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RESEARCH ARTICLE

Growth and bioactive compounds of baby-leaf chicory on colored cultivation benches with different wavelengths

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ABSTRACT

Care for human well-being and health requires the consumption of healthy foods, and therefore, the "baby-leaf" gains increased space in the market as they have higher concentrations of bioactive compounds. The present study aimed to evaluate the growth and bioactive compounds of baby-leaf chicory (*Cichorium intybus* L.) according to the color of cultivation benches with different wavelengths. The experiment was conducted in a non-acclimatized greenhouse, covered with low density polyethylene film with an aluminized screen of 50% shading under the film and 50% shading screens on the sides. In a completely randomized design, five types of benches were evaluated without and with bright reflective colored materials: Control bench without reflective material, a bench with reflective bright white laminate, reflective bright dark red laminate, reflective bright dark blue laminate, and reflective bright yellow laminate. The bright white and yellow laminates amplified the most photosynthetically active radiation. All the laminates enhanced yield of baby-leaf chicory plants, where white, yellow, red, and dark blue enhanced the fresh matter by 102.6%, 50.2%, 60.6%, and 37.4% compared to the control, respectively. The bright dark blue laminates enhanced the bioactive compounds of baby-leaf chicory plants. The bright dark blue laminate enhanced the carotenoid production of baby-leaf chicory plants by 11.0%. The use of colored laminates was effective in the cultivation of baby-leaf chicory, both in terms of yield and quality, with significant increases in fresh matter and bioactive compounds. To provide baby-leaf chicory with higher bioactive content with antioxidant effect (carotenoids) the use of dark blue laminates is necessary; however, if the objective is greater shoot DM it will be white laminates.

Key words: *Cichorium intybus*, light supplementation, phytochemicals, reflected photosynthetically active radiation.

INTRODUCTION

Chicory (*Cichorium intybus* L.) is an annual herbaceous *Asteraceae* (Compositae) vegetable. This leafy vegetable has a bitter taste and is very nutritious, with medicinal (Al-Snafi, 2016) and pharmacological (Street et al., 2013; Qadir et al., 2022) properties and bioactive compounds (Qadir et al., 2022). Studies and laboratory tests reveal the beneficial effects of *C. intybus* extracts as health promoters (Janda et al., 2021) and in combating diseases such as osteoporosis (Hozayen et al., 2016), diabetes (Rezagholizadeh et al., 2016), and fungi (Mares et al., 2005), as these extracts promote the maturation and activation of dendritic cells (Karimi et al., 2014). The commercialization of *C. intybus* can be in microgreens, baby-leaf, or adult plants.

Baby-leaf vegetables are consumed in their early stages of growth and are part of a growing market in Brazil and worldwide. These foods are smaller, easier to consume and prepare, and have higher bioactive compounds essential to human health. In addition to being small, baby-leaves obtained through early harvesting provide better practicality and stand out for the modern and colorful aspects they give to dishes, making them tastier and more attractive to children and adults.

Baby-leaf as a food with high added value, young leaves are grown in protected environments where provide better the development and biosynthesis of pigments. Species grown in ideal light conditions promote increased photosynthesis, show greater efficiency in converting solar radiation into dry matter, have a higher lipid content, increased *a*, *b* and total chlorophyll indices, as well as lower leaf thickness, acid detergent fiber, cellulose and lignin content, thus presenting more attractive bromatological characteristics for commercial production, which contributes to obtaining better characteristics from the chicory produced (Schwerz et al., 2017; Kang et al., 2023; Silva et al., 2023).

An adequate and uniform distribution of photosynthetically active radiation (PAR) inside protected environments allows for an increase in photosynthetic gains and, consequently, alters the production and/or alter the production rates of bioactive compounds since this light radiation is the main source of chemical energy in physiological and biochemical, being its intensity and quality can alter leaf structure, chloroplasts, and plant pigments (Taiz et al., 2014).

