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Nitrogen and chlorophyll status in romaine lettuce using spectral indices from RGB digital images

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Abstract

Destructive methods for crop nutrition estimation are accurate and standardized but costly and limited by spatial scale. Non-destructive techniques such as the use of digital imaging provide fast and reliable results *in situ*; however, there is limited information on these non-destructive techniques in leafy vegetable crops. During the study it was estimated the concentration of nitrogen (N) and chlorophyll (Chl) in romaine lettuce using spectral indices derived from the RGB (red, green and blue) digital images. The lettuce crop was grown in plastic tunnels and irrigated with five N levels: 0, 4, 8, 12, and 16 mEq L^{-1} NO_3-N (corresponding to 0, 248, 496 and 744 mg L^{-1}, respectively) based on a modified Steiner solution. The treatment of 16 mEq L^{-1} NO_3-N showed the highest growth at 42 days after transplanting (DAT). Digital images of the plants were acquired weekly with a RGB camera and processed to obtain scale mosaics and twelve spectral vegetation indices. Correlation analysis of the spectral indices indicated that normalized green-red difference index (NGRDI), excess green (ExG) and green channel (g) indices showed a positive linear correlation with the concentration of N ($r > 0.93$) and Chl ($r > 0.93$). Besides, an exponential correlation with leaf area ($r > 0.86$) was founded, which was stronger in the last 21 DAT due to the acceleration in leaf growth during the vegetative stage of the crop. These results show that RGB digital images are a low cost, non-destructive, reliable and accurate method to estimate N and Chl concentration and leaf area in romaine lettuce during production. Therefore, this technique could be an affordable alternative that combined with portable meters (i.e. SPAD) provides real-time monitoring of the nutritional status of the lettuce crop, especially in crop factories.

Key words: *Lactuca sativa* var. *longifolia*, leaf area, non-destructive techniques, nitrogen, chlorophyll, spectral index.

Introduction

Chlorophyll (Chl) and nitrogen (N) are essential components of plants because of their role in protein production and photosynthesis. An excess or deficiency of these can cause toxicity or low yield (Taiz et al., 2014). Precise assessment and evaluation of the nutritional status of the crop *in situ* and at different time scales is required to optimize the use of fertilizers and reduce costs (Muñoz-Huerta et al., 2013).

Nitrogen concentration in plants is usually determined by two approaches – the destructive or non-destructive methods. Destructive methods such as Kjeldahl and Dumas are based on tissue analysis and although they are accurate and standardized, they are often limited by the size of the plots, high cost and efforts required for sampling (Paz Pellat et al., 2015; Baresel et al., 2017). Non-destructive methods allow for remote information collection in less time and do not require highly specialized personnel (Vollmann et al., 2011). Besides, measurements may be applied frequently and repeatedly on the same plant; thus allowing the monitoring of leaf area and crop N status for efficiently adjusting fertilization rates throughout the growing

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season (Sandmann et al., 2013). These methods use the relationship of reflectance / transmittance properties of some leaf pigments such as Chl and polyphenols with their chemical characteristics (Parks et al., 2012).

It is well known that changes in plant spectral characteristics are closely related to changes in nutrient concentration in the visible (~550 nm) and near-infrared (~750 nm) regions (Muñoz-Huerta et al., 2013). If they are combined as spectral indices, it is possible to obtain functional relations that estimate the concentration of N and Chl (Cammarano et al., 2014). Remote sensing data of crops are acquired at leaf level from Chl meters (e.g., SPAD) or at canopy level from reflectance sensors (e.g., digital cameras). In the first case, the approach is the point sampling with a limited spatial scale, while in the second, it allows monitoring of spatial and temporal variability (Muñoz-Huerta et al., 2013).

Using digital cameras and image processing (e.g., visible spectral indices) is less expensive than other techniques (Elsayed et al., 2018) and have been reported to be a powerful and low-cost tool in assessing leaf area and nutrient status, especially in field crops (Liu et al., 2018; Prey et al., 2018; Zheng et al., 2018). In horticulture, the processing images have been used to estimate the N and Chl concentration in tomato (Mercado-Luna et al., 2010), potato (Yadav et al., 2010), pepper (Yuzhu, 2011) and sweet pepper (Horgan et al., 2015) seedlings, but there are few studies in leafy vegetables (Jung et al., 2015; Mao et al., 2015).

