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DIRETORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS AGRÁRIAS – AGRONOMIA

MORPHOPHYSIOLOGICAL CHARACTERISTICS OF *CAMPOMANESIA PUBESCENS* ON PROPAGATION UNDER DIFFERENT LIGHT QUALITY

Autora: Agda Rabelo Centofante
Orientador: Prof. Dr. Aurélio Rubio Neto

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TITULAÇÃO: Doutorado em Ciências Agrárias-Agronomia - Área de Concentração em Produção Vegetal Sustentável no Cerrado

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“Ainda que a minha mente e o meu corpo enfraqueçam, Deus é a minha força, ele é tudo o que eu preciso”.

Salmo 73:26

BIOGRAFIA DA AUTORA

AGDA RABELO CENTOFANTE, filha de Joaquim Rabelo Sobrinho (*in memoriam)* e Sebastiana Rabelo Pereira, nasceu na cidade de Itapurango-GO.

Em 1990/2 ingressou no Curso de Biologia (Licenciatura) na Universidade Federal de Mato Grosso (UFMT) – Campus Pontal do Araguaia - MT, concluiu em 1994. Em agosto de 2006, iniciou no curso de mestrado no Programa de Pós-Graduação em Agronomia (Fisiologia Vegetal/Agronomia) na Universidade Federal de Lavras- MG (UFLA), sob orientação do Professor Dr. Evaristo Mauro de Castro. Foi bolsista da Fundação de Amparo à Pesquisa do Estado de Minas Gerais, FAPEMIG, e, em julho de 2008, defendeu a dissertação intitulada por "Descrição morfo-anatômica e germinação de *Merremia tomentosa* (Choisy) Hall". Em março de 2016 ingressou no Programa de Pós-graduação em Ciências Agrárias – Agronomia do IF Goiano Campus Rio Verde – GO, como estudante de Doutorado, sob orientação do Professor Dr. Aurélio Rubio Neto.

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LISTA DE SÍMBOLOS, SIGLAS, ABREVIAÇÕES E UNIDADES

cm^2	Centímetro ao quadrado	-
ºC	Graus Celsius	-
CO_2	Dióxido de Carbono	-
DIC	Delineamento inteiramente ao acaso	-
µm	Micrômetro	-
µmol	Micromol	-
µmol $m^{-2}s^{-1}$	Micromol por metro quadrado por segundo	-
NaO Cl	Hipoclorito de sódio	-
GENES	Sofware de análise estatística	-
SISVAR	Sofware de análise estatística	-
pH	Potencial de hidrogênio	-
PVC	Polivinilcloreto	-
FL	Fluprescência	-
LED	Diodos de emissão de luz	-
BL	LED azul	-
RL	LED vermelho	-
WL	LED branca	-
GL	LED verde	-
PL	LED púrpura	-
Fo	Fluorescência inicial	-
F_v/F_m	Rendimento quântico máximo do fotossistema II	-
NPQ	Dissipação não fotoquímica	
Y(II)	Rendimento quântico efetivo do fotossistema II	-
ETR	Taxa relativa de transporte de elétrons	-
Y(NPQ)	Rendimento quântico da dissipação regulada	-
Y(NO)	Rendimento quântico da dissipação não regulada	-
qP	Quenching fotoquímico	-
Ab Ep	Espessura da epiderme abaxial	µm
Ad Ep	Espessura da epiderme adaxial	µm
Esp P Clor	Espessura do parênquima clorofiliano	µm
SP	Parênquima esponjoso	µm
PP	Parênquima paliçádico	µm
Dens Est	Densidade estomática	mm^{-2}
D Polar Est	Diâmetro Polar Estomático	µm
D Eq Est	Diâmetro Equatorial Estomático	µm
D Pol/D Eq	Razão Diâmetro Equatorial Estomático	-

RESUMO

CENTOFANTE, AGDA RABELO. Instituto Federal de Educação, Ciência e Tecnologia Goiano – IF Goiano - Campus Rio Verde. Março de 2019. **Morphophysiological Characteristics of *Campomanesia pubescens* on Propagation Under Different Light Quality.** Orientador: Dr. Aurélio Rubio Neto, Coorientadores: Dr. Fabiano Guimarães Silva, Dr. Fernando Higino de Lima e Silva e Dr. Sebastião C. Vasconcelos Filho.

A *Campomanesia pubescens* (gabiroba), uma espécie frutífera nativa do Cerrado com potencialidade para uso alimentício, ornamental, melíferas e medicinal. Não há registro de estudos dessa espécie na área de propagação utilizando qualidade de luz, sendo importante para obtenção de plântulas de melhor qualidade, assim como fornecer subsídios para futuros estudos das possibilidades de utilizar LEDs para aclimatização e obtenção de metabólitos secundários. Assim, objetivou-se com este trabalho avaliar o crescimento, as características anatômicas e fisiológicas de *C. pubescens* sob condições de cultivo *ex vitro* e *in vitro* sob diferentes qualidades de luz. No primeiro capítulo, avaliou-se respostas morfoanatômicas e fisiológicas das plântulas de *C. pubescens* cultivadas em diferentes qualidades de luz. Utilizou-se diodos emissores de luz (LEDs), nos comprimentos de onda monocromática vermelha (v) (600 - 700 nm), monocromática azul (a) (400 - 490 nm), combinação azul/vermelha (1:1) e branca (b) (400 - 700nm) com 50 ± 5 μmol m^{-2} s^{-1}, fotoperíodo de 16 horas. No segundo capítulo, observou-se alterações anatômicas e fisiológica em plântulas de *C. pubescens* cultivadas *in vitro* sobre diferentes qualidades de luz. Utilizou-se diodos emissores de luz (LEDs), nos comprimentos de onda branca (W), azul em combinação com vermelha nas seguintes proporções: (BR 1:1); (BR 1:3) e (BR 3:1) a 100 ± 5 μmol m^{-2} s^{-1}, fotoperíodo de 16 horas. Em todos os ensaios

experimentais utilizou-se delineamento inteiramente ao acaso, os dados foram submetidos análise de normalidade utilizando o Shapiro-Wilk, posteriormente a análise de variância e em seguida comparou-se as médias através do teste Tukey a 5% de probabilidade. A Correlação network foi feita utilizando o *software* R versão 3.1.2 e o gráfico utilizando o pacote "qgraph". Verificou-se que quando as plântulas foram cultivas sob LED vermelha, este proporcionou danos oxidativos, como visto pelo aumento significativo no MDA. Esse dano repercutiu negativamente nas respostas biométricas das plantas, afetando até mesmo sua fisiologia. Por outro lado, quando as plântulas foram cultivadas em LEDs brancas e combinações de azul/vermelha, observou-se maiores biometrias. Entretanto, embora proporcionem comportamento semelhante em termos de biometria, verificou-se que maior densidade estomática, funcionalidade dos estômatos e, consequentemente, maior F_v/F_m, YII, repercutindo em maior massa seca das folhas e caules quando cultivas em branco e vermelhor/azul. Entretando, verificou-se que quando as plântulas foram cultivadas *in vitro* em combinações de LEDs azul/vermelha 1:1 e 3:1 proporcionaram maior biomassa, apresentando valores superiores das epidermes e parênquima clorofiliano, com maior densidade estomática e funcionalidade, obtendo melhor eficiência fotossintética, com valores superiores de Y(II), qP e maiores concentrações de clorofila total, não proporcionando danos oxidativos em relação aos LEDs branco.

PALAVRAS-CHAVE: Cerrado, LEDs, Myrtaceae, Gabiroba, Cultura de tecidos vegetais.

ABSTRACT

CENTOFANTE, AGDA RABELO. Goiano Federal Institute of Education, Science, and Technology (IF Goiano) Rio Verde Campus. March 2019**.** . **Morphophysiological Characteristics of *Campomanesia pubescens* on Propagation Under Different Light Quality.** Advisor: Dr. Aurélio Rubio Neto; Co-advisor: Dr. Fabiano Guimarães Silva, Dr. Fernando Higino de Lima e Silva e Dr. Sebastião C. Vasconcelos Filho.

Campomanesia pubescens (gabiroba), a fruit species native from Cerrado with potential for food, ornamental, honey and medicinal use. There is no studies record of this species in the propagation area using light quality, being important to obtain better quality seedlings, as well as providing subsidies for future studies about the possibilities of using LEDs for acclimatization and obtaining secondary metabolites. Thus, the objective of this work was to evaluate the growth, anatomical and physiological characteristics of *C. pubescens* under *ex vitro* and *in vitro* culture conditions under different light qualities. In the first chapter, there were evaluated morpho-anatomical and physiological responses of *C. pubescens* seedlings grown in different light qualities. Light-emitting diodes (LEDs) were used at the monochromatic red (v) (600 - 700 nm), monochromatic blue (a) (400 - 490 nm), blue/red (1: 1) and white (b) (400-700nm) at 50 ± 5 μmol m^{-2} s^{-1}, photoperiod of 16 hours. In the second chapter, it was observed anatomical and physiological changes in seedlings of *C. pubescens* grown *in vitro* on different light qualities. Light-emitting diodes (LEDs), at white wavelengths (W), were used in combination with red in the following proportions: (BR 1: 1); (BR 1: 3) and (BR 3: 1) at 100 ± 5 μmol m^{-2} s^{-1}, photoperiod of 16 hours. In all experimental trials, a completely randomized design was used and data were submitted to normality analysis using Shapiro-Wilk, after analysis of

variance and the means were then compared using the Tukey test at 5% probability. The network correlation was done using the R version 3.1.2 software and the graph using the "qgraph" package. It was verified that when seedlings were cultivated under red LED, this provided oxidative damages, as seen by the significant increase in MDA. This damage had negative repercussions on the plants biometric responses, affecting even their physiology. On the other hand, when seedlings were cultivated in white LEDs and blue/red combinations, larger biometrics were observed. However, although they provide similar behavior in terms of biometrics, it was found that higher stomatal density, stomatal functionality and, consequently, higher F_v/F_m, YII, reflected in a higher dry mass of leaves and stems when grown in white and red/blue . However, it was verified that when seedlings were cultivated in combinations of 1: 1 and 3: 1 blue/red LEDs they provided higher biomass, presenting higher values of epidermis and chlorophyllic parenchyma, with greater stomatal density and functionality, obtaining better photosynthetic efficiency, with higher Y (II) values, qP and higher total chlorophyll concentrations, not providing oxidative damages in relation to white LEDs.

KEYWORDS: Cerrado, LEDs, Myrtaceae, Gabiroba, Vegetable tissue culture.

1. INTRODUÇÃO GERAL

O Cerrado é o segundo maior bioma da América do Sul, ocupando a área de 2.036.448 km^2, cerca de 22% do território nacional. A sua área contínua incide sobre os estados de Goiás, Tocantins, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Bahia, Maranhão, Piauí, Rondônia, Paraná, São Paulo e Distrito Federal. Considerado como um hotspots mundiais de biodiversidade, o Cerrado apresenta extrema abundância de espécies endêmicas e sofre uma excepcional perda de habitat. Do ponto de vista da diversidade biológica, o Cerrado brasileiro é reconhecido como a savana mais rica do mundo, abrigando 11.627 espécies de plantas nativas já catalogadas (MMA, 2018).

Considerado como um *hotspots* mundiais de biodiversidade, o Cerrado apresenta extrema abundância de espécies endêmicas e sofre uma excepcional perda de *habitat*. Do ponto de vista da diversidade biológica, o Cerrado brasileiro é reconhecido como a savana mais rica do mundo, abrigando 11.627 espécies de plantas nativas já catalogadas. Além dos aspectos ambientais, o Cerrado tem grande importância social. Muitas populações sobrevivem de seus recursos naturais, incluindo etnias indígenas, quilombolas, raizeiros, ribeirinhos, babaçueiras e comunidades quilombolas que, juntas, fazem parte do patrimônio histórico e cultural brasileiro, e detêm um conhecimento tradicional de sua biodiversidade (MMA, 2018).

Mais de 220 espécies têm uso medicinal e mais 416 podem ser usadas na recuperação de solos degradados, como barreiras contra o vento, proteção contra a erosão, ou para criar habitat de predadores naturais de pragas. Mais de 10 tipos de frutos comestíveis são regularmente consumidos pela população local e vendidos nos centros urbanos, como os frutos do Pequi (*Caryocar brasiliense*), Buriti (*Mauritia flexuosa*), Mangaba (*Hancornia speciosa*), Cagaita (*Eugenia dysenterica*), Bacupari (*Salacia*

crassifolia), Cajuzinho do cerrado (*Anacardium humile*), Araticum (*Annona crassifolia*) e as sementes do Baru (*Dipteryx alata*)(MMA, 2018).

A exploração da gabiroba dá-se pelo extrativismo e, também pelo cultivo em pequenos pomares familiares, sendo uma fonte de renda para muitas famílias. Nos últimos anos, observa-se a importância socioeconômica da utilização da gabiroba, em especial, na região centro-oeste do país, onde seus frutos são consumidos *in natura*, vendidos para fábricas de picolés, licores e doces, porém o desenvolvimento lento da plântula e a alta predação de seus frutos dificulta o cultivo comercial (Oliveira et al., 2011). Além disso, suas sementes são consideradas recalcitrantes, ou seja, apresentam alto teor de água no momento da dispersão e não suportam desidratação, sendo por isso, de difícil armazenamento, uma vez que a metodologia convencional de conservação de sementes frequentemente utiliza a secagem e o armazenamento em câmaras com baixas temperaturas como forma de preservar a viabilidade das mesmas (Dosseau et al., 2011).

A propagação *in vitro* refere-se ao crescimento e multiplicação de células, tecidos e órgãos em um meio de cultura específico semissólido ou líquido em condições ambientais controladas, e na ausência de patógenos (Chandra et al. 2010). Técnicas de cultura de tecidos têm sido desenvolvidas para algumas espécies frutíferas do Cerrado, já que muitas delas apresentam dificuldades de propagação, como a produção de sementes recalcitrantes ou com certo grau de dormência e heterogeneidade na maturação dos frutos, além de fornecer intercâmbio do material genético, o resgate de germoplasma ameaçado e sua conservação (Pinha et al., 2011).

A luz promove respostas morfológicas, anatômicas e fisiológicas diferentes entre as espécie . A utilização dos LEDs em cultura de tecidos parece ser vantajoso em relação ao uso clássico de lâmpadas fluorescente, uma vez que os LEDs podem ser mais eficientes fornecendo luz em comprimentos de onda específico dentro de espectro. Devido a esta especificidade, nos últimos anos os LEDs têm sido utilizado em câmera de crescimento e biorreatores para melhorar o desenvolvimento de plantas *in vitro* com respostas fisiológicas desejadas (Gupta e Jatothu, 2013). Os trabalhos desenvolvidos nesta pesquisa serão de suma importância para área de cultura de tecidos, assim como o conhecimento dos efeitos da qualidade de luz na morfoanatomia e fisiologia em planta nativa, sendo este pioneiro para esta espécie.

2. REVISÃO DE LITERATURA

2.1. Aspectos Gerais da Campomanesia pubescens (DC.) O. Berg

A espécie *Campomanesia pubescens* (DC.) O. Berg, conhecida popularmente como gabirobeira, guabiroba, guabiroba-do-campo ou guavira, é um arbusto que pode atingir 60-80 cm de altura e sua frutificação dá-se de setembro a novembro. Nativa do Cerrado brasileiro, pertence à família Myrtaceae, na qual estão incluídos cerca de 130 gêneros e mais de 4.000 espécies e podem ser encontradas na América do Sul, sudeste da Ásia e Austrália, sendo que no Brasil são encontradas no Sudeste e na região Centro Oeste (Chang et al, 2011). Espécies do gênero *Campomanesia* podem ser encontradas na forma de subarbustos ou arbustos de 0,3 m até 2 m de altura ou árvores de 8 a 15 metros (Oliveira et al. 2011).

As flores são brancas e os frutos são verdes e globosos possuindo de 2,0 a 2,5 cm de diâmetro com polpa amarelada quando madura. As sementes são pequenas, pardas e possuem mucilagem intimamente aderida, porém não foram encontradas substâncias inibidoras da germinação nessa mucilagem, não sendo, portanto um componente que interfira nesse processo (Silva et al. 2009).

Figure 1. *C. pubescens* (DC.) O. Berg. Flor (A); plantas com frutos (B); frutos (C) e sementes (D).

A *C. pubescens* (DC.) O. Berg, é uma espécie que tem boas perspectivas socioeconômico, podendo ser utilizada em reflorestamento de áreas contaminadas e degradadas, pois são consideradas como pioneiras; melífera e pela beleza de suas flores são utilizadas em ornamentações (Gogosz, 2010).

A polpa e o resíduo de *C. pubescens* possuem conteúdo elevado de umidade, baixo teor calórico e alta concentração de fibra alimentar, constituindo-se um alimento que pode reduzir os níveis de triglicerídeos e glicose no sangue. Além disso, a polpa apresenta um

teor considerável de ferro, mineral essencial para o funcionamento do organismo dos seres humanos, pois atua na produção de células vermelhas do sangue e no transporte de oxigênio para todo o corpo. Possuem compostos fenólicos e alta capacidade antioxidante. No organismo humano, os compostos fenólicos atuam na eliminação de radicais livres e na proteção de antioxidantes alimentares, como as vitaminas C e E, promovendo benefícios adicionais à saúde, (Cardoso et al. 2010; Klafke et al. 2010; Alves et al. 2013).

O teor de vitamina C observado no suco de gabiroba (*Campomanesia xanthocarpa* Berg) foi maior do que o encontrado em bacuri (*Platonia insignis*), carnaúba (*Copernicia Prunifera*), cajá (*Spondias mombin*), jabuticaba (*Myrciaria cauliflora*) e jambolão (*Syzygium cumini*), todas frutas tropicais consideradas exóticas (Santos et al. 2013). Cardoso et al. (2010) confirmaram a ação do extrato das folhas contra seis espécies diferentes de bactérias. O chá das folhas *Campomanesia* spp. mostraram atividades gastroprotetora, antiúlcera e anti-inflamatória (Madalosso et al. 2012; Ferreira et al. 2013).

Estudo fitoquímico da espécie *C. pubescens* (DC.) O. Berg, sobre a composição do óleo essencial, em diferentes partes da planta (raiz, caule, folhas e frutos), identificando compostos fenólicos (monoterpenos e sesquiterpenos) e proantocianidinas com atividades antioxidante e antimicrobiana contra agentes patogênicos orais, incluindo bactérias aeróbias e anaeróbias, o que os tornam particularmente interessantes para futuros desenvolvimentos de novos agentes antimicrobianos. Importante ressaltar que o estudo sobrea atividade antioxidante dos extratos etanólicos de *C. pubescens* poderia ser considerado como uma fonte de antioxidante natural para aplicações medicinais e alimentares (Chang et al. 2011).

Atualmente, há grande interesse em identificar princípios ativos em plantas nativas, levando vários grupos de pesquisadores a estudarem a atividade biológica de plantas medicinais de diversas regiões, mas pouco se tem conhecimento relacionado ao cultivo e propagação de espécies nativas do Cerrado, informações sobre propagação são imprescindíveis para o sucesso do cultivo. Trabalhos com qualidades de luz na propagação de *C. pubescens*, sobretudo relacionada com a influência da qualidade de luz na anatomia e fisiologia são inexistentes, exemplos de trabalhos realizados com esta espécie estão descritos abaixo (Tabela 1).

