

INSTITUTO FEDERAL DE EDUCAÇÃO, CIÊNCIA E TECNOLOGIA
GOIANO CAMPUS RIO VERDE – GO
DIRETORIA DE PESQUISA E PÓS-GRADUAÇÃO E INOVAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS
AGRÁRIAS-AGRONOMIA

**PROPOSTA PARA AVALIAÇÃO DA QUALIDADE
DE MUDAS DE CAGAITEIRA (*Eugenia dysenterica*
MART. ex DC.) EM DIFERENTES SUBSTRATOS**

Autor: Paulo Dornelles
Orientador: Prof. DSc. Fabiano Guimarães Silva

RIO VERDE – GO
Dezembro- 2016

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Prof. Dr. Clenilso Sehnem Mota
Avaliador externo
IF Catarinense/Rio do Sul

Prof. Dr. Gilson Dourado da Silva
Avaliador interno
IF Goiano/Urutaí

Prof. Dr. Aurélio Rubio Neto
Avaliador interno
IF Goiano/RV

Dr. Daniele Nogueira dos Reis
Avaliador interno
IF Goiano/RV

Prof. Dr. Fabiano Guimarães Silva
Orientador
IF Goiano/RV

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BIOGRAFIA DO AUTOR

Paulo Dornelles, filho de Luiz Dornelles e Gercir Dornelles, nasceu em Erechim, Estado do Rio Grande do Sul.

Em 1990, concluiu o curso de Técnico em Agropecuária, concedido pela Escola Agrotécnica Federal, atual Instituto Federal Goiano – Campus Rio Verde.

Em 1995, foi aprovado em concurso público na Escola Agrotécnica Federal de Rio Verde, atual Instituto Federal Goiano – Campus Rio Verde - GO.

Em 1999, recebeu grau de Licenciatura em Ciências Biológicas, conferido pela Universidade de Rio Verde.

Em 2002, concluiu a Especialização em Biologia Geral pela Universidade Federal de Lavras (UFLA) - MG.

Em 2012, concluiu o curso de Mestrado em Ciências Agrárias pelo Instituto Federal Goiano, Campus Rio Verde-GO.

Em 2016, concluiu o curso de Doutorado em Ciências Agrárias - Agronomia pelo Instituto Federal Goiano, Campus Rio Verde-GO.

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LISTA DE SÍMBOLOS E ABREVIACÕES

| Símbolo/Sigla Symbol /Acronym | Significado Meaning |
|----------------------------------|---|
| A | Net carbon assimilation |
| A/E | Water use efficiency |
| AG | Areia grossa |
| Al | Alumínio |
| am | Before dawn |
| ATP | Adenosina trifosfato |
| ATP | Adenosine triphosphate |
| BC | Bagaço de cana |
| C | Carbon |
| C | Carbono |
| CA | Casca de arroz |
| Ca | Cálcio |
| Ca | Calcium |
| CD | Collar diameter |
| CE | Composto de esterco bovino com silagem de milho |
| Ci/Ca | Relationship internal and external of CO ₂ |
| cm | Centimeter |
| cm | Centímetro |
| cm ³ | Centímetro cúbico |
| cm ³ | Cubic centimeter |
| CM | Tanned cattle manure |
| CMC | Cattle manure compost |
| CO ₂ | Dióxido de carbono |
| CO ₂ | Carbon dioxide |
| CS | Coarse sand |
| CS | Decomposed corn silage |
| CTC | Capacidade de troca de cátions |
| CTC | Cation exchange capacity |
| Cu | Copper |
| CV | Vegetation House |
| DAS | Days after sowing |
| DQI | Dickson quality index |
| DW | Dry weight |
| DWL | Dry weight of the leaves |
| DWR | Dry weight of the roots |
| DWS | Dry weight of the stems |
| E | Transpiration |

| | |
|------------------|---|
| EB | Esterco de bovino curtido |
| ESI | Emergence speed index |
| ETR | Taxa de transporte de elétrons |
| ETR | Electron transport rate |
| F | Fluorescence |
| FCM | Fermented cattle manure |
| FC | Filter cake from sugar-alcohol mills |
| Fe | Iron |
| Fm | Maximum fluorescence |
| A | Fotossíntese líquida |
| F0 | Minimum fluorescence |
| Fv/Fm | Razão entre a fluorescência variável e a fluorescência máxima |
| ϕ PSII | Maximum photochemical efficiency of PSII |
| FV | Fine vermiculite |
| FW | Fresh weight |
| g | Grams |
| gg ⁻¹ | Grams per gram |
| gs | Stomatal conductance |
| H ₂ O | Água |
| H ₂ O | Water |
| IVE | Índice de velocidade de emergência |
| K | Potássio |
| K | Potassium |
| Kg | Kilogram |
| LA | Leaf area |
| LCTV | Plant Tissues Culture Laboratory |
| m | Meters |
| Mg ¹ | Megagrama |
| Mg | Magnesium |
| mg | Milligram |
| mm | Millimeter |
| Mn | Manganês |
| MO | Matéria orgânica |
| OM | Organic matter |
| MP | MecPlant [®] |
| N | Nitrogen |
| Na | Sodium |
| NADPH | Nicotinamida adenina difosfato |
| NADPH | Nicotinamide adenine diphosphate |
| NL | Number of leaves |
| LDW | Leaf dry weight |
| NPQ | Non-photochemical energy |
| ϕ NPQ | Non-photochemical energy dissipation of PSII |
| ϕ NO | Yield of unregulated non-photochemical energy dissipation of PSII |
| O | Oxygen |
| P | Phosphor |
| PES | Percentage of emerged seedlings |
| pH | Potencial de hidrogênio |

| | |
|------------------|--|
| pH | Potential of hydrogen |
| PNPD | National Program Postdoctoral |
| PPE | Porcentagem de plantas emergidas |
| PSE | Percent seedling emergence |
| PSI | Photosystem I |
| PSII | Fotossistema II |
| PSII | Photosystem II |
| ϕ PSII | Effective quantum yield of PSII (ϕ PSII) |
| RDW | Root dry weight |
| RH | Rice husks |
| R/S | Root and shoot dry weight ratio |
| RMC _L | Relative leaf moisture content |
| RMC _S | Relative moisture content |
| RWC | Relative water content |
| S | Sulfur |
| s | Second |
| SB | subsoil |
| SB | Subsolo |
| SCB | Sugarcane bagasse |
| SDW | Stem dry weight |
| SL | Stem length |
| SL/CD | Ratios between Stem length and Collar diameter |
| SL/RCD | Ratio between stem length and root collar diameter |
| SL/S | Ratio between stem length and shoot dry weight |
| SL/SD | Ratios between Stem length and stem diameter |
| SM | Silagem de milho decomposta |
| SN | Soil collected from around parent plants |
| SP | Solo de redor das plantas matrizes |
| TDW | Total dry weight |
| TF | Torta de filtro de usina |
| TW | Turgid weight |
| V | Base saturation |
| VF | Vermiculita fina |
| WW | Wet weight |
| v:v | Volume ratio volume |
| WCDS | Water content in the dry base substrates |
| Zn | Zinc |
| μ | Micro |
| $^{\circ}$ C | Degree Celsius |

RESUMO

DORNELLES PAULO, Instituto Federal Goiano – Campus Rio Verde – GO, dezembro de 2016. **Proposta para avaliação da qualidade de mudas de cagaita (*Eugenia dysenterica* Mart. ex DC.) em diferentes substratos.** Orientador, Fabiano Guimarães Silva, e coorientadores: Aurélio Rúbio Neto e Giselle Camargo Mendes.

A *Eugenia dysenterica* Mart. ex DC. (cagaiteira), é uma frutífera nativa do Cerrado com utilização alimentícia e farmacológica, a manutenção da espécie requer plantios de mudas de qualidade. Esta conquista é determinada a partir da fase de viveiro, sendo o substrato um dos mais importantes insumos para o crescimento das plântulas. Além da fixação, a nutrição, hidratação e oxigenação são fatores essenciais atribuídos ao substrato. O aproveitamento de resíduos agroindustriais é uma alternativa bastante valiosa, principalmente quando utilizados em misturas de diferentes componentes. Objetivou-se com este estudo avaliar a qualidade fisiológica e o crescimento das mudas de cagaiteira cultivadas em diferentes substratos. Com isso, recomendar aos produtores de mudas um método, prático e rápido, para avaliação da qualidade de mudas e, também, para a produção em larga escala, utilizando substratos alternativos. O trabalho foi dividido em três experimentos, sendo os seguintes componentes utilizados para a formação dos substratos: subsolo (SB), solo de redor das plantas matrizes (SP), areia grossa (AG) vermiculita fina (VF), casca de arroz carbonizada (CA), esterco bovino curtido (EB), silagem de milho decomposta (SM), composto de esterco bovino com silagem de milho (CE), bagaço de cana (BC), torta de filtro de usina (TF) e os substratos comerciais Bioplant[®], MecPlant[®] (MP) e Tri-mix[®]. Foram realizadas análises biométricas e

fisiológicas, bem como, a correlação de Pearson entre as variáveis analisadas. Verificou-se no primeiro experimento, que as análises fisiológicas de fluorescência da clorofila *a* foram bastante eficazes na avaliação da qualidade das plântulas. O uso de resíduos orgânicos, EB e SM como substratos foram eficientes na promoção do crescimento e nutrição das plântulas de *E. dysenterica*. No segundo experimento, verificou-se que a vermiculita fina promoveu maior índice de qualidade de Dickson, bem como maior diâmetro do caule das plantas. Maior teor de N ocorreu nas folhas das mudas crescidas no Tri-Mix[®] e VF e Mg na VF e VF+CA, respectivamente. Os substratos não alteraram os atributos fisiológicos das mudas. No terceiro experimento, evidenciou-se que as características biométricas correlacionaram-se positivamente com a fotossíntese, com destaque para o rendimento quântico máximo do PSII (F_v/F_m), a taxa de transporte de elétrons (ETR) e o rendimento quântico efetivo do fotossistema II (ϕ PSII), mostrando que essas características podem ser alternativas aos índices de qualidade tradicionais utilizados para as mudas. O uso de CE, BC e TF mostraram-se adequados para a produção e suprimento nutricional de mudas de *E. dysenterica*.

PALAVRAS-CHAVE: cerrado, cagaita, resíduos de agroindústria, trocas gasosas, fluorescência.

ABSTRACT

DORNELLES PAULO, Goiano Federal Institute - Rio Verde Campus - GO, December 2016. Proposal to evaluate the quality of cagaíta seedlings (*Eugenia dysenterica* Mart. Ex DC.) on different substrates. Advisor, Fabiano Guimarães Silva, and Co-Advisors Giselle Camargo Mendes and Aurélio Rúbio Neto.

Eugenia dysenterica Mart. Ex DC. (Cagaíteira), is a native fruit of the Cerrado with food and pharmacological use, the maintenance of the species requires plantings of quality seedlings. This achievement is determined from the nursery stage, the substrate being one of the most important inputs for the seedlings growth. Besides fixation, nutrition, hydration and oxygenation are essential factors attributed to the substrate. The use of agroindustrial waste is a very valuable alternative, especially when used in mixtures of different components. The objective of this study was to evaluate the physiological quality and growth of cagaíteira seedlings grown on different substrates. With this, to recommend to the seedlings producers a method, practical and fast, to evaluate the quality of seedlings and also for the production in large scale, using alternative substrates. The work was divided in three experiments, the following components being used for substrate formation: subsoil (SB), soil around matrix plants (SN), coarse sand (CS) fine vermiculite (FV), Carbonized rice husk (RH), tanned cattle manure (CM) decomposed corn silage (CS), cattle manure compost (Corn silage + fermented cattle manure) (CMC), sugarcane bagasse (SCB), plant filter cake (FC) and commercial substrates Bioplant[®], MecPlant[®] (MP) and Tri-mix[®]. Biometric and physiological analyzes were performed, as well as the Pearson correlation between the analyzed variables. It was verified in the first experiment that the physiological analyzes of chlorophyll α fluorescence were quite effective in the evaluation

of seedling quality. The use of organic residues, FCM and CS as substrates were efficient in promoting the growth and nutrition of *E. dysenterica* seedlings. In the second experiment, it was verified that the fine vermiculite promoted a higher quality index of Dickson, as well as a larger diameter of the stem of the plants. Higher N content occurred in the leaves of the seedlings grown in Tri-Mix[®] and FV and Mg in FV and FV+RH, respectively. The substrates did not alter the physiological attributes of the seedlings. In the third experiment it was observed that the biometric characteristics were positively correlated with photosynthesis, with emphasis on the maximum quantum yield of PSII (Fv/Fm), electron transport rate (ETR) and the effective quantum yield of photosystem II (ϕ PSII), showing that these characteristics may be alternatives to the traditional quality indexes used for seedlings. The use of CMC, SCB and FC was adequate for the production and nutritional supply of *E. dysenterica* seedlings.

KEY WORDS: cerrado, cagaita, agroindustrial residues, gas exchange, fluorescence.

INTRODUÇÃO GERAL

O Cerrado é um Domínio com grande extensão territorial, as diferentes fitofisionomias vegetal e a quantidade de espécies, fazem jus a ser a savana mais diversificada do planeta. Essa riqueza do Cerrado possui destacada importância socioeconômica, por atender as diversas finalidades de uso pelo homem (KLINK & MACHADO 2005; OLIVEIRA et al., 2016; PASA & ÁVILA, 2010). O Cerrado também é considerado o berço das águas, suas nascentes alimentam rios das principais bacias hidrográficas do Brasil (BERNARDI et al., 2003; BORGES et al., 2016).

Espécies como a *Eugenia dysenterica* Mart. (cagaiteira), *Hancornia speciosa* Gomes (mangabeira), *Annona crassiflora* Mart. (araticunzeiro), *Caryocar brasiliense* Camb. (pequizeiro), são algumas das dezenas de plantas nativas do Cerrado que produzem frutos de ótima qualidade, que são coletados nas épocas de maturação (CARDOSO et al., 2014). Esta atividade tem importância socioeconômica e ambiental, por manter o agricultor no campo retirando parte de seu sustento da natureza sem grandes despesas, enquanto mantém as plantas no ambiente (ARAKAKI et al., 2009).

A escassez de vegetação nativa causada pelos desmatamentos reduziu muito a prática de colheita dos frutos, assim como o sistema extrativista predatório. Esses fatores tornaram ainda mais valorizados os frutos e produtos dessas plantas e é, portanto, compreensível o interesse de muitos agricultores por mudas destas espécies. A demanda é diversificada de acordo com as necessidades e interesse do produtor rural, sendo que muitos desses necessitam fazer reconstituição de áreas de preservação permanente, ou investir na recuperação de solos degradados e outros ainda procuram mudas para plantios com objetivos de exploração comercial (NEGREIROS et al., 2009). Portanto, há necessidade que o setor viveirista atenda a essa demanda, produzindo mudas com boa

qualidade, utilizando insumos adequados, promovendo alta germinação e crescimento precoce das mudas (LIMA et al., 2014).

A falta de trabalhos científicos relacionados ao comportamento de crescimento e desenvolvimento de mudas, para a maioria das espécies do Cerrado é realidade, sendo um desafio ao setor viveirista trabalhar com plantas nativas. Os estudos ajudarão entre outros fatores, conhecer melhor a biologia e fisiologia, que até o momento é quase inexistente, assim como na domesticação destas espécies (MOTA et al., 2016).

O plantio das espécies nativas vem minimizar as condições perturbadas do Cerrado, que é um dos mais ameaçados do país. Por isso, serão abordados nesta tese temas como produção, manejo e avaliação da qualidade de mudas nativas, bem como as boas características do substrato, e desta forma contribuir com o setor viveirista.

Até o presente momento a propagação mais utilizada para espécies nativas ainda é a sementeira, por isso, os índices de emergência e a qualidade das plântulas, estão diretamente relacionada à boa procedência, como o porte e o vigor das plantas matrizes, tamanho da semente e ausência de danos. Normalmente é comum, as sementes florestais possuírem características que exigem cuidados e conhecimentos, como a recalcitrância ou a dormência. Por isso, são necessários procedimentos apropriados durante e após as coletas com objetivos de se manterem viáveis à germinação e emergência e quando possível realizar análises laboratoriais de qualidades das sementes (MATOS et al., 2009).

Durante a fase de germinação as sementes exigem umidade, oxigenação e temperatura adequadas, estas necessidades são supridas por meio do substrato. Para a maioria das espécies o substrato mais adequado é o resultado de misturas de diferentes componentes. Densidade, porosidade, disponibilidade e retenção de água, capacidade de troca de cátions (CTC), potencial de hidrogênio (pH), ausência de agentes contaminantes estão entre as propriedades mais importantes do substrato (FERMINO et al., 2010). Portanto ao dispor de um substrato ou de materiais que são passivos de gerar substratos, é interessante verificar se o produto atende às condições recomendadas anteriormente, em vistas de proporcionar o crescimento das mudas com qualidade (CALDEIRA et al., 2014).

1 - REVISÃO DE LITERATURA

1.1 – Cagaita (*Eugenia dysenterica* Mart. ex DC)

A cagaiteira (*E. dysenterica*) é planta nativa frutífera do cerrado, pertencente à família Myrtaceae, com maior ocorrência nos estados de Goiás, Minas Gerais e Bahia (MARTINOTTO et al., 2008). A árvore tem crescimento lento, mede entre 4 e 10 m de altura, tronco vistoso, cascas grossas, galhos tortuosos e copa bastante desenvolvida. As folhas caem no período seco e o surgimento das folhas novas ocorre durante e após a floração (CAMILO et al., 2013). A espécie possui baixa exigência nutricional, desenvolvendo inclusive em solos mais pobres, podendo ocupar também áreas que foram degradadas pela ação antrópica e assim contribuir no manejo sustentável (RIBEIRO & RODRIGUES 2006).

Durante o período das flores, a *Eugenia dysenterica* Mart. possui mais um tipo de exploração, as abelhas colhem néctar e pólen para produzir mel, sendo importante para o agricultor aproveitar esse potencial (Figura 1). A madeira serve para construção, lenha e carvão, as folhas e cascas são utilizadas como remédios para diversos problemas de saúde, a árvore muito vistosa, permite o aproveitamento ornamental de ruas e praças (MARTINOTTO et al., 2007). Porém, o maior potencial econômico da espécie está em seus frutos, com média variável, de 500 a 2000 frutos por árvore (OLIVEIRA et al. 2011).