The advantages of using non-destructive optical methods have been widely studied in field crops (Elsayed et al., 2018). Furthermore, although studies in closed environment systems are few, they show promising results that have allowed, according to Jung et al. (2015), quick and successful estimation of the N and Chl concentration in lettuce. Therefore, these methods could be incorporated into high-tech closed production systems for the measurement of nutritional status in less time, allowing the necessary adjustments in fertilization schemes to optimize resources, reduction in production costs and increase in yields (Gruda et al., 2019). In the countries such as Mexico, lettuce production in greenhouses is a continuously growing area that could benefit from a precise diagnosis and monitoring system of the nutritional status of N with high spatio-temporal precision, considering that N is the most limiting nutrient to the yield (Zhou et al., 2020).

This paper explores the possibility of estimating the N and Chl concentration and leaf area of romaine lettuce using a non-destructive method based on digital images from a visible RGB camera and spectral indices. It could be used as a practical and low-cost alternative to traditional destructive methods and providing accurate information with higher spatial resolution than measurements with proximal sensors such as SPAD, which could be useful for application in the greenhouse vegetable production industry.

Materials and methods

Cultural conditions and crop management. This experiment was conducted from April to May of 2018 in high tunnels, at the Faculty of the Agricultural Sciences (18°58′51″ N, 99°13′74 55″ W, alt. 1,866 m) in the Autonomous University of the State of Morelos, Mexico. Air temperature, relative humidity and light intensity were recorded every 5 minutes with a Hobo® datalogger U12-012 (Onset Computer Co., USA) throughout the experiment. The average values of temperature, relative humidity and light intensity were 26°C, 45% and 518 µmol m^{-2} s^{-1}, respectively.

Romaine lettuce (*Lactuca sativa* var. *longifolia*) cultivar 'Green Star' (Johnny's Seeds, USA) was sown on 13 March 2018, in 200-cavity polystyrene trays with commercial BM2 Berger® substrate. After 30 days, lettuce seedlings were transplanted into 10 L polyethylene bags containing volcanic rock (locally called tezontle, with 1 to 7 mm grain size) as substrate. The plants were watered daily with 0.5 L (first 21 days) and 1.5 L (last 21 days) of a Steiner (1984) nutrient solution, modified to supply five nitrate nitrogen (N) levels: 0, 4, 8, 12 and 16 mEq L^{-1} NO_3-N (corresponding to 0, 248, 496, 744 and 992 mg L^{-1} NO_3-N, respectively), as recommended by Mercado-Luna et al. (2010). The chemical composition of the original Steiner nutrient solution was: 1.062 $Ca(NO_3)_2$ (calcium nitrate), 303 KNO_3 (potassium nitrate), 492 $MgSO_4$ (magnesium sulphate), 261 K_2SO_4 (potassium sulphate) and 136 KH_2PO_4 (monopotassium phosphate), in mg L^{-1}. Macronutrients were supplied from soluble fertilizers depending on the treatments. When necessary, calcium sulphate (272.3 mg L^{-1} $CaSO_4$) and calcium chloride (55.5 mg L^{-1} $CaCl_2$) were added to maintain a balance of 20 mEq L^{-1} anions-cations in the nutrient solutions. The micronutrients were applied through a commercial chelate mixture Ultrasol Micro Mix (SQM, Chile), in a dose of 80 g m^{-3} of nutrient solution. To calculate that dose, the supply of 3 mg L^{-1} Fe was considered. The commercial mixture of micronutrients contained the following concentrations in percentage: Fe (7.5), Mn (3.7), B (0.4), Zn (0.6), Cu (0.3) and Mo (0.2). After preparing the nutrient solutions, the pH was adjusted between 5.6 and 6.0 with H_2SO_4 (sulfuric acid). Plants were watered to provide a 15% to 20% leachate fraction to prevent salt accumulation.

Experimental design and reference data. A completely randomized experimental design with 180 pots in total (36 pots per treatment) distributed in five N treatments: 0, 4, 8, 12 and 16 mEq L^{-1} NO_3-N, was used. Sampling was done weekly from 7 to 42 days after transplantation (DAT), for analysis, 6 pots of each treatment with one plant per pot as the experimental unit were used. The leaf area of each plant in cm^2 was measured with a portable area meter LI-3100 (LI-COR Biosciences, USA). Chlorophyll concentration (mg g^{-1}) was estimated from the 18 recently matured (fully expanded) leaves by treatment (3 leaves per plant) with the spectrophotometric method described by Mackinney (1941) and Wettstein (1957). Leaves used to measure leaf area were dried in a forced-air oven at 70 °C temperature for 72 h and used to determine the total N by the micro-Kjeldahl method (Kalra, 1997).

