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LUDYMILLA CÉLIA SOUSA SANTOS

ESTIMATING BIOMETRIC, PHYSIOLOGICAL AND NUTRITIONAL VARIABLES IN LETTUCE AND CABBAGE SEEDLINGS USING MULTISPECTRAL IMAGES

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Dissertation presented to the Graduate Plant Production Program of the Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro –Campus Uberaba, as a requirement for obtaining a Master's Degree in Plant Production.

Advisor:
Dr. Hamilton César de Oliveira Charlo

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RESUMO

A alface (*Lactuca sativa* L.) e o repolho (*Brassica oleracea* L. var. *capitata*) são hortaliças cultivadas extensivamente e consumidas em todo mundo. De forma inovadora, o uso de imagens digitais para definir parâmetros fisiológicos e nutricionais vem ganhando espaço na pesquisa. Todavia, em hortaliças, sobretudo em mudas, são poucos os estudos que tenham avaliado parâmetros fisiológicos, nutricionais e biométricos, baseados nas respostas de imagens multiespectrais. Diante disto, este estudo teve por objetivo estimar quantitativamente parâmetros morfométricos, fisiológicos e nutricionais em mudas de alface e repolho, a partir de modelos paramétricos e não paramétricos (aprendizado de máquina) baseados na resposta de imagens multiespectrais. O estudo foi composto por três experimentos, sendo dois com a cultura da alface e um com a cultura do repolho. A variabilidade dos dados foi alcançada através da produção de plântulas de repolho e alface, com variações no tempo para início da fertirrigação e/ou intervalos entre fertirrigações nas mudas. Para tanto, foram conduzidos dois experimentos com seis tempos de início da fertirrigação nas culturas da alface e repolho e um experimento de intervalos entre aplicações da fertirrigação na cultura da alface. Os três experimentos foram conduzidos em delineamento de blocos casualizados, com 6 repetições cada. Aos 20 dias após a semeadura, foram capturadas imagens das plantas utilizando câmera MAPIR Survey 3, que tem uma resolução de 12 bits, 19 mm de distância focal, 2,3 cm GSD e bandas verdes (550 nm), vermelho (660 nm) e infravermelho próximo (850 nm), bem como os parâmetros biométricos, fisiológicos e nutricionais das mudas. As imagens foram capturadas de 10 plantas de cada parcela experimental, totalizando 360 imagens no estudo com repolho e 660 imagens no estudo com alface. As fotos foram tiradas das 11h30 às 12h30 sem sombra. Tanto para o repolho quanto para a alface a banda do vermelho (B_{660}) da câmera Mapir, exibiu a maior variabilidade, mostrando que a gama vermelha é mais sensível aos diferentes tratamentos, com exceção da variável área foliar da banda B_{555} e índices derivados. Foi possível estimar todas as variáveis agronômicas utilizando os modelos gerados pelo algoritmo M5.

Palavras-chave: *Lactuca sativa* L. *Brassica oleracea* var. *capitata*. Imagens Multiespectrais. Sensoriamento remoto. Variáveis Morfofisiológicas.

ABSTRACT

Lettuce (*Lactuca sativa* L.) and cabbage (*Brassica oleracea* L. var. *capitata*) are vegetables grown and consumed extensively, worldwide. The innovative use of digital images to define physiological and nutritional parameters is gaining ground in the field of research. However, there are few studies that assess the physiological, nutritional and biometric parameters of vegetables based on multispectral images, particularly in seedlings. As such, the present study aimed to quantitatively estimate morphometric, physiological and nutritional parameters in lettuce and cabbage seedlings based on multispectral images, using parametric and nonparametric models (machine learning). Three individual experiments were conducted, two with lettuce and the other with cabbage. Data variability was achieved by growing lettuce and cabbage seedlings using different fertigation start times and/or intervals. To that end, two of the experiments were carried out using six irrigation start times in lettuce and cabbage, and one with different fertigation intervals in lettuce. All experiments used a randomized block design with six repetitions each. At 20 days after sowing, images of the plants were captured using a MAPIR Survey 3 camera, with 12-bit resolution, focal length of 19 mm, a 2.3 cm ground sampling distance (GSD) sensor and green (550 nm), red (660 nm) and near-infrared (NIR) bands (850 nm), and the biometric, physiological and nutritional parameters of the seedlings were assessed. Images were taken of 10 plants from each experimental plot, totaling 360 images in the cabbage experiment and 660 for lettuce. The photographs were taken between 11:30 a.m. and 12:30 p.m., with no shade. For the lettuce and cabbage experiment, the B660 band of the Mapir camera showed the greatest variability, indicating that the red band is more sensitive to the different treatments, except for leaf area in the B555 band and the resulting indices. All the agronomic variables were estimated using the models generated by the M5 algorithm.

Keywords: *Lactuca sativa* L. *Brassica oleracea* L. var. *capitata*. Multispectral Images. Remote Sensing. Morphophysiological Variables.

SUMMARY

CHAPTER I

GENERAL CONSIDERATIONS

1.1 GENERAL INTRODUCTION

Lettuce (*Lactuca sativa* L.) and cabbage (*Brassica oleracea* var. *capitata*) are grown extensively and consumed worldwide in salads, with high levels of vitamins essential to human health (ROSA et al., 2014).

The progressive increase in the global population has led to a growing demand for food, with studies indicating that agricultural production will need to expand by 70 to 100% by 2050. (MAGGIO et al., 2018).

These data highlight the need for continued research in the seed and seedling sectors, since successful production is related to this field (PERALTA, 2018)

Seedling production is one of the most important phases in lettuce and cabbage production systems, since the final nutritional and developmental performance of plants depends on their “start” (LIMA et al., 2019).

Given the importance of these crops, they are frequently studied in the fields of breeding, mineral nutrition and irrigation management. However, new technologies for fertilizer and water management are still scarce when compared to crops such as soybean and corn. Optical sensors are among the most widely used technologies in modern agriculture, particularly for grasses, using spectral reflectance from the canopy to determine different parameters (SERRANO et al., 2018). Advances in these sensors have enabled better data management in the field because destructive sampling, which is generally time-consuming and costly, is not required.

The main advantage of multispectral cameras in relation to active optical sensors is their ability to map horizontal variability and be mounted onto unmanned aerial vehicles (UAVs), also known as drones (BARETH; SCHELLBERG, 2018).

The innovative use of digital images to define physiological and nutritional parameters is gaining ground in the field of research. Images have been used in crops such as acerola and forage grasses (LUCENA et al., 2011; SERRANO et al., 2016). However, few studies have assessed the physiological, nutritional and biometric parameters of vegetables based on multispectral images, particularly in seedlings.

In light of the above, the present study aimed to quantitatively estimate morphometric, physiological and nutritional parameters in lettuce and cabbage seedlings based on multispectral images, using parametric and nonparametric models (machine learning).

1.2 LITERATURE REVIEW

1.2.1 Lettuce and cabbage crops in Brazil

Lettuce (*Lactuca sativa* L.) is an herbaceous leafy vegetable, with a short stem containing smooth or ruffly leaves. Originating in the Mediterranean basin, it belongs to the family Asteraceae and is one of the most popular and widely consumed vegetables in Brazil and the world (DALASTRA et al., 2016; ECHER et al., 2016; SALA; COSTA, 2012).

The different cultivation techniques available mean lettuce can be grown throughout the year, supplying the year-round demand for the crop across Brazil (CARVALHO; SILVEIRA, 2017).

The main types of lettuce grown in the country, in order of importance, are looseleaf, iceberg, butterhead and romaine (SALA and COSTA, 2012) According to Maluf (2001), lettuce can be classified into five groups: 1 – romaine, which grows into a tall head of sturdy, dark green leaves with firm white ribs down their centers; 2- crispleaf, with smooth, delicate leaves that develop into a rosette; 3 – butterhead, characterized by a loose head of smooth, tender, light green oily-textured leaves; 4 - iceberg, with a dense head of broad, thick and crisp leaves, similar to cabbage; 5 – curly endive, with delicate “ruffly” leaves.

Due to its organoleptic properties, lettuce is consumed fresh and is an excellent source of nutrients such as vitamin A, B1, B2, B6, potassium, calcium and iron, in addition to having a high fiber content (SILVEIRA, 2016; MEIRELLES, 2016).

Cabbage (*Brassica oleracea var. capitata*), which belongs to the family Brassicaceae, is a leafy vegetable with thick waxy leaves that form into a round, compact head. Highly nutritious, with a high fiber content, it is a source of vitamin C, B1, B2, E and K; mineral salts, especially calcium and phosphorus, and is also known for its high β-carotene content (MOREIRA et al., 2011, ALMEIDA et al., 2015, REIS et al., 2017).

Although cabbage is adaptable to different growing conditions, it develops better under mild temperatures, especially during head formation. However, the existence of cultivars that are better adapted to tropical conditions means the crop can be grown in tropical climates (MOREIRA et al., 2011). According to the Food and Agriculture Organization (FAO, 2021), 69.4 million metric tons of cabbage were produced in 2018, in an area of approximately 2.4 million hectares.

Seedling production is the most important phase for most crops because seedling quality directly affects crop development, influencing yield and the final quality of the product (GONÇALVES et al., 2018).

1.2.2 Agronomic aspects in seedling production

The final quality of vegetables begins with the selection of certified seeds or seedlings and includes using varieties adapted to the soil and climate conditions of the growing region that are resistant to physiological disorders and pathogen attack (MORAES, 2006).

Producing quality vegetables requires careful attention from the seedling production stage to postharvest treatment. Quality seedlings are essential to producing plants that perform well in the field and failures at this stage can result in yield losses. Superior seedlings show high growth and survival potential after planting, reducing the need for replanting (FERREIRA et al., 2017) According to Echer et al. (2000), low quality seedlings compromise crop development, that is, a poorly developed seedling lengthens the phenological cycle of the crop.

Producing high-quality seedlings depends on a number of factors, such as the location of the nursery and the quality of the seed, substrate, container and management practices adopted (ANDRADE et al., 2015; MODOLO, 2015).

