Relationship between spectral response and manganese concentrations for assessment of the nutrient status in rose crop

Relación entre la respuesta espectral y las concentraciones de manganeso para evaluar el estado nutricional en el cultivo de rosa

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ABSTRACT

The present research was conducted on a Freedom rose (*Rosa* sp.) variety grown in greenhouses in the municipality of Tocancipá, Cundinamarca department (Colombia), to assess the relationship between reflectance and manganese content in leaves. A randomized complete block design was implemented, including five treatments with different manganese doses (0%, 25%, 50%, 75%, and 100% of the commercial dose, which is 2 mg L^{-1}), each with five replicates. Samplings at five phenological stages were carried out, with 10 plants analyzed per treatment for each sampling, totaling 50 plants per sampling. Spectral responses were taken from the adaxial surface of the leaves using a FieldSpec® 4 spectroradiometer, covering a wavelength range from 350 to 2500 nm. As the concentration of manganese in the leaves decreased, the reflectance values increased, showing an inverse relationship between these two parameters. The increase in reflectance values was particularly pronounced in the spectral regions between 560 nm and 840 nm. Among the vegetation indices evaluated, GNDVI, DATT4, DATT2, and D1 stood out; DATT4 and GNDVI showed the most promising results. DATT4 exhibited correlations greater than 0.6 during the "palmiche" (induction of the floral primordium) and "rice" (flower bud less than 4 mm in diameter) phenological stages, while GNDVI presented correlations of 0.64 in the "chickpea" (peduncle with an average length of 4 cm) phenological stage and 0.52 in the "scratch color" (the color of the petals could be observed) phenological stages.

Key words: crop nutrition, reflectance spectra, spectral indices, spectroradiometer, simple linear regression.

RESUMEN

La presente investigación se realizó en rosa (*Rosa* sp.), variedad Freedom, cultivada bajo invernaderos en el municipio de Tocancipá, departamento de Cundinamarca (Colombia), para evaluar la relación entre la reflectancia y el contenido de manganeso en las hojas. Se implementó un diseño experimental de bloques completos al azar, que incluyó cinco tratamientos con diferentes dosis de manganeso (0%, 25%, 50%, 75% y 100% de la dosis comercial, que es de 2 mg L^{-1}), cada uno con cinco repeticiones. Se realizaron muestreos en cinco etapas fenológicas, analizándose 10 plantas por tratamiento para cada muestreo, totalizando 50 plantas por muestreo. Las respuestas espectrales se tomaron de la superficie adaxial de las hojas utilizando el espectrorradiómetro FieldSpec® 4, cubriendo un rango de longitud de onda de 350 nm a 2500 nm. A medida que disminuía la concentración de manganeso en las hojas, los valores de reflectancia aumentaban, mostrando una relación inversa entre estos dos parámetros. El aumento de los valores de reflectancia se observó particularmente en las regiones espectrales entre 560 nm y 840 nm. Entre los índices de vegetación evaluados destacaron GNDVI, DATT4, DATT2 y D1; DATT4 y GNDVI mostraron los resultados más prometedores. DATT4 exhibió correlaciones superiores a 0,6 durante las etapas fenológicas de "palmiche" (inducción del primordio floral) y "arroz" (botón floral de menos de 4 mm de diámetro), mientras que GNDVI presentó correlaciones de 0,64 en el estado fenológico "garbanzo" (pedúnculo con una longitud media de 4 cm) y de 0,52 en los estados fenológicos "color de rayado" (el color de los pétalos podía observarse).

Palabras clave: nutrición de cultivos, espectro de reflectancia, índices espectrales, espectroradiómetro, regresión lineal simple.

Introduction

The flower industry arrived in Colombia in the 1960s through an alliance between foreign investors and local entrepreneurs. Gradually, it consolidated, becoming one of the main sectors in the agricultural economy and a strong export product in Colombia, competing with major flower producers worldwide. Colombia is now the leading flower exporter in Latin America and the second in the world, surpassed only by the Netherlands (ICA, 2024).

The increase in flower exports, improvement of sector competitiveness, and the demands of international markets, such as consistency in stem quality, size of flower buds, uniformity in the cutting point, and intensity of color, have elevated quality standards. To meet these parameters, an

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integrated crop management approach is essential. This includes an appropriate technological package which supplies nutrients in quantities required by the crops to obtain high-quality flowers (Ruppenthal & Castro, 2005).

Mineral nutrient supply is indispensable for obtaining commercially valuable flowers. Nutrients are categorized as macronutrients and micronutrients, depending on their concentrations in leaf tissue, with macronutrients present in higher concentrations. Despite their lower concentrations, micronutrients such as iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), boron (B), chlorine (Cl), and molybdenum (Mo) are equally crucial for the growth and development of crops and for producing flowers that meet the market quality standards (Rashed *et al*., 2019).

One of the nutrients present in low concentrations in rose plants is Manganese (Mn), which is essential for floral development and differentiation. However, it is prone to losses, such as through leaching, making it important to incorporate Mn into the soil or substrate at the right time and correct amounts (González Hurtado *et al*., 2007). If not managed appropriately, deficiency of this nutrient can lead to nutritional problems.

Manganese is crucial for plant growth; its deficiency affects the oxygen-evolving complex of photosystem II (PSII). This leads to detrimental effects on the photosynthetic apparatus, oxidative stress and reduced water-use efficiency (Schmidt *et al*., 2016). This is because the Mn-cluster that splits water molecules is anchored to PSII, and its deficiency reduces this binding, leading to the disintegration of PSII complexes. Consequently, there is a marked reduction in the D1 protein and intrinsic PsbP and PsbQ proteins, affecting the PSII core due to oxidative damage and leading to reduced carbon assimilation and lower plant yield (Schmidt *et al*., 2016).

Distinctive foliar symptoms of manganese deficiency become unmistakable when the growth rate is significantly restricted. These symptoms include diffuse interveinal chlorosis in fully expanded leaves. Severe necrotic spots may also form. Symptoms often first appear in middle leaves, unlike magnesium deficiency symptoms that appear in older leaves. In roses specifically, deficiency of Mn halts cellular growth in the leaf tissues between the veins, leading to cell death overtime and the appearance of brown spots (Humphries *et al*., 2006).

The diagnosis of the nutritional status of the rose crop is of fundamental importance as it influences decision-making regarding the formulation of exogenous fertilizer applications. These applications can be made via foliar spraying, but mainly through fertigation systems. The formulations, interactions, and relationships between soil/substrate, plant, and water, along with the timing of these applications, are factors that determine the quality and productivity indicators of the crop. These indicators are closely related to the sustainability and viability of the crop, since adequate supplementation of micronutrients substantially increases crop productivity. For this reason, it is essential to maintain plant nutrition in optimal balance, which requires the timely diagnosis of the nutritional status of the crop (Hariyadi *et al*., 2019).