Esses quando maduros possuem coloração amarelo clara ou arroxeadada, polpa carnosa, muito aquosa, variando entre 2 e 4 cm de diâmetro, com 1 a 4 sementes em seu interior (SILVA et al., 2008). Com aspecto visual atrativo e sabor peculiar agradável e levemente ácido, os frutos possuem teores de vitaminas A e C, fonte de folatos, além de sais minerais. Também são observados baixos níveis de lipídeos, energia e açúcares, que influenciam a importância nutricional saudável (CAMILO et al., 2014). A polpa serve para sucos, licores, sorvetes, picolés, bebidas e doces diversos, propriedades que comprovam capacidade para a agroindustrialização (OLIVEIRA et al., 2011; CAMILO et al., 2014).

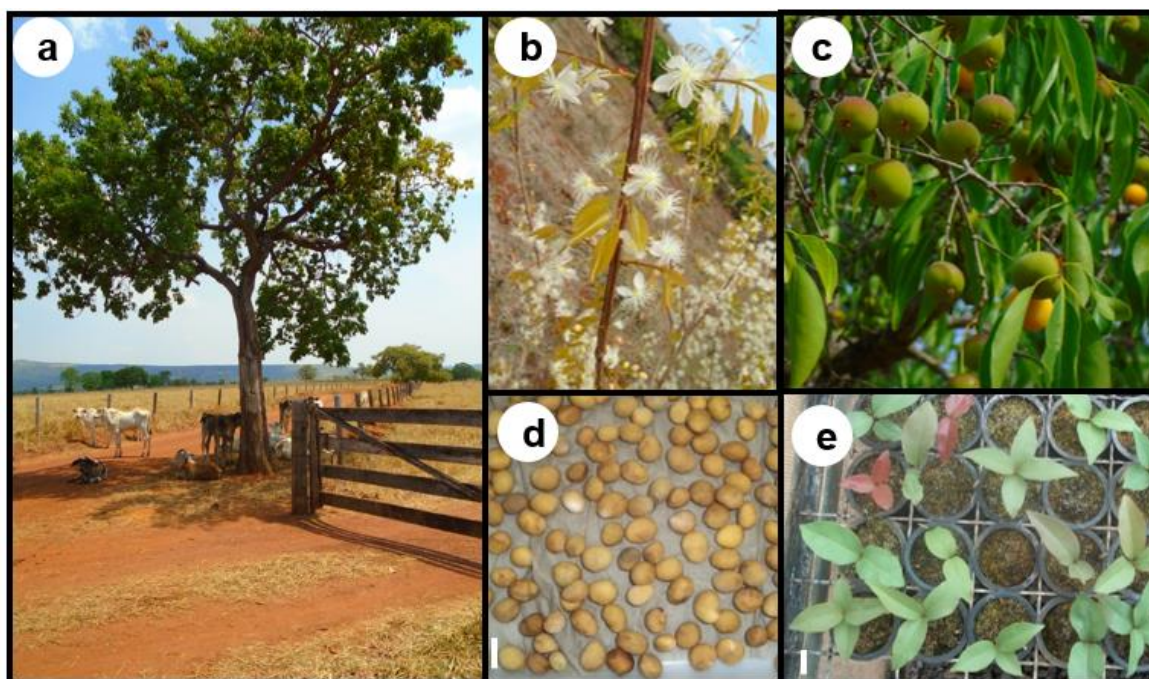


Figura 1 - *Eugenia dysenterica* (Cagaiteira): a) Planta adulta; b) Planta jovem aos quatro anos florida; c) Frutificação; d) Sementes; e) Plântulas em viveiro. Imagens: Paulo Dornelles/Rio Verde/2016. Barra = 3 cm.

Para o consumo natural, deve-se dar preferência aos frutos colhidos diretamente da árvore, após, caídos ao chão podem ter efeito laxativo. Nesses casos é recomendado o aproveitamento para o processamento de bebidas como vinagre e álcool, que elimina o efeito laxante. Os frutos também contribuem para a alimentação silvestre; além de nutrientes, a quantidade de água na polpa é uma alternativa para algumas espécies, pela escassez hídrica no período seco (Tabela 1).

Tabela 1- Principais estudos da cagaiteira, planta frutífera do Cerrado pertencente à família Myrtaceae do gênero *Eugenia*, publicados no período de 2011 a 2017 (dados obtidos na Web of Science e Science direct). Rio Verde, 2016.

| Objetivo da pesquisa | Utilização | Autor |
|--|-----------------|-------------------------|
| Recuperação do solo | Mudas | Venturoli et al. (2013) |
| Caracterização física e química | Frutos | Cardozo et al. (2011) |
| Viabilidade quanto a secagem | Sementes | Silva et al. (2016) |
| Caracterização da geleia de cagaita | Frutos | Santos et al. (2012) |
| Avaliação sensorial de frutos do Cerrado | Frutos | Arruda et al. (2016) |
| Produção e conservação de extratos | Polpa | Daza et al. (2016) |
| Desenvolvimento fisiológico | Frutos | Silva et al. (2017) |
| Efeito curativo da cagaita | Frutos e folhas | Silva et al. (2015) |
| Análise química | Polpa | Pestana et al. (2015) |

1.2 – Estudos para a produção de mudas nativas do cerrado

As espécies florestais nativas possuem capacidade de adequar-se melhor às condições de sua própria região, pelas peculiaridades adquiridas no tempo. Quando o objetivo é a recuperação de áreas degradadas, em que as condições de sobrevivência são mais difíceis, essas espécies são as mais propensas a serem cultivadas. Parâmetros como a rusticidade e a desenvoltura do sistema radicular das espécies nativas do Cerrado conseguem alcançar maior profundidade no solo. Entre os benefícios ao ambiente destacam-se a recuperação dos nutrientes situados nas camadas profundas e a descompactação do solo, possibilitando maior infiltração da água e a manutenção da biodiversidade das espécies. Quando o reflorestamento é realizado com as frutíferas nativas, além dos benefícios citados ao ambiente, garantem alimento e geração de renda aos produtores rurais (PAIVA SOBRINHO et al., 2010).

A procura por frutos e seus derivados tornou interessante o plantio das frutíferas nativas na forma de pomar, assim como também existem muito interesse por mudas para a reposição de áreas abertas. Entretanto, mudas destas espécies dificilmente são encontradas em viveiros, a razão de tal limitação é decorrente da falta de estudos das plantas nativas do Cerrado em relação à produção destas e seu cultivo. Portanto, é importante a realização de trabalhos científicos, com vistas às metodologias de produção de mudas, a fim de aumentar sua produção, com baixos custos e alta qualidade (Dantas et al., 2009).

A identificação das exigências nutricionais possibilita o desenvolvimento de metodologias capazes de alcançar um padrão de qualidade das mudas em relação ao crescimento, robustez, sanidade e uniformidade (SOUZA et al., 2013). E a realização de análises biométricas, morfofisiológicas e nutricionais nas mudas, torna-se essenciais. Estas informações são ferramentas preciosas, que irão favorecer a linha de produção de mudas nativas, como também no processo de domesticação destas espécies (ZANELLA et al., 2006; FERREIRA et al., 2008).

O crescimento das plantas está diretamente relacionado com o acúmulo de carboidratos via fotossíntese, que permite, por meio de avaliações dos seus órgãos, como folhas, caule e raízes, mensurar o grau de desenvolvimento e o potencial de produtividade (CAMPOS et al., 2008). Por outro lado, os fatores ecofisiológicos igualmente permitem o acompanhamento durante o ciclo de crescimento e precisam ser mais utilizados e estudados em espécies arbóreas florestais. Os resultados destes parâmetros reforçam a

eficiência das atividades experimentais destas espécies, a ecofisiologia relaciona os fatores do ambiente e as respostas metabólicas das plantas (SOUZA et al., 2011).

1.3 - Substrato

O substrato encontra-se entre os insumos mais importantes na produção de mudas, sendo necessário possuir características que atendam às exigências da espécie. Para isso, é imprescindível a presença de propriedades que promovam além da sustentação da muda, o seu crescimento, disponibilizando umidade, aeração e nutrientes (EHLERS & ARRUDA, 2014).

Após o processamento o substrato deve ter preferencialmente média densidade, para facilitar o crescimento radicular, manejo e transporte das mudas. Ser suficientemente firme para sustentar estacas, sementes e mudas, manter-se inerte, sem alterar seu volume quando molhado, não sofrer compactação. Deve umedecer com facilidade e de maneira homogênea, possuir boa retenção de água, disponibilizando água de acordo com a necessidade da planta. A granulometria variada contribui para a boa textura e estrutura, enquanto a alta porosidade facilita a drenagem do excesso de água e entrada de oxigênio em seu interior (SILVA et al., 2011).

Quanto às propriedades químicas do substrato possuir bons teores de matéria orgânica e elevada (CTC), favorece o fornecimento dos nutrientes às mudas, também é importante possuir baixa salinidade e pH levemente ácido (SIBALDELLI et al., 2015). Entre as propriedades biológicas, é interessante a ausência de sementes de espécies invasoras e organismos patogênicos, e por fim, estar disponível para aquisição a preço condizente com o mercado (DIAS et al., 2009; KRATZ & WENDLING, 2013).

É compreensível que um só componente não possua todos os requisitos exigidos ao substrato, havendo a necessidade de ocorrer misturas de materiais diferentes, as propriedades de um único material isolado podem não atender às necessidades das plantas (KRATZ et al., 2013; GONÇALVES et al., 2014).

1.3.1 – Aproveitamento de materiais orgânicos residuais regionais

Com a adoção de novas técnicas de cultivo de plantas em viveiro, surgiram avanços diversos, como ambiente protegido e suspenso, recipientes de menor volume e retornáveis e o melhor aproveitamento de resíduos agroindustriais para substratos (HERRERA et al., 2008; KRATZ & WENDLING 2013).

Os resíduos agroindustriais tornaram-se interessantes pela proximidade, disponibilidade, facilidade de manejo e qualidades próprias, permitindo assim, ao viveirista e ao agricultor produzir seu próprio substrato. São evidenciadas outras vantagens como o menor custo e níveis representativos de nutrientes, além do destino adequado desses resíduos e a preservação dos recursos não renováveis (VIEIRA et al., 2014).

Estercos, bagaços, tortas, palhadas, serragens, cascas, restos de frutas e verduras são originados das mais variadas fontes agroindustriais, possuem diferentes composições químicas e físicas, podendo ser utilizados de preferência em misturas (Figura 2), sendo importante que estes resíduos, antes do uso passem por compostagem, que consiste da ação dos microrganismos na quebra das partículas maiores, melhorando a estrutura do material (ORRICO JÚNIOR et al., 2009; LEAL et al., 2013; BLANK et al., 2014).



Figura 2 – Materiais residuais disponíveis na região do Cerrado e com potencial para a produção de mudas nativas de *Eugenia dysenterica* Mart.. a) Bagaço de cana; b) Torta de filtro; c) Silagem de milho; d) Mistura de ingredientes para substratos; e) Plântulas de cagaita em fase de crescimento. Barra = 3 cm

Para as propriedades químicas ocorre a elevação da fração da matéria orgânica e aumento da CTC, assim as plantas podem obter maiores teores de nutrientes que são disponibilizados, podendo reduzir o uso de fertilizantes químicos (ANDRADE et al., 2013). Os benefícios de ordem física são notados quanto à maior retenção de água e equilíbrio da densidade; biologicamente, o material não possui cheiro desagradável e pode ser o habitat de microrganismos benéficos às plantas. Também é interessante, quando

necessário permitir a esterilização, sem sofrer danos às suas características originais. Estudos realizados com o uso destes materiais na constituição de substratos mostraram resultados de qualidade, iguais ou até superiores aos encontrados no comércio, além das vantagens econômica e sustentável (SCHNEIDER et al., 2012; BARROS et al., 2014).

Bagaço de cana e torta de filtro são resíduos derivados das usinas sucroalcooleiras, com grande oferta na região dos cerrados. O uso desses materiais como componentes de substratos constitui uma alternativa interessante, diminuindo os impactos ambientais e contribuindo com o cultivo sustentável de mudas (DUTRA et al., 2013).

O bagaço de cana de açúcar é uma alternativa para o preparo de substrato, subproduto de grande rendimento, durante o processamento da cana são gerados aproximadamente 250 kg Mg⁻¹, que necessitam ser conduzidos adequadamente. Possui boa composição química e alto percentual de MO, leveza e aeração, cuja utilização permite redução no consumo de fertilizantes químicos, sugerindo uma opção atraente como substrato para mudas (CUNHA et al., 2005).

A torta de filtro é outro subproduto da cana de açúcar que surge no momento da filtração da moagem, portanto é um material renovável, com rendimento médio de 20 kg Mg⁻¹ de cana processada (FIGUEIREDO & SCALA JUNIOR, 2011). Possui quantidades expressivas de nutrientes e matéria orgânica (MO), elevado pH e ausência de Al tóxico, com conceito de fertilizante orgânico e detém condições promissoras para atender às exigências como componente de substratos. A utilização da torta de filtro após compostagem foi comprovadamente eficiente em cultivos diversos, revertendo benefícios de notável crescimento e produtividade das plantas e hortaliças (BARROS et al., 2014).

A mistura do bagaço de cana e torta de filtro reflete na maior adequação como substrato, pois o bagaço de cana possui partículas maiores e com formato de fibras longas, enquanto a torta de filtro possui partículas finas. Juntos esses componentes melhoram as propriedades físicas e químicas, resultando em substrato capaz de produzir mudas com qualidade, conforme visto em diversos trabalhos e em diferentes espécies, como na produção de mudas de citros (SERRANO et al., 2004), maracujazeiro-amarelo (SERRANO et al., 2006) e *Anacardium othonianum* Rizz. (DORNELLES et al., 2014).

Os esterco são muito requisitados como materiais constituintes de substratos, principalmente o bovino pela grande disponibilidade e praticidade de uso. Este resíduo orgânico possui variada composição química de acordo com a nutrição dos animais, porém é considerado fonte de nutrientes e principalmente matéria orgânica. A matéria orgânica possui capacidade de melhorar as condições físicas, químicas e biológicas do substrato

como a CTC, umidade e densidade, além de favorecer a presença da microfauna. A adição de esterco bovino favoreceu as propriedades do substrato utilizado durante o crescimento do eucalipto, sendo observado qualidade das mudas e redução do valor do substrato (MELO et al., 2014; MOTA et al., 2016).

A casca de arroz possui atributos que favorecem a utilização como componente de substrato, principalmente na forma carbonizada, em que se verifica ausência de contaminantes, inércia, leveza, pH alcalino e teores de nutrientes como Ca e K. De acordo com Kratz & Wendling (2016), a casca de arroz após passar pelo processo de carbonização reduz o tamanho de suas partículas, aumentando a capacidade de retenção de água, reunindo maiores atributos para ser utilizada principalmente em misturas com outros componentes para substratos.

De origem mineral, inerte, alta capacidade de absorção de água e com baixa densidade, a vermiculita destaca-se pela utilização como componente de substratos no cultivo de mudas. De granulometria variada contém poucos teores de nutrientes, sendo ideal balancear de acordo com as necessidades da espécie, com misturas de outros componentes proporcionando melhores propriedades ao substrato (CALDEIRA et al., 2013).

A areia é um material mineral, possui alta densidade e baixos teores nutricionais, normalmente de fácil obtenção. A areia e o solo foram os materiais tradicionais no uso para multiplicação de plantas. Quando misturada ao solo a areia melhora a drenagem da água por possuir partículas maiores aumentando a porosidade do substrato. Muito utilizado sozinha em emergências e testes de emergências de sementes. No entanto, para o melhor crescimento das mudas recomenda-se misturá-la a outros componentes, que possuem propriedades físicas e químicas diferentes, como os teores nutricionais e a densidade (ANDRADE et al., 2013; PIMENTEL et al., 2016).

A utilização do subsolo (camadas mais profundas do solo) oferece menores variações de pH, MO, e ausência de sementes de plantas invasoras. Naturalmente sua densidade é elevada e os parâmetros de umidade e oxigenação podem não ser os mais adequados. Atualmente é comum a mistura de materiais residuais ao solo e, os resíduos além de reduzirem a densidade, melhoram os níveis nutricionais (PIMENTEL et al., 2016). Saidelles et al. (2009), observaram no trabalho com espécies florestais, que a mistura de até 50% de casca de arroz carbonizada ao solo representou o melhor substrato em benefícios na qualidade das mudas.

1.4 - Ecofisiologia aliada à produção de mudas de qualidade

Alguns fatores edafoclimáticos nas regiões tropicais são responsáveis por causar desconfortos nas plantas, assim na região do Cerrado a baixa umidade no período de estiagem, a intensa luminosidade e altas temperaturas promovem estresses nas plantas (LARCHER, 2006; ZHANG et al., 2011). No entanto, as plantas nativas do Cerrado desenvolveram alguns mecanismos de adaptações a estas causas desfavoráveis, como o rápido e vultoso crescimento do sistema radicular, capaz de encontrar água em camadas mais profundas do solo, o fechamento dos estômatos nas horas quentes do dia, evita a desidratação das plantas através da transpiração, porém impede a absorção do CO₂, e a dissipação do excesso de energia, são formas de suportar as condições adversas do clima. Esse comportamento adaptativo pode ser observado através de avaliações em *Hancornia speciosa* Gomes, em que os parâmetros reduziram seus valores, porém foram recuperados sem danificar o aparato fotossintético em cinco dias após sessar o estresse hídrico (SCALON et al., 2015).

1.4.1 – Fluorescência da clorofila *a*

A irradiância quando em excesso proporciona desgastes no processo de fotossíntese das plantas que prejudicam seu desenvolvimento. Para amenizar os danos, as plantas através de mecanismos adaptativos dissipam parte desta energia. Uma forma de dissipação ocorre na forma de fluorescência da clorofila *a*, principalmente no PS II (OUKARROUM et al., 2007). A obtenção da fluorescência da clorofila *a* através de medidas com a utilização de equipamentos adequados, contribui para os estudos relacionados às estruturas fotossintéticas. Essas análises além de eficientes não causam danos às mudas, podendo ser repetidas durante o período de acompanhamento (KONRAD et al, 2005; GOLÇALVES et al., 2007).

1.4.2 – Trocas gasosas

As trocas gasosas ocorrem entre as células estomáticas das folhas e a atmosfera, envolvendo a fixação do CO₂ e a liberação do O₂ e da H₂O por transpiração. A assimilação do CO₂ tem por finalidade a formação de estruturas maiores, denominados carboidratos, responsáveis pelo crescimento e produtividade das plantas. As quantidades de CO₂ consumido na fotossíntese ou liberado na respiração, podem ser obtidos através das medições de trocas gasosas (SILVA et al., 2015).