The length of the greenhouse should face east-west to ensure a longer period of sunlight, with a high ceiling and zenith windows to allow better air circulation (OLIVEIRA et al., 2016; FARIA JÚNIOR, 2004).

Nascimento, Dias and Silva (2011) found that low quality seedlings tend to produce poor crop stands, whereby unevenly emerging seedlings compromise not only yield, but the quality and standardization of the final product. The quality of a seed is defined by the combination of its genetic, physical, health and physiological qualities (NASCIMENTO; SILVA; CANTLIFFE, 2016).

Silva et al (2008) studied the effect of five substrates on the initial development of three lettuce cultivars and found that poor substrate management compromised the germination speed and rate of seedlings. Six substrates were assessed in tomato seedling production and the one that resulted in the best seedling development also produced the lowest incidence of leaning (SUMIDA et al., 2014).

Modolo (2015) reported that the ideal substrate for vegetable seedlings should have the following physical characteristics: a) apparent density: 100 to 300 kg m^{-3}, b) 75 to 85% porosity, c) aeration: pore space of 20 to 30%. In regard to chemical properties, substrates with low salinity and neutral or slightly acidic pH have been favored (SORIA, OLIVERT, 2002).

According to Cavallaro Júnior (2016), the seed tray selected and, consequently, the substrate available per seed, should enable healthy root growth, a key factor in good plant development in the field. Nurseries currently use trays with small cells in order to optimize production costs and space (more seedlings per m^2) (GODOY; CARDOSO, 2005). However, competition for light and space can compromise final production in vegetables (PURQUERIO; CARNEIRO JÚNIOR; GOTO, 2004).

Marques et al (2003) found that lettuce seedlings grown in 120- and 200-cell trays performed satisfactorily in the field, whereas those produced in 288-cell trays exhibited poor performance in both the initial growth and adult phases.

Fertilization and water management are the most intensive activities in any seedling production system. Water quality is a key factor, and the water used should have: a) pH between 5 and 7; b) electrical conductivity (EC) less than 0.75 dS m^{-1}; c) sodium absorption ratio (SAR) lower than 2.0; c) sodium, boron and fluoride content below 40, 0.5 and 0.75 ppm, respectively (LESKOVAR; SHARMA (2016). Viana, Fernandes and Gheyi (2001) reported that increased salinity in irrigation water caused a decline in the shoot and root development of lettuce seedlings.

Although some authors have investigated the effect of nutrient supply times and techniques on lettuce and cabbage development in the field, these studies are rare and focus on adult plants (SUÁREZ-REY et al., 2019; CALABRIA; LENS; YEH, 2019; CHEN et al., 2019; OLIVEIRA et al., 2019; CARDOSO et al., 2020; SCHMITT et al., 2020).

1.2.3 Remoting sensing in agriculture using multispectral cameras

Remote sensing (RS) is defined as the recording of reliable information from the ultraviolet, visible, infrared and microwave bands of the electromagnetic spectrum, with no physical contact, by coupled sensors and platforms such as aircraft and satellites, with the resulting information being either visually analyzed or submitted to digital image processing (JENSEN, 2009).

The characteristics, components and constituents of the targets are defined according to the behavior of their spectral response. Spectral behavior is defined as the reflectance curve as a function of wavelength in the electromagnetic spectrum.

According to Del'Arcosanches et al. (2003), leaves are the main plant organ exposed to electromagnetic radiation when sunlight strikes the surface of the canopy. Each leaf intercepts the incident radiant flux and this electromagnetic energy interacts with leaf pigments, water and intercellular spaces, triggering three physical pathways: reflection, absorption and transmission. These energies differ depending on leaf structure (LIU, 2015).

Ponzoni et al. (2012) reported that reflectance is the property whereby an object reflects electromagnetic radiation (EMR) and is determined by the proportion of reflected (ascending flux) and incident radiation (descending flux), an important parameter in monitoring agricultural crops (LIANG, 2005).

Interaction between EMR and the leaves is interpreted considering absorption, transmission and reflection phenomena (CAMPELO, 2018). Ponzoni et al. (2007) emphasizes that changes in the content of photosynthetic pigments and their internal morphology are the main characteristics of interest.

In the literature, the most widely described spectra in the electromagnetic spectrum are the visible (VIS) and near-infrared (NIR) regions, which have the greatest chance of detecting and correlating changes (NIR) in vegetation reflectance as a function of mineral nutrition. Changes in the visible spectrum band are largely due to chlorosis, that is, the absence of chlorophyll pigments, expressed by the gradual yellowing of the leaves, visible to the naked eye (CILIA et al., 2014).

According to Novo (2010), the NIR spectrum, between 700 and 1,300 nm, is influenced by the cellular structure of the mesophile, where considerable light scattering occurs. Leaf growth and increased biomass in well-fertilized plants interfere heavily in NIR reflectance. Additionally, the strong association between reflectance changes and structural modifications means that NIR shows considerable potential in crop yield (SCHLEMMER et al., 2013). Spongy mesophiles are typically responsible for scattering NIR light. When the number of vacuoles rises, NIR reflectance increases (LIU, 2015).

In the 1,300 to 2,500 nm region, which is predominantly mid-infrared, vegetation reflectance is governed by the water content of leaves. Ponzoni et al. (2012) found that water absorbs a significant amount of incident radiation, with peaks concentrated between 1,450 nm and 2,700nm, which correspond to the atmospheric absorption bands.

Remote sensing systems consist of platforms and sensors that capture the EMR emitted or reflected by objects on the earth's surface (FORMAGIO and SANCHES, 2017).

Artificial satellites emerged in the mid-20th century, using imaging sensors that did not rely on filming and encompassed a larger number of spectral bands, known as multispectral sensors. Multispectral technology advanced in the 1980s, enabling imaging sensors to obtain images in hundreds of narrow bands, denominated hyperspectral sensors.

According to Inamasu and Castro Jorge (2014), sensors that operate in the visible spectrum function as the "farmer's eyes" in evaluating the crop. However, certain responses to nutrient stress, physiological indicators and canopy structure are better assessed in the infrared or NIR regions, which are used in multispectral cameras, where the blue filter typically present in a visible spectrum camera (RGB) is substituted for an infrared filter.

Multispectral data from ETM + (Enhanced Thematic Mapper Plus) or OLI/Landsat sensors (Operational Land Imager/Land Remote Sensing Satellite), among others, are widely used in remote sensing for agriculture (FORMAGIO and SANCHES, 2017). Furlanetto et al., (2017) found that multispectral cameras mounted in remotely piloted aircraft (RPA) minimize operating costs and provide fast, accurate measurements to assess the growth cycle of crops.

Scientific studies correlate vegetation reflectance with agronomic characteristics in soybean (IOST FILHO et al., 2020), sugarcane (SILVEIRA et al., 2020) and lettuce (MACIAL et al., 2019), using multispectral and hyperspectral sensors.

Seedling analysis via imaging optimizes assessment times and improves the accuracy of results, whereby the data obtained are calculated in image processing software (JEROMINI et al., 2019).

Watson (1947) defined the leaf area index (LAI) as the total one-sided area of leaf tissue per unit ground surface (m^2/m^2), calculated based on the leaf area of the plant. The LAI is a key variable in understanding vegetation dynamics in land-based ecosystems and characterizes the canopy-atmosphere interface where most energy fluxes exchange, in addition to affecting important ecological aspects, such as inter and intraspecific competition between plants (BRÉDA, 2003).

The index can be measured using different direct or indirect "destructive" methods. It is important to obtain a real measurement of the leaf canopy via representative sampling depending on the type of plant, considered a standard method by some authors (FAVARIN et al., 2002; LIMA et al., 2019).

Indirect methods estimate the LAI while maintaining leaf integrity. Tools used to obtain these estimates include those that operate according to the radiation transmitted within the canopy and are based on the probability distribution and arrangement of leaf elements (JONES, 1992).

Vegetation indices have been widely used to monitor plant cover on a global or local scale (MIURA et al., 2001). These indices are a combination of spectral data from two or more selected bands aimed at synthesizing and improving the relationship between the data and plant biophysical parameters. In order to minimize the variability caused by external factors, spectral reflectance has been converted into different vegetation indices (PONZONI, 2001).

The best-known vegetation index is the Normalized Difference Vegetation Index (NDVI) (DORIGO et al., 2007). According to Cohen et al. (2003), the NDVI can be analyzed by interpreting remote sensing images obtained on different dates, making it possible to assess the variation in vegetation over a certain time period.

Given its association with nitrogen and chlorophyll content in plants, Oliveira (2015) reported that the NDVI is used in the application of different rates of nitrogen, in addition to being correlated with agronomic (yield and grain mass, among others) and plant pathology parameters (disease incidence and severity). Its rapid saturation makes the NDVI unsuitable for detecting variations in plant biomass from a certain growth stage onwards (ASRAR et al., 1984).

Yang et al. (2008) monitored cabbage crops based on aerial images taken with a near-infrared camera and found that the NDVI is highly correlated with plant diameter and area, while Jensen (2009) found that NDVI and GNDVI (Green Normalized Difference Vegetation Index) are strongly correlated with plant weight. Thus, as reported for parameters related to leaf development, Yang et al. (2008) associated the GNDV with parameters such as plant and head weight in cabbage.

The GNDVI was proposed by Gitelson et al. (1996), who used orbital images of mature and senescing plants to determine chlorophyll concentration and nutrient deficiencies in plants. According to the author, the GNDVI can be used as a substitute for the combination of red and near-infrared channels, providing information on plant health and chlorophyll quality in the leaves.

ImageJ® software is a public domain image processing program that can be used for a variety of different fields, including leaf area analysis and medicine. In seed analysis, radiometric images are used to obtain biometric data, foregoing traditional techniques such as pachymeters and rulers in order to ensure more accurate results (ANDRADE et al., 2010).

