

Production and phenotypic characterization through multispectral imaging of strawberry cultivars¹

Produção e caracterização fenotípica de cultivares de morango por meio de imagem multiespectral

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HIGHLIGHTS:

The Cerrado influences the production of different strawberry cultivars.

In remote sensing it is possible to calculate vegetation indices as an indirect measure of agronomic characteristics.

This manuscript will guide future research to speed up the high-throughput phenotyping of strawberries.

ABSTRACT: Technologies assisted by remote sensing have been developed to quantify the morphophysiological characteristics of plants in a timely and rapid manner. Thus, the purpose of the present study was to evaluate the behavior of strawberry cultivars in terms of production and their phenotypic characterization using multispectral imaging under the environmental conditions of the Brazilian Cerrado biome. The experiment was conducted in the field, at the Federal University of Uberlândia, Campus Monte Carmelo, Minas Gerais state, Brazil. A randomized block experimental design was used with six strawberry cultivars (San Andreas, Albion, PR, Festival, Oso Grande, and Guarani), with four replicates. The variables evaluated were number and mass of fruits per plant; total productivity SPAD index; number, length, and width of leaves, soluble solids content, titratable acidity, vitamin C, anthocyanins, fruit firmness, soil temperature under and above the mulch, leaf temperature, air temperature, and relative air humidity. Multispectral imaging was obtained with unmanned aerial vehicle with a Mapiir Survey 3W camera attached to it. The normalized difference vegetation index was calculated, and Pearson's correlation was performed between the agronomic variables and the spectral bands. The obtained results indicated that temperature and relative air humidity affect strawberry production. Estimation of agronomic variables using multispectral imaging at 78 days after planting is feasible and can help in the selection of new genetic materials.

Key words: *Fragaria ananassa*, reflectance, temperature, normalized difference vegetation index

RESUMO: Tecnologias assistidas por sensoriamento remoto têm sido desenvolvidas para quantificar as características morfofisiológicas das plantas de forma oportuna e rápida. Assim, o objetivo do presente estudo foi avaliar o comportamento de cultivares de morango em termos de produção e sua caracterização fenotípica usando imagens multiespectrais sob as condições ambientais do bioma Cerrado brasileiro. O experimento foi conduzido em campo, na Universidade Federal de Uberlândia, Campus Monte Carmelo, Minas Gerais, Brasil. O delineamento experimental foi em blocos casualizados com seis cultivares de morango (San Andreas, Albion, PR, Festival, Oso Grande e Guarani), com quatro repetições. As variáveis avaliadas foram número e massa de frutos por planta; índice SPAD de produtividade total; número, comprimento e largura de folhas, teor de sólidos solúveis, acidez titulável, vitamina C, antocianinas, firmeza dos frutos, temperatura do solo abaixo e acima da cobertura morta, temperatura da folha, temperatura do ar e umidade relativa do ar. As imagens multiespectrais foram obtidas com veículo aéreo não tripulado com uma câmera Mapiir Survey 3W acoplada a ele. Foi calculado o índice de vegetação por diferença normalizada e realizada a correlação de Pearson entre as variáveis agrônomicas e as bandas espectrais. Os resultados obtidos indicaram que a temperatura e a umidade relativa do ar afetam a produção de morango. A estimativa de variáveis agrônomicas por meio de imagens multiespectrais aos 78 dias após o plantio é viável e pode auxiliar na seleção de novos materiais genéticos.

Palavras-chave: *Fragaria ananassa*, refletância, temperatura, índice de vegetação por diferença normalizada

INTRODUCTION

The production of Brazilian strawberries (*Fragaria ananassa*) does not meet domestic demand, therefore requiring imports, especially as frozen fruits (AGROSTAT, 2022). Importation is required because the strawberry vegetative and reproductive development are affected by environmental factors, especially photoperiod and temperature, specific to each genetic material (Sonstebly et al., 2016).

The development of new cultivars and cultivation techniques capable to reduce the seasonality of production (Fagherazzi et al., 2017) is critical, especially in areas with high temperatures, to supply the production chain and meet the demands of the consumer market for fruits in natura. However, one of the limitations for the efficiency and speed of this process is the extended periods of time required by the researcher to select plants with high vigor and production (Chaudhary & Sandhu, 2024). To address this limitation, the use of high and medium spatial resolution multispectral imaging phenotyping techniques to generate production estimation and mapping models has increased (Gano et al., 2021; Ganeva et al., 2023). Among the techniques applied, those that use the relationships between vegetation indices, near infrared wavelength reflectance, and production are highlighted.

Although several studies presented satisfactory results using remote sensing, the combined analysis of data from remote sensing with agronomic variables, which aims at estimating vegetable production, especially that of strawberry, requires more research to increase the accuracy of estimates. Thus, the purpose of the present study was to evaluate the behavior of strawberry cultivars in terms of production and their phenotypic characterization using multispectral imaging under the environmental conditions of the Brazilian Cerrado biome.

MATERIAL AND METHODS

The experiment was performed in the field conditions, from March 16 to August 28, 2020, at the Federal University

of Uberlândia, Campus Monte Carmelo, MG, Brazil, located in the Triângulo Mineiro and Alto Paranaíba mesoregion, with 18° 43' 36" South latitude, 47° 31' 31" North longitude geographic coordinates, and 900 m of altitude (Figure 1). The strawberry growing season was defined as the period when this region has a decreased number of sunlight hours, that is, the planting was conducted in the beginning of fall, and the harvests were performed until the end of winter.

The region's climate, according to the Köppen classification, is humid temperate, characterized by hot summers and dry winters. The temperature and rainfall data (Figure 2) was obtained from the meteorological station of the Water and Soil Engineering Laboratory (Laboratório de Engenharia de Água e Solo – ENGAS), located at 350 m from the experimental field. The experiment total area was 266 m².

This study used a randomized block experimental design with six treatments and four replicates, totaling 24 experimental plots. The treatments consisted of six commercial strawberry cultivars: San Andreas, Albion, PR, Festival, Oso Grande, and Guarani. Each experimental plot consisted of 18 plants, distributed in two rows, spaced 0.3 m between rows and 0.3

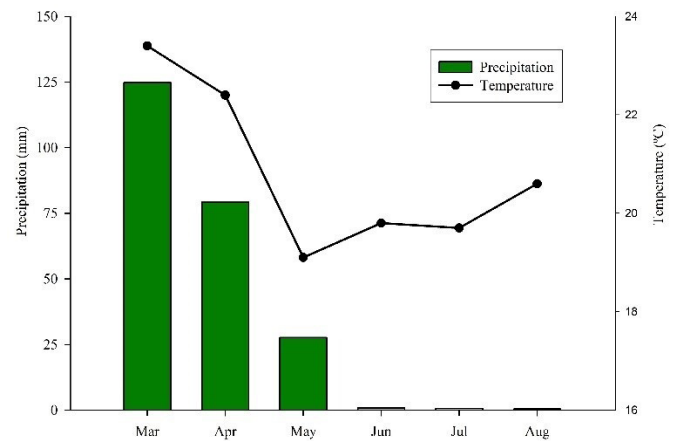


Figure 2. Variation in mean air temperature (°C) and rainfall (mm) during the experimental months

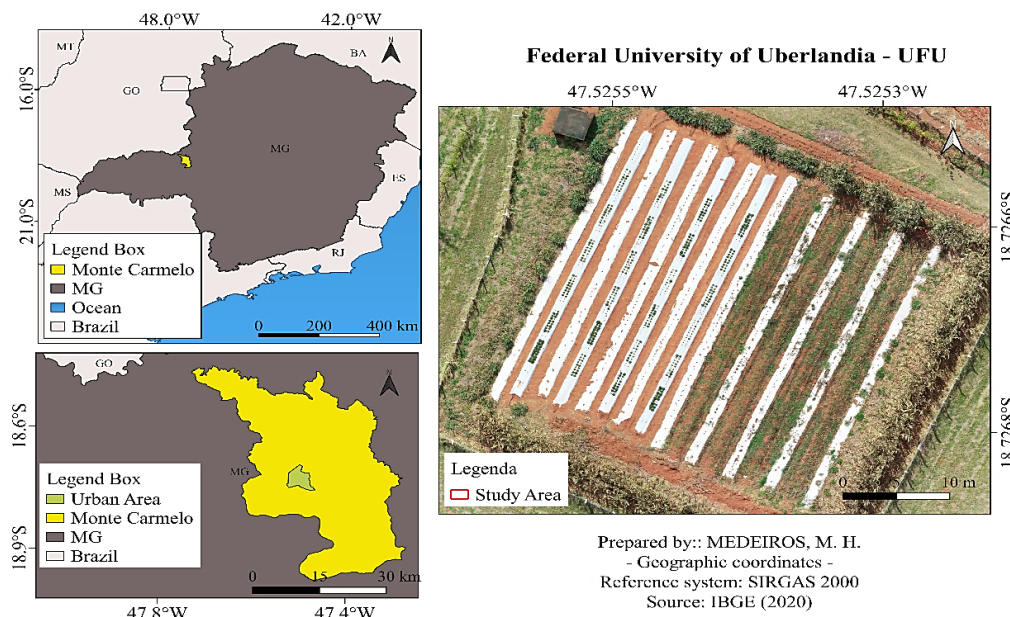


Figure 1. Location map of the experiment in Monte Carmelo, MG

m between plants, considering 10 central plants in each plot for evaluation (Figure 3).