The use of reflective materials on cultivation benches in protected environments aims to improve efficiency light incidence by increasing reflecting light to the leaves. Recent studies have proven the efficiency of using a mirror on the growing benches as a reflective material in the initial growth of *Passiflora edulis* (Santos et al., 2017) and pepper production (Silva et al., 2021), as well as the material consisting of aluminized mesh in the quality of seedlings of *Syzygium cumini* (Salles et al., 2017), *Schizolobium amazonicum* (Mortate et al., 2019), and *Dipteryx alata* (Costa et al., 2020).

Colored reflective materials (white and red) on growing benches increased the reflectance of PAR, improving the use of available light in environments with greater shading, positively influencing the development of basil (Cavalcante et al., 2021). Plant growth and fruit production of cherry tomatoes were favored with the use of aluminized material and red laminate on the growing benches, promoting desirable characteristics of ornamental plants (Campos et al., 2023).

White light irradiance has been shown to increase the photosynthetic rate compared to monochromatic lighting. However, blue light favors the accumulation of chlorophyll and carotenoids. It was observed that the concentration of carotenoids was higher in buckwheat seedlings grown under white light when compared to those under exposure to blue or red light (Zheng et al., 2019).

With regard to plant development, blue light stands out because it has an inhibitory effect on stem elongation in several species, including both ornamental and medicinal plants, exemplified by chrysanthemum and stevia. However, it is assumed that the different responses to the quality of light may be due to the specific characteristics of the species and genotype in question (Zheng et al., 2019). In addition, in pepper plants (*Capsicum frutescens*), the blue, red and aluminum reflector benches did not influence plant growth and production (Lima et al., 2018).

In view of the above, this experiment aims to address the following hypotheses: 1) Benches with reflective coatings increase the photosynthetic efficiency of chicory; 2) benches with colored reflective coatings increase the biomass and growth of chicory; 3) chicory produces more chlorophyll and carotenoids when grown on blue reflective benches.

Given the above, this study aimed to evaluate the growth and production of bioactive compounds of chicory baby-leaf in colored reflective cultivation benches with different wavelengths.

MATERIALS AND METHODS

The experiment with baby-leaf chicory (*Cichorium intybus* L.) was conducted at the Mato Grosso do Sul State University (UEMS), Unit of Cassilândia, Cassilândia-MS, Brazil, from 18 October to 17 November 2022. The site is at 19°07'21" S, 51°43'15" W; 516 m a.s.l. (Automatic Station Cassilandia-A742).

The experiments were conducted in an agricultural greenhouse of 18.0 m length × 8.0 m width × 4.0 m height under the gutter (area of 144 m^2), covered with low density polyethylene (LDPE) film of 150 µm, light diffuser, anti-drip, with the zenithal opening sealed with 50% white screen, with side and front monofilament screen of 50% shading. Under the LDPE film, an aluminized thermo-reflective mobile screen of 50% shading remained extended under the film.

Inside the protected environment, bright colored laminated reflective materials (Formicas™), they are called laminated mulches, were tested on the cultivation benches with different wavelengths, in a completely randomized design, with five treatments and five replicates, designated as: Control without material on the bench surface, a bench covered with bright white laminate, a bench covered with bright dark red laminate, a bench covered with bright dark blue laminate, and a bench covered with bright yellow laminate (Figure 1).

The cultivation benches had dimensions of 1.40 m wide × 3.50 m long × 0.80 m high, and each reflective material covered an area of 1.03 m × 1.25 m (1.29 m^2), where each replica was 80 cm away from each other, so that there was no interference from one treatment to the other. The laminates were 308 cm long, 125 cm wide and 0.8 mm thick. According to the manufacturers, they are manufactured at high pressure, contain a surface protection film (overlay), contain 100% melamine resin, decorative veneer, special kraft, and phenolic resin.