For nutritional variables, the Simple Ratio (SR) proposed by Jordan (1969) is the ratio between the reflectance in the red and NIR bands. It was used by Mao et al. (2015) to monitor nutrients in cabbage, and by Martins et al. (2021) for the same purpose in lettuce. According to Usha et al. (2013), other horticultural studies have also indicated the SR as the ideal spectral component for modeling N.

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CHAPTER II

MULTISPECTRAL IMAGES FOR ESTIMATING MORPHOPHYSIOLOGIAL AND NUTRICIONAL PARAMETERS IN CABBAGE SEEDLINGS

ABSTRACT

Remote sensing data have been used to monitor numerous agricultural crops. However, few studies have combined biometric, nutritional and physiological parameters in a cabbage crop, especially in seedlings, using low-cost multispectral sensors. Thus, the objective of this study was to estimate the biometric variables of cabbage from parametric and non-parametric models based on the response of multispectral images taken by a multispectral camera. The experiment was conducted in a greenhouse. Data variability was achieved by producing cabbage seedlings with variations in the fertigation start time. Twenty days after sowing, multispectral 360 images of the plants were captured using a MAPIR Survey 3 camera. To compose the estimation models, along with the original bands of the camera, the multispectral vegetation indices. It was possible to quantify the estimated biometric, physiological, and nutritional variables of cabbage using multispectral cameras. Among the Mapir camera bands, the banda do vermelho exhibited the greatest variability, showing that the red range was the most sensitive to the different treatments. Except for leaf area, all the parameters measured could be estimated by linear models with deterministic coefficients of up to 72%. Neural network models were more accurate in estimating parameters, but the maximum quantum yield of photosystem II (Fv/Fm) was most accurately estimated (1.2%) by the linear model generated by algorithm M5. This study highlights the integrated use of different tools to assist in monitoring the evaluated parameters.

Key words: *Brassica oleracea* var. *capitata*. Morphobiometrical Variables. Low Cost Multispectral Camera. Prediction Models.

2.1 INTRODUCTION

Monitoring physiological and nutritional parameters quickly, early and non-destructively of plants can help in the adopted management and, consequently, in improving the development of crops. Agricultural production requires applying numerous technologies to raise yield, reduce environmental damage, achieve food security and increase productive process profitability.

In leafy vegetables, seedling production is one of the most relevant phases to achieve high yields. Poorly formed seedlings may compromise plant development and prolong their cycle, causing production losses (CHRYSARGYRIS et al., 2019; LIMA et al., 2019).

Among leafy vegetables, whose seedling formation is an essential stage for high yields, cabbage (*Brassica oleracea* L. var. *capitata*) stands out for its high nutritional value, and is produced and consumed worldwide (CHRYSARGYRIS et al., 2019; LIU et al., 2020). In Brazil, the crop is economically important due to the high levels of production and consumption (REIS et al., 2017), and socioeconomically significant because of the demand for intensive labor and growing crops in small areas (CASSOL et al., 2017).

Because of its relevance, the species is widely researched in different study areas, including breeding programs, mineral nutrition and irrigation management. Thus, improving crop breeding techniques such as nutritional diagnosis and water status is essential to reaching the desired yield and savings in production inputs, primarily water and fertilizers in general. In this respect, remote sensing has a number of applications in agriculture (KHABAL et al., 2020; WEISS et al., 2020) data have been used to monitor agricultural crops and in decision making, in order to improve management practices (MARIN et al., 2019).

Remote sensing typically uses airplanes and satellites, but can also be performed with drones equipped with different types of sensors, such as RGB, thermal, multispectral and hyperspectral cameras. These sensors have different wavelengths, which encompass the visible, near-infrared and short-wave infrared regions (MAES & STEPPE, 2019).

The spectral remote sensing technique enables early, efficient, objective and non-destructive assessment of plant responses to different environmental stress factors (LI et al., 2010), since plants react to biotic and abiotic stresses via biophysical and biochemical changes such as reduced biomass, lower chlorophyll content and changes in internal leaf structures (MAHAJAN et al., 2014).

These changes can be easily detected from the difference in reflected energy in visible and near-infrared regions, which allow the development of vegetation indices

(HILLNHUTTER et al., 2011), based on various combinations of bands of the electromagnetic spectrum (CARNEIRO et al. , 2020). These indices show high correlation with changes in plant physiology and chemistry and low sensitivity to the factors responsible for hindering interpretation of remote sensing data, such as shading, canopy cover, condition and geometry of the atmosphere, solar angle and bottom soil (GABRIEL et al., 2017).Passive sensors such as multispectral cameras are capable of detecting the NDVI (Normalized Difference Vegetation Index) of crops, which is the most used vegetation index. However, there are other vegetation indices used in remote sensing, and their combination can improve prediction models.

Literature studies correlate the reflectance of vegetation with agronomic characteristics, using multispectral and hyperspectral sensors, particularly in soybean (IOST FILHO et al., 2020); sugarcane (SILVEIRA at al., 2020) and lettuce (MACIEL et al., 2019). However, few studies have combined biometric, nutritional and physiological parameters in cabbage crops, especially seedlings, using low-cost multispectral sensors.

In light of the above, the aim of the present study was to quantitatively estimate the biometric, physiological and nutritional parameters of cabbage seedlings, applying parametric and nonparametric parameters (machine learning) based on the response of multispectral images.

2.2 MATERIAL AND METHODS

The study was conducted in the municipality of Uberaba, MG, Brazil, which is in the Mesoregion of Triângulo Mineiro and Alto Paranaíba, between December 8 and 28, 2019, with a cultivar adapted to tropical conditions in late spring and early summer, when there is an increasing market demand for cabbage and seedling production.

The arch-type greenhouse where the study was conducted was constructed in double span plastic and measured 14 m wide and 51 m long, with eave and arc heights of 3.00 and 1.5 m, respectively, in the east-west direction and lies at coordinates 19° 39’ 44’’ S and 47° 58’ 02’’ W, at an altitude of 790 m. It was covered in 150-µm light-diffusing plastic film with closed sides and 50% shade cloth.

Temperature and air relative humidity were collected daily in the greenhouse using a thermohygrometer (Testo 174 H data logger) installed 1.20 m above ground level, to assess the environmental conditions just above the plants.

Data variability was achieved by producing cabbage seedlings with variations in the fertigation start time. The experiment was conducted in randomized block design, with six repetitions. Each experimental unit consisted of 128 plants. The treatments consisted of six fertigation start times (T_1 = 0, T_2 = 3, T_3 = 6, T_4 = 9, T_5 = 12, and T_6 = 15 days after emergence). After the first application of each treatment, fertigation was repeated in five-day intervals. Thus, depending on the start time of the first application, the number of applications was different for the various treatments, with four applications for T_1, three for T_2 and T_3, two for T_4 and T_5, and one for T_6.

The nutrient solution used in this study was proposed by Furlani et al. (1999), and and has the following composition: N (198,0 g $1000L^{-1}$); P (39,0 g $1000L^{-1}$); K (183,0 g $1000L^{-1}$); Ca (142,0 g $1000L^{-1}$); Mg (38,0 g $1000L^{-1}$); S-SO_4 (52,0 g $1000L^{-1}$); B (300 mg $1000L^{-1}$); Cu (20 mg $1000L^{-1}$); Fe (2000 mg $1000L^{-1}$); Mn (400 mg $1000L^{-1}$); Mo (60 mg $1000L^{-1}$) e Zn (60 mg $1000L^{-1}$). Fertigation depth varied as a function of seedling development stage. More advanced stages required higher fertigation depths. Nutrient solution was applied as follows: 0.4 L from emergence to 3 days after emergence (DAE); 0.5 L from 4 to 7 DAE; 0.6 L from 8 to 11 DAE; 0.7 L from 12 to 14 DAE and 0.8 L from 15 to 18 DAE.

The cabbage cultivar used to produce the seedlings was Astrus Plus from Seminis®. The seeds were sown in polyethylene trays, with 22 cm^3 cells, filled with the Bioplant Plus® substrate. The experiment was conducted on wire benches 90 cm above the ground.

The seedlings were irrigated four times a day (8:00 am, 12:00 pm, 2:00 pm, and 4:30 pm) using an automated sprinkler irrigation system without drainage to maintain the substrate in the containers. From sowing to emergence (three days after sowing) all treatments were irrigated with water only. Emergence was considered to be established when at least 90% of the tray cells contained emerged seedlings. Fertigation was then initiated within the time frames proposed in the experimental design, and performed only once a day (8:00 am).

The sprinkler irrigation system installed inside the greenhouse provides an average depth of 3.98 mm h^{-1}, which corresponds to 3.98 L of water for each m^2 of greenhouse in one hour. Water depth was measured every three days by weighing the trays. The trays were irrigated until the water began to drain, that is, until container capacity was reached, corresponding to field capacity concept, when they were weighed. They were weighed again after 2.5 hours and the procedure was repeated four times throughout the day. The difference between weight measurements was attributed to crop evapotranspiration (ETc), which was subsequently converted into the amount of water to be applied daily via irrigation or fertigation.

Irrigation depth varied as a function of seedling development stage. More advanced stages require greater irrigation depths. The depth applied up to 3 days after emergence was 4.98 mm day^{-1}, from 4 to 7 DAE, 5.24 mm day^{-1}, from 8 to 11 DAE, 5.44 mm day^{-1}, from 12 to 14 DAE, 5.70 mm day^{-1} and from 15 to 18 DAE, 5.97 mm day^{-1}.

Each day, all the treatments received the same amount of water. However, when fertigation was applied, the water was replaced by the same volume of nutrient solution for the first irrigation of the day (8:00 am). Fertigation was applied using a backpack sprayer with a capacity of 20 L and flow rate of 0.8 L h^{-1}. After this application, a small volume of water was sprayed on the seedlings to prevent the nutrient solution from accumulating on the leaves. The other irrigations (12:00 pm, 2:00 pm, and 4:30 pm) were performed with water only for all treatments.

At 15 DAE, the number of leaves (NF15) was evaluated by counting the fully developed leaves of 10 plants from each plot.