Seedlings were produced in polyethylene trays of 50 cells, containing 100 mL of commercial substrate per cell, in the city of Pouso Alegre, MG, Brazil, by a producer of commercial strawberry seedlings. Before setting up the experiment, the soil was sampled at a 0 to 20 cm depth and transported to the Soil Analysis Laboratory to determine its chemical and physical properties. The results are described in Table 1.

Liming was performed 60 days before transplanting the seedlings to raise the base saturation to 80%, using 1.7 t ha^{-1} of limestone distributed in the total area. The limestone mineral composition was 30% CaO, 8% MgO, and 80% total relative neutralizing power.

At 50 days after liming, it was used a 1.20 m-wide rotavator bedformer coupled to a tractor to prepare four beds, 21.4 m in length and 1.2 m in width, where the planting fertilizers were applied. Fertilization application during the entire crop cycle followed the recommendations by Ribeiro et al. (1999), with $220 \text{ kg ha}^{-1} \text{ N}$, $200 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$, and $80 \text{ kg ha}^{-1} \text{ K}_2\text{O}$. The planting fertilization represented 16% N, 100% P_2O_5 , and 70% K_2O total, using urea, simple superphosphate, and potassium nitrate as sources, respectively. Topdressing was weekly conducted through fertigation, adding urea and potassium nitrate in the irrigation system, to distribute 84 and 30% of the remaining N and K_2O , respectively, totaling 23 fertilizer applications via fertigation during the 164 days of the experiment.

On March 16, 2020, the seedlings were transplanted to the beds covered with mulch film, whose black color was on the internal side (in contact with the soil) and the white color on the external side. The seedlings were placed in holes with 75 mm in diameter, all along the mulching, according to the spatial distribution of the plants in the experimental plots.

The experiment used a drip irrigation system with two drip tubes per plot, and a daily irrigation shift was applied following the recommendations of Kc for strawberry crop

and the daily evapotranspiration measurements from the meteorological station installed 200 m from the site. Self-compensating drippers with 25 mm in diameter and a 1.6 L h^{-1} flow rate were used.

Insect pest control was performed using the insecticide Abamectin Nortox[®] 400 WG, applied with a 20-L manual back pump at a 75 mL product dose for every 100 L of spray. For disease control, Methyliofoan[®] products were applied monthly at a dose of 70 g of the product for every 100 L of spray, expecting the maximum number of applications described in the package insert. Folicur[®] 200 EC was applied at a dose of 75 g of the product for every 100 L of spray, both applied with a manual back pump.

Harvest was performed weekly, starting at 40 days after planting (DAP) of the seedlings and ending at 164 DAP, with the harvest point for fruit defined when fruits attained 75% of the total size and presented a red color. After harvest, the fruits were transported to the Botany Laboratory of the Federal University of Uberlândia for production evaluations. The number of fruits per plant was determined, and mean fruit mass was calculated as the ratio between the total mass of fruits per plant and the number of fruits per plant. Values were expressed in grams (g), and total production per plant was measured by the cumulative weighing of the fruits from each plant of the useful plot, using a digital scale, with the values expressed in g per plant.

In addition, biweekly, between 35 and 132 DAP, the SPAD index was evaluated, with measurements performed in the morning in 10 plants within the useful area from each plot, at four points in the middle third of each leaflet, in three leaflets, from the newly developed trifoliate from each plant, using the SPAD-502 Plus[®] Chlorophyll Meter (Konica Minolta, Brazil). The number of fully developed leaves and the leaf length and width of the newly expanded trifoliate were evaluated using a graduated ruler, with values expressed in centimeters. Leaf length corresponded to the measurement between the

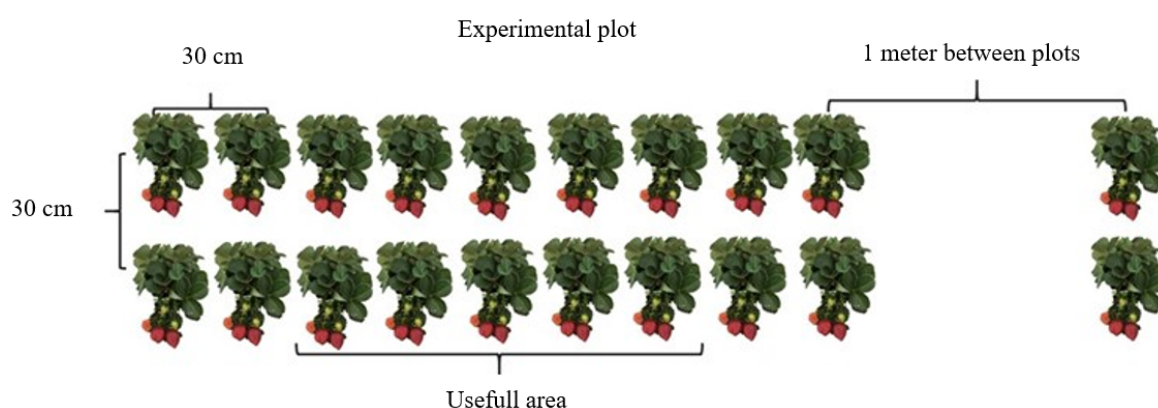


Figure 3. Details of the experimental plot and useful area containing 10 central plants

Table 1. Results of soil physical-chemical sampling carried out from 0-20 cm

Sand	Silt (%)	Clay	pH CaCl_2	P meh (Mg dm^{-3})	K	Ca (cmol dm^{-3})	Mg	B (mg dm^{-3})
25.5	7.5	67.0	5.4	9.8	0.34	2.65	0.58	0.15
Cu	Fe (mg dm^{-3})	Mn	Zn	H+Al	SB (cmol dm^{-3})	T	V (%)	
1.3	20.0	1.7	1.0	1.9	3.57	5.47	65	

SB - Sum of bases; V - Base saturation; T - Potential total cation exchange capacity. Analyses carried out at Laboratório Brasileiro de Análises Ambientais e Agrícolas L.T.D.A. (LABRAS), Monte Carmelo, MG, Brazil

beginning of the leaf, at the base of the petiole, to the end of the leaf, following the midrib. Leaf width corresponded to the leaf size in the central portion, perpendicular to the central vein.

At 80 and 115 DAP, fruit samples from each experimental plot were collected and sent to the Laboratory of Bromatology and Sensory Analysis at the Federal Institute of Education, Science and Technology of Triângulo Mineiro, Campus Uberaba, Minas Gerais state, where the physicochemical characteristics of strawberry were evaluated. The evaluated characteristics were soluble solids content, titratable acidity, maturity index given by the relation SS/TA (Ratio), vitamin C, anthocyanins, and fruit firmness. The soluble solids content (°Brix) was quantified by extracting the cellular juice from 100 g of strawberries, crushing it with a Viva Walitta® 400W mixer, and the values were read using a Reichert® digital refractometer. Titratable acidity (expressed in % citric acid) was determined according to the methodology described by Ryan & Dupont (1973). Vitamin C (expressed in mg 100 g⁻¹ of fresh mass) was determined according to the Tillmans' method (Instituto Adolfo Lutz, 1985). Anthocyanin (expressed in mg 100 g⁻¹ of fresh mass) was quantified according to the differential pH method, described by Giusti & Wrostad (2001), and fruit firmness (expressed in Newton) was measured in whole fruits, using the Stable Micro System texture analyzer, model TAXT Plus Texture Analyzer®.

Soil temperature under and above the mulch, leaf temperature, air temperature, and relative air humidity were evaluated weekly, from 35 to 132 DAP. Data was collected from 8 a.m. to 4 p.m., with 2-hour intervals.

The temperature of the soil under the mulch was measured by a Minipa MT-350A (Minipa, Brazil) digital portable infrared thermometer, placed 3 cm from the neck of two plants, in exposed soil, in the hole made in the plastic. The temperature above the mulch was measured at 15 cm from the plant base, at a 10 cm distance from the height at which the surface was fully exposed to solar radiation.

Leaf temperature was measured in the plant's most developed trifoliolate, always from two plants of each experimental plot (always the same plants throughout all evaluations) at a 10 cm distance from the target object, using a Minipa MT-350A digital infrared portable thermometer. Air temperature and relative air humidity were measured using a Lutron LM-8000A anemometer, humidity, light meter, and thermometer, placed 30 cm above the ground.

