under different tested substrates. Each substrate exhibited specific characteristics (Fig 2). The soil surrounding the parent plants (SN) resulted in the lowest percentage of seedling emergence (PSE). This was the only substrate where the PSE was less than 95%, with a mean value of 80% (Table 2). This substrate also resulted in the lowest vigor, but this value was only different from the MP+RH substrate (0.29). In general, the best shoot development and adventitious root formation were found in *cagaitera* seedlings grown in the SB+FV+CS and SB+CS+CM substrates.

The SN substrate exhibited the lowest relative moisture content ( $\text{RMC}_s$ ), followed by the SB+CS+CM substrate (0.24 and  $0.34 \text{ g g}^{-1}$ , respectively). The highest  $\text{RMC}_s$  was observed in MP+RH substrate ( $1.97 \text{ g g}^{-1}$ ), followed by SB+FV+CS ( $1.65 \text{ g g}^{-1}$ ) (Table 3). The  $\text{RMC}_s$  and emergence speed index (ESI) were positively correlated (0.66), indicating that ESI was negatively affected by decreasing  $\text{RMC}_s$ . The relative leaf moisture content ( $\text{RMC}_L$ ) remained unaffected (no correlation) by  $\text{RMC}_s$  (data not shown). However, the substrates resulted in different  $\text{RMC}_L$  values, with higher values observed for the SB+FV+RH (1:1:1) and SB+FV+CS (1:3:6) substrates ( $0.97 \text{ g g}^{-1}$ ) compared with the MP+RH (7:3) substrate ( $0.95 \text{ g g}^{-1}$ ).

### *Physiological metabolism of the seedlings of Eugenia dysenterica DC*

Despite the difference observed in  $\text{RMC}_L$ , this characteristic did not affect (there was no correlation) leaf gas exchange (data not shown). However, the leaf gas exchange characteristics were affected by the substrates tested, and the soil collected from around the parent plants resulted in a lower net  $\text{CO}_2$  assimilation rate ( $A$ ) ( $1.44 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ), compared with the plants grown in the MP+RH (7:3), SB+FV+CS (1:3:6), and SB+CS+CM (2:2:1) substrates ( $5.21$ ;  $5.74$ , and  $5.61 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , respectively) (Table 4). With respect to transpiration rate ( $E$ ) there was a significant difference only between the SB+CS+CM (2:2:1) ( $2.10 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) and SN ( $0.55 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) substrates. The stomatal conductance values ( $g_s$ ) were also lower in the plants grown in the SN substrate ( $0.02 \text{ mol m}^{-2} \text{ s}^{-1}$ ), but only compared with the seedlings grown in the SB+FV+CS (1:3:6) substrate ( $0.11 \text{ mol m}^{-2} \text{ s}^{-1}$ ). However, the relationship between internal and external  $\text{CO}_2$  levels ( $C_i/C_a$ ) in the plants remain unaffected by the substrates tested (Table 4).

The electron transport rate (ETR) showed difference in the seedlings grown in the different substrates, in which the SB+FV+CS (1:3:6) ( $111.77 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) and SB+CS+CM (2:2:1) ( $122.61 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) substrates resulted in the highest ETR values compared with the plants grown in the SN ( $46.10 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) substrate (Table 5). The maximum photochemical efficiency of PSII ( $F_v/F_m$ ) and the effective quantum yield of PSII ( $\phi\text{PSII}$ ) were lower in the plants grown in the SN substrate (0.70 and 0.12, respectively) compared with the SB+FV+CS (1:3:6) and SB+CS+CM (2:2:1) substrates, and also compared with the  $F_v/F_m$  of plants grown in the MP+RH (7:3) substrate (Table 5).

### *Morphological characteristics*

The biometric characteristics of stem length (SL), collar diameter (CD), number of leaves (NL), leaf dry weight (LDW), and stem dry weight (SDW), were higher in the plants grown in the SB+FV+CS (1:3:6) (4.78 cm, 1.26 mm, and 4.3 for SL, CD and NL, respectively) and SB+CS+CM (2:2:1) (4.46 cm, 1.26 mm, and 4 for SL, CD and NL, respectively) substrates compared with the other tested substrates (Table 6). The SN substrate resulted in the lowest mean root dry weight (RDW) and total dry weight (TDW) values ( $0.276 \text{ g}$  and  $0.447 \text{ g}$ , respectively) compared with the other substrates (Table 6).

The net  $\text{CO}_2$  assimilation ( $A$ ) was positively correlated with some of the biometric characteristics, including SL, NL, RDW, and TDW (0.41, 0.38, 0.36, and 0.47, respectively). The effective quantum yield ( $\phi\text{PSII}$ ) and electron transport rate (ETR) were positively correlated with SL, CD, SDW, LDW, and TDW (0.50, 0.42, 0.44, 0.49, and 0.46, respectively). The maximum photochemical efficiency of PSII ( $F_v/F_m$ ) was positively correlated with TDW (0.38) (Table 7). The SB+FV+CS (1:3:6) substrate resulted in the highest ratio between stem length and collar diameter (SL/CD) ( $3.73 \text{ cm mm}^{-1}$ ) compared with plants grown in the MP+RH (7:3), SN,

and SB+FV+RH (1:1:1) substrates (2.74, 2.73 and 2.78 cm mm<sup>-1</sup>, respectively) (Table 8). However, the root and shoot dry weight ratio (R/S) was higher in the plants grown in the MP+RH (7:3) and SB+FV+RH (1:1:1) substrates (3.91 and 3.81 g g<sup>-1</sup>, respectively). The Dickson quality index (DQI) was lower in the plants grown in the SN (0.14) substrate compared with those grown in the MP+RH (7:3) (0.22) and SB+FV+RH (1:1:1) (0.20) substrates (Table 8). In addition, the SL/CD quality indices were correlated positively with the gas exchange and fluorescence characteristics *A*, *F<sub>v</sub>/F<sub>m</sub>*, and  $\phi$ PSII (Table 7).