At 17 DAE, the physiological variables of the seedlings were evaluated through the OJIP transient fluorescence of chlorophyll a, including the initial fluorescence (F_0), variable fluorescence (F_v), maximum fluorescence (F_m), maximum quantum yield (F_v/F_m), amount of photons absorbed by the antenna complex (ABS/RC), amount of energy flowing through the antenna complex and captured by the PSII reaction center (TRo/RC), and amount of energy dissipated by non-photochemical dissipation (DIo/RC). These variables were measured using PSI's (Photon Systems Instruments) Fluorometer FP100 model, performing readings on the second true leaf of five plants in each plot between 12:00 am and 3:00 am so that the plants were adapted to the dark. Chlorophyll a index was evaluated on the same day using Falker's CFL1030 Clorofilog model, taking five readings per plot on the second true leaf from 11:00 am to 3:00 pm.

At 21 days after sowing, or 18 days after the beginning of the treatments, when the seedlings reached the commercial point of transplanting, the following variables were assessed in 40 plants from each experimental plot: leaf area (Area), expressed in cm² plant^{-1}; shoot dry mass (SDM), expressed in g plant^{-1}; and root dry mass (RDM), expressed in g plant^{-1}.

Shoot N, P, K, Ca, Mg, and S (g kg^{-1}) content was also determined in 40 seedlings from each experimental plot.

After obtaining the data, dispersion analysis was performed and the variables were submitted to descriptive statistical analysis, obtaining the mean, standard deviation, and coefficient of variation.

At 20 days after sowing, multispectral images of the plants were captured using a MAPIR Survey 3 camera, which has 12-bit resolution, 19 mm focal length, 2.3 cm GSD (Ground Sample Distance), and green (550 nm) (B_{550}), red (660 nm) (B_{660}), and near infrared (850 nm)(B_{850}) bands.

The images were captured of ten plants from each experimental plot, totaling 360 images. The camera was installed on a horizontally and vertically levelled support 1.20 m from the ground, in the nadir position. The plants were placed under a black nonwoven fabric to mitigate the effect of reflectance from neighboring targets.

To ensure maximum absorption and reflection conditions of solar electromagnetic radiation (Jensen 2009), the images were taken from 11:30 am to 12:30 pm without shading, which could be caused by clouds or anthropic features near the frame of the capture area. The radiometric calibration of the images was performed using the Mapir Camera Control software. The process was possible because the images were calibrated with the reflected radiance of the calibration plate, provided by the Survey 3 camera manufacturer, at the same time as the images were taken.

After calibration, radiometric normalization of all the images was performed to compensate for the lighting effects from the first to the last shot, where the image taken at 12:00 pm was set as the reference. Normalization was performed in ENVI 5.1 software, according to the methodology proposed by Jensen (2009).

During the normalization process, in the reference image, as well as the images to be normalized, the radiance values were manually extracted from the set of light and dark pixels in all the bands of the camera. In all the cases, the pixels were extracted from the calibration plate.

The following equation was used to determine the coefficients of linear transformation:

$$Ti = mi \times xi + bi \tag{1}$$

where:

mi - (Bri - Dri)/(Bsi - Dsi);

bi - (Dri × Bsi - Dsi × Bri)/(Bsi - Dsi);

Ti - Radiance for the reference image;

xi - Radiance for the image to be normalized;

Bri - mean of the light reference set;

Dri - mean of the dark reference set;

Bsi - mean of the light set to be normalized;

Dsi - mean of the dark set to be normalized; and,

i - bands of the sensor under study.

The multispectral vegetation indices were calculated [eqs. (2), (3) and (4)] to compose the estimation models, along with the original camera bands, from the original calibrated Survey 3 camera bands (Table 1), using the ENVI 5.1 software.

Table 1. Equations an references for calculations of vegetation indices derived from the original bands of the Survey 3 Camera

Index	Equation	Reference
Simple Ratio (SR)	SR = $(B_{850})/(B_{660})$ **(2)**	(Birth & McVey, 1968)
Normalized Difference Vegetation Index (NDVI)	NDVI = $(B_{850} - B_{660})/(B_{850} + B_{660})$ **(3)**	(Rouse et al., 1973)
Green Normalized Difference Vegetation Index (GNDVI)	GNDVI = $(B_{850} - B_{550})/(B_{850} + B_{550})$ **(4)**	(Gitelson et al., 1996)

The individual mean brightness values of the original bands and derived multispectral indices were extracted for all the plants. The average brightness values were extracted from the irregular polygons surrounding the plant crowns through the regions of interest tool created in ENVI 5.1 software. The mean zonal method was used for data extraction.

The estimation process used in this study is based on parametric and non-parametric regression methods. The non-parametric Multilayer Perceptron (neural network) and the parametric Multiple Linear Regression (MLR) algorithms were used as estimators, and the results were compared.

Neural network implementation was used to estimate and validate the cabbage variables (biometric, physiological, and nutritional), in Weka software (Weka 3.9.4, University of Waikato).

The neural network variables were set to their defaults (two neurons and one layer). It is important to underscore that several tests were conducted to optimize the network variables, but the standard architecture of the software always exhibited more accurate estimates.

This study used the MLR method, which attempts to estimate the variables of the model and describe the relationship between two or more independent variables and a response variable by fitting a linear equation to the observed data, often using the least squares method in Weka (Weka 3.9.4, University of Waikato). In Weka, the selection of the features model was completed using a backward elimination method called “M5”, wherein the attribute with the smallest standardized coefficient is removed until no improvements are observed in the Akaike information criterion (Akaike, 1974).

All the estimation models were created from the combination of the original bands and derived multispectral indices. For model training, the radiometric values of 288 randomly defined plants (80% of the sample set) were considered. Root mean squared errors (RMSE) [eq. (5)] and normalized RMSE (RMSE%) [eq. (6)] were calculated to validate the accuracy of the models, considering the residue of the difference between the estimated and measured agronomic variables for 72 plants (20 % of the sample set).

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(x_o - x_e)^2}{n}} \tag{5}$$

$$\mathrm{RMSE}(\%) = \sqrt{\frac{\sum_{i=1}^{n}(x_o - x_e)^2}{n}} * \frac{100*n}{\sum_{i=1}^{n} x_e} \tag{6}$$

where:

x_o – observed value;

x_e – estimated value; and,

n – number of samples.

2.3 RESULTS AND DISCUSSION

The average temperature and air relative humidity during the experiment were 27.51 °C and 67.16%, respectively. Maximum and minimum temperatures of 46.10 and 18.10 °C were recorded on 12/24/2019 and 12/22/2019, respectively, and maximum (94.6%) and minimum air relative humidity (27.9%) on 12/19/2019 and 12/24/2020, respectively.

The means obtained for the evaluated parameters are listed in Table 2. The standard deviation varied from 0.0051 (RDM) to 1,866.10 (Fm). This shows that the higher the value, the greater the data dispersion. The coefficients of variation (CV) ranged from 0.08% (B_{850}) to 33.50 % (leaf area), which represents low data dispersion for all the evaluated parameters (Table 2).

For average reflectance, the average spectral response of cabbage exhibited higher radiance for the B_{850} band (252.91) and lower for the B_{660} band (239.76). This characteristic represents the normal behavior of a plant in full development, where chlorophyll *a* and *b* act in the red spectral range and the cell structure of a healthy leaf in the near-infrared spectral range. By contrast, given the similarity in the average radiance values of the B_{550} (239.77) and B_{660} bands (239.76), no classic feature was established for electromagnetic radiation absorption in the red spectrum range. However, this condition is natural for cabbage given its yellow color and the pigment patterns on the leaves (YANGet al., 2008).

Table 2. Means, standard deviations, and coefficients of variation (CV) of biometric, physiological, and nutritional variables evaluated in cabbage seedlings.

Variables	**Means**	**Standard Deviations**	**CV (%)**
B_{850}	252.91	0.20	0.08
B_{550}	239.77	4.89	2.04
B_{660}	239.76	9.58	4.00
	Biometric		
NF15	3.09	0.42	13.51
SDM	0.1276	0.0330	25.87
RDM	0.0193	0.0051	26.32
Area	20.26	6.79	33.50
	Physiological		
F0	5,919.89	451.07	7.62
Fm	28,851.45	1866.10	6.47
Fv	22,931.56	1567.18	6.83
Fv/Fm	0.79	0.01	1.41
ABS/RC	1,825.21	120.25	6.59
TRo/RC	1,444.05	77.75	5.38
DIo/RC	0.37	0.03	8.02
Chlorophyll *a*	37.41	3.08	8.24
	Nutritional		
N	16.11	1.86	11.54
P	13.37	1.70	12.72
K	31.25	3.12	9.99
Ca	19.05	1.05	5.48
Mg	4.79	0.23	4.80
S	2.48	0.36	14.36

NF15 - Number of leaves at 15 DAE; SDM – Shoot dry mass (g plant^{-1}); RDM – Root dry mass (g·plant^{-1}); Chlorophyll a - Chlorophyll a index; Area - Leaf area (cm^2 plant^{-1}); F0 - Initial fluorescence; Fm - Maximum fluorescence; Fv - Variable fluorescence; Fv/Fm - Maximum quantum yield; ABS/RC - Absorption flow/center of reaction; TRo/RC - Captured energy flow/center of reaction; Dio/RC - Non photochemical energy flow/center of reaction; N, P, K, Ca, Mg, and S leaf concentrations (g kg^{-1}); CV - Coefficient of variation, n = 36.

For the data extracted from the image, the highest CV was for B_{660} (4%), meaning that the red spectrum is the best region to discriminate between the different types of treatment. For this experiment, the greater spectral variability in this range is associated with the high variability of the biometric and physiological variables of cabbage, given that reflected energy in the 630-680 nm spectral range is influenced by photosynthesis-related variables, such as NF15 and chlorophyll a (JENSEN, 2009).

The low CV values for the B_{550} (2.04 %) and B_{850} (0.08 %) bands show spectral regions with less potential for discriminating between the different treatments applied to the cabbage samples. The low variability of the B_{550} band is associated with the condition that all the samples exhibited the typical appearance of healthy green vegetation, that is, the same phycocyanin concentrations.