Estudos relacionados às trocas gasosas esclarecem o mecanismo de abertura e fechamento dos estômatos que regem a fotoproteção. Esse movimento da água absorvida do solo pela planta promove a hidratação, absorção de minerais e sua saída ameniza o excesso de calor. O déficit hídrico é um dos principais fatores estressantes nas plantas, para evitar colapso por desidratação, as plantas mantêm seus estômatos fechados (ENNAHLI & EARL, 2005; COSTA et al., 2015). Durante o período de déficit hídrico, os estômatos fechados, por meio de avaliações, observaram-se o decréscimo dos valores da condutância estomática, transpiração, concentração interna de CO₂, eficiência quântica fotoquímica e eficiência instantânea de carboxilação (ARCOVERDE et al., 2011).

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OBJETIVO

Objetivou-se com este estudo avaliar a qualidade fisiológica e o crescimento das mudas de cagaiteira cultivadas em diferentes substratos. Com isso, recomendar aos produtores um método, prático e rápido, para a produção de mudas em larga escala utilizando substratos alternativos.

CATÍTULO I - Use of physiological parameters to assess seedlings quality of *Eugenia dysenterica* DC. grown in different substrates¹

(Artigo formatado de acordo com normas da Revista Australian Journal of Crop Science)

Abstract: The *cagaiteira* tree, which belongs to the Myrtaceae family, is a fruit tree that is native to the Cerrado. Its fruits are of economic interest mainly because is used in cuisine. Studies on the conservation and propagation of native species may aid reduce biodiversity loss. The purpose of this study was to evaluate the physiological quality of *Eugenia dysenterica* DC. seedlings grown in different substrates. The following substrates were used: MecPlant[®] (MP), rice husk (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. The analysis of percent seedling emergence and vigor, biometric characteristics, gas exchange, chlorophyll *a* fluorescence, quality indices, and leaf mineral nutrient levels were evaluated 127 days after sowing. Overall, the SB+CS+CM substrate resulted in higher values for all of the characteristics analyzed, except for leaf nutrient levels, but this substrate also resulted in high content of minerals. Likewise, the SB+FV+CS substrate showed second high content of nutrients. Based on the results of this study, it is possible to conclude that using tanned cattle manure and decomposed corn silage resulted in the best *Eugenia dysenterica* seedling quality.

Key words: *Eugenia dysenterica*; mineral nutrition; physiological quality; quality index; seedling production.

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1.1 - Introduction

The Cerrado (savanna) is the second largest Brazilian biome that is located in centre of Brazil. This region occupies an area of 2,036 million kilometers, which is equivalent to 22% of the area of the country. Native fruit species are unique in this biome because they exhibit high content of sugars, vitamins, proteins, and mineral salts; the fruit is eaten fresh or is used as a raw material to produce sweets, jams, ice-creams, and liqueurs. Among these plants, the native Cerrado fruit species known as *cagaiteira* (*Eugenia dysenterica* DC.), belonging to the family Myrtaceae, is distinctive because of the unique flavor of its fruits, which are characterized as peculiar taste. In addition to its use as food, the fruit has pharmacological potential and nutraceutical properties.

Studies on the conservation and propagation of native species may aid reduce biodiversity loss. Fruit trees native to the Cerrado are mainly exploited through extraction (Donadio et al., 2002), and even though rational and technologically advanced plantings exist, little is known about many species, despite their high potential (Lederman et al., 2000). Studies related to the domestication, genetic selection, and development of planting systems are way to avoid genetic erosion, the extinction of superior individuals, and the extraction pressure placed on nature. The case is no different for *E. dysenterica*, and it is necessary to develop appropriate techniques that must be initiated using high-quality seedlings to establish successful orchards. Thus, the seedlings used to establish orchards must be robust, healthy, well nourished, highly adaptable, and fast growing to ensure orchard success. The first step in commercial planting is to obtain healthy seedlings that can be established in nurseries, which would ensure that the plants remain healthy in the field. Knowledge of the genetic diversity of a species and aspects related to its propagation is necessary and must precede orchard establishment (Rosa et al., 2005).

A high-quality seedling exhibits the attributes necessary for survival and development after being transplanted into the field (Duryea, 1985). Thus, it is essential that seedling production have high quality standards, as the seedlings must resist adverse field conditions after planting, resulting in trees with desirable growth characteristics. 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 an important factor that directly affects seedling quality, considering that it provides water, nutrients, and support for the roots (Ferraz et al., 2005). It is important that the substrate components be easily sourced

close to the greenhouse and at reasonable prices (Dantas et al., 2009). The ideal substrate that will result in high-quality seedlings varies with the plant species to be propagated, and few studies exist on the quality of fruit tree seedlings. To contribute to information on the seedling production and quality of native Cerrado species, the main purpose of this study was to evaluate the growth, quality, mineral nutrition, and physiology of *Eugenia dysenterica* DC. seedlings grown in different substrates.

1.2 - Results

Chemical analysis of nutrients of the substrates

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



Fig 1 – substrates tested: 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). Bar = 3 cm.

Table 1 – Chemical analysis and macro- and micronutrients of the substrates used in the study. Rio Verde, 2016.

| Sample | pH (H ₂ O) | V (%) | OM | (t) | Ca | Mg | P | K | Zn | Fe | Mn | Cu | B |
|---|--------------------------|----------|----------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | | | dag kg ⁻¹ | cmol _c dm ⁻³ | cmol _c dm ⁻³ | cmol _c dm ⁻³ | cmol _c dm ⁻³ | cmol _c dm ⁻³ | mg dm ⁻³ | mg dm ⁻³ | mg dm ⁻³ | mg dm ⁻³ | mg dm ⁻³ |
| MP ^y +RH ^x (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 ^w +FV ^v +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 ^u (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 ^t +CM ^s (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 ^r | 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 |

^yMecPlant[®]; ^xRice husks; ^wSoil; ^vFine vermiculite; ^uDecomposed corn silage; ^tCoarse sand; ^rTanned cattle manure;

^sSoil collected from around the parent plants. V=Base saturation index. OM = organic matter.

Seedling quality evaluation

The quality of the seedlings produced was affected by the different substrates tested, and each substrate exhibited unique characteristics (Fig. 2).

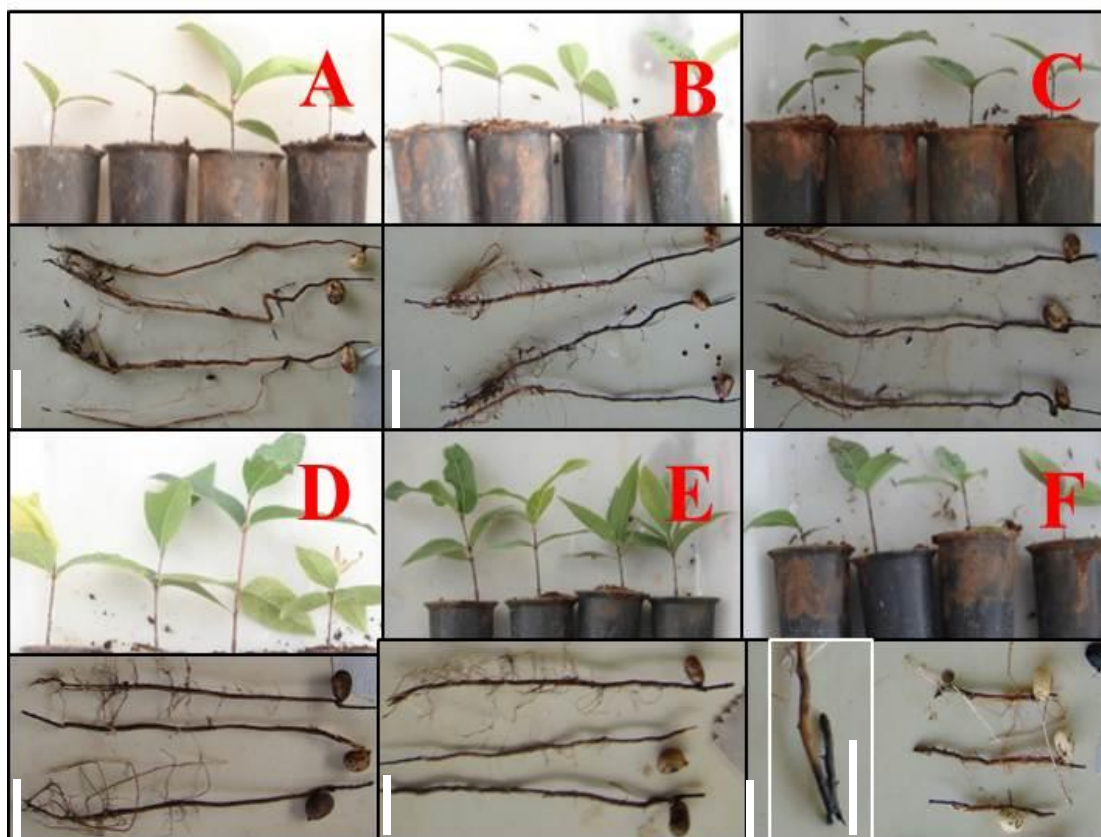


Fig 2 – *Eugenia dysenterica* seedlings produced in the following substrates: MecPlant® (MP)+rice husks (RH; 7:3; A); subsoil (SB)+fine vermiculite (FV)+RH (1:2:2; B); SB+FV+RH (1:1:1; C); SB+FV+ decomposed corn silage (CS; 1:3:6; D); SB+ coarse sand (CS)+tanned cattle manure (CM; 2:2:1; E); soil collected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

The soil surrounding the parent plants (SN) resulted in the lowest percent 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 that of the MP+RH substrate (0.29). The best shoot development and adventitious root formation were found in cagaiteira seedlings grown in the SB+FV+CS and SB+CS+CM substrates.

Table 2 - Percent seedling emergence (PSE) and emergence speed index (ESI) of *Eugenia dysenterica* DC. seedlings in different substrates. Rio Verde, 2016.

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

^zMeans followed by the same letter do not differ according to Tukey's test ($p>0.05$); ^yMecPlant[®]; ^xRice husks; ^wSoil; ^vFine vermiculite; ^uDecomposed corn silage; ^tCoarse sand; ^sTanned cattle manure; ^fSoil collected from around the parent plants. ^qMinimum significant difference.

Relative moisture content in the substrate and in the leaf

The SN substrate exhibited the lowest relative moisture content (RMC_S), followed by the SB+CS+CM substrate (0.24 and 0.34 g g⁻¹, respectively). The highest RMC_S was recorded in the MP+RH substrate (1.97 g g⁻¹), followed by SB+FV+CS (1.65 g g⁻¹) (Table 3).

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. Rio Verde, 2016.

| Substrate | RMC _S (g g ⁻¹) | RMC _L |
|---|--|------------------|
| MP ^y +RH ^x (7:3) | 1.97 a | 0.95 b |
| SB ^w +FV ^v +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 ^u (1:3:6) | 1.65 b | 0.97 a |
| SB+CS ^t +CM ^s (2:2:1) | 0.34 d | 0.97 a |
| SN ^f | 0.24 d | 0.96 ab |
| MSD ^q | 16.98 | 0.02 |

^zMeans followed by the same letter do not differ according to Tukey's test ($p<0.05$); ^yMecPlant[®]; ^xRice husks; ^wSoil; ^vFine vermiculite; ^uDecomposed corn silage; ^tCoarse sand; ^sTanned cattle manure; ^fSoil collected from around the parent plants. ^qMinimum significant difference.

The RMC_S and emergence speed index (ESI) were positively correlated (0.66), indicating that ESI was negatively affected by decreasing RMC_S. The relative leaf moisture

content (RMC_L) remained unaffected (i.e., no correlation) by RMC_S (data not shown). However, the substrates resulted in different values of RMC_L , with higher values observed for substrates SB + VF + CA (1: 1: 1), SB + VF + SM (1: 3: 6) and SB + AG + EB (2: 2: 1) (0.97 g g^{-1}) compared to the MP + CA substrate (7:3) (0.95 g g^{-1}).

Gas exchange

Despite the difference found in RMC_L , this characteristic did not affect (there was no correlation) gas exchange (data not shown). However, the gas exchange characteristics were affected by the substrates tested, and the soil collected from around the parent plants (SN) resulted in a lower net carbon 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 CO_2 levels (C_i/C_a) in the plants remain unaffected by the substrates tested (Table 4).

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. Rio Verde, 2016.

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

^zMeans followed by the same letter do not differ according to Tukey's test ($p < 0.05$); ^yMecPlant[®]; ^xRice husks; ^wSoil; ^vFine vermiculite; ^uDecomposed corn silage; ^tCoarse sand; ^sTanned cattle manure; ^rSoil collected from around the parent plants. ^qMinimum significant difference.

Chlorophyll a fluorescence

The electron transport rate (ETR) was affected by the substrates studied, where 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; the ETR values measured in the other substrates did not differ significantly from these values (Table 5). The maximum photochemical efficiency of PSII (F_v/F_m) and the effective quantum yield of PSII (ϕPSII) 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).

Table 5 – Maximum photochemical efficiency of PSII (F_v/F_m), effective quantum yield of PSII (ϕPSII), and the electron transport rate (ETR) of *Eugenia dysenterica* DC. seedlings grown in different substrates. Rio Verde, 2016.

| Substrate | F_v/F_m | ϕPSII | ETR $\mu\text{mol m}^{-2} \text{s}^{-1}$ |
|---|-----------|-------------------|---|
| MP ^y +RH ^x (7:3) | 0.75 a | 0.21 ab | 78.84 ab |
| SB ^w +FV ^v +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 ^u (1:3:6) | 0.76 a | 0.30 a | 111.77 a |
| SB+CS ^t +CM ^s (2:2:1) | 0.77 a | 0.31 a | 122.61 a |
| SN ^f | 0.70 b | 0.12 b | 46.10 b |
| MSD ^q | 0.053 | 0.133 | 51.96 |

^zMeans followed by the same letter do not differ according to Tukey's test ($p < 0.05$); ^yMecPlant@; ^xRice husks; ^wSoil; ^vFine vermiculite; ^uDecomposed corn silage; ^tCoarse sand; ^sTanned cattle manure; ^fSoil collected from around the parent plants. ^qMinimum significant difference.

Morphological characteristics

The biometric characteristics, i.e., 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, 4.3, 0,242g, 0,042g e 0,458g for SL, CD and NL, SDW and LDW respectively) and SB+CS+CM (2:2:1) (4.46 cm, 1.26 mm, 4,00, 0,042g and 0,233g for SL, CD, NL, SDW and LDW respectively) substrates compared with the other substrates tested (Table 6). The SN substrate resulted in the lowest mean root dry weight (RDW) and total dry weight (TDW) values (0.276 g and 0.447 g, respectively) compared with the other substrates (Table 6).

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. Rio Verde, 2016.

| Substrate | SL (cm) | CD (mm) | NL (-) | SDW (g) | LDW | RDW | TDW |
|---|---------------------|------------|-----------|------------|---------|---------|---------|
| MP ^y +RH ^x (7:3) | 2.72 b ^z | 0.99 b | 2.1 b | 0.019 b | 0.116 b | 0.499 a | 0.634 a |
| SB ^w +FV ^v +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 ^u (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 ^t +CM ^s (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 ^f | 2.78 b | 1.04 b | 2.2 b | 0.024 b | 0.147 b | 0.276 b | 0.447 b |
| MSD ^q | 1.12 | 0.15 | 1.13 | 0.011 | 0.068 | 0.108 | 0.144 |

^zMeans followed by the same letter do not differ according to Tukey's test ($p < 0.05$); ^yMecPlant@; ^xRice husks; ^wSoil; ^vFine vermiculite; ^uDecomposed corn silage; ^tCoarse sand; ^sTanned cattle manure; ^fSoil collected from around the parent plants. ^qMinimum significant difference.

Relationship between the variables

Net carbon assimilation (*A*) was the only gas exchange characteristic that was correlated (positively) 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 (ϕ 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). Of the quality indices, only SL/CD was correlated (positively) with the gas exchange and fluorescence characteristics *A*, F_v/F_m , and ϕ PSII (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 cm mm^{-1}) compared with those of the 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^{-1} , 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^{-1} , 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).

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. Rio Verde, 2016.

| | A^z | E^y | g_s^x | Ci/Ca^w | F_v/F_m^u | $\phi PSII^t$ | ETR^s |
|--------------------|-------|-------|---------|-----------|-------------|---------------|---------|
| SL ^r | 0.41* | 0.24 | 0.28 | -0.20 | 0.34 | 0.50* | 0.48* |
| CD ^q | 0.25 | 0.15 | 0.19 | -0.10 | 0.14 | 0.42* | 0.46* |
| NL ^p | 0.38* | 0.30 | 0.33 | 0.03 | 0.33 | 0.32 | 0.36 |
| SDW ^o | 0.27 | 0.13 | 0.13 | -0.32 | 0.33 | 0.44* | 0.45* |
| LDW ⁿ | 0.33 | 0.20 | 0.22 | -0.18 | 0.28 | 0.49* | 0.48* |
| RDW ^m | 0.36* | 0.27 | 0.27 | -0.14 | 0.27 | 0.23 | 0.23 |
| TDW ^l | 0.47* | 0.32 | 0.33 | -0.23 | 0.38* | 0.46* | 0.46* |
| SL/CD ^k | 0.38* | 0.21 | 0.23 | -0.25 | 0.38* | 0.40* | 0.35 |
| R/S ^j | 0.13 | 0.15 | 0.16 | 0.12 | 0.05 | -0.15 | -0.16 |
| DQI ⁱ | 0.22 | 0.18 | 0.20 | 0.01 | 0.13 | 0.08 | 0.12 |

*Pearson correlation significant at the 5% probability level; ^zNet CO₂ assimilation; ^yTranspiration; ^xStomatal conductance; ^w*Ci/Ca* ratio; ^uMaximum quantum yield of PSII; ^tEffective quantum yield of PSII; ^sElectron transport rate; ^rStem length; ^qCollar diameter; ^pNumber of leaves; ^oStem dry weight; ⁿLeaf dry weight; ^mRoot dry weight; ^lTotal dry weight; ^kRatio between stem length and diameter; ^jRatio between root and shoot dry weight; ⁱDickson 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. Rio Verde, 2016.