In flower crops, as well as in other crops, nutrient analyses are traditionally performed in the laboratory using methods that are destructive and demanding in terms of time and labor, both for sample collection and processing. This leads to delays in the nutrient diagnosis of the crop. Hence, there is an interest in methods such as the use of sensors estimating certain parameters of the plants, such as the nutrient content in leaves. In this regard, the measurement of crop reflectance is a non-destructive, rapid, and integrative alternative for characterizing the nutrient status. Schmidt *et al*. (2016) emphasize that timely and efficient diagnosis and remediation of Mn deficiency in plants is a significant challenge in plant production systems.

In this regard, various studies have established relationships between spectral information and Mn content in plants, such as in cotton seed flour, where near-infrared spectroscopy (NIRS) and chemometrics were employed (Yu *et al*., 2019). These authors carried out the spectral corrections using the standard normal variable along with the first derivative, as well as applying Monte Carlo methods (MCUVE) and the successive projections algorithm to extract informative variables from complete NIR spectra (Yu *et al*., 2019). As a result, optimal models were developed to predict Mn content in cotton seeds, with a root mean square error of prediction of 1.99, a coefficient of determination R^2 of 0.94, and a residual predictive deviation of 4.37 (Yu *et al*., 2019).

Researchers assessed mineral element contents in common beans using spectral reflectance techniques, as these are affected in plants under abiotic stress such as salinity and drought (Boshkovski *et al*., 2020). Multivariate regression identified a relationship between reflectance in specific regions of the spectrum with foliar concentration of phosphorus (P) and the Normalized Difference Vegetation Index (NDVI), indicating significant correlation with foliar concentrations of B, Fe, K, Mn, P, and Zn. Furthermore, these authors developed customized spectral indices that show significantly high correlation with B, Fe, K, Mg (magnesium), Mn, Na (sodium), P, Zn, and N (nitrogen) (Boshkovski *et al*., 2020).

Other researchers studied the nutrient content of oil palm leaves, using spectral reflectance data to determine suitable wavelengths for predicting the levels of the most important leaf nutrients: N, P, K, Ca, Mg, B, Cu, and Zn (Santoso *et al*., 2019). These authors have built prediction models through stepwise regression followed by principal component regression. The resulting models showed strong positive correlations with foliar contents of N ($R^2 = 0.53$) and Ca ($R^2 = 0.50$). The contents of P, K, Mg, B, Cu, and Zn exhibited moderately positive correlations, with R^2 values ranging from 0.33 to 0.49 (Santoso *et al*., 2019).

Based on the above, this research was conducted to evaluate the relationship between spectral reflectance and foliar manganese content compared to chemical analysis of foliar tissue to diagnose the nutrient status of the rose crop.

Materials and methods

Experimental design

The research was conducted on a Freedom rose (*Rosa* sp.) variety grown under greenhouse conditions in the municipality of Tocancipá, Cundinamarca department (Colombia) at an altitude of 2605 m a.s.l. with coordinates 4°58'40.1" N, 73°59'06.6" W. The experimental field covered an approximate area of 176 m^2. In a randomized complete block design (RCBD), five doses of manganese applied through fertigation were evaluated in plants grown in a coconut fiber substrate. Five treatments with five replicates were carried out, consisting of Mn doses relative to the recommended dose of 2 mg L^{-1} with the product MF ACTIVA Mn 12% EDTA, as shown in Table 1, ensuring pH of the fertilizer solution between 5.3 and 5.8 and an electrical conductivity between 1.5 and 1.8 dS m^{-1}. These nutrients were supplied through drip fertigation based on the amount of water used by the crop, which was 120 L per standard 32 m bench, corresponding to 4 pulses of 17 L per plot per day, i.e., 4.25 L per plot per pulse, distributed throughout the day during all days of the experiment.

Each of the 25 plots had an area of 1.35 m^2 (0.3 m x 4.5 m) and contained a total of 60 rose plants. Five plots were arranged per each of the five hydroponic benches, avoiding the first and last plots to minimize edge effects. Each bench received the five treatments through a drip irrigation system.

TABLE 1. Nutrient composition of solutions (mg L^{-1}) used in the experiment.

	T1 (Mn 0%)	T2 (Mn 25%)	T3 (Mn 50%)	T4 (Mn 75%)	T5 (Mn 100%)
N	160	160	160	160	160
P	10	10	10	10	10
K	180	180	180	180	180
Ca	100	100	100	100	100
Mg	40	40	40	40	40
S	14	14	14	14	14
Mn	0	0.5	1	1.5	2
Zn	0.7	0.7	0.7	0.7	0.7
Cu	1.2	1.2	1.2	1.2	1.2
Fe	2	2	2	2	2
B	0.2	0.2	0.2	0.2	0.2
Mo	0.09	0.09	0.09	0.09	0.09

T – treatment.

Irrigation system

To apply each treatment to its corresponding plot, each nutrient solution was prepared in a separate tank connected to a half-horsepower centrifugal pump (0.5 hp), resulting in five pumps. Each pump was connected to a system of pipes and drippers that worked independently and distributed the nutrient solutions differentially to each plot.

The irrigation was remotely activated through the implementation of smart power outlets connected to a Wi-Fi network via a router located at the top of the greenhouse. The irrigation system was controlled from a mobile device with an Android operating system, using a downloaded app that allowed for the programming of the irrigation schedule.

Leaf chemical analysis

The chemical analysis consisted of determining the contents of Mn in rose leaves. The analysis was conducted using the AGRILAB laboratory, and the Mn content was analyzed using Microwave-Assisted Extraction methodology with the use of HNO_3 and H_2O_2 and read through Inductively Coupled Plasma Optical Emission Spectroscopy (Ghosh *et al*., 2013).

Measurement of spectral responses in the laboratory

To measure spectral responses, the FieldSpec® 4 spectroradiometer (Malvern Panalytical Ltd, UK (ASD, 2012)) was utilized in the Geomatics Laboratory, Faculty of Agricultural Sciences of the Universidad Nacional de Colombia (Bogotá campus). The reflectance was measured using the leaf clip of the spectroradiometer, specifically in the fourth and fifth fully developed leaves (with five or more leaflets), using the floral bud as a reference and counting the leaf number from the top down towards the base of the stem. The spectroradiometer recorded readings between 350 and 2500 nm with spectral resolutions of 1.4 nm from 350 to 1050 nm and 2 nm from 1050 to 2500 nm. This device operates by measuring light at different wavelengths, converting photons into electrons, and recording a signal over a defined integration time, typically 17 ms, which can increase to 34 ms under low-light conditions (ASD, 2012). The spectral reflectance of each leaf was measured using the leaf clip with the plant probe, which utilizes an internal broad-spectrum halogen light source. Prior to reflectance measurement, the spectroradiometer was standardized using a white reference standard (Spectralon, Labsphere Inc., Sutton, NH, USA).

The leaves used for measuring spectral responses were obtained in different phenological stages of plant development. The following sequential phenological stages were assessed, which were named according to Valencia *et al.* (2018): "palmiche" (induction of the floral primordium) followed by "rice" (flower bud less than 4 mm in diameter), "chickpea" (peduncle with an average length of 4 cm and approximately thirteen pairs of leaves), "scratch color" (the color of the petals could be observed due to the advance in flower development), and "straight sepals". In each phenological stage, a total of 50 leaflets were evaluated per treatment. The leaves were counted from the top downwards (from the youngest leaves to the mature ones), and a destructive sampling method was employed by removing the 4th and 5th leaves.