The variability of the B_{850} band, slightly lower than that of B_{550}, was directly influenced by the low variability of the nutritional variables of cabbage (JENSEN, 2009), which had a maximum CV for S (14,36%, Table 2). In this case, in addition to the influence of nutritional variables, a low CV is associated with the calibration process and radiometric normalization, which contributed significantly to the lower range and adherence of the values as a function of the mean.

Table 3 shows that R² values varied between 22 and 72%, with the lowest value for leaf area and the highest for chlorophyll *a* index. The variables estimated with the highest number of predictive variables obtained higher R² values, which did not necessarily result in more accurate predictive models, as observed in Table 2.

Table 3. Linear models of regression and coefficient of determination (R^2) for the estimation of biometric, physiological, and nutritional variables

Variables	Model	R^2 (%)
	Biometric	
Number of leaves at 15 DAE	$NF15 = -2.68 * NDVI + 3.16$	31
Shoot dry mass (g plant^{-1})	$SDM = 0.075 * B_{850} - 0.0059 * B_{550} + 0.9102 * GNDVI - 18.28$	41
Root dry mass (g plant^{-1})	$RDM = 0.0123 * B_{850} + 0.1723 * GNDVI + 0.607 * SR - 3.2605$	52
Leaf area (cm^2 plant^{-1})	$Area = -53.26 * NDVI + 21.7$	22
	Physiological	
Initial fluorescence	$F_0 = 6.78 * B_{550} + 4295.42$	39
Maximum fluorescence	$F_m = 34.5 * B_{660} + 20580.26$	26
Variable fluorescence	$F_v = 315.06 \times B_{550} - 43630.2$	25
Maximum quantum yield	$F_v/F_m = -0.01 * B_{850} + 2.82$	43
Absorption flow/center of reaction	$ABS/RC = 6.67 * B_{550} + 223.92$	35
Captured energy flow/center of reaction	$TRo/RC = 6.43 \times B_{550} - 100$	38
Non photochemical energy flow/center of reaction	$DIo/RC = -6.94 * NDVI - 3.94 * GNDVI - 2.59 * SR + 4.36$	58
Chlorophyll *a* index	$Chlorophyll\ a = 6.01 * B_{880} + 0.29 * B_{660} + 208.87 * SR - 1561.53$	72
	Nutritional	
Nitrogen leaf concentrations (g kg^{-1})	$N = 11.92 * SR + 15.79$	32
Phosphorus leaf concentrations (g kg^{-1})	$P = 33 * SR + 12.48$	58
Potassium leaf concentrations (g kg^{-1})	$K = 0.22 * B_{550} - 22.17$	34
Calcium leaf concentrations (g kg^{-1})	$Ca = 0.02 * B_{660} + 14.35$	36
Magnesium leaf concentrations (g kg^{-1})	$Mg = 0.62 * B_{880} - 0.03 * B_{660} - 11.46 * NDVI - 143.95$	60
Sulfur leaf concentrations (g kg^{-1})	$S = 5.3108 * GNDVI + 18.5792 * SR - 3.0504$	44

SR - Simle Ratio; GNDVI - Green normalized difference vegetation index

As in Martins et al. (2021), the models that estimate agricultural variables, when composed of a single predictive variable, tend to have a low coefficient of determination, as shown in Table 3. However, this does not necessarily result in an inaccurate estimate of the variable because if the validation data are normally distributed, the errors in data variability around the mean will be null and decrease the RMSE.

The B_{550}, B_{660} and B_{850} bands and the indices derived from the near infrared contribute mostly to the estimation models of the physiological variables (Table 3). For the biometric parameters, the multispectral indices derived are present in all the models. The NDVI index is used in the models to estimate the variables related to leaf development, such as the number of leaves at 15 days (NF15) and leaf area (Table 3), and the GNDVI index in those related to plant mass, as observed for shoot (SDM) and root dry mass (RDM).

For parameters such as NF15 and leaf area, the use of NDVI in the models is justified by the fact that this index is strongly correlated with the physical variables of vegetation in general (Jensen, 2009). This was also observed by Yang et al. (2008), who monitored cabbage crops from aerial images taken with a near-infrared camera, observing that the NDVI index is highly correlated with plant diameter and area and cabbage canopy.

Similarly to the NDVI index, the composition of the GNDVI index in the prediction models of plant mass variables can also be justified by the fact that the index is highly correlated with plant volume and mass (JENSEN, 2009). Thus, as with the parameters related to leaf development in Yang et al. (2008), the GNDVI was highly correlated with cabbage plant parameters, such as plant mass and head mass. Martins et al (2021) observed the same behavior in leafy vegetables such as lettuce, applying the same methodology to obtain the images used in this study. They found that biometric parameters were estimated from compound simple regression models, primarily according to the GNDVI and SR vegetation indices derived from the Mapir3 camera.

For the physiological variables, band B_{550} was present in most prediction models, except for the variables Fm, Fv/Fm, Dio/RC and chlorophyll *a* (Table 3). The fact that the other physiological variables are predicted only by the B_{550} band is justified because they are related to plant pigmentation and therefore sensitive to the green region of the spectrum (Jensen, 2009). For the other physiological parameters, the B_{660} and B_{850} bands and SR vegetation index were the predictive values for chlorophyll, corroborating the concepts that the red spectrum and near-infrared ranges and the SR index are sensitive to the amount and absorption of chlorophylls *a* and *b* and photosynthesis activity, respectively (MARTINS et al., 2021).

Other studies also exhibit red and near-infrared spectrum ranges, given that multiscale remote sensing has been widely used to monitor horticulture (USHA et al., 2013). Based on these ranges, the linear regression model based on vegetation were used to estimate the leaf chlorophyll content, some nonlinear regression methods such as principal component analysis, exponential model (LELONG et al., 2008), clustering algorithm (GIANNINI et al., 2018).

For the nutritional variables, the predominance of the SR index (Table 3) in the prediction models reflects the direct relationship between the red and near infrared with cabbage nutrients, as observed by Mao et al. (2015) and Martins et al. (2021), in the monitoring of nutrients in lettuce crops. According to Usha et al. (2013), other studies related to horticulture also found the SR vegetation index to be an optimal spectral component for modeling the nutrient N.

Table 4 shows the performance of the algorithms (RMSE and RMSE%) for estimating biometric, physiological, and nutritional variables. For the biometric variables, algorithms M5 and the neural networks were highly accurate in estimating NF15 (around 15%) and RDM (22.79%). Martins et al. (2021) used the radiance extracted from images taken with the Mapir3 camera of lettuce samples, and the M5 algorithm was also more accurate in estimating NF15, where the error did not exceed 11%.

The estimated leaf area was not satisfactory because the errors were 29.30 and 30.77% for the neural networks models and M5, respectively (Table 4). By contrast, when monitoring cabbage area, Yang et al. (2008) verified that the estimation of leaf area by remotely piloted aircraft cameras was possible and determinant for the development of a methodology capable of detecting plant volume.

Similarly, in monitoring lettuce health, Ren et al. (2017) verified that the estimation of leaf area by remotely piloted aircraft cameras was possible and determinant for the development of a methodology capable of detecting leaf deterioration. Burmgarner et al. (2012) also highlighted the possibility of monitoring the leaf area index using RGB images and machine learning algorithms. The authors were able to estimate the leaf area index of lettuce samples under different treatments with an accuracy ranging from 71 to 95%.

In regard to the physiological variables, the neural network model showed higher precision in the estimation of F_0 (6.8%), Fm (6.25%), Fv (13.37%), ABS/RC (5.54%) and Dio/RC (7.83%). The M5 model had higher precision in the estimate of Fv/Fm (1.2%) and Chlorophyll a (6.43%).

Specifically for chlorophyll *a*, hyperspectral data could also be used in similar studies. Simko et al. (2015) determined that lettuce leaf deterioration can be detected using hyperspectral images. In the same study, using hyperspectral indices, chlorophyll was classified according to leaf health with an accuracy of 97%. Hyperspectral curves obtained by field spectroradiometers can also provide spectral models capable of estimating biometric variables. In Kizil et al. (2012), chlorophyll was estimated with an accuracy of 97%, and the authors built the models using neural networks from hyperspectral indices derived from field radiometric measurements.

Table 4. Performance of algorithms in estimating biometric, physiological, and nutritional variables

Variables	Neural Network		M5	
	RMSE	RMSE%	RMSE	RMSE%
		Biometric		
NF15	0.47	15.66	0.46	15.33
SDM	0.03	26.61	0.04	29.27
RDM	0.004	22.79	0.004	22.79
Area	6.30	29.30	6.48	30.77
		Physiological		
F_0	392.74	6.80	431.45	7.41
F_m	1772.96	6.25	1888.73	6.67
F_v	4087	13.37	4538	14.85
F_v/F_m	0.01	1.8	0.009	1.2
ABS/RC	100.21	5.54	104.88	5,80
TRo/RC	76.82	5.35	76.98	5.36
DIo/RC	0.029	7.83	0.031	8.37
Chlorophyll a	2.49	6.49	2.40	6.43
		Nutritional		
N	1.53	8.92	1.98	11.55
P	2.15	14.07	2.7	15.6
K	3.57	11.29	3.44	10.88
Ca	1.06	5.67	1.03	5.31
Mg	0.32	6.57	0.33	6.78
S	0.19	7.69	0.26	9.98

NF15 - Number of leaves at 15 DAE; SDM – Shoot dry mass (g $plant^{-1}$); RDM – Root dry mass (g·$plant^{-1}$); Chlorophyll a - Chlorophyll a index; Area - Leaf area (cm^2 $plant^{-1}$); F_0 - Initial fluorescence; F_m - Maximum fluorescence; F_v - Variable fluorescence; F_v/F_m - Maximum quantum yield; ABS/RC - Absorption flow/center of reaction; TRo/RC - Captured energy flow/center of reaction; Dio/RC - Non photochemical energy flow/center of reaction; N, P, K, Ca, Mg, and S leaf concentrations (g kg^{-1})

Among the nutritional variables, the neural network model was more accurate in estimating N (8.92%), P (14.7%), Mg (6.57%), and S (7.69%). The M5 model had higher precision in the estimation to K (10.88%) and Ca (5.31%) foliar content (Table 4).