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

^zMeans followed by the same letter do not differ according to Tukey's test ($p < 0.05$); ^yMecPlant®; ^xRice husks; ^wSoil; ^vFine vermiculite; ^uDecomposed corn silage; ^tCoarse sand; ^sTanned cattle manure; ^fSoil collected from around the parent plants. ^qMinimum significant difference.

Analysis of nutrient content in the tissues

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

Table 9 – Macro- and micronutrient contents in the leaf tissues of *Eugenia dysenterica* DC. seedlings grown in different substrates. Rio Verde, 2016.

| Substrate | N | P | K | Ca | Mg | S | B | Fe | Cu | Mn | Zn |
|---|--------------------|-------|--------|-------|--------|-------|---------------------|---------|-------|--------|--------|
| | mg g ⁻¹ | | | | | | mg kg ⁻¹ | | | | |
| MP ^y +RH ^x (7:3) | 19.6b ^z | 5.00a | 9.28a | 11.0b | 3.86c | 0.52b | 142.8a | 127.4b | 5.4ab | 312.6a | 12.2bc |
| SB ^w +FV ^v +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 ^u (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 ^t +CM ^s (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 ^r | 19.0b | 0.88c | 3.52d | 8.9b | 4.16c | 0.28b | 124.4c | 217.0a | 4.6b | 341.4a | 11.0c |
| MSD ^q | 1.68 | 0.853 | 1.475 | 5.27 | 2.339 | 0.453 | 5.13 | 69.12 | 1.16 | 149.29 | 4.38 |

^zMeans followed by the same letter do not differ according to Tukey's test (p<0.05); ^yMecPlant@; ^xRice husks; ^wSoil; ^vFine vermiculite; ^uDecomposed corn silage; ^tCoarse sand; ^sTanned cattle manure; ^rSoil collected from around the parent plants. ^qMinimum significant difference.

The N leaf levels were higher in the plants from the SB+FV+CS (1:3:6) (25.2 mg g⁻¹) and SB+CS+CM (2:2:1) (24.8 mg g⁻¹) substrates; the lowest values were measured in plants grown in the SB+FV+RH (1:1:1) and (1:2:2) (11.4 mg g⁻¹ and 12 mg g⁻¹, respectively) substrates. The P content was highest in the plants grown in the MP+RH (7:3) (5 mg g⁻¹) and SB+CS+CM (2:2:1) (4.80 mg g⁻¹) substrates, followed by SB+FV+CS (1:3:6) (3.80 mg g⁻¹) substrate.

Higher K contents were observed in the plants grown in MP+RH (7:3) (9.28 mg g⁻¹), followed by SB+FV+RH (1:1:1) (7.36 mg g⁻¹) and SB+FV+CS (1:3:6) (6.08 mg g⁻¹). The Ca and S contents were highest in the plants grown in SB+FV+CS (1:3:6) (20 and 1.02 mg g⁻¹, respectively) and SB+CS+CM (2:2:1) (17.8 and 1.34 mg g⁻¹, respectively). The highest Mg contents were observed in the plants grown in the SB+CS+CM (2:2:1) (9.76 mg g⁻¹) and SB+FV+RH (1:2:2) (5.84 mg g⁻¹) substrates. The boron content exhibited the following values in decreasing order in the respective substrates: MP+RH (7:3) (142 mg kg⁻¹) > SB+CS+CM (2:2:1) (135.2 mg kg⁻¹) > SN (124.4 mg kg⁻¹) > SB+FV+RH(1:1:1) (117.6 mg kg⁻¹) > SB+FV+RH(1:2:2) (98.2 mg kg⁻¹) = SB+FV+CS (96 mg kg⁻¹).

The SN substrate resulted in the highest Fe content in the plant leaves (217 mg kg⁻¹) compared with the MP+RH (7:3) (127.4 mg kg⁻¹) and SB+FV+RH (1:2:2) (144.6 mg kg⁻¹) substrates; the mean Fe contents 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⁻¹), SB+CS+CM (2:2:1) (4.4 mg kg⁻¹) and SN (4.6 mg kg⁻¹) 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⁻¹) and SN (341.4 mg kg⁻¹) substrates. The highest Zn contents were measured in the plants grown in SB+FV+CS (1:3:6) (18.2 mg kg⁻¹) and SB+CS+CM (2:2:1) (15.4 mg kg⁻¹), whereas the latter did not differ from MP+RH (7:3) and SB+FV+RH (1:2:2); a value of 12.2 mg kg⁻¹ was measured in the plants grown in these two substrates (Table 9).

The correlation analysis between the leaf mineral nutrient contents and gas exchange, chlorophyll *a* fluorescence, biometrics, and quality of *Eugenia dysenterica* seedlings presented in Table 10 shows 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 ϕ 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*, ϕ PSII, ETR, SL, CD, NL, SDW, LDW, and TDW.

Among the macronutrients, K exhibited the lowest number of correlations, i.e., 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 , ϕ PSII, ETR, SL, CD, NL, SDW, LDW, TDW, and SL/CD and a negative correlation with R/S. Mg and S exhibited positive correlations with A , E , g_s , F_v/F_m , ϕ 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 evaluated that exhibited only negative correlations, with the following: A , E , g_s , F_v/F_m , and RDW. Cu 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 , ϕ PSII, ETR, SL, CD, NL, SDW, LDW, TDW, and SL/CD (Table 10).

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. Rio Verde, 2016.

| | 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 ^r | 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; ^zNet CO₂ assimilation; ^yTranspiration; ^xStomatal conductance; ^w*Ci/Ca* ratio; ^vMaximum quantum yield of PSII; ^uEffective quantum yield of PSII; ^tElectron transport rate (ETR); ^sStem length; ^rCollar diameter; ^qNumber of leaves; ^pStem dry weight; ^oLeaf dry weight; ⁿRoot dry weight; ^mTotal dry weight; ^lRatio between stem length and diameter; ^kRatio between root and shoot dry weight; ^jDickson quality index

1.3 - Discussion

The high seedling emergence rate of 100% in three treatments and above 95% in five treatments (Figure 2 and Table 2) has not been previously reported for *Eugenia dysenterica* plants. Souza et al. (2001) reported 81% seedling emergence, a rate lower than most of the treatments in the present study and only similar to that of the soil collected from around the parent plants (SN). Removing the seed coat that surrounds the *cagaiteira* seeds, which may contain substances that inhibit germination or hinder water absorption or gas exchange, may have helped 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 smaller amount of subsoil in the substrates led to an increase in the relative substrate moisture content (RMC_S) (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 capacity. 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.

Despite the differences in gas exchange between the plants, they remained unaffected by RMC_S and RMC_L , as indicated by the lack of correlation (data not shown). The low stomatal conductance (g_s) of the plants grown in SN resulted in the lowest transpiration (E) and net CO_2 assimilation (A) values because stomatal opening regulates CO_2 and water entry and exit into/from leaves. 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 phase of the photosynthesis (Table 4).

In this experiment, did not had water restriction, the observed RMC_L values indicate that water restriction occurred due to damage to the root system, as observed in Figure 2, where death occurred in the main root apex and adventitious roots were reduced. The cause of death is assumed to be lack of oxygen, considering that the structure of the soil used in the SN substrate was destroyed when preparing the material to fill the tubes, thereby leading to a lack of macropores, which enable gas exchange between the root system and the atmosphere.

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) and 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 $12 \mu\text{mol m}^{-2} \text{ s}^{-1}$, 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. 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 ($122.61 \mu\text{mol m}^{-2} \text{ s}^{-1}$) was lower than that observed by Lemos-Filho (2000) during the rainy season, which was $160 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Table 5).

When comparing, the value observed by the author during the dry season ($90 \mu\text{mol m}^{-2} \text{ s}^{-1}$), 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) in the plants grown in the NS (0.70) indicates the occurrence of photoinhibitory damages. However, F_v / F_m value of 0.77 obtained in SB+FV+CS (1:3:6) and SB+CS+CM (2:2:1) did not indicate such damage according to a study by Lemos-Filho (2000) during dry and rainy seasons, respectively.

The ϕPSII 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 a study on *Eugenia uniflora*, Neves et al. (2009) recorded an F_v/F_m value around 0.7 in leaves under actinic light of $1,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$, 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 seedling 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), among various others.

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*, *gs*, *Fv/Fm* 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 & Zeigher, 2013).

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 ϕ 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^{-1} , Table 8) was lower than that reported by Souza et al. (2001), (6.0 cm mm^{-1}), which indicates that the seedlings in the present study had a higher quality, i.e., lower etiolation. 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, the 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 ϕPSII indicates that the plants were not etiolated and that an increased SL/CD resulted in increases in these characteristics (Table 7).

The higher R/S ratio 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, i.e., 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.

A higher DQI should represent a seedling with higher quality (Hunt, 1990), and the DQI for conifers should exceed 0.20. 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.

The SB+CS+CM (2:2:1) substrate resulted in the highest leaf levels of N, P, Ca, Mg, S, Zn, and Fe, i.e., the greatest number of mineral elements at high concentration in leaves compared with the other substrates (Table 9). As a consequence of this high mineral content, this substrate also resulted in the highest values for gas exchange, 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) and can not be used to measure efficient in seedling quality. Another substrate that exhibited high mineral element levels was SB+FV+CS (1:3:6), which similar to SB+CS+CM (2:2:1),

resulted in good seedling growth, gas exchange, and fluorescence. However, the SN substrate exhibited high levels of only Fe and Mn, and these elements did not exhibit any correlations, or exhibited negative correlations, such as in the case of Fe, indicating possible plant toxicity (Table 8).

When comparing the leaf levels of N, P, K, Ca, Mg, and S observed in the present study with those obtained by Melo and Haridasan (2009), only the SB+FV+CS (1:3:6) and SB+CS+CM (2:2:1) substrates exhibited higher levels than those found by these authors. 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.

The positive correlations found in the present study between mineral nutrients and analysis as biometric, gas exchange, and chlorophyll *a* fluorescence characteristics 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 possible phytotoxicity.

Finally, it was showed that the correlations between the mineral nutrients and the other characteristics indicate that good mineral nutrition is necessary for the seedlings and that the use of agricultural correctives may be required. 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*.

1.4 - 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 (17° 48' 16'' S, 50° 54' 19'' W, altitude of 753 m), during the 2011/2012 crop season. Parental plants of *Eugenia dysenterica* DC. were grown at the Gameleira farm (19°53'S, 44°25'W, altitude of 749 m), Montes Claros of Goiás, Goiás (GO), Brazil. Fruits were collected from plants in full production, after removing the pulp from the seeds, the seed coat was completely removed using a scalpel; care was taken to avoid damaging the other seed tissues to ensure germination.

The following components were used to formulate the substrates: MecPlant® (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 (Figure 1). Cartridges (288 cm³) were used as containers to provide support for the substrates. One seed was sown per cartridge. 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 6 mm at each interval.

Chemical analysis of the substrates

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).

Evaluation of percent seedling emergence (PSE)

Percent seedling emergence (PSE) was determined at two-day intervals after the first seedling emerged. Emergence speed index (ESI) was obtained according to Maguire (1962).

Relative moisture content (RMC)

The relative leaf moisture content (RMC_L) and relative substrate moisture content (dry basis) (RMC_S) were recorded before dawn. To determine RMC_L, 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). RMC_L was calculated using the following equation: $RMC_L = (FW - DW) / (TW - DW)$ according to Weatherley (1950).

To determine RMC_S, substrate samples from the tubes that contained the plants used to determine RMC_L were collected. 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_S : $RMC_S = (WW-DW)/DW$. These analyses were performed 127 days after sowing the seeds.

Physiological measurements

The gas exchange parameters evaluated was CO_2 assimilation (A , $\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$); transpiration (E , $\text{mmol m}^{-2} \text{ s}^{-1}$); stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$); and the ratio between the intercellular and atmospheric CO_2 concentration (C_i/C_a , $\mu\text{mol } CO_2 \text{ mol}^{-1}$). 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 ($1,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$) throughout the entire experiment, in an open system and under a high environmental CO_2 concentration.

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

Morphological characteristics

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) was determined according to Dickson et al. (1960). All of the morphological characteristics were evaluated 127 days after sowing the seeds.

Analysis of tissue nutrient content

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 was in a block design with five replicates and 20 experimental units (tubes/plants) per replicate for PSE and ESI. Two plants were used per replicate for RMC_L and RMCs; A plant for physiological analysis; And five plants were used for the other parameters. Data were submitted to analysis of variance and Tukey's test. A significance probability level of 5% was used to discriminate significant differences between means. The Pearson correlation method was used to detect possible correlations between the characteristics analyzed. Statistical procedures were performed using SAEG 9.

1.5 - Conclusions

Eugenia dysenterica seedlings grown in rice husks exhibited inadequate root and shoot growth with lower aerial growth than root.

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 was efficient in promoting plant growth and meeting the nutritional requirements of the *E. dysenterica* seedlings.

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CATÍTULO II - Physiological and nutritional evaluation of *Eugenia dysenterica* DC seedlings grown in vermiculite and rice husk-based substrates

(Artigo formatado de acordo com as normas da Revista Brasileira de Fruticultura)

ABSTRACT - The aim of this study was to evaluate the growth, quality, and physiology of *E. dysenterica* seedlings grown in vermiculite (FV) and rice husk-based (RH) substrate with the following combinations: 1:0, 3:1, 1:1, and 1:3 ratios (v:v), beyond the commercial substrate Tri-Mix[®]. Was evaluated: percent seedling emergence (PSE), emergence speed index (ESI), gas exchange, chlorophyll *a* fluorescence, relative seedling water content, relative substrate moisture content, plant biometric growth characteristics, accumulated dry weight and the nutritional status of leaf macronutrient content 128 days after emergence. The increase in the proportion of rice husk mixed with vermiculite results in reduction of the water in the substrates. The FV substrate promotes the highest quality index of Dickson, as well as the greatest stem diameter of the plants. Higher content of N and Mg occurred in the leaves of the seedlings grown in the FV in relation to the seedlings of the mixtures of FV and RH and FV + RH 1: 3, respectively. Higher N content occurred in the leaves of the seedlings grown in Tri-Mix[®] and FV and Mg in FV and FV + RH, respectively. The substrates did not alter the physiological attributes of the seedlings.

Key words: Cagaita, gas exchange, chlorophyll *a*, fluorescence, index of quality.

RESUMO - O objetivo deste estudo foi avaliar o crescimento, qualidade e fisiologia de mudas de *E. dysenterica* cultivadas em substratos à base de vermiculita (VF) e casca de arroz (CA), com as seguintes combinações: proporções 1:0, 3:1, 1:1 e 1:3 (v:v), além do substrato comercial Tri-Mix[®]. Foi avaliada a porcentagem de emergência de plântulas (PPE), índice de velocidade de emergência (IVE), trocas gasosas, fluorescência da clorofila *a*, teor de água relativa das mudas, teor de umidade relativa do substrato, características de crescimento biométrico da planta, peso seco acumulado e teor nutricional de macronutrientes foliares aos 128 dias após a emergência. O aumento da proporção de casca de arroz misturada com vermiculita resulta na redução da água nos substratos. O substrato de VF promoveu o maior índice de qualidade de Dickson, bem como o maior diâmetro do caule das plantas. Maior teor de N e de Mg ocorreu nas folhas das mudas crescidas na VF em relação às mudas das misturas de VF e CA e VF+CA 1:3, respectivamente. Maior teor de N ocorreu nas folhas das mudas crescidas no Tri-Mix[®] e VF e Mg na VF e VF+CA, respectivamente. Os substratos não alteraram os atributos fisiológicos das mudas.

Palavras-chave: Cagaita, trocas gasosas, clorofila *a*, fluorescência, índice de qualidade.

2.1 – Introduction

The Cerrado (tropical savanna) is considered the second largest Brazilian domain and is home to a great diversity of plant species (PAIVA SOBRINHO et al., 2010). *Eugenia dysenterica* DC is a fruiting species endemic to the Cerrado (SOUZA et al., 2013), belongs to the Myrtaceae family and is popularly known as cagaiteira. The fruit of *E. dysenterica* is considered an important source of vitamins C and A (CARDOSO et al., 2011) and the extract of its leaves have shown anti-diarrheal activity, so that the plant offers promise as a new medication (LIMA et al., 2010).

The cagaita leaf extracts are also known for their antifungal properties (COSTA et al., 2000; SOUZA et al., 2012) have shown that the leaf extracts inhibit α -amylase and α -glycosidase activity, which can be important in controlling diabetes. Although the socioeconomic and ecologic importance of *E. dysenterica*, (BAILÃO et al., 2015), there are few studies on the maintenance of the species. The cultivation of native species through seedlings propagation can be an effective way to avoid biodiversity loss in the Cerrado.

The substrate where the roots develop is one of the factors that influence seedling quality through the ability to supply structural support for the seedlings and by serving as a source of water, oxygen, and nutrients. The production of seedlings can be an alternative to

the spread of *E. dysenterica*. The substrate choice can affect its morphophysiological characteristics because the substrate quality used is considered one of the main factors affecting seedling quality, and should exhibit adequate physical and chemical characteristics (FERRAZ et al., 2005).

The production of healthy, robust, well-nourished and high-quality seedlings is required for the propagation of woody plant species after transplantation in the field, resulting in successful establishment of fruit orchards and even reforested areas (GOMES et al., 2003). The substrate increases the rates of germination and emergence when it has porosity that allows the hydration and aeration of the seed, since it does not require nutrients to germinate and emerge, but water and oxygen to its metabolism (NOGUEIRA et al., 2003).

It is also interesting that the substrate is resistant to decomposition, as well as high cation exchange capacity, absence of pathogens and undesirable plant seeds, and are available in the market at affordable prices (DANTAS et al., 2009). To obtain these characteristics is often necessary mix two or more components, which together will form a suitable substrate for the formation of seedlings (ARAÚJO NETO et al., 2009).

The use of such waste for seedling production may be a feasible alternative because large volumes of these waste products are generated and create environmental problems when not disposed of adequately. Single materials and combinations of different material types are used for seedling production, and these can be produced in a greenhouse or acquired from specialized companies. The use of expanded vermiculite and rice husks is common in the production of seedlings of forest species (SILVA et al., 2012; DELAMERLINA et al., 2014). However, the use of these components in substrates for seedling production of Cerrado native species is still incipient.

Biometric parameters that consider the balance between the root and shoot weights, measurements that provide a robustness quotient, indexes, like IQD, which consider seedling total dry weight, root to shoot ratio, and stem length/stem diameter (SILVEIRA et al., 2013). Measures of gas exchange and chlorophyll a fluorescence are potential indicators of seedling quality and of stress (BARALDI et al., 2008; NEVES et al., 2009). Thus, to contribute to information on the seedling production and quality of native Cerrado species, the main purpose of this study were to evaluate the growth, quality, mineral content in leaves and physiology of *Eugenia dysenterica* DC. seedlings produced in cartridges using vermiculite and rice husk-based substrates.