Table 1. Estudos realizados do gênero Campomanesia (dados obtidos na Web of Science e Sciencedirect)

Espécie de planta	Título	Objetivos	Referência
Campomanesia pubescens	Diferentes substratos e recipientes na produção de mudas de gabiroba (*Campomanesia pubescens* O. Berg.).	Avaliar o desempenho da produção de mudas de guabiroba em função de diferentes recipientes e substratos.	Bardiviesso et al. (2011)
Campomanesia pubescens	Essential oil composition and antioxidant and antimicrobial properties of *Campomanesia pubescens* O. Berg, Native of Brazilian Cerrado.	Investigar a atividade antimicrobiana dos óleos essenciais e avaliar a atividade antioxidante de extratos etanólicos e os teores de fenólicos e proantocianidinas do extrato etanólico de diferentes partes de C. *pubescens*.	Chang et al. (2011)
Campomanesia species	Antimicrobial activity of the extracts and fractions of hexanic fruits of *Campomanesia* species (Myrtaceae).	Avaliar a atividade antimicrobiana de extratos de hexânicos Campomanesia espécies obtido a partir de seus frutos.	Cardoso et al. (2010)

Campomanesia xanthocarpa	Morfoanatomia da plântula de *Campomanesia xanthocarpa* O. Berg., (Myrtaceae)..	Descrever a morfologia e anatomia da plântula, sementes *Campomanesia xanthocarpa*.	Gogosz et al. (2010)
Campomanesia xanthocarpa	Anti-diabetic effects of *Campomanesia xanthocarpa* (Berg) leaf decoction.	Identificar os efeitos do tratamento com o decocto das folhas de *Campomanesia xanthocarpa* Berg. (20 g/L), durante 3 semanas, sobre parâmetros fisiológicos, bioquímicos e histológicos de ratos normais e diabéticos induzidos por estreptozotocina.	Vinagre et al. (2010)
Campomanesia xanthocarpa	Effects of *Campomanesia xanthocarpa* on biochemical, hematological and oxidative stress parameters in hypercholesterolemic patients.	Investigar o efeito de *Campomanesia xanthocarpa* sobre os parâmetros bioquímicos, hematológicos, antropométricos e de estresse oxidativo em pacientes hipercolesterolêmicos.	Klafke et al. (2010)

2.2. Influência da qualidade de luz na morfoanatômia e fisiologia das plântulas de Campomanesia pubescens (DC.) O. Berg

Todos os organismos para crescerem e desenvolverem precisam perceber e interpretar informações de seu ambiente. Portanto, as plantas têm de adaptar-se ao seu padrão de desenvolvimento com as mudanças ambientais para garantir a sobrevivência e reprodução (Morelli e Ruberti, 2000). A influência da qualidade da luz sobre a morfologia das plantas compreende muitas respostas, entre elas o alongamento da haste, expansão e ângulo foliar (Warpeha e Montgomery, 2016). A luz como fonte primária de energia é um dos fatores ambientais mais importantes para o crescimento, influenciando diretamente no desenvolvimento de mecanismos morfofisiológicos, para se adaptar as variações de luz (Li e Kubota, 2009).

Diodos de emissão de luz (LEDs) tem sido recomendado como fonte luminosa em ambientes controlados, pois apresentam características desejáveis, tais como: capacidade de controlar a composição espectral emitindo comprimentos de ondas específicos, longa durabilidade, baixa emissão de calor, eficiência na conversão de energia e especificidade do comprimento de onda (Massa et al., 2008; Morrow et al., 2008). A influência da qualidade da luz tem forte impacto na horticultura e plantas ornamentais, influenciando no crescimento e desenvolvimento das plantas, além de induzir respostas variadas dos metabólicos secundários, tais como: quantidade de pigmentos e capacidade antioxidante das plantas, alterando o conteúdo fitoquímico, valor nutricional, controle de floração, sucesso do transplante e produção de material de regeneração (Bantis et al., 2018).

Os LEDs proporcionam um sistema de iluminação eficiente nas plantas em câmeras de crescimento, melhorando várias características qualitativas e quantitativas em diferentes espécies de cultura (Tabela 2), sendo indicado como a próxima geração de fonte de luz para o cultivo em ambiente controlado (Agarwal e Gupta, 2016).

Table 2. Efeitos de LEDs no crescimento e desenvolvimento de planta em ambientes controlados, publicados no período de 2013 a 2018 (dados obtidos na Web of Science e Sciencedirect).

Espécie de planta	Título	LEDs utilizados	Conclusão	Referência
Brassica napus L. (colza)	The effects of different light qualities on rapeseed (*Brassica napus* L.) plantlet growth and morphogenesis in vitro	FL, BL, RL, BL+ RL(3:1; 1:1; 1:3)	A taxa de diferenciação, proliferação, crescimento e sobrevivência foram superiores quando utilizadas LEDs B:R = 3:1.	Li et al.(2013)
Solanum lycopersicum L. (tomate)	Response of photosynthetic capacity of tomato leaves to different LED light wavelength	WL, BL+ RL(1:1), PL, RL	Os LEDs W e B+R exibiu maior eficiência fotossintética.	Yang et al. (2018)
Brassica napus L. (colza)	Morphological, photosynthetic, and physiological responses of rapeseed leaf to different combinations of red and blue lights at the Rosette stage.	BL, RL, BL+ RL (3:1; 1:1; 1:3)	A luz azul proporcionou a morfogênese e a luz vermelha é benéfica ao aparato fotossintético. Combinações de LEDs B + R foram adequadas para taxa fotossintética líquida.	Shengxin et al. (2016)

Platycodon grandiflorum (Jacq.) A. DC. (flor de balão)	Evaluation Of leaf morphology, structure and biochemical substance of balloon flower (*Platycodon grandiflorum* (Jacq.) A. DC.) plantlets in vitro under different light spectra.	FL, BL, RL, BL+ RL (3:1; 1:1; 1:3)	Luz azul e combinação azul e vermelha (3:1), aumentaram o número de folha, área foliar, estômatos elíptico e produziu maior massa seca da planta.	Liu et al. (2014)
Cucumber sativus (pepino)	Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs	BL, RL, (50B:50R%; 10B:90R%; 75B:25R%; 20B:28G:52R%; 30B:70R%)	Respostas , tais como: Pn, gs e concentração de clorofila aumentou com o aumento da luz B nas combinações, enquanto a luz vermelha monocromática diminuiu a taxa de crescimento.	Hernández and Kubota (2015)
Chrysanthemum (crisântemo)	Effects of different irradiation levels of light quality on Chrysanthemum.	RL, BL, RB (75:25%), WL	A luz azul potencializou o desenvolvimento anatômicos da folha, assim como o desenvolvimento e movimentos dos estômatos.	Zheng et al. (2018)

Mesembryanthemum crystallinum (planta de gelo)	Plant Growth and photosynthetic characteristics of *Mesembryanthemum crystallinum* grown aeroponically under diferente blue and red-LEDs.	BL, RL, (50B:50R%; 10B:90R%; 20B:80R%; 30B:70R%	Combinação apropriada de LEDs azul e vermelha, favorece o crescimento das plantas e capacidade fotossintética.	He et al. (2017)
Solaneum lycopersicum (tomate)	Tomato seedling physiological responses under different percentages of blue and red photon flux ratios using LEDs and cool white fluorescent lamps.	100R, 10B:90R, 20B:28G: 52R, 30B:70R, 50B:50R, 75B:25R and 100B	Para a produção de mudas de tomate em fazendas ve rticais 30B: 70R e 50B: 50R qualidades de luz de fluxo de fótons são recomendados.	Hernández et al. (2016)
Lactuca sativa L (alface)	Influence of Green, Red and Blue Light Emitting Diodes on Multiprotein Complex Proteins and Photosynthetic Activity under Different Light Intensities in Lettuce Leaves (*Lactuca sativa* L.).	GL, BL, RL	os LEDs azuis em alta intensidade promovem o crescimento das plantas, controlando a integridade de proteínas dos cloroplastos que elevam o	Muneer et al. (2014)

	desempenho fotossintético.

FL: fluorescente, BL: LED azul, RL: LED vermelho, WL: LED branca, GL: LED verde, PL: LED púrpura

A intensidade e a qualidade da luz são fatores fundamentais que interferem na morfologia e fisiologia das plantas (Fukuda et al., 2008; Li e Kubota, 2009). Quando a intensidade luminosa for alta pode-se reduzir a eficiência fotossintética por causa da não capacidade do aparato fotossintético em dissipar o excesso de energia em forma de calor, podendo ocorrer fotoinibição e danos nos centros de reação dos fotossistema (Fan et al., 2013), sendo prejudicial para a planta.

3. REFERÊNCIAS BIBLIOGRÁFICAS

Agarwal, A., Dutta Gupta, S. D., 2016. Impact of Light-Emitting Diodes (LEDs) and its Potential on Plant Growth and Development in Controlled-Environment Plant Production System. Current Biotechnology. 5 (1): 28-43.

Alves, A. M.; Alves, M. S. O.; Fernandes, T. O.; Naves, R. V.; Naves, M. M. V., 2013. Caracterização física e química, fenólicos totais e atividade antioxidante da polpa e resíduo de gabiroba. Revista Brasileira de Fruticultura, Jaboticabal. 35 (3): 837-844.

Bantis, F., Smirnakoub, S., Ouzounis, T., Koukounaras, A., Ntagkas, N., Kalliopi Radoglou, K., 2018. Current status and recent achievements in the field of horticulture with the use of light-emitting diodes (LEDs). Sci Hortic-amsterdam. 235: 437-451. https://doi.org/10.1016/j.scienta.2018.02.058.

Bardiviesso, D. M., Wilson Itamar Maruyama, W. I., Reis, L.L., Modesto, J. H., Rezende, W. E., 2011. Diferentes substratos e recipientes na produção de mudas de guabiroba (*Campomanesia pubescens* o.berg). Revista Científica Eletrônica de Agronomia, Garça.18 (1): 52-59.

Cardoso, C. A. L. Salmazzo, G. R., Honda, N. K., Prates, C. B., Vieira, M. D. C., Coelho, R. G., 2010. Antimicrobial activity of the extracts and fractions of hexanic fruits

of *Campomanesia* species (Myrtaceae). Journal of Medicinal Food. 13(5), 1273-1276. http://dx.doi:10.1089/jmf.2009.0047.

Chang, R., Morais, S. A. L., Nascimento, E. A., Cunha, L. C., Rocha, E. D. O., Aquino, F. J. T., Souza M. G. M, Cunha W.R., Martins, C. H., 2011. Essential oil composition and antioxidant and antimicrobial properties of *Campomanesia pubescens* O. Berg, Native of Brazilian Cerrado. Latin American Journal of Pharmacy. 30(9), 1843-1848.

Dousseau, S., Amauri Alves de Alvarenga, A. A., Guimarães, R.M., Túlio Silva Lara, T. S., Custódio, T. N., Chaves, I. S., 2011. Ecophysiology of Campomanesia pubescens seed germination. 1362 Dousseau et al. Ciência Rural. 41(8): 1362-1368.

Fan, X., Xu, z. G., Liu,X. Y., Tang, C. M., Wang, L. W., Han, X. L., 2013. Effects of light intensity on the growth and leaf development of young tomato plants grown under a combination of red and blue light. Sci Hortic. 153, 50-55. https://doi.org/10.1016/j.scienta.2013.01.017.

Ferreira, L. C., Grabe-Guimaraes, A., Paula, C. A., Michel, M .C. P., Guimaraes, R. G., Rezende, S. A., Souza Filho, J. D., Saúde-Guimaraes, D. A., 2013. Anti-inflammatory and antinociceptive activities of *Campomanesia adamantium.* J Ethnopharmacology, v. 145, p. 100–108.

Fukuda, N., Fujita, M., Ohta, Y., Sase, S., Nishimura, S., Ezura, H., 2008. Directional blue light irradiation triggers epidermal cell elongation of abaxial side resulting in inhibition of leaf epinasty in geranium under red light condition. Sci Hort. 115(2):176-182.

Gupta, S. D., Jatothu, B., 2013. Fundamentals and applications of light-emitting diodes (LEDs) in in vitro plant growth and morphogenesis**.** Plant Biotechnol Rep 7:211–220

Gogosz, A. M., Cosmo, N. L., Bona, C., Souza, L. A., 2010. Morfoanatomia da plântula de *Campomanesia xanthocarpa* O. Berg., (Myrtaceae). *Acta Botanica Brasilica* 24(3), 613-623. http://dx.doi.org/10.1590/S0102-33062010000300003.

He, J., Qin, L., Chong, E.L.C.,Choong, T.-W., Lee, S. K., 2017. Plant Growth and photosynthetic characteristics of *Mesembryanthemum crystallinum* grown aeroponically under diferente blue and red-LEDs. Front Plant Sci. 8, 361. doi:10.3389/fpls.2017.00361.

Hernández, R., Kubota, C., 2015. Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs . Environ Exp Bot. 173(8), 92-99. http://dx.doi.org/10.1016/j.envexpbot.2015.04.001

Hernández, R., Eguchi, T., Deveci, M., Kubota, C., 2016. Tomato seedling physiological responses under different percentages of blue and red photon flux ratios using LEDs and cool white fluorescent lamps. Sci Hortic-amsterdam. 213, 270–280. https://doi.org/10.1016/j.scienta.2016.11.005.

Klafke J. Z, da Silva M. A, Panigas T. F, Belli K. C, de Oliveira M. F, Barichello M. M, Rigo F. K, Rossato M. F, Soares dos Santos A. R, Pizzolatti M. G, Ferreira J, Viecili P. R. 2010. Effects of *Campomanesia xanthocarpa* on biochemical, hematological and oxidative stress parameters in hypercholesterolemic patients. J Ethnopharmacol. 3;127(2):299-305. doi: 10.1016/j.jep.2009.11.004.

Li Q, Kubota C (2009) Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Env Exp Botany. 67(1):59-64.

Li, H., Tang, C., Xu, Z. (2013). The effects of different light qualities on rapeseed (*Brassica napus* L.) plantlet growth and morphogenesis in vitro. Scientia Horticulturae. 150, 117–124. http://dx.doi.org/10.1016/j.scienta.2012.10.009.

Liu, M., Xu, Z., Guo, S., Tanga, C., Liu, X., Jao, X. (2014). Evaluation Of leaf morphology, structure and biochemical substance of balloon flower (*Platycodon grandiflorum* (Jacq.) A. DC.) plantlets in vitro under different light spectra. Scientia Horticulturae. 174, 112–118. https://doi.org/10.1016/j.scienta.2014.05.006.

Madalosso, R. C., Oliveira, G. C., Martins, M. T., Vieira, A. E. D., Barbosa, J., Caliari, M.V., Castilho, R. O., Tagliati, A. C. A., 2012. *Campomanesia lineatifolia* Ruiz & Pav. as a gastroprotective agente. J Ethnopharmacology. 139 (3): 772–779. 10.1016/j.jep.2011.12.014.

Massa G. D., Kim H. H., Wheeler R. M., Mitchell C. A., 2008. Plant productivity in response to LED lighting. *HortScience*. 43(7), 1951-1956.

Ministério do Meio Ambiente (MMA), 2018. Site do Ministério do Meio Ambiente. Bioma Cerrado. http://www.mma.gov.br/biomas/cerrado.html.

Morelli, G., Ruberti, I., 2000. Shade avoidance responses. Driving auxin along lateral routes. Plant Physiol. 122(3), 621-626. https://doi.org/10.1104/pp.122.3.621

Morrow R.C., 2008. LED lighting in horticulture. *HortScience*. 43(7), 1947-1950.

Muneer, S., Kim, E. J., Park, J. S., Lee, J. H., 2014. Influence of Green, Red and Blue Light Emitting Diodes on Multiprotein Complex Proteins and Photosynthetic

Activity under Different Light Intensities in Lettuce Leaves (*Lactuca sativa* L.). Int. J. Mol. Sci. 15, 4657-4670. doi:10.3390/ijms15034657.

Oliveira, L. J., Silva, S. M., Netto A. P. C., Silva, S. M., Mariano-da-Silva, F. Mª. S., 2011. Características agronômicas e atividade da redutase do nitrato em plantas de Campomanesia sp. Sob estresse hídrico. Revista Agrarian, Dourados. 4 (11): 43-53.

Pinha, H. F., Anastácio, M. R., Carneiro, P. A. P., Silva, V. J. D., Morais, T. P. D., Luz, J. M. Q., 2011. Aplicações da cultura de tecidos vegetais em fruteiras do Cerrado. Ciência Rural. 41(37): 1136-1142.

Santos, M. S., Correia, C. H., Petkowicz, C. L. de O., Cândido, L. M. B., 2013. Quantification of the major bioactive phytochemical in the gabiroba (*Campomanesia xanthocarpa* Beg) juice. Acta Scientiarum Technology, Maringá.35 (4): 783-787.

Silva, E. D., Vilas, B., Rodrigues, L. J., Siqueira, H. H., 2009. Caracterização física, química e fisiológica de gabiroba (*Campomanesia pubescens*) durante o desenvolvimento. Ciência e Tecnologia de Alimentos**,** Campinas. 29(4): 803-809.

Shengxin, C., Chunxia, L., Xuyang, Y., Song, C., Xuelei, J., Xiaoying, L., Zhigang, X., Rongzhan, G., 2016. Morphological, photosynthetic, and physiological responses of rapeseed leaf to different combinations of red and blue lights at the Rosette stage. Front Plant Sci. 7, 1144. doi: 10.3389/fpls.2016.01144.

Vinagre, A. S., Rönnau, Â. D. S. R. O., Pereira, S. F., Silveira, L. U. D., Wiilland, E. D. F., Suyenaga, E. S. (2010). Anti-diabetic effects of *Campomanesia xanthocarpa* (Berg) leaf decoction. Brazilian Journal of Pharmaceutical Sciences. 46(2): 169-177. http://dx.doi.org/10.1590/S1984-82502010000200002.

Yang, X. Y., Xu, X. M., Cui, J., 2018. Response of photosynthetic capacity of tomato leaves to different LED light wavelength. Environ Exp Bot. *150*, 161–171. https://doi.org/10.1016/j.envexpbot.2018.03.013.

Warpeha, K. M., Beronda L.Montgomery, B.L., 2016. Light and hormone interactions in the seed-to-seedling transition. Environmental and Experimental Botany. 121, 56-65. https://doi.org/10.1016/j.envexpbot.2015.05.004.

Zheng, L., Van Labek, M.-C., 2018. Effects of different irradiation levels of light quality on Chrysanthemum. Sci Hortic-amsterdam. 233, 124-131. https://doi.org/10.1016/j.scienta.2018.01.033.

OBJETIVOS

Geral

Avaliar respostas morfoanatômicas, fisiológicas e de crescimento em plântulas de *Campomanesia pubescens* (D.C) O. Berg, *ex vitro e in vitro* cultivadas em diferentes qualidades de luzes (LEDs) para obtenção de mudas de melhor qualidade.

Específicos

- Analisar as respostas morfoanatômicas e fisiológicas das plântulas de *C. pubescens* cultivadas em diferentes qualidades de luz subsidiando futuros estudos com propagação *ex vitro e in vitro* dessa espécie;

- Avaliar as alterações anatômicas e fisiológica em plântulas de *C. pubescens* em cultivo *in vitro* sobre diferentes qualidades de luz.

CAPÍTULO I

Light quality on the morphanatomy and physiology of *Campomanesia pubescens* (DC.) O. Berg. seedlings

ABSTRACT: Light quality can change the morphogenesis of seedlings through receptors. They absorb and interpret light in the red and blue regions of the spectrum, being a viable way to increase the gabiroba seedlings [*Campomanesia pubescens* (DC.) O. Berg] morphophysiological quality. This species is native from Cerrado with great ornamental, medicinal and food potential. The aim of this study was to analyze the morphological and physiological responses of *C. pubescens* seedlings grown in different light qualities, subsidizing future studies with *ex vitro* and *in vitro* propagation of this species. Light-emitting diodes (LEDs) were used at the monochromatic red (v) (600-700 nm), blue monochromatic (a) (400-490 nm), blue/red (1:1) and white (b) (400-700nm) at 50 ± 5 μmol m^{-2} s^{-1}, in a photoperiod of 16 hours. Biometry, dry matter, leaf area, foliar anatomy, chlorophyll *a* fluorescence, chloroplast pigments and malonaldehyde content (MDA) were evaluated. Variations in growth and physiological parameters were observed in response to different light qualities. When seedlings were grown under red LED, there were oxidative damages as can be observed by the significant increase in MDA. This damage had negative repercussions on the plants biometric responses, affecting their physiology. On the other hand, when the plants were grown in white and blue-red LEDs, higher biometrics were observed. Higher stomatal density, stomatal functionality and, consequently, higher Fv/Fm, YII, reflected in a higher leaf and stem dry mass when grown in white and blue/red LEDs. The present study contributed to a better understanding of the impact of different light qualities on growth and development of *C. pubescens* seedlings and may contribute with more detailed studies to produce seedlings with better quality.

Keywords: Cerrado, LEDs, Myrtaceae, Fluorescence of chlorophyll *a*

1. Introduction

Campomanesia pubescens (DC.) O. Berg. (family Myrtaceae) is native to the Cerrado, popularly known as "*gabirobeira*, *guabiroba* and *guabiroba-do-campo"*. These plants are used in reforestation of degraded areas for being considered pioneers and can be used as ornamental due to the exuberance of their flowers (Gogosz et al., 2010; Souza and Lorenzi, 2008). The fruit presents high content of vitamin C so it is indicated against colds and flu. It is also attributed an effect in the control of diabetes, in the fight against cholesterol and obesity (Vinagre et al., 2010; Cardoso et al., 2010; Klafke et al., 2010).