With respect to N, Mao et al. (2015) discussed the potential of composing N prediction models in lettuce from hyperspectral images, where it was possible to characterize in detail the spectral response of the plant and detect nutritional characteristics omitted by multispectral cameras.

Furthermore, although it is possible to estimate Ca by neural network, the architecture created by the M5 proved to be more efficient. Various studies aiming to monitor Ca in horticulture have already been evidenced in the literature. Story et al. (2010) determined that it was possible to detect Ca deficiency from computational vision algorithms by considering the integration between images composed by visible RGB bands, hue-saturation-luminance (HSL), and morphological features.

It is important to underscore that based on the results obtained, except for leaf area, it is possible to estimate the biometric, physiological and nutritional variables of cabbage in absolute values, using multispectral cameras to monitor seedling yield without the need for destructive analyses, in the shortest time possible and without complex architectures, given that the simplest models generated by the M5 parametric algorithm were efficient in estimating the variables assessed.

Despite the possibility of accurately estimating these cabbage variables, it is important to underscore that the methodology exhibits implementation limitations in situ. The challenges lie mainly in the radiometric data acquisition protocol, since under ideal electromagnetic radiation incidence, the interval between images should be restricted to a short time frame, between 11 am and 1 pm.

Another point to highlight is the possibility of continuous calibration of coefficients contained in the regression models and the configuration of neural network architecture, since the models presented here are calibrated for specific seasonal conditions at the time and place where the images were taken. Thus, with the confirmed possibility of estimating the quality variables cabbage using the Mapir camera, it is recommended that models be recalibrated at every field campaign.

2.4 CONCLUSIONS

It was possible to quantify the estimated biometric, physiological, and nutritional variables of cabbage using multispectral cameras.

Among the Mapir camera bands, the B_{660} exhibited the greatest variability, showing that the red range was the most sensitive to the different treatments.Greater band sensitivity promotes greater power in estimation.

Except for the variable leaf area from the B_{555} band and derived indices, it was possible to estimate all the agronomic variables using the models generated by the M5 algorithm.

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CHAPTER III

ESTIMATION OF BIOMETRIC, PHYSIOLOGICAL, AND NUTRICIONAL VARIABLES IN LETTUCE SEEDLINGS USING MULTIESPECTRAL IMAGES

ABSTRACT

The formation of seedlings is one of the most important phases of lettuce cultivation. Therefore, any strategy that aims to obtain high-quality seedlings can increase productivity. One of these strategies is the prediction of morphophysiological attributes based on optical properties. The objective of this study was to quantitatively estimate the biometric variables of lettuce from parametric and non-parametric models based on the response of multispectral camera images. The experiment was conducted in a greenhouse in the municipality of Uberaba, Minas Gerais State, Brazil. Twenty days after sowing, multispectral images of the plants were captured using a MAPIR Survey 3 camera. To compose the estimation models, along with the original bands of the camera, the multispectral vegetation indices were calculated using the calibrated original camera bands. Bands B550, B660, and B850 and the near-infrared indices contributed significantly to estimating the physiological variable models, with B850 contributing the most to the biometric and nutritional variables. From the near-infrared band (B850) and derived indices, it was possible to estimate all the agronomic variables from the models generated by the M5 algorithm, with an accuracy of up to 1.6% for the maximum quantum yield. Thus, it was possible to quantify the biometric, physiological, and nutritional variables of lettuce using a multispectral camera. Among the Mapir camera bands, B660 exhibited the greatest variability, showing that the red range was the most sensitive.

Key words: *Lactuca sativa*. Morphophysiological Variables. Prediction Models.

3.1 INTRODUCTION

The development of seedlings is one of the most critical phases of the crop cycle because it directly influences final plant performance from both a nutritional and productive perspective (GUSATT et al., 2019; DESAI et al., 2020).

The purchase price of lettuce seeds represents 8% of the input cost and 4.15% of the total production cost of this vegetable (OLIVA et al., 2016). Thus, any strategy aimed at obtaining high-quality seedlings may represent an increase in productivity and, consequently, higher profitability for the producer.

However, one of the limitations is the lack of rapid and efficient technologies to select high-vigor seedlings that result in more productive plants.

Remote sensing has several agricultural applications (WEISS et al., 2020) and uses leaf or canopy reflectance to calculate vegetation indices to assess agronomic variables such as nutritional status, biomass, leaf area, and drought resistance (GOGOI et al., 2018).

The use of optical sensors has been found to be efficient for the assessment of several plant species (PICOLI et al., 2013; MAKANZA et al., 2018; MACIEL et al., 2019); however, there are few reports on *Lactuca sativa* seedlings. These sensors can cover an extensive area in a very short time, providing rapid assessment of all the seedlings and minimizing the effect of rapidly changing environmental conditions, such as wind speed, cloud cover, and solar radiation.

Thus, the present study aimed to quantitatively estimate the biometric, physiological, and nutritional variables of lettuce, using parametric and non-parametric models (machine learning) based on images taken using a multispectral camera.

3.2 MATERIAL AND METHODS

The study was conducted in the municipality of Uberaba, MG, Brazil, which is in the mesoregion of Triângulo Mineiro and Alto Paranaíba, between December 8 and 28, 2019, with a cultivar adapted to tropical conditions in late spring and early summer, when there is an increasing market demand for lettuce and seedling production.

The arch-type greenhouse where the study was conducted was constructed in double-span plastic and measured 14 m wide and 51 m long, with eaves and arc heights of 3.00 and 1.5 m, respectively, in the east-west direction and lies at coordinates 19° 39’ 44″’’ S and 47°

58’ 02’’″ W, at an altitude of 790 m. It was covered in a 150-µm light-diffusing plastic film with closed sides and 50% shade cloth (Figure 1).

Temperature and relative humidity were collected daily in the greenhouse using a thermohygrometer (Testo 174 H data logger) installed 1.20 m above ground level to assess the environmental conditions just above the plants.

Figure 1. Greenhouse view where the experiments were conducted

Data variability was achieved by producing lettuce seedlings with variations in the fertigation start time and application intervals. Two experiments were performed. For both experiments, a randomized block design was used with six repetitions. Each experimental unit consisted of 140 plants.

The treatments in the first experiment consisted of six fertigation start times (T_1 = 0, T_2 = 3, T_3 = 6, T_4 = 9, T_5 = 12, and T_6 = 15 days after emergence). After the first application of each treatment, fertigation was repeated at five-day intervals. Thus, depending on the start time of the first application, the number of applications differed between treatments, with four applications for T_1, three for T_2 and T_3, two for T_4 and T_5, and one for T_6.

The treatments in the second experiment consisted of five fertigation application intervals (T_1 = 3, T_2 = 4, T_3 = 5, T_4 = 6, and T_5 = 7 days). In this experiment, the first application occurred three days after plant emergence, with five applications in T_1, four in T_2, three in T_3 and T_4, and three in T_5.

Fertigation depth varied as a function of seedling development stage. More advanced stages required increased fertigation depths. Nutrient solution was applied as follows: 0.4 L from emergence to 3 days after emergence (DAE); 0.5 L from 4 to 7 DAE; 0.6 L from 8 to 11 DAE; 0.7 L from 12 to 14 DAE and 0.8 L from 15 to 18 DAE.

The lettuce cultivar used to produce seedlings was Vanda (from Sakata®). The seeds were sown in polyethylene trays, with 12.5 cm^3 cells filled with Bioplant Plus® substrate. The experiment was conducted on wire benches, 90 cm above the ground.

The seedlings were irrigated four times a day (8:00 a.m., 12:00 p.m., 2:00 p.m., and 4:30 p.m.) using an automated sprinkler irrigation system without drainage to maintain the substrate in the containers. From sowing to emergence (3 days after sowing), all treatments were irrigated with water only. Emergence was considered to be established when at least 90% of the tray cells contained emerged seedlings. Fertigation was then initiated within the time frames proposed in the experimental design and performed only once a day (8:00 a.m.).

The sprinkler irrigation system installed inside the greenhouse provides an average depth of 3.98 mm h^{-1}, which corresponds to 3.98 L of water for each m^2 of greenhouse in 1 h. Water depth was measured every 3 days by weighing the trays. The trays were irrigated until the water began to drain, that is, until the container capacity was reached, when they were weighed. They were weighed again after 2.5 h and the procedure was repeated four times throughout the day. The difference between weight measurements was attributed to crop evapotranspiration (ETc), which was subsequently converted into the amount of water to be applied daily via irrigation or fertigation.

The irrigation depth varied as a function of the seedling development stage. More advanced stages required greater irrigation depth. The depth applied up to 3 days after emergence was 4.98 mm day^{-1}, from 4 to 7 DAE, 5.24 mm day^{-1}, from 8 to 11 DAE, 5.44 mm day^{-1}, from 12 to 14 DAE, 5.70 mm day^{-1} and from 15 to 18 DAE, 5.97 mm day^{-1}.

Each day, all treatments received the same amount of water. However, when fertigation was applied, the water was replaced with the same volume of nutrient solution for the first irrigation of the day (8:00 a.m.). Fertigation was applied using a backpack sprayer with a capacity of 20 L and flow rate of 0.8 L h^{-1}. After this application, a small volume of water was sprayed on the seedlings to prevent the nutrient solution from accumulating on the leaves. The other irrigations (12:00, 2:00, and 4:30 p.m.) were performed with water only for all treatments.

At 15 DAE, the number of leaves (NF15) was evaluated by counting the fully developed leaves of 10 plants from each plot.