2.2 - Material and methods

The *E. dysenterica* DC fruits were harvested from healthy adult plants, in full production, at Gameleira Farm located in Montes Claros de Goiás, GO, Brazil. The fruits were transported to the Laboratory of Plant Tissue Culture (LCTV) of the Goiano Federal Institute, Campus Rio Verde, where were pulped and the seed coats were removed to facilitate germination. The study was conducted in a greenhouse at the LCTV. Temperature and relative humidity inside the greenhouse were recorded using a data logger. The mean temperature and relative humidity were 25.5°C and 76%, respectively.

Substrates were formulated based on fine vermiculite (FV), carbonized rice husks (RH), and Tri-mix[®] (comprising FV, carbonized RH and coconut fiber, according to the manufacturer's information), by testing the Trimix[®], FV and the FV+RH at 3:1, 1:1, and 1:3 ratios (v/v), whose chemical characteristics are shown in Table 1.

Table 1. The chemical analysis of macro and micronutrients of the substrates. Rio Verde, 2016.

| Substrate | pH H ₂ O | V % | Ca | | Mg | |
|--------------------------|------------------------|--------|-----------------------|------|-----------------------|--|
| | | | cmol dm ⁻³ | | cmol dm ⁻³ | |
| Tri-mix [®] | 6.42 | 79.0 | 0.33 | 2.92 | | |
| FV ^x | 6.76 | 88.9 | 0.20 | 7.76 | | |
| FV+RH ^w (3:1) | 6.41 | 86.6 | 0.34 | 7.69 | | |
| FV+RH (1:1) | 6.79 | 83.6 | 0.21 | 6.10 | | |
| FV+RH (1:3) | 6.79 | 71.0 | 0.26 | 3.49 | | |

| Substrate | P | K | Na | Zn | Fe | Mn | Cu | B |
|--------------------------|------|-----|-------|------|-------|------|------|------|
| | | | | | | | | |
| Tri-mix [®] | 2.8 | 447 | 114.8 | 1.49 | 145.1 | 14.7 | 0.65 | 0.29 |
| FV ^x | 9.9 | 11 | 2.1 | 0.42 | 65.7 | 7.2 | 0.43 | 0.12 |
| FV+RH ^w (3:1) | 5.8 | 125 | 10.2 | 0.92 | 107.5 | 15.5 | 0.56 | 0.14 |
| FV+RH (1:1) | 8.1 | 119 | 6.1 | 0.97 | 88.0 | 12.8 | 0.45 | 0.22 |
| FV+RH (1:3) | 15.4 | 227 | 16.2 | 8.47 | 80.9 | 17.4 | 0.33 | 0.45 |

^xFine-grained vermiculite; ^wRice husks.

Cartridges of 288 cm³ were filled with the substrates, in which one seed were sowed for germination. The spray irrigation was 12 mm day⁻¹, divided into two irrigations diary. Percent seedling emergence (PSE) was tested at two day intervals between emergence of the first seedling and the end of emergence; the emergence speed index (ESI) was calculated according to Maguire (1962).

Seedling gas exchange, fluorescence, growth, quality, and water content, as well as substrate moisture content, were evaluated at 128 days after the seeding. Gas exchange was evaluated in the morning between 7:30 AM and 11:30 AM with an LCi portable

photosynthesis meter (ADC BioScientific, Great Amwell, United Kingdom), yielding the following variables: net CO₂ assimilation (A), transpiration (E), water use efficiency (A/E) obtained from the ratio between A and E , and stomatal conductance (g_s).

The chlorophyll a fluorescence variables were obtained using a Mini-PAM modulated fluorometer (Walz, Effeltrich, Germany). The maximum quantum yield of photosystem II (PSII) (F_v/F_m) was calculated using the darkness parameters with the equation, $F_v / F_m = (F_m - F_0) / F_m$, where F_0 and F_m are the minimum and maximum fluorescence, respectively, of the dark-adapted plant tissue obtained before dawn. F_0 was obtained by lighting the plant tissue with a low-intensity modulated red light ($0.03 \mu\text{mol m}^{-2} \text{s}^{-1}$), and F_m was obtained with a saturating light pulse ($6,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) lasting 0.8 seconds. The effective quantum yield of PSII (ϕPSII) was calculated using the equation $\phi\text{PSII} = (F_m' - F) / F_m'$, on what F_m' and F are the maximum fluorescence and fluorescence, respectively, when the plant tissue is under actinic light with an intensity of $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 50 seconds. The quantum yield of non-photochemical energy dissipation of PSII (ϕNPQ) and the quantum yield of unregulated non-photochemical energy dissipation of PSII (ϕNO) were obtained according to Hendrickson et al. (2004). The apparent electron transport rate of PSII (ETR) was obtained from Bilger et al. (1995).

The relative water content (RWC) and substrate moisture content were measured before dawn at 4:30 am. To determine RWC, one leaf was collected per plant using a scalpel and was then immediately weighed on an analytical balance to obtain the fresh weight (FW). After obtaining the FW, the leaves were placed in a humid chamber, and the petioles were immersed in distilled water, remaining under these conditions for 24 hours at 25°C, with compensation irradiance, to allow the leaves to reach maximum turgor.

After the leaves reached maximum turgor, they were weighed to obtain the turgid weight (TW) and were then dried in a forced-air oven at 65°C to constant weight, yielding the dry weight (DW). The RWC was calculated using the equation $\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW})$ according to Weatherley (1950).

To determine the relative moisture of the substrates studied, substrate samples were collected from the same cartridges containing the plants used for determining the RWC. Immediately after collecting the substrates, they were weighed on a semi-analytical balance to obtain wet weight (WW) and then dried in a forced-air oven at 105°C to constant weight, yielding the substrate dry weight (DWs). To obtain the relative moisture

content of the substrate (RMC) on a dry basis, the equation $RMC = (WW - DWs) / DWs$ was used.

After 128 days of seeding, were measured diameter stem diameter (SD) and count the number of seedlings leaves (NL), which were separated in stems, leaves, and roots. Was obtained the stem length (SL) and the leaf area (LA). The stems, leaves, and roots were dried in a forced-air oven at 65°C to constant weight, to obtain weights separately. The ratio between the SL and SD (SL/SD), the ratio between the root dry weight and shoot dry weight (R/S), and the Dickson quality index (DQI) were used as seedling quality parameters according to Dickson et al. (1960). Leaves from the plants of each replicate were combined and ground in a Willey mill to determine the macronutrient content according to Malavolta et al. (1997).

The experiment was a randomized block design with five replicates and 20 experimental units (tubes/plants) per replicate for PPE and IVE. Two plants (tubes/plants) were used per repetition for RMC and RWC; A plant for physiological analysis; And five plants were used for the other parameters. Data were submitted to analysis of variance and Tukey's test. A significance probability level of 5% was used to discriminate the minimum significant differences between the means. The Pearson correlation method was used to detect possible correlations between the characteristics analyzed. Statistical procedures were performed using SAEG 9.1.

2.3 - Results and discussion

The seedling emergence percentage (PSE) of *E. dysenterica* had no differences between the substrates, with an average emergence value of 99%, expressive value in woody plants. Souza et al. (2001) reported emergence values of 80.6% in *E. dysenterica* seedlings grown in different substrates. The emergence speed index (ESI) was lower in the substrate containing only vermiculite (0.30) compared to Tri-Mix[®] (0.34) (Table 2).

The vermiculite mineral substrate has some interesting characteristics such as slightly acidic pH, Mg and P contents, high water absorption and low density. However, vermiculite is more efficient when it is combined with materials containing organic matter, such as carbonized rice husks, increasing the cation exchange capacity, emergence, growth and quality of the seedlings (SANTOS et al., 2014; (SOUZA et al., 2015).

Table 2. Percent seedling emergence (PSE) and emergence speed index (ESI) in *Eugenia dysenterica* DC seedlings as a function of the different proportions of vermiculite and rice husks. Rio Verde, 2016.

| Substrate | PSE | ESI |
|--------------------------|-------------------|---------------------|
| | % | |
| Tri-Mix [®] | 100 ^{ns} | 0.34 a ^z |
| FV ^x | 96 | 0.30 b |
| FV+RH ^w (3:1) | 98 | 0.32 ab |
| FV+RH(1:1) | 100 | 0.32 ab |
| FV+RH(1:3) | 100 | 0.33 ab |
| MSD ^v | 5.65 | 0.033 |

^zMeans followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$); ^xFine-grained vermiculite; ^wRice husks; ^vMinimum significant difference; ^{ns}Means do not differ according to Tukey's test ($p > 0.05$).

According to Guerrini & Trigueiro (2004), adding carbonized rice husk to other materials is important for better physical structuring of the substrate, as it is a light material inert to hydration and is able to increase substrate porosity proportionally to its percentage in the mixture. The ESIs found in the present study were higher than those found by Nietzsche et al. (2004) of 0.08 and 0.1 for small and large cagaita seeds grown in different substrates, respectively. However, those authors did not report removing the coats from the seeds, a procedure that is believed to reduce the time to germination and emergence.

The substrate comprising only vermiculite was able to accumulate a higher amount of water and exhibited the highest moisture content on a dry basis before dawn compared to the two substrates with a higher percentage of rice husks, this is, FV+RH 1:1 and 1:3 ratios (Table 3).

Table 3. Relative moisture content (RMC) of the substrates on a dry basis and leaf relative water content (RWC) in *Eugenia dysenterica* DC seedlings as a function of the different proportions of vermiculite and rice husks. Rio Verde, 2016.

| Substrate | RMC | RWC |
|--------------------------|-------------------------------|--------------------|
| | ----- g g ⁻¹ ----- | |
| Tri-mix [®] | 2.69 ab ^z | 0.97 ^{ns} |
| FV ^x | 3.49 a | 0.97 |
| FV+RH ^w (3:1) | 2.99 ab | 0.96 |
| FV+RH (1:1) | 2.17 bc | 0.97 |
| FV+RH (1:3) | 1,49 c | 0.96 |
| MSD ^v | 0.84 | 0.040 |

^zMeans followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$); ^xFine-grained vermiculite; ^wRice husks; ^vMinimum significant difference; ^{ns}Means do not differ according to Tukey's test ($p > 0.05$).

In this case, the occurrence of lower water retention values is associated with the higher amount of macropores inherent to the carbonized rice husk. However, leaf relative

water content remained unaffected by the substrates, indicating that all the substrates have the ability to adequately provide water to the plants. The similarity in leaf relative water content of the plants grown in the different substrates tested suggests that they were under the same water conditions and that the differences in the substrates' moisture levels did not affect the water availability to the plants.

The net carbon assimilation (A), transpiration (E), water use efficiency (A/E), and stomatal conductance (g_s) parameters remained unaffected by the substrates used. The A , E , A/E , and g_s values were on average $4.36 \mu\text{mol m}^{-2} \text{s}^{-1}$, $1.52 \text{mmol m}^{-2} \text{s}^{-1}$, $2.85 \mu\text{mol mmol}^{-1}$, and $0.082 \text{mol m}^{-2} \text{s}^{-1}$, respectively (Table 4). Therefore, the different substrates were unable to interfere in the biochemical and physiological aspects of the photosynthetic apparatus of the cagaita seedlings. Chlorophyll a fluorescence variables remained unaffected by the substrates tested, exhibiting a mean of 219 for minimum fluorescence (F_0).

Table 4. Net CO_2 assimilation rate (A), transpiration (E), water use efficiency (A/E), and stomatal conductance (g_s) in *Eugenia dysenterica* DC seedlings as a function of different proportions of vermiculite and rice husks. Rio Verde, 2016.

| Substrate | A $\mu\text{mol m}^{-2} \text{s}^{-1}$ | E $\text{mmol m}^{-2} \text{s}^{-1}$ | AE $\mu\text{mol mmol}^{-1}$ | g_s $\text{mol m}^{-2} \text{s}^{-1}$ |
|--------------------------|---|---|-----------------------------------|--|
| Tri-mix [®] | 3.94 ^{ns} | 1.74 ^{ns} | 2.46 ^{ns} | 0.08 ^{ns} |
| FV ^x | 4.92 | 1.85 | 2.93 | 0.12 |
| FV+RH ^w (3:1) | 2.89 | 0.91 | 3.29 | 0.05 |
| FV+RH (1:1) | 4.91 | 1.83 | 2.73 | 0.12 |
| FV+RH (1:3) | 4.12 | 1.59 | 2.83 | 0.08 |
| MSD ^v | 2.92 | 1.18 | 1.44 | 0.108 |

^{ns}Means do not differ in the column according to Tukey's test ($p < 0.05$); ^xFine-grained vermiculite; ^wRice husks; ^vMinimum significant difference.

The mean maximum quantum yield of PSII (F_v/F_m), effective quantum yield of PSII (ϕ_{PSII}), regulated non-photochemical energy dissipation (ϕ_{NPQ}), and unregulated non-photochemical energy dissipation of PSII (ϕ_{NO}) values were 0.74, 0.227, 0.733, and 0.340, respectively. The effective quantum yield of PSII (ϕ_{PSII}) was lower than the ϕ_{NPQ} and ϕ_{NO} . Therefore, the photosynthetic yield, i.e., the amount of the energy absorbed by chlorophyll that is directed to the generation of reducing power (ATP and NADPH), was lower than the regulated and unregulated non-photochemical energy dissipation of PSII (Table 5).

Table 5. Maximum quantum yield of PSII (F_v/F_m), effective quantum yield of PSII (ϕ_{PSII}), quantum yield of regulated non-photochemical energy dissipation (ϕ_{NPQ}), quantum yield of unregulated non-photochemical energy dissipation in the PSII (ϕ_{NO}), and electron transport rate (ETR) in *Eugenia dysenterica* DC seedlings in relation to different proportions of vermiculite and rice husks. Rio Verde, 2016.

| Substrate | F_v/F_m | ϕ_{PSII} | ϕ_{NPQ} | ϕ_{NO} | ETR $\mu\text{mol m}^{-2} \text{s}^{-1}$ |
|--------------------------|--------------------|--------------------|--------------------|--------------------|---|
| Tri-mix [®] | 0.73 ^{ns} | 0.24 ^{ns} | 0.44 ^{ns} | 0.32 ^{ns} | 97.60 ^{ns} |
| FV ^x | 0.68 | 0.19 | 0.48 | 0.33 | 76.14 |
| FV+RH ^w (3:1) | 0.69 | 0.20 | 0.43 | 0.37 | 77.47 |
| FV+RH (1:1) | 0.73 | 0.31 | 0.34 | 0.35 | 118.31 |
| FV+RH (1:3) | 0.73 | 0.27 | 0.40 | 0.33 | 107.85 |
| MSD ^y | 0.113 | 0.151 | 0.212 | 0.082 | 60.44 |

^{ns}Means do not differ in the column according to Tukey's test ($p>0.05$); ^yFine-grained vermiculite; ^xRice husks; ^wMinimum significant difference.

This indicates the existence of photoinhibition, most likely caused by some stress factor(s), because the high ϕ_{NPQ} indicates dissipation of energy absorbed in the form of heat by the xanthophyll cycle (BARALDI et al., 2008).

The substrates did not affect the electron transport rate (ETR), exhibiting a mean of 86.63 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Lemos-Filho (2000) found ETR of approximately 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the dry season and approximately 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the rainy season for tree seedling of Cerrado, including cagaita. The stomatal conductance values (g_s) found in this study are lower than those observed by Lemos-Filho (2000), which found g_s of 0.26 $\text{mol m}^{-2} \text{s}^{-1}$ in plants under field conditions during the rainy season. Neves et al. (2009) found a g_s of 0.14 $\text{mol m}^{-2} \text{s}^{-1}$, A of 12 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and E of 1.2 $\text{mmol m}^{-2} \text{s}^{-1}$ in young *E. uniflora* plants grown hydroponically, i.e., under optimal nutritional and water conditions. Water use efficiency (A/E) in the present study was 2.85 μmol of CO_2 mmol^{-1} of H_2O , i.e., for each mol of CO_2 fixed, 351 mol of H_2O were transpired, which is an intermediate value between C4 and C3 plants (BACON, 2004). The F_v/F_m values being lower than 0.8, is indicative of stress (Neves et al., 2009). In the current study, the F_v/F_m values were less than 0.73, suggesting the presence of at least one stress factor.

For the growth characteristics evaluated, just stem diameter (SD) was affected by the substrates. FV exhibited higher seedlings with SD compared to FV+RH (1:3), with values of 1.06 and 0.92 mm, respectively; the other substrates did not differ from these. The stem length (SL), number of leaves (NL), and leaf area (LA) exhibited mean values of 2.51 cm, 2.2 leaves, and 14.7 cm^2 , respectively. Leaf, stem, root, and total

accumulated dry weights were also unaffected by the substrates, exhibiting means of 0.12, 0.018, 0.44, and 0.59 g, respectively (Table 6).

The lack of differences in the biometric characteristics of the *E. dysenterica* seedlings as a function of the substrates may also be due to the lack of differences in gas exchange. Although low, the SL and SD values were similar to those found by Melo & Haridasan (2009) in *E. dysenterica* at a similar age; however, the SL and NL values found were lower than those observed by Nietsche et al. (2004).

Table 6. Stem length (*SL*); stem diameter (*SD*); number of leaves (*NL*); leaf area (*LA*); and stem (*SDW*), leaf (*LDW*), root (*RDW*), and total dry weights (*TDW*) in *Eugenia dysenterica* DC seedlings as a function of the different proportions of vermiculite and rice husks. Rio Verde, 2016.

| Substrate | <i>SL</i> (cm) | <i>SD</i> (mm) | <i>NL</i> | <i>LA</i> (cm ²) | <i>SDW</i> | <i>LDW</i> | <i>RDW</i> | <i>TDW</i> |
|--------------------------|--------------------|----------------------|--------------------|---------------------------------|---------------------|---------------------|---------------------|---------------------|
| | | | | | ----- g ----- | | | |
| Tri-mix [®] | 2.42 ^{ns} | 1.00 ab ^z | 2.20 ^{ns} | 15.63 ^{ns} | 0.018 ^{ns} | 0.140 ^{ns} | 0.449 ^{ns} | 0.639 ^{ns} |
| FV ^x | 2.46 | 1.06 a | 2.24 | 15.87 | 0.019 | 0.129 | 0.491 | 0.607 |
| FV+RH ^w (3:1) | 2.52 | 0.98 ab | 2.36 | 14.52 | 0.018 | 0.130 | 0.419 | 0.591 |
| FV+RH (1:1) | 2.69 | 0.96 ab | 2.16 | 15.51 | 0.020 | 0.128 | 0.444 | 0.567 |
| FV+RH (1:3) | 2.50 | 0.92 b | 2.12 | 12.20 | 0.018 | 0.101 | 0.429 | 0.547 |
| MSD ^v | 0.504 | 0.106 | 0.467 | 5.101 | 0.007 | 0.058 | 0.092 | 0.110 |

^zMeans followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$); ^xFine-grained vermiculite; ^wRice husks; ^vMinimum significant difference; ^{ns}Means do not differ according to Tukey's test ($p < 0.05$).