Phytochemical studies of the essential oil of *C. pubescens* developed by Chang et al. (2011) demonstrated the presence of terpenes (monoterpenes and sesquiterpenes) with antimicrobial activity against oral pathogens, including aerobic and anaerobic bacteria which make them particularly interesting for the development of new antimicrobial agents. Research on the antioxidant activity of ethanolic extracts from different parts of the plant suggests that *C. pubescens* is considered a source of natural antioxidant for medicinal and food applications.

Light is an environmental factor that influences several responses in the development of plants such as: seed germination, carbon assimilation, stem elongation, leaf morphology, flowering, among others. For this reason, the detection and measurement of light quality is essential for plants growth and development (Carvalho et al., 2011). Changes in light quality induce and trigger a series of morphological, physiological, biochemical and molecular variations (Ali and Abbasi, 2014). Plants have developed at least three photoreceptor families that specifically recognize different wavelengths of light: phytochromes, cryptochromes and phototropines which activate a signaling cascade culminating in different physiological responses (Morelli and Ruberti, 2000).

Phytochromes are photoreceptors related to the qualitative and quantitative perception of light by plants, triggering several and complex physiological responses. When the inactive form (Pr) absorbs red light, a conformational alteration of the chromophore protein converts it into the active form (Prf). Researches have shown that these molecules are also related to a range of responses to abiotic and biotic stress due to

their role in regulating transcription of specific genes, influencing biochemical and molecular mechanisms of cell signaling (Carvalho et al., 2011). The control of abiotic factors such as light quality may represent a strategy to optimize morphophysiological characteristics of the plants, increasing the efficiency to *in vitro* environment, since this factor affects the growth and development of *ex vitro* seedlings (Fogaça et al., 2007).

The influence of light quality on crops has a strong economic impact, changing crop yield, phytochemical profile, nutritional value, flowering control, transplant success, preharvest, post-harvest quality and production of regeneration material (Carvalho et al., 2011). Such changes can be observed in studies with *Lycopersicon esculentum* (Fan et al., 2013), *Brassica napus* L. (Carvalho and Folta, 2014) and *Triticum aestivum* L (Dong et al., 2014). In addition, the influence of wavelength acts on plant growth and induces different metabolic responses and changes such as pigment concentrations and antioxidant capacity of the plant (Liu et al., 2014).

Although light is the primary source of energy for the plant and activates different responses, its excess can cause oxidative stress (Shohael et al., 2006). One of the consequences is the accumulation of reactive oxygen species, which causes lipid peroxidation of the membranes through the increase of malonaldehyde causing a decrease in the photosynthetic efficiency (Li et al., 2017). The evaluation of chlorophyll fluorescence is a useful tool for understanding the efficiency of the photosynthetic apparatus, the state of PSII, how the energy absorbed by chlorophylls is being used and their damages due to excess under several environmental conditions, including *in vitro* seedlings (Costa et al., 2014; Leite et al., 2017).

Technologies using light emitting diodes (LEDs) are useful for light optimization in horticulture and in the cultivation of ornamental plants, demanding knowledge of the morphophysiological, genetic and biochemical responses of plants exposed to these environments (Carvalho et al., 2011). LEDs have several advantages including long life, low heat emission, energy conversion efficiency and wavelength specificity (Massa et al., 2008; Morrow et al., 2008). Promising results such as growth and morphogenesis are observed with the use of LEDs in the cultivation of several plant species such as: *Ocimum basilicum* L. and *Melissa officinalis* L. (Fraszczak et al., 2014), *Cucumis sativus* and *Solanum lycopersicum* (Brazaityte et al., 2010, 2009), *Lactuca sativa* (Kim et al., 2004; Li and Kubota 2009; Lin et al., 2013; Ouzounis et al., 2015) and *Brassica napus* (Li et al., 2013).

In Cerrado plants cultivated *ex vitro*, there are few works with the application of LEDs, especially with an approach related to the effect of light quality. This evaluation associated with the determination of chloroplastidic pigment concentration, membrane integrity and anatomical profile of the stomata can provide a broader view of plant responses to environmental variations and their relationship to growth and development. In this context, the aim of this study was to analyze the morphological and physiological responses of *C. pubescens* seedlings cultivated with different light qualities and their possible impacts on the seedlings quality, subsidizing future studies with *ex vitro* and *in vitro* propagation of this species.

2. Materials and methods

2.1. Plant material and growth conditions

The experiment was carried out in the Laboratory of Culture and Vegetable Tissues of the Goiano Federal Institute, Campus Rio Verde-GO, Brazil.. The exsiccate is deposited in the Herbarium of the Goiano IF, campus Rio Verde, under collection number 1022A.

Fruits of *C. pubescens* were collected from 35 matrices in the municipality of Iporá-GO, Brazil, located in the western region of Goiania between 16° 24' 00" and 16° 28' 00" S, 51° 04' 00' and 51° 09' 00" W, (IBGE, 2010), altitude of 587m, from November 2017 to January 2018. The pulps were removed with a sieve, then a 5% sodium hydroxide solution was used for 10 minutes to facilitate seeds removal. The sowing was performed in plastic trays (53x37x8 cm) with washed and sieved coarse sand as a substrate. The initial growth was in a growth room with fluorescent light at an average temperature of 25 ± 3 and 55 ± 5% of relative humidity.

2.2. Light environment

Thirty days after sowing, trays containing 80 seedlings with one pair of leaves were transferred to the growth room and cultivated under metal structures (1.10 x 0.90 x 0.60m - length, width and height, respectively) with LED tubes of 20W (Lanao series Tubes, China) with different spectral ranges: monochromatic red (600-700 nm), monochromatic blue (400-490 nm), white (400-700 nm) and blue-red (1:1). Photosynthetic photon flux density (PPFD) of 50 ± 5 μmol m^{-2} s^{-1} was used with

photoperiod of 16 hours, 500 ± 50 μmol mol^{-1} CO_2, temperature of 24 ± 3°C and 60 ± 5% of relative humidity. The spectral quality was determined using a USB 2000 spectroradiometer (Ocean Optics, Dunedin, FL, USA). The light intensity was adjusted using a PAR sensor (QSO-S; Decagon Devices, Pullman, WA, EUA). Every 15 days, the trays were irrigated with 400 mL of MS medium (Murashige and Skoog, 1962) at 50%, and weekly irrigated maintaining a relative humidity of 55% in the substrate. The LED structures containing the seedlings were sealed using a black nonwoven fabric to avoid interference from the external light. After 45 days, all the evaluations were performed using 6 seedlings from the center of the trays. Each seedling was considered the replicate.

2.3. Biometric analysis

Seedling height (cm), number of leaves, number of segments, number of roots, leaf area (cm^2), leaf, stem and root dry mass (g) were evaluated. Length measurements were obtained with millimeter ruler. The leaf area was obtained from the integration of images by ImageJ® software (National Institutes of Health, Bethesda, MD, USA). The total dry mass was determined in a digital analytical balance, after drying the material in a forced ventilation oven at 65 ºC for 72 hours until constant weight.

2.4. Anatomical analyzes

For these evaluations, *C. pubescens* leaves diaphanization and fixation were carried out. Samples of fully expanded leaves, about 2 cm^2, were immersed in 5% sodium hydroxide for 24 hours, clarified with hydrochloric acid 1:6:1 (w / v) for another 24 hours and stained with 1% safranin in ethanol 50% (Arnott, 1959). Soon afterwards, slides were made and covered with coverslips using Canada Balsam. The characteristics observed were: stomatal location, morphology and density.

At fixation, samples of the middle third of 6 leaves, about 1 cm^2, were fixed in Karnovsky's solution (Karnovsky, 1965) for 48 hours. Then, the leaves were dehydrated in a growing ethylic series, pre-infiltrated and infiltrated with historesin to obtain historesin blocks with plant material. After drying the blocks on silica gel, the material was transversely sectioned at 5 μm thickness, in a rotary microtome (model RM 2155, Leica). The sections were stained with 0.05% toluidine blue pH 4.0 (O'Brien et al., 1964) for evaluation of palisade and spongy parenchyma thicknesses, epidermal thickness of both leaf faces and mesophyll thickness.

The images were obtained under an optical microscope (model BX61, Olympus) with U-photo system, at the Anatomy Laboratory of the Goiano Federal Institute – campus Rio Verde. The images were processed using the softwareImageJ®.

2.5. Evaluation of chlorophyll a fluorescence

The images and fluorescence parameters of chlorophyll *a* were obtained using the modulated fluorometer IMAGING PAM (MAXI version) and the software Imaging Win (Heinz Walz GmbH, Effeltrich, Alemanha). To obtain the images (640 × 480 pixels), the fully expanded leaves were individually fixed in a holder 18.5 cm away from the coupled charge device camera (CCD) to the fluorescence device. Measurement of the adaxial portion of leaves, which were adapted to dark for 30 minutes, so the reaction centers were fully opened. Under this condition, the leaf tissues were exposed to low intensity light (0.03 μmol $m^{-2}s^{-1}$) to determine the initial fluorescence (Fo). Then, a pulse of saturation light (> 6000 μmol m^{-2} s^{-1}) was applied for 0.8 s to determine the maximum fluorescence (Fm). Then the maximum quantum yield of photosystem II (FSII) (Fv/Fm = (Fm-Fo)/Fm) (Genty et al., 1989). After illuminating the sample for 40 s at 21 μmol m^{-2} s^{-1}, the fluorescence of the sample adapted to the light before the saturation pulse (F) and the maximum fluorescence in the light-adapted sample (Fm') after the pulse saturation. Then the effective quantum yield of FSII, ΔF/Fm'= (Fm'-F)/Fm' was calculated according to Genty et al. (1989). The photochemical extinction coefficient, qP = (Fm'-F)/(Fm'-Fo ') was estimated according to Genty et al. (1989) and Stern-Volmer non-photochemical extinction coefficient, NPQ = (Fm-Fm')/Fm') was estimated according to Bilger and Björkmann (1990) and Kooten and Snel, (1990). The results obtained from the median region of the leaves were processed.

2.6. Determination of Chloroplastidic Pigments

The concentrations of pigments (chlorophyll *a*, *b* and carotenoids) were determined using dimethylsulfoxide (DMSO) as described by Wellburn (1994). Three leaf discs with 5 mm diameter were placed in containers with 5 mL of DMSO, saturated with 50 g L^{-1} $CaCO_3$ (calcium carbonate) and kept in the dark. After 6 hours in water bath at 65°C, the absorbance of the extract was determined through a UV-VIS spectrophotometer Model 60S (Thermo Fischer Scientific, Madison - USA), read at 665.1 and 649.1; 480 nm using DMSO saturated with $CaCO_3$.

The concentrations of the pigments were expressed in μg cm^{-2}. The wavelengths, equations and calculations for the determination of pigment concentration were based on the work of Wellburn (1994).

2.7. Malonaldehyde content (MDA)

Cell damage was assessed by lipid peroxidation through the increase of MDA as described by Cakmak and Horst (1991). Samples of 100 mg of leaf tissue were macerated in liquid N_2 in mortar. The powder was homogenized in 2 mL 1% trichloroacetic acid (TCA) and centrifuged at 12,000×g for 15 min at 4°C. After centrifugation, 0.5 mL of the supernatant was added to 1.5 mL of the 0.5% (m/v) thiobarbituric acid solution (prepared in 20% (w/v) of TCA) and incubated in water bath at 95° C for 30 min. After this period, the reaction was stopped in an ice bath. Samples were centrifuged at 9000×g for 10 min and the specific absorbance of the supernatant was determined at 532 nm. Non-specific absorbance was measured at 600 nm and subtracted from the specific absorbance value. The concentration of MDA was calculated using the extinction coefficient of 155 $mM^{-1}cm^{-1}$ and was expressed in μmol g^{-1} of fresh mass (Heath and Packer, 1968).

2.8. Experimental design

The experiment was performed adopting an unifactorial completely randomized design. The data were submitted to normality test using the Shapiro-Wilk, ANOVA applying the F test, and then Tukey and Pearson's correlation. The discrete variables were transformed into $\sqrt{x}$. SISVAR software was used to analyze the data (Ferreira, 2011).

3. Results

3.1. Spectral quality and growth characteristics of Campomanesia pubescens seedlings grown for 45 days in different light qualities

It was observed purity in the spectral quality of the different LEDs used to stimulate different morpho-anatomic and physiological responses in the seedlings of *C. pubescens* (Fig. 1).

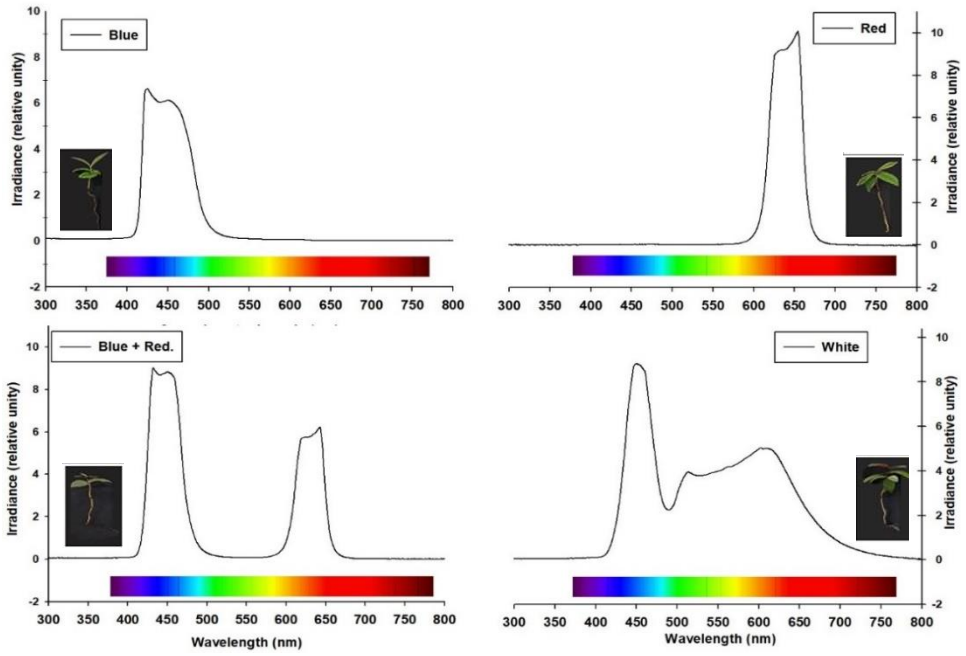

Fig. 1. Relative distribution of different spectral LEDs (monochromatic blue, monochromatic red, blue/red (1:1) and white used during seedlings production.

The different light conditions influenced several growth parameters of *C. pubescens* seedlings: seedlings cultivated under blue/red LEDs (1:1) reached an average height of 4.3 cm, exceeding the height of seedlings cultivated in monochromatic red light that reached a mean of 3.5 cm (Fig. 2A). It was observed higher values in the number of nodal segments and consequently number of leaves when seedlings were cultivated under white and blue /red (1:1) LEDs with a mean of 2.1 and 1.9 that provided an increase in the number of leaves with means of 2.8 and 2.6, respectively (Fig. 2B and C).

The number of secondary roots was higher in the white LED, which provided a mean of 5.5 new roots, but a larger length of these roots was observed in seedlings grown in blue/red (1:1) LEDs with a mean of 4.0 cm (Fig. 2E). Regarding the dry mass of leaves and stems, seedlings grown on white, red and blue/red LEDs were superior (Fig. 2F). There was no statistically significant difference in root dry mass among the different LEDs, with mean of 0.22 g obtained under white LED (Fig. 2 F).

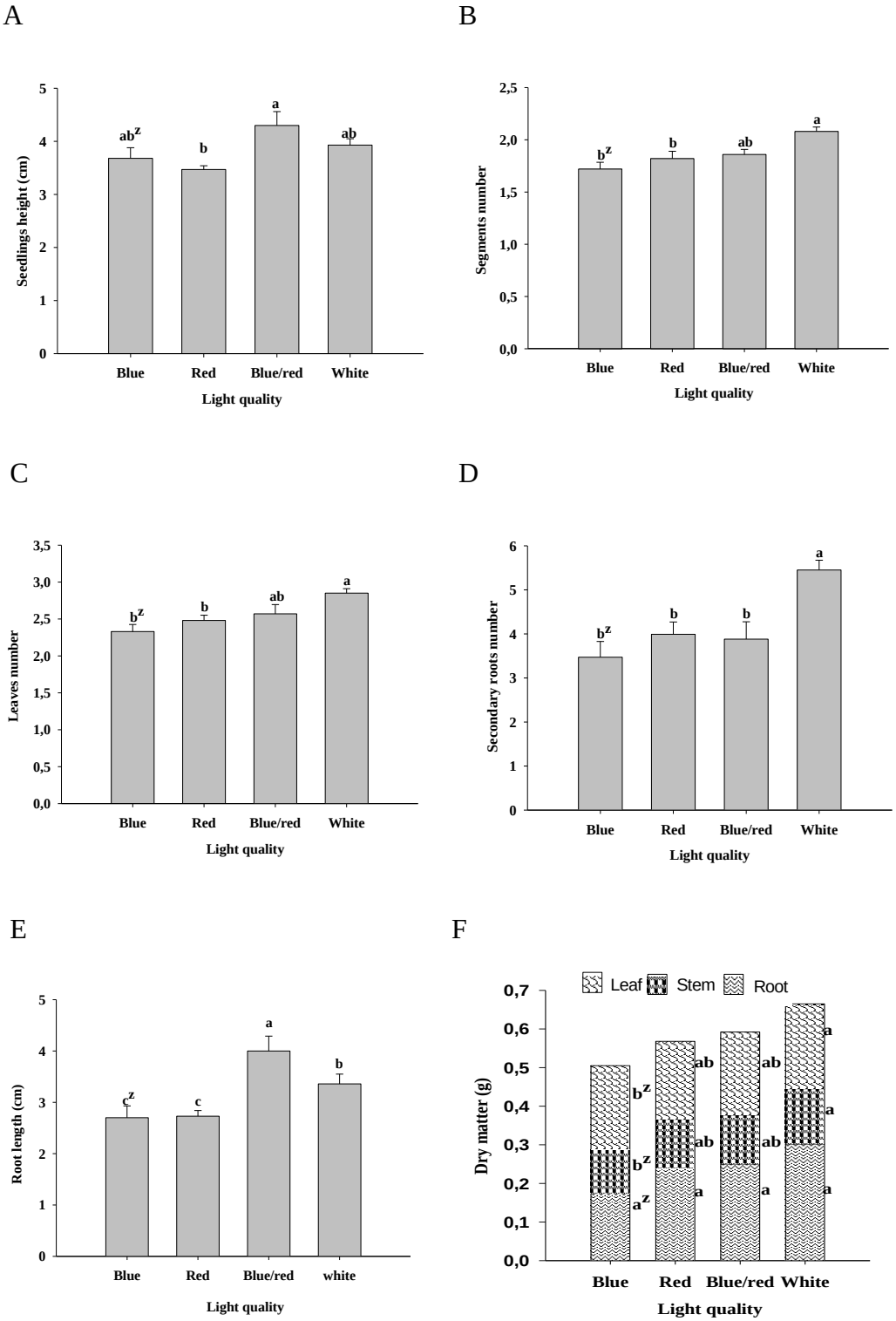

Fig. 2. Seedlings height (A), segments number (B), leaves number (C), secondary roots number (D), root length (E) and dry mass (F) of *C. pubescens* after 45 days under different LED qualities. [Z]Means followed by the same letter do not differ statistically among themselves using the Tukey's test at 5% probability.

It was observed a larger leaf area in seedlings cultivated under white LED, with a mean of 18.56 cm^2 when compared to the blue monochromatic LED that provided the mean of 9.03 cm^2 (Fig. 3).

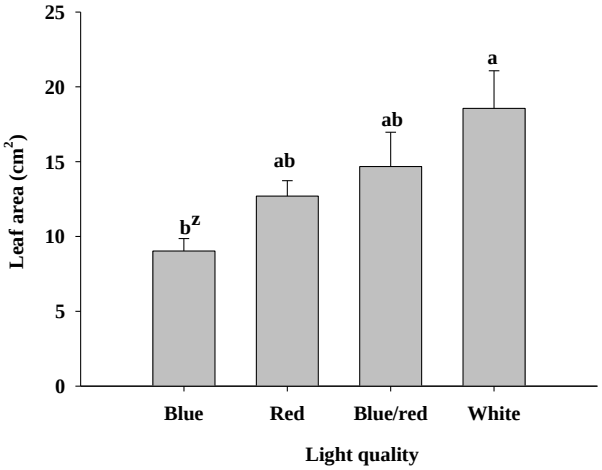

Fig. 3. Leaf area of *C. pubescens* after 45 days under different light qualities. [Z]Means followed by the same letter do not differ statistically among themselves using the Tukey's test at 5% probability.