Image acquisition and processing. Coded targets were placed as ground control points (GCPs) at a distance of 45 by 45 cm. The digital images were acquired using RGB camera WB250F 16 Mpx (Samsung) mounted on a goniometer (Sandmeier, 2000) in an inclination range of 15° to 165° every 25°, taking at least 50 pictures in each session with a minimum 70% overlap. A photogrammetric restitution process using software *Agisoft Photoscan*, version 1.2 (Agisoft LLC, Russia) was performed according to Grenzdörffer (2014) to generate scaled ortho-mosaics and three-dimensional digital models (Fig. 1).

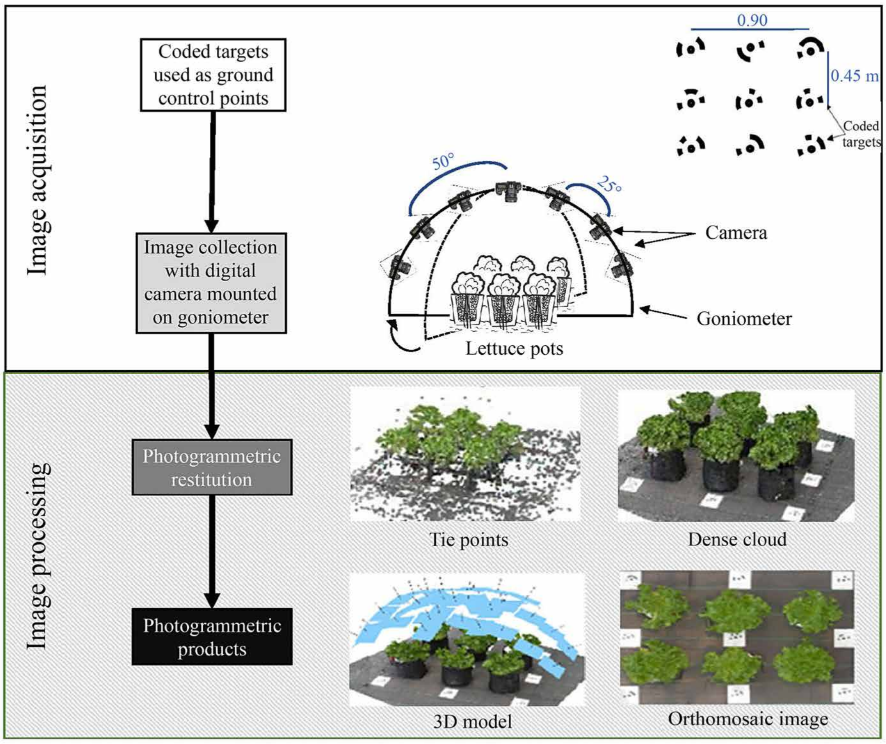


Figure 1. The process to generate scaled orthomosaic images and 3D models

Spectral indices calculation and classification images. Twelve spectral vegetation indices based on the three RGB (red, green and blue) visible region bands (Table 1) were calculated from the orthomosaics through a script in the statistical software *R*, version 3.5.0 (R Core Team, 2019).

Each spectral vegetation index image was subjected to a supervised classification process using the maximum likelihood method to separate the pixels corresponding to the lettuce crop, obtaining images classified with three classes: leaf area, substrate and soil. The pixel values of the lettuce crop in the classified images were compared with the reference data (N, Chl and leaf area) using a simple correlation coefficients matrix, as reported by Chung et al. (2017). Therefore, those spectral indices that are statistically different and have the highest coefficient of correlation (r) and the lowest root-mean-square error (RMSE) were identified and selected (Fig. 2).

Table 1. Spectral indices calculated from the RGB (red, green and blue) digital images

Index name	Application	Equation	References
1. Normalized green-red difference index (NGRDI)	biomass, N, phenology	$(G – R)/(G + R)$	Jannoura et al., 2015
2. Normalized difference index (NDI)	N, Chl	$(R – G)/(R + G + 0.01)$	Lee, Lee, 2013
3. Intensity (INT)	colour, canopy	$(R + G + B)/3$	
4. Saturation (SAT)		$1 – (3 × R × G × B)$	
5. Excess green index (ExG)	segmentation, Chl, N	$((2 × G) – (R + B))/(R + G + B)$	Baresel et al., 2017
6. Channel index R (r)	greenness, Chl, N	$R/(R + G + B)$	
7. Channel index G (g)		$G/(R + G + B)$	
8. Channel index B (b)		$B/(R + G + B)$	
9. Binary channel index (rb)		R/B	
10. Binary channel index (rg)		R/G	
11. Binary channel index (bg)		B/G	
12. Triangular green index (TGI)	Chl	$G – (0.39 × R) – (0.61 × B)$	Hunt et al., 2013