The identification of the accumulated dry mass was a notable feature, and the root system accounted for 70% to 80% of the accumulated MST. According to Silveira et al. (2013), *E. dysenterica* commonly prioritizes root system development to the detriment of the shoot, and the initial growth of the shoot is slow, which corroborates the resulting of the present study.

Regarding the indices used to evaluate *E. dysenterica* seedling quality, only the DQI was affected by the substrates, and the pure vermiculite led to higher values compared to the substrates comprising vermiculite mixed with rice husk at all ratios. Tri-Mix[®] yielded a DQI similar to the other substrates used for *E. dysenterica* seedling production. The other quality indices, such as the ratio between stem length and stem diameter (SL/SD), ratio between stem length and shoot dry weight (SL/SD), and ratio between root dry weight and shoot dry weight (R/S), exhibited means of 3.09 cm mm⁻¹, 19.48 cm g⁻¹, and 2.76 g g⁻¹, respectively (Table 7).

The SL /SD ratio found was lower than that observed by Souza et al. (2001), who reported values close to 6.0 cm mm⁻¹, suggesting that the plants of the present study have higher quality because they exhibited lower etiolation; however, further studies are necessary to support this assumption. Another quality index, the R/S ratio, indicates that the substrates tested provided higher photoassimilate allocation to the root system, a characteristic usually exhibited by plants under water or nutritional stress.

It is important to verify the type of container used in each work, substrate qualities, growth environment and evaluation time of the seedlings. This type of photoassimilation may be characteristic of this species, prioritizing the development of the root system. However, these values may vary with the nutrient contents of the substrate, since higher concentrations of nutrients decrease the R / PA ratio (MELO & HARIDASAN 2009). Characteristic also observed by Paiva Sobrinho et al. (2010) found values for R / PA, close to 1.4, but the Ca and P contents were higher than the present work.

Table 7. Ratio between stem length (*SL*) and stem diameter (*SL/SD*), ratio between root and shoot dry weight (*R/S*), and Dickson quality index (*DQI*) in *Eugenia dysenterica* DC seedlings as a function of the different proportions of vermiculite and rice husks. Rio Verde, 2016.

| Substrate | <i>SL/SD</i> (cm mm ⁻¹) | <i>R/S</i> (g g ⁻¹) | <i>DQI</i> |
|--------------------------|--|------------------------------------|----------------------|
| Tri-mix [®] | 2.49 ^{ns} | 3.51 ^{ns} | 0.22 ab ^z |
| FV ^x | 2.37 | 3.56 | 0.25 a |
| FV+RH ^w (3:1) | 2.58 | 3.34 | 0.20 b |
| FV+RH(1:1) | 2.81 | 3.19 | 0.20 b |
| FV+RH(1:3) | 2.76 | 3.89 | 0.19 b |
| MSD ^v | 0.55 | 0.88 | 0.054 |

^zMeans followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$); ^xFine-grained vermiculite; ^wRice husks; ^vMinimum significant difference; ^{ns}Means do not differ according to Tukey's test ($p > 0.05$).

The P, Ca, and S levels in the leaves did not differ among the substrates tested, exhibiting means of 1.0, 15.9, and 0.6 mg g⁻¹, respectively (Table 8). The leaf Mg levels only differed between the Tri-mix[®] and FV substrates, 7.9 and 12.0 mg g⁻¹, respectively.

Table 8. Macronutrients in leaf tissue of *Eugenia dysenterica* DC seedlings as a function of the different proportions of vermiculite and rice husks. Rio Verde, 2016.

| Substrate | N | P | K | Ca | Mg | S |
|--------------------------|--------------------------------|--------------------|-------|--------------------|--------|--------------------|
| | ----- mg g ⁻¹ ----- | | | | | |
| Tri-mix [®] | 17.0ab ^z | 1.08 ^{ns} | 11.0a | 18.4 ^{ns} | 7.9b | 0.44 ^{ns} |
| FV ^x | 17.6a | 1.12 | 2.4d | 18.9 | 12.0a | 0.74 |
| FV+RH ^w (3:1) | 16.0b | 0.96 | 3.8cd | 10.4 | 9.4ab | 0.52 |
| FV+RH(1:1) | 10.8c | 0.88 | 5.4bc | 12.2 | 10.3ab | 0.60 |
| FV+RH(1:3) | 16.0b | 0.96 | 7.2b | 19.4 | 9.4ab | 0.62 |
| MSD ^y | 1.41 | 0.28 | 2.99 | 11.57 | 4.08 | 0.42 |

^zMeans followed by the same letter in the column do not differ according to Tukey's test ($p < 0.05$); ^xFine-grained vermiculite; ^wRice husks; ^yMinimum significant difference; ^{ns}Means do not differ according to Tukey's test ($p > 0.05$).

The FV substrate exhibited the highest leaf N content 17.6 mg g⁻¹ relative to FV+RH. Regarding the K leaf content, higher variation was observed (Table 8). Tri-mix[®] exhibited the highest value (11.0 mg g⁻¹), followed by FV+RH (1:3) with a value of 7.2 mg g⁻¹; the lowest value was found in FV (2.4 mg g⁻¹); and the other substrates had values intermediate between FV and FV+RH (1:3), with no significant differences. Melo & Haridasan (2009) found leaf N, P, K, Ca, and Mg contents of 15.0, 1.3, 6.8, 16.9, and 3.1 mg g⁻¹, respectively, for the highest doses of each nutrient in the soil. The S leaf content was 0.4 mg g⁻¹ at all the doses, and the measurements were made at 345 days after sowing; the values found by this authors for N, Ca, S, and P are similar to those found in the present study. The mean leaf K content was similar, but ranged from 2.4 to 11.0 in the present study in the FV and Tri-mix[®] substrates, respectively. The leaf K content increased according to increasing proportion of rice husk, indicating that rice husk can be a source of K for the plants. The leaf Mg content was approximately four times higher than that found by Melo & Haridasan (2009).

2.4 - CONCLUSION

The increase in the proportion of rice husk mixed with vermiculite results in reduction of the water in the substrates.

The fine vermiculite substrate promotes the highest quality index of Dickson, as well as the greatest stem diameter of the plants.

Higher content of N and Mg occurred in the leaves of the seedlings grown in the VF in relation to the seedlings of the mixtures of FV and RH and FV+RH 1:3, respectively.

The substrates did not alter the physiological attributes of the seedlings.

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CATÍTULO III - CORRELATION BETWEEN PHYSIOLOGICAL AND BIOMETRIC ANALYSES FOR EVALUATING *EUGENIA DYSENTERICA* SEEDLINGS GROWN IN ALTERNATIVE SUBSTRATES BASED ON ORGANIC WASTE FROM THE AGRICULTURAL INDUSTRY

(Artigo formatado de acordo com as normas da Revista New Forest)

Abstract: The objective of this study was to evaluate the physiology, nutrition and quality of *Eugenia dysenterica* DC. seedlings that were grown on substrates derived from the organic waste of agricultural industries. The wastes used here were as follows: rice husk (RH); fermented cattle manure (FCM); cattle manure compost (CMC), which contained corn silage and FCM; sugarcane bagasse (SCB); filter cake from sugar-alcohol mills (FC); and subsoil (SB). Four substrates were formulated from the wastes, namely SB+RH (1:1; v:v), SB+CMC (1:1), SB+FCM (3:1) and SCB+FC (3:2). Bioplant[®] substrate was also used. The following characteristics were evaluated: the emergence and vigor percentages, biometric characteristics, seedling quality indices, gas exchange, chlorophyll *a* fluorescence and leaf nutrient levels. The seedling emergence was similar for all the substrates. Of the alternative substrates, the best vigor was found in seedlings that were cultivated in SCB+FC, which was equal to the vigor observed in Bioplant[®]. In general, the use of the SB+CMC substrate gave better seedling results for the biometric and nutritional characteristics, followed by SCB+FC. Most of the biometric characteristics showed a correlation with photosynthesis, electron transport rate (ETR) and effective quantum yield of photosystem II, thus showing that these characteristics can be alternatives to the traditional quality indexes used for seedlings. The use of CMC, SCB and FC was shown to be appropriate for the production and nutritional supply of *E. dysenterica* seedlings.

Key words: Cagaita tree (*Eugenia dysenterica* Mart.); Seedling quality; Mineral nutrition; Gas exchanges; Chlorophyll *a* fluorescence; Seedling physiology.

3.1 – Introduction

Various native fruit species of the Cerrado region produce nutraceutical fruits with peculiar flavor characteristics, and they have important roles related to their consumption and nutritional properties. *Eugenia dysenterica* DC. is among these fruits, which is a species in the Myrtaceae family and a native fruit of the Cerrado with high socioeconomic value. The fruit is consumed in whole form or processed into sweet items and jams, and it is also used in ice creams, popsicles and beverages. This fruit is considered to be an important source of vitamins C and A (Cardoso et al., 2011). Souza et al. (2012) reported the high inhibitory activity of *E. dysenterica* leaf extracts on α -amylase and α -glucosidase, with the latter contributing to the control of diabetes.

The correct, rational use of this species must be achieved through the establishment of commercial orchards because this species' current exploitation is performed in an extractive manner. Thus, in view of the importance of the native fruit of the Cerrado, studies that aim to propagate this species should be conducted. The propagation of woody plant species is achieved by producing high quality seedlings; that is, healthy, well-nourished and robust plants. These characteristics contribute to the survival of the plants after transplantation to the field, thus resulting in the success of orchard installations, even in reforestation situations (Rosa et al., 2005).

The quality indices that are used to evaluate the seedlings are ratios, such as the one between the lengths and diameters of the stem, between the dry weights (DWs) of the root system and the shoot and other possible ratios that indicate the robustness of the seedlings. Dickson et al. (1960) created a quality index that considers the ratios mentioned above as well as the accumulation of total DWs. Additionally, analyses of physiological parameters have been used to complement the growth and development parameters of native seedlings from the Cerrado (Mota et al., 2016). Nevertheless, in addition to the quality indices of the seedlings, it is important to consider their nutritional status and photosynthetic capacity.

The starting point for obtaining quality seedlings lies in using combinations of substrates with appropriate chemical and physical characteristics, such as the organic matter and water contents, which will provide the best conditions for development. These conditions will vary according to the species (Boene et al., 2013). In addition to being a sustainable way of allocating waste from milling processes, the use of substrates derived from the organic waste of the agricultural industry for the production of seedlings

represents a reduction in the cost of seedling production for farmers, given that these wastes are inputs that are easily obtained.

There are few or no studies on the seedling production of Cerrado fruit species that evaluate their quality through these indices, or that evaluate their nutritional content, photosynthesis and chlorophyll *a* fluorescence as possible indicators of seedling quality. Thus, the objective of this study was to evaluate the growth, Seedling quality, nutrition, gas exchange and chlorophyll *a* fluorescence in *E. dysenterica* DC. seedlings that were produced in substrates containing different organic wastes from agricultural industries.

3.2 - Material and Methods

Field conditions, plant material and substrate chemical properties

This experiment was conducted in a greenhouse in the Laboratory of Plant Tissue Culture (LCTV) Laboratory at the Rio Verde Campus of the Goiano Federal Institute (Instituto Federal Goiano) (17°48' 16'' S latitude, 50°54' 19'' W longitude and 753 m altitude). *E. dysenterica* DC. fruits were collected from healthy adult plants in full production stage (Figure 1).

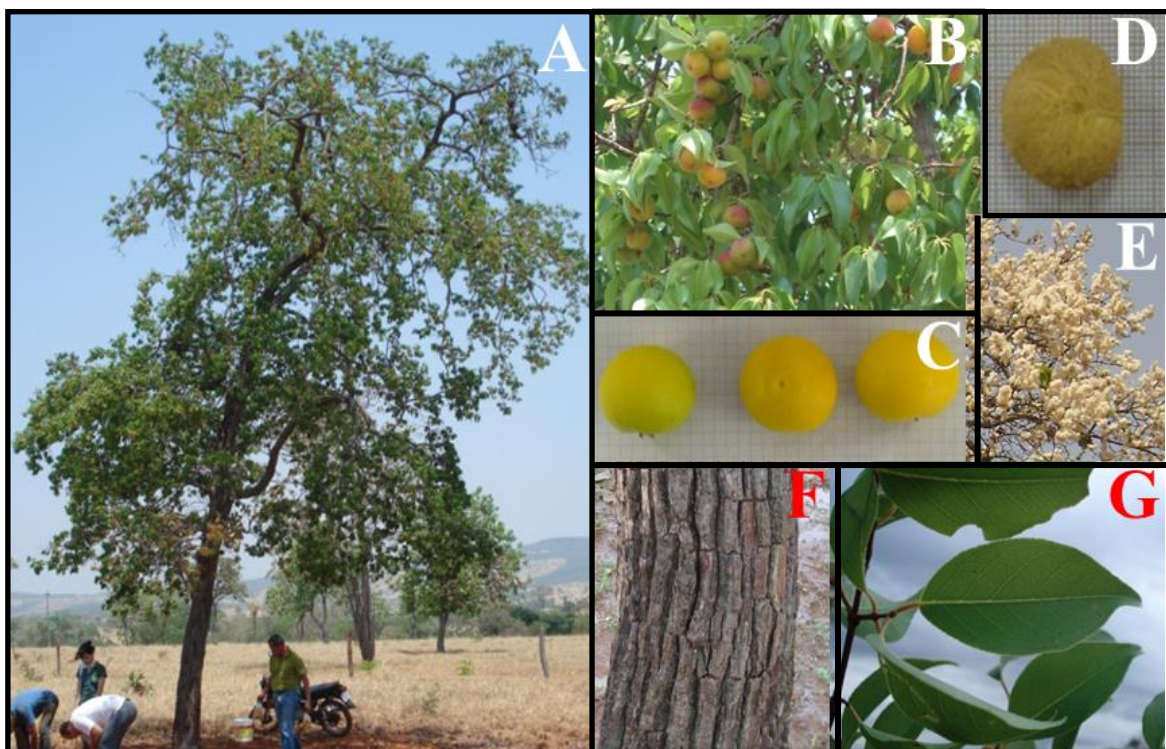


Figure 1. A) *Eugenia dysenterica* DC. mother plant in an open field, B) fruits on the plant and C) fruits in detail, D) seed in detail, E) flowering and F) details of the stem and G) the leaves. Bar = 3 cm.

The mother plants are located at the Gameleira farm, which is situated in the municipality of Montes Claros de Goiás, GO (19°53'S, 44°25'W, altitude of 749 m).

Following fruit pulping, the integuments of the seeds were removed for germination (Martinotto et al., 2007).

The tested substrates were formulated from sugarcane bagasse (SCB), filter cake from sugar-alcohol mills (FC), fermented cattle manure (FCM), rice husks (RHs), cattle manure compost (CMC) (which contained corn silage and FCM), subsoil (SB) from soil classified as a dystroferic oxisol and the commercial substrate Bioplant[®]. According to information from the manufacturer, this last substrate is composed of *Pinus* bark, vermiculite, coir, aggregating agents and mineral supplements (NPK+micro). Five substrates were formulated and tested as follows: Bioplant[®], SCB+FC at a 3:2 proportion, SB+RH (1:1), SB+CMC (1:1) and SB+FCM (3:1). The proportions were based on substrate volumes (Figure 2). Substrates chemical analysis are presented on Table 1.



Figure 2. Tested substrates: A) Bioplant[®], B) subsoil (SB) + rice husks (RHs) (1:1, v:v), C) SB + cattle manure compost (CMC) consisting of corn silage + fermented cattle manure (1:1; v:v), D) SB + fermented cattle manure (FCM) (3:1, v:v) and E) sugarcane bagasse (SCB) + filter cake from sugar-alcohol mills (FC) (3:2; v:v). *Eugenia dysenterica* seedlings produced in the following substrates: F) Bioplant[®], G) SB+RH (1:1), H) SB+CMC (1:1), I) SB+FCM (3:1) and J) SCB+FC (3:2). Bar = 3 cm.

Table 1. Chemical analysis of macro- and micronutrients for the substrates before used in the study. Rio Verde, 2016.

| Sample | pH (H ₂ O) | V (%) | MO (dag kg ⁻¹) | C | | | P | K | Zn | Fe | Mn | Cu | B |
|---|--------------------------|----------|----------------------------------|------------------------------------|-------|------|---------------------|------|------|-----|----|------|------|
| | | | | (t) | Ca | Mg | | | | | | | |
| | | | | cmol _c dm ⁻³ | | | mg dm ⁻³ | | | | | | |
| Bioplant [®] | 4.97 | 56 | 43.0 | 18.7 | 10.35 | 4.04 | 3.7 | 1207 | 24.5 | 132 | 52 | 1.66 | 2.14 |
| SB ^y +RH ^x (1:1) | 6.42 | 55 | 2.2 | 2.6 | 1.13 | 0.37 | 20.8 | 3.3 | 3.8 | 39 | 24 | 1.76 | 0.17 |
| SB+CMC ^w (1:1) | 6.60 | 86 | 11.8 | 12.6 | 4.3 | 6.90 | 170.0 | 950 | 22.6 | 103 | 62 | 2.33 | 0.56 |
| SB+FCM ^v (3:1) | 6.40 | 82 | 2.5 | 8.5 | 3.6 | 3.5 | 82.3 | 543 | 17.2 | 58 | 35 | 4.75 | 0.53 |
| SCB ^u +FC ^t (3:2) | 7.02 | 70 | 60.0 | 5.3 | 2.30 | 0.61 | 21.9 | 667 | 30.8 | 153 | 81 | 2.32 | 2.29 |

^ySubsoil; ^xRice husks; ^wCattle manure compost (corn silage + fermented cattle manure); ^vFermented cattle manure; ^uSugarcane bagasse; ^tFilter cake from sugar-alcohol mill; ^sBase saturation index; ^rOrganic matter; and ^qEffective cation exchange capacity.