3.2. Foliar anatomy of Campomanesia pubescens seedlings grown for 45 days in different light qualities

Stomata were identified only on the abaxial face of the epidermis, characterizing the leaves as hypoestomatic. According to the organization of the subsidiary cells, the stomata were classified as paracytic (Fig. 4).

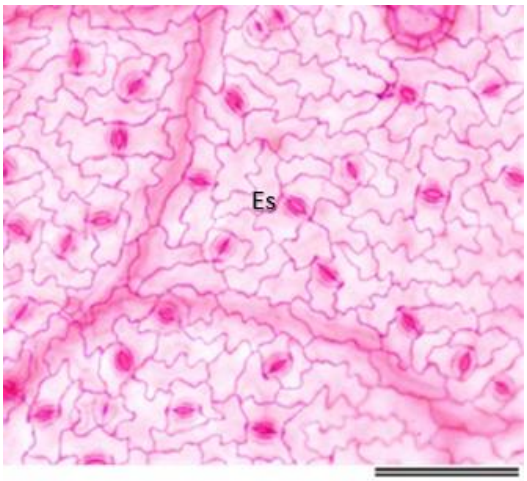

Fig. 4. Photomicrography of *C. pubescens* leaf with 45 days of cultivation. Abaxial face of the epidermis with stomata (Es). Scale bar = 100 μm.

Regarding the micromorphometry characteristics of *C. pubescens*, there were some leaf tissue alterations with different light qualities (Fig. 5).

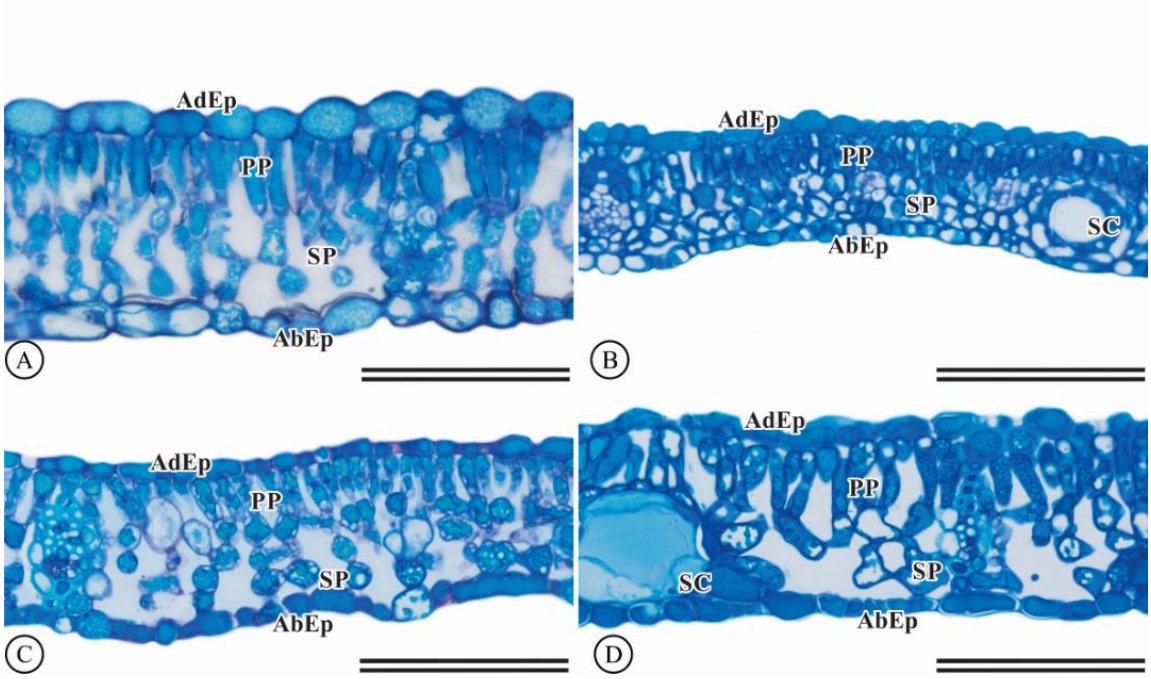

Fig. 5. Photomicrograph of *C. Pubescens* leaves showing anatomical changes after cultivation under monochromatic blue light, monochromatic red, blue/red (1:1) and white for 45 days. (A) Blue, (B) Red, (C) Blue/red, (D) White. (AdEp) adaxial epidermis. (AbEp)abaxial epidermis. (PP) palisade parenchyma. (SP) spongy parenchyma. (SC) cavity secretory. Scale bar 100 μm.

Seedlings of *C. pusbescens* cultivated under blue monochromatic LED had greater investment in leaf tissue with values for Ep Ad and Ep Ab overlapping in relation to other light conditions. There were no differences among the monochromatic blue and white LEDs in relation to the Esp P and Chlor, being superior to the other light qualities (Table 1).

Table 1. Leaf micromorphometry anatomical characteristics: adaxial epidermis (Ad Ep), abaxial epidermis (Ep Ab), chlorophyllic parenchyma thickness (Esp P Clor) of *C. pubescens* after 45 days under different light qualities

Light quality (LEDs)	Leaf anatomical characteristics		
	Ep Ad (µm)	Ep Ab (µm)	Esp P Clor (µm)
Blue	16.20 ± 0.41a	13.20 ± 0.38a[z]	78.46 ± 0.39a
Red	11.03 ± 0.25c	8.20 ± 0.26c	54.18 ± 1.14c
Blue/red	12.51 ± 0.42bc	11,21 ± 0,53bc	67.37 ± 1.02b
White	14.00 ± 0.62b	11.30 ± 0.79ab	84.00 ± 2.80a

[z]Means followed by the same letter in each column do not differ statistically among themselves using Tukey's test at 5% probability. [±]Standard error.

Greater stomatal density (278.79 stomata mm^{-2}) was observed in seedlings grown under blue monochromatic LEDs than in the combination blue/ red (221.66 stomata mm^{-2}). In the blue monochromatic LEDs, the stomata presented lower values for D Polar Est (17.35 µm) and D Eq Est (11.58 µm). On the other hand, stomata with more ellipsoid characteristics were observed in plants grown under white LEDs, with higher value (1.65) for the ratio between polar and equatorial diameters (Table 2).

Table 2. Leaf anatomy: stomatal density (Dens Est), stomata polar diameter (D Polar Est), stomata equatorial diameter (D Eq Est) and ratio between polar and equatorial diameter (D Polar / D Eq) of *C. pubescens* after 45 days under different light qualities

Light quality (LEDs)	Leaf anatomical characteristics			
	Dens Est (Stomata mm^{-2})	D Polar Est (µm)	D Eq Est (µm)	D Pol/D Eq
Blue	278.79 ± 24.04a	17.35 ± 0.20b	11.58 ± 0.30c	1.50 ± 0.04b
Red	240.69 ± 06.21ab	19.51 ± 0.48ab	12.77 ± 0.23ab	1.52 ± 0.01b
Blue/red	221.66 ± 09.62b	20.68 ± 0.13a	13.82 ± 0.23a	1.50 ± 0.02b
White	236.37 ± 07.44ab	19.94 ± 0.26ab	12.33 ± 0.14ab	1.65 ± 0.02a

[z]Means followed by the same letter in each column do not differ statistically among themselves using the Tukey's test at 5% probability. [±]Standard error.

3.3. Physiological response of Campomanesia pubescens after 45 days under different light qualities

Data obtained in the chlorophyll fluorescence evaluations showed changes among the light qualities (Table 3 and Fig. 6). F_v/F_m values were higher in seedlings grown in white LEDs (0.77) and reduced in the red monochromatic LED (0.66). The effective quantum yield of photosystem II [Y(II)] was higher in white and monochromatic blue LEDs (0.27 and 0.22 respectively) and lower in the red one (0.12). The mean values of non-photochemical quenching (NPQ) were higher in white, monochromatic blue and blue/red LEDs (0.34, 0.30 and 0.30, respectively) and lower in the red monochromatic LED (0.21). Photochemical quenching (qP) was higher in the white, blue and blue/red LEDs (0.42, 0.42 and 0.33 respectively) and lower in the red (0.22) (Table 3).

Table 3. Characteristics of chlorophyll *a* fluorescence: maximum quantum yield (F_v/F_m), effective quantum yield Y(II), non-photochemical quenching (NPQ) and photochemical quenching (qP) on leaves of *C. pubescens* after 45 days under different light qualities

Light quality (LEDs)	Physiological characteristics			
	F_v/F_m	Y(II)	NPQ	qP
Blue	0.71 ± 0.03ab	0.22 ± 0.02a	0.30 ± 0.02a	0.42 ± 0.01a
Red	0.66 ± 0.02b	0.12 ± 0.01b	0.21 ± 0.02b	0.22 ± 0.01b
Blue/red	0.71 ± 0.00ab	0.18 ± 0.02ab	0.30 ± 0.02a	0.33 ± 0.03a
White	0.77 ± 0.00a	0.27 ± 0.02a	0.34 ± 0.00a	0.42 ± 0.02a

[Z]Means followed by the same letter in each column do not differ statistically among themselves using the Tukey's test at 5% probability. [±]Standard error.

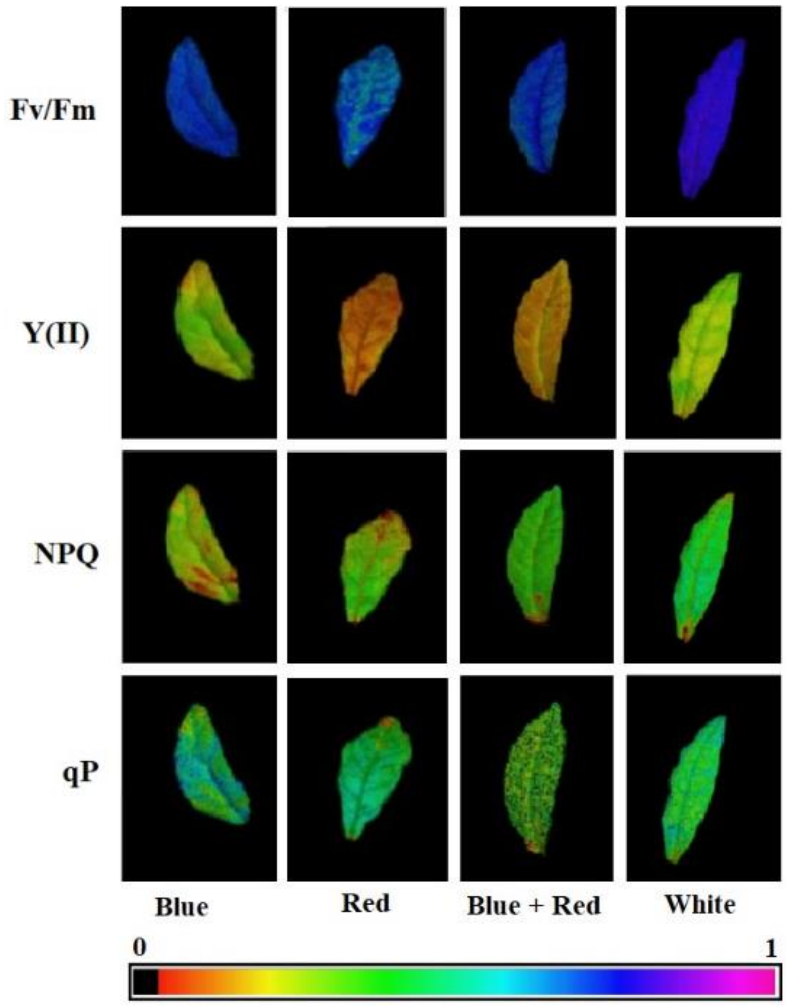

Fig. 6. Maximum quantum yield (F_v/F_m), effective quantum yield (YII), non-photochemical quenching (NPQ) and photochemical quenching (qP) on leaves of *C. pubescens*, after 45 days under different light qualities. Image obtained by evaluating the fluorescence of chlorophyll *a* through the fluorescence modulated Imaging-PAM (Heinz Walz, Effeltrich, Germany).

3.4. Chloroplastidic pigments of Campomanesia pubescens after 45 days under different light qualities

Levels of chlorophyll *a*, *b*, carotenoids and total chlorophyll were significantly higher in the white LED with 40.85 μg cm^{-2}, 19.26 μg cm^{-2}, 14.50 μg cm^{-2} and 60.12 μg cm^{-2} respectively, while in the red monochromatic LED the lowest values of chlorophyll and carotenoids were 7.72 μg cm^{-2} and 12.06 μg cm^{-2}, respectively (Table 4).

Table 4. Chloroplastidic pigments: chlorophyll *a*, chlorophyll *b*, carotenoids, total chlorophyll and of *C. pubescens* after 45days under different light qualities

Light quality (LEDs)	Chloroplastidic pigments			
	chlorophyll *a* (µg cm^{-2})	chlorophyll *b* (µg cm^{-2})	carotenoids (µg cm^{-2})	total chlorophyll (µg cm^{-2})
Blue	28.28±1.53b[z]	14.52±0.41bc	9.40±0.22bc	42.81±1.84b
Red	20.15±2.11b	12.06±1.39c	7.72±0.42c	32.22±3.49b
Blue-Red	27.56±1.79b	16.00±0.95ab	10.48±0.81b	43.56±2.68b
White	40.85±3.41a	19.26±0.96a	14.50±0.45a	60.12±4.26a

[Z]Means followed by the same letter in each column do not differ statistically among themselves using the Tukey's test at 1% probability. [±]Standard error.

3.5. Malondialdehyde content (MDA) of Campomanesia pubescens after 45 days under different light qualities

Seedlings *C. pubescens* cultivated under red monochromatic LED presented higher values of MDA in their leaves compared to the other LEDs (Fig. 7).

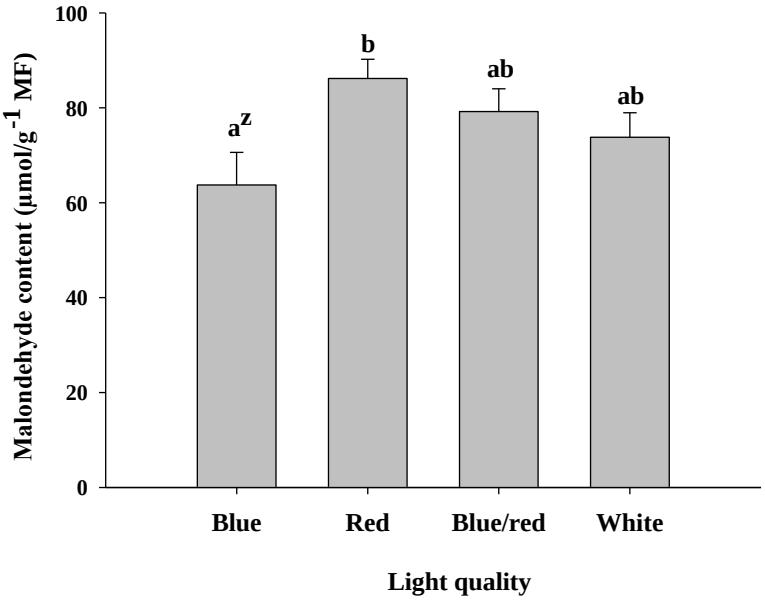

Fig. 7. Malondialdehyde contents in leaves of *C. pubescens* after 45 days under different light qualities. [Z]Means followed by the same letter do not differ statistically among themselves using the Tukey's test at 5% probability.

3.6. Correlation coefficient (r) between the anatomy and physiology of Campomanesia pubescens after 45 days under blue/red and white LEDs

There was a correlation between the anatomical and physiological characters evaluated in the blue/red LEDs, a strong positive correlation ($0.8 \leq r < 1$) occurred between the variables Abe p and NPQ; Ad ep and CP; DE and Fv/Fm; Chla and Chlb; Chla and ChlT; Chlb and ChlT; Y(II) and qP. Lower and negative correlations were observed (Table 5).

Table 5. Estimates of correlation coefficients for anatomic[1] and physiological[2] variables in leaves of *C. pubescens*, cultivated under blue/red light.

	Ab ep	Ad ep	CP	Dens	DP	DE	DP/E	Chla	Chlb	Carot	ChlT	Fv/Fm	Y(II)	NPQ	qP
Ab ep	1														
Ad ep	0.55**	1													
CP	0.71**	0.88**	1												
Dens	-0.30^{ns}	0.61**	0.38^{ns}	1											
DP	0.16^{ns}	0.53**	0.66**	0.44^{ns}	1										
DE	0.06^{ns}	0.17^{ns}	-0.16^{ns}	-0.04^{ns}	-0.01^{ns}	1									
DP/E	0.72**	0.54**	0.35^{ns}	-0.12^{ns}	-0.28^{ns}	0.48^{ns}	1								
Chla	-0.93**	-0.82**	-0.87**	-0.05^{ns}	-0.39^{ns}	-0.16^{ns}	-0.71**	1							
Chlb	-0.92**	-0.55**	-0.57**	0.31^{ns}	-0.15^{ns}	-0.44^{ns}	-0.82**	0.89**	1						
Carot	-0.71**	-0.60**	-0.90**	-0.08^{ns}	-0.72*	0.33^{ns}	-0.11^{ns}	0.76**	0.52**	1					
ChlT	-0.95**	-0.74**	-0.79**	0.08^{ns}	-0.32^{ns}	-0.26^{ns}	-0.77**	0.99**	0.95**	0.69**	1				
Fv/Fm	0.15^{ns}	-0.05^{ns}	-0.18^{ns}	-0.38^{ns}	0.11^{ns}	0.88**	0.29^{ns}	-0.15^{ns}	-0.49^{ns}	0.14^{ns}	-0.27^{ns}	1			
Y(II)	0.30^{ns}	-0.15^{ns}	0.23^{ns}	-0.47^{ns}	0.57**	-0.14^{ns}	-0.35^{ns}	-0.19^{ns}	-0.24^{ns}	-0.60**	-0.22^{ns}	0.31^{ns}	1		
NPQ	0.84**	0.44^{ns}	0.60**	-0.19^{ns}	-0.16^{ns}	-0.33^{ns}	0.65**	-0.72**	-0.61**	-0.54**	-0.70**	-0.35^{ns}	-0.05^{ns}	1	
qP	0.57**	0.03^{ns}	0.47^{ns}	-0.46^{ns}	0.50**	-0.34^{ns}	-0.13^{ns}	-0.44^{ns}	-0.41^{ns}	-0.80**	-0.44^{ns}	0.07^{ns}	0.92**	0.34^{ns}	1

[1]Ab ep: abaxial epidermis; Ad ep: adaxial epidermis; CP: chlorophyllic parenchyma; Dens: stomatal density; SD: polar diameter of the stomata; DE: equatorial diameter of the stomata; DP/E: ratio of polar / equatorial diameter. [2] Chla: chlorophyll a; Chlb: chlorophyll b; Carot: carotenoids; ChlT: total chlorophyll; Fv/Fm: maximum quantum yield; Y(II): effective quantum yield; NPQ: non-photochemical dissipation; qP: photochemical dissipation. ns, * and **: non-significant, significant at 5% and 1%, respectively, by the F test

Regarding the correlations between the anatomical and physiological characters evaluated in the white LED, there was a strong positive correlation ($0.8 \leq r < 1$) between the variables Abe p and CP; Dens and Y (II); Dens and qP; DP and NPQ; DP/E and Chla; DP/E and Chlb; DP/E and Carot; DP/E and ChlT; Chla and Chla; Chla and ChlT; Chlb and Carot; Chlb and ChlT; Chlb and Y(II); Chlb and qP; Carot and ChlT; Carot and Y(II); Carot and qP. Positive perfect correlation ($r = 1$) between the variables Y(II) and qP. Lower and negative correlations were observed (Table 6).

Table 6. Estimates of correlation coefficients for anatomical[1] and physiological[2] variables evaluated in leaves of *C. pubescens* cultivated under white light.