Spectral band regions: R – red, 650–700 nm, G – green, 500–550 nm, B – blue, 450–500 nm

Statistical analysis. Analysis of variance and Tukey's test for pairwise comparison was performed using the statistical software *SAS*, version 9.1 (SAS Institute, USA) with a one-way factor (N level) and statistical significance at $P \leq 0.01$. Arithmetic mean ($\bar{x}$), standard error (SE), coefficient of variation (CV) and the least significant difference (LSD) were calculated. The simple linear regression model in the statistical software *SigmaPlot*, version 12 (SyStat Software Inc., USA) was used to assess the correlation between spectral indices and reference data values.

Results and discussion

Crop response to nitrogen (N) levels. At 42 DAT, the treatment of 16 mEq L^{-1} NO_3-N had the maximum values (statistically significant difference) of Chl, N concentration and leaf area compared to the rest of the treatments (Table 2).

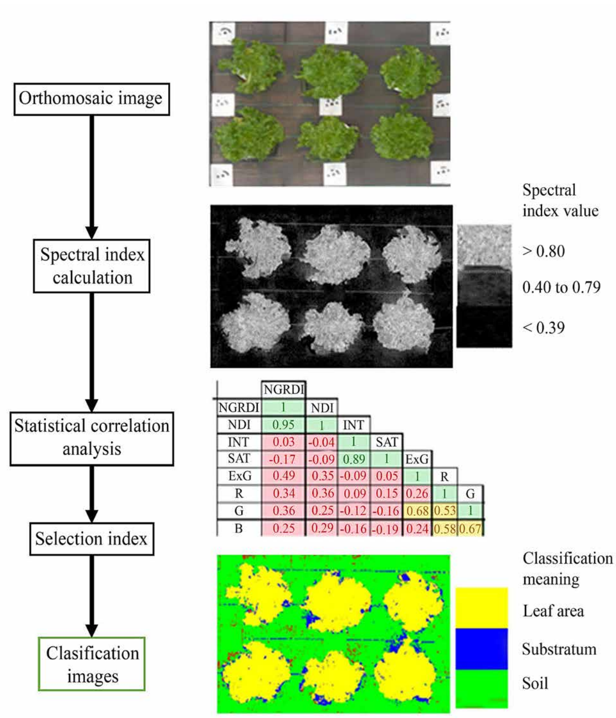


Figure 2. The process for spectral analysis of scaled orthomosaic images

Table 2. Response to different NO_3-N levels at 42 days after transplanting (DAT) in romaine lettuce

Treatment mEq L^{-1} NO_3-N	$\bar{X}$		
	Chl mg g^{-1} FW	N % DW	leaf area cm^2
0	0.387 b	2.48 b	49.3 d
4	0.396 b	2.51 b	51.0 cd
8	0.402 b	2.54 b	53.9 c
12	0.411 b	2.75 b	65.6 b
16	0.453 a	3.76 a	73.1 a
CV	0.067	0.085	0.070
LSD	0.038	0.906	4.437

Note. FW – fresh weight, DW – dry weight; letters between columns indicate statistical difference ($P \leq 0.01$).

There was a positive relationship between the N concentration of the nutrient solution and the N concentration in the plant tissue. This trend was reported by Zandvakili et al. (2019) in four cultivars of lettuce and by Fallovo et al. (2009) in lettuce 'Green Salad Bowl' crop. Similarly, there was a positive relationship between the concentration of N applied versus the concentration of Chl and leaf area. These same trends were reported for Chl in lettuce 'Parris Island' (Konstantopoulou et al., 2012). Nitrogen is the most important nutrient for leafy vegetables, especially at the end of the vegetative stage, which in lettuce coincides with its harvest. As a yield component of crop lettuce, leaf area was also positively influenced by the concentration of N in the nutrient solution. Besides, similar results have been reported in rocket salad (*Eruca vesicaria* L.) by Santamaria et al. (2002).

Correlation analysis between spectral indices values and reference data. The comparison between indices showed that NGRDI, NDI and TGI have a strong correlation between them, likewise INT and SAT. In our study, a moderate correlation ($r < 0.74$) between NGRDI with R, G and B was found, and these results agree with those obtained in tomato by Ali et al. (2013), who had correlation coefficients between 0.48 and 0.59. The rest of the spectral indices had none or weak correlation between themselves (Table 3).

