Revista de Ciencias Agrícolas
e-ISSN 2256-2273
https://doi.org/10.22267/rcia.20244102.3242

Research article: Agronomy

Impact of colored shade netting on growth and physiological traits in avocado creole rootstock seedling

Impacto de mallas de colores sobre el crecimiento y fisiología de plántulas de aguacate criollo

Sara Isabel Bedoya-Ramírez [1]; Ruby Alejandra Loaiza-Ruiz [2]; Carlos Felipe Barrera-Sánchez [3]; Oscar de Jesús Córdoba-Gaona [4*]

Authors Data

1. Student, Ph.D., Universidad Nacional de Colombia, Medellín, Colombia, sibedoyar@unal.edu.co https://orcid.org/0000-0002-3371-8268
2. Researcher, M.Sc., Universidad Nacional de Colombia, Medellín, Colombia, raloaiza@unal.edu.co, https://orcid.org/0000-0003-0074-1186
3. Professor, Ph.D., Universidad Nacional de Colombia, Medellín, Colombia, cfbarreras@unal.edu.co, https://orcid.org/0000-0002-5015-2956
4. Professor, Ph.D., Universidad Nacional de Colombia, Medellín, Colombia, ojcordobag@unal.edu.co, https://orcid.org/0000-0003-2642-4146
(*) Correspondence

Cite: Bedoya-Ramírez, S.I.; Loaiza-Ruiz, R.A.; Barrera-Sánchez, C.F.; Córdoba-Gaona, O.J. (2024). Impact of colored shade netting on growth and physiological traits in avocado creole rootstock seedling. *Revista de Ciencias Agrícolas*. 41(3): e3242.
https://doi.org/10.22267/rcia.20244103.242

Received: October 25 2023 Accepted: October 31 2024

ABSTRACT

Photoselective netting is well-known for filtering the intercepted solar radiation and affecting light quality. However, more information is needed about how horticulture manipulation of light quantity and quality affects avocado rootstock seedlings' growth and physiological traits. Therefore, this study aimed to investigate the effects of colored shading on the growth and development of avocado creole for planting production. A completely randomized experimental design with four treatments and five repetitions was used. The treatments included colored nets (red, black, white, and control – without a net). Net shading treatments affected leaf number, leaf area, leaf area index, rootstock diameter and length, dry matter, Dickson quality index, and all gas exchange variables. In contrast, specific leaf area and root length were not affected by the

colored net. The red net generally increased the rootstock diameter and length, Dickson quality index, and net photosynthesis and water use efficiency more than the black, white net, and full sunlight growth conditions. This study reveals that changing the light intensity allows rootstock growth and physiological performance to be manipulated using different colored shade nets.
Keywords: avocado cv. Hass; development; leaf gas exchange; nursery; photoselective colored net; radiation.

RESUMEN

Las mallas fotoselectivas son bien conocidas por filtrar la radiación solar interceptada y afectar la calidad de la luz. Sin embargo, es necesario más información sobre cómo la manipulación hortícola de la luz, la cantidad y la calidad, afectan el crecimiento y los rasgos fisiológicos de las plántulas de portainjertos de aguacate. Por lo tanto, este estudio tuvo como objetivo investigar los efectos de polisombras coloreadas en el crecimiento y desarrollo del aguacate criollo para la producción de material de siembra. Se utilizó un diseño experimental completamente al azar, con cuatro tratamientos y cinco repeticiones. Los tratamientos consistieron en mallas de colores (rojo, negro, blanco y control sin malla). Las polisombras afectaron el número de hojas, el área foliar, el índice de área foliar, el diámetro y longitud del portainjerto, la materia seca, el índice de calidad de Dickson y todas las variables de intercambio de gases. Por el contrario, el área foliar específica y la longitud de la raíz no se vieron afectados por la malla roja. En general, la malla roja favoreció el diámetro, la longitud del portainjerto, y el índice de calidad de Dickson, así como la fotosíntesis neta y la eficiencia en el uso del agua más que las mallas blancas y negras, y las condiciones de crecimiento a completa exposición solar. Este estudio revela que, al cambiar la intensidad de la luz, el crecimiento del portainjerto y el rendimiento fisiológico se pueden manipular con el uso de polisombras de diferentes colores.

Palabras clave: vivero; aguacate cv. Hass; desarrollo; intercambio gaseoso foliar; radiación; Red de color fotoselectiva.

INTRODUCTION

The avocado (*Persea americana* Mill.) has become a commercially crucial subtropical fruit crop in many countries worldwide, with Colombia second in terms of production, achieving 10.88% of the total produced worldwide, with about 876,754 t behind Mexico (29.70%) (FAO, 2022). Most commercial avocado orchards are planted with grafted plants (Zafar & Sidhu, 2018). The scions are of several common edible cultivars, where cv. 'Hass' is the most used and consumed variety (Carman *et al*., 2009) in the world. Grafting is an important and valuable horticultural technique for commercial fruit production (Barón *et al*., 2019; Hartmann *et al*., 2002), with many different applications, including plant propagation, water-use efficiency, and stress tolerance contribution (Cantero-Navarro *et al*., 2016; Melnyk, 2017). In a grafted plant, the scion becomes the new shoot, and the rootstock is the root system and conducts nutrients across the graft union into the shoot. The success and growth of grafted plants as a new individual are influenced by environmental conditions (light, temperature, rainfall, and humidity), grafting method, and selection of cultivar (scion) and rootstock (Bhandari, 2021).