The Shapiro-Wilk, Levene, and Tukey tests were used to determine whether the data met the analysis of variance assumptions regarding residual normality, homogeneity of variances, and additivity at 5% probability, respectively.

After meeting the assumptions, the Scott-Knott's test ($p < 0.05$) was performed to compare the means, using the R

Core Team statistical software, version 4.1.2 (R Core Team, 2022). SigmaPlot 12.0 (2012) was used to prepare graphs of soil temperature under and above the mulch, leaf temperature, and air temperature and relative air humidity.

At 60 days after planting the seedlings, a topographic survey was performed using the GNSS Promark 200 receiver, to georeference the 24 experimental plots and subsequent correction in the phototriangulation step. The aerial flight was performed at 78 DAP (Table 2). Flight planning was performed using the DroneDeploy® software, establishing five lanes, with a 30 m altitude, 3 m s⁻¹ speed, longitudinal and lateral overlap of 80%, and a flight time of approximately 4 min.

Images were obtained at noon, using the unmanned aerial vehicle DJI Phantom 4 Pro Plus (Dà-Jiāng Innovations Science and Technology Co.), with an attached Mapir Survey 3W camera with 12-megapixel resolution, with channels corresponding to green (550 nm), red (660 nm), and near infrared (850 nm) (RGN) bands, and full width at half maximum of 40, 60 and 80 nm, respectively.

After obtaining the images using the RGN sensor, the mosaic was built using the AgisoftPhotoScan Professional software. In this software, the images were aligned and then combined based on the camera position and the common points of each image, forming a cloud of sparse points. Subsequently, a dense point cloud was constructed, and based on this, the surface was generated using the 3D polygonal mesh model, generating the flight mosaic.

Atmospheric correction was performed with Mapir Survey 3W images, using the Mapir Camera Control software. For this, before the flight, an image of the target, which has four pallets (black, dark gray, light gray, and white) of radiances was captured with the sensor. Subsequently, the model, lens, and filter of the Mapir Survey 3W camera were specified in the software. Next, the target image was selected and its QR code was detected. Hence, the necessary atmospheric correction values for the camera were calculated, and the input folder containing the mosaic was loaded. After the atmospheric correction process, the calibrated mosaic folder was created automatically.

For all images, the reflection values for the set of light pixels and the set of dark pixels in the red (R), green (G), and near infrared (NIR) bands were manually extracted. Subsequently, the coefficients of a linear transformation for each spectral band were determined using the Eqs. 1 to 3.

$$T_i = m_i \times x_i + b_i \quad (1)$$

where:

T_i - reflectance (RGN) of the normalized image,

Table 2. Biometric assessment timing and frequency summary for strawberry cultivation

Date	Assessment	Frequency
35 DAP until 132 DAP	SPAD index, number of fully developed leaves, leaf length, and width of the newly expanded trifoliolate	Biweekly
35 DAP until 132 DAP	Soil temperature under and above the mulch, leaf temperature, air temperature, and relative air humidity	Weekly
40 DAP until 164 DAP	Number of fruits per plant, mean fruit mass	Weekly
80 and 115 DAP	Soluble solids content, titratable acidity, maturity index given by the relation SS/AT, vitamin C, anthocyanins, and fruit firmness	Two evaluations
78 DAP	Aerial flight for collecting multispectral images	One evaluation

DAP - Days after planting

$$m_i = \frac{B_{ri} - D_{ri}}{B_{si} - D_{si}} \quad (2)$$

m_i - spectral band to be normalized, and,

$$b_i = \frac{(D_{ri} \times B_{si}) - (D_{si} \times B_{ri})}{B_{si} - D_{si}} \quad (3)$$

B_{ri} - mean clear reference set;

D_{ri} - mean dark reference set;

B_{si} - mean of the clear set to be normalized; and,

D_{si} - mean of the dark set to be normalized.

In the ENVI 5.1 software (Environment for Visualizing Images), using the Band Math tool, the equation was applied to each band of the images, generating layers. In the Layer Stacking tool, the three layers generated from each image were combined to form a single image. Radiometric data was extracted from each mosaic using the Region of Interest (ROI) function of the ENVI 5.1 software.

The normalized difference vegetation index (NDVI) was calculated using the mean values of reflectance (RGN), with the mathematical formula that uses the spectral data of the red and near infrared bands (Eq. 4):

$$NDVI = \frac{(B_{850} - B_{660})}{(B_{850} + B_{660})} \quad (4)$$

where:

B_{660} and B_{850} - are the red and near infrared bands, respectively, both of the RGN sensor channels.

After obtaining all the images and calculating the NDVI, the Pearson's correlation coefficient was calculated for statistical analysis of the intensity and direction on the relationship between the SPAD index, number of leaves, leaf length, leaf width, production per plant, number of fruits per plant, and mean fruit mass versus NDVI, red (R), green (G) and near infrared (NIR) bands.

The agronomic variables were estimated from the multispectral vegetation index and sensor bands using the Weka software. A sample set containing 80% (192 plants) of radiometric value data was used as a training model. The correlation coefficient and the root mean square error (RMSE) between the observed and predicted values were calculated to validate the accuracy of the models, considering the difference between the measured and estimated agronomic variables for 48 plants (20% of the sample set).

Finally, maps of the agronomic variables were constructed from the respective linear regression equations, generated with the highest Pearson's correlations, between the agronomic variables and spectral bands (Figure 4).

RESULTS AND DISCUSSION

According to Table 3, the higher number of fruits per plant presented in the Guarani cultivar (59.25) did not favor

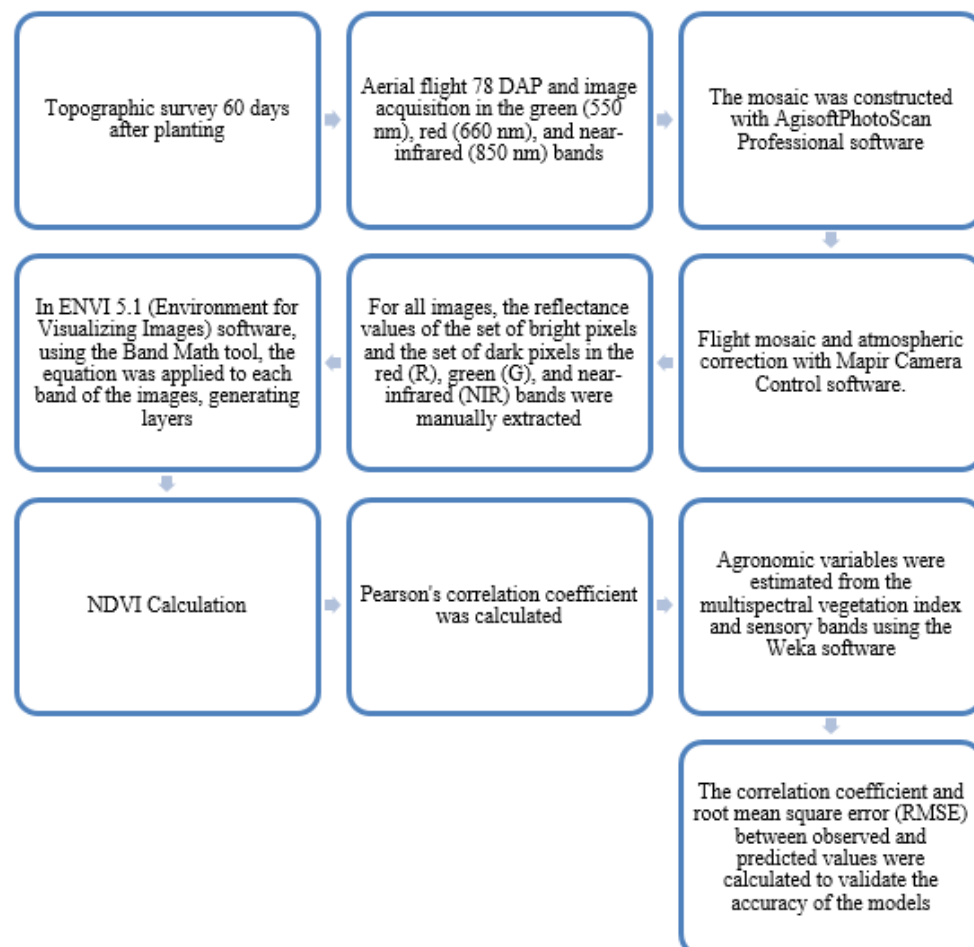


Figure 4. Summary of image processing performed on six strawberry cultivars

the biomass deposition in the fruits owing to the substantial competition for photoassimilates, which resulted in this cultivar having fruits with lower mean mass (6.38 g). However, the high number of fruits in Guarani reflected an intermediate production per plant (377.75 g per plant). Furthermore, this cultivar showed a SPAD index 8.4% lower than the average of the five cultivars (44.34 SPAD), and the number of leaves per plant was 43.6% higher than the average (12.29 leaves) value in the other cultivars evaluated. All cultivars produced statistically equal mean fruit mass, ranging from 11.46 to 13.25 g per plant, except Guarani, which obtained a mean fruit mass approximately 49% lower than the average of the other five cultivars (12.598 g) (Table 3).