We ensured that the leaves were in good condition (without pest and disease damage), free from visual traces of agrochemicals, and kept hydrated during transportation to the laboratory. Once the leaves were collected in the field, they were stored in a styrofoam cooler with a refrigerant gel and transported within 5 h after recollection to the Geomatics Laboratory of the Universidad Nacional de Colombia for spectral response measurements. Each leaf underwent five readings of spectral responses corresponding to the five individual leaflets.

Data analysis

The evaluated spectral indices are presented in Table 2, where "R" represents Reflectance and "D" represents the First Derivative of reflectance. An analysis using a box plot was conducted to identify outliers. Shapiro-Wilk test was employed to assess the normality of the data, while the Bartlett test was used to evaluate the equality of variance across different populations. If the statistical assumptions were met, an analysis of variance (ANOVA) was performed. If these assumptions were not met, the Kruskal-Wallis test was conducted to determine potential differences between treatments. Finally, the Tukey test was applied to the data with a significance level of alpha=0.05.

TABLE 2. Vegetation spectral indices evaluated in rose plants. Adapted from Lehnert *et al.* (2016).

Index	Formula
CARTER4	$R710 / R760$
DATT	$(R850 - R710) / (R850 - R680)$
DATT2	$R850 / R710$
DATT3	$D754 / D704$
DATT4	$R672 / (R550 * R708)$
D1	$D730 / D706$
D2	$D705 / D722$
MI	$(R780 - R710) / R7(80 - R680)$
MCARI	$((R700 - R670) - 2 * (R780 - R710)) / (R780 - R680))$
MCARI/OSAVI	$MCARI / ((1 + 0.16) * (R780 - R710) / (R800 - R670 + 0.16))$
mND705	$(R750 - R705) / (R750 + R705 - 2 * R445)$
MTCI	$(R754 - R709) / (R709 - R681)$
REP _ Li	$700 + 40 * (RE - R700 / R740 - R700)$ where $RE = (R670 + R780) / 2$
VOG	$R740 / R720$
VOG2	$(R734 - R747) / (R715 + R726)$
VOG3	$D715 / D705$
VOG4	$(R734 - R747) / (R715 + R720)$
NDVI	*(NIR - Red) / (NIR + Red)*
NDRE	*(NIR – Red edge) / (NIR + Red edge)*
GNDVI	*(R840-R560) / (R840 + R560)*

MCARI: Modified chlorophyll absorption ratio index; NIR: Near-infrared; Red: Red band; Red edge: the region of the spectrum between red and near-infrared.

Reflectance data was smoothed using the Savitzky-Golay filter (Savitzky & Golay, 1964) with a second-order polynomial and a seven-band window. Subsequently, the first derivative was calculated. Other transformations of reflectance data were explored, such as Range Normalization (RN), where each row is divided by its range (*i.e.*, the difference between the maximum and minimum values), ensuring that the curve segment is normalized to 1 (Camo, 2006). Finally, Pearson's linear correlation coefficients were calculated, and regression analyses were conducted.

Results and discussion

The Mn concentration in the leaves throughout the different phenological states increased due to the applications made through fertigation (Fig. 1). In the "palmiche" stage, all treatments started with similar concentrations of Mn in leaves, with values ranging from 142 to 149 mg kg^{-1}, except for T1, which presented the lowest value (104 mg kg^{-1}). From the "rice" stage and throughout all subsequent stages, differences in Mn concentrations were observed due to the treatments. These results suggest that rose plants responded to fertilization, as treatments affected Mn concentrations in leaves.

In the "palmiche" stage, there were differences between the control treatment (without Mn) and the other treatments, forming two groups, one ("group a") included treatments T2, T3, T4, and T5, while the other ("group b") corresponded to T1 (without Mn). There were no significant differences between the treatments in "group a" while T1 showed significant differences compared to treatments in "group a".

In the "rice" stage, the treatments formed three groups: "group a" (T4 and T5), "group b" (T3), and "group c" (T1 and T2). This suggests that treatments had a clearer impact on Mn concentrations in the leaves at this stage, with low Mn doses related to lower foliar contents of Mn and high Mn doses related to higher concentrations of Mn.

From the " chickpea" stage onwards (including "scratch color" and "straight sepals"), four response groups were observed, indicating that Mn doses affected foliar Mn concentration, as shown in Figure 1.