Grafted materials production through sustainable-friendly practices is an alternative to meet the biotic and abiotic stresses impending fruit production under climate change (Manja & Aoun, 2019). Thus, protected cultivation may be defined as a graft-growing method where the microclimate is controlled as required by the grafted plant for optimum growth and development (Lenka, 2020). Colored nets are being used to protect fruit trees against stressful environmental conditions, such as wind and excess sunlight while improving the quality of grafted plants (Manja & Aoun, 2019). Thus, it is necessary to use current knowledge about how plants respond to light in horticulture. Manipulation of light quantity and quality is used in horticulture production via photo-selective netting to promote physiological and plant growth responses, improving cultivated plants' quality (Oliveira *et al*., 2016; Bastías & Corelli-Grappadelli, 2012). Additionally, to protect crops from stressful environmental conditions (solar radiation, heat, and drought), along with improvements in the thermal climate, resulted in various changes in both crop microclimate and crop activity (Kittas *et al*., 2009; Incesu *et al*., 2016; Ilić *et al*., 2018).

The use of protective coverings has gained popularity worldwide and is being applied as a new technology due to the positive effects on the growth of fruit plants (Anushma *et al*., 2014; Ilić *et al*., 2018). This practice is an economical option for fruit nurseries. In protected nurseries, photo-selective nets influence grafting success, rootstock, and budding growth of a new plant (Brar *et al*., 2020).

Solar radiation is the main climatic parameter affected by the covering net. Photo-selective nets modify the solar radiation quality with significant alterations in the blue (400–500 nm) and red (600–700 nm) light spectrums and promote desired physiological and morphological plant responses (Costa *et al*., 2010). Also, covering nets modify climatic variables inside the plant canopy, such as temperature, relative humidity, and wind speed (Arthurs *et al*., 2013). Colored nets affect light quality by increasing the relative proportion of diffuse light (scattering by 50% or more) as well as absorbing various spectral bands (infra-red radiation) (Milenković *et al*., 2020). Colored covering nets can also alter the radiation spectra reaching the crops below. Thus, black shade nets reduce light intensity without affecting light quality (Shahak *et al*., 2008), and red and yellow nets stimulate the grafted vegetative growth and canopy vigor, while the blue-colored nets stimuli dwarfing growth and the grey-colored nets enhance plant branching and bushiness (Ilić & Fallik, 2017). Spectral manipulation by colored covering nets can trigger a wide range of physiological and morphological responses, mainly over above-ground (scion) tissues of plants.

Physiologically, the covering net effects have been more variable, depending on net color, fruit crops, and cultivars (Costa *et al*., 2010). Much research on netting (black, white, and dark green-colored shade nets are widely utilized) has focused on radiation reduction percentage (shading) by nets. Nevertheless, detailed information about using colored nets in fruit nurseries is currently limited (Aras & Eșitken, 2019).

Therefore, it is essential to determine the responses of plants under photoselective colored nets (Aras & Eşitken, 2019). Numerous studies have addressed the influence of photoselective netting on the growth of fruit tree species, including citrus (Zhou *et al*., 2018), mango (Scuderi *et al*., 2022), tomato (Sotelo-Cardona *et al*., 2021), and avocado (Tinyane et al., 2018). Colored netting is not used routinely in avocado grafting nurseries. However, using a covering net is a quiet way to change the radiation quality favorably for vegetative plant growth to manipulate seed germination and shoot development (Anushma *et al*., 2014); little is known about the effect of different colored netting on avocado-rootstock seedling production. Thus, the interaction between the shading and grafting processes for avocado planting material production is unclear. This research aimed to evaluate rootstock seedling growth and physiological traits in response to the combined utilization of shading for planting avocado production.

MATERIAL AND METHODS

Location. This work was carried out under plastic house conditions at the CARTAMA nursery in the department of Caldas, municipality of Supía (5°26’39’’N; 75°38’56’’W, at 1185 m asl) with a mean temperature between 22 and 24 °C. Annual rainfall of 1,612 mm, and maximum and minimum relative humidity of 94% and 30%, respectively. The mean temperature (°C) was monitored with a portable thermohydrometer CEM DT-172® (CEM Instruments. Kolkata, India) and photosynthetic active radiation (PAR) using a LightScout Quantum Meters® (Spectrum Technologies, Inc. Aurora IL, USA).

Experimental design. A completely randomized experimental design was used, with four treatments and five repetitions. The treatments consisted of colored nets (red, black, white, and control – without a net), and each experimental unit consisted of 60 rootstock plants. Each rootstock was grown from seed extracted from fruits previously disinfected and sown in a seedbed. At 18 days, the seeds were transplanted into 3.7-caliber black bags with 50 cm high by 15 cm wide with perforations. The substrate was a mixture of 70% soil, 20% sand, and 10% coffee beans dry**,** disinfected with 50 g of basamid (Dazomet ®98%).