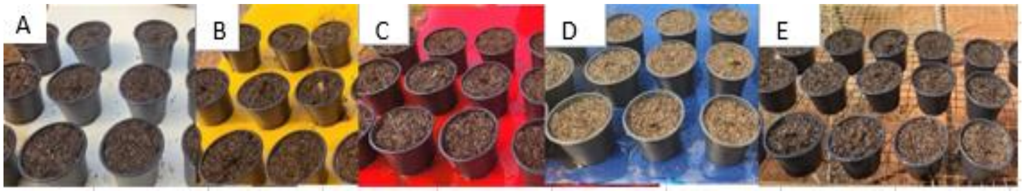

Figure 1. Cultivation on colored benches, white (A), yellow (B), red (C), dark blue (D) and control (E).

Black pots (1.0 L) containing commercial substrate (Carolina Soil, Carolina Soil Company, Santa Cruz do Sul, Rio Grande do Sul, Brazil) were used to produce baby-leaf chicory. The black pots measure 10.5 cm in height, 9.5 cm in diameter at the bottom and 13.5 cm in diameter at the top. Three seeds were sown per pot, after emergence, the thinning was performed (with scissors), leaving only one plant per pot. Irrigation of the plants occurred twice daily, in the morning and afternoon, when necessary. The irrigation was with manual watering cans and no supplementary fertilizer was applied, as at the baby leaf stage the commercial substrate has sufficient nutrients.

At 30 d after sowing, when the young leaves of all the treatments reached between 13 and 16 cm in length, the following assessments were performed: Number of leaves (NL), plant height (PH), shoot fresh matter (SFM), shoot DM (SDM), root DM (RDM), total DM (TDM), chlorophyll *a* (CLA), chlorophyll *b* (CLB), total chlorophyll (CLT) and carotenoids (CRT) contents. Internal CO_2 concentration (C_i), transpiration (E), stomatal conductance (g_S), CO_2 assimilation rate or net photosynthesis (A), water use efficiency (WUE), and instantaneous carboxylation efficiency (A/C_i) were also evaluated, portable infrared gas exchange meter (LCi, ADC Bioscientific, Hertfordshire, UK).

Plant height (PH) was measured with the aid of a ruler, measuring the distance from the base to the apex of the stem, and the number of leaves was obtained by counting (NL). The determinations of shoot fresh matter (SFM), shoot DM (SDM), and root DM (RDM) were made on an analytical balance (four decimal places). Plant material was dried using forced air at 65 °C for 72 h, and then weighed on an analytical balance.

Extractions of chlorophylls (*a* and *b*) and carotenoids were performed following the methodology of Lichtenthaler (1987). A sample of 0.5 g fresh plant material was weighed, 5 mL 80% acetone was added, and the material was placed in 14 mL test tubes for 48 h in a refrigerator at 25 °C. After this period, the test tubes were centrifuged for 15 min at 4000 rpm, and then the supernatant of the extract was diluted in the ratio of 0.3 mL extract to 1.7 mL 80% acetone. Measurements were performed in a UV/VIS spectrophotometer (IL-226-NM, Kasuaki, Tokyo, Japan), at wavelengths of 470, 647, 653, 663, and 665 nm.

The portable infrared gas exchange meter (LCi, ADC Bioscientific) was used to determine the internal CO_2 concentration (C_i), transpiration (E), stomatal conductance (g_S), and CO_2 assimilation rate or net photosynthesis (A) at 09:00 h. Subsequently, water use efficiency (WUE) (A/E ratio) and instantaneous carboxylation efficiency (A/C_i ratio) were calculated.