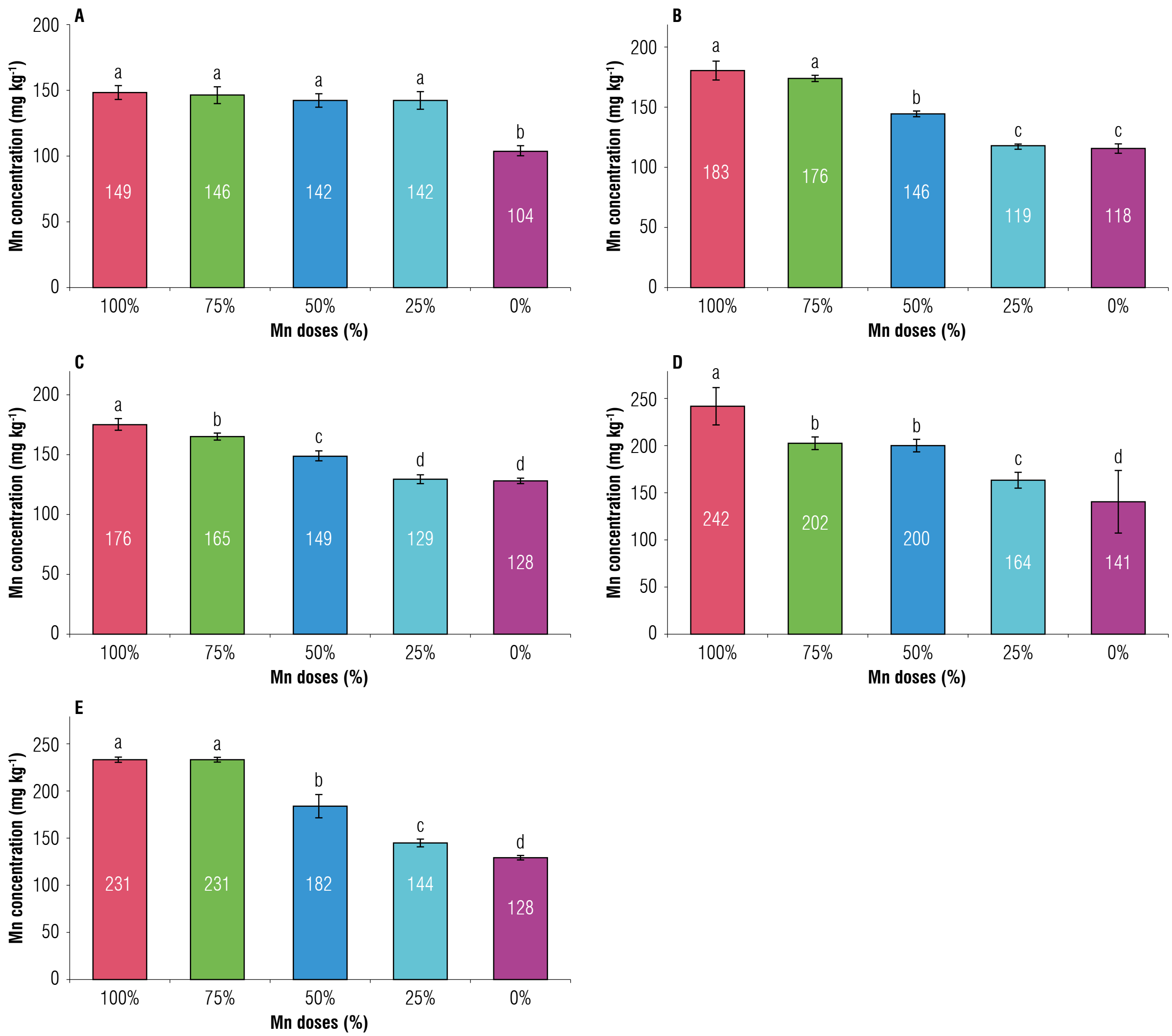

FIGURE 1. Multiple comparisons of the sample mean for the Mn concentration in rose leaves according to different Mn doses (T1: 0% Mn, T2: 25% Mn, T3: 50% Mn, T4: 75% Mn, T5: 100% Mn of the commercial dose, which is 2 mg L^{-1}) and phenological stages: A) palmiche - induction of the floral primordium, B) rice - flower bud less than 4 mm in diameter, C) chickpea - peduncle with an average length of 4 cm, D) scratch color - the color of the petals could be observed, and E) straight sepals. Averages with different letters indicate significant differences according to the Tukey test (alpha = 0.05). Values are expressed as mean ± SE.

The ANOVA indicated highly significant differences in foliar Mn content due to the treatments. The Tukey test showed that as the plants grew, the Mn content in leaves accumulated differentially based on the applied doses. This accumulation became more noticeable starting from the "chickpea" stage, which presented four statistically significant response groups.

Effects of treatments on spectral responses

Figure 2 illustrates the reflectances of the different treatments across the five phenological stages, highlighting the areas of the spectrum with the greatest differences (500-630 nm, 1500-1750 nm, 2000-2400 nm). In Figure 2A, corresponding to the phenological stage of "palmiche," T2 shows the highest reflectance, followed by T3, T1, T5, and

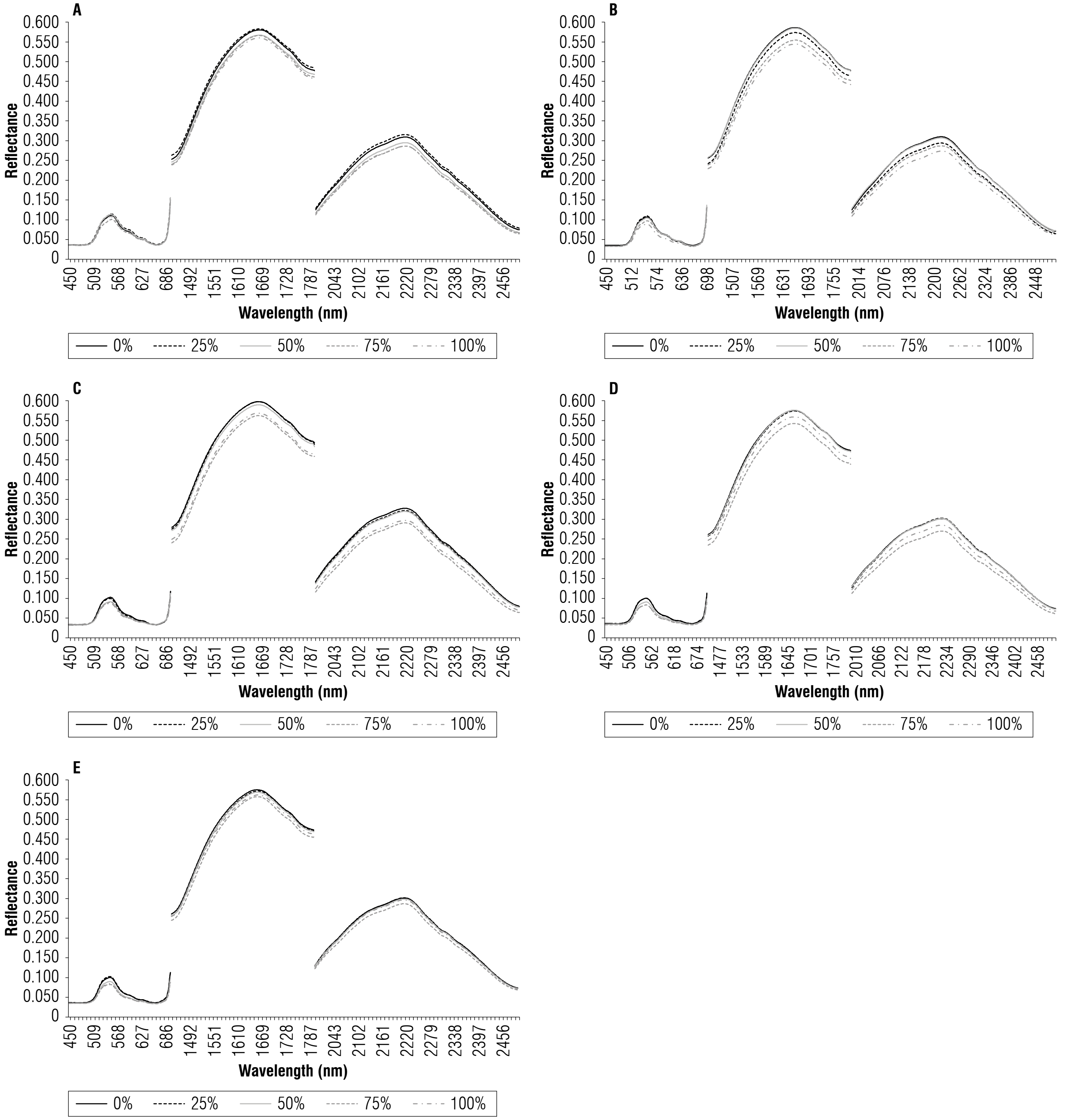

FIGURE 2. Relative reflectance in rose leaves with Savitzky-Golay preprocessing combined with range normalization. A) palmiche - induction of the floral primordium, B) rice - flower bud less than 4 mm in diameter, C) chickpea - peduncle with an average length of 4 cm, D) scratch color - the color of the petals could be observed, and E) straight sepals.

T4, respectively. This pattern suggests an inverse relationship between reflectance and foliar Mn content, with lower Mn content associated with higher reflectance. A similar pattern is observed in Figure 2B for the "rice" stage, where T2 presents the highest reflectance, followed by T1, while T3, T4, and T5 show decreasing values.