The containers used in this study were small tubes with a capacity of 288 cm³. One seed was sown per tube. The temperature and humidity data that were recorded by a DataLogger (NOVUS, Porto Alegre, Brazil) inside the greenhouse included maximum, minimum and mean temperatures of 35, 20 and 25 °C, respectively; and maximum,

minimum and mean relative air humidity values of 96, 44 and 77%, respectively. The irrigation was 12 mm daily, and it was divided into two periods.

Seedling growth and morphological characteristics

The percentage of emerged seedlings (PES) was verified at intervals of two days between the emergence of the first seedling and the end of the emergence, while the vigor was calculated in accordance with Maguire (1962) via the emergence speed index (ESI). The biometric evaluations, gas exchange, chlorophyll *a* fluorescence, seedling quality, hydric ratios and the water contents of the substrates were determined at 126 days after sowing.

The non-destructive biometric characteristics evaluated here were the stem length (SL), root collar diameter (RCD) and number of leaves (NL). After the plants were separated into their stems, leaves and roots, they were dried in a forced-air-circulating oven at 65 °C until reaching a constant weight; the DW values were obtained separately. The ratio between the SL and RCD (SL/RCD), the ratio between the DWs of the roots and shoots (R/S) and the Dickson quality index (DQI) (Dickson et al., 1960) were used as seedling quality parameters. For the nutritional evaluations of the leaf tissues, the dry material was ground in a Willey type grinder, and it was quantified in accordance with methodologies described by Malavolta et al. (1997).

Relative water content (RWC)

The leaf relative water content (RWC_L) and the water contents of the substrates were determined before dawn, at 4:30 a.m. To determine the RWC, one leaf per plant was collected with the aid of a cutting blade, and immediately after collection, each leaf was weighed on an analytical balance to find its fresh weight (FW). After the FW values were obtained, the leaves were placed in a humid chamber, with each petiole was immersed in distilled water. The leaves remained in this environment for 24 h at a temperature of 25 °C, and the compensation irradiance needed to enable the leaves to reach the maximum turgidity was provided. After the leaves reached maximum turgidity, they were weighed to obtain the turgid weight (TW) and then placed in a forced-air-circulating oven at 65 °C until reaching a constant weight, at which point the DW was obtained. The RWC was calculated using the equation $RWC = (FW - DW) / (TW - DW)$ according to Barrs and Weatherley, 1962.

To determine the substrate water contents substrate samples were collected from the same containers (the ones containing the plants) that were used to determine the RWC_S

values. Immediately after collection, the substrates were weighed on a semi-analytical balance to obtain the wet weight (WW) values, and then dried in a forced-air-circulating oven at 105 °C until reaching a constant weight. At that point, the DW values were obtained. To find the water content in the dry base substrates (WCDS), the equation $WCDS = (WW - DW) / DW$ was used.

Physiological measurements and analyses of the tissue nutrient content

Gas exchange evaluations were performed in the morning, with the aid of an LCi portable photosynthesis meter (ADC-BioScientific, England) under $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ of actinic light, which was supplied by an artificial light source. The following variables were obtained: liquid assimilation of CO_2 , (A); transpiration (E); stomatal conductance (g_s); and the ratio between the intercellular and atmospheric concentration of CO_2 (C_i/C_a).

The chlorophyll a fluorescence variables were obtained with a Mini-PAM modulated fluorometer (Walz, Germany). The maximum quantum yield of the PSII (F_v/F_m) was calculated on the basis of the so-called dark parameters, using the equation $F_v/F_m = (F_m - F_0) / F_m$, in which F_0 and F_m are the minimum and maximum fluorescence values of the plant tissue that was adapted to the dark, respectively, when obtained before dawn. The F_0 was obtained by illuminating the plant tissue with a low-intensity modulated red light ($0.03 \mu\text{mol m}^{-2} \text{s}^{-1}$), while the F_m was obtained with a saturating light pulse ($6,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) with a 0.8 s duration. The effective quantum yield of the PSII (ϕPSII) was calculated via the equation $\phi\text{PSII} = (F_m' - F) / F_m'$, in which F_m' and F are the maximum fluorescence and fluorescence, respectively, when the plant tissue was under actinic light with an intensity of $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a duration of 50 s. The apparent electron transport rate (ETR) of the PSII was obtained in accordance with Bilger et al. (1995).

Experimental design and statistical analysis

The experimental design used here consisted of randomized blocks with 5 repetitions and 20 small tubes per repetition for the PES and ESI. The following were randomized: two plants (small tubes) per repetition for RWC and WCDS, one plant for gas exchange and fluorescence and five plants for the biometric evaluations. To quantify the nutrients in the leaf tissue, the leaves from each repetition were combined. The data were subjected to an analysis of variance, and Tukey's test at 5% probability was used to discriminate among the minimum significant differences between the means. For the correlations, Pearson's

method was used. The statistical procedures were performed using the SAEG 9.1 computer program.

3.3 - Results

The ability of the substrates to absorb water, that is, the water content, differed among the substrates. The water content was greatest in the SCB+FC, followed by the others in the following order: Bioplant[®] > SB+CMC > SB+RH, and the substrate SB+FCM did not differ from the last two items (Table 2). Despite the difference in the water contents of the substrates, this characteristic did not influence the RWC of the *E. dysenterica* seedlings.

Table 2. Water content in the dry base substrates (WCD_S) and leaf relative water content (RWC_L) in *Eugenia dysenterica* DC. seedlings grown in different substrates. Rio Verde, 2016.

| Substrate | WCD _S | | RWC _L |
|---|----------------------|--|--------------------|
| | (g g ⁻¹) | | |
| Bioplant [®] | 1.73b ^z | | 0.95 ^{ns} |
| SB ^y +RH ^x (1:1) | 0.35d | | 0.96 |
| SB+CMC ^w (1:1) | 0.60c | | 0.96 |
| SB+FCM ^v (3:1) | 0.51cd | | 0.93 |
| SCB ^u +FC ^t (3:2) | 2.22a | | 0.96 |
| MSD ^s | 0.205 | | 0.064 |

^zMeans followed by the same letter do not differ from one another according to Tukey's test ($p > 0.05$); ^ySubsoil; ^xRice husk; ^wCattle manure compost (Corn silage + fermented cattle manure); ^vFermented cattle manure; ^uSugarcane bagasse; ^tFilter cake from sugar-alcohol mill; ^s Minimum significant difference; and ^{ns}Non-significant difference.

The PSE, which had a mean value of 97.2%, was not influenced by the tested substrates (Table 3). The cagaita tree seedlings had greater vigor when grown in the Bioplant[®] and SCB+FC substrates, followed by the SB+FCM and SB+RH substrates. Less vigor was observed in the SB+CMC treatment.

Table 3. Percent seedling emergence (PSE) and emergence speed index (ESI) in *Eugenia dysenterica* DC. seedlings cultivated in different substrates. Rio Verde, 2016.

| Substrate | PSE | ESI |
|---|------------------|---------------------|
| | % | (-) |
| Bioplant [®] | 98 ^{ns} | 0,36 a ^z |
| SB ^y +RH ^x (1:1) | 98 | 0,29 b |
| SB+CMC ^w (1:1) | 92 | 0,23 c |
| SB+FCM ^v (3:1) | 98 | 0,30 b |
| SCB ^u +FC ^t (3:2) | 100 | 0,35 a |
| MSD ^s | 9,88 | 0,044 |

^zMeans followed by the same letter do not differ between one another according to Tukey's test ($p > 0.05$); ^ySubsoil; ^xRice husk; ^wCattle manure compost (Corn silage + fermented cattle manure); ^vFermented cattle manure; ^uSugarcane bagasse; ^tFilter cake from sugar-alcohol mill; ^sMinimum significant difference; and ^{ns}Non-significant difference.

The gas exchange characteristics of the *E. dysenterica* seedlings, such as the liquid assimilation of CO₂ (*A*), transpiration (*E*), stomatal conductance (*g_s*), and the ratio between the intercellular and atmospheric concentration (*C_i/C_a*), did not differ in accordance with the tested substrates (Table 4). The mean values of *A*, *E*, *g_s* and *C_i/C_a* were 6.31 μmol m⁻² s⁻¹, 2.23 mmol m⁻² s⁻¹, 0.16 mol m⁻² s⁻¹ and 0.69 mol mol⁻¹, respectively.

Table 4. Rate of liquid assimilation for CO₂ (*A*), transpiration (*E*), stomatal conductance (*g_s*) and the ratio between intercellular and atmospheric concentrations (*C_i/C_a*) in *Eugenia dysenterica* DC seedlings that were cultivated in different substrates. Rio Verde, 2016.

| Substrate | <i>A</i> μmol m ⁻² s ⁻¹ | <i>E</i> mmol m ⁻² s ⁻¹ | <i>g_s</i> mol m ⁻² s ⁻¹ | <i>C_i/C_a</i> mol mol ⁻¹ |
|---|--|--|---|---|
| Bioplant [®] | 5.34 ^{ns} | 2.06 ^{ns} | 0.14 ^{ns} | 0.72 ^{ns} |
| SB ^y +RH ^x (1:1) | 4.02 | 1.56 | 0.09 | 0.71 |
| SB+CMC ^w (1:1) | 7.79 | 2.39 | 0.17 | 0.65 |
| SB+FCM ^v (3:1) | 7.15 | 2.65 | 0.23 | 0.69 |
| SCB ^u +FC ^t (3:2) | 7.23 | 2.48 | 0.18 | 0.68 |
| MSD ^l | 4.50 | 1.94 | 0.187 | 0.105 |

^ySubsoil; ^xRice husk; ^wCattle manure compost (Corn silage + fermented cattle manure); ^vFermented cattle manure; ^uSugarcane bagasse; ^tFilter cake from sugar-alcohol mill; ^sMinimum significant difference; and ^{ns}Non-significant difference.

The characteristics of chlorophyll *a* fluorescence in the *E. dysenterica* DC seedlings, as indicated by the minimum fluorescence adapted to the dark (*F₀*), maximum quantum yield of the PSII (*F_v/F_m*) and effective quantum yield of the PSII (*φPSII*), were not influenced by the tested substrates (Table 5). Their means were 212, 0.76 and 0.28, respectively. The ETR differed in two of the tested substrates; it was higher in plants that were cultivated in the SCB+FC substrate and lower in the SB+RH substrate, with means of 140.7 and 75.5 μmol m⁻² s⁻¹, respectively.

Table 5. Minimum fluorescence adapted to the dark (*F₀*), maximum quantum yield of the PSII (*F_v/F_m*), effective quantum yield of the PSII (*φPSII*) and electron transport rate (ETR) in *Eugenia dysenterica* DC seedlings cultivated in different substrates. Rio Verde, 2016.

| Substrate | <i>F₀</i> | <i>F_v/F_m</i> (-) | <i>φPSII</i> | ETR μmol m ⁻² s ⁻¹ |
|---|----------------------|---|--------------------|---|
| Bioplant [®] | 209.4 ^{ns} | 0.76 ^{ns} | 0.23 ^{ns} | 90.90 ab ^z |
| SB ^y +RH ^x (1:1) | 219.8 | 0.74 | 0.20 | 75.45 b |
| SB+CMC ^w (1:1) | 205.8 | 0.78 | 0.33 | 125.13 ab |
| SB+FCM ^v (3:1) | 221.6 | 0.74 | 0.32 | 123.58 ab |
| SCB ^u +FC ^t (3:2) | 203.4 | 0.78 | 0.34 | 140.65 a |
| MSD ^s | 25.68 | 0.043 | 0.154 | 59.54 |

^zMeans followed by the same letter do not differ between one another according to Tukey's test (*p* > 0.05); ^ySubsoil; ^xRice husk; ^wCattle manure compost (Corn silage + fermented cattle manure); ^vFermented cattle manure; ^uSugarcane bagasse; ^tFilter cake from sugar-alcohol mill; ^sMinimum significant difference; and ^{ns}Non-significant difference.

The cultivation substrates had an influence on all the biometric characteristics that were evaluated in the *E. dysenterica* seedlings (Table 6). The SB+CMC substrate gave the seedlings the highest values in all of the biometric characteristics, except for the DW of the roots (DWR), which had lower values than those of the Bioplant[®] substrate. In relation to the evaluated biometric characteristics, the seedlings cultivated in the SCB+FC substrate did not differ from those grown in the SB+CMC substrate.

Table 6. Stem length (SL), root collar diameter (RCD), number of leaves (NL), dry weight of stem (DWS), dry weight of leaves (DWL), dry weight of roots (DWR) and total dry weight (TDW) in *Eugenia dysenterica* DC seedlings cultivated in different substrates. Rio Verde, 2016.

| Substrate | SL | RCD | NL | DWS | DWL | DWR | TDW |
|---|---------------------|---------|--------|---------|----------|----------|----------|
| | (cm) | (mm) | (-) | | (g) | | |
| Bioplant [®] | 3.04 c ^z | 0.98 c | 2.08 b | 0.020 b | 0.145 cd | 0.599 a | 0.765 a |
| SB ^y +RH ^x (1:1) | 3.02 c | 0.95 c | 2.44 b | 0.018 b | 0.129 d | 0.466 b | 0.613 ab |
| SB+CMC ^w (1:1) | 5.21 a | 1.26 ab | 5.04 a | 0.038 a | 0.348 a | 0.367 bc | 0.753 ab |
| SB+FCM ^v (3:1) | 3.96 bc | 1.33 a | 3.88 a | 0.037 a | 0.226 bc | 0.339 c | 0.602 b |
| SCB ^u +FC ^t (3:2) | 4.96 ab | 1.13 b | 4.76 a | 0.041 a | 0.272 ab | 0.404 bc | 0.716 a |
| MSD ^s | 1.17 | 0.15 | 1.20 | 0.0095 | 0.084 | 0.110 | 0.16 |

^zMeans followed by the same letter do not differ between one another according to Tukey's test ($p > 0.05$); ^ySubsoil; ^xRice husk; ^wCattle manure compost (Corn silage + fermented cattle manure); ^vFermented cattle manure; ^uSugarcane bagasse; ^tFilter cake from sugar-alcohol mill; and ^sMinimum significant difference.

The lowest values were observed in the seedlings grown in the SB+RH substrate, except for the total dry weight (TDW), which showed no difference in the other substrates; and the dry weight of the roots (DWR), which was greater than that of the SB+FCM and less than that of the Bioplant[®].

The Bioplant[®] and SB + CA substrates provided lower values in the cultivated seedlings for the biometric characteristics except for DWR, which was superior to the others and TDW that was superior to the SB + EB substrate.

It is observed that there was a correlation between the biometric characteristics evaluated and the gas exchange and fluorescence of chlorophyll a in the seedlings of *E. dysenterica* (Table 7). The SL, NL, dry weight of the stems (DWS) and dry weight of the leaves (DWL) showed a positive correlation with the A, ϕ PSII and ETR. The F_v/F_m showed a positive correlation with the biometric characteristics of SL, NL, DWL and the TDW. The latter characteristic also showed a negative correlation with the F₀. Among the quality indexes evaluated, IQD, R / PA and CC / DC, only CC / DC showed a positive correlation with A, Fv/Fm, ϕ PSII and ETR (Table 7).

Table 7. Matrix of Pearson's linear correlation between the characteristics of gas exchange and chlorophyll *a* fluorescence and the biometric characteristics and quality of *Eugenia dysenterica* DC seedlings grown in different substrates. Rio Verde, 2016.

| | A^z | E^y | g_s^x | Ci/Ca^w | F_0^v | F_v/F_m^u | $\phi PSII^t$ | ETR^s |
|---------------------|--------|--------|---------|-----------|---------|-------------|---------------|---------|
| SL ^r | 0.452* | 0.303 | 0.255 | -0.264 | -0.332 | 0.491* | 0.548* | 0.529* |
| RCD ^q | 0.212 | 0.074 | 0.100 | -0.386 | 0.249 | -0.077 | 0.322 | 0.326 |
| NL ^p | 0.530* | 0.399 | 0.382 | -0.187 | -0.351 | 0.524* | 0.632* | 0.593* |
| DWS ^o | 0.444* | 0.317 | 0.313 | -0.246 | -0.151 | 0.323 | 0.527* | 0.519* |
| DWL ⁿ | 0.436* | 0.277 | 0.240 | -0.326 | -0.272 | 0.482* | 0.511* | 0.487* |
| DWR ^m | -0.156 | -0.080 | -0.162 | 0.167 | -0.205 | 0.076 | -0.225 | -0.231 |
| TDW ^l | 0.228 | 0.169 | 0.051 | -0.108 | -0.453* | 0.501* | 0.223 | 0.196 |
| SL/RCD ^k | 0.409* | 0.310 | 0.232 | -0.105 | -0.559* | 0.616* | 0.437* | 0.418* |
| R/S ^j | -0.346 | -0.212 | -0.235 | 0.346 | 0.024 | -0.194 | -0.410* | -0.429* |
| DQI ⁱ | -0.253 | -0.156 | -0.214 | 0.110 | 0.055 | -0.128 | -0.296 | -0.285 |

*Significant Pearson's correlation at the 5% probability level; ^zLiquid assimilation of CO₂; ^yTranspiration; ^xStomatal conductance; ^w*Ci/Ca* ratio; ^vMinimum fluorescence adapted to the dark; ^uMaximum quantum yield of the PSII; ^tEffective quantum yield of the PSII; ^sElectron transport rate; ^rStem length; ^qRoot collar diameter; ^pNumber of leaves; ^oDry weight of stem; ⁿDry weight of leaves; ^mDry weight of roots; ^lTotal dry weight; ^kRatio between length and diameter of stem; ^jRatio between dry weight of the shoot and the root system; and ⁱDickson quality index.

The DQI was higher in plants cultivated in the Bioplant[®] substrate than in plants cultivated in the SB+CMC, SB+FCM and SCB+FC substrates (Table 8). In relation to the other substrates, the Bioplant[®] and the SB+RH substrate provided seedlings that were cultivated with a higher ratio between the dry weight of the root system and the shoot (R/S). The SL/RCD was greater in the seedlings cultivated in the SCB+FC and SB+CMC substrates than in those cultivated in the other substrates.