Variables evaluated. Dry biomass - 90 days after seed transplanting (DAT), sixty rootstock plants were taken by treatment and repetition. Each plant was determined: seed (SeDM), root (RDM), stem (SDM), and leaf (LDM) dry matter and shoot dry matter (ShDM = SDM + LDM). At the same plants, leaves number (LN), rootstock height (RPH), rootstock stem diameter at 10 cm (RD), rootstock height (RH), and leaf area (LA: using a portable leaf area meter Li3000® (LI-COR Environmental. Lincoln, NE, USA) were measured. The samples’ dry leaf weight and measured leaf area were used to calculate the specific leaf area as $cm^2 g^{-1}$. The leaf area index (LAI - m^2/m^2) was determined as the leaf area per unit of horizontal ground surface area (Fang *et al*., 2019).

The Dickson quality index (DQI) was determined, according to Binotto *et al*. (2010), by the expression:

$$DQI = \frac{TDM}{\frac{RH\ (cm)}{RD\ (mm)} + \frac{ShDM\ (g)}{RDM\ (g)}}$$

Where: TDM (total dry matter), RH (rootstock height), RD (rootstock base diameter), ShDM (shoot dry matter), and root dry matter (RDM).

Leaf gas exchange. Gas exchange traits were measured with a portable infrared gas analyzer, LCpro open mode (ADC BioScientific Ltd., UK), over ten plants for each treatment. Net photosynthesis rate (Pn – μmol CO_2 m^{-2} s^{-1}), transpiration rate (E - mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs - mmol H_2O m^{-2} s^{-1}), and leaf temperature (Tl - °C) were determined. The leaflet arranged in the fifth position from the apical meristem of each plant was measured between 09:00 to 12:00 hours. Water use efficiency (WUE) and intrinsic water use efficiency (WUEi) were determined by Pn/E and Pn/gs, respectively.

Statistical analysis. A two-way analysis of variance (ANOVA) was carried out after the validation of normality and homoscedasticity (Shapiro-Wilk and Bartlett tests). Using the R project "agricolae" package, the Tukey's honestly significant difference (HSD) test was used for the mean comparison with a significance level of 5%. To determine the multivariate ordination of gas exchange traits (net photosynthesis rate, transpiration rate, stomatal conductance, leaf temperature. water use efficiency and intrinsic water use efficiency) a principal component analysis (PCA) was calculated using packages "ggplot2, plotly, WriteXLS, and ggfortify" in the statistical environment of the R project. The variables used were net photosynthesis rate (Pn – μmol CO_2 m^{-2} s^{-1}), transpiration rate (E - mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs - mmol H_2O m^{-2} s^{-1}), leaf temperature (Tleaf - °C) Water use efficiency (WUE) and intrinsic water use efficiency (WUEi) were determined using Pn/E and Pn/gs, respectively, and growth variables. and development.

RESULTS AND DISCUSION

Light and temperature. The highest values of photosynthetic photon flux density (PPFD) obtained during the study time were 1,452 μmol m^{-2} s^{-1} (Control), 938 μmol m^{-2} s^{-1} (under 35.4% white net), 802 μmol m^{-2} s^{-1} (under 45% red net), and 658 μmol m^{-2} s^{-1} (under 55% black net) (Figure 1A). Regarding temperature, the red and white shading net showed similar mean air temperatures recorded throughout the day, with maximums of 33 °C; however, under the black net, the air registered temperatures lower by about 2 °C, related to the red and white colored net (Figure 1B). The maximum temperature was recorded under control conditions – without a net at 39.7°C, exceeding

6.4°C for the red and white nets and 8.2°C for the black net. Another aspect to highlight is that between 16:00 and 05:00 hours, the temperature under plastic house conditions was about 2 °C lower compared to the other colored nets.

The primary radiometric property for colored covering nets is altering the photosynthetic active radiation (PAR, 400–700 nm) transmission due to the ability to modify various spectral properties. These light changes impact photosynthesis, plant growth, and development (Manja & Aoun, 2019; Lenka, 2020). Colored covering nets can reduce light intensity (at least 50%) relative to open field conditions (2000 µmol m^{-2} s^{-1}) (Ilić *et al*., 2017). However, although black nets are entirely opaque, this covering does not modify the spectral quality of radiation but decreases light intensity (Manja & Aoun, 2019; Brar *et al*., 2020). Red nets mainly alter the spectral quality in PAR, decreasing blue and increasing red and far-red spectral (590-760 nm) regions or visible ranges (Costa *et al*., 2010; Brar *et al*., 2020).

The effect of nets on temperature still needs to be better understood. Some work reports a thermal increase, whereas others report a thermal decrease, depending on local microclimates, the types of nets used, the method of net application, and the regional location of the orchard (Manja & Aoun, 2019). Some nets reduce the maximum temperature during the day due to their ‘shading effect’ (Manja & Aoun, 2019). In warm climates, a temperature reduction is beneficial, increasing the photosynthesis rate during the hotter hours of the day and protecting against sunburn: here is higher photosynthesis, higher light, and heat excess dissipation by photochemical and transpiration means.