The inverse relationship between reflectance and Mn content in leaves becomes more pronounced in the "chickpea" stage (Fig. 2C), where the reflectance values clearly decrease as the Mn content increases. This pattern persists in the subsequent phenological states, "scratch color" and "straight sepals" (Figs. 2D and 2E). In summary, lower concentrations of Mn in the leaves were consistently related to higher reflectance.

Figure 3 presents the first derivative of reflectance in the spectral regions most sensitive to the varying

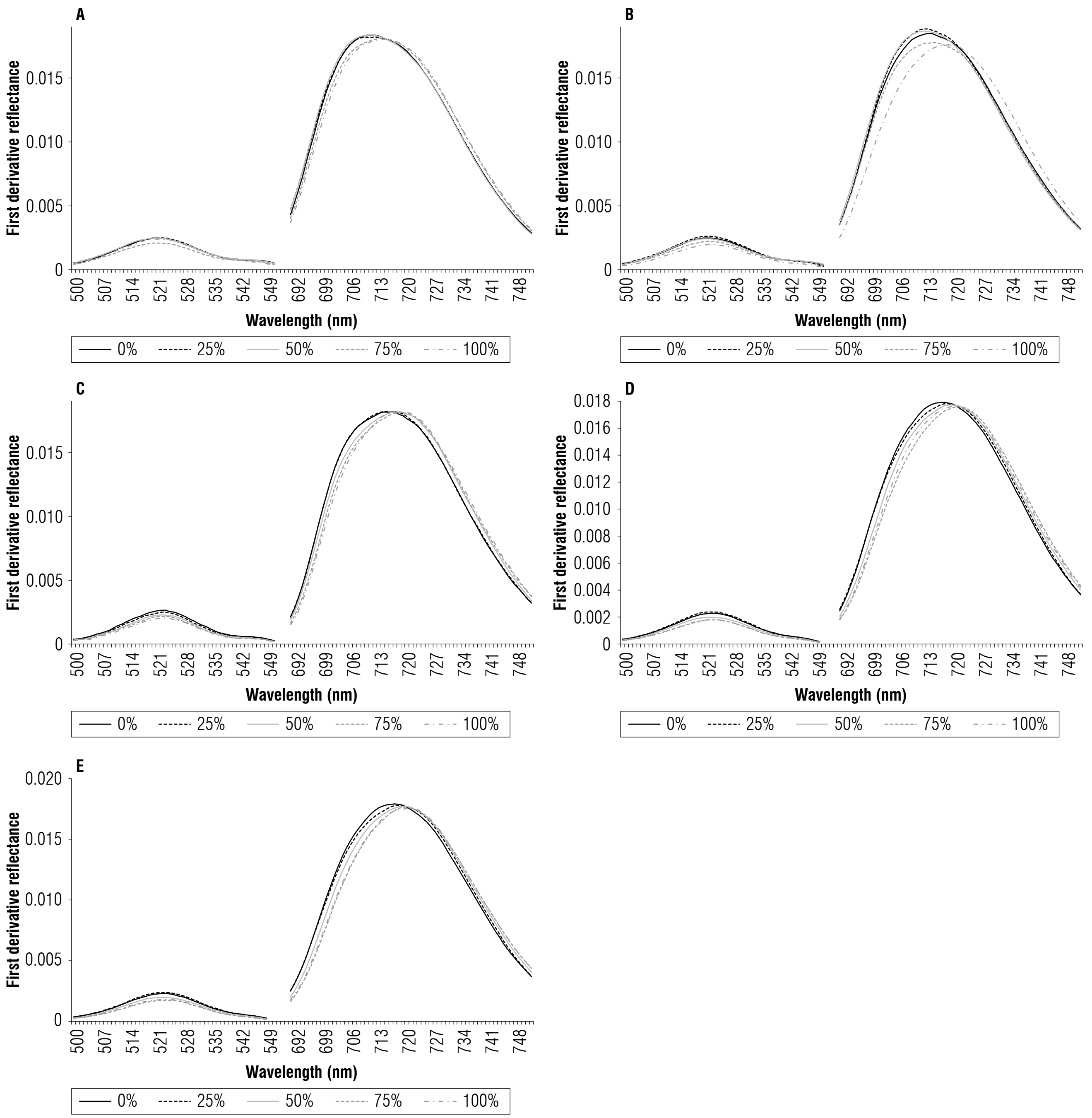

FIGURE 3. Median of the first derivative (n=500, 50 plants x 10 leaflets). A) palmiche - induction of the floral primordium, B) rice - flower bud less than 4 mm in diameter, C) chickpea - peduncle with an average length of 4 cm, D) scratch color - the color of the petals could be observed, and E) straight sepals.

concentrations of Mn in the leaves across the sampled phenological stages. The areas between 500-550 nm and 680-750 nm are particularly highlighted. In the 500 nm to 550 nm region, an inverse relationship is observed between the values of the first derivative of reflectance and the Mn content in the leaves. Conversely, in the 680 nm to 750 nm region, the effect of Mn concentrations is manifested as a rightward shift of the curves, with the treatments with the lowest Mn doses reaching their peak first. As the foliar Mn concentrations increase, the curves shift progressively to the right.

The phenological stage that most clearly demonstrates these results in the 500 nm to 550 nm region is "chickpea" (Fig. 3C). In this case, the highest value of the first derivative corresponds to treatment T1, which had the lowest Mn content in the leaves, followed by T2, T3, and T4. In contrast, T5, which received the highest Mn concentration, showed the lowest value of the first derivative. This clearly illustrates the inverse relationship between the foliar Mn content and the values of the first derivative of reflectance, emphasizing how a lower Mn content is associated with higher reflectance in this phenological stage.

In the 680 nm to 750 nm region, the most relevant phenological stage was "scratch color" (Fig. 3D), due to the behavior of the curves based on foliar Mn concentration. In this area of the spectrum, the values of the first derivative are similar for all treatments; however, a rightward shift is observed, starting with T1 at 715 nm and ending with T4 at 720 nm. This suggests that, in this sampling, the treatments do not significantly affect the values of the first derivative. Instead, as the Mn content increases, the maximum values shift to the right within the spectrum.

Differences in Mn concentration in the leaves had an impact on reflectance. Higher doses of Mn were associated with lower reflectance, whereas treatments with lower doses showed higher reflectance values. These differences were observed from the "palmiche" phenological stage and became more evident progressing from the "chickpea" to "straight sepals" phenological stages. Although there are no specific studies on Mn in this context, these results can be compared with research on other elements, such as nitrogen (N). For instance, Schepers *et al.* (1996) found a strong inverse relationship between the N concentration in corn leaves and reflectance values at 550 nm, attributing this to the N present in chlorophyll (Peng *et al.*, 2017). Chlorophyll, as an absorbing pigment, is associated with reflectance reduction, as leaves with higher chlorophyll levels absorb more light in the visible region (Mulyadi *et al.*, 2017). These findings can be linked to those obtained in this study, as Mn, as mentioned earlier, is essential in the water oxidation system in photosystem II and plays an important role in chlorophyll synthesis (Rashed *et al.*, 2019).