	Ad ep	CP	Dens	DP	DE	DP/E	Chl*a*	Chl*b*	Carot	ChlT	Fv/Fm	Y(II)	NPQ	qP
Ab ep														
Ad ep	1													
CP	0.43^{ns}	1												
Dens	-0.23^{ns}	-0.66^{**}	1											
DP	0.53^{**}	0.07^{ns}	-0.62^{**}	1										
DE	0.37^{ns}	-0.65^{**}	0.31^{ns}	0.54^{**}	1									
DP/E	-0.23^{ns}	0.25^{ns}	0.44^{ns}	-0.93^{**}	-0.64^{**}	1								
Chla	-0.52^{**}	0.27^{ns}	0.26^{ns}	-0.89^{**}	-0.83^{**}	0.88^{**}	1							
Chlb	-0.27^{ns}	0.07^{ns}	0.61^{**}	-0.96^{**}	-0.50^{**}	0.98^{**}	0.83^{**}	1						
Carot	-0.21^{ns}	0.01^{ns}	0.70^{**}	-0.94^{**}	-0.39^{ns}	0.94^{**}	0.79^{**}	0.98^{**}	1					
ChlT	-0.48^{ns}	0.23^{ns}	0.35^{ns}	-0.93^{**}	-0.78^{**}	0.93^{**}	0.99^{**}	0.89^{**}	0.86^{**}	1				
Cla/Clb	-0.61^{**}	0.38^{ns}	-0.22^{ns}	-0.47^{ns}	-0.88^{**}	0.43^{ns}	0.79^{**}	0.32^{ns}	0.27^{ns}	0.71^{**}				
Fv/Fm	0.31^{ns}	-0.53^{**}	0.65^{**}	-0.08^{ns}	0.69^{**}	0.06^{ns}	-0.36^{ns}	0.21^{ns}	0.25^{ns}	-0.24^{ns}	1			

Y(II)	-0.32^{ns}	-0.29^{ns}	0.86**	-0.84**	-0.16^{ns}	0.73**	0.67**	0.82**	0.90**	0.73**	0.25^{ns}	1	
NPQ	0.39^{ns}	0.27^{ns}	-0.84**	0.90**	0.24^{ns}	-0.78**	-0.73**	-0.87**	-0.93**	-0.79**	-0.22^{ns}	-0.99**	1
qP	-0.35^{ns}	-0.24^{ns}	0.81**	-0.85**	-0.22^{ns}	0.73**	0.72**	0.82**	0.89**	0.76**	0.16^{ns}	1.00**	-0.99**	1

[1]Ab ep: abaxial epidermis; Ad ep: adaxial epidermis; CP: chlorophyllic parenchyma; Dens: stomatal density; SD: polar diameter of the stomata; DE: equatorial diameter of the stomata; DP/E: ratio of polar/equatorial diameter[2]. Chl*a*: chlorophyll *a*; Chl*b*: chlorophyll *b*; Carot: carotenoids; ChlT: total chlorophyll; Fv/Fm: maximum quantum yield; Y(II): effective quantum yield; NPQ: non-photochemical dissipation; qP: photochemical dissipation. ns, * and **: non-significant, significant at 5% and 1%, respectively, by the F test

4. Discussion

4.1. Growth characteristics of C. pubescens seedlings in different light qualities.

Light modulates growth and development of plants, interacting with the physiological and morphological processes in several ways, depending on the species (Simlat et al., 2016). Different light qualities affected the growth of *C. pubescens* seedlings as observed in white and blue/red LEDs (1:1) with higher values for all the growth variables analyzed in this study (Fig. 2 and 3). The results showed higher photochemical efficiency of FSII in these bands of visible light spectrum (400-700 nm) and better photochemical quenching (qP) (Table 3). The perception of these wavelengths is performed by photoreceptors and pigments that correspond to blue (400-500 nm), and red (600-700 nm) (Huché-Thélier et al., 2016). These wavelengths are more effective in stimulating FSII activity (Zienkiewiewicz et al., 2015). Results obtained in this study showed that when using white and blue/red combination LEDs for all analyzed growth variables (Fig. 2) there was a greater growth in *C. pubescens* seedlings.

There were morphoanatomic and physiological variations in *C. pubescens* seedlings, depending on the type of LED used. There was an increase in the photosynthetic rate and synthesis of chlorophylls *a*, *b* and carotenoids when white LED was used, influencing seedlings growth (Tables 3 and 4), suggesting a relationship between the cryptochrome and the phytochrome (Hernandéz et al., 2016). Similar results were described by Liu et al. (2011b) in plants of *Solanum lycopersicum*, and Li et al. (2012) analyzing the effects of different light qualities on *Brassica campestris* L.. In seedlings of *Cucumis sativus*, Li and Kubota (2009) and Hernández and Kubota (2014) verified that in leaves with greater leaf area there is more light interception, promoting a significant biomass increase. Greater leaf area was obtained with white, blue/red (1:1) and red on *C. pubescens* seedlings (Fig. 3). Macedo et al. (2011) have recommended studies using combinations of the blue and red bands to obtain higher-quality of *Alternanthera brasiliana* Kuntze seedlings due to the influence of these wavelengths on the responses of cryptochromes and phytochromes.

4.2. Foliar morphoanatomic modifications of C. pubescens seedlings in different light qualities

Different light qualities provided variations in the thickness of the foliar tissues, providing greater capacity of using the light for photosynthesis. The blue light causes phosphorylation and activation of H^+-ATPases of the plasma membrane, keeping stomata open to facilitate the gases exchange between the plant and the atmosphere. This gases exchange is necessary for photosynthesis and for water captation through the roots. To date, important components involved in the signaling pathway of blue light in stomatal guard cells such as phototropines, BLUS1, BHP, PP1, and PM H^+ -ATPase have been identified. However, the signaling mechanism is not fully understood (Inouea and Kinoshita, 2017). In the blue monochromatic LED, a greater stomatal density was observed, favoring the acclimatization, providing better control in gas exchanges and reduction of water loss. The stomatal density was lower in blue/red LEDs than in the blue. However, in the blue/red LEDs there were larger stomata in relation to the blue one. Elliptic stomata were found on the white LED, improving gas exchange, CO_2 fixation and providing more raw material for photosynthesis, resulting in a superior leaf and stem dry mass and in the seedlings under blue LED (Table 2F). A similar result was found by Kim et al. (2004) with *Chrysanthemum*.

In this study, a greater thickness of the epidermis and the chlorophyll parenchyma in the blue LED was observed, indicating that the photoassimilates were directed to increase epidermis thickness and to present differences in the cells of the mesophyll making them heterogeneous, while in the red LED there was a smaller thickness of foliar tissues with homogeneous mesophyll cells. Variations in leaf thickness are mainly attributed to different proportions of leaf mesophyll, which are influenced by light. Terfa et al. (2013) analyzing the leaf formation of Rosa x hybrida showed that the addition of blue light increased leaf thickness from 5 to 20%.

4.3. Physiological responses of C. pubescens seedlings in different light qualities

The reduction of the F_v/F_m ratio may indicate possible oxidative damage to the light collecting complex and to the FSII reaction center. The maximum quantum yield of photosystem II - Fv/Fm in the *C. pubescens* seedlings was above 0.7 in the white, blue and blue/red LEDs (Table 3), increasing the photosynthetic efficiency due to the distribution of spectral energy which coincides with the peaks of chlorophyll absorption, increasing seedling growth and development. Similar results were described by Yang et

al. (2018) in *Solanum lycopersicum* L. In the red LED this value was relatively lower and was observed in plants under light and water stress conditions, such as in *Hymenaea stigonocarpa* Mart. (Costa et al., 2015). Prolonged exposure to red LEDs may result in poor photosynthetic performance, causing damage to the thylakoid membrane, being deficient in chlorophyll, reducing photosynthetic efficiency (Hogewoning et al., 2010).

Photoinhibition is characterized by the degradation of D1 protein at the FSII reaction center, interfering the electron transport, reducing the maximum quantum efficiency (Santos et al., 2013). Thus, reductions in the Fv/Fm ratio may be indicative of damage to the photosynthetic machinery in relation to the influence of some stress (Araújo and Deminicis, 2009). In this study, there was a reduction of both Fv/Fm and effective quantum yield, YII and an increase of NPQ in *C. pubescens* seedlings grown under red monochromatic LEDs, with lower values of chlorophyll *a, b* and carotenoids and could indicate excessive damage induced by the time of exposure to this condition, increasing the MDA value indicating a peroxidation of membrane lipids induced by stress at the cellular level. Therefore, the level of malondialdehyde (MDA) may be an indicator of oxidative damage (Shohael et al., 2006).

It was verified that when white and blue/red LEDs were used, there was a correlation between the anatomical and physiological characteristics evaluated in seedlings of *C. pubescens*. In the blue/red LED, there was a strong and significant correlation between the abaxial epidermis with the NPQ and adaxial epidermis with increase of the chlorophyllic parenchyma. The increase of chlorophylls *a, b*, total chlorophyll and carotenoids was correlated with the capture of light and protection of photosystem II. Y (II) was correlated with qP with better use of energy in the photochemical dissipation. In the white LED correlations between abaxial epidermis, there was an increase in the chlorophilic parenchyma. It was observed a correlation between the polar and equatorial diameter of the stomata, increasing the content of chlorophyll *a, b*, carotenoids and total chlorophyll, providing better photochemical efficiency of photosystem II. The effects and mechanisms associated with light quality in plants may vary according to species. The adaptive capacity of seedlings to adjust morphologically and physiologically according to changes in light quality may be decisive for their survival in different environments.

5. Conclusion

C. pubescens seedlings presented varied morphological and physiological characteristics depending on the type of light. White and blue/red (1:1) LEDs are promising alternatives for the propagation of the species, due to their positive influence on most of the characteristics under study.

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References

Ali M., Abbasi B. H., 2014. Light-induced fluctuations in biomass accumulation, secondary metabolites production and antioxidant activity in cell suspension cultures of *Artemisia absinthium* L. J Photochem Photobiol B. 140, 223-227. http://dx.doi:10.1016/j.jphotobiol.2014.08.008.

Araújo, S. A. C., Deminicis, B. B., 2009. Fotoinibição da Fotossíntese. Rev Bras Biocienc. 7(4), 463-472.

Arnott, H. J., 1959. Leaf clearings. Turtox News 37(8), 192-194.

Bilger, W., Björkman, O., 1990. Role of xanthophyll cycle in photoprotection elucidated by measurements of light induced absorbance changes, fluorescence and photosynthesis in leaves of Hedera canariensis. Photosynth Res. 25, 173-185. http://dx.doi:10.1007/BF00033159.

Brazaityte, A., Duchovskis, P., Urbonavičiute, A., Samuoliene. G., Jankauskiene J., Kasiulevičiute-Bonakere, A., Bliznikas, Z., Novčkovas, A., Bree K., Žukauskas, A., 2009. The effect of light-emitting diodes lighting on cucumber transplants and after-effect on yield. Zemdirbyste Agriculture. 96(3), 102-118. UDK 635.6:581.132.

Brazaityte, A., Duchovskis, P., 2010. The effect of light-emitting diodes lighting on the growth of tomato transplants. Zemdirbyste-Agriculture. 97(2), 89-98. UDK 635.64.531.031.027.34.

Cakmak, L., Horst W. J., 1991. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxide activity in root tip of soybean (*Glycine max*). Plant Physiol. 83(3), 463-468. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x

Cardoso, C. A. L. Salmazzo, G. R., Honda, N. K., Prates, C. B., Vieira, M. D. C., Coelho, R. G., 2010. Antimicrobial activity of the extracts and fractions of hexanic fruits of *Campomanesia* species (Myrtaceae). J Med Food. 13(5), 1273-1276. http://dx.doi:10.1089/jmf.2009.0047.

Carvalho, R. F., Campos, M. L., Azevedo, R. A., 2011. The role of phytochrome in stress tolerance. J Integr Plant Biol. 53(12), 920-92. http://dx.doi.org/10.1111/j.1744-7909.2011.01081.x.

Carvalho, S. D., Folta, K. M., 2014. Sequential light programs shape kale (*Brassica napus*) sprout appearance and alter metabolic and nutrient content. Hortic Res. 8(1), 1-13. http://doi.org/10.1038/hortres.2014.8.

Chang, R., Morais, S. A. L., Nascimento, E. A., Cunha, L. C., Rocha, E. D. O., Aquino, F. J. T., Souza M. G. M, Cunha W.R., Martins, C. H.. 2011. Essential oil composition and antioxidant and antimicrobial properties of *Campomanesia pubescens* O. Berg, Native of Brazilian Cerrado. Lat Am J Pharm. 30(9), 1843-1848.

Costa, A . C., Rosa, M., Megguer, C. A., Silva, F. G., Pereira, F. D., Otoni, W. C., 2014. A reliable methodology for assessing the in vitro photosynthetic competence of two Brazilian savanna species: Hyptis marrubioides and Hancornia speciosa. Plant Cell Tiss Organ Cult. 117(3), 443-454. http://dx.doi 10.1007/s11240-014-0455-y.

Costa, A. C., Resende-Silva, S. L., Megguer, C. A., Moura, L. M. F., Rosa, M., Silva, A. A., 2015. The effect of irradiance and water restriction on photosynthesis in young jatobá-do-cerrado (*Hymenaea stigonocarpa*) plants. Photosynthetica 53(1), 118-127. http://dx.doi:10.1007/s11099-015-0085-6.

Dong, C., Fu, Y., Liu, G., Liu, H., 2014. Low light intensity effects on the growth, photosynthetic characteristics, antioxidant capacity, yield and quality of wheat (*Triticum aestivum* L.) at different growth stages in BLSS. Adv Space Res. *53*(11), 1557-1566. http://dx.doi:10.1016/j.asr.2014.02.004.

Fan, X., Xu, z. G., Liu,X. Y., Tang, C. M., Wang, L. W., Han, X. L., 2013. Effects of light intensity on the growth and leaf development of young tomato plants grown under a combination of red and blue light. Sci Hortic. 153, 50-55. https://doi.org/10.1016/j.scienta.2013.01.017.

Ferreira, D. F., 2011. Sisvar: a computer statistical analysis system. Ciência e agrotecnologia. 35(6), 1039-1042. http://dx.doi.org/10.1590/S1413-70542011000600001.

Fogaça, L. A., Dortzbach, D., Alves, A. C., Pedrotti, E. L., 2007. Características morfofisiológicas de brotos micropropagados de agapantho sob diferentes intensidades luminosas e concentrações de sacarose. Scientia Agraria. 8(4), 371-378. http://dx.doi.org/10.5380/rsa.v8i4.9882.

Fraszczak, B., Golcz, A., Zawirska-Wojtasiak, R., Janowska, B., 2014. Growth rate of sweet basil and lemon balm plants grown under fluorescent lamps and LED modules. Acta Sci. *Pol*. 13(2), 3-13.

Genty, B., Briantais, J. M., Baker, N. R., 1989. A relação entre o rendimento quântico de transporte de electrões fotossintéticos e extinção de fluorescência da clorofila. Biochim. Biophys. *Acta*. 990, 87-92.

Gogosz, A. M., Cosmo, N. L., Bona, C., Souza, L. A., 2010. Morfoanatomia da plântula de *Campomanesia xanthocarpa* O. Berg., (Myrtaceae). Acta Bot Bras. 24(3), 613-623. http://dx.doi.org/10.1590/S0102-33062010000300003.

Heath, R. L., Packer, L., 1968. . Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives in Biochemistry and Biophysics. 125, 189–198. https://doi.org/10.1016/0003-9861(68)90654-1.

Hernández, R., Kubota, C., 2014. Growth and morphological response of cucumber seedlings to supplemental red and blue photon flux ratios under varied solar daily light integrals. Sci Hortic-amsterdam. 173(8), 92-99. http://dx.doi.org/10.1016%2Fj.scienta.2014.04.035.

Hernández, R., Eguchi, T., Deveci, M., Kubota, C., 2016. Tomato seedling physiological responses under different percentages of blue and red photon flux ratios using

LEDs and cool white fluorescent lamps. Sci Hortic-amsterdam. 213, 270–280. https://doi.org/10.1016/j.scienta.2016.11.005.

Huché-Thélier, L., Crespel, L., Gourrierec, J. L., Morel, P., Sakr, S., Leduc, N., 2016. Light signaling and plant responses to blue and UV radiations - Perspectives for applications in horticilture. Environmental and Experimental Botany. 121, 22-38. https://doi.org/10.1016/j.envexpbot.2015.06.009.

Hogewoning, S. W., Trouwborst, G., Maljaars, H., Poorter, H., van Ieperen, W., Harbinson, J., 2010. Blue light dose-responses o leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. J. Exp.Bot. 61(11), 3107–3117. doi.org/10.1093%2Fjxb%2Ferq132.

IBGE - Instituto Brasileiro de Geografia e Estatística, 2010. Divisão Territorial do Brasil e Limites Territoriais.

Inouea, S., Kinoshita, T., 2017. Blue light regulation of stomatal opening and the plasma membrane H^+-ATPase. Plant Physiology, 172(2), 531-538. https://doi.org/10.1104/pp.17.00166

Karnovsky, M. J., 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J Cell Biol. 27, 137-8A.

Kim S.J., Hahn E.J., Paek K.Y., 2004. Effects of LEDs on net photosynthetic rate, growth ande leaf stomata of chrysanthemum plantlets *in vitro*. Sci Hortic-amsterdam. 101(1), 43-51. https://doi.org/10.1016%2Fj.scienta.2003.10.003.

Klafke, J. Z., da Silva, M. A., Panigas, T. F., Belli, K. C., de Oliveira, M. F., Barichello, M. M., Ferreira, J., 2010. Effects of *Campomanesia xanthocarpa* on biochemical, hematological and oxidative stress parameters in hypercholesterolemic patients. J Ethnopharmacol. 127(2), 299-305. https://doi. 10.1016/j.jep.2009.11.004.

Kooten, O., Snel, F. H., 1990. Photosynth Res. 25: 147

Leite, M. S., Silva, F. G., Assis, E. S., Rubio Neto, A., Mendes, G. C., Rosa, M., 2017. Morphoanatomy and physiology of Pouteria gardneriana Radlk plantlets grown in vitro at varied photosynthetic photon flux densities. Acta Sci-Agron. 39(2), 217-224. http://dx.doi.org/10.4025/actasciagron.v39i2.32515.

Li, Q., Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ *Exp Bot*. 67(1): 59–64. https://doi.org/10.1016/j.envexpbot.2009.06.011.

Li, H., Tang, C., Xu, Z., Liu, X., Han, X., 2012. Effects of different light sources on the growth of non-heading Chinese cabbage (*Brassica campestris* L.).J. Agri. Sci. 4(4), 262-270. https://doi.org/10.5539/jas.v4n4p262.

Li, H., Tang, C., Xu, Z., 2013. The effects of different light qualities on rapeseed (*Brassica napus* L.) plantlet growth and morphogenesis in vitro. Sci Hortic-amsterdam. 150, 117–124. http://dx.doi.org/10.1016/j.scienta.2012.10.009.

Li, J., Cang Z., Jião F., Bai X., Zhang D., 2017. Influence of drought stress on photosynthetic characteristics and protective enzymes of potato at seedling stage. Journal of the Saudi Society of Agricultural Sciencehttps. 16(1), 82-88. http://dx.doi.org/10.1016/j.jssas.2015.03.001.

Lin, K. H., Huang, M. Y., Huang, W. D., Hsu, M. H., Yang, Z.W., Yang, C.M., 2013. The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (Lactuca sativa L. var. capitata). Sci Hortic-amsterdam. 150, 86–91. http://dx.doi: 10.1016/j.scienta.2012.10.002

Liu, X. Y., Guo, S. R., Xu, Z. G., Jiao, X. L., Tezuka, T., 2011b. Regulation of chloroplast ultrastructure, cross-section anatomy of leaves, and morphology of stomata of cherry tomato by different light irradiations of light-emitting diodes. HortScience. 46(2), 217-221.

Liu, M., Xu, Z., Guo, S., Tanga, C., Liu, X., Jao, X., 2014. Evaluation Of leaf morphology, structure and biochemical substance of balloon flower (*Platycodon grandiflorum* (Jacq.) A. DC.) plantlets in vitro under different light spectra. Sci Hortic-amsterdam. 174, 112–118. https://doi.org/10.1016/j.scienta.2014.05.006.

Macedo, A. F., Tavares, E., Leal-Costa M., Esquibel, M. A., 2011. The effect of light quality on leaf production and development of *in vitro*-cultured plants of *Alternanthera brasiliana* Kuntze. Environ Exp Bot. 70(1), 43-50. https://doi.10.1016/j.envexpbot.2010.05.012.

Massa G. D., Kim H. H., Wheeler R. M., Mitchell C. A., 2008. Plant productivity in response to LED lighting. HortScience. 43(7), 1951-1956.