All colored nets reduced about 35-55% of transmitted light photosynthetically active radiation (PAR) compared with uncovered plots (Figure 1A); therefore, net shading affects the environmental temperature at about 2°C under black nets. Contrary to what Zhou *et al*. (2018) found in a study evaluating the effects of colored netting on root growth and development of young, grafted orange trees. It was stated that PPFD measured in the control treatment (complete exposition) was 15.7, up to 61.8% higher than under photo-selective nets (pearl, red, and yellow). At the same time, the leaf temperature differences (0.4 °C) were not significant among the colored net treatments.

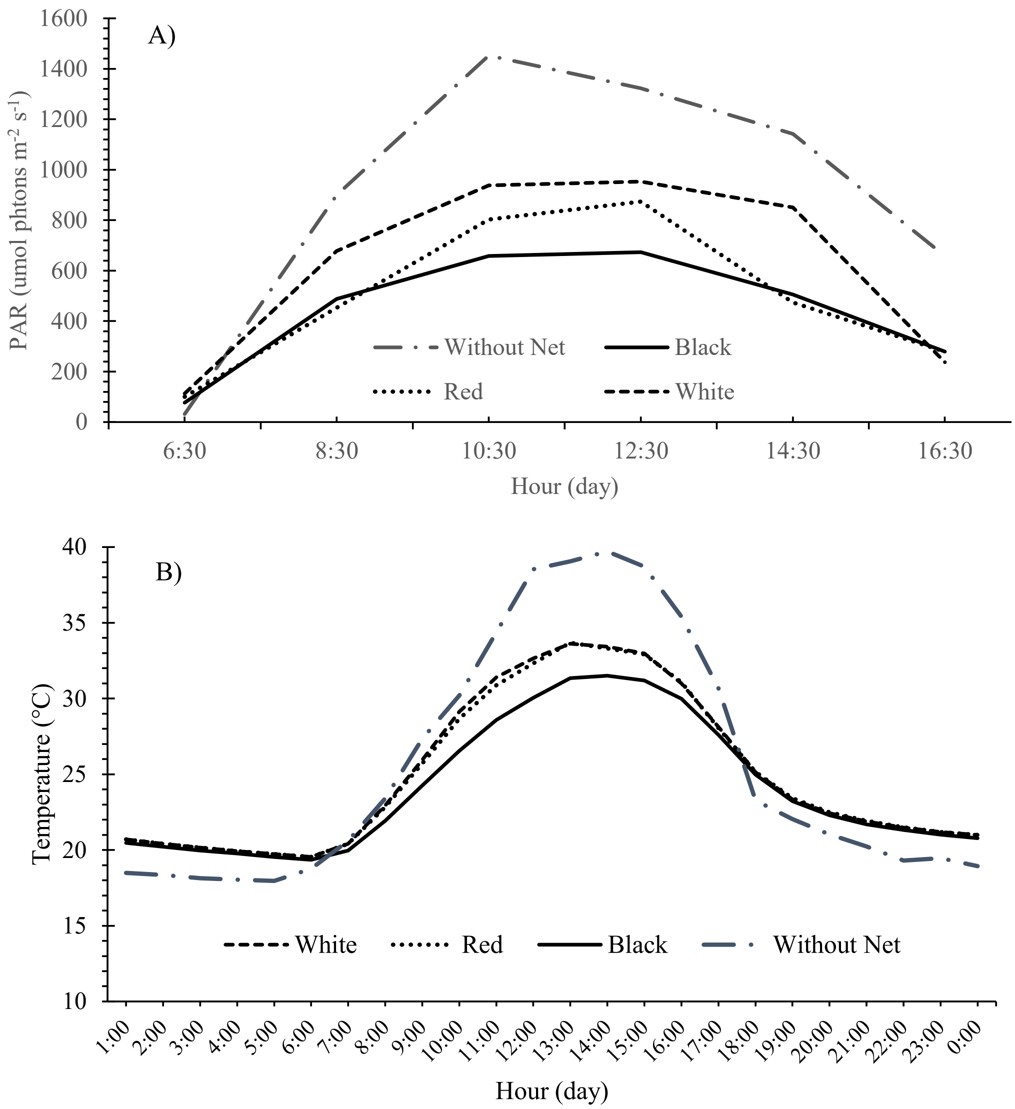


Figure 1. Photosynthetic photon flux density (PPFD -µmol m^{-2} s^{-1}) measured under different colored nets (white, Red, Black and Without net) (A) and Air temperature (°C) over avocado creole rootstock canopy (B).

Rootstock growth. The leaf number (p= 0.000756), leaf area (p= 0.000818), and leaf area index (p= 0.00519) were statistically influenced by net shading treatments (colored net); however, specific leaf area (p= 0.0743) was not different between avocado rootstock seedling growth under the different shade-grown conditions. Red shade-grown rootstocks significantly issued more leaves and leaf area than those grown without-net and black and white nets (Table 1). Moreover, the light intensity management did not affect the avocado creole rootstock's specific leaf area. The LAI was significantly higher by combining light-scattering (red, black, and white) than LAI in plant growth under plastic growth conditions (Table 1).