Vegetation spectral indices

The results of the ANOVA for vegetation spectral indices indicate that in the "palmiche" phenological stage, 17 out of the 20 analyzed indices showed highly significant differences (P<0.01). In the "rice" phenological stage, 18 indices exhibited highly significant differences, while in "chickpea," 17 indices showed highly significant differences, and one index had significant differences. For the "scratch color" phenological stage, 16 indices displayed highly significant differences and 2 had significant differences. In the "straight sepals" phenological stage, 17 indices had highly significant differences, and 1 had significant differences. These results, summarized in Table 3, indicate that the treatments had a significant effect on the vegetation spectral indices, suggesting that these indices may estimate the Mn nutritional status in rose cultivation.

Table 4 displays the results of the Tukey test for vegetation spectral indices. In the "palmiche" phenological stage, the NDRE, DATT3, and DATT4 indices showed statistically significant differences in foliar Mn content, forming three groups. For the "rice" phenological stage, 18 indices exhibited highly significant differences, while MCARI and MCARI.OSAVI were not significantly different. The NDRE, DATT4, and D1 indices stood out for forming three significant groups. In the "chickpea" phenological stage, the DATT4 index showed the most significant differences, forming four groups, positioning it as a potential index for estimating Mn in leaves. Other indices such as GNDVI, CARTER4, DATT, D1, D2, MI, mND705, MTCI, VOG2, VOG3, and VOG4 also formed three groups. In the "scratch color" phenological stage, the D1, D2, VOG, VOG2, VOG3, and VOG4 indices stood out, forming four significant groups, making them potential indices for estimating Mn concentration in leaves in this stage. Other indices, including GNDVI, CARTER4, DATT, DATT2, DAFF4, MI, MCARI, MCARI.OSAVI, mND705, MTCI, and REP_Li, formed three groups. Finally, in the "straight sepals" stage, the D1 and D2 indices formed four significant groups, while NDVI, GNDVI, CARTER4, DATT, DATT2, DATT3, DATT4, MI, mND705, MTCI, REP_Li, VOG, VOG2, VOG3, and VOG4 formed three groups. NDRE and MCARI showed no significant differences and MCARI.OSAVI formed two groups. As the plants progressed through the phenological stages, statistical differences increased among the spectral indices.

TABLE 3. Summary of analysis of variance for spectral indices.

Variable	GL	Palmiche	Rice	Chickpea	Scratch color	Straight sepals
NDVI	4	0.05806 *	0.000651 ***	0.189	5.29e-06 ***	0.000281***
NDRE	4	2.48e-05 ***	3.33e-13 ***	4.76e-06 ***	0.0129 *	0.518
GNDVI	4	0.000673 ***	1.23e-08 ***	6.58e-11 ***	1.8e-13 ***	1.66e-13 ***
CARTER4	4	2.3e-06 ***	5.89e-11 ***	2.09e-08 ***	6.03e-13 ***	<2e-16 ***
DATT	4	1.18e-06 ***	7.78e-12 ***	3.72e-08 ***	1.08e-12 ***	<2e-16 ***
DATT 2	4	5.76e-07 ***	5.76e-07 ***	1.15e-08 ***	5.77e-14 ***	<2e-16 ***
DATT 3	4	<2e-16 ***	4.28e-14 ***	1.7e-08 ***	2.92e-05 ***	4.28e-14 ***
DATT 4	4	<2e-16 ***	1.32e-05 ***	4.39e-10 ***	8.01e-14 ***	1.22e-14 ***
D1	4	6.58e-07 ***	2.77e-14 ***	3.43e-08 ***	0.302	2.7e-12 ***
D2	4	4.37e-06 ***	4.37e-06 ***	6.11e-08 ***	0.2503	1.93e-13 ***
MI	4	1.35e-06 ***	2.16e-11 ***	2.45e-12 ***	2.45e-12 ***	2.86e-16 ***
MCARI	4	0.137466	0.053	0.0536	0.000163 ***	0.0378 *
MCARI.OSAVI	4	0.186827	0.186827	0.01267 *	1.09e-05 ***	0.00181 **
mND705	4	1.6e-06 ***	9.35e-11 ***	2.1e-08 ***	1.28e-12 ***	<2e-16 ***
MTCI	4	3.09e-07 ***	8.41e-09 ***	8.41e-09 ***	3e-13 ***	3.08e-16 ***
REP _ Li	4	4.08e-06 ***	1.57e-10 ***	6.7e-08 ***	7.43e-13 ***	<2e-16 ***
VOG1	4	1.08e-06 ***	3.82e-13 ***	2.75e-08 ***	7.24e-13 ***	3.65e-14 ***
VOG2	4	3.21e-07 ***	1.59e-13 ***	1.87e-08 ***	2.16e-13 ***	3.46e-15 ***
VOG3	4	4.55e-07 ***	6.63e-13 ***	9.87e-09 ***	0.0341 *	7.24e-16 ***
VOG4	4	3.21e-07 ***	1.59e-13 ***	3.21e-07 ***	2.16e-13 ***	3.46e-15 ***

*Significant difference at the 0.05 level, ***highly significant difference at the 0.01 level according to ANOVA.

TABLE 4. Multiple comparisons of the sample mean for vegetation indices in rose crop.

INDEX	Palmiche					Rice					Chickpea					Scratch color					Straight sepals				
	Treatments (% Mn)																								
	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100
NDVI	ab	b	a	ab	ab	b	a	ab	b	b	a	a	a	a	a	b	b	a	a	a	ab	bc	c	ab	a
NDRE	ab	abc	a	bc	c	ab	a	a	b	c	a	a	ab	b	b	a	ab	ab	b	ab	a	a	a	a	a
GNDVI	ab	b	b	a	ab	b	b	b	b	a	c	bc	ab	a	a	c	c	b	a	ab	c	c	b	a	ab
CARTER4	a	a	a	b	b	a	a	a	a	b	a	ab	bc	c	c	a	a	b	c	bc	a	a	b	c	c
DATT	b	b	b	a	a	b	b	b	b	a	c	bc	ab	a	a	c	c	b	a	ab	c	c	b	a	a
DATT2	b	b	b	a	a	b	b	b	b	a	c	bc	ab	a	a	c	c	b	a	ab	c	c	b	a	a
DATT3	c	c	b	a	a	b	b	b	b	a	b	b	b	a	a	a	a	a	a	a	c	c	b	a	a
DATT4	c	bc	b	a	a	bc	c	c	b	a	d	cd	bc	ab	a	c	bc	b	a	a	c	bc	b	a	a
D1	b	b	b	a	a	bc	c	c	b	a	c	bc	ab	a	a	d	cd	bc	a	ab	d	cd	bc	ab	a
D2	a	a	a	b	b	a	a	a	a	b	a	ab	bc	c	c	a	ab	bc	d	cd	a	ab	bc	cd	d
MI	a	a	a	b	b	b	b	b	b	a	c	bc	ab	a	a	c	c	b	a	ab	c	c	b	a	a
MCARI	a	a	a	a	a	ab	a	ab	b	ab	a	ab	ab	ab	b	a	ab	ab	bc	c	a	a	a	a	a
MCARI.OSAVI	a	a	a	a	a	ab	a	ab	b	b	a	ab	ab	ab	b	a	a	ab	bc	c	a	ab	ab	b	b
mND705	a	a	a	a	a	b	b	b	b	a	c	bc	ab	a	a	c	c	b	a	ab	c	c	b	a	a
MTCI	a	a	a	a	a	b	b	b	b	a	c	bc	ab	a	a	c	c	b	a	ab	c	c	b	a	a
REP _ Li	a	a	a	a	a	b	b	b	b	a	b	b	ab	a	a	c	c	b	a	ab	c	c	b	a	a
VOG	a	a	a	a	a	b	b	b	b	a	b	b	ab	a	a	d	cd	bc	a	ab	c	bc	b	a	a
VOG2	a	a	a	a	a	a	a	a	a	b	a	ab	bc	c	c	a	ab	bc	d	cd	a	a	b	c	c
VOG3	a	a	a	a	a	b	b	b	b	a	c	bc	ab	a	a	d	cd	bc	a	ab	c	c	b	a	a
VOG4	a	a	a	a	a	a	a	a	a	b	a	ab	bc	c	c	a	ab	bc	d	cd	a	a	b	c	c