Reflected photosynthetically active radiation (RPAR) (µmol m^{-2} s^{-1}) was monitored from the cultivation benches with a portable digital pyranometer (Apogee Instruments, Logan, Utah, USA) daily at 09:30 h (MS). The RPAR data were compared in a randomized block design with five replicates of 7 d. The mean external and internal photosynthetic radiation were 2014.8 and 624.8 µmol m^{-2} s^{-1}, respectively. The external radiation (full sun) and the internal radiation in the environment were collected with the sensor facing upwards and the reflected radiation by the benches the sensor was facing downwards. The internal incident radiation was measured and this was considered 100%. The reflected radiation of the colors was a percentage of the internal incident radiation, being obtained by the rule of three.

The reflectance spectra of the colored laminate reflective materials (Formica) were obtained using a UV-Vis-NIR spectrophotometer (Lambda 1050, PerkinElmer, Waltham, Massachusetts, USA) with a step size of 1 nm at 100 nm min^{-1}. Small disks of the laminates (diameter = 1 cm) were inserted into the sample holder of a 150 mm radius integrating sphere.

The statistical software Sisvar 5.3 (Ferreira, 2011) was used, and the means were submitted to the LSD test at 5% probability.

RESULTS

The reflectance spectra of baby-leaves were obtained in the range of 400-700 nm (Figure 2) to follow the contribution of the colors of the laminates used in cultivation. As expected, the white laminate reflects almost all visible light portions, with maximum reflectance (~ 90%) around the green region. The yellow laminate exhibits a reflectance cut off in the yellow region, thus preventing much of the solar radiation from being reflected and consequently absorbed by the chlorophylls *a* and *b* and the carotenoids of the leaves, which also absorb between 400 and 500 nm. The maximum solar reflectance by the yellow laminate is around 82% and is in the region between orange and red. On the other hand, red laminate has a reflectance cut-off around the orange of the electromagnetic spectrum, allowing only the visible light between 600 and 700 nm to be reflected to the plant. The maximum reflectance reaches 68% in this region, which can be useful for absorbing the plant's chlorophylls *a* and *b*. Finally, the dark blue laminate has very low reflectance in the visible region (10%-15%), with higher reflectance in the violet-blue regions (Figure 2).

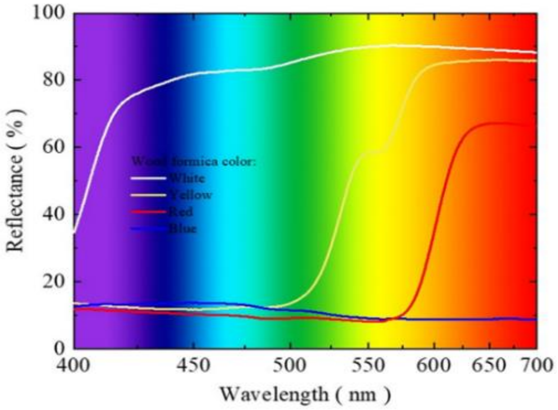

Figure 2. Reflectance of the laminates used on the cultivation benches with different wavelengths for growing baby-leaf chicory.

The mean external and internal photosynthetic radiation were 2014.8 and 624.8 µmol m^{-2} s^{-1}, respectively. The white and yellow laminates were the most reflective of reflected photosynthetically active radiation (RPAR) (Figures 3A and 3B). In percentage, all laminates reflected more RPAR than the control bench, as the white, yellow, red, and dark blue laminates amplified 487%, 295%, 110%, and 16% respectively the RPAR compared to the control (Figure 3A). On average, the RPAR of the white and yellow benches reflected 22.5% and 15.0% of the initial RPAR inside the environment (Figure 3B).