#### Correlation between nutrient content of tissues and different substrates

None of the tested substrates consistently resulted in the highest or lowest leaf nutrient levels analyzed (Table 9). The

N leaf levels were higher in the plants from the SB+FV+CS (1:3:6) (25.2 mg g<sup>-1</sup>) and SB+CS+CM (2:2:1) (24.8 mg g<sup>-1</sup>) substrates. The lowest values were measured in plants grown in the SB+FV+RH (1:2:2) (12 mg g<sup>-1</sup>) substrate. The P content was highest in the plants grown in the MP+RH (7:3) (5 mg g<sup>-1</sup>) and SB+CS+CM (2:2:1) (4.80 mg g<sup>-1</sup>) substrates. The K contents were higher in the plants grown in MP+RH (7:3) (9.28 mg g<sup>-1</sup>), followed by SB+FV+RH (1:1:1) (7.36 mg g<sup>-1</sup>) and SB+FV+CS (1:3:6) (6.08 mg g<sup>-1</sup>). The Ca and S contents were highest in the plants grown in SB+FV+CS (1:3:6) (20 and 1.02 mg g<sup>-1</sup>, respectively) and SB+CS+CM (2:2:1) (17.8 and 1.34 mg g<sup>-1</sup>, respectively). The highest Mg contents were observed in the plants grown in the SB+CS+CM (2:2:1) (9.76 mg g<sup>-1</sup>) and SB+FV+RH (1:2:2) (5.84 mg g<sup>-1</sup>) substrates. The boron content exhibited the following values in decreasing order as following substrates:

**Table 1.** Chemical analysis and macro- and micronutrients of the substrates used in the study.

Sample	pH (H <sub>2</sub> O)	V (%)	OM dag kg <sup>-1</sup>	(t)		Mg	P	K	Zn	Fe	Mn	Cu	B
				Ca	cmol <sub>c</sub> dm <sup>-3</sup>								
MP <sup>y</sup> +RH <sup>x</sup> (7:3)	4.10	30.10	61.95	9.41	4.70	2.42	195.30	487.00	9.35	163.30	57.30	6.20	1.23
SB <sup>w</sup> +FV <sup>v</sup> +RH(1:2:2)	6.72	81.30	3.65	5.67	6.00	4.50	39.20	171.00	2.50	79.20	18.40	1.47	0.13
SB+FV+RH(1:1:1)	6.41	72.00	4.56	4.64	9.00	3.11	7.70	175.00	2.64	71.50	19.60	1.82	0.19
SB+FV+CS <sup>u</sup> (1:3:6)	7.05	89.80	13.17	9.69	4.11	4.39	35.90	347.00	10.93	87.10	39.10	1.52	0.48
SB+CS <sup>t</sup> +CM <sup>s</sup> (2:2:1)	5.70	72.67	2.36	4.96	2.30	1.60	61.47	411.84	10.04	56.93	30.84	2.31	0.56
SN <sup>f</sup>	5.77	50.70	3.91	5.96	4.27	1.03	10.80	155.00	2.07	79.30	97.30	1.85	0.19

<sup>y</sup>MecPlant<sup>®</sup>; <sup>x</sup>Rice husks; <sup>w</sup>Soil; <sup>v</sup>Fine vermiculite; <sup>u</sup>Decomposed corn silage; <sup>t</sup>Coarse sand; <sup>s</sup>Tanned cattle manure; <sup>f</sup>Soil collected from around the parent plants. V=Base saturation index. OM = organic matter.

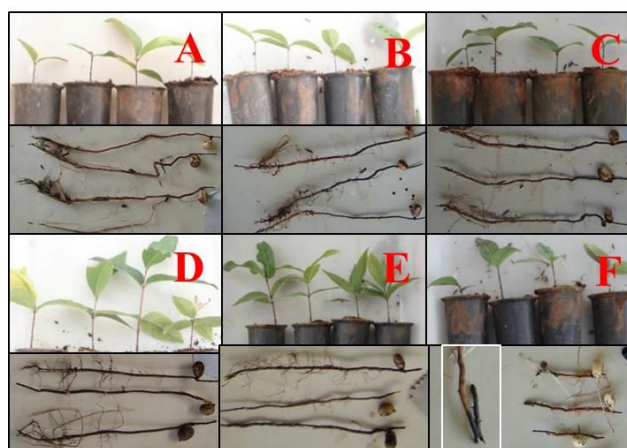


**Fig 1.** substrates tested: MecPlant<sup>®</sup> (MP)+rice husks (RH; 7:3; A); subsoil (SB)+fine vermiculite (FV)+RH (1:2:2; B); SB+FV+RH (1:1:1; C); SB+FV+ decomposed corn silage (CS; 1:3:6; D); SB+ coarse sand (CS)+tanned cattle manure (CM; 2:2:1; E); soil collected from around the parent plant (SN; F).

**Table 2.** Percent seedling emergence (PSE) and emergence speed index (ESI) of *Eugenia dysenterica* DC. seedlings in different substrates.

Substrate	PSE (%)	ESI
MP <sup>y</sup> +RH <sup>x</sup> (7:3)	100 a <sup>z</sup>	0.34 a
SB <sup>w</sup> +FV <sup>v</sup> +RH(1:2:2)	100 a	0.31 ab
SB+FV+RH(1:1:1)	100 a	0.31 ab
SB+FV+CS <sup>u</sup> (1:3:6)	98 a	0.30 ab
SB+CS <sup>t</sup> +CM <sup>s</sup> (2:2:1)	96 a	0.31 ab
SN <sup>f</sup>	80b	0.29 b
MSD <sup>q</sup>	8.28	0.048

<sup>z</sup>Means followed by the same letter do not differ according to Tukey's test (p<0.05); <sup>y</sup>MecPlant<sup>®</sup>; <sup>x</sup>Rice husks; <sup>w</sup>Soil; <sup>v</sup>Fine vermiculite; <sup>u</sup>Decomposed corn silage; <sup>t</sup>Coarse sand; <sup>s</sup>Tanned cattle manure; <sup>f</sup>Soil collected from around the parent plants. <sup>q</sup>Minimum significant difference.



**Fig 2.** *Eugenia dysenterica* seedlings produced in the following substrates: MecPlant® (MP)+rice husks (RH; 7:3; A); subsoil (SB)+fine vermiculite (FV)+RH (1:2:2; B); SB+FV+RH (1:1:1; C); SB+FV+ decomposed corn silage (CS; 1:3:6; D); SB+ coarse sand (CS)+tanned cattle manure (CM; 2:2:1; E); soil collected from around the parent plant (SN; F).The root systems from each substrate are shown in detail. The photos were taken 127 days after germination.

**Table 3.** Relative moisture content of the substrates ( $RMC_S$ ), and the relative leaf moisture content ( $RMC_L$ ) of *Eugenia dysenterica* DC. seedlings grown in different substrates.