Different lower-case letters (a-d) represent significant differences between treatments according to the Tukey test ($P \leq 0.05$).

Simple linear regression for Mn concentrations in leaves

In the different phenological stages, linear regression analyses between the Mn concentration and the spectral indices showed statistically significant differences. During the "palmiche" phenological stage, the DATT4 index exhibited the most significant correlation, with a value of 0.6, indicating a strong relationship. Therefore, this index has a high potential for estimating Mn content in leaves during this phase.

In the "rice" phenological stage, significant correlations were also observed between Mn concentration and several spectral indices. As shown in Figure 4A, the DATT4 index exhibited the highest correlation, with a value of 0.6, followed by the D1 index in Figure 4B, with a correlation of 0.541; both were classified as strong correlations. Additionally, in Figure 4C, the NDRE index exhibited a strong negative correlation.

These results suggest that the DATT4, D1, and NDRE indices have the potential to estimate the Mn nutritional status in the rose crop during the "rice" phenological stage.

Figure 5 shows the linear regressions of the three indices with the highest correlations for estimating the Mn nutritional status in the "chickpea" phenological stage. In Figure 5A, the GNDVI index exhibited the highest correlation, with a coefficient of 0.646. In Figure 5B, the DATT4 index presented a correlation coefficient of 0.63, while in Figure 5C, the mND705 index recorded a coefficient of 0.571. These results suggest that these three indices have significant potential for estimating the Mn nutritional status in the rose crop during the "chickpea" phenological stage.

Figure 6 presents the linear regressions of the three indices that achieved the highest correlation coefficients in the "scratch color" phenological stage. In Figure 6A, the GNDVI index had the highest correlation, reaching 0.52. In Figure 6B, the DATT index showed a correlation of 0.481, while in Figure 6C, the DATT2 index exhibited a correlation of 0.488. These findings suggest that the GNDVI and DATT indices have the potential for estimating the Mn nutritional status in the rose crop during the "scratch color" phenological stage, while the DATT2 index showed a moderate correlation.

Figure 7 presents the linear regressions of the three indices with the highest correlations for estimating Mn content in the "straight sepals" phenological stage. In Figure 7A, the

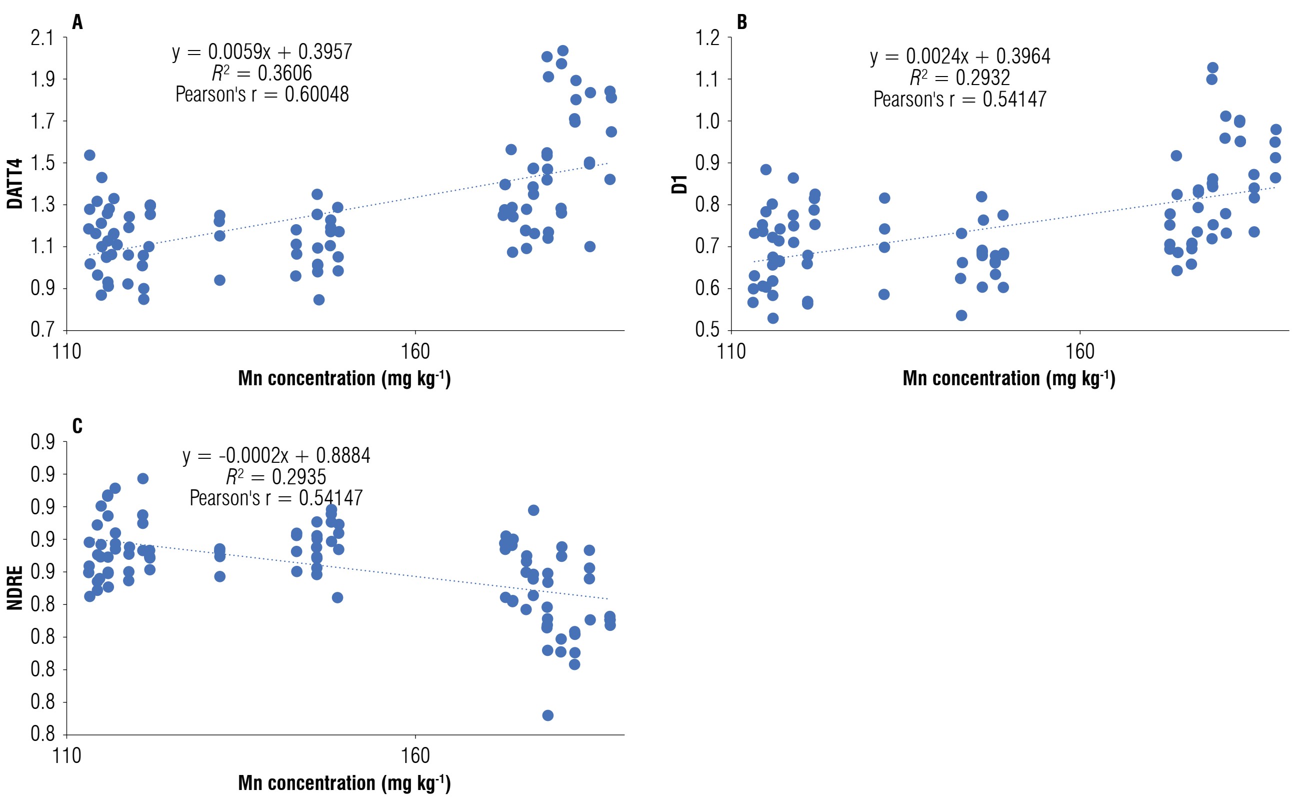

FIGURE 4. Linear regressions between the Mn concentration in rose leaves and spectral indices. A) DATT4 index, B) D1 index, C) NDRE index.