The highest strawberry fruit production was obtained in the Festival cultivar, with 519.7 g per plant, with this value being 47.7, 63.8, and 68.2% higher than that of San Andreas, PR, and Albion cultivars, respectively, which presented the lowest yields. Festival cultivar fruits had a mean mass of 12.37 g and 41.83 fruits per plant. The immense potential of this genotype in crops under high temperatures was highlighted, with high resistance to both foliar and root diseases. It maintains fruit shape and size throughout the production cycle (Antunes, 2021).

Cultivar Oso Grande exhibited production similar to that of Guarani; however, with a lower number of fruits per plant and a higher mean fruit mass.

Low leaf emission did not facilitate the development and expansion of the leaf blade, resulting in a small leaf length and

width in Albion and PR cultivars. These cultivars also attained a low number of fruits and production per plant, as they did not develop a vegetative structure capable of supporting the fruit load (Table 3).

Owing to the high number of fruits per plant and strong competition for photoassimilates, Guarani had the lowest content of soluble solids (6.68 °Brix), that is, fruits with 17.43% less soluble solids than the average (8.09 °Brix) for the other five cultivars (Table 4). As for titratable acidity, San Andreas cultivar presented the highest value (1.01% citric acid), statistically differing from the other cultivars.

As to Ratio, which represents the degree of fruit maturation, the genotypes showing superior values were PR, Festival, and Oso Grande (Table 4). The high values found for this characteristic are due to the low titratable acidity verified since Ratio is the relation between soluble solids and the titratable acidity.

Ascorbic acid or vitamin C is another important acid in fruit and vegetables because of its several functions in human health. Vitamin C content was statistically the same for the cultivars San Andreas, Albion, PR, Festival, and Oso Grande, ranging from 49.39 to 60.96 mg of ascorbic acid 100 g⁻¹ (Table 4). Albion exhibited the highest content of anthocyanins (Table 4), which are pigments present in various fruits, flowers, leaves, stems, and roots that have antioxidant, anticarcinogenic, antiviral, and anti-inflammatory activity (Braga et al., 2018). Festival, which showed the highest production (519.70 g per plant), also had higher levels of soluble solids (8.76°Brix),

Table 3. Mean values of number of fruits per plant (NFP), mean fruit mass (MFM, in g), production per plant (PP, g per plant), SPAD index (SPAD, in SPAD index), number of leaves per plant (NL), leaf length (LL, in cm), and leaf width (LW, in cm) of six different strawberry cultivars

Cultivars	NFP	MFM	PP	SPAD	NL	LL	LW
San Andreas	21.05 c	12.91 a	272.04 c	44.77 a	13.76 c	5.73 a	5.34 a
Albion	12.33 d	13.25 a	165.36 c	42.36 b	8.19 d	4.85 b	4.93 b
PR	16.28 d	11.46 a	188.24 c	44.29 a	9.14 d	4.68 b	4.55 b
Festival	41.83 b	12.37 a	519.70 a	45.80 a	17.29 b	6.23 a	6.01 a
Oso Grande	26.38 c	13.00 a	345.34 b	44.46 a	13.06 c	5.62 a	5.91 a
Guarani	59.25 a	6.38 b	377.75 b	40.61 c	21.75 a	5.35 a	5.54 a
Sw	0.43	0.19	0.39	0.43	0.57	0.24	0.12
F	0.98	0.06	0.57	0.25	0.89	0.40	0.27
F ^o	0.44	0.71	0.26	0.24	0.74	0.82	0.60
CV (%)	13.7	10.09	21.52	2.4	19.6	10.8	10.1

Means followed by the same letter do not differ from one another by the Scott-Knott test at $p \leq 0.05$. Sw, F, F^o: Shapiro-Wilk, Levene, and Additivity statistics test, respectively; CV - Coefficient of variation

Table 4. Mean values of soluble solids (SS), titratable acidity (TA), ratio (SS/TA), vitamin C (Vit. C), anthocyanin, and fruit firmness from different strawberry cultivars

CV	SS (°Brix)	TA (% citric acid)	Ratio (SS/TA)	Vit. C (mg ascorbic acid 100 g ⁻¹)	Anthocyanin (mg of cyanidin-3-glycoside 100 g ⁻¹)	Firmness (N)
San Andreas	8.32 a	1.01 a	8.32 b	51.99 a	26.42 b	0.58 a
Albion	7.90 a	0.81 c	9.75 b	49.39 a	47.47 a	0.25 b
PR	7.74 a	0.68 d	11.68 a	56.41 a	28.55 b	0.51 a
Festival	8.76 a	0.82 c	10.68 a	60.96 a	30.89 b	0.54 a
Oso Grande	7.73 a	0.71 d	10.89 a	58.02 a	29.36 b	0.23 b
Guarani	6.68 b	0.87 b	7.68 b	21.98 b	26.42 b	0.17 c
Sw	0.71	0.95	0.90	0.17	0.93	0.48
F	0.50	0.26	0.35	0.93	0.07	0.67
F ^o	0.05	0.17	0.57	0.63	0.79	0.43
CV (%)	7.02	7.62	4.32	11.54	8.73	12.15

Means followed by the same letter do not differ from one another by the Scott-Knott test at $p \leq 0.05$. Sw, F, F^o: Shapiro-Wilk, Levene, and Additivity statistics test, respectively; values in bold indicate residuals with normal distribution, homogeneous variances, and blocks with an additive effect at a significance level of 0.05, CV - Coefficient of variation

vitamin C (60.96 mg ascorbic acid 100 g⁻¹), and firmness (0.54 N). Firmness is a very important variable when analyzing fruit and vegetables, as it is linked to the postharvest period duration and resistance to the transport over long distances. Guarani showed lower fruit firmness (0.17 N); in contrast, San Andreas, PR, and Festival produced firmer fruit (0.58, 0.51, and 0.54 N, respectively).

Strawberry production is affected by the cultivation biotic and abiotic conditions cultivation (Antunes et al., 2010). Analysis of the abiotic variables measured throughout this study indicated that the temperature of the soil under the mulch had the greatest variation between 04/20/2020 and 05/18/2020 (Figure 5) because the plants were still young, with a poorly developed canopy, increasing the incidence of solar radiation on the basal part of the plant. The highest soil temperature under the mulch was reached in plots with Albion plants (46.5 °C) at noon. The lowest soil temperature recorded under the mulch was 12.6 °C at 10 a.m., in the plots with the PR plants. Throughout the evaluated period, the highest soil temperatures under the mulch were reached in the plots with San Andreas and Albion plants (Figure 5), and these two cultivars were among the three cultivars with low fruit yields (Table 3).

The temperature above the mulch was higher than that under the mulch (Figure 6). The highest temperature variation above the mulch between cultivars was observed from 8 a.m. to 2 p.m. The highest temperature above the mulch was 51.0 °C at noon, on 05/04/2020, in the plots with Festival plants. The lowest temperature above the mulch was 13.2 °C, on 06/01/2020 at 8 a.m., in the plots with Oso Grande plants (Figure 6).

Leaf temperature is an important characteristic to observe from a physiological point of view, as it influences transpiration, the photosynthesis process, and cellular respiration (Figure 7). Festival was highly productive and managed to dissipate heat efficiently, reaching low leaf temperatures. Similarly, Oso Grande also presented lower leaf temperatures than the other cultivars, especially in the hottest hours of the day. In contrast, PR, Albion, and San Andreas had high leaf temperatures, which may have resulted in low yields; however, the fruits contained high levels of soluble solids (Tables 3 and 4). Leaf temperature in Albion reached 40.2 °C and that in Festival was 11.6 °C, that is, the highest and lowest temperatures observed in the study, respectively (Figure 7).

The maximum and minimum air temperatures were 41.2 and 12.9 °C, respectively (Figure 8). The mean temperature recorded between 8 a.m. and 4 p.m. was 28.6 °C. The rank regarding air temperature at 8 a.m. among the cultivars remained constant in the evaluation period, evidencing the existence of a microclimate in each experimental plot. However, the air temperature was higher than the culture basal temperature (7 °C). The experimental plots with Festival showed the highest air temperature, followed by the plots with Albion and PR. The plots with Oso Grande reached lower temperatures and had greater amount of fruit than the plots with Albion and PR (Table 3).