Table 1. Leaf number (LN), leaf area (LA), leaf area index (LAI), specific leaf area (SLA), Rootstock diameter (RD), Rootstock height, Root length (RoL), Dickson quality index (DQI) of avocado creole rootstock grown under different colored shading nets.

Variable	Without net	Black	Red	White
LN*	16.4 ± 0.6 b	18.8 ± 0.97 b	26.0 ± 1.95 a	21.4 ± 0.51 ab
LA (cm^2)	1458.8 ± 139.6 b	2057.5 ± 126.6 ab	2530.8 ± 184.3 a	1528.6 ± 140.6 b
LAI	0.73 ± 0.04 b	1.33 ± 0.11 a	1.24 ± 0.11 a	1.19 ± 0.07 a
SLA	187.95 ± 10.30 a	164.67 ± 4.75 a	160.78 ± 7.27 a	167.74 ± 10.67 a
RD (cm)	6.54 ± 0.39 b	7.67 ± 0.28 ab	8.17 ± 0.33 a	7.79 ± 0.14 a
RH (cm)	93.6 ± 3.41 a	63.2 ± 3.10 b	90.6 ± 2.34 a	62.4 ± 1.94 b
RoL (cm)	34.8 ± 2.97 a	37 ± 3.49 a	34.4 ± 2.68 a	37.2 ± 2.01 a
DQI	1.83 ± 0.15 ab	2.36 ± 0.20 ab	2.45 ± 0.25 a	1.56 ± 0.11 b

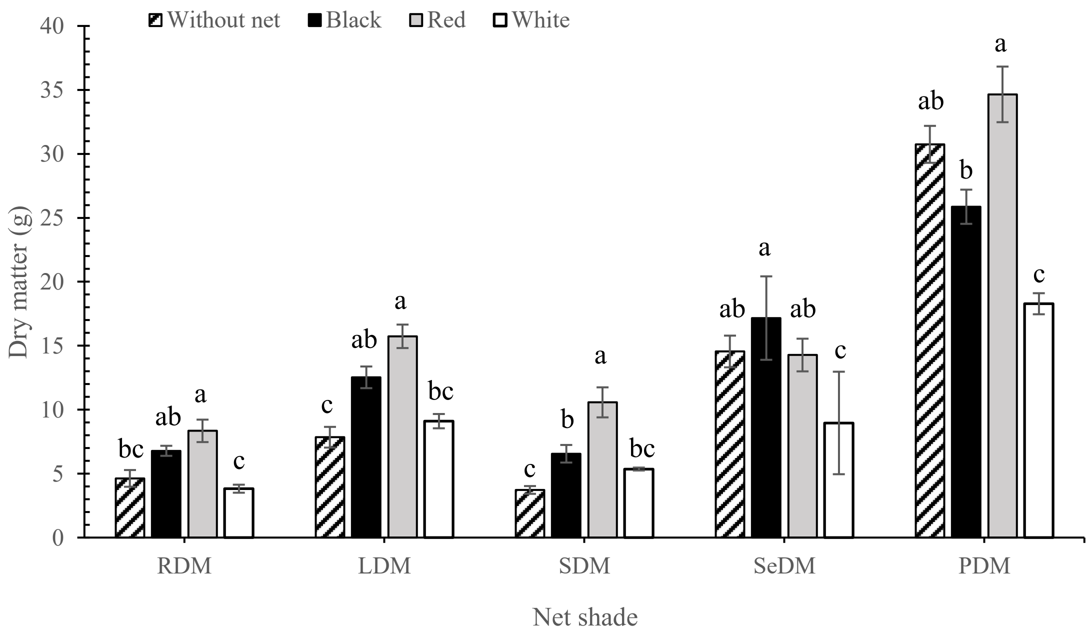
* Mean values ± standard error (n=10) in each followed by different letters are significantly different ($P \leq 0.05$) according to Tukey's honestly significant difference (HSD).

Similarly, other growth parameters like rootstock diameter (p= 0.007), rootstock height (p= 0.000), and Dickson quality index - DQI (p= 0.0217) were also significantly influenced by different growing structures, while root length was not affected (p= 0.886). The rootstock seedlings with the greatest stem diameter were recorded in the red, black, and white nets treatment, exceeding the rootstocks grown in full exposure. On the other hand, net- and full-exposition-grown plants were significantly taller than those grown under black-and-white nets shading. As for the Dickson quality index, the lowest value was registered in rootstocks developed in modified environments with white net shading. Finally, the root length was similar for all shade-grown conditions (Table 1).

Compared to the control, the red, black, and white nets significantly influenced rootstock dry matter accumulation. Root (p= 0.001), leaf (p= 0.000), stem (p= 0.000), seed (p= 0.044), and total (p= 0.000) dry matter of avocado creole rootstock were significantly influenced by different growing structures. Stem dry matter was significantly higher from avocado rootstock seedlings under the red-colored netting (39, 50, and 65%) when compared with those under the black, white, and control, respectively. At the same time, rootstock growth in red (55 and 45%) and black (44 and 32%) showed higher root and leaf dry matter than reported for rootstocks under a white net and control. The highest rootstock total dry matter was recorded in red net and control; at the same time, the lowest total plant dry matter was reported for under-white netting (Figure 1).