At 17 DAE, the physiological variables of the seedlings were evaluated through the OJIP transient fluorescence of chlorophyll a, including the initial fluorescence (F_0), variable fluorescence (F_v), maximum fluorescence (F_m), maximum quantum yield (F_v/F_m), number of photons absorbed by the antenna complex (ABS/RC), amount of energy flowing through the antenna complex and captured by the PSII reaction center (TRo/RC), forward electron flow from the reaction center (ETo/RC), and amount of energy dissipated by non-photochemical dissipation (DIo/RC). These variables were measured using PSI's (Photon Systems Instruments) Fluorometer FP100 model, performing readings on the second true leaf of five plants in each plot between 12:00 and 3:00 a.m. so that the plants were adapted to the dark. The chlorophyll a index was evaluated on the same day using Falker's CFL1030 Clorofilog model, taking five readings per plot on the second true leaf from 11:00 a.m. to 3:00 p.m.

At 21 days after sowing, or 18 days after the beginning of the treatments, when the seedlings reached the commercial point of transplanting, the following variables were assessed in 40 plants from each experimental plot: leaf area (Area), expressed in cm^2 $plant^{-1}$; shoot dry mass (SDM), expressed in g $plant^{-1}$; and root dry mass (RDM), expressed in g $plant^{1}$.

Shoot N, P, K, Ca, Mg, and S (g kg^{-1}) concentrations were also determined in 40 seedlings from each experimental plot.

After obtaining the data, dispersion analysis was performed, and the variables were subjected to descriptive statistical analysis to obtain the mean, standard deviation, and coefficient of variation.

At 20 days after sowing, multispectral images of the plants were captured using a MAPIR Survey 3 camera, which has a 12-bit resolution, 19 mm focal length, 2.3 cm ground sample distance (GSD), and green (550 nm), red (660 nm), and near-infrared (850 nm) bands.

Images of ten plants from each experimental plot were captured for each experiment, totaling 660 images. The camera was installed on a horizontally and vertically leveled support (1.20 m) from the ground in the nadir position. The plants were placed under a black nonwoven fabric to mitigate the effects of reflectance from neighboring targets.

To ensure maximum absorption and reflection conditions of solar electromagnetic radiation, the images were taken from 11:30 a.m. to 12:30 p.m. without shading, which could be caused by clouds or anthropic features near the frame of the capture area.

Radiometric calibration of the images was performed using the Mapir Camera Control software. This process was possible because the images were calibrated with the reflected radiance of the calibration plate, provided by the Survey 3 camera manufacturer, at the same time as the images were taken.

After calibration, radiometric normalization of all the images was performed to compensate for the lighting effects from the first to the last shot, where the image taken at 12:00 p.m. was set as the reference. Normalization was performed using ENVI 5.1 software, according to the methodology proposed by Jensen (2009).

During the normalization process, in the reference image, as well as the images to be normalized, the radiance values were manually extracted from the set of light and dark pixels in all the bands of the camera. In all cases, the pixels were extracted from the calibration plate.

The following equation was used to determine the coefficients of linear transformation:

$$\mathrm{Ti} = \mathrm{mi} \times \mathrm{xi} + \mathrm{bi}, \quad (1)$$

where:

mi - (Bri - Dri)/(Bsi - Dsi);

bi - (Dri × Bsi - Dsi × Bri)/(Bsi - Dsi);

Ti - Radiance of the reference image

xi: Radiance of the image to be normalized.

Bri - mean of the light reference set;

Dri - mean of the dark reference set;

Bsi - mean of the light set to be normalized;

Dsi - mean of the dark set to be normalized; and,

i - bands of the sensor under study.

The multispectral vegetation indices were calculated using Eqns. 2, 3, and 4 to compose the estimation models, along with the original camera bands, from the original calibrated Survey 3 camera bands (Table 1), using ENVI 5.1 software.

Table 1. Equations an references for calculations of vegetation indices derived from the original bands of the Survey 3 Camera

Index	Equation	Reference
Simple Ratio (SR)	$\mathrm{SR} = (\mathrm{B}_{850})/(\mathrm{B}_{660})$ **(2)**	(Birth & McVey, 1968)
Normalized Difference Vegetation Index (NDVI)	$\mathrm{NDVI} = (\mathrm{B}_{850} - \mathrm{B}_{660})/(\mathrm{B}_{850} + \mathrm{B}_{660})$ **(3)**	(Rouse et al., 1973)
Green Normalized Difference Vegetation Index (GNDVI)	$\mathrm{GNDVI} = (\mathrm{B}_{850} - \mathrm{B}_{550})/(\mathrm{B}_{850} + \mathrm{B}_{550})$ **(4)**	(Gitelson et al., 1996)

The individual mean brightness values of the original bands and the derived multispectral indices were extracted for all plants. The average brightness values were extracted from the irregular polygons surrounding the plant crowns using the regions of interest tool created in the ENVI 5.1 software. The mean zonal method was used for data extraction.

The estimation process used in this study is based on parametric and non-parametric regression methods. The non-parametric multilayer perceptron (neural network) and parametric multiple linear regression (MLR) algorithms were used as estimators, and the results were compared.

Neural network implementation was used to estimate and validate the lettuce variables (biometric, physiological, and nutritional), in Weka software (Weka 3.9.4, University of Waikato).

The neural network variables were set to their default values (two neurons and one layer). It is important to emphasize that several tests were conducted to optimize the network variables, but the standard architecture of the software always exhibited more accurate estimates.

This study used the MLR method, which attempts to estimate the variables of the model and describe the relationship between two or more independent variables and a response variable by fitting a linear equation to the observed data, often using the least squares method in Weka (Weka 3.9.4, University of Waikato). In Weka, the selection of the features model was completed using a backward elimination method called “M5,” where in the attribute with the smallest standardized coefficient is removed until no improvements are observed in the Akaike information criterion (AKAIKE, 1974).

All estimation models were created from the combination of the original bands and the derived multispectral indices. For model training, the radiometric values of 528 randomly defined plants (80% of the sample set) were considered. The root mean squared error (RMSE) (Eq. 5), and the normalized RMSE (RMSE%) (Eq. 6) were calculated to validate the accuracy of the models, considering the residue of the difference between the estimated and measured agronomic variables for 132 plants (20% of the sample set).

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(x_o - x_e)^2}{n}}, \tag{5}$$

$$\mathrm{RMSE}(\%) = \sqrt{\frac{\sum_{i=1}^{n}(x_o - x_e)^2}{n}} * \frac{100 * n}{\sum_{i=1}^{n} x_e} \tag{6}$$

where:

x_o – observed value;
x_e – estimated value; and
n – number of samples.

3.3 RESULTS AND DISCUSSION

The average temperature and air relative humidity during the experiment were 27.51 °C and 67.16%, respectively. Maximum and minimum temperatures of 46.10 °C and 18.10 °C were recorded on December 22 and 24, 2019, respectively, and maximum (94.6%) and minimum air relative humidity (27.9%) on December 19 and 24, 2019, respectively.

The standard deviation varied from 0.006 (RDM) to 4,184.01 (Fm). This shows that the higher the value, the greater is the data dispersion (Table 2).

Table 2. Means, standard deviations, and coefficients of variation (CV) of biometric, physiological, and nutritional variables evaluated in lettuce seedlings

Variables	Means	Standard deviations	CV (%)
B_{850}	252.89	6.59	2.26
B_{550}	244.06	1.96	0.81
B_{660}	225.10	12.05	5.35
Biometric			
NF15	3.57	0.41	11.45
SDM	0.08	0.02	25.30
RDM	0.02	0.006	27.06
Area	32.35	10.41	32.17
Physiological			
F0	7,281.91	696.98	9.57
Fm	39,588.33	4,184.01	10.57
Fv	32,306.42	3,603.00	11.15
Fv/Fm	0.82	0.01	1.25
ABS/RC	2,309.99	148.94	6.45
TRo/RC	1,880.77	110.03	5.85
ETo/RC	446.30	277.55	62.19
DIo/RC	0.43	0.05	10.62
Chlorophyll *a*	17.36	1.55	8.95
Nutritional			
N	13.39	2.34	17.47
P	6.49	0.80	12.37
K	31.54	3.39	10.75
Ca	9.23	1.05	11.37
Mg	2.35	0.16	7.01
S	1.20	0.18	15.07

NF15 - number of leaves at 15 DAE; SDM: shoot dry mass (g plant^{-1}); RDM: root dry mass (g·plant^{-1}); Chlorophyll a - chlorophyll a index; Area - leaf area (cm^2 plant^{-1}); F_0 - initial fluorescence; F_m - maximum fluorescence; F_v - variable fluorescence; F_v/F_m - maximum quantum yield; ABS/RC - absorption flow/center of reaction; TRo/RC - captured energy flow/center of reaction; ETo/RC - electron transport flow/center of reaction; Dio/RC - non-photochemical energy flow/center of reaction; N, P, K, Ca, Mg, and S - leaf concentrations (g kg^{-1}); CV - coefficient of variation, n = 66

The coefficients of variation (CV) ranged from 0.81% (B_{550}) to 62.19% (ETo/RC), which represents low data dispersion for all the evaluated variables (CANTELLI et al., 2016), except for ETo/RC and Area (Table 2).

For the data extracted from the image, the highest CV was for B_{660} (5.35%), indicating that the red spectrum is the best region for discriminating between the different types of treatment. For this experiment, the greater spectral variability in this range is associated with the high variability of the biometric and physiological variables of lettuce, given that reflected energy in the 630-680 nm spectral range is influenced by photosynthesis-related variables, such as NF15 and chlorophyll a (JENSEN, 2009).

The low CV values for the B_{550} (0.81%) and B_{850} (2.26%) bands show spectral regions with less potential for discriminating between the different treatments applied to the lettuce samples. The low variability of the B_{550} band is associated with the condition that all the samples exhibited the typical appearance of healthy green vegetation, that is, the same phycocyanin concentrations.

The variability of the B_{850} band, which was slightly higher than that of B_{550}, was directly influenced by the low variability of the nutritional variables of lettuce (JENSEN, 2009), which had a maximum CV for N (17.47%). In this case, in addition to the influence of nutritional variables, a low CV was associated with the calibration process and radiometric normalization, which contributed significantly to the lower range and adherence of the values as a function of the mean.