The climatic characteristic that exhibited the lowest variation among cultivars was relative air humidity (Figure 9). The plots with Oso Grande, Guarani, and San Andreas showed high relative air humidity, whereas those with Festival had the lowest relative air humidity over time. The highest relative air

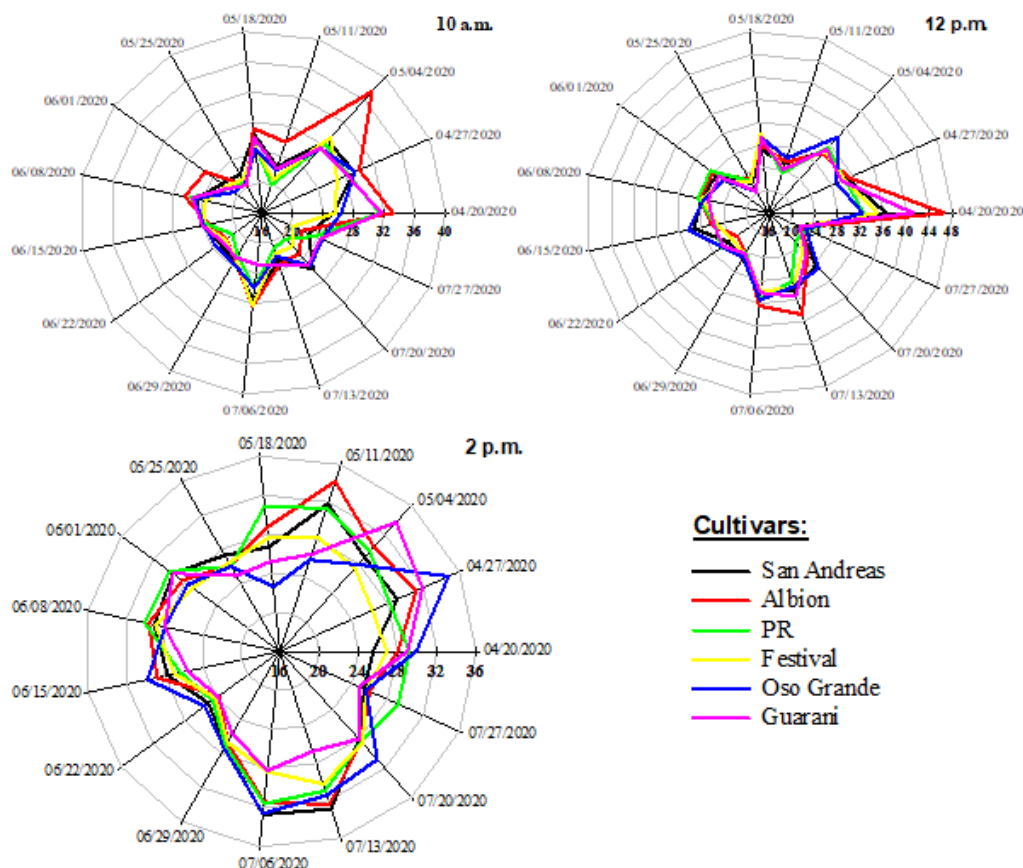


Figure 5. Soil temperature under the mulch (°C) of six different strawberry cultivars from April 20 to July 27, 2020

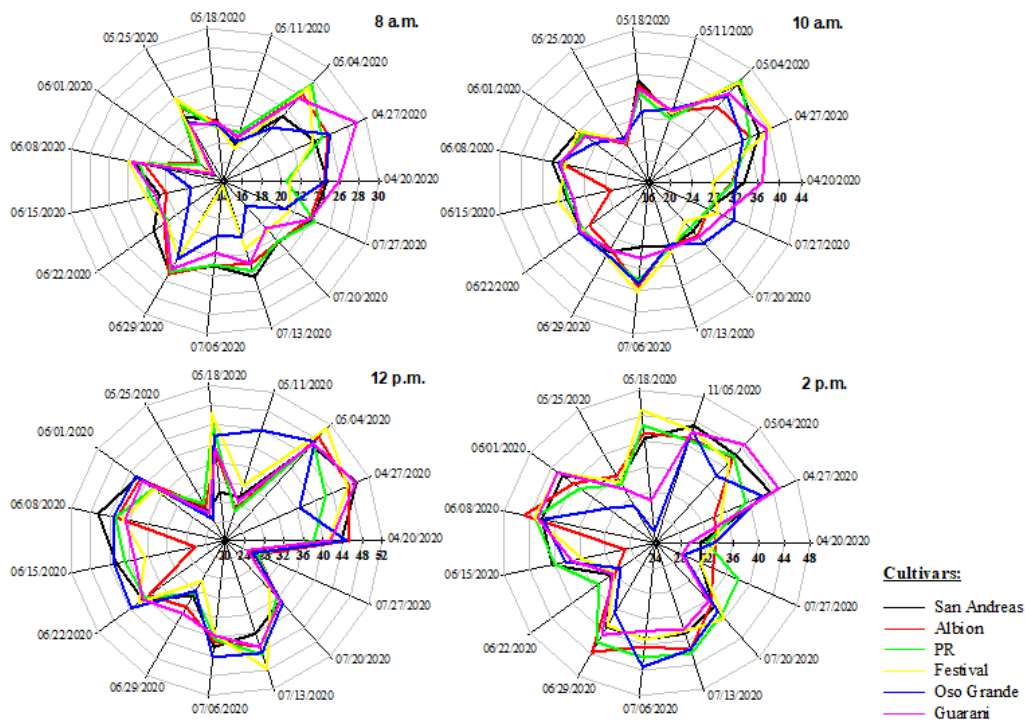


Figure 6. Temperature above the mulch (°C) of six different strawberry cultivars from April 20 to July 27, 2020

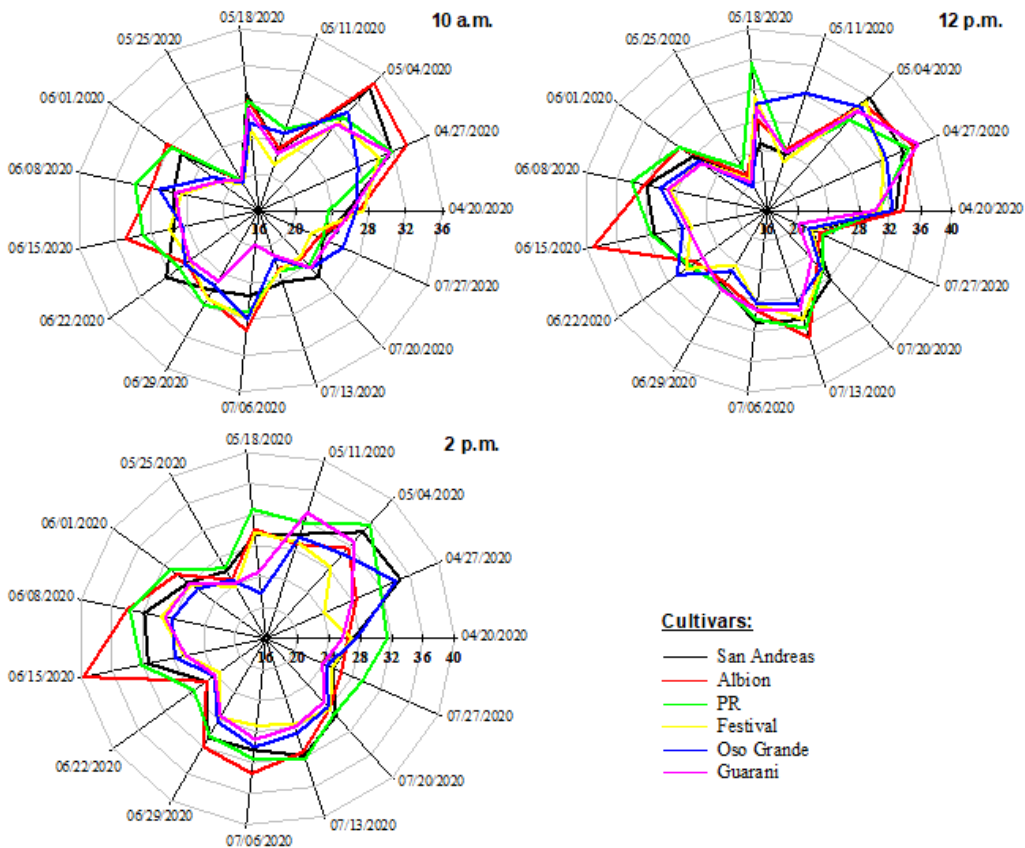


Figure 7. Leaf temperature (°C) of six different strawberry cultivars from April 20 to July 27, 2020

humidity (85.4%) occurred on 06/01/2020 at 8 a.m. in the plots with Oso Grande. The lowest relative air humidity (20.7% at 8 a.m. on 06/15/2020) was detected in the plots with Albion (Figure 9).