Strong and significant correlations ($r > 0.80$) were found between NGRDI, NDI, ExG and *g* indices and reference data (N, Chl and LA). These indices were developed initially to detect the green parts of the plants, so they are known as greenness indices. Later on, given their ability to highlight the spectral characteristics of the green and red bands, they were used as an indicator of the quantity and quality of the photosynthetic material of the plants (Rasmussen et al., 2016), so in leafy vegetables such as lettuce could be an indicator of Chl, leaf area and biomass. In lettuce crop, Jung et al. (2015) reported that the *g* index had the highest correlation for leaf area estimation ($r = 0.93$). The results of our experiment are similar to those reported by Xue and Yang (2008), where NGRDI and NDI could be used for the calculation of N concentration in the vegetative stage of rice ($r = 0.95$). It allows using these indices for the estimation of leaf area

Table 3. Correlation matrix between spectral indices and reference data values

		NGRDI	NDI	INT	SAT	ExG	*r*	*g*	*b*	*rb*	*rg*	*bg*	TGI	Chl	N	LA
Spectral indices from the RGB digital images	NGRDI	1														
	NDI	0.95	1													
	INT	0.03	−0.04	1												
	SAT	−0.17	−0.09	0.89	1											
	ExG	0.49	0.35	−0.09	0.08	1										
	r	0.34	0.36	0.09	0.15	0.26	1									
	g	0.36	0.25	−0.12	−0.16	0.68	0.53	1								
	b	0.25	0.29	−0.16	−0.19	0.24	0.58	0.67	1							
	rb	0.60	0.44	0.28	0.14	0.05	0.57	0.34	0.57	1						
	rg	0.54	0.53	0.29	0.16	0.07	0.27	0.47	0.18	0.28	1					
	bg	0.33	0.39	0.31	0.14	−0.06	0.35	0.47	0.14	0.55	0.58	1				
	TGI	0.90	0.89	−0.18	−0.14	0.14	0.24	0.17	0.17	0.49	0.63	0.27	1			
Reference data	Chl	0.94	0.93	0.16	0.08	0.91	0.58	0.80	0.15	0.05	0.16	0.14	0.73	1		
	N	0.93	0.91	0.09	0.13	0.86	0.36	0.86	0.58	0.18	0.28	0.37	0.68	0.94	1	
	LA	0.95	0.93	0.22	−0.36	0.89	0.74	0.85	0.36	−0.18	−0.18	−0.18	0.57	0.92	0.93	1

0.01 0.50 0.75 0.99

Note. Explanation of acronyms in Table 1; significant values are those marked with green colour ($P \leq 0.01$).

accurately and quickly similar to reported in horticultural crops by Usha and Singh (2013).

The TGI index showed a moderate and significant correlation ($r > 0.60$) with Chl and N. In contrast, the INT, SAT, *r*, *b*, *rb*, *bg* and *rg* indices produced a weak to moderate non-significant correlation ($r < 0.60$) with reference data.

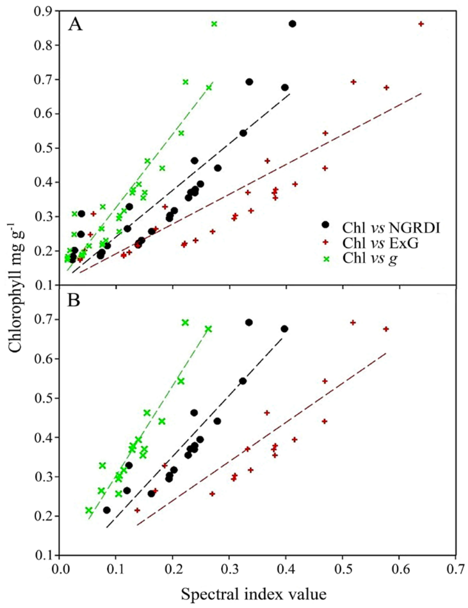
In summary, it was found that the most efficient spectral indices are those that consider the spectral bands of the green and red region. On the other hand, the ExG and *g* indices that incorporate the blue band have lower performance in the prediction of Chl, N and leaf area, which may be due to the low sensitivity of plant tissue in the blue channel (~450 nm).