MMA, 2018. Site do Ministério do Meio Ambiente. Bioma Cerrado. http://www.mma.gov.br/biomas/cerrado.

Morelli, G., Ruberti, I., 2000. Shade avoidance responses. Driving auxin along lateral routes. Plant Physiol. 122(3), 621-626. https://doi.org/10.1104/pp.122.3.621.

Morrow R. C., 2008. LED lighting in horticulture. HortScience. 43(7), 1947-1950.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plantarum. 15(3), 473-497. https://doi.10.1111/j.1399-3054.1962.tb08052.x.

O'Brien, T. P., Feder, N., McCully, M. E., 1964. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma. 59(1), 368-373.

Ouzounis, T., Parjikolaei, R., B., Fretté, X., Rosenqvist, E., Ottosen, C. O., 2015. Predawn and high intensity application of supplemental blue light decreases the quantum yield of PSII and enhances the amount of phenolic acids, flavonoids end pigments in *Lactuca sativa*. Front Plant Sci. 6, 1-14. https://dx.doi.org/10.3389%2Ffpls.2015.00019.

Ronquim, C. C., Prado, C. H. B. A.; Souza, J. P., 2009. Growth, photosynthesis and leaf water potential in Young plants of *Capaifera langsdorffii* Desf. (Caesalpiniaceae) under contrasting irradiances. Braz Society Plant Phys. 21(3), 197- 208. http://dx.doi.org/10.1590/S1677-04202009000300004.

Santos, C. M., Verissimo, V., Wanderley Filho, H. C. L., Ferreira, V. M., Cavalcante, P. G. S., Rolim, E. V., Endres, L., 2013. Seasonal variations of photosyntesis gas exchange, quantum efficiency of photosystem II and biochemical responses of *Jatropha curcas* L. grow in semi-humid and semi-arid áreas subjetict to water stress. Ind Crop Prod. 41(6), 203-213. http://dx.doi.org/10.1016/j.indcrop.2012.04.003.

Shohael, A. M., Ali, M. B., Yu, K. W., Hahn, E. J., Islam, R., Paek, K.Y., 2006. Effect of light on oxidative stress, secondary metabolites and induction of antioxidant enzymes in Eleutherococcus senticosus somatic embryos in bioreactor. *Process Biochemistr*y. 41(5), 179-1185.

Simlat, M., Slezak P., Mos, M., Warchoł, M., Skrzypek, E.,Ptak, A., 2016. The effect of light quality on seed germination, seedling growth and selected biochemical properties of Stevia rebaudiana Bertoni. Sci Hortic-amsterdam. 211, 295–304. http://dx.doi.org/10.1016/j.scienta.2016.09.009.

Souza, V. C., Lorenzi, H., 2008. Botânica sistemática: guia ilustrado para identificação das famílias de fanerógamas nativas e exóticas no Brasil, baseado em APG II. 2ª ed. Instituto Plantarum, Nova Odessa.

Terfa, M.T., Solhaug, K. A., Gislerød, H. R., Olsen, J. E., Torre, S., 2013. A high proportion of blue light increases the photosynthesis capacity and leaf formation

rate of Rosa × hybrida but does not affect time to flower opening. Plant Physiol. 148(1), 146-159. https://doi.10.1111/j.1399-3054.2012.01698.x.

Vinagre, A. S., Rönnau, Â. D. S. R. O., Pereira, S. F., Silveira, L. U. D., Wiilland, E. D. F., Suyenaga, E. S., 2010. Anti-diabetic effects of *Campomanesia xanthocarpa* (Berg) leaf decoction. Braz J of Pharma Sci. 46(2), 169-177. http://dx.doi.org/10.1590/S1984-82502010000200002.

Wellburn, A. R., 1994. The spectral determination of Chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol. 144(3), 307-313. https://doi.org/10.1016/S0176-1617(11).

Yang, X. Y., Xu, X. M., Cui, J., 2018. Response of photosynthetic capacity of tomato leaves to different https://doi.org/10.1016/j.envexpbot.2015.06.012 LED light wavelength. Environ Exp Bot. *150*, 161–171. https://doi.org/10.1016/j.envexpbot.2018.03.013.

Zienkiewicz, M., Drozak, A., Wasilewska, W., Bacławska, I., Przedpełska-Wasowicz, E., Romanowska, E., 2015. The short-term response of *Arabidopsis thaliana* (C3) and Zea mays (C4) chloroplasts to red and far red light. Planta. 242(6), 1479-1493. https://doi.10.1007/s00425-015-2392-3.

CAPÍTULO II

In vitro culture of *Campomanesia pubescens* under different light qualities: a morphoanatomical and physiological characterization

Abstract

Campomanesia pubescens (gabiroba) is a fruit tree that is native to the Cerrado and that has commercial potential and medicinal properties. It has recalcitrant seeds that do not tolerate desiccation and storage. Here, *C. pubescens* plants were grown *in vitro* under different light qualities. Light-emitting diodes (LEDs) were used at wavelengths for white (W) and blue in combination with red in the proportions BR (1:1), BR (1:3) and BR (3:1), at 100 ± 5 μmol m^{-2} s^{-1} over a 16-hour photoperiod. The leaf anatomy, *chlorophyll a* fluorescence, chloroplast pigments and malondialdehyde (MDA) content were evaluated. When the plants were cultivated in combinations of blue/red LEDs 1:1 and 3:1, they yielded higher biomass, presenting higher epidermis and chlorenchyma values with greater stomatal density and functionality, better photosynthetic efficiency, higher Y(II) values, qP and higher total chlorophyll concentrations, and thus these lights did not cause oxidative damage compared to the white (control) LEDs. This study contributed to a better understanding of the anatomical and physiological changes in *C. pubescens* plants grown *in vitro* under different light qualities.

Key words: Light-emitting diodes, Myrtaceae, Chlorophyll *a* fluorescence, Tissue culture, Correlation network

1 Introduction

Campomanesia pubescens (DC.) O. Berg. is native to the Cerrado, and it is found in South America, Southeast Asia and Australia, and in Brazil, it is found in the Southeast and Midwest regions (Chang et al., 2011). This plant belongs to the Myrtaceae family, and its genus has 25 species, including 15 species native to Brazil. Known popularly as "gabirobeira", "guabiroba" and "guabiroba-do-campo", this species stands out for its economic potential because its fruits are consumed *in natura* or as juices, jams, jellies and ice cream (Bardiviesso et al., 2011). The fruit contains high levels of vitamin C and is indicated for use against flus and colds; it is also used in the control of diabetes and for combating cholesterol and obesity (Cardoso et al., 2010; Klafke et al., 2010; Vinagre et al., 2010).

Studies on native species are scarce and restricted to a few species, and there is a need for expansion to other species of Cerrado fruit trees, especially those that pose difficulties during propagation by other methods (traditional) or those that are limited by natural genetic variability (Pinhal et al., 2011). Nondomesticated plants, such as gabiroba, tend to have several tissue responses when they are grown *in vitro* due to their genetic variability. However, this genetic variability is very interesting because it allows for the production of products with different characteristics, such as pharmaceuticals and dyes, within the same species (Hartmann et al., 2010).

Using tissue culture to propagate Cerrado fruit trees can minimize the loss of genetic material by destruction from the environment through systematized plant multiplication (Pinhal et al., 2011). The challenge of tissue culture is to provide a

sufficient controlled light intensity and quality for plant development (Dong et al., 2014; Samuoliene et al., 2013). Light-emitting diodes (LEDs) have been proposed as a light source for controlled environments because they have desirable characteristics, such as the ability to control the spectral composition, a long durability, the ability to emit specific wavelengths, and relatively cold emission surfaces, in addition to their reduced size, which facilitates their management and installation in growth chambers (Li et al., 2013; Muneer et al., 2014).

The development and physiology of plants are strongly influenced by the lights in their growth environment, specifically the intensity, wavelength, and direction in which the lights are emitted and how they are perceived by plant photoreceptors, such as phytochromes, cryptochromes, and phototropins, which generates a series of specific physiological responses (Li and Kubota, 2009). Blue light is involved in the processes of phototropism, photomorphogenesis, and stomatal opening, whereas red light promotes germination, de-etiolation, and growth and has great potential as a source of light for photosynthesis because it emits a light spectrum close to the maximum absorbance of both chlorophylls and phytochromes (Muneer et al., 2014).

Studies on Cerrado plants grown *in vitro* focusing on light quality and its effects on plant development and growth are scarce. Records of anatomical and physiological *C. pubescens* characteristics when grown *in vitro* under different lighting qualities using LEDs are nonexistent. In this context, the aim of this study was to analyze the anatomical and physiological responses of *C. pubescens* seedlings from *in vitro* cultivation under different light qualities, to support future studies on the propagation and conservation of this species.

2 Materials and methods

2.1 Plant material and establishment of seedlings under different light qualities

The fruits of *C. pubescens* were collected from November to December of 2017 in the municipality of Iporá, GO, Brazil, at the geographic locations (latitude, longitude and altitude) of 16°25`32.16 ", 51°07'37.86" and 587 m. The experimental assays were performed at the Laboratory of Plant Tissue Culture, Ecophysiology and Plant Productivity, Seeds and Plant Anatomy of the Federal Institute of Goiano - Rio Verde Campus, GO.

After the fruit collection, the fruit's pulp was stripped to remove the mucilage. For this purpose, 5% sodium hydroxide was applied for 5 min and subsequently washed away with running water, and the samples were dried on paper towels. The seeds were germinated in plastic trays under washed coarse sand. Each tray contained 100 seeds and remained in the growth room at an average temperature of 25 ± 3°C.

The phytosanitary management of the seedlings was performed 24 hours before inoculation by spraying a systemic fungicidal solution of Derosal ® (carbendazim 500 g L^{-1}) to 0.2% of the commercial product. A nutrient solution made up of 50% MS medium salts was applied every two weeks (Murashige and Skoog, 1962). At 60 days after sowing, the nodal segments measuring 2 cm and two axillary buds were collected and used as a source of explants for the experiments.

For the disinfestation, the explants were coated with gauze and placed in running water with three drops of neutral Tween detergent for 15 min, and then they were immersed in 70% alcohol for 1 min. They were then subjected to 20% commercial sodium hypochlorite solution (NaClO, commercial bleach, 2.5% active chlorine) and washed 3 times with sterile water in a laminar flow hood. The explants were inoculated in test tubes (25 x 150 mm) containing 20 mL of MS medium at a 50% salt concentration(Murashige

and Skoog, 1962), 5 g L^{-1} sucrose, and 2.0 g L^{-1} charcoal and then supplemented with 3.5 g L^{-1} agar, and the pH was adjusted to 5.7 ± 0.03. Subsequently, the medium was autoclaved at a temperature of 121°C for 20 min.

The explants were transferred to a growth chamber with ventilated shelves under 20 W light emitting diode (LED) tubes (Lanao series Tubes, China). Under these conditions, the following different spectral ranges were used: white (W), blue/red (BR 1:1), blue/red (BR 1:3), and blue/red (BR 3:1) under a photosynthetic photon flux density (PPFD) of 100 ± 5 μmol m^{-2} s^{-1}, a photoperiod of 16 hours and a temperature of 24 ± 3°C. The spectral quality was determined using a USB 2000 spectroradiometer (Ocean Optics, Dunedin, FL, USA), and the light intensity was adjusted using a PAR sensor (QSO-S; Decagon Devices, Pullman, WA, USA). The evaluations were performed after 45 days of *in vitro* culture, and the anatomical and physiological characteristics were considered (Fig. 1).

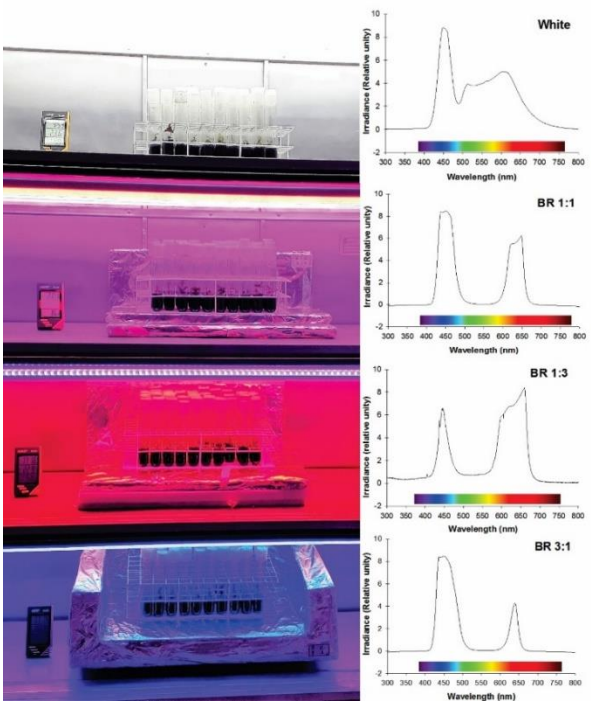

Figure 1. Relative distribution of different light qualities used during the growth of C. pubescens: white, BR (1:1), BR (1:3), and BR (3:1).

2.2 Biomass analysis

Ten (10) plants were randomly selected for biomass analysis within each treatment. Their fresh matter (FM) mass was determined on a digital analytical balance.

2.3 Leaf morphoanatomic characterization

For these evaluations, the leaves of *C. pubescens* were diaphanized and fixed. For this purpose, the samples of fully expanded leaves measuring approximately 2 cm^2 each were immersed in 5% sodium hydroxide for 24 hours, clarified with chloral hydrate 1:6:1 (w/v) for another 24 hours, and stained with safranin 1% in 50% ethanol (Arnott, 1959). Next, slides were prepared and covered with a coverslip using Canada balsam. The following characteristics were observed: stomatal localization, stomatal morphology, and stomatal density.

During the fixation of the samples, 3 cm^2 of the central region from each fully expanded leaf was collected from all the replicates (n = 10) of each treatment (n = 4) of *C. pubescens* seedlings. The samples were initially fixed in a Karnovsky (1965) solution for 24 hours. After this period, the plant material was prewashed in phosphate buffer (0.1 M, pH 7.2) and dehydrated in an ethanol series of increasing concentrations (30% to 100%), and then it was preinfiltrated and infiltrated with historesin (Leica, Germany) as recommended by the manufacturer. The samples were subsequently sectioned transversely into 5 μm slices in a rotary microtome (Model 1508R, Logen Scientific, China), and the sections were stained with toluidine blue-polychromatic color (0.05% 0.1 M phosphate buffer, pH 6, 8) (O'Brien et al., 1964). Images were obtained using an Olympus microscope (BX61, Tokyo, Japan) coupled to a DP-72 camera using a clear field option.

Subsequently, morphoanatomical observations of the epidermis were performed on the adaxial and abaxial surfaces and the palisade, spongy, and mesophyll parenchyma. To perform micromorphometry analyses, the data were obtained with the aid of ImageJ Software Image Processing and Analysis in Java, version 1.47, for a total of 10 observations/repetition for each evaluated structure.

2.4 Evaluation of chlorophyll a fluorescence

The fluorescence images and parameters for chlorophyll *a* were obtained using Imaging PAM (MAXI version) and Imaging Win software (Heinz Walz GmbH, Effeltrich, Germany). To obtain the images (640 × 480 pixels), fully expanded leaves were fixed individually on a support that was 18.5 cm away from a camera with a coupled charge device (CCD) attached to the fluorescence device. Measurements of the adaxial portions of the leaves were performed, and they were adjusted in the dark for 30 min so that the reaction centers remained fully open. Under this condition, the leaf tissues were exposed to low intensity light (0.03 μmol m^{-2} s^{-1}) to determine the initial fluorescence (Fo). Then, a pulse of saturation light (> 6000 μmol m^{-2} s^{-1}) was applied for 0.8 s to determine the maximum fluorescence (Fm); from these results, the maximum quantum yield of photosystem II (FSII) was calculated. (Fv/Fm = (Fm-Fo)/Fm) (Genty et al., 1989). Following the illumination of each sample for 40 s at 21 μmol m^{-2} s^{-1}, the fluorescence of samples that were adapted to light before the saturation pulse (F) and maximum fluorescence in a sample exposed to the light (Fm') after the pulse were determined at saturation. Subsequently, the effective quantum yield of PSII, or ΔF/Fm'= (Fm'-F)/ Fm'), was calculated in accordance with Genty et al. (1989). The regulated nonphotochemical dissipation (YNPQ) and nonregulated dissipation (YNO) yields were calculated as described by Kramer et al. (2004). The results obtained from the median regions of the leaves were processed.

2.5 Determination of chloroplastid pigments

The concentrations of the pigments (chlorophyll *a* and *b* and carotenoids) were determined using dimethyl sulfoxide (DMSO) as an extractor, as described by Wellburn (1994). Three 5 mm-diameter leaf disks were placed in containers in 5 mL of DMSO, saturated with 50 g L^{-1} $CaCO_3$ (calcium carbonate) and kept in the dark. After 6 hours in a water bath at 65°C, the extract absorbance was determined using an Evolution 60S UV-VIS spectrophotometer (Thermo Fischer Scientific, Madison, USA) at 665.1, 649.1 and 480 nm, with DMSO saturated with $CaCO_3$ as the blank.

The pigment concentrations were expressed in μg cm^{-2}. The wavelengths, equations, and calculations for determining the pigment concentration were based on the work of Wellburn (1994).

2.6 Malonaldehyde content (MDA)

Cell damage was assessed by measuring lipid peroxidation with increased MDA as described by Cakmak and Horst (1991). Samples consisting of 100 mg of leaf tissue were macerated in liquid N_2 in a mortar to obtain a fine powder. The resulting powder was homogenized in 2 mL of 1% trichloroacetic acid (TCA) (w/v) and centrifuged at 12,000 × *g* for 15 min at 4°C. Following the centrifugation, 0.5 mL of the supernatant was added to 1.5 mL of the 0.5% (m/v) thiobarbituric acid solution (prepared in 20% (m/v) TCA) and incubated in a 95°C water bath for 30 min. After this period, the reaction was stopped in an ice bath. The samples were centrifuged at 9000 × *g* for 10 min, and the specific absorbance of the supernatant was determined at 532 nm. The nonspecific absorbance was measured at 600 nm and subtracted from the specific absorbance value. The MDA concentration was calculated using the extinction coefficient 155 mM^{-1} cm^{-1} and expressed in μmol g^{-1} fresh weight (Heath and Packer, 1968).

2.7 Experimental design

The experimental design was completely randomized (CRD), and the data were subjected to a normality analysis using the Shapiro-Wilk test followed by analysis of variance. The means were compared by Tukey's test at 5% probability. For these analyses, Sisvar software was used (Ferreira, 2011).

The network correlation was performed using R software version 3.1.2 (R Core Team, 2019) and the graph was produced using the "qgraph" package (Epskamp et al., 2012).

3 Results

3.1 Seedling biomass, C. pubescens

There was a difference in the BR 1:1 combination; the mean values were 31% higher than the BR 1:3 combination, with no differences between the other wavelengths (Fig. 2).

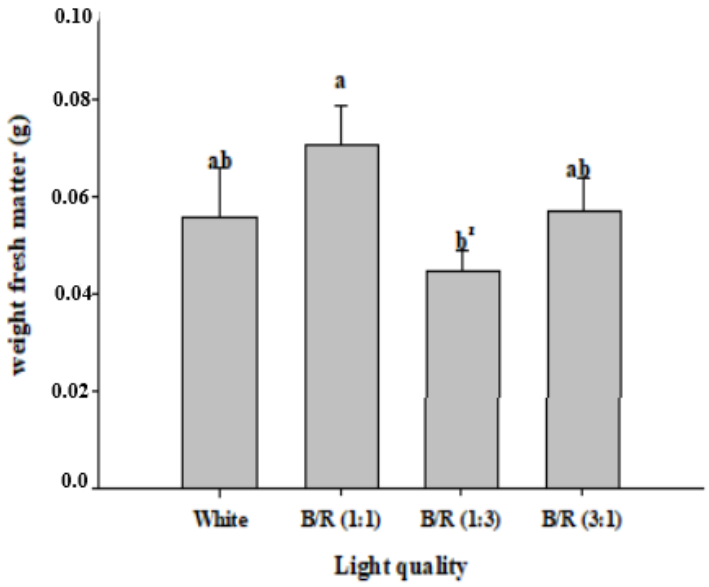

Figure 2. Fresh weights of C. pubescens seedlings after being grown in vitro for 45 days under different light qualities. ZMeans followed by the same letter do not differ statistically according to Tukey's test at 5% probability.