These abiotic variables are important for understanding the ecophysiological characteristics of the different cultivars under study. According to the Pearson's correlation coefficient

analysis, strawberry yield showed a high correlation with leaf number ($r = 0.79$), fruit number ($r = 0.75$), leaf length ($r = 0.82$), and leaf width ($r = 0.84$ (Table 5)). These results demonstrate the influence of plant architecture and leaf shape on strawberry production. A similar result was observed by Rosa et al. (2013), when studying two strawberry cultivars, Arazá and Yvapitá, obtained through simple measurements

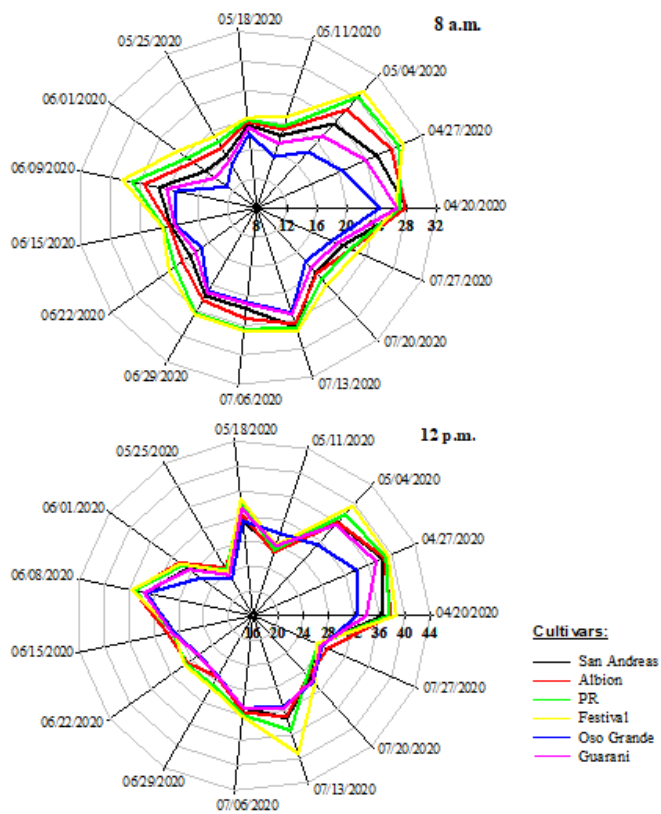


Figure 8. Air temperature (°C) of six different strawberry cultivars from April 20 to July 27, 2020

of the length and width of the central leaflet, as performed in this study for the six cultivars.

The SPAD index, or Soil Plant Analysis Development, is a measure of chlorophyll concentration in leaves and is used to

estimate nitrogen content in plants. It is a non-destructive value obtained using a portable chlorophyll meter, which facilitates real-time monitoring of nitrogen nutrition and plant health (Fontes & Araújo, 2007). This variable only correlated with mean fruit mass and leaf length. Interestingly, an increase in the SPAD index resulted in larger fruit mass and longer leaves (Table 5). The SPAD index and mean fruit mass did not correlate with NDVI, as they have little relationship with plant biomass.

The values of the near-infrared band decreased with increasing leaf length and width and total production per plant (Table 5). Similar results were observed for the green band, showing a decrease in reflectance values as the number of leaves, leaf length and width, production per plant, and number of fruits increased. Therefore, the NIR band and the green band followed the same trend. The red band showed the highest Pearson correlations for number of fruits, total production per plant, number of leaves, leaf length and width, concluding that this band is the most suitable for further image processing (Table 5).

Table 6 shows the performance of the algorithms and lists the estimated correlation coefficients and RMSE values of the proposed models among the agronomic variables. This study found that all correlation coefficients estimated through artificial intelligence reached high values compared to the Pearson coefficients, as the software trains the model with 80% of the data, resulting in a better fit.

The SPAD index, leaf length, and leaf width showed the lowest RMSE in the model estimation using the NDVI (1.06, 0.54, and 0.21, respectively). For the variables number of leaves and number of fruits per plant, the red band showed

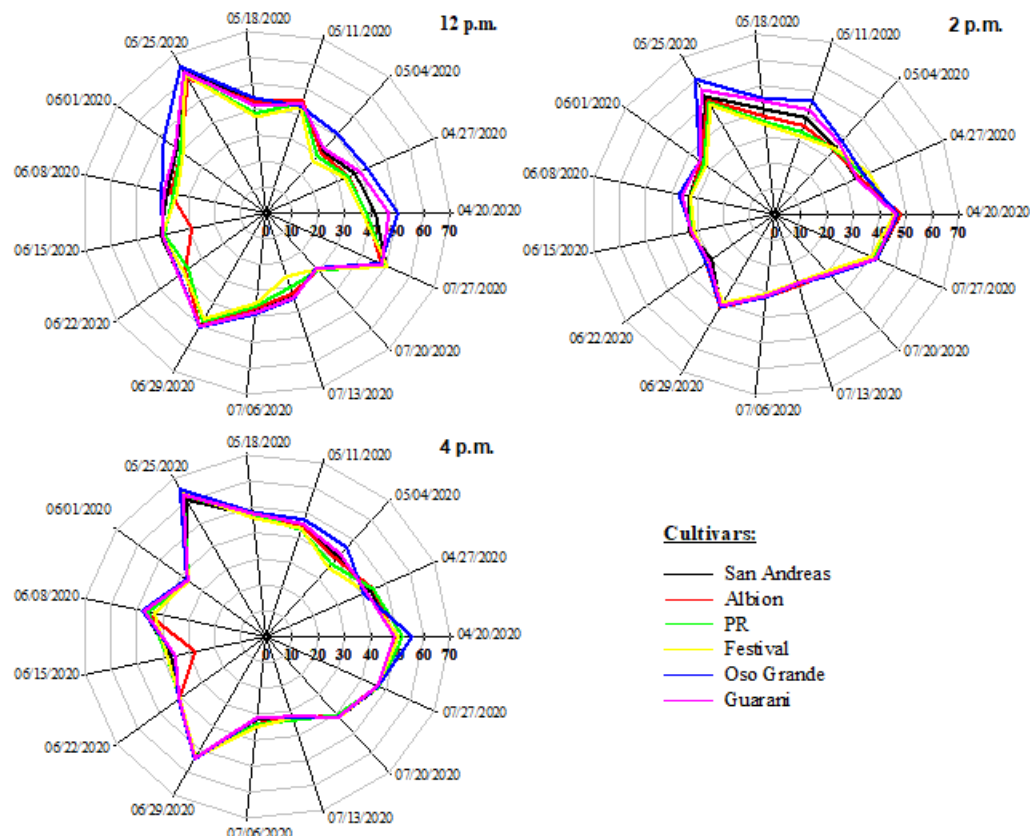


Figure 9. Relative air humidity (%) of different strawberry cultivars from April 20 to July 27, 2020

Table 5. Simple correlation coefficients (r) – determined using Pearson’s correlation – between agronomic variables, vegetation index, and spectral bands of six strawberry cultivars

AC	NDVI	R	G	NIR	MFM	NFP	PP	LW	LL	NL
SPAD	0.30 ^{ns}	0.34 ^{ns}	-0.39 ^{ns}	-0.08 ^{ns}	0.67 ^{**}	-0.29 ^{ns}	0.30 ^{ns}	0.33 ^{ns}	0.46 *	-0.16 ^{ns}
NL	0.80 ^{**}	0.81 ^{**}	-0.68 ^{**}	-0.30 ^{ns}	-0.51*	0.91 ^{**}	0.79 ^{**}	0.68 ^{**}	0.65 ^{**}	
LL	0.87 ^{**}	0.88 ^{**}	-0.88 ^{**}	-0.42*	0.22 ^{ns}	0.45*	0.82 ^{**}	0.93 ^{**}		
LW	0.91 ^{**}	0.92 ^{**}	-0.89 ^{**}	-0.48*	0.11 ^{ns}	0.54 ^{**}	0.84 ^{**}			
PP	0.89 ^{**}	0.93 ^{**}	-0.89 ^{**}	-0.43*	-0.09 ^{ns}	0.75 ^{**}				
NFP	0.68 ^{**}	0.70 ^{**}	-0.54 ^{**}	-0.25 ^{ns}	-0.69 ^{**}					
MFM	-0.05 ^{ns}	-0.02 ^{ns}	-0.13 ^{ns}	-0.09 ^{ns}						

** , * and ns - Significant at $p \leq 0.01$ and $p \leq 0.05$, and not significant by the F test, respectively; AC - Agronomic characteristics; NDVI - Normalized difference vegetation index; R - Red band; G - Green band; NIR - Near infrared band; MFM: Mean fruit mass; NFP - Number of fruits per plant; PP - Total production per plant; LW - Leaf width; LL - Leaf length; NF - Number of leaves; SPAD - Soil Plant Analysis Development index

Table 6. Estimated correlation coefficients (C.C.) and root mean square error (RMSE) between agronomic variables and the model estimation