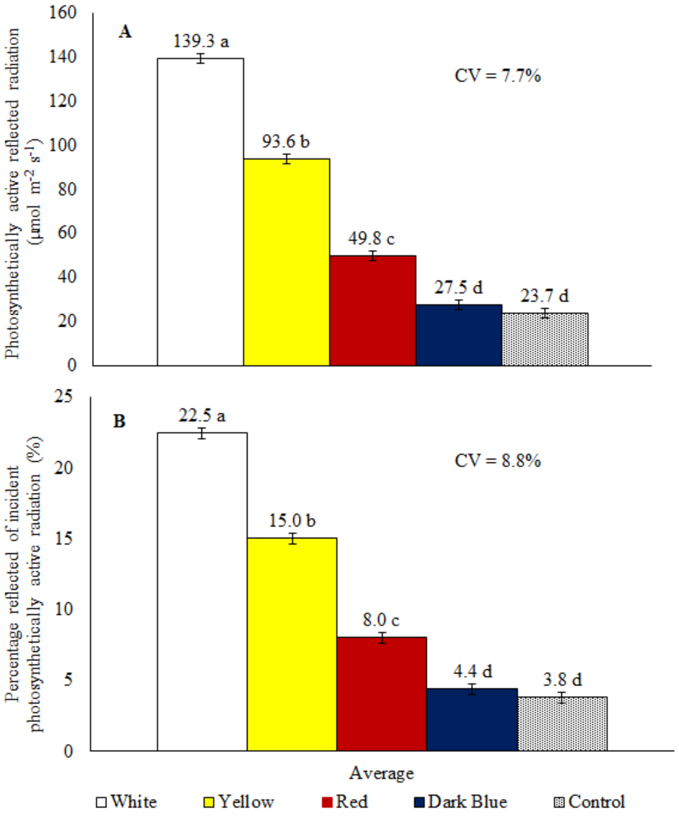

Figure 3. Reflected photosynthetically active radiation by the cultivation benches (A) and percentage of incident photosynthetically active radiation reflected by the cultivation benches (B) with different wavelengths for growing baby-leaf chicory from 18 October to 17 November 2022. CV: Coefficient of variation. Means followed by the same letter do not differ by the LSD test. Vertical bars indicate standard error.

The colors of the laminates on the cultivation benches increased the number of leaves, height, and shoot fresh and DM, emphasizing the white and yellow laminates (Figures 4A, 4B, 4C, 4D, 4E, and 4F). The white, red, yellow, and dark blue laminates increased the fresh matter of the shoot by 103%, 61%, 50%, and 35% (Figure 4C). The white color of the laminate promoted plants with higher DM, followed by the yellow and red laminates, and increased 82%, 43%, and 40% of the shoot DM compared to the control (Figure 4D).

The dark blue color of the cultivation bench increased the chlorophyll production along with the red bench (Figures 5A, 5B, and 5C). The red cultivation bench increased the chlorophyll by 10% (Figure 5C) compared to the control bench. The dark blue cultivation bench promoted baby-leaf chicory plants with higher carotenoid content, increasing by 11% (Figure 5D).

There were no differences between treatments for C_i, E, g_S, A, WUE, and as shown in Figures 6A to 6F, the plants showed instantaneous carboxylation efficiency (A/C_i), demonstrating that the plants were under similar conditions.