3.2 Leaf morphoanatomic characterization of C. pubescens

In vitro cultivation under different light qualities led to modifications in the anatomy of *C. pubescens* (Fig. 3). During cultivation using the BR combination (1:1), there was an expansion of the palisade parenchyma cells and larger intercellular spaces, while under the BR combination (3:1), the parenchyma cells were compacted, and the intercellular spaces were reduced compared to the other light qualities (Fig. 3).

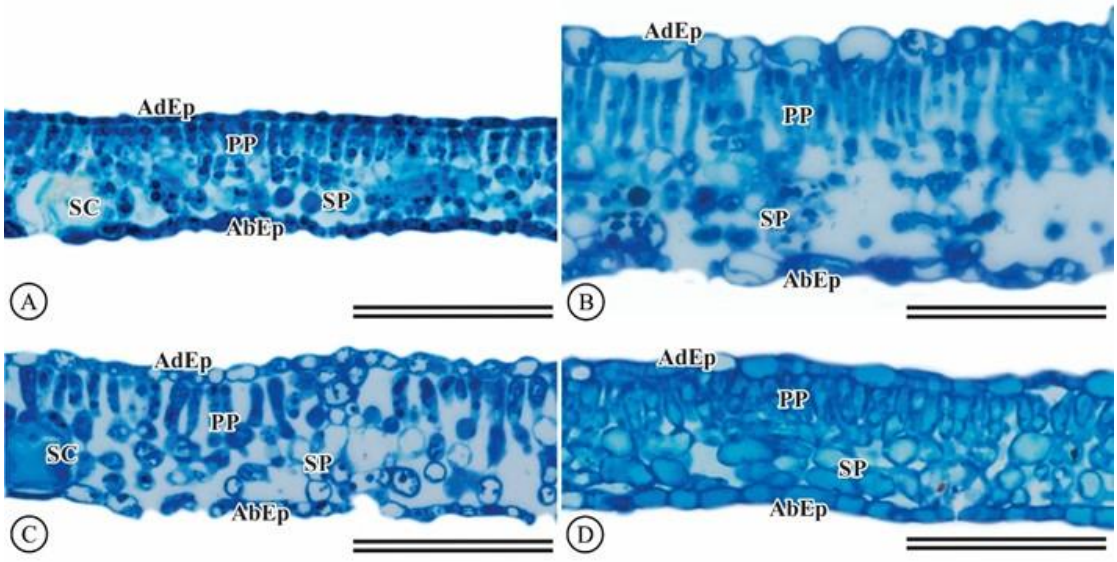

Figure 3. Photomicrographs of C. pubescens leaves, with anatomical changes showing after 45 days of cultivation. (A) White, (B) BR (1:1), (C) BR (1:3), and (D) BR (3:1). (Ad Ep) Adaxial epidermis. (Ab Ep) Abaxial epidermis. (PP) Palisade parenchyma. (SP) Spongy

C. pubescens grown under BR light combination (1:1) showed the adaxial epidermis (13.88 μm), abaxial epidermis (18.00 μm), and chlorenchyma (97.79 μm) overlapping compared to the other wavelengths (Table 1).

Table 1. Anatomical characteristics of leaf micromorphometry: adaxial epidermis (Ad Ep), abaxial epidermis (Ep Ab), and the thickness of the chlorophyllic parenchyma (CP) from C. pubescens after 45 days under different light qualities.

Light quality (LEDs)	Leaf anatomical characteristics		
	Ep Ad (μm)	Ep Ab (μm)	Esp P Clor (μm)
White	07.53 ± 0.16d	09.25 ± 0.17c[z]	59.04 ± 1.69c
BR(1:1)	13.88 ± 0.28a	18.00 ± 0.61a	97.79 ± 2.46a
BR (1:3)	11.58 ± 0.12c	11.78 ± 0.32b	56.28 ± 3.72c
BR (3:1)	11.86 ± 0.36b	12.98 ± 0.30b	73.30 ± 2.70b

[Z]Means followed by the same letter in each column do not differ statistically according to Tukey's test at 5% probability. [±]Standard error.

There was a higher stomatal density (132.00 mm^{-2} stomata) in seedlings cultured in combinations of BR LEDs (1:1) in relation to the other wavelengths. Under the LEDs providing the BR combination (1:3), the stomata had lower values for Polar D Est (16.50 μm) and white LEDs for D Eq Est (12.61 μm). However, stomata with more ellipsoidal characteristics were observed in plants grown under white LEDs and combinations of BR (3:1), with values of 1.38 and 1.41, respectively, between the polar and equatorial diameter ratio (Table 2).

Table 2. Leaf anatomy: stomatal density (Dens Est), stomata polar diameter (D Polar Est), stomata equatorial diameter (D Eq Est), and the ratio between the polar and equatorial diameter (D Pol / D Eq) of C. pubescens after 45 days of cultivation under different.

Light quality (LEDs)	Leaf anatomical characteristics			
	Dens Est (Stomata mm^{-2})	D Polar Est (µm)	D Eq Est (µm)	D Pol/D Eq
White	108.60 ± 23.38b[z]	17.28 ± 1.01bc	12.61 ± 1.44b	1.38 ± 0.08a
BR (1:1)	132.00 ± 11.94a	18.14 ± 0.66ab	15.23 ± 0.70a	1.21 ± 0.08b
BR (1:3)	104.20 ± 5.31b	16.50 ± 0.34c	14.30 ± 0.59ab	1.16 ± 0.05b
BR (3:1)	108.60 ± 23.38b	18.76 ± 0.55a	13.34 ± 0.29ab	1.41 ± 0.04a

[Z]Means followed by the same letter in each column did not differ statistically according to Tukey's test at 5% probability. [±]Standard error.

Regarding the photomicrograph of *C. pubescens*, changes in the stomata were observed according to the light quality (Fig. 4).

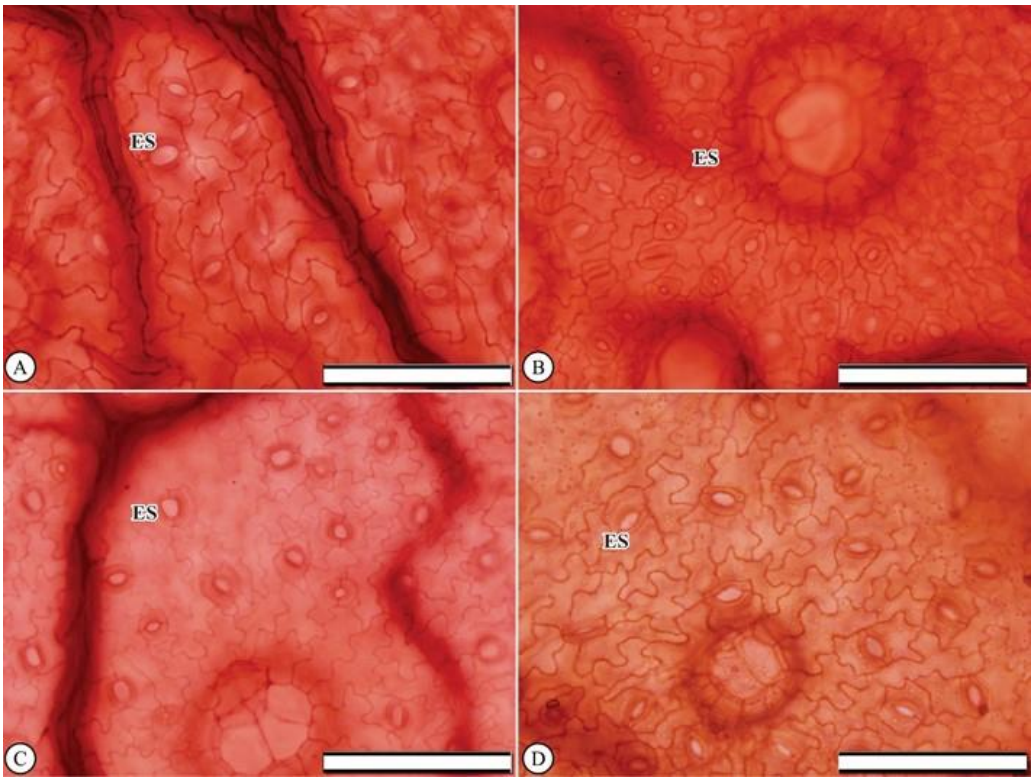

Figure 4. Photomicrographs of C. pubescens leaves, with anatomical changes after 45 days in cultivation. (A) White, (B) BR (1:1), (C) BR (1:3), (D) BR (3:1), and (ES) stomata. Scale bar 100 µm.

3.3 Characteristics of chlorophyll a fluorescence in C. pubescens

There were no significant differences between the light qualities for the Fv/Fm ratio.

However, the mean values for the Y(II) of BR (3:1) were higher than that of BR (1:3). The seedlings under white light and BR (1:1) did not exhibit significant differences under the other wavelengths.

Under combinations of BR lights (1:3), lower Y(NPQ) values than white and BR combination (3:1) were observed; however, the opposite result was observed for Y(NO). For both variables, the means of the BR association (1:1) did not differ from those of the other treatments. The average qP of the white LEDs did not differ from the other evaluated wavelengths, but the plants under combinations of BR lights (1:1) and (3:1) had higher indices than did the BR combination (1:3) (Table 3 and Fig. 5).

Table 3. Characteristics of fluorescence a: maximum quantum yield (Fv/Fm), effective quantum yield [Y(II)], nonphotochemical quenching yield [Y(NPQ)], nonregulated photochemical dissipation [Y(NO)], and photochemical quenching (qP) in the leaves of C. Pubescens.

Light condition (LED)	Physiological characteristics				
	Fv/Fm	Y(II)	Y(NPQ)	Y(NO)	qP
White	0.72±0.01a[z]	0.150±0.05ab	0.258±0.04a	0.590±0.06a	0.238±0.09ab
BR (1:1)	0.67±0.01a	0.154±0.05ab	0.166±0.01ab	0.680±0.08ab	0.260±0.01a
BR (1:3)	0.72±0.04a	0.110±0.02b	0.130±0.05b	0.760±0.07b	0.162±0.05b
BR (3:1)	0.73±0.02a	0.188±0.02a	0.252±0.07a	0.560±0.09a	0.290±0.04a

[z]Means followed by the same letter do not differ statistically according to Tukey's test at 5% probability. [±]Standard error of the mean.

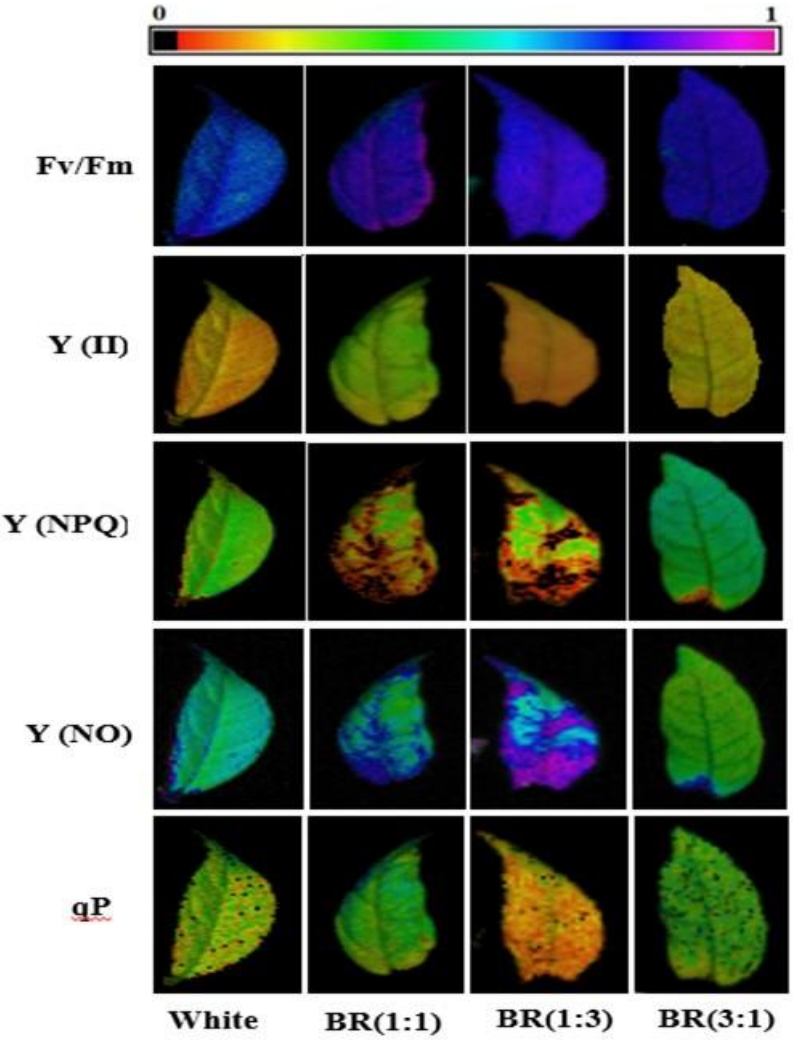

Figure 5. Maximum quantum yield (Fv/Fm), effective quantum yield [Y(II)], regulated nonphotochemical quenching yield [Y(NPQ)], nonregulated photochemical dissipation [Y(NO)], and photochemical quenching (qP) for leaves of C. pubescens after 45 days of cultivatio.

3.4 C. pubescens chloroplastid pigments

Higher concentrations of chlorophyll *a* were observed under the BR (1:1) light combinations. Regarding chlorophyll *b*, the light combinations for BR (3:1) were greater than those of BR (1:3). The other wavelengths did not differ.

A greater carotenoid content was observed for BR (1:1), with 10.48 μg cm^{-2}, compared to other lights, but they displayed no differences between them. The total

chlorophyll was significantly higher in the BR (3:1) and (1:1) combinations, with 45.14 μg cm^{-2} and 43.18 μg cm^{-2}, respectively, whereas for the BR 1:3 combinations, the lowest value was 34.60 μg cm^{-2} (Table 4).

Table 4. Chloroplastid pigments:chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll from Campomanesia pubescens as affected by light conditions after 60 days under cultivation.

Light condition (LED)	Chloroplastid pigments			
	Chlorophyll *a* (μg cm^{-2})	Chlorophyll *b* (μg cm^{-2})	Carotenoids (μg cm^{-2})	Total Chlorophyll (μg cm^{-2})
White	19.50±0.74b[z]	16.96±0.10ab	6.36±0.86b	36.54±0.63bc
Blue/red (1:1)	24.58±0.90a	18.66±0.07ab	10.48±0.60a	43.18±0.64ab
Blue/red (1:3)	19.12±0.92b	15.30±0.98b	6.00±0.08b	34.60±1.01c
Blue/red (3:1)	22.60±0.65ab	22.48±0.49a	6.70±0.16b	45.14±0.85a

[Z]Means followed by the same letter in each column do not differ statistically according to Tukey's test at 5% probability. [±]Standard error.

3.5 Malonaldehyde content (MDA) of C. pubescens

C. pubescens plants cultivated under the BR 3:1 combination reached lower values, at 24.5% MDA in their leaves, compared to the control light (white) and in relation to the other combinations (Fig. 6).

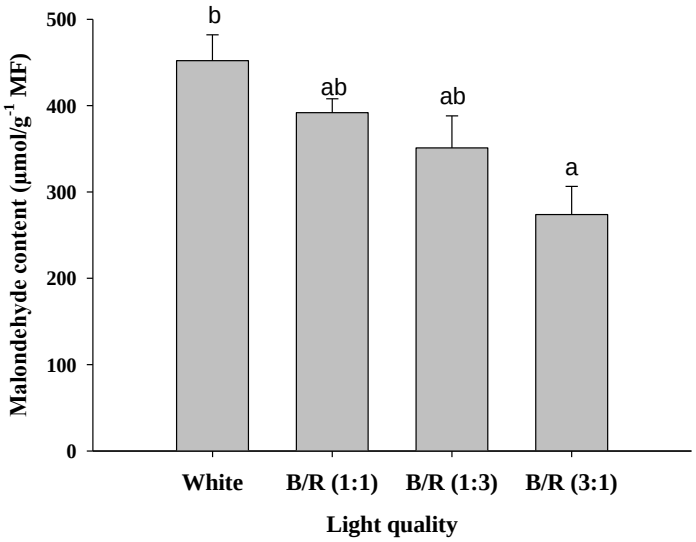

Figure 6. Malondialdehyde contents of leaves from C. pubescens after 45 days of cultivation under different light qualities. ZMeans followed by the same letter do not differ statistically according to Tukey's test at 5% probability.

In relation to the estimated correlations between anatomical and physiological characteristics, positive and significant correlations ($p < 0.01$) were observed between the variables AbE x AdE (0.95), C x Cla (0.97), C x Car (0.96), PDm x Clb (0.95), PDm x TC (0.97), and SFM x Y(II) (0.98), and for the variables SD x Car (0.98) and DES x MDA (1.0), positive and significant correlations ($p < 0.05$) were observed. Estimated negative and significant correlations ($p < 0.05$) were observed between Y(NPQ) and Y(NO) (Fig. 7).

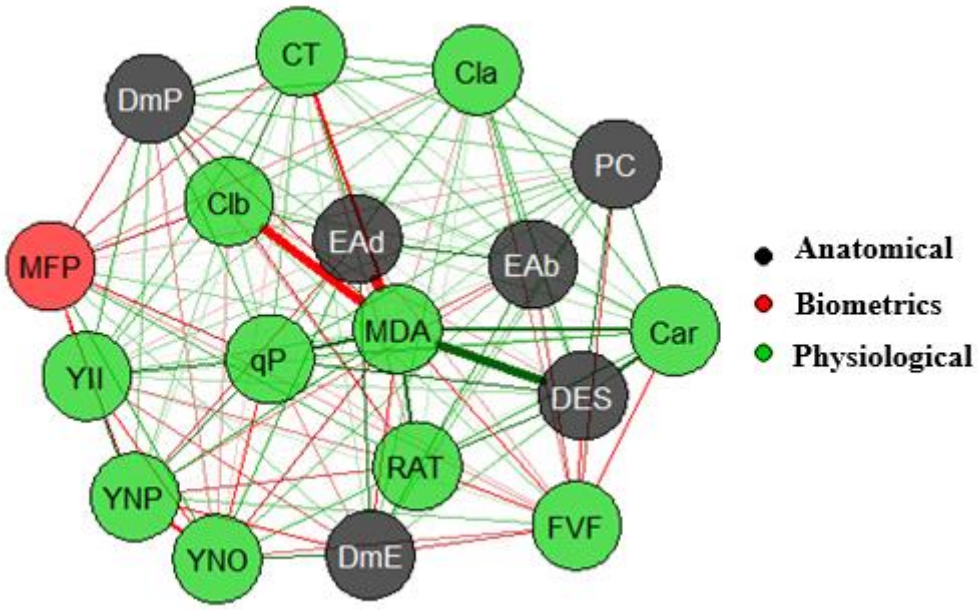

Figure 7. Correlation network constructed using phenotypic variables of C. pubescens. The red and green lines represent negative and positive correlations, respectively. The thickness of the line is proportional to the strength of the correlation. Plantling descriptors, **Anatomical**: adaxial epidermis (EAd), abaxial epidermis (EAb), chlorenchyma (PC), stomatal density (DES), polar diameter (DmP), and equatorial diameter (DmE). **Biometrics:** seedling fresh matter (MFP). **Physiological:** maximum quantum yield (FVF), effective quantum yield (YII), regulated quantum dissipation yield (YNP), nonregulated quantum yield (YNO), and photochemical quenching (qP), chlorophyll a (Cla), chlorophyll b (Clb), total chlorophyll (CT), ratio between chlorophyll a and chlorophyll b (RAT), carotenoids (Car), and malondialdehyde (MDA).

4 Discussion

4.1 Biomasses of C. pubescens seedlings under different light qualities

The biomasses increased with the use of BR LEDs (1:1) compared to the BR 1:3 combination, and they did not differ from the other LEDs (Fig. 2). These results show the importance of blue light for photosynthesis and subsequent biomass production, and it

should be combined with red light in equal proportions or up to 75% blue light (3:1). The addition of blue light improves leaf development, stomatal development and biomass accumulation (Zheng and van Labeke, 2018). Blue light is important for the synthesis of pigments, enzymes, the opening and closing of stomata, the activation of the circadian rhythm (Kapoor et al., 2018), promoting signaling in stomatal cells, and regulating the phosphorylation mechanisms of H^+-ATPases in the plasma membrane, as mediated by cryptochromes and phototropins (Inoue and Kinoshita, 2017). These results demonstrate the importance of blue light in photosynthesis and the subsequent biomass production (Piovene et al., 2015), which is driven by photosynthetic activity (Gerovac et al., 2016).