Substrate	$RMC_S$ ( $g\ g^{-1}$ )	$RMC_L$
MP <sup>y</sup> +RH <sup>x</sup> (7:3)	1.97 a	0.95 b
SB <sup>w</sup> +FV <sup>v</sup> +RH(1:2:2)	0.70 c	0.96 ab
SB+FV+RH(1:1:1)	0.56 c	0.97 a
SB+FV+CS <sup>u</sup> (1:3:6)	1.65 b	0.97 a
SB+CS <sup>t</sup> +CM <sup>s</sup> (2:2:1)	0.34 d	0.97 ab
SN <sup>r</sup>	0.24 d	0.96 ab
MSD <sup>q</sup>	16.98	0.02

<sup>a</sup>Means followed by the same letter do not differ according to Tukey's test ( $p<0.05$ ); <sup>y</sup>MecPlant®; <sup>x</sup>Rice husks; <sup>w</sup>Soil; <sup>v</sup>Fine vermiculite; <sup>u</sup>Decomposed corn silage; <sup>t</sup>Coarse sand; <sup>s</sup>Tanned cattle manure; <sup>r</sup>Soil collected from around the parent plants. <sup>q</sup>Minimum significant difference.

**Table 4.**  $CO_2$  net assimilation rate ( $A$ ), transpiration ( $E$ ), stomatal conductance ( $g_s$ ), and ratio between intercellular and atmospheric  $CO_2$  levels ( $C_i/C_a$ ) in *Eugenia dysenterica* DC. seedlings grown in different substrates.

Substrate	$A$ $\mu mol\ m^{-2}\ s^{-1}$	$E$ $mmol\ m^{-2}\ s^{-1}$	$g_s$ $mol\ m^{-2}\ s^{-1}$	$C_i/C_a$ $mol\ mol^{-1}$
MP <sup>y</sup> +RH <sup>x</sup> (7:3)	5.21 a <sup>z</sup>	1.72 ab	0.10 ab	0.69 a
SB <sup>w</sup> +FV <sup>v</sup> +RH(1:2:2)	4.02 ab	1.47 ab	0.07 ab	0.68 a
SB+FV+RH(1:1:1)	4.16 ab	1.53 ab	0.08 ab	0.69 a
SB+FV+CS <sup>u</sup> (1:3:6)	5.74 a	1.74 ab	0.11 a	0.68 a
SB+CS <sup>t</sup> +CM <sup>s</sup> (2:2:1)	5.61 a	2.10 a	0.11 ab	0.66 a
SN <sup>r</sup>	1.44 b	0.55 b	0.02 b	0.68 a
MSD <sup>q</sup>	2.89	1.25	0.087	0.10

<sup>a</sup>Means followed by the same letter do not differ according to Tukey's test ( $p<0.05$ ); <sup>y</sup>MecPlant®; <sup>x</sup>Rice husks; <sup>w</sup>Soil; <sup>v</sup>Fine vermiculite; <sup>u</sup>Decomposed corn silage; <sup>t</sup>Coarse sand; <sup>s</sup>Tanned cattle manure; <sup>r</sup>Soil collected from around the parent plants. <sup>q</sup>Minimum significant difference.

**Table 5.** Maximum photochemical efficiency of PSII ( $F_v/F_m$ ), effective quantum yield of PSII ( $\phi_{PSII}$ ), and the electron transport rate (ETR) of *Eugenia dysenterica* DC. seedlings grown in different substrates.

Substrate	$F_v/F_m$	$\phi_{PSII}$	ETR $\mu mol\ m^{-2}\ s^{-1}$
MP <sup>y</sup> +RH <sup>x</sup> (7:3)	0.75 a	0.21 ab	78.84 ab
SB <sup>w</sup> +FV <sup>v</sup> +RH(1:2:2)	0.74 ab	0.24 ab	87.98 ab
SB+FV+RH(1:1:1)	0.74 ab	0.19 ab	72.47 ab
SB+FV+CS <sup>u</sup> (1:3:6)	0.76 a	0.30 a	111.77 a
SB+CS <sup>t</sup> +CM <sup>s</sup> (2:2:1)	0.77 a	0.31 a	122.61 a
SN <sup>r</sup>	0.70 b	0.12 b	46.10 b
MSD <sup>q</sup>	0.053	0.133	51.96

<sup>a</sup>Means followed by the same letter do not differ according to Tukey's test ( $p<0.05$ ); <sup>y</sup>MecPlant®; <sup>x</sup>Rice husks; <sup>w</sup>Soil; <sup>v</sup>Fine vermiculite; <sup>u</sup>Decomposed corn silage; <sup>t</sup>Coarse sand; <sup>s</sup>Tanned cattle manure; <sup>r</sup>Soil collected from around the parent plants. <sup>q</sup>Minimum significant difference.

**Table 6.** Stem length (SL), collar diameter (CD), number of leaves (NL), stem dry weight (SDW), leaf dry weight (LDW), root dry weight (RDW), and total dry weight (TDW) of *Eugenia dysenterica* DC. seedlings grown in different substrates.

Substrate	SL (cm)	CD (mm)	NL (-)	SDW (g)	LDW	RDW	TDW
MP <sup>y</sup> +RH <sup>x</sup> (7:3)	2.72 b <sup>z</sup>	0.99 b	2.1 b	0.019 b	0.116 b	0.499 a	0.634 a
SB <sup>w</sup> +FV <sup>v</sup> +RH(1:2:2)	3.05 b	0.99 b	2.0 b	0.020 b	0.128 b	0.453 a	0.601 a
SB+FV+RH(1:1:1)	2.62 b	0.94 b	2.3 b	0.022 b	0.110 b	0.471 a	0.603 a
SB+FV+CS <sup>u</sup> (1:3:6)	4.78 a	1.26 a	4.3 a	0.042 a	0.242 a	0.458 a	0.742 a
SB+CS <sup>t</sup> +CM <sup>s</sup> (2:2:1)	4.46 a	1.26 a	4.0 a	0.042 a	0.233 a	0.401 a	0.676 a
SN <sup>f</sup>	2.78 b	1.04 b	2.2 b	0.024 b	0.147 b	0.276 b	0.447 b
MSD <sup>q</sup>	1.12	0.15	1.13	0.011	0.068	0.108	0.144

<sup>z</sup>Means followed by the same letter do not differ according to Tukey's test (p<0.05); <sup>w</sup>MecPlant@; <sup>x</sup>Rice husks; <sup>y</sup>Soil; <sup>v</sup>Fine vermiculite; <sup>u</sup>Decomposed corn silage; <sup>t</sup>Coarse sand; <sup>f</sup>Tanned cattle manure; <sup>s</sup>Soil collected from around the parent plants. <sup>q</sup>Minimum significant difference.

**Table 7.** Pearson linear correlation matrix of the gas exchange, chlorophyll *a* fluorescence and biometric characteristics and the qualities of the *Eugenia dysenterica* DC. seedlings.