Table 8. Ratio between stem length and root collar diameter (SL/RCD) and between the dry weights of the roots and shoots (R/S) as well as the Dickson quality index (DQI) in *Eugenia dysenterica* DC. seedlings grown in different substrates. Rio Verde, 2016.

| Substrate | SL/RCD (cm mm ⁻¹) | R/S (g g ⁻¹) | DQI (-) |
|---|----------------------------------|-----------------------------|------------|
| Bioplant [®] | 3.12 b ^z | 3.80 a | 0.23 a |
| SB ^y +RH ^x (1:1) | 3.18 b | 3.34 a | 0.19 ab |
| SB+CMC ^w (1:1) | 4.11 a | 1.16 b | 0.15 b |
| SB+FCM ^v (3:1) | 3.03 b | 1.44 b | 0.17 b |
| SCB ^u +FC ^t (3:2) | 4.42 a | 1.59 b | 0.15 b |
| MSD ^l | 0.91 | 0.74 | 0.05 |

^zMeans followed by the same letter do not differ between one another according to Tukey's test ($p > 0.05$); ^ySubsoil; ^xRice husk; ^wCattle manure compost (Corn silage + fermented cattle manure); ^vFermented cattle manure; ^uSugarcane bagasse; ^tFilter cake from sugar-alcohol mill; ^lMinimum significant difference; and ^{ns}Non-significant difference.

The nitrogen (N) was higher in the leaves of the seedlings grown on the substrate SB + CE, but did not differ from the BC + TF. The phosphorus (P) contents were higher in the leaves of the seedlings grown on the Bioplant ® and BC + TF substrate, While for potassium (K) the highest leaf contents occurred in Bioplant® substrate seedlings, being similar to SB + CA seedlings. The other macros and micro are in Table 9.

Table 9. Levels of macro and micronutrients in the leaf tissue of *Eugenia dysenterica* DC seedlings that were cultivated in different substrates. Rio Verde, 2016.

| Substrate | N | P | K | Ca | Mg | S | B | Fe | Cu | Mn | Zn |
|---|---------------------|--------|---------|--------|-------|-------|--------------------|---------------------|-------------------|--------|--------------------|
| | mg kg ⁻¹ | | | | | | mg g ⁻¹ | | | | |
| Bioplant [®] | 19.0c ^z | 5.28a | 13.76a | 10.78b | 2.98b | 1.02a | 171.2a | 162.8 ^{ns} | 5.2 ^{ns} | 67.8b | 16.2 ^{ns} |
| SB ^y +RH ^x (1:1) | 15.6d | 0.88c | 10.24ab | 11.12b | 3.10b | 0.24b | 129.4c | 168.4 | 5.0 | 53.4b | 11.8 |
| SB+CMC ^w (1:1) | 26.4a | 1.92bc | 7.04bc | 25.68a | 7.04a | 1.06a | 138.4b | 164.0 | 4.8 | 38.4b | 12.6 |
| SB+FCM ^v (3:1) | 24.4b | 2.80b | 9.44bc | 14.82b | 7.56a | 1.32a | 122.6d | 165.0 | 4.0 | 30.2b | 12.8 |
| SCB ^u +FC ^t (3:2) | 26.0ab | 4.24a | 5.92c | 35.98a | 6.98a | 1.18a | 106.4e | 121.8 | 5.0 | 170.8a | 17.6 |
| MSD ^s | 1.95 | 1.35 | 4.04 | 10.32 | 2.12 | 0.33 | 4.83 | 80.12 | 1.23 | 42.50 | 7.00 |

^zMeans followed by the same letter do not differ between one another according to Tukey's test ($p > 0.05$); ^ySubsoil; ^xRice husk; ^wCattle manure compost (Corn silage + fermented cattle manure); ^vFermented cattle manure; ^uSugarcane bagasse; ^tFilter cake from sugar-alcohol mill; ^sMinimum significant difference; and ^{ns}Non-significant difference.

Compared to the other substrates, there were higher leaf levels of calcium (Ca) in seedlings cultivated in the SCB+FC and SB+CMC substrates. In relation to the other substrates, the foliar magnesium (Mg) levels were lower in the plants cultivated in the Bioplant[®] and SB+RH substrates; the leaf sulfur (S) concentration was lower in the seedlings cultivated in the SB+RH substrate. The SCB+FC substrate provided the highest leaf concentration of manganese (Mn). For boron (B), the leaf concentrations were different in all of the substrates (which are arranged in descending order) as follows: Bioplant[®] > SB+CMC > SB+RH > SB+FCM > SCB+FC. In the cagaita seedlings, the leaf concentrations of iron (Fe), copper (Cu) and zinc (Zn) were not influenced by the tested substrates.

The analyses of correlations between the leaf nutrient levels and the biometric characteristics and seedling quality show that N had the highest number of correlations with the following characteristics (Table 10): positive for SL, RCD, NL, DWS, DWL and SL/RCD; negative for DWR, R/S and DQI; and there was no correlation for TDW.

After N, the other elements that had the highest number of correlations were K and Mg. K had a positive correlation with DWR, R/S and DQI, and a negative correlation with SL, NL, DWS, DWL and SL/RCD. Mg had a positive correlation with SL, RCD, NL, DWS, DWL and SL/RCD. The leaf Ca levels had a negative correlation with the quality indices of R/S and DQI, and a positive correlation with SL, NL, DWS, DWL and SL/RCD. The S levels generally had positive correlations (with SL, RCD, NL, DWS and DWL), but there was one negative correlation (with R/S).

Of the micronutrients, B had the highest number of correlations, which included negative correlations with SL, NL and DWS and positive correlations with DWR, R/S and DQI. After B, Cu had the highest number of correlations; the only negative ones were with RCD, NL and DWS. The nutrients P, Mn and Zn had a correlation with DWR, SL/RCD and TDW, respectively, while Fe displayed no correlations.

Table 10. Matrix of the Pearson's linear correlation between the leaf mineral levels and the biometric characteristics and quality of *Eugenia dysenterica* DC seedlings according to different substrates. Rio Verde, 2016.

| | N | P | K | Ca | Mg | S | B | Cu | Fe | Mn | Zn |
|---------------------|---------|--------|---------|---------|---------|---------|---------|---------|--------|--------|--------|
| SL ^z | 0.736* | -0.025 | -0.638* | 0.685* | 0.641* | 0.467* | -0.442* | -0.342 | -0.085 | 0.286 | 0.193 |
| RCD ^y | 0.670* | -0.034 | -0.342 | 0.369 | 0.805* | 0.599* | -0.331 | -0.502* | 0.229 | -0.162 | 0.157 |
| NL ^x | 0.724* | -0.095 | -0.715* | 0.576* | 0.621* | 0.444* | -0.519* | -0.447* | -0.221 | 0.209 | 0.032 |
| DWS ^w | 0.772* | 0.088 | -0.626* | 0.601* | 0.724* | 0.592* | -0.522* | -0.404* | -0.093 | 0.229 | 0.202 |
| DWL ^v | 0.781* | -0.060 | -0.665* | 0.572* | 0.638* | 0.476* | -0.339 | -0.286 | -0.126 | 0.096 | 0.086 |
| DWR ^u | -0.580* | 0.406* | 0.570* | -0.391 | -0.705* | -0.170 | 0.603* | 0.275 | 0.132 | 0.088 | 0.371 |
| TDW ^t | 0.083 | 0.396 | 0.014 | 0.100 | -0.169 | 0.259 | 0.321 | 0.023 | 0.031 | 0.195 | 0.487* |
| SL/RCD ^s | 0.502* | 0.036 | -0.582* | 0.667* | 0.321 | 0.212 | -0.382 | -0.114 | -0.259 | 0.518* | 0.188 |
| R/S ^r | -0.841* | 0.192 | 0.686* | -0.595* | -0.851* | -0.458* | 0.607* | 0.345 | 0.100 | -0.044 | 0.044 |
| DQI ^q | -0.579* | 0.394 | 0.629* | -0.507* | -0.618* | -0.080 | 0.604* | 0.229 | 0.211 | -0.095 | 0.373 |

*Significant Pearson's correlation at the 5% probability level; ^zStem length; ^yRoot collar diameter; ^xNumber of leaves; ^wDry weight of stem; ^vDry weight of leaves; ^uDry weight of roots; ^tTotal dry weight; ^sRatio between length and diameter of stem; ^rRatio between dry weight of the root system and the shoot; and ^qDickson quality index.

3.4 - Discussion

The tested substrates influenced the biometric characteristics that indicated the quality and nutrition. The SCB+FC and SB+CMC substrates gave the best results, while the SB+RH result was the least interesting.

The Bioplant[®] and SCB+FC substrates, which had the highest water content values, also had the highest levels of organic matter (Table 1). Organic matter is a component that contributes to water absorption, and among its benefits are improvements to the soil structure. The presence of subsoil in the substrates contributed to a reduction in the water content, together with the fact that the substrates had low levels of organic matter in relation to the Bioplant[®] and SCB+FC. Another component that contributes to the water absorption capacity comprises RH. The substrate containing this material had the lowest RWC. According to Guerrini and Trigueiro (2004), the increase in the RH content in the substrates was inversely proportional to the water retention capacity in the same substrates. The lack of difference in the RWC in the *E. dysenterica* DC leaves shows that, despite the difference in the RWC in the substrates, they were able to satisfy the water requirements of the plants.

The positive correlation between the emergence speed index (ESI) of the seedlings and the water content in the substrates indicates that the amount of water absorbed by the substrate influenced the seedling vigor. For small and large *E. dysenterica* seeds, Nietsche et al. (2004) obtained ESI values of 0.08 and 0.10, respectively, in this work was observed 0,39. The percentage of emerged plants was higher than that observed by Souza et al. (2001), who reported an emergence of 80.6%. Martinotto et al. (2007) reported that the removal of the integuments from the *E. dysenterica* seeds favored *in vitro* germination, reaching close to 90% as well as functioned as indicators for *in vitro* germination. The removal of the seed coat from the seeds in the present study favored emergence, which reached 97% and is higher than the values reported in the literature for this specie. Removal of the tegument by scarification with a scalpel contributed to the rapid and uniform seed germination (MARTINOTTO et al., 2007).

As observed for the RWC, the evaluated substrates did not have an influence on gas exchange, and even variations in the water contents of the substrates were not enough to influence the A , E , g_s and Ci/Ca . Lemos-Filho (2000) reported g_s values of $0.26 \text{ mol m}^{-2} \text{ s}^{-1}$ during the wet season, which are higher than those observed in the present study. The value closest to the one observed by the author was provided by the SB+FCM substrate ($0.23 \text{ mol m}^{-2} \text{ s}^{-1}$). Neves et al. (2009) observed an A value of $12 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ and an E of

1.2 mmol m⁻² s⁻¹ in *Eugenia uniflora* plants that were cultivated hydroponically. In comparison to the values reported in the present study, the *A* values were higher and the *E* values were lower, and even the *Ci/Ca* values were lower in Neves et al. (2009).

These results may indicate that the plants in the present study had chronic damage in the biochemical phase of photosynthesis due to the higher *Ci/Ca*, higher *E* and lower *A*. However, the absence of a difference between the *F_v/F_m* values in the means in the present study and the similarity with the values observed (0.78) by Lemos-Filho (2000) do not indicate evidence of damage in the photochemical phase. Nevertheless, when we compared these results with the results observed (0.81) by Neves et al. (2009) in the *E. uniflora* species, there was damage in the photochemical phase. However, despite evidence of possible damage in the photochemical phase, the ϕ PSII of the present study was similar to that observed (0.30) by Lemos-Filho (2000) and was much higher than that observed (0.5) by Neves et al. (2009), which occurred all under the same intensity of actinic light. The similarity between the *F₀* mean values counters possible damage to photosystem II.

The differences between the ETR values of the plants grown in the SCB+FC and SB+RH substrates may have contributed to the difference in the photosynthesis values between them, although the result was not significantly different. Lemos-Filho (2000) found an ETR value of 200 μ mol m⁻² s⁻¹, while in the present study, the ETR was no more than 140 μ mol m⁻² s⁻¹.

The stem growth in length and diameter as well as the NL, and consequently the higher values for the dry weights of the stems and leaves, were favored by the substrates containing CMC, SCB and FC in relation to the RH component and Bioplant[®]. By contrast, the growth of the root system was greater in the Bioplant[®], and it was influenced by the greater accumulation of the DWR; the TDW was also greater in this substrate. Morgado et al. (2009) found superior biometric characteristics when using SCB+FC, regardless of the ratio between them, during the production of sugarcane seedlings. The use of cattle manure has also shown good results in species such as *Acacia sp.* (CUNHA et al., 2006), *Enterolobium contortisiliquum* (ARAÚJO and PAIVA SOBRINHO, 2011) and *Harconia speciosa* (SILVA et al., 2009); and *E. dysenterica* seedlings grown in combined substrates in the presence of cattle manure have shown better values for *A*, *E*, *gs*, *F_v/F_m* and ETR as well as better biometric characteristics (MOTA et al., 2016). However, in the present study, cattle manure was more promising when it was composted with corn silage.

Despite A not being influenced by the tested substrates, it was the only gas exchange characteristic to show a correlation with most of the biometric characteristics, and all were positive. The positive correlations between the biometric characteristics and the chlorophyll a fluorescence characteristics, which are linked to the photosynthetic potential with ETR, ϕ PSII and Fv/Fm, together with A , demonstrate that the alterations of these characteristics influence the growth of the *E. dysenterica* seedlings.

In the analysis of the seedling quality indices, the Bioplant[®] substrate was observed to be associated with higher values for these indices, which suggests better quality and a better chance of survival after transplant to the field, given that it had the highest DQI and R/S and the lowest SL/RCD. The higher R/S shows that the plants invested photoassimilates into root system growth. According to Hunt (1990), the DQI for conifer seedlings should be greater than 0.20; values higher than this level were observed only for seedlings that were grown in Bioplant[®]. The lower SL/RCD indicates that the seedlings that were grown in the Bioplant[®] substrate are robust and less likely to suffer from etiolation. The correlation between SL/RCD and A as well as with the chlorophyll a fluorescence characteristics demonstrates that these values can be used to evaluate the quality of *E. dysenterica* seedlings, especially fluorescence due to its status as a quick and simple evaluation method. The correlation between R/S, ϕ PSII and ETR also contributed to this method, strengthening the use of chlorophyll a fluorescence.

However, when analyzing SL/RCD to account for the NL and the plant height, the seedlings grown in the Bioplant[®] only had two leaves, while those grown in SB+CMC and SCB+FC had approximately five leaves. If we consider that *E. dysenterica* has two opposite leaves per node (Silveira et al., 2013), the plants show one node in the Bioplant[®] substrate and three nodes in SB+CMC and SCB+FC. In addition, the seedlings grown in the Bioplant[®] have a height close to 60% of the height of those grown, for example, in SB+CMC, which produced the greatest height in absolute terms. If we also calculate the NL per centimeter of stem, we have 0.7 leaves/cm for plants grown in Bioplant[®], while for the SB+CMC and SCB+FC substrates, which had higher SL/RCD values, we find 1 leaf/cm, thus confirming that these latter substrates were not responsible for etiolation in the seedlings. A similar characteristic occurs in the R/S, given that Bioplant[®] provided 3.8 times greater accumulation of dry weight in the root system than in the shoot, considering that the species prioritizes the development of the root system to the detriment of the shoot when young (Silveira et al., 2013).

Because the SCB+FC and SB+CMC substrates showed high leaf nutrient levels such as N, Ca, Mg, S as well as P and Mn in the case of the former, a larger NL and greater height and better equilibrium in the distribution of photoassimilates and growth of the shoot, these substrates had the capacity to meet the nutritional needs of the plants more efficiently than the Bioplant[®] and SB+RH substrates. However, the correlations between the leaf nutrient levels and the biometric characteristics show that the nutrients N, Ca, Mg and S were the most limiting for the growth of the *E. dysenterica* plants. By contrast, when the foliar levels of the nutrients K, B and Cu increased, there were reductions in plant growth.

The foliar levels of N, Mg and S of 15, 3 and 0.4 g kg⁻¹, respectively, that were found by Melo and Haridasan (2009) in *E. dysenterica* plants are less than the levels observed in the present study, regardless of the treatment. In the comparison between the leaf macronutrient levels found by those authors and those for the plants grown in the SCB+FC and SB+CMC substrates, the levels are lower, except for the K level in the SCB+FC substrate. The superior performance of the *E. dysenterica* plants in the SCB+FC and SB+CMC substrates may be related to the mineralization of the sources of organic matter.

The influence of the leaf nutrient levels on the quality indices given by the positive and negative correlations, although they were not clearly defined and, despite the effect of the nutritional levels found here (especially when they were individualized) on the quality of the seedlings, demonstrates the importance of proper nutrition for obtaining quality seedlings.

3.5 - Conclusions

E. dysenterica seedlings that were grown in different substrates derived from the agricultural industry, particularly the substrates containing SCB, FC and CMC, were more efficient at meeting the nutritional needs of *E. dysenterica* seedlings.

Chlorophyll *a* fluorescence techniques can be used as indicators of seedling quality, as can the photosynthesis rates. These uses are especially true for Fv/Fm, ϕ PSII and ETR because they show good correlations with the biometric characteristics, thus indicating their potential for use as quality indicators.

In general, the SB+CMC substrate proved to be the most suitable for the production of *E. dysenterica* seedlings.

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CONCLUSÃO GERAL

A expressiva emergência das plântulas de *Eugenia dysenterica*, deve-se à retirada do tegumento das sementes antes da semeadura, sendo considerada uma prática recomendada.

A espécie demonstrou afinidade por substratos que tinham além de misturas de diferentes componentes, conteúdos representativos de MO e nutrientes, com destaque para os substratos que possuíam em sua composição os resíduos EB, SM, CE, BC e TF.

De acordo com os resultados obtidos, os substratos SB+VF+SM (1:3:6), SB+AG+EB (2:2:1), SB+CE (1:1) e BC+TF (3:2), são os mais promissores para a produção de mudas de *Eugenia dysenterica*.

As técnicas de análises de fluorescência da clorofila *a* e de trocas gasosas demonstraram eficiência para a qualidade das mudas, especialmente para as variáveis de rendimento quântico máximo do PSII (F_v/F_m), rendimento quântico efetivo do fotossistema II (ϕ PSII) e taxa de transporte de elétrons (ETR), por demonstrar correlações positivas com as características biométricas, indicando assim o seu potencial de utilização como indicadores de qualidade.

O resultado positivo referente à qualidade das mudas da *Eugenia dysenterica*, através do aproveitamento de materiais residuais, vai além do resgate desta espécie. Proporciona mais uma forma de utilização desses resíduos, tornando menor acúmulo no ambiente e ao mesmo tempo, oferece ao setor produtor de mudas uma forma mais barata e sustentável de cultivo.