Red light, via phytochromes, triggers various and complex physiological responses. When the inactive form (Pr) absorbs red light, there is a conformational alteration in the protein of the chromophore itself, which converts it to the active form (Prf) (Carvalho et al., 2011). Red light stimulated the maximum accumulation of biomass in *Rhodiola imbricata* (Kapoor et al., 2018), and these wavelengths coincide with the chlorophyll uptake peaks and with better photochemical efficiency. Similar results were observed for *in vitro* seedlings of *Brassica napus L*., (Li et al., 2013), rosette leaves (Shengxin et al., 2016), *Cucumis sativus* seedlings (Hernández and Kubota, 2015; Trouwborst et al., 2016), and *Mesembryanthemum crystallinum* plants (He et al., 2017). Macedo et al. (2011) obtained seedlings of *z* Kuntze that were cultivated *in vitro*, and they suggested performing studies using combinations of blue and red due to the influence of these wavelengths on the responses of cryptochromes and phytochromes. Hogewoning et al. (2012) confirmed that the appropriate balance of the components of the red and blue light spectra would be beneficial to plants.

4.2 Leaf anatomical modifications of C. pubescens under different light qualities

Variations in leaf thickness are primarily attributed to the increase and shape of the palisade parenchyma. Liu et al. (2014) studied *Platycodon grandiflorum in vitro* under different light spectra, and they concluded that blue monochromatic light and BR (3:1) treatments promoted an increase in the palisade parenchyma with almost rectangular shapes. In this study, a greater thickness of the epidermis and a palisade parenchyma elongation were observed in the combination BR LEDs (1:1). This effect is important because it leads to lower transpiration rates in leaves, which increases the chances of plant survival in the *ex vitro* environment.

In relation to the spongy parenchyma, larger intercellular spaces were observed, which would allow for the greater use of incident light in the inner lower portion of the leaves, promoting the diffusion of light. The increased leaf thickness promotes an increase in heat dissipation, which is an important factor for plant survival in drier habitats with high irradiance because overheating and high transpiration rates are harmful (Chiamolera et al., 2010).

Increased stomatal density favors CO_2 uptake, resulting in higher mass (Liu et al., 2014). In our study, the combination BR (1:1) LEDs led to an increase in the stomatal density of 29.11%, which was higher than that of the other LEDs, resulting in higher biomass. The white and BR (3:1) LED had more elliptical stomata (normal functions) compared to the other LEDs; these treatments showed a higher qP and lower Y(NO) than the blue and red (1:3) LEDs. Similar results were described by Liu et al. (2014) in *P. grandi* seedlings *in vitro*, in which the use of blue light and the BR (3:1) combination were beneficial for stomatal development, inducing greater qP and lower NPQ and producing more dry mass. Stomata will open in response to blue light, facilitating the exchange of gases between the plant and the atmosphere and leading to an increase in biomass and photosynthetic efficiency (Inoue and Kinoshita, 2017).

4.3 Physiological responses of C. pubescens seedlings under different light qualities

Under stress conditions, thylakoids can be affected by interfering with the efficiency of photosynthesis and inactivating photosystem II and the electron transport chain that originates ATP and $NADPH_2$ (Martinazzo et al., 2013). The Fv/Fm ratio is viewed as a measure for estimating the maximum quantum efficiency of the photochemical activity and the effects of stress on the integrity of the PSII, and its values may vary depending on the species (Santos et al., 2013). In the *C. pubescens* seedlings, the maximum quantum yield of photosystem II, Fv/Fm, showed results of 0.67 to 0.73 in the LED combinations BR (1:1) and (3:1), respectively, with no significant differences between the light qualities.

However, the effective quantum yield of PSII (Y(II)) was higher in the BR (3:1) LED combination compared to the BR (1:3) LEDs (Table 3), with no significant differences in the other LEDs. Similar results were observed for the Y values (NPQ). The increase in Y(NPQ) alleviates the photosystems through the xanthophyll cycle, which plays an important role in protecting the photosynthetic machinery by dissipating the excess energy by interconverting the forms of violaxanthin to zeaxanthin with energy expenditure (Marín-Guirao et al., 2013).

The quantum yield of nonregulated dissipation Y(NO) was lower in the BR (3:1) and white LEDs compared to BR (1:3), indicating that the spectral ratios favoring red provide greater environmental pressure because this parameter reflects the fraction of the energy that is dissipated in the form of constitutive heat loss, especially when the PSII reaction centers are closed or damaged (Schreiber and Klughammer, 2008). Photochemical quenching (qP) represents the proportion of photon energy captured by the open photosystem II reaction centers that is dissipated by photochemistry (Juneau et al., 2005). In *C. pubescens* seedlings grown under BR (1:1), (3:1) and white LEDs, there

were higher values compared to BR (1:3) LEDs (Table 3), showing their higher photosynthetic efficiency.

Higher concentrations of chlorophylls a and b were observed in the BR (1:1) and BR (3:1) LEDs, correlating with the accumulation of biomass and better photosynthetic performance, which demonstrated the greater use of light in these regions of the visible spectrum and stimulated the activity of PSII. Furthermore, wavelengths in the blue band (approximately 480 nm) induce greater PSII excitation (Hogewoning et al., 2012). The chlorophyll concentration increased with increasing blue light, showing that blue light is essential for the initial chlorophyll biosynthesis, a result also found by Hernández and Kubota (2015) when they were evaluating the physiological and morphological responses of cucumber (*Cucumis sativus*) seedlings under blue and red LEDs.

In this study, the results indicate that *C. pubescens* seedlings grown under 3:1 AV LEDs have more efficient mechanisms of protection against oxidative damage than plants grown under white LEDs. Malondialdehyde (MDA) is one of the primary aldehydes formed by the oxidation of polyunsaturated fatty acids and one of the secondary products of lipid peroxidation; thus, an increase in the MDA level indicates peroxidation of the lipids in the membrane induced by stress at the cellular level. Therefore, the MDA level may be an indicator of oxidative damage (Shohael et al., 2006).

Network analysis becomes interesting when working with distinct groups of variables. However, through the correlation network graph, it is possible to identify the variables and the ways in which they are connected. The correlation between the anatomical and physiological characteristics assessed in the *C. pubescens* seedlings was verified (Fig. 7). There was a positive correlation between the stomatal density and carotenoids as well as the chlorenchyma with chlorophyll a and carotenoids, with an increase in the uptake of energy for photosystem II. This observation shows an increase

in the chlorophyll *a/b* ratio, which may have influenced the uptake of light and increased the MDA level. Similarly, Y(II) correlated with qP, with a better use of energy in photochemical dissipation, providing better photochemical efficiency in photosystem II.

For Y(NPQ), a significant negative ($p < 0.05$) correlation was observed with Y(NO), which is expected because with increased regulated dissipation, there is a decrease in the quantum yield of nonregulated dissipation, thus reflecting the fraction of energy that is dissipated in the form of heat and fluorescence, especially when the PSII reaction centers are closed (Schreiber and Klughammer, 2008).

5 Conclusions

C. pubescens plants exhibited varied anatomical and physiological characteristics depending on the spectrum of light used here. The combinations of BR (1:1) or BR (3:1) LEDs are promising for the propagation of the species, given their positive influence on most of the studied traits. Knowledge of the correlation between the variables is important to identify the traits that contribute the most to the desired goal during the cultivation, propagation, and acclimatization of *C. pubescens*.

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References

Arnott, H.J., 1959. Leaf clearings. Turtox News 37, 192–194. https://doi.org/10.1021/cen-v037n009.p192.

Bardiviesso, D.M., Maruyama, W.I., Reis, L., Modesto, J., Rezende, W.E., 2011. Diferentes substratos e recipientes na produção de mudas de guabiroba (*Campomanesia pubescens* O. Berg). Revista Científica Eletrônica de Agronomia 18, 52–59.

Cakmak, I., Horst, W.J., 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiol. Plant. 83, 463–468. https://doi.org/10.1111/j.1399-3054.1991.tb00121.x.

Cardoso, C.A., Salmazzo, G.R., Honda, N.K., Prates, C.B., Mdo, C.V., Coelho, R.G., 2010. Antimicrobial activity of the extracts and fractions of hexanic fruits of *Campomanesia* species (*Myrtaceae*). J. Med. Food 13, 1273–1276. https://doi.org/10.1089/jmf.2009.0047.

Carvalho, R.F., Campos, M.L., Azevedo, R.A., 2011. The role of phytochrome in stress tolerance. J. Integr. Plant Biol. 53, 920–929. https://doi.org/10.1111/j.1744-7909.2011.01081.x.

Chang, R., Morais, S., Nascimento, E., Cunha, L.C., Rocha, E.O., Aquino, F., Souza, M., Cunha, W.R., Martins, C.H., 2011. Essential oil composition and antioxidant and antimicrobial properties of *Campomanesia pubescens* O. Berg, native of Brazilian Cerrado. Lat. Am. J. Pharm. 30, 1843–1848.

Chiamolera, L.D.B., Ângelo, A.C., Boeger, M.R., 2010. Resposta morfoanatômica da folha de *Podocarpus lambertii* Klotzsch ex Endl (*Podocarpaceae*) implantado em

duas áreas com diferentes graus de sucessão às margens do Reservatório Iraí–PR. Biotemas 23, 1–11. https://doi.org/10.5007/2175-7925.2010v23n2p1.

Dong, C., Fu, Y., Liu, G., Liu, H., 2014. Low light intensity effects on the growth, photosynthetic characteristics, antioxidant capacity, yield and quality of wheat (*Triticum aestivum* L.) at different growth stages in BLSS. Adv. Space Res. 53, 1557–1566. https://doi.org/10.1016/j.asr.2014.02.004.

Epskamp, S., Cramer, A.O.J., Waldorp, L.J., Schmittmann, V.D., Borsboom, D., 2012. qgraph: network visualizations of relationships in psychometric data. Journal of Statistical Software 48, 1–18. https://doi.org/10.18637/jss.v048.i04.

Ferreira, D.F., 2011. Sisvar: a computer statistical analysis system. Ciênc. Agrotecnol. 35, 1039–1042. https://doi.org/10.1590/S1413-70542011000600001.

Genty, B., Briantais, J.M., Baker, N.R., 1989. A relação entre o rendimento quântico de transporte de electrões fotossintéticos e extinção de fluorescência da clorofila. Biochim. Biophys. Acta 990, 87–92.

Gerovac, J.R., Craver, J.K., Boldt, J.K., Lopez, R.G., 2016. Light intensity and quality from sole-source light-emitting diodes impact growth, morphology, and nutrient content of Brassica microgreens. HortScience 51, 497–503. https://doi.org/10.21273/HORTSCI.51.5.497.

Hartmann, H., Daoust, G., Bigué, B., 2010. Negative or positive effects of plantation and intensive forestry on biodiversity: a matter of scale and perspective. The Forestry Chronicle 86, 354–364. https://doi.org/10.21273/HORTSCI.51.5.497.

He, J., Qin, L., Chong, E.L., Choong, T.W., Lee, S.K., 2017. Plant growth and photosynthetic characteristics of *Mesembryanthemum crystallinum* grown aeroponically under different blue- and red-LEDs. Frontiers in Plant Science 8, 361. https://doi.org/10.3389/fpls.2017.00361.

Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189–198. https://doi.org/10.1016/0003-9861(68)90654-1.

Hernández, R., Kubota, C., 2015. Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. Environ. Exp. Bot. 173, 92–99. https://doi.org/10.1016/j.envexpbot.2015.04.001.

Hogewoning, S.W., Wientjes, E., Douwstra, P., Trouwborst, G., van Ieperen, W., Croce, R., Harbinson, J., 2012. Photosynthetic quantum yield dynamics: from photosystems to leaves. Plant Cell 24, 1921–1935. https://doi.org/10.1105/tpc.112.097972.

Inoue, S.-I., Kinoshita, T., 2017. Blue light regulation of stomatal opening and the plasma membrane H+-ATPase. Plant Physiol. 43, 531–538. https://doi.org/10.1104/pp.17.00166.

Juneau, P., Green, B.R., Harrison, P.J., 2005. Simulation of pulse-amplitude-modulated (PAM) fluorescence: limitations of some PAM-parameters in studying environmental stress effects. Photosynthetica 43, 75–83. https://doi.org/10.1007/s11099-005-5083-7.

Kapoor, S., Raghuvanshi, R., Bhardwaj, P., Sood, H., Saxena, S., Chaurasia, O.P., 2018. Influence of light quality on growth, secondary metabolites production and antioxidant activity in callus culture of *Rhodiola imbricata* Edgew. Journal of Photochemistry and Photobiology B, Biology 183, 258–265. https://doi.org/10.1016/j.jphotobiol.2018.04.018.

Karunovsky, M., 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. The Journal of Cell Biology 27, 137A.

Klafke, J.Z., da Silva, M.A., Panigas, T.F., Belli, K.C., de Oliveira, M.F., Barichello, M.M., Rigo, F.K., Rossato, M.F., Santos, A.R.S.D., Pizzolatti, M.G., Ferreira, J., Viecili, P.R., 2010. Effects of *Campomanesia xanthocarpa* on biochemical, hematological and oxidative stress parameters in hypercholesterolemic patients. J. Ethnopharmacol. 127, 299–305. https://doi.org/10.1016/j.jep.2009.11.004.

Kramer, D.M., Johnson, G., Kiirats, O., Edwards, G.E., 2004. New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. Photosynth. Res. 79, 209–218. https://doi.org/10.1023/B:PRES.0000015391.99477.0d.

Li, H., Tang, C., Xu, Z., 2013. The effects of different light qualities on rapeseed (*Brassica napus L.*) plantlet growth and morphogenesis *in vitro*. Scientia Horticulturae 150, 117–124. https://doi.org/10.1016/j.scienta.2012.10.009.

Li, Q., Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ. Exp. Bot. 67, 59–64. https://doi.org/10.1016/j.envexpbot.2009.06.011.

Liu, M., Xu, Z., Guo, S., Tang, C., Liu, X., Jao, X., 2014. Evaluation of leaf morphology, structure and biochemical substance of balloon flower (*Platycodon grandiflorum* (Jacq.) A. DC.) plantlets *in vitro* under different light spectra. Scientia Horticulturae 174, 112–118. https://doi.org/10.1016/j.scienta.2014.05.006.

Macedo, A.F., Leal-Costa, M.V., Tavares, E.S., Lage, C.L.S., Esquibel, M.A., 2011. The effect of light quality on leaf production and development of *in vitro*-cultured plants of *Alternanthera brasiliana* Kuntze. Environ. Exp. Bot. 70, 43–50. https://doi.org/10.1016/j.envexpbot.2010.05.012.

Marín-Guirao, L., Ruiz, J.M., Sandoval-Gil, J.M., Bernardeau-Esteller, J., Stinco, C.M., Meléndez-Martínez, A., 2013. Xanthophyll cycle-related photoprotective

mechanism in the *Mediterranean seagrasses Posidonia oceanica* and *Cymodocea* nodosa under normal and stressful hypersaline conditions. Aquat. Bot. 109, 14–24. https://doi.org/10.1016/j.aquabot.2013.03.006.

Martinazzo, E.G., Perboni, A.T., de Oliveirai, P.V., Bianchi, V.J., Bacarin, M.A., 2013. Atividade fotossintética em plantas de ameixeira submetidas ao déficit hídrico e ao alagamento. Ciênc. Rural 43, 35–41. https://doi.org/10.1590/S0103-84782012005000126.

Muneer, S., Kim, E.J., Park, J.S., Lee, J.H., 2014. Influence of green, red and blue light emitting diodes on multiprotein complex proteins and photosynthetic activity under different light intensities in lettuce leaves (*Lactuca sativa* L.). Int. J. Mol. Sci. 15, 4657–4670. https://doi.org/10.3390/ijms15034657.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15, 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

O'Brien, T., Feder, N., McCully, M.E., 1964. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 59, 368–373. https://doi.org/10.1007/BF01248568.

Pinhal, H.F., Anastácio, M.R., Carneiro, P.A.P., da Silva, V.J., de Morais, T.P., Luz, J.M.Q., 2011. Aplicações da cultura de tecidos vegetais em fruteiras do Cerrado. Ciênc. Rural 41, 1136–1142. https://doi.org/10.1590/S0103-84782011005000089.

Piovene, C., Orsini, F., Bosi, S., Sanoubar, R., Bregola, V., Dinelli, G., Gianquinto, G., 2015. Optimal red:blue ratio in led lighting for nutraceutical indoor horticulture. Scietia Horticulturae 193, 202–208. https://doi.org/10.1016/j.scienta.2015.07.015.

R Core Team, 2019. R: a language and environment for statistical computing. https://www.gbif.org/en/tool/81287/r-a-language-and-environment-for-statistical-computing (accessed 10 February 2015).

Samuoliene, G., Brazaityte, A., Sirtautas, R., Virsile, A., Sakalauskaite, J., Sakalauskiene, S., Duchovskis, P., 2013. LED illumination affects bioactive compounds in romaine baby leaf lettuce. J. Sci. Food Agric. 93, 3286–3291. https://doi.org/10.1002/jsfa.6173.

Santos, C.M.D., Verissimo, V., Filho, H.C.L.W., Ferreira, V.M., Cavalcante, P.G.S., Rolim, E.V., Endres, L., 2013. Seasonal variations of photosynthesis, gas exchange, quantum efficiency of photosystem II and biochemical responses of *Jatropha curcas* L. grown in semi-humid and semi-arid areas subject to water stress. Indus. Crops Prod. 41, 203–213. https://doi.org/10.1016/j.indcrop.2012.04.003.

Schreiber, U., Klughammer, C., 2008. Non-photochemical fluorescence quenching and quantum yields in PS I and PS II: analysis of heat-induced limitations using maxi-imaging-PAM and Dual-PAM-100. PAM Application Notes 1, 15–18.

Shengxin, C., Chunxia, L., Xuyang, Y., Song, C., Xuelei, J., Xiaoying, L., Zhigang, X., Rongzhan, G., 2016. Morphological, photosynthetic, and physiological responses of rapeseed leaf to different combinations of red and blue lights at the rosette stage. Frontiers in Plant Science 7, 1144. https://doi.org/10.3389/fpls.2016.01144.

Shohael, A.M., Ali, M.B., Yu, K.W., Hahn, E.J., Islam, R., Paek, K.Y., 2006. Effect of light on oxidative stress, secondary metabolites and induction of antioxidant enzymes in *Eleutherococcus senticosus* somatic embryos in bioreactor. Process Biochemistry 41, 1179–1185. https://doi.org/10.1016/j.procbio.2005.12.015.

Trouwborst, G., Hogewoning, S.W., van Kooten, O., Harbinson, J., van Ieperen, W., 2016. Plasticity of photosynthesis after the 'red light syndrome' in cucumber. Environ. Exp. Bot. 121, 75–82. https://doi.org/10.1016/j.envexpbot.2015.05.002.

Vinagre, A.S., Rönnau, Â.D.S.R.O., Pereira, S.F., Silveira, L.U.D., Wiilland, E.D.F., Suyenaga, E.S., 2010. Anti-diabetic effects of *Campomanesia xanthocarpa* (Berg) leaf decoction. Braz. J. Pharm. Sci. 46, 169–177. https://doi.org/10.1590/S1984-82502010000200002.

Wellburn, A.R., 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307–313. https://doi.org/10.1016/S0176-1617(11)81192-2.

Zheng, L., van Labeke, M.-C., 2018. Effects of different irradiation levels of light quality on *Chrysanthemum*. Scientia Horticulturae 233, 124–131. https://doi.org/10.1016/j.scienta.2018.01.033.

Conclusões geral

Plântulas de *C. pubescens* exibiram característica morfoanatômicas e fisiológicas variadas de acordo com a qualidade de luz utilizada. LEDs branca e combinação azul/vermelha são alternativas promissoras para propagação da espécie, visto, sua influência positiva para a maioria das características em estudo.

LEDs azul combinada com vermelha 1:1 ou 3:1, são promissoras para propagação da espécie, visto, sua influência positiva para a maioria das características em estudo e suas correlações.

Estudos adicionais são necessários com qualidades de luz, tanto monocromática, assim como combinações de azul e vermelha para aclimatização da espécie, visando diminuir o estresse ocasionado pelo processo com perdas das plântulas. Além de ser ferramenta valiosa para aplicações biotecnológicas para induzir metabólitos secundários.