Agronomic variables		NDVI	R	G	NIR
SPAD	C. C.	0.94	0.92	0.96	0.91
	RMSE	1.06	1.10	1.42	1.29
NFP	C. C.	0.91	0.93	0.93	0.88
	RMSE	8.04	6.32	6.36	10.36
PP	C. C.	0.96	0.97	0.97	0.0
	RMSE	52.54	52.54	52.10	151.07
NL	C. C.	0.97	0.96	0.99	0.95
	RMSE	2.66	2.23	2.24	2268.0
LL	C. C.	0.84	0.77	0.86	0.82
	RMSE	0.54	0.62	0.57	0.71
LW	C. C.	0.97	0.93	0.96	0.98
	RMSE	0.21	0.31	0.29	0.44
MFM	C. C.	-0.74	-0.67	0.74	0.76
	RMSE	2.21	2.02	1.75	1.78

MFM - Mean fruit mass; NFP - Number of fruits per plant; PP - Total production per plant; SPAD - SPAD index; NL - Number of leaves; LL - Leaf length; LW - Leaf width; NDVI - Normalized difference vegetation index; R - Red band; G - Green band; NIR - Near infrared band

the lowest RMSE, with values of 2.23 and 6.32, respectively. Regarding total production per plant (52.10 g per plant) and mean fruit mass (1.75 g), the green band had the lowest RMSE value (Table 6).

Distribution class maps of strawberry agronomic variables were prepared according to linear regression equations, as a function of the red band (Figure 10). When observing the map, only the pixel clouds that correspond to the plant canopy should be analyzed, as it is the place where the extraction of pixels occurred. It was not possible to distinguish the tested cultivars through the red, green, and near infrared bands, as they all showed the same reflectance pattern. This may have been due to the low phenotypic variability among the materials used. However, it was possible to correlate the red band values with plant yield, which is of great importance in image-based phenotyping.

In this case, through a drone flight 78 days after planting, plant yield was estimated for the different cultivars. In the field, this would be an important tool for rural producers to make investment, fertilization, and crop management decisions.

The pixel distribution classes varied according to each agronomic variable and are shown in Figure 10. Festival, Guarani, and Oso Grande had the highest pixel cloud, whereas San Andreas, Albion and PR had low pixel clouds for all agronomic variables.

The cultivars with large pixels clouds and canopies showed increased variation in the color distribution of the pixel classes. The increased variation in pixel color in plants with large

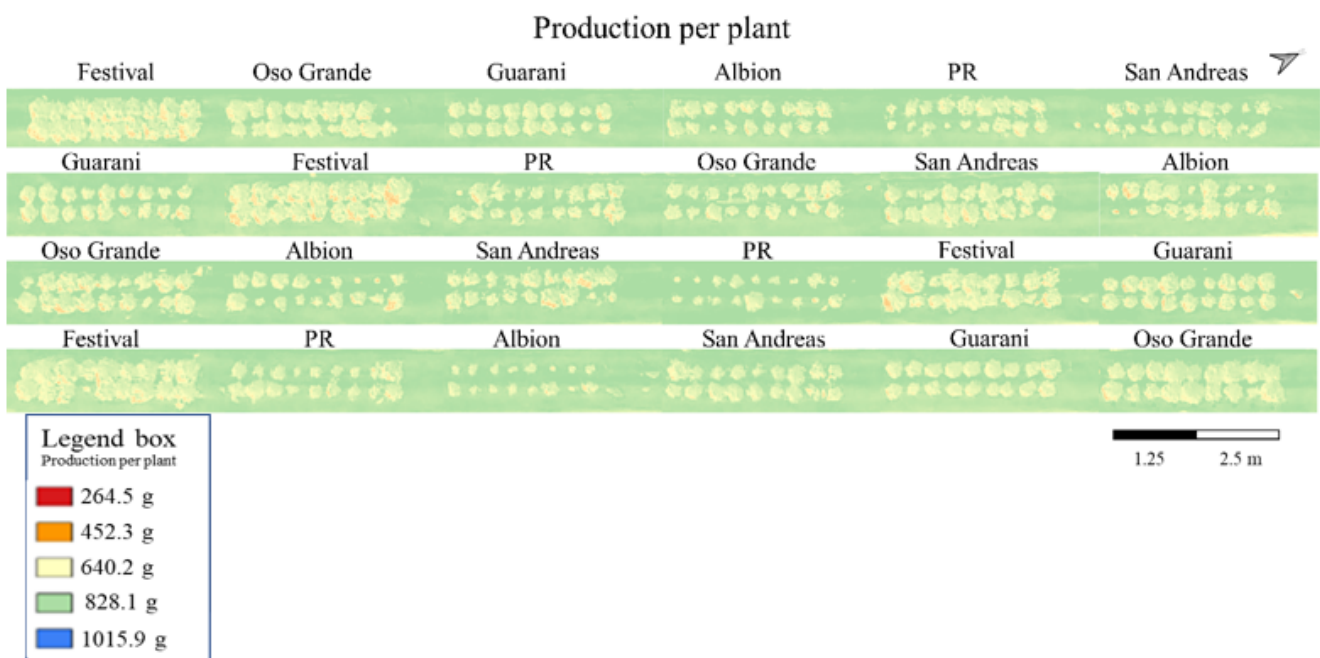


Figure 10. Class map of production per plant for six strawberry cultivars, generated as a function of the red band (R), at 78 days after planting

point clouds is justified by the sensor's ease of acquiring the varying reflectance of the bands in large objects on the ground, as the resolution from each pixel indicates 1 cm² on the land according to the flight characteristics.

This experiment resulted in interesting findings. The maximum production of strawberries under the climatic conditions of the Cerrado in the Triângulo Mineiro was 519.70 g per plant, obtained in the Festival cultivar. In Santa Catarina state, Tazzo et al. (2015) obtained the highest yields in crops with mulching in San Andreas and Albion cultivars, with 260.0 and 180.0 g per plant, respectively, that is, yields close to those obtained with the same cultivars (272.04 g per plant and 165.36 g per plant) in the current study but lower than the maximum production obtained in the current study.

Although cultivar San Andreas dominates the Brazilian market, accounting for 65% of the total cultivated area (Chiomento et al., 2021), it appears that for the evaluated region, it presented the lowest yield. The low yields obtained by San Andreas, Albion, and PR (Table 3) may be related to the inadequacy of these cultivars to the growing conditions, as they were the cultivars that presented high temperatures above the mulch and on the leaf throughout the whole cycle (Figures 4 and 5). This indicates that production may vary owing to the plant's response to the agroecosystem edaphoclimatic factors (Zanin et al., 2019) and the crop ecophysiology (Costa et al., 2016). Temperature and photoperiod are the two main environmental factors that regulate the ontogeny of strawberry flowers (Durner, 2015).

In the present experiment, although air temperatures occurred around 41.2 °C in some experimental plots (Figure 8), this generally occurred at the beginning of vegetative growth, a time when the strawberry plant requires high temperatures to emit stolons and expand the stem (Strand, 2008). Regarding the photoperiod, short-day cultivars showed higher fruit production and a relative increase in the number of fruits per plant than neutral-day cultivars, as clearly observed among the analyzed cultivars (Table 3).

The development of leaves and the entire stem during the vegetative period have a strong influence on the number of floral buds, and therefore, on the number of fruits that will be formed. Hence, the greater the number of leaves, the greater is the number of inflorescences (Strand, 2008). Albion and PR presented low number of leaves, leaf length and width, and consequently a small photosynthetically active area for photoassimilates production, as reflected in the low productive performance.

The number of fruits obtained in the current study (12.33–59.25 fruits) were not consistent with those obtained in the study by Santos et al. (2019) (2.28–5.70 fruits), who evaluated different strawberry cultivars in a protected environment and in full sun. This can be explained by the high air temperatures (28.2 °C) at the time of flowering, as occurred in the study by Santos et al. (2019), which can cause flower abortion, and consequently, reduce the total number of fruits (Silva et al., 2021).

Guarani presented a high number of fruits per plant, an agronomic characteristic of interest, whether for the in natura market or the industry. However, the high fruit load

on the plant did not allow good distribution and deposition of photoassimilates in the fruits, making them 49% lighter than the fruits of the most productive cultivar (Table 3). In addition, the fruits had a low content of soluble solids due to the imbalance in the source/sink ratio. Although the low soluble solids content would not be an impediment for the industry, which aims at good pulp yields, it would be a disadvantage for the fruit intended for in natura trade.

When analyzing environmental conditions, it appears that the plots with Guarani plants showed high relative air humidity values, which may have been caused by the shape of the closed canopy, which provided increased retention of moisture on the plant.