Comparison between reference data and spectral indices from RGB digital images. *Chlorophyll concentration.* The Chl values were compared for the two periods: (1) the whole crop cycle from 1 to 42 DAT and (2) from 22 to 42 DAT (Table 4). For the entire cycle, NGRDI (r = 0.94) and *g* (r = 0.86) indices had a strong linear correlation (Fig. 3).

Table 4. Linear regression coefficients and standard error (SE) for the relationship between the chlorophyll (Chl) concentration and spectral indices

Period evaluated	Spectral index	Linear regression coefficient		SE
		a	b	
1 to 42 DAT	NGRDI**	0.1052	1.3601	0.07
	ExG**	0.1054	0.8673	0.08
	*g***	0.1133	2.1406	0.06
22 to 42 DAT	NGRDI**	1.4536	0.1804	0.06
	ExG**	1.4524	0.6623	0.07
	*g***	1.0622	0.2043	0.05

Explanation of acronyms in Table 1; ** – high significant correlation between the variables ($P \leq 0.01$)



Explanation of acronyms in Table 1

Figure 3. Correlation between the chlorophyll (Chl) concentration and spectral indices (NGRDI, ExG and *g*) at 42 DAT (A) and the last 21 DAT (B)

These results are similar to those obtained by Álvarez-Bermejo et al. (2017) in tomato and cucumber using the NGRDI (r = 0.90), and De la Cruz et al. (2011) in wheat using the *g* index (r = 0.97). Besides, there is a strong linear correlation with the ExG index (r = 0.88, RMSE = 0.03), a value comparable to that reported by Elazab et al. (2016) in wheat (r > 0.93). On the other hand, Lin et al. (2013) obtained correlation coefficients of 0.85 between ExG and Chl concentration in cucumber plants during their vegetative stage.

A greater adjustment to the linear regression model was obtained if the values from 22 to 42 DAT are considered (Fig. 3). The index that presented the highest correlation was ExG (r = 0.92, RMSE = 0.03) compared to NGRDI (r = 0.93, RMSE = 0.04) and *g* (r = 0.93, RMSE = 0.02). Similar results were obtained by Sun et al. (2016), who recorded strong correlations ($r \geq 0.88$) between ExG index and Chl concentration in cucumber. In this sense, Baresel et al. (2017) suggest that NGRDI and ExG indices can determine the Chl concentration in crops, because when using the *g* band (500 to 550 nm) it highlights the pigments responsible for the green colour of plants such as Chl allowing its quantification. On the other hand, Hunt et al. (2013) found that the relationship between NGRDI and Chl is not well established for all stages of the crop but could be more pronounced in early stages due to the existence of an underlying correlation with leaf area.

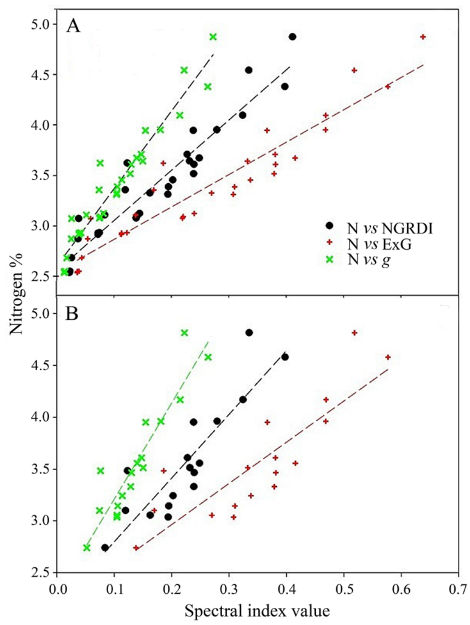
Nitrogen (N) concentration. Table 5 shows the correlation analysis and regression coefficients between N concentration and spectral indices values.

Table 5. Linear regression coefficients for the relationship between the nitrogen (N) concentration and spectral indices

Period evaluated	Spectral index	Linear regression coefficient		SE
		a	b	
1 to 42 DAT	NGRDI**	7.9238	1.8789	0.06
	ExG**	5.1094	1.8646	0.07
	*g***	12.2480	0.1133	0.07
22 to 42 DAT	NGRDI**	3.6735	1.5245	0.08
	ExG**	2.3247	1.9866	0.06
	*g***	6.6737	0.1535	0.05

Explanation of acronyms in Table 1; ** – high significant correlation between the variables ($P \leq 0.01$)

The concentration values of N had a high linear correlation with the NGRDI (r = 0.95, RMSE = 0.06), ExG (r = 0.94, RMSE = 0.04) and *g* index (r = 0.96, RMSE = 0.04) throughout the 42 DAT (Fig. 4).