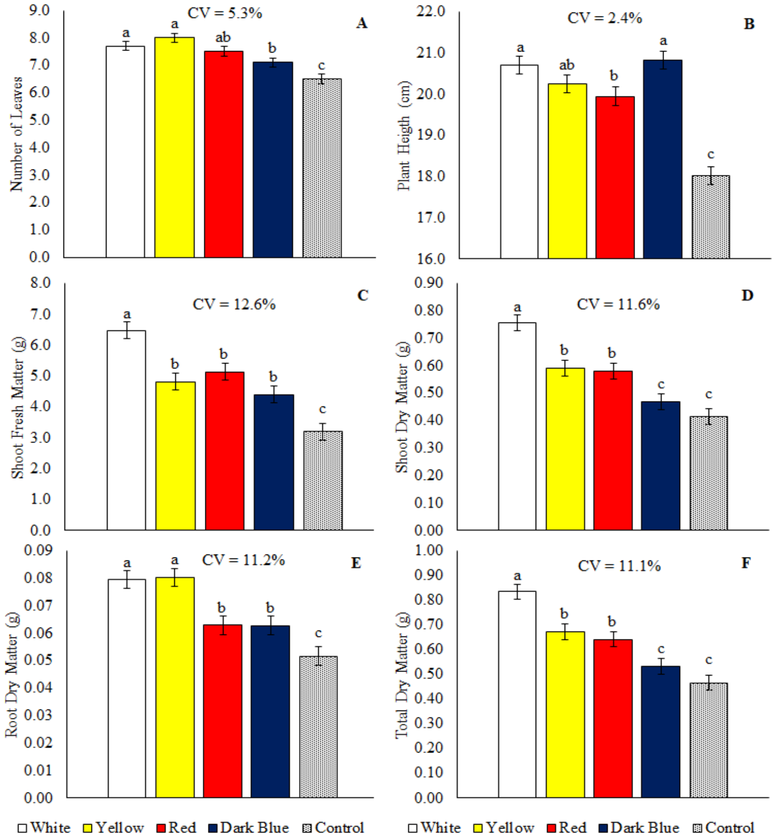

Figure 4. Number of leaves (A), plant height (B), shoot fresh matter (C), shoot DM (D), root DM (E), and total DM (F) of baby-leaf chicory grown on cultivation benches with different colors and different wavelengths from 18 October to 17 November 2022. CV: Coefficient of variation. Means followed by the same letter for each variable do not differ by the LSD test. Vertical bars indicate standard error.

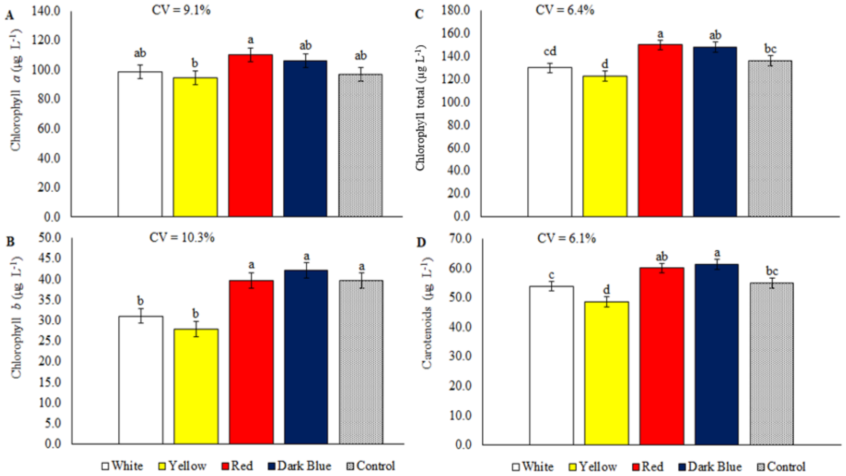

Figure 5. Chlorophyll *a* (A), chlorophyll *b* (B), total chlorophyll (C), and carotenoids (D) of baby-leaf chicory grown on cultivation benches with different colors and different wavelengths from 18 October to 17 November 2022. CV: Coefficient of variation. Means followed by the same letter for each variable do not differ by the LSD test. Vertical bars indicate standard error.