Temperature directly affects the speed of chemical reactions and enzymatic activity that occur in plant metabolism (Larcher, 2000). The studies by Cui et al. (2021) showed that the strawberry subjected to extreme temperatures (2 and 35 °C), in a short period of time, affected the formation of flowers and fruits. Although the place where Festival and Oso Grande plants were located showed the highest and lowest air temperatures, respectively, both cultivars presented low leaf temperatures, and were among the most productive cultivars in the present study. This may have occurred because of the greater number of morphophysiological mechanisms; among them, better control of stomatal opening and closing, lower transpiration rate, and greater cuticular thickness, which provide better adaptability to adverse conditions than those in the other cultivars evaluated, reducing the negative impacts on plant development caused by very high or low air temperature.

The soluble solids content ranged from 6.68 to 8.76 °Brix, results that were similar to those found by Camargo et al. (2018) (6.3–9.7 °Brix), Antunes et al. (2010) (6.8–8.7 °Brix), and Temocico et al. (2019) (6.3–13.2°Brix).

According to Mancini et al. (2023), the levels of total soluble solids change with the species, cultivar, harvest season (harvest year but also different dates across the same year), and environmental conditions (light exposure, temperature, radiation, and water management). High values of soluble solids are associated with high temperatures, as reported by Resende et al. (2010), because high temperatures increase the synthesis of secondary compounds, in addition to allowing the plant to accumulate high concentrations of soluble sugars.

Titrateable acidity decreases after the fruit is harvested, serving as a substrate for cellular respiration. The most flavorful fruits should have at least 7% soluble solids and 0.8% acidity (Kader, 2002). San Andreas, Albion, and Festival met these characteristics proposed by Kader (2002), producing fruits with a pleasant sensory quality.

San Andreas fruits had a 23% higher acidity than the average of the other cultivars, in addition to exhibiting good results in terms of soluble solids, vitamin C, and firmness. The high temperature of the soil above the mulch and in the air did not affect the fruit characteristics of San Andreas, the most planted cultivar in Brazil, but negatively affected production and number of fruits per plant. Extreme environmental conditions presented an adverse environment for vegetative growth, which did not allow the expression of the vegetative potential of this important cultivar. The mean titrateable acidity

value of 0.82% citric acid is consistent with the results by Camargo et al. (2018).

There is a growing market of consumers looking for nutraceuticals for health promotion. Thus, physical-chemical characterization is extremely important for the positioning of genetic materials that produce high quality fruits with high concentration of vitamin C, anthocyanins, polyphenols, and other antioxidants (Chiomento et al., 2021).

Rocha et al. (2008) and Musa et al. (2015) noted that cultivars influence the vitamin C content, with variations of up to two to three times in its concentration. However, the present study did not find a significant difference in the content of vitamin C. The average content of vitamin C was 49.79 mg 100 g⁻¹, a result superior to that reported by Pinheiro et al. (2021), when evaluating different strawberry genetic materials commonly planted in southern Minas Gerais, including San Andreas, Oso Grande, Albion, and Festival.

According to the chemical composition data from the USDA (2021), the average vitamin C content for the strawberry crop is 56 mg 100 g⁻¹, that is, a value higher than the values found in the present study for San Andreas, Albion, and Guarani. According to Gabas et al. (2003), low values of vitamin C content may be explained by several factors, such as oxygen, pH, light, temperature, and moisture content or water activity.

Albion was the cultivar with the highest anthocyanin content, with 47.47 mg of cyanidin-3-glycoside 100 g⁻¹. The synthesis of anthocyanins is directly related to the incidence of light, nutritional balance, temperature changes during growth and ripening, and to the cultivation system itself (Portela et al., 2012), and agronomic practices that increase the vegetative growth result in decreased anthocyanin synthesis (Severo et al., 2010). The results from the present study are consistent with these findings, as the plots with Albion presented, in general, high temperatures under the mulch and on the leaf, and low relative air humidity (Figures 3, 5, and 6), probably inducing less vegetative growth, and consequently, increased accumulation of anthocyanins (Table 4).

Firm fruits allow long postharvest shelf life, and less damage when handling and transporting over long distances (Pinheiro et al., 2021). Together, these factors enable production at strategic points to reach new consumer markets. San Andreas, PR, and Festival produced fruits with increased firmness and mean mass. The superior deposition of fruit biomass reflected in better structural organization and solidity, resulting in increased firmness for these cultivars. In contrast, Guarani, which showed low mean fruit mass, produced less firm fruits. This cultivar is destined for the industry and would need fruit with good firmness to tolerate possible damage caused during transportation.

Considering the spectral bands and agronomic variables, this study found that the red, green, and NDVI bands had high correlations with the number of leaves, leaf length, leaf width, production per plant, and number of fruits (Table 3).

As in the present study, infrared-based indices were applied to predict dry biomass in strawberry (Zheng et al., 2022), phenotyping and management, with an emphasis on applications in strawberry farming (Zheng et al., 2021) and yield forecasting by satellite remote sensing and machine-learning models (Pinto et al., 2022).

The spectral radiance extracted from the polygons of the crowns of the strawberry plants showed a high correlation between the red band and the agronomic variables, but a low correlation between the near infrared band and the agronomic variables, as it captures only the beginning of the thermal region frequency range (850 nm), a region where vegetation does not have the maximum reflected energy (Martins et al., 2017).

The near infrared band is related to plant morphology, leaf tissue structure, and leaf area index; the red and green bands are more sensitive to the concentration of chlorophyll (Resende et al., 2020). However, the results of this study are not consistent with those reported by Resende et al. (2020), as the near infrared band showed a low capacity to estimate variables related to leaf structure, such as number of leaves and leaf length and width.

In the present study, the number of leaves, leaf length, width, and production, and number of fruits increased with the addition of NDVI, which is partly consistent with the results of Salvador-Castillo et al. (2021), who stated that NDVI is related to plant cover and biomass. The SPAD index and the fruit mass showed no correlation with the NDVI, as they have little relationship with plant biomass, which is difficult to detect in the pixel resolution generated in the flights performed in the present study.

The results from this study demonstrated that phenotyping through high-performance imaging is an efficient method to select strawberry cultivars based on the number of fruits, total production per plant, leaf width and length, and number of leaves. The studies by Maciel et al. (2019) and Maciel et al. (2020) showed that multispectral imaging can be useful tools for detecting carotenoid levels in lettuce leaves (which are highly correlated with the SPAD index). However, the present study could not verify this using the unmanned aerial vehicle.

In general, the RMSE was low, except for the near infrared band (Table 6). The mean RMSE for the red, green, and NDVI bands were 1.19 (for the SPAD index), 6.91 (for the number of fruits per plant), 52.39 (for production), 2.38 (for the number of leaves), 0.58 (for leaf length), 0.27 (for leaf width), and 1.99 (for mean fruit mass). Considering the speed of data acquisition and the reduction in manpower, the results were satisfactory and consistent with the data determined in the field, validating the model estimated by the artificial intelligence software. Resende et al. (2020) found an RMSE value of 885.07 cm² when evaluating the leaf area index in corn and concluded that the results were satisfactory according to the data collected in the field in the conventional manner.

All correlation coefficients estimated through artificial intelligence attained higher values than the Pearson's coefficients, as the software trains the model with 80% of the data, causing a better adjustment. The present study could not distinguish the tested cultivars through the red, green, and near infrared bands, as all cultivars showed the same reflectance pattern. This may have occurred because the materials used have low variability. According to Maciel et al. (2020), using genetic materials with high variability facilitates the differentiation of cultivars through phenotyping using multispectral images.

However, this study was able to relate the red band values with agronomic characteristics, which is of great importance in phenotyping through images (Figure 10).

The pixel distribution classes varied according to each agronomic variable and are represented in each map caption (Figure 10). Festival, Guarani, and Oso Grande had the highest pixel cloud, whereas San Andreas, Albion, and PR had low pixel clouds for all agronomic variables.

The cultivars with the largest pixel clouds and largest canopies (Festival, Guarani, and Oso Grande) showed considerable variation in the pixel class color distribution. In contrast, San Andreas, PR, and Albion presented small plant canopies, and consequently, small pixel clouds.

According to Furbank & Tester (2011), a bottleneck in the phenotyping process is that it requires great human effort, in addition to some evaluations in certain environments, thereby limiting the performance of this process. Imaging evaluation has been shown to be effective to solve this challenge, as it is rapid and has a high correlation to various anatomical, physiological, and biochemical characteristics of plants (Walter et al., 2015), as demonstrated by the present experiment.

CONCLUSIONS

1. The environmental conditions of the Cerrado influence the production of different strawberry cultivars.
2. Cultivars that reached high soil temperature under and above the mulch and on the leaves were the least productive.
3. Festival was the cultivar most adapted to the local conditions, with the best productive performance and fruit quality.
4. Agronomic variables were estimated using multispectral images collected at 78 days after planting.
5. The red, green, and NDVI spectral bands can be used to estimate the number of leaves, leaf length, leaf width, and yield and number of fruits in strawberry cultivars.

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