	A <sup>z</sup>	E <sup>y</sup>	g <sub>s</sub> <sup>x</sup>	Ci/Ca <sup>w</sup>	F <sub>v</sub> /F <sub>m</sub> <sup>u</sup>	ϕPSII <sup>t</sup>	ETR <sup>s</sup>
SL <sup>f</sup>	0.41*	0.24	0.28	-0.20	0.34	0.50*	0.48*
CD <sup>q</sup>	0.25	0.15	0.19	-0.10	0.14	0.42*	0.46*
NL <sup>p</sup>	0.38*	0.30	0.33	0.03	0.33	0.32	0.36
SDW <sup>o</sup>	0.27	0.13	0.13	-0.32	0.33	0.44*	0.45*
LDW <sup>n</sup>	0.33	0.20	0.22	-0.18	0.28	0.49*	0.48*
RDW <sup>m</sup>	0.36*	0.27	0.27	-0.14	0.27	0.23	0.23
TDW <sup>l</sup>	0.47*	0.32	0.33	-0.23	0.38*	0.46*	0.46*
SL/CD <sup>k</sup>	0.38*	0.21	0.23	-0.25	0.38*	0.40*	0.35
R/S <sup>j</sup>	0.13	0.15	0.16	0.12	0.05	-0.15	-0.16
DQI <sup>i</sup>	0.22	0.18	0.20	0.01	0.13	0.08	0.12

\*Pearson correlation significant at the 5% probability level; <sup>z</sup>Net CO<sub>2</sub> assimilation; <sup>y</sup>Transpiration; <sup>x</sup>Stomatal conductance; <sup>w</sup>Ci/Ca ratio; <sup>u</sup>Maximum quantum yield of PSII; <sup>t</sup>Effective quantum yield of PSII; <sup>s</sup>Electron transport rate; <sup>r</sup>Stem length; <sup>q</sup>Collar diameter; <sup>p</sup>Number of leaves; <sup>o</sup>Stem dry weight; <sup>n</sup>Leaf dry weight; <sup>m</sup>Root dry weight; <sup>l</sup>Total dry weight; <sup>k</sup>Ratio between stem length and diameter; <sup>j</sup>Ratio between root and shoot dry weight; <sup>i</sup>Dickson quality index.

**Table 8.** Ratio between stem length and collar diameter (SL/CD), the root and shoot dry weight ratio (R/S), and the Dickson quality index (DQI) of *Eugenia dysenterica* DC. seedlings grown in different substrates.

Substrate	SL/CD (cm mm <sup>-1</sup> )	R/S (g g <sup>-1</sup> )	DQI
MP <sup>y</sup> +RH <sup>x</sup> (7:3)	2.74b <sup>z</sup>	3.91 a	0.22 a
SB <sup>w</sup> +FV <sup>v</sup> +RH(1:2:2)	3.06 ab	3.28 a	0.18 ab
SB+FV+RH(1:1:1)	2.78 b	3.81 a	0.20 a
SB+FV+CS <sup>u</sup> (1:3:6)	3.73 a	2.00 b	0.19 ab
SB+CS <sup>t</sup> +CM <sup>s</sup> (2:2:1)	3.50 ab	1.80 b	0.17 ab
SN <sup>f</sup>	2.73 b	1.75 b	0.14 b
MSD <sup>q</sup>	0.80	0.91	0.054

<sup>z</sup>Means followed by the same letter do not differ according to Tukey's test (p<0.05); <sup>w</sup>MecPlant@; <sup>x</sup>Rice husks; <sup>y</sup>Soil; <sup>v</sup>Fine vermiculite; <sup>u</sup>Decomposed corn silage; <sup>t</sup>Coarse sand; <sup>f</sup>Tanned cattle manure; <sup>s</sup>Soil collected from around the parent plants. <sup>q</sup>Minimum significant difference.

**Table 9.** Macro- and micronutrient contents in the leaf tissues of *Eugenia dysenterica* DC. seedlings grown in different substrates.

Substrate	mg g <sup>-1</sup>						mg kg <sup>-1</sup>				
	N	P	K	Ca	Mg	S	B	Fe	Cu	Mn	Zn
MP <sup>y</sup> +RH <sup>x</sup> (7:3)	19.6b <sup>z</sup>	5.00a	9.28a	11.0b	3.86c	0.52b	142.8a	127.4b	5.4ab	312.6a	12.2bc
SB <sup>w</sup> +FV <sup>v</sup> +RH(1:2:2)	12.0c	0.88c	6.56b	8.9b	7.58ab	0.48b	98.2e	144.6b	5.4ab	35.4b	12.2bc
SB+FV+RH(1:1:1)	11.4c	0.96c	7.36b	9.2b	5.84bc	0.42b	117.6d	157.8ab	5.8a	44.8b	10.8c
SB+FV+CS <sup>u</sup> (1:3:6)	25.2a	3.80b	6.08bc	20.0a	7.40b	1.02a	96.0e	158.6ab	4.8ab	105.6b	18.2a
SB+CS <sup>t</sup> +CM <sup>s</sup> (2:2:1)	24.8a	4.80a	4.96cd	17.8a	9.76a	1.34a	135.2b	153.2ab	4.4b	64.0b	15.4ab
SN <sup>f</sup>	19.0b	0.88c	3.52d	8.9b	4.16c	0.28b	124.4c	217.0a	4.6b	341.4a	11.0c
MSD <sup>q</sup>	1.68	0.853	1.475	5.27	2.339	0.453	5.13	69.12	1.16	149.29	4.38

<sup>z</sup>Means followed by the same letter do not differ according to Tukey's test (p<0.05); <sup>w</sup>MecPlant@; <sup>x</sup>Rice husks; <sup>y</sup>Soil; <sup>v</sup>Fine vermiculite; <sup>u</sup>Decomposed corn silage; <sup>t</sup>Coarse sand; <sup>f</sup>Tanned cattle manure; <sup>s</sup>Soil collected from around the parent plants. <sup>q</sup>Minimum significant difference.

**Table 10.** Pearson linear correlation matrix between the leaf nutrient contents and the gas exchange, chlorophyll *a* fluorescence, biometric, and quality characteristics of the *Eugenia dysenterica* DC. seedlings.