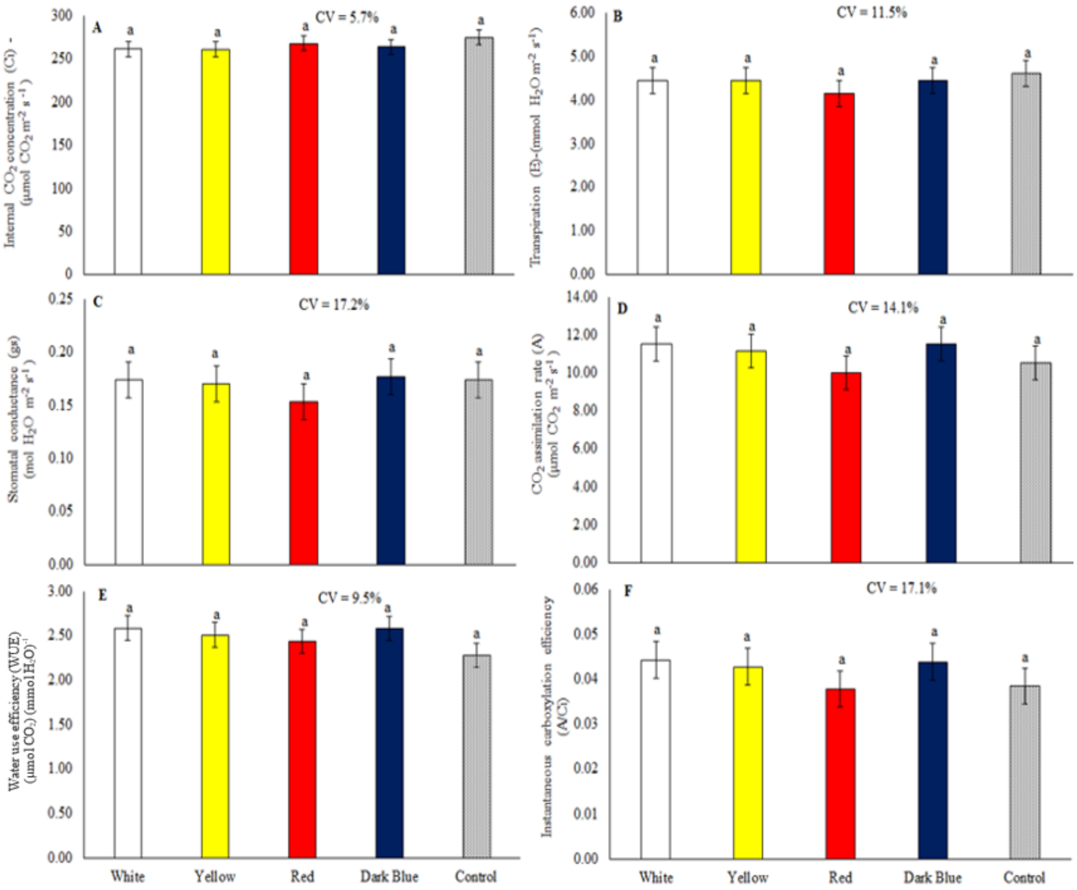

Figure 6. Internal CO_2 concentration (A), transpiration (B), stomatal conductance (C), CO_2 assimilation rate (D), water use efficiency (E), and instantaneous carboxylation efficiency (F) of baby-leaf chicory grown on cultivation benches with different colors and different wavelengths from 18 October to 17 November 2022. CV: Coefficient of variation. Means followed by the same letter for each variable do not differ by the LSD test. Vertical bars indicate standard error.

DISCUSSION

According to the results found in this study, the hypotheses about the positive effects of reflective growing benches on the growth and biomass of chicory are clear. The cultivation benches with reflective material are a technique for plant production that makes it possible to return the radiation present in the environment to the leaves of the plants, presenting greater efficiency and use of energy, providing an increase in plant growth both qualitatively and quantitatively (Salles et al., 2017). The efficiency of using reflective materials in increasing the growth rate (Santos et al., 2017) presented significant results for the quality of the baby-leaf plants produced in agricultural greenhouses (Mortate et al., 2019).

Photosynthesis and photomorphogenesis of plants are influenced by light wavelength, intensity, and photoperiod via photoreceptors that control plant growth (Bilodeau et al., 2019). The use of cultivation benches with white and yellow reflective materials offers better conditions for the growth of baby-leaf chicory. According to Wu et al. (2014), plants monitor the environment for photosynthetically active radiation and articulate their growth and development to enhance solar energy capture, ensuring production.