Colored nets increase the dispersed light penetrating the plant canopy, improving radiation efficiency (Stamps, 2009), influencing plant development and growth, enhancing photo morphogenetic and physiological responses (Ilić & Fallik, 2017). Shading nets promote leaf carbon assimilation by decreasing midday leaf temperature, stimulating vegetative growth, and increasing branching and plant compactness

(Gimeno *et al.,* 2015; Sivakumar *et al.,* 2017). Considering all variables analyzed, the colored net treatments strongly impacted shoot and root growth. This effect can be explained by changing both lights (Figure 2, Table 1).



Error bars correspond to the standard error (SE) of the mean (n=20). Bars of the same color (same tissue type) across categories followed by different letters are significantly different (P ≤ 0.05) according to Tukey's honestly significant difference (HSD).

Figure 2. Root (RDM), Leaf (LDM), Stem (SDM), Seed (SeDM), and total (PDM) dry matter of avocado creole rootstock grown under different colored shading nets.

The diameter and seedling length are fundamental for the grafting success (Güney *et al*., 2020; Tinyane *et al*., 2018). In this study, the colored nets have been shown to stimulate vegetative seedling growth in some cases. The stem diameter and plant height under red nets were the highest among the different colored nets (black and white). However, Mejía-Jaramillo *et al*. (2022) refer to the fact that in the Cartama nursery, the criterion for selecting rootstocks for grafting is that the stem exceeds 5 mm in diameter and 30 cm in length at the time of grafting. Other authors, such as Gálvez-Cendegui *et al*. (2016), Garbanzo & Coto (2017), and Maradiaga (2017), indicated that homogeneous seedlings must reach at least 0.45–1.0 cm in diameter and 20-50 cm in length at the grafting moment. In this sense, all shading net conditions favored stem diameter and plant height for the grafting process. Contrasting results were recorded by Tinyane *et al*. (2018) in citrus rootstock seedlings, where the highest stem diameter was recorded in open conditions. Despite the above, an aspect to be analyzed in future work should be the speed of growth to achieve favorable morphological attributes in the rootstock for grafting since the shorter time spent in the nursery, the greater the efficiency in rootstock production as a part of the grafting processes to produce planting material for avocado cultivation.

Root growth potential is one of the most accurate traits in terms of land performance. Nonetheless, less effort has been made to understand how colored nets affect plant root development. The high root growth potential of seedlings that rapidly develop after planting is important in obtaining quality rootstocks. Nissim-Levi *et al*. (2014) reported increased root length of ornamental plants grown under yellow nets, and for peach rootstocks, red radiation reduced the root growth (Zhou *et al*., 2018). Contrary to those reported in previous work, our results are consistent. The root length was not affected and remained consistent among light-scattering treatments. Despite this, dry matter variability was recorded for rootstock roots, and significant differences were not observed among colored net treatments. The roots' highest dry matter accumulation was found for rootstocks grown under the red net, compared to the lowest dry matter in the white net.

Leaf area index (LAI) and specific leaf area (SLA) depend on the radiation quality. Under shading conditions with colored nets, an increase in SLA and reductions in the thickness of the leaf are common morphological alterations that confer functional advantages to plants grown in environments with low radiation intensity. Thus, it is essential to understand how anatomical and ultra-structural leaf changes can be related to biomass production (Aras & Eșitken, 2019). As previously indicated, reductions in temperature can favor plant compactness. In this sense, black, red, and white nets increase by 70% of LAI compared to plants in plastic growth conditions. In other words, a greater leaf area about an area of occupation on the ground could be associated with a greater number of leaves emitted in rootstocks under the red net.

In contrast, in the black net, it was related, although with a lower number of leaves and a greater leaf area (larger leaves). In the white net, although the LAI was the same as the other colored net, the rootstocks presented a lower leaf area. This allows us to explain plants' phenotypic plasticity and ability to adapt the canopy structure to the light supply they develop (Table 1).

The DQI is a selection criterion that integrates morphological parameters and is often used during rootstock breeding selection programs. A high DQI value indicates better rootstock-seedling vigor, which is a more desirable phenotype, indicating robustness and balance in biomass distribution in the seedling (Scalon *et al*., 2014). The DQI values of rootstock seedlings varied between 1.56 and 2.45, with the highest values consistently observed in the red net and control treatments. According to Mejía-Jaramillo *et al*. (2022), the DQI for creole rootstocks is related to the seedlings originating from seeds weighing. Kuan-Hung *et al*. (2019) state that seedling vigor influences nursery growth, survival, and plant quality. Andrade *et al*. (2013) and Jaenicke (1999) suggest that highly vigorous seedlings exhibit a balance in growth, dominant stems, large root zones, balanced shoot/root ratio, tolerance to moderate drought, and high irradiation. In this sense, avocado rootstock growth under red netting shows the highest leaf number, leaf area, stem diameter, plant height, and plant dry matter compared to black and white netting but is no different from rootstock growth under plastic growth conditions (Table 1).