	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
$A^z$	0.33	0.55*	0.30	0.45*	0.48*	0.55*	0.01	-0.10	-0.53*	-0.32	0.38*
$E^y$	0.21	0.44*	0.19	0.31	0.47*	0.44*	0.05	-0.15	-0.51*	-0.31	0.22
$g_s^x$	0.24	0.44*	0.26	0.36	0.41*	0.41*	0.00	-0.03	-0.47*	-0.29	0.27
$Ci/Ca^w$	-0.13	-0.05	0.11	-0.07	-0.18	-0.19	-0.10	0.30	0.01	0.04	-0.06
$F_v/F_m^v$	0.33	0.53*	0.23	0.39*	0.47*	0.66*	0.07	-0.08	-0.53*	-0.29	0.33
$\phi PSII^u$	0.39*	0.45*	-0.01	0.54*	0.57*	0.54*	-0.16	-0.18	-0.30	-0.33	0.45*
$ETR^t$	0.42*	0.51*	-0.02	0.53*	0.59*	0.56*	-0.11	-0.21	-0.24	-0.34	0.50*
$SL^s$	0.62*	0.41*	-0.24	0.70*	0.50*	0.58*	-0.20	-0.43*	-0.19	-0.27	0.68*
$CD^f$	0.66*	0.48*	-0.24	0.65*	0.44*	0.49*	-0.08	-0.31	0.15	-0.20	0.69*
$NL^q$	0.62*	0.45*	-0.29	0.75*	0.48*	0.57*	-0.14	-0.38*	-0.19	-0.25	0.65*
$SDW^p$	0.66*	0.42*	-0.35	0.70*	0.51*	0.63*	-0.12	-0.46*	-0.05	-0.28	0.64*
$LDW^o$	0.71*	0.42*	-0.40*	0.71*	0.47*	0.63*	-0.15	-0.59*	-0.12	-0.18	0.59*
$RDW^n$	-0.12	0.27	0.66*	0.15	0.05	0.06	-0.04	0.22	-0.37*	-0.29	0.23
$TDW^m$	0.31	0.46*	0.30	0.53*	0.31	0.41*	-0.12	-0.16	-0.35	-0.35	0.53*
$SL/CD^l$	0.44*	0.25	-0.19	0.54*	0.41*	0.49*	-0.23	-0.40*	-0.35	-0.24	0.51*
$R/S^k$	-0.61*	-0.10	0.76*	-0.39*	-0.30	-0.37*	0.12	0.56*	-0.30	-0.06	-0.33
$DQI^j$	-0.14	0.23	0.61*	0.02	-0.12	-0.05	0.09	0.28	-0.17	-0.10	0.07

\*Pearson correlation significant at the 5% probability level; <sup>z</sup>Net CO<sub>2</sub> assimilation; <sup>y</sup>Transpiration; <sup>x</sup>Stomatal conductance; <sup>w</sup>*Ci/Ca* ratio; <sup>v</sup>Maximum quantum yield of PSII; <sup>u</sup>Effective quantum yield of PSII; <sup>t</sup>Electron transport rate (ETR); <sup>s</sup>Stem length; <sup>r</sup>Collar diameter; <sup>q</sup>Number of leaves; <sup>p</sup>Stem dry weight; <sup>o</sup>Leaf dry weight; <sup>n</sup>Root dry weight; <sup>m</sup>Total dry weight; <sup>l</sup>Ratio between stem length and diameter; <sup>k</sup>Ratio between root and shoot dry weight; <sup>j</sup>Dickson quality index;

MP+RH (7:3) (142 mg kg<sup>-1</sup>) > SB+CS+CM (2:2:1) (135.2 mg kg<sup>-1</sup>) > SN (124.4 mg kg<sup>-1</sup>) > SB+FV+RH(1:1:1) (117.6 mg kg<sup>-1</sup>) > SB+FV+RH(1:2:2) (98.2 mg kg<sup>-1</sup>) = SB+FV+CS (96 mg kg<sup>-1</sup>). The SN substrate resulted in the highest Fe content in the plant leaves (217 mg kg<sup>-1</sup>) compared with the MP+RH (7:3) (127.4 mg kg<sup>-1</sup>) and SB+FV+RH (1:2:2) (144.6 mg kg<sup>-1</sup>) substrates. The Fe content measured in the plants from the other substrates did not differ from these values. The leaf Cu contents only differed between the SB+FV+RH (1:1:1) (5.8 mg kg<sup>-1</sup>), SB+CS+CM (2:2:1) (4.4 mg kg<sup>-1</sup>) and SN (4.6 mg kg<sup>-1</sup>) substrates, whereas the latter two values were lower. The highest leaf Mn contents were measured in the plants grown in the MP+RH (7:3) (312.6 mg kg<sup>-1</sup>) and SN (341.4 mg kg<sup>-1</sup>) substrates. The highest Zn contents were measured in the plants grown in SB+FV+CS (1:3:6) (18.2 mg kg<sup>-1</sup>) and SB+CS+CM (2:2:1) (15.4 mg kg<sup>-1</sup>) which did not differ from MP+RH (7:3) and SB+FV+RH (1:2:2). A value of 12.2 mg kg<sup>-1</sup> was measured in the plants grown in these two substrates (Table 9).

The correlation analysis between the leaf mineral nutrient contents and leaf gas exchange, chlorophyll *a* fluorescence, biometrics, and quality of *E. dysenterica* seedlings was presented in Table 10. The results show that S, followed by P, Ca, and Mg, exhibited a higher number of correlations, whereas B and Mn did not exhibit any correlations, and *Ci/Ca* was not correlated with any of the minerals quantified. The N content was negatively correlated with R/S and positively correlated with  $\phi PSII$ , ETR, SL, CD, NL, SDW, LDW, and SL/CD. The P content was only positively correlated with A, E,  $g_s$ ,  $F_v/F_m$ ,  $\phi PSII$ , ETR, SL, CD, NL, SDW, LDW, and TDW. Among the macronutrients, the K content exhibited the lowest number of correlations. It was positively correlated with RDW, R/S, and DQI and negatively correlated with LDW. The Ca levels exhibited positive correlations with A,  $F_v/F_m$ ,  $\phi PSII$ , ETR, SL, CD, NL,

SDW, LDW, TDW, and SL/CD and a negative correlation with R/S. The Mg and S contents exhibited positive correlations with A, E,  $g_s$ ,  $F_v/F_m$ ,  $\phi PSII$ , ETR, SL, CD, NL, SDW, LDW, and SL/CD, and S exhibited a positive correlation with TDW and a negative correlation with R/S. The Fe was the only mineral that exhibited negative correlations with the following: A, E,  $g_s$ ,  $F_v/F_m$ , and RDW. The Cu content was negatively correlated with SL, NL, SDW, LDW, and SL/CD, and positively correlated with R/S. Among the micronutrients, Zn exhibited the largest number of correlations (all positive), with the following: A,  $\phi PSII$ , ETR, SL, CD, NL, SDW, LDW, TDW, and SL/CD (Table 10).