Explanation of acronyms in Table 1

Figure 4. Correlation between the nitrogen (N) concentration and spectral indices (NGRDI, ExG and *g*) at 42 DAT (A) and the last 21 DAT (B)

During the vegetative stage of crop lettuce (last 21 DAT of the crop cycle), the relationship between the concentration of N and the NGRDI (r = 0.97, RMSE = 0.05), ExG (r = 0.97, RMSE = 0.04) and g (r = 0.97, RMSE = 0.06) showed greater adjustment to the linear regression model (Fig. 4). These results agree with those obtained by Meyer and Neto (2008), who reported coefficients of correlation ≥ 0.85 for the ExG index in the quantification of N in stages of vegetative growth in soybean.

Few studies use the NGRDI to determine the concentration of N in vegetables; however, the use of this spectral index has shown strong correlation (≥0.84) in the vegetative stage of maize (Hunt et al., 2013; Elazab et al., 2016), rice (Zheng et al., 2018) and wheat (Yang et al., 2020). Besides, spectral indices of the RGB visible region were shown to be efficient in determining N concentration in bean plants due to their effect on the amount and colouration of pigments within plant tissues (Lee, Lee, 2013), which indicates that the use of spectral indices represents a reliable and accurate methodology for quantifying the concentration of N in leafy crops.

Leaf area. Table 6 shows the regression coefficients and the results of the statistical analysis.

Table 6. Exponential regression coefficients for the relationship between the leaf area and spectral indices

Period evaluated	Spectral index	Exponential regression coefficient a	Exponential regression coefficient b	SE
1 to 42 DAT	NGRDI**	1.2705	2.4701	0.06
	ExG**	2.6704	2.1802	0.07
	g**	4.1901	1.8506	0.07
22 to 42 DAT	NGRDI**	1.5974	1.0563	0.08
	ExG**	1.2445	1.4357	0.06
	g**	2.1352	1.0406	0.05

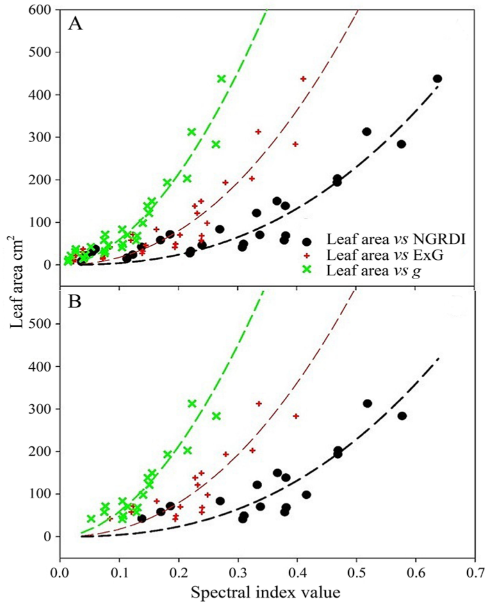
Explanation of acronyms in Table 1; ** – high significant correlation between the variables ($P \leq 0.01$)

The indices NGRDI (r = 0.94, RMSE = 0.05), ExG (r = 0.95, RMSE = 0.04) and g (r = 0.96, RMSE = 0.03) showed best fit to an exponential model to estimate leaf area (Fig. 5).

This mathematical model is different from the one reported by Campillo et al. (2010) for the g index, because they found a better fit to a linear model (r ≥ 0.92) in tomato. Besides, Horgan et al. (2015) and Beniaich et al. (2019) reported values with fit to linear models between leaf area and NGRDI in sweet pepper (r = 0.83) and basil (r = 0.85), respectively.

As is well known, the biomass can be used as a predictor of leaf area, especially in the early stages of crop growth. Studies in onion (Ballesteros et al., 2018) and tomato (Sun, Wang, 2019) report high correlations (r ≥ 0.87) between the g spectral index and leaf area; this is attributed to the growth rate of the crop. Furthermore, Hunt et al. (2013) found that the NGRDI is highly correlated with the amount of biomass in maize and soybean crops, especially in early stages, but tends to become saturated as the crop develops. Therefore, NGRDI can be useful in short-cycle plants such as leafy vegetables.