Gas exchange. In general, red, black, and white nets and without net caused statistical differences in gas exchange variables: Pn (p <2e-16), E (p= 0.000027), gs (p= 9.68e-05), Tl (p <2e-16), WUE (p <2e-16), and WUEi (p=5.59e-14) in avocado leaves of rootstock seedlings. Under red nets and full exposition, the avocado leaves presented the highest net photosynthesis. Black (41 and 48%) and white (49 and 55%) nets decreased Pn more than rootstock seedling growth under red netting and plastic growth conditions. The highest stomatal conductance (gs) was recorded under full exposition, which increased from 32% from the combination of light scattering (red, black, and white). Additionally, the gs was not altered with red, black, or white nets. The lowest transpiration rates (E) were observed in red netting, which decreased from 16, 23, and 12% to the black and white net and complete exposition conditions. Rootstock seedlings under black and white nets recorded an increase in leaf temperature (Tl) of about 3.7 and 5°C compared with those reported in the red net and control. Finally, the instantaneous WUE and WUEi of avocado creole rootstock under the red-colored shading and plastic growth conditions almost doubled significantly from the black and white netting (Table 2).

Table 2. Physiological characteristics of avocado creole rootstock grown under different colored shading nets.

Variable	Without net	Black	Red	White
Pn*	4.96 ± 0.22 a	2.61 ± 0.16 b	4.37 ± 0.26 a	2.27 ± 0.16 b
g_s	0.116 ± 0.004 a	0.079 ± 0.005 b	0.082 ± 0.004 b	0.078 ± 0.001 b
E	4.59± 0.20 a	4.56 ± 0.37 a	3.85 ± 0.22 b	4.97 ± 0.11 a
Tleaf	35.2 ± 0.38 c	38.9 ± 0.04 b	35.4 ± 0.28 c	40.2 ± 0.06 a
PAR	1438.54 ± 67.78 a	291.32 ± 12.96 c	394.86 ± 18.99 bc	496.52 ± 21.24 b
WUE	1.12 ± 0.06 a	0.57 ± 0.02 b	1.11 ± 0.04 a	0.45 ± 0.03 b
WUEi	46.61 ± 3.11 a	34.48 ± 1.33 b	54.77 ± 2.25 a	29.11 ± 1.97 b

Net photosynthesis (Pn - μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s - mmol H_2O m^{-2} s^{-1}), transpiration rate (E- mol H_2O m^{-2} s^{-1}), Leaf temperature (Tleaf - °C), photosynthetically active radiation (PAR -μmol m^{-2} s^{-1}), water use efficiency (WUE - μmol CO_2 / mol H_2O), and intrinsic water use efficiency (WUEi - μmol CO_2 / mmol H_2O). * Mean values ± standard error (n=10) in each followed by different letters are significantly different (P ≤ 0.05) according to Tukey's honestly significant difference (HSD).

The first two principal components (PCs) from the PCA of gas exchange variables for avocado rootstock seedling growth under different light conditions in the present study accounted for 74.97% of the variation in the data (Figure 3). The PCA showed variation for different physiological gas exchange traits studied under net shading conditions. The Tleaf positively correlated with the white net, while Pn, gs, E, WUE, and WUEi were negatively correlated with the white net in PC1 (Figure 3). Tl in avocado rootstock seedling leaves was higher in black and white net shading conditions, while the gs, E, and Qleaf over rootstock were higher under full light conditions. Red net shading favored the highest Pn, WUE, and WUEi (Figure 3).

The analysis of the principal components revealed a clear differentiation between the treatments. The first principal component (PC1), closely related to growth and development variables, indicated that the red net promoted significantly higher vegetative development than the other treatments. The second principal component (PC2), associated with physiological variables (Pn, WUE, WUEi, gs, E, and Qleaf), showed that the red net optimized water use efficiency and the photosynthetic rate. The black and white net treatments were grouped in regions of the PCA space that reflected a lower capacity of rootstock plants to grow and photosynthesize efficiently, suggesting limiting effects on light capture and utilization.