The ratio between root DM to total DM is increased when grown in environments with reflective material, which also verifies that the reflective material has higher reflected photosynthetically active radiation. Reflective materials in cultivation benches can increase the reflection of radiation used by the metabolic process and enable an increase in biomass (Cabral et al., 2020), which highlights the positive effect on the greater distribution of photosynthetically active radiation in the leaf area of plants, through the use of benches with reflective material.

Through the increase in total DM in the production of baby-leaf chicory, the efficiency of colored materials in cultivation benches can be proven (Cavalcante et al., 2021; Campos et al., 2023), which act as supplementary radiation, similar to that provided by LEDs, since blue and red LEDs increase the length of plant shoots and the accumulation of dry and fresh matter (Li et al., 2013; Silva et al., 2016; 2017).

The total chlorophyll from the dark blue laminate presented higher values when compared to the other treatments, evidencing the superiority over the other laminates since dark blue light is strongly absorbed by plants and thus leads to greater production of chlorophylls (Galvão and Fankhauser, 2015; Carvalho and Folta, 2016; Klem et al., 2019). In addition to the higher production of chlorophylls *a* and *b* with the dark blue color, it was also found the highest index of carotenoids in the present study due to the high correlation between the pigments (Cassetari et al., 2015). In plants grown under dark blue light, the stimulation of chloroplast development, chlorophyll synthesis, stomata opening, and regulation of leaf expansion is evident (Liu et al., 2014; Hung et al., 2016) in responses directed by cryptochromes and phototropin (Landi et al., 2020).

In addition, the ratio of total chlorophyll and carotenoids in the reflective white color of the cultivation bench increased the process of photosynthesis, which shows that the radiation was better used by the plant photosynthetic apparatus, contributing to the accumulation of reserves for chlorophyll, consequently plant growth and development.

Using reflective materials, alternative methods provide plants with high conditions to capture photosynthetic radiation, consequently increasing development and yield. Colored laminate materials aim to improve the reflectance of sunlight and, consequently, the absorption rates that will generate by-products for photosynthesis. Being this oxidation-reduction reaction between CO_2 (oxidizing agent) and H_2O (reducing agent); in this reaction, the electrons are transferred against an electrochemical gradient (Taiz et al., 2014). The transpiration is measured through the difference in water vapor concentration in the leaf intercellular spaces (Long et al., 1996).

CONCLUSIONS

All laminates increased the yield of baby-leaf chicory plants. The white, yellow, red, and dark blue laminates increased the fresh matter by 102.6%, 50.2%, 60.6%, and 37.4% compared to the control, respectively. The bright dark blue laminate increased the carotenoid production of baby-leaf chicory plants by 11%.

The use of colored laminates has been shown to be effective in the cultivation of baby-leaf chicory, both in terms of yield and quality, with significant increases in fresh matter and bioactive compounds. To provide baby-leaf chicory with higher bioactive content with antioxidant effect (carotenoids) the use of dark blue laminates is necessary, however if the objective is greater shoot dry matter it will be white laminates.

Author contribution

Conceptualization: T.A.N.A., F.F.S.B., T.D., E.C. Methodology: T.A.N.A., T.D., F.F.S.B., E.C. Investigation: T.A.N.A., T.D., F.F.S.B., E.C. Resources: G.H.C.V., E.C., F.F.S.B. Data curation: T.A.N.A., T.D., F.F.S.B., E.C., S.M.L, L.H.C.A. Writing-original draft: T.A.N.A., T.D., F.F.S.B., E.C., E.P.V., F.C.S.R., S.M.L., L.H.C.A. Writing-review & editing: T.A.N.A., T.D., F.F.S.B., E.C., E.P.V., F.C.S.R., S.M.L., L.H.C.A. Project administration: F.F.S.B., E.C., G.H.C.V. Funding acquisition: F.F.S.B., E.C., G.H.C.V. All co-authors reviewed the final version and approved the manuscript before submission.

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