## Discussion

### *Morphological and physiological quality of the E. dysenterica seedlings on different substrates*

The high seedling emergence rate of 100% was observed in three treatments and above 95% in five treatments (Fig 2 and Table 2), which has not been previously reported for *Eugenia dysenterica* DC plants. Souza et al. (2001) reported 81% seedling emergence, a rate lower than most of treatments applied in the present study and only similar to that of the soil collected from around the parent plants (SN). Removing the seed coat surrounding the *cagaiteira* seeds, which may contain substances that inhibit germination or hinder water absorption or gas exchange, possibly have contributed to the higher emergence observed in the present study. Removing the coat may have also contributed to the high seedling vigor observed in the present study compared with the mean value (0.10) reported by Nietsche et al. (2004). The reduced amount of subsoil in the substrates led to an increase in the relative substrate moisture content (RMC<sub>S</sub>) (Table 3). The

increase in  $RMC_S$  was also linked to an increased proportion of organic residues and/or vermiculite, as both exhibit good water retention capacities. The positive correlation between  $RMC_S$  and vigor indicates that there may have been a lower amount of water available for absorption by the seeds. However, there was no correlation between  $RMC_S$  and relative leaf moisture content ( $RMC_L$ ), which shows that the plant roots were able to absorb water from the substrates after emergence. The low stomatal conductance ( $g_s$ ) of the plants grown in SN substrate resulted in the lowest transpiration ( $E$ ) and net  $CO_2$  assimilation ( $A$ ) values. The stomatal pores regulate the amount of water and solutes by opening and closing. The ratio between internal and external carbon dioxide concentration ( $C_i/C_a$ ) was very similar among the plants, indicating that  $g_s$  regulated  $A$  and  $E$  and that there was no damage to the biochemical pathway of the photosynthesis (Table 4). The data presented in this study show the importance of using mixtures of components with different characteristics when formulating substrates to obtain better emergence indices and physiological traits of *cagaitera* seedlings. The  $g_s$  values found in the present study are lower ( $\sim 0.10 \text{ mol m}^{-2} \text{ s}^{-1}$ ) than those observed by Lemos-Filho (2000) which do not reach 50% of the value observed by this author during the rainy season ( $0.26 \text{ mol m}^{-2} \text{ s}^{-1}$ ). During the dry season, this author reported a  $g_s$  value of  $0.054 \text{ mol m}^{-2} \text{ s}^{-1}$ , which is lower than the values observed in the present study except for the plants grown in the SN substrate. Neves et al. (2009) studied *Eugenia uniflora* and reported an  $A$  value of  $12 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ , an  $E$  of  $1.2 \text{ mmol m}^{-2} \text{ s}^{-1}$ , and a  $C_i/C_a$  value of  $0.58 \text{ mol mol}^{-1}$ . High ETR values related to low  $A$  values may generate excess reducing power, which can be used to form reactive oxygen species, leading to photoinhibitory damage (Table 5). The differences in ETR values among the plants grown in the different substrates contributed to the differences observed in the  $A$  values (Table 4). The maximum ETR value recorded in the present study ( $122.61 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) was lower than that observed by Lemos-Filho (2000) during the rainy season, which was  $160 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  (Table 5). When comparing the value observed by the author during the dry season ( $90 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ ), only the plants grown in the SB+FV+CS (1:3:6) and SB+CS+CM (2:2:1) substrates exhibited higher ETR values, and the plants grown in the SB+FV+RH (1:2:2) substrate exhibited a similar value (Table 4). For the plants grown in the SN substrate, the ETR value was 50% of that observed by Lemos-Filho (2000) during the dry season. Thus, the correlation between ETR and  $A$  in the *E. dysenterica* seedlings obtained in the present study indicates normal development of the seedlings. The maximum photochemical efficiency of PSII ( $F_v/F_m$ ) (0.70) indicates the occurrence of photoinhibitory damage. However, in comparison to the  $F_v/F_m$  value of 0.78 obtained by Lemos-Filho (2000) during the rainy season, the  $F_v/F_m$  values of the plants grown in the SB+FV+CS (1:3:6) and SB+CS+CM (2:2:1) substrates did not indicate such damage (Table 5). The  $\phi PSII$  values in the SB+FV+CS (1:3:6) and SB+CS+CM (2:2:1) substrates, the highest in the present study, are similar to those observed by Lemos-Filho (2000) under the same actinic light intensity. In study of *Eugenia*

*uniflora* plants, Neves et al. (2009) showed an  $F_v/F_m$  value around 0.7 in leaves under actinic light of  $1,000 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ , which is the same value found in the present study in the plants grown in the SN substrate. Thus, it is concluded that  $F_v/F_m$  values close to 0.7 may be a particular characteristic of the species. The presence of decomposed corn silage and tanned cattle manure led to better plant performance compared with the other substrates, for all biometric characteristics except the root (RDW) and total (TDW) dry weight (Table 6). The benefits of using cattle manure as a substrate component for forest tree production has been demonstrated for several species, including *Acacia sp.* (Cunha et al., 2006), *Enterolobium contortisiliquum* (Vell. Morong) (Araújo and Paiva Sobrinho, 2011), *Genipa americana* L. (Costa et al., 2005), and *Harconia speciosa* Gomes (Silva et al., 2009). These benefits may be related to its rapid decomposition compared with rice husks, for example, and the release of nutrients for absorption by plants. However, cattle manure availability and quality are irregular and vary by region, and quality is related to pasture and livestock management (Lekasi et al., 2003). Decomposed corn silage, similar to tanned cattle manure is a good source of nutrients and; thus, promotes plant growth because corn is harvested before plant senescence begins. Seedling of *E. dysenterica* DC grown with organic residues, such as tanned cattle manure and decomposed corn silage in the substrates (SB+FV+CS and SB+CS+CM) led to better physiological parameters ( $A$ ,  $E$ ,  $g_s$ ,  $F_v/F_m$  and ETR) and biometric characteristics (SL, CD, NL, SDW, LDW, RDW and TDW). This occurs because adding organic matter to the substrate improves their physical characteristics and it is important to stimulate development of the shoot, in term of height and leaf area besides retain more moisture throughout the day with maintains higher water availability to the plant (Graciano et al., 2006). The seedlings grown with this substrate showed more macronutrients content (N, P, Ca, Mg and S – Table 9) in the leaves. The N content is essential nutrient for plant growth and development. It is a major constituent of amino acids, the building blocks of proteins, nucleotides, nucleic acids, coenzymes, enzymes and is an essential constituent of chlorophyll. Chlorophyll is vital for photosynthesis, which allows plants to absorb energy from light and produce more biomass (Taiz and Zeigler, 2009).