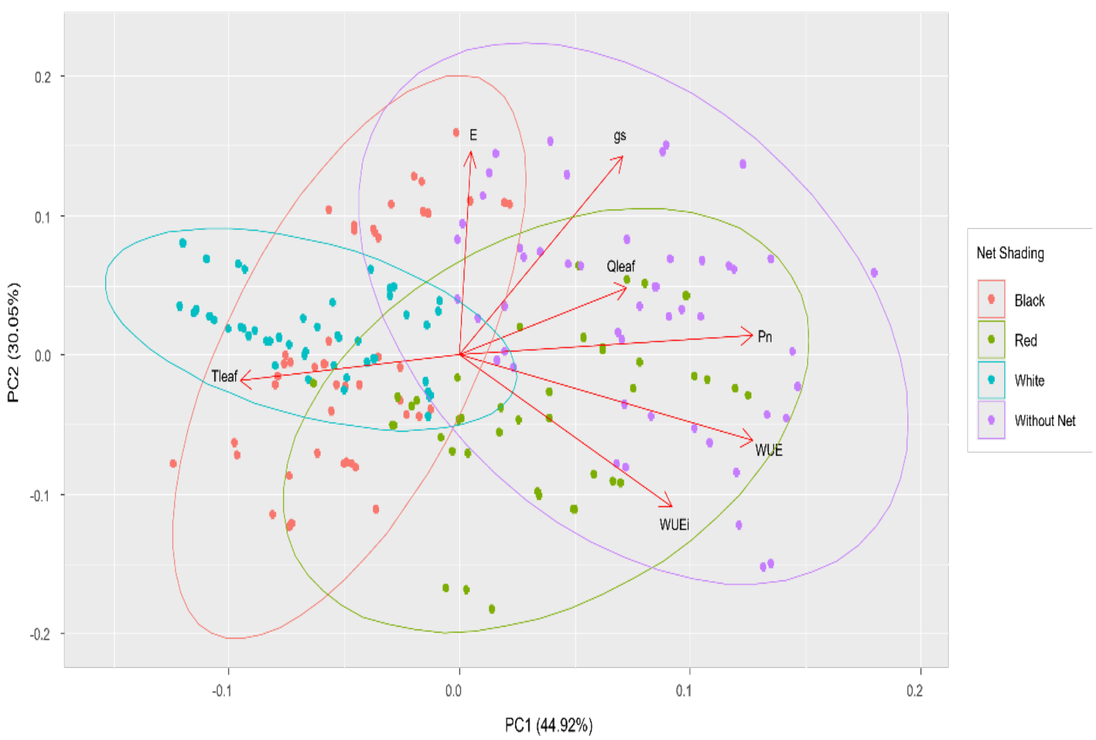


Figure 3. Principal component analysis of gas exchange traits of avocado creole rootstock growth under different light conditions by colored net shading. Net photosynthesis rate (Pn), transpiration rate (E), stomatal conductance (gs), leaf temperature (Tleaf), water use efficiency (WUE) and intrinsic water use efficiency (WUEi).

Enhanced microclimate under photo-selective covering nets characterized by reduced air velocity and climatic variations explain improved net photosynthetic and water use efficiency (Shahak *et al*., 2008). Manja & Aoun (2019) state that with increasing red and blue light, plants increase net photosynthesis. In this sense, red nets antagonistically affect blue (less) and red (greater) light transmission. When red-light intensity increases, the synthesis of enzymes related to photosynthesis rate increases, thus increasing chlorophyll content per unit leaf area (Sivakumar *et al*., 2017).

Lower gs under red-colored nets were also linked to higher Pn and lower E, contrary to black and white nets, where despite having the same gs compared to the red net, the Pn was lower and E was higher in these treatments (Table 2). These results are contrary to those reported in apple and citrus, where exposure to red shading nets increased not only Pn but also gs and E, and Pn did not differ between colored nets (blue and red) (Medina *et al*., 2002; Bastías *et al.,* 2021). These results suggest that even though seedling leaves under red net develop as "shade leaves", these conditions limit the photosynthetic process, decreasing palisade cell development and stomata density. Even so, these morphological changes did not affect their gas exchange performance (Takemiya *et al*., 2005). Leaf gas exchange in plants at plastic growth conditions indicated that while red, black, and white nets reduced PPFD compared to the control treatment (Without net) (Figure 1A), Pn was higher under normal light conditions than black and white covering nets, and there were no differences between gs and E (Table 2). These results can be explained by the differences in spectral light quality transmission between black and white nets, which are associated with these responses (Bastías *et al*., 2021).

Within the physiological gas exchange traits, a relevant aspect of the analysis is the leaf temperature (Tl). The highest Tl was recorded in leaves under black and white nets, while the lowest was in red nets and complete light, equally associated with the higher Pn. The higher the photosynthetic rate, the greater the capacity to dissipate excess energy from radiation, metabolic heat or environmental heat. In this sense, increases in Pn favor photochemical quenching processes in the leaves of the rootstocks, which tends to improve the efficiency of water use since transpiration does not become the main quenching mechanism; therefore, the leaves under the red net presented the lower E.

On the other hand, shading has been used to mitigate extreme climatic fluctuation, increasing WUE by reducing the transpiration rate as long as maintaining the photosynthesis rate (Alarcón *et al.,* 2006; Nicolás *et al*., 2008). Manja & Aoun (2019) state that WUE under colored nets is generally lower than in open-field conditions. However, this approach cannot be generalized since WUE depends on transpiration and CO_2 fixation. Therefore, in the present study, although black and white nets did not affect E compared to plastic growth conditions, Pn was reduced by 50% in these lighting environments, contrary to what was observed with red-net, where E was reduced and Pn was higher, causing a greater WUE.

CONCLUSIONS

Photoselective shade nets offer an effective strategy to mitigate the adverse effects of high solar radiation in avocado nurseries. The results show that using a net of different colors significantly impacts the growth and physiology of the plants used as avocado rootstocks. In particular, the red net promoted greater vegetative growth and better water use efficiency, suggesting its potential to optimize rootstock quality under conditions of high solar irradiation.

ACKNOWLEDGMENTS

We thank the Avofruit S.A.S for their trust and support in the research area. To the Universidad Nacional de Colombia Medellin Campus for facilitating the alliance and providing knowledge. To the Laboratorio de Ecofisiología de Plantaciones en el Trópico - EPAT for their trust and willingness. To David Saavedra Burbano and Juan Pablo Roldan Eusse.

Conflict of interest: The authors declare that there is no conflict of interest.

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