In the early stages of lettuce cultivation, the growth rate of leaf area is lower than in the later stages; therefore, the relationship with the indices with this parameter is not linear. Besides, results of our experiment show better fit to the exponential model in the last 21 days of the crop. This can be explained, because in early stages the RGB camera has spatial limitation to detect



Explanation of acronyms in Table 1

Figure 5. Correlation between the concentration of leaf area and spectral indices (NGRDI, ExG and g) at 42 DAT (A) and the last 21 DAT (B)

leaf area in small leaves, this limitation is reduced as the crop grows. Therefore, the efficiency of this tool using the spectral indices derived from RGB digital images depends on the phenology and plant growth habit.

Conclusions

1. The normalized green-red difference index (NGRDI) had the highest correlation coefficient with the values of Chl (r = 0.95) and N (r = 0.93). Besides, the excess green (ExG) index obtained the highest correlation with leaf area (r = 0.92). These indices highlight the spectral response in the green and red regions and are more accurate than the indices using the blue band.

2. Romaine lettuce has an accelerated leaf growth in the last stages of its vegetative cycle. In this study, the values of NGRDI, ExG and green channel (g) indices compared to leaf area are better adjusted to an exponential model. So, the use of this technique requires considering the type of crop and its phenology or growth habit.

3. The accuracy of the spectral indices derived from RGB (red, green and blue) digital images for estimating N, Chl status and leaf area increases in the later stages of romaine lettuce cultivation. It is because in the early stages of growth the leaves are small in size, which is a spatial limitation for detection by the RGB camera.

4. The use of spectral indices derived from RGB digital images is useful and accurate for the estimation of N, Chl status and leaf area during the production of lettuce. It could be a low-cost alternative to destructive methods, and it can be part of a combined system with portable meters (SPAD, atLEAF and others) for real-time monitoring of vegetables grown in a crop factory.

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Azoto ir chlorofilo kiekis sėjamųjų salotų lapuose, nustatytas naudojant raudonai žaliai mėlyno (RŽM) skaitmeninio vaizdo spektrinius indeksus

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Santrauka

Destruktyvūs augalų mitybos įvertinimo metodai yra tikslūs ir standartizuoti, tačiau brangūs ir apriboti erdvinės skalės. Nedestruktyvių metodų, pavyzdžiui, skaitmeninio vaizdo naudojimas, duoda greitus ir patikimus rezultatus *in situ*. Tačiau yra nedaug informacijos apie šių nedestruktyvių metodų naudojimą tiriant lapines daržoves. Tyrimo metu buvo vertinta azoto (N) ir chlorofilo (Chl) koncentracija sėjamųjų salotų lapuose naudojant spektrinius indeksus, gautus iš raudonai žaliai mėlyno (RŽM) skaitmeninio vaizdo. Salotos augintos plastikiniuose šiltnaminiuose tuneliuose modifikuotame Steinerio tirpale ir laistytos penkių koncentracijų N tirpalu: 0, 4, 8, 12 ir 16 mEq L^{-1} NO_3-N (atitinkamai 0, 248, 496 ir 744 mg L^{-1}). Laistant 16 mEq L^{-1} NO_3-N, salotų augimas buvo didžiausias 42 dieną po persodinimo. Augalų skaitmeniniai vaizdai kas savaitę buvo fotografuojami RŽM fotoaparatu ir apdoroti, kad būtų gauta mastelio matrica ir dvylika augalų spektrinių indeksų. Spektrinių indeksų koreliacijos analizė parodė, kad normalizuotas žaliai raudonos komponentės skirtumo indeksas, perteklinis žalios komponentės (ExG) ir žaliojo kanalo (g) indeksai sudarė teigiamą tiesinę koreliaciją su N (r > 0,93) ir Chl (r > 0,93) kiekiu. Taip pat buvo nustatyta eksponentinė koreliacija su lapų plotu (r > 0,86), kuri po persodinimo praėjus 21 dienai buvo stipresnė dėl greitesnio lapų augimo vegetacijos metu.

Tyrimo duomenimis, RŽM skaitmeninio vaizdo taikymas yra nebrangus, nedestruktyvus, patikimas ir tikslus metodas, siekiant įvertinti sėjamųjų salotų N bei Chl koncentraciją ir lapų plotą augimo metu. Šis metodas galėtų būti veiksminga alternatyva, kartu su nešiojamais chlorofilo matavimo prietaisais (SPAD) realiuoju laiku leidžianti stebėti salotų mitybinę būklę, ypač daržoves auginant pramoniniu būdu.

Reikšminiai žodžiai: *Lactuca sativa* var. *longifolia*, lapų plotas, nedestruktyvus metodas, azotas, chlorofilas, spektrinis indeksas.