#### **Correlation between the leaf nutrients contents and morpho-physiological characteristics**

The SB+CS+CM (2:2:1) substrate resulted in the highest of N, P, Ca, Mg, S, Zn, and Fe levels in the leaves, compared with other substrates (Table 9). As a consequence of this high mineral content, this substrate also resulted in the highest values of leaf gas exchange parameters, chlorophyll *a* fluorescence, and all biometric parameters. This shows that the substrate was efficient in providing nutrients to meet plant requirements, although there were possible deficiencies of some elements, such as K, B, Cu, and Mn. However, these elements did not exhibit any correlation (such as B and Mn), or exhibited mostly negative correlations (such as Cu and K

with the quality indices) which cannot be used to measure efficient in seedling quality. Another substrate that exhibited high mineral element levels was SB+FV+CS (1:3:6), which was similar to SB+CS+CM (2:2:1), resulted in good seedling growth, leaf gas exchange and fluorescence parameters. However, the SN substrate exhibited high levels of only Fe and Mn nutrients. These elements did not exhibit any correlations, or exhibited negative correlations, such as those observed in the case of Fe, which might indicate possible plant toxicity (Table 8).

Comparing the N, P, K, Ca, Mg, and S levels in leaf obtained in our study with those obtained by Melo and Haridasan (2009), shows that only the SB+FV+CS (1:3:6) and SB+CS+CM (2:2:1) substrates produce higher values. The SN and SB+FV+RH (1:1:1) substrates exhibited the lowest values for P and C and also SN exhibited the lowest K and S values. The data obtained in the present study show that the presence of tanned cattle manure or corn silage is beneficial for seedling nutrition. However, the availability as well as the quality of cattle manure are irregular and vary among regions (Lekasi et al., 2003).

The positive correlations between mineral nutrients and analysis as biometric, leaf gas exchange, and chlorophyll *a* fluorescence characteristics found in the present study indicated that low leaf levels of N, P, Ca, Mg, S, and Zn may be the limiting factors for seedling growth and may lead to low-quality seedlings. However, the negative correlations observed for Fe may indicate a possible phytotoxicity. Finally, it was observed that the correlations between the mineral nutrients and the other characteristics such as good mineral nutrition are necessary for the seedlings and that the use of agricultural nutrient management may be required.

#### **Correlation between the morpho-physiological characteristics and different substrate**

The positive correlation between *A* and most of the biometric characteristics indicates that the photosynthetic abilities of the plants improved their growth, and this variable can; thus, be used as a physiological trait to evaluate seedling quality (Table 7). Other physiological traits that exhibited correlations with the biometric parameters were  $\phi$ PSII and ETR, both were correlated with five of the seven biometric parameters (Table 7). The data presented in this study indicate that physiological parameters can be used to determine the quality of *Eugenia dysenterica* seedlings.

The SL/CD ratio observed in the present study (3.73 cm mm<sup>-1</sup>, Table 8) was lower than that reported by Souza et al. (2001) (6.0 cm mm<sup>-1</sup>) which indicates that the seedlings in the present study had a higher quality. However, when considering the number of leaves and stem length, except for the plants grown in the SB+FV+CS (1:3:6) and SB+CS+CM (2:2:1) substrates, plants had approximately two leaves. Considering that *cagaiteira* has two opposite leaves per node, the plants had only a single node, except those grown in the SB+FV+CS (1:3:6) and SB+CS+CM (2:2:1) substrates, which had two nodes. The positive correlation between

SL/CD and *A*,  $F_v/F_m$ , and  $\phi$ PSII indicates that the plants were not etiolated (Table 7).

The R/S ratio was higher in the plants grown in the MP+RH (7:3) and SB+FV+RH (1:1:1) substrates, in both proportions, may be an indicator that these plants invested more photoassimilates into the root system, approximately over three times more investment in roots than shoots. This characteristic indicates possible nutritional stress (Table 8). However, this is a characteristic of the species under study, which initially prioritizes root system development (Silveira et al., 2013). Melo and Haridasan (2009) observed an R/S above 5.0 and concluded that reduced nutrient content in the culture medium increased the R/S value. However, lower values (1.0 and 1.4) have been observed by Souza et al. (2001) and Paiva Sobrinho et al. (2010), respectively, which are both, lower than the lowest R/S value observed in the present study.

The DQI is a balance formula which includes the morphological characteristics. A higher DQI should represent a better seedlings quality, indicating the robustness and balance of biomass in the seedling (Fonseca et al., 2002). A value similar to or higher than 0.20 was only observed for the MP+RH (7:3) and SB+FV+RH (1:1:1) substrates. However, analyzing the three quality indices, such as SL/CD, R/S, and DQI together, it was not possible to confirm that these indices represent quality indicators for *E. dysenterica* (Table 8) because they indicated that plants with lower growth and dry weight were of a better quality (Fig 2). The native species of the Midwest, such as the mangaba (*H. speciosa*) and baru (*Dipteryx alata* Vog.) were developed better when the substrate was devoid of any source of organic matter (Paiva e Sobrinho et al., 2010).

The results in this study show the importance of using mixtures of components in substrates to obtain better emergence indices and physiological traits of *cagaiteira* seedlings, compare with SN substrate as control. Based on the results of this study, it is concluded that the use of organic residues, tanned cattle manure and decomposed corn silage as substrates can produce better PSE and ESI, high macronutrients content in the leaves, resulting to more efficient photosynthesis to improve the seedling development. These results showed that using mixture of component is an important tool to produce *Eugenia dysenterica* plants with high-quality. Although this study found significant physiological quality values for the *E. dysenterica* seedlings grown in different substrates, additional studies are necessary to determine the adequate levels of mineral nutrients for *E. dysenterica*.

#### **Materials and Methods**

##### **Field conditions, plant material and substrate chemical properties**

The experiment was conducted in a greenhouse located at the Goiano Federal Institute (Instituto Federal Goiano - IFG), Rio Verde campus (latitude 17° 48' 16'' S, longitude 50° 54' 19'' W and altitude of 753 m), during the 2011/2012 crop season.



Parental plants of *Eugenia dysenterica* DC. were grown at the Gameleira farm (latitude 19°53'S, longitude 44°25'W and altitude of 749 m) in the town of Montes Claros, Goias, Brazil. Fruits were collected from plants in full production (Fig. 1). The fruits were processed under running water, the pulp was removed and whole seeds with no signs of injury were selected and left in the Laboratory of Tissue Culture at IF Goiano Campus Rio Verde, Goias, Brazil.