The multiple linear models presented higher coefficients of determination (R^2) because they incorporated more index bands in the regression equations, as verified for the variables F_v/F_m (43%), DIo/RC (34%), and P (58%) (Table 3). However, it is evident that the greater number of predictor variables did not guarantee that these models were the most accurate (Table 4).

As in Oliveira et al. (2020), the models that estimate agricultural variables, when composed of a single predictive variable, tend to have a low coefficient of determination, as shown in Table 3. However, this does not necessarily result in an inaccurate estimate of the variable because if the validation data are normally distributed, the errors in data variability around the mean will be null and decrease the RMSE.

The B_{550}, B_{660}, and B_{850} bands and the indices derived from the near-infrared region contribute mostly to the estimation models of the physiological variables (Table 3). Band B_{850} contributed the most to the biometric and nutritional variables (Table 3). The variables NF15, SDM, RDM, and Area represent the vegetation biomass, which may explain these results because multispectral indices such as SR and GNDVI are highly correlated with the biomass of canopies of several crops (JENSEN, 2009).

Table 3. Linear models and coefficient of determination (R^2) for the estimation of biometric, physiological, and nutritional variables

Variables	**Model**	**R^2 (%)**
Biometric		
Number of leaves at 15 DAE	$NF15 = -3.44 \times GNDVI + 3.7$	22
Shoot dry mass (g plant^{-1})	$SDM = -0.01 \times B_{850} + 2.7$	18
Roor dry mass (g plant^{-1})	$RDM = 0.00002 \times B_{850} + 0.2$	20
Leaf area (cm^2 plant^{-1})	$Area = -5.7 \times B_{850} + 1341.6$	2
Physiological		
Initial fluorescence	$F_0 = -99.7 \times B_{850} + 32647.2$	19
Maximum fluorescence	$F_m = 328.48 \times B_{550} - 39469.7$	26
Variable fluorescence	$F_v = 315.06 \times B_{550} - 43630.2$	15
Maximum quantum yield	$F_v/F_m = 0.0095 \times B_{850} - 0.16 \times GNDVI - 1.58$	43
Absorption flow/center of reaction	$ABS/RC = -47.51 \times B_{850} + 14330.4$	7.8
Captured energy flow/center of reaction	$TRo/RC = 33.67 \times B_{550} - 7735.06$	12
Electron transport flow/center of reaction	$ETo/RC = 33.67 \times B_{660} - 7735.06$	15
Non-photochemical energy flow/center of reaction	$DIo/RC = -0.0336 \times B_{850} + 0.5251 \times GNDVI + 8.909$	34
Chlorophyll *a* index	Chlorophyll *a* $= -0.054 \times B_{660} + 29.4$	15
Nutritional		
Nitrogen leaf concentrations (g kg^{-1})	$N = 1.33 \times B_{850} - 323.42$	11
Phosphorus leaf concentrations (g kg^{-1})	$P = 0.46 \times B_{850} - 41.1 \times GNDVI - 21.31 \times SR - 89.07$	58
Potassium leaf concentrations (g kg^{-1})	$K = 1.18 \times B_{850} - 269.55$	14
Calcium leaf concentrations (g kg^{-1})	$Ca = 0.18 \times B_{850} - 37.37$	10
Magnesium leaf concentrations (g kg^{-1})	$Mg = 0.06 \times B_{850} - 12.28$	16
Sulfur leaf concentrations (g kg^{-1})	$S = 0.06 \times B_{850} - 14.62$	12

SR - simple ratio; GNDVI - green normalized difference vegetation index

For the physiological variables, bands B_{550} and B_{850} were present in most prediction models, except for the variables chlorophyll a and ETo/RC (Table 3). For chlorophyll a, the B_{660} red spectrum band was the predictor variable because this specific portion of the reflective spectrum is causally related to chlorophyll a and b absorption (JENSEN, 2009).

For the nutritional variables, the predominance of the B_{850} band in the prediction models reflects the direct relationship between the near-infrared and lettuce nutrients, as observed by MAO et al. (2015).

Table 4 shows the performance of the algorithms (RMSE and RMSE%) for estimating the biometric, physiological, and nutritional variables.

Table 4. Performance of algorithms in estimating biometric, physiological, and nutritional variables

Variables	Neural Network		M5	
	RMSE	RMSE%	RMSE	RMSE%
		Biometric		
NF15	0.421	11.79	0.382	10.7
SDM	0.022	27	0.025	32
RDM	0.005	15.6	0.007	22
Area	11.94	43	12.2	44
		Physiological		
F_0	742	10.2	826	11.38
F_m	4700	12.5	5253	14.26
F_v	4087	13.37	4538	14.85
F_v/F_m	0.013	1.7	0.012	1.6
ABS/RC	154	6.74	148.32	6.5
TRo/RC	112.38	6.06	107	5.8
ETo/RC	300	77	265	63
DIo/RC	0.050	12	0.052	12.1
Chlorophyll a	1.305	5.9	1.31	7.5
		Nutritional		
N	3.4	23.5	3.19	21.9
P	1.11	16.2	1.07	15.6
K	3.11	9.2	3.32	10.7
Ca	1.09	11.2	1.34	13.7
Mg	0.21	8.6	0.18	7.5
S	0.15	12.4	0.14	11.5

NF15 - number of leaves at 15 DAE; SDM: shoot dry mass (g plant^{-1}); RDM: root dry mass (g·plant^{-1}); Chlorophyll a - chlorophyll a index; Area - leaf area (cm^2 plant^{-1}); F_0 - initial fluorescence; F_m - maximum fluorescence; F_v - variable fluorescence; F_v/F_m - maximum quantum yield; ABS/RC - absorption flow/center of reaction; TRo/RC - captured energy flow/center of reaction; ETo/RC - electron transport flow/center of reaction; Dio/RC – non-photochemical energy flow/center of reaction; N, P, K, Ca, Mg, and S leaf concentrations (g kg^{-1})

Regarding the biometric variables, the M5 model had higher precision in the NF15 estimate (10.7%), while the neural networks provided more precise estimates of SDM (27%) and RDM (15.6%) (Table 4).

The estimated leaf area was not satisfactory because the errors were 43% and 44% for the neural networks and M5 models, respectively (Table 4). In contrast, when monitoring lettuce health, Ren et al. (2017) verified that the estimation of leaf area by remotely piloted aircraft cameras was possible and determinant for the development of a methodology capable of detecting leaf deterioration.

Burmgarder et al. (2012) also highlighted the possibility of monitoring the leaf area index (LAI) using RGB images and machine learning algorithms. The authors were able to estimate the LAI of lettuce samples under different treatments with an accuracy ranging from 71% to 95%.

As shown in Table 3, the performance of the B_{660} chlorophyll estimate regression model stands out. The composition of the red spectrum response may be associated with the fact that this range is sensitive to chlorophyll a and b (JENSEN, 2009). Hyperspectral data can also be used in similar studies. Simko et al. (2015) determined that lettuce leaf deterioration can be detected using hyperspectral images. In the same study, using hyperspectral indices, chlorophyll was classified according to leaf health with an accuracy of 97%.

Hyperspectral curves obtained by field spectroradiometers can also provide spectral models capable of estimating biometric variables. In Kizil et al. (2012), chlorophyll was estimated with an accuracy of 97%, and the authors built models using neural networks from hyperspectral indices derived from field radiometric measurements.

With regard to the physiological variables, the M5 model showed higher precision in the estimates of F_v/F_m (1.66%), ABS/RC (6.5%), TRo/RC (5.8%), and Dio/RC (12%), while the neural networks had higher precision in the estimates of F_0 (10.2%), F_m (12.5%), F_v (13.37%), and Chlorophyll a (5.9%). The estimate of the ETo/RC parameter was not satisfactory because the errors were 77% and 63% for the neural networks and M5 models, respectively (Table 4).

Among the nutritional variables, the M5 model was more accurate in estimating N (21.9%), P (15.6%), Mg (7.5%), and S (11.5%), while the neural networks had higher precision in the estimation of K (9.2%) and Ca (11.2%) (Table 4).

With respect to N, Mao et al. (2015) discussed the potential of composing N prediction models in lettuce from hyperspectral images, where it was possible to characterize in detail the spectral response of the plant and detect nutritional characteristics omitted by multispectral cameras.

Furthermore, although it is possible to estimate Ca by M5, the architecture created by the neural networks proved to be more efficient. Various studies aimed at monitoring Ca in lettuce have already been reported. Story et al. (2010) determined that it was possible to detect Ca deficiency using computational vision algorithms by considering the integration between images composed by visible RGB bands, hue-saturation-luminance (HSL), and morphological features.

It is important to emphasize that, based on the results obtained, except for chlorophyll a, TRo/RC, and ETo/RC contents, it is possible to estimate the biometric, physiological, and nutritional variables of lettuce in absolute values, using multispectral cameras to monitor seedling yield without the need for destructive analyses, in the shortest possible time and without complex architectures, given that the simplest models generated by the M5 parametric algorithm were efficient in estimating the variables assessed.

Despite the possibility of accurately estimating these lettuce variables, it is important to emphasize that the methodology exhibits implementation limitations in situ. The challenges lie mainly in the radiometric data acquisition protocol, because under ideal electromagnetic radiation incidence, the interval between images should be restricted to a short timeframe, between 11 a.m. and 1 p.m.

Another point to highlight is the possibility of continuous calibration of coefficients contained in the regression models and the configuration of neural network architecture, since the models presented here are calibrated for specific seasonal conditions at the time and place where the images were taken. Thus, with the confirmed possibility of estimating quality variables in lettuce using the Mapir camera, it is recommended that models be recalibrated for every field campaign.

3.4 CONCLUSIONS

In this study, it was possible to quantify the estimated biometric, physiological, and nutritional variables of lettuce using multispectral cameras.

Among the Mapir camera bands, B_{660} exhibited the greatest variability, showing that the red range was the most sensitive to different treatments. Except for the variables Chlorophyll a, TRo/RC, and ETo/RC from the B_{850} band and derived indices, it was possible to estimate all the agronomic variables using the models generated by the M5 algorithm.

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