The seeds were sown in tubes (288 cm<sup>3</sup>) in the following substrates: MecPlant<sup>®</sup> (MP); fine vermiculite (FV); rice husks (RH); coarse sand (CS); subsoil (SB); tanned cattle manure (CM); decomposed corn silage (CS); and soil collected from around the parent plants at a depth of 0–20 cm (SN). The following substrates were formulated using volume-based proportions: MP+RH (7:3), SB+FV+CS (1:3:6), SB+CS+CM (2:2:1), SB+FV+RH (proportions of 1:2:2 and 1:1:1) and SN (Fig.1). Chemical analysis of the substrates was performed according to the method described in the Embrapa (1997) Manual. After preparing the substrate formulations, they were subjected to the analysis (Table 1).

Relative humidity and air temperature were recorded at 30-minute intervals using a DataLogger (NOVUS, Brazil); the daily mean values were 76% and 26°C, respectively. The plants were spray irrigated twice daily, applying 6mm at each interval.

#### **Seedlings growth and morphological characteristics**

Percent of seedling emergence (PSE) was determined at two-day intervals after the first seedling emerged. Emergence speed index (ESI) was obtained according to Maguire (1962). The following growth characteristics were evaluated: stem length (SL), collar diameter (CD), and number of leaves (NL). The plants were divided into stems, leaves, and roots to obtain the dry weight, and these components were dried separately in a forced air oven at 65°C to constant weight. The seedling quality parameters evaluated were the ratios between SL and CD (SL/CD) and between root dry weight and shoot dry weight (R/S). The Dickson quality index (DQI) is a tool to evaluate seedling quality as a function of total dry matter (TDM) (g), shoot height (SH) (cm), stem base diameter (SBD) (mm), shoot dry matter (SDM) (g) – sum of stem base dry matter and leaf dry matter – and root dry matter (RDM) (g), and is given by the expression:  $DQI = TDM \text{ g} / [ (SH \text{ cm} / SBD \text{ mm} + (SDM \text{ g} / RDM \text{ g})) ]$  (Dickson et al., 1960). All of the morphological characteristics were evaluated 127 days after sowing the seeds.

#### **Relative moisture content (RMC)**

The relative leaf moisture content (RMC<sub>L</sub>) and relative substrate moisture content (dry basis) (RMC<sub>S</sub>) were recorded before dawn. To determine RMC<sub>L</sub>, one leaf per plant was collected using a sharp blade, and the leaf was weighed on an analytical balance immediately after collection to obtain the fresh weight (FW). Next, the leaves were placed in a humid chamber with the petiole immersed in distilled water at 25°C, and compensation irradiance was permitted to take place for

24 hours for the leaves to reach maximum turgor. Then, the turgid weight (TW) was obtained, and the leaves were dried in a forced air oven at 65°C to a constant weight to obtain the dry weight (DW). The RMC<sub>L</sub> was calculated using the following equation:  $RMC_L = (FW - DW) / (TW - DW)$ .

To determine the RMC<sub>S</sub>, substrate samples from the tubes that contained the plants were collected and used to determine RMC<sub>L</sub>. Immediately after collecting the substrates, they were weighed on a semi-analytical balance to obtain the wet weight (WW) and then dried in a forced air oven at 105°C to a constant weight to obtain the dry weight (DW). The following equation was used to obtain RMC<sub>S</sub>:  $RMC_S = (WW - DW) / DW$ . These analyses were performed 127 days after sowing the seeds.

#### **Physiological measurements and analysis of tissue nutrient content**

The leaf gas exchange parameters evaluated were net CO<sub>2</sub> assimilation (*A*, μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>); transpiration (*E*, mmol m<sup>-2</sup> s<sup>-1</sup>); stomatal conductance (*g<sub>s</sub>*, mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>); and the ratio between the intercellular and atmospheric CO<sub>2</sub> concentration (*C<sub>i</sub>/C<sub>a</sub>*, μmol CO<sub>2</sub> mol<sup>-1</sup>). An LCi infrared gas analyzer (IRGA) (ADC-BioScientific, United Kingdom) was used to determine the gas concentrations. The evaluations were performed at 8:00 AM and 11:30 AM, using artificial actinic light (1,000 μmol m<sup>-2</sup> s<sup>-1</sup>) throughout the entire experiment, in an open system and under a high environmental CO<sub>2</sub> concentration.

Chlorophyll *a* fluorescence was determined using a Mini-PAM modulated fluorometer (Walz, Germany). The maximum photochemical efficiency of PSII (*F<sub>v</sub>/F<sub>m</sub>*) was calculated using the equation  $F_v/F_m = (F_m - F_0) / F_m$ , where; *F<sub>0</sub>* and *F<sub>m</sub>* are the minimum and maximum fluorescence, respectively, of dark-adapted plant tissue. The effective quantum yield of PSII ( $\phi$ PSII) was obtained using the equation  $\phi$ PSII = (*F<sub>m</sub>' - F*) / *F<sub>m</sub>'*, where *F<sub>m</sub>'* and *F* are the maximum fluorescence and fluorescence, respectively, when the plant tissue is under artificial actinic light with an intensity of 1,000 μmol m<sup>-2</sup> s<sup>-1</sup> for a duration of 50 s. The apparent electron transport rate of PSII (ETR) was obtained according to Bilber et al. (1995). The parameters of *F<sub>0</sub>* and *F<sub>m</sub>* were evaluated before dawn and *F* and *F<sub>m</sub>'* were evaluated between 8:00 AM and 11:30 AM. These analyses were performed 127 days after sowing the seeds.

The fresh plant material was dried in an oven at 75°C to constant weight. Then, the material was ground in a Willey mill. The mineral composition of the leaf tissue was determined according to Malavolta et al. (1997).

#### **Experimental design and statistical analysis**

The experiment design was a completely randomized-block with five replicates. The results were submitted to analysis of variance (ANOVA) by the F tests, and the means were compared using Tukey tests at 5% probability level. The Pearson correlation method was used to detect possible

correlations between the characteristics analyzed. The statistical procedures were performed using SAEG 9.1.

## Conclusions

*Eugenia dysenterica* seedlings grown in rice husks exhibited inadequate root and shoot growth. The physiological and chlorophyll *a* fluorescence analyses were quite effective in evaluating seedling quality. Based on the results of this study, it is concluded that the use of organic residues, tanned cattle manure and decomposed corn silage as substrates are efficient in promoting plant growth and meeting the nutritional requirements of the *E. dysenterica* seedlings. Therefore, the method proposed in this study can be used as an alternative for large-scale production of high-quality *E. dysenterica* seedlings.

## Acknowledgement

The authors thank to the CNPq, CAPES and FAPEG for financial supports. The authors also thank Mr. Arlindo Thomáz Da Silva and family for providing the plant material.

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