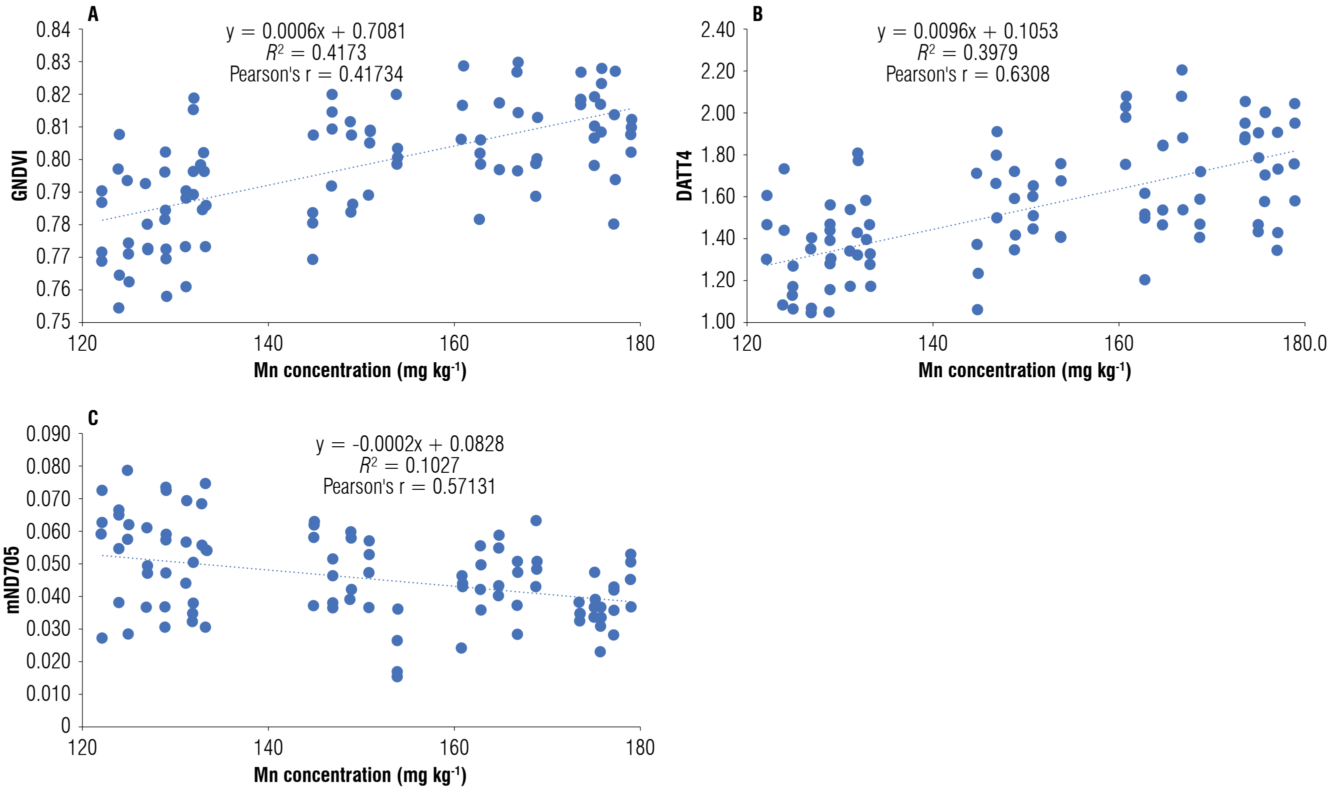

FIGURE 5. Linear regressions between the Mn concentration in rose leaves and spectral indices. A) GNDVI index, B) DATT4 index, C) mND705 index.

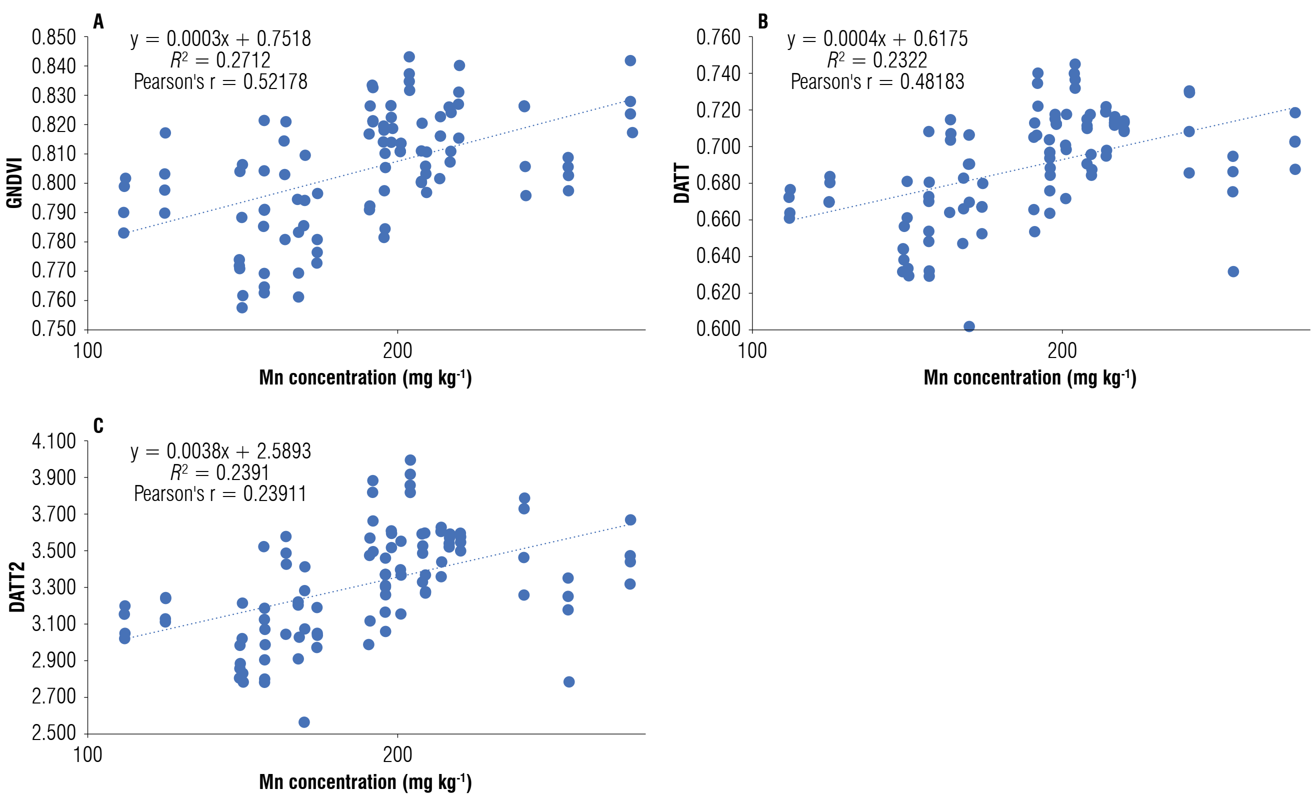

FIGURE 6. Linear regressions between the Mn concentration in rose leaves and spectral indices. A) GNDVI index, B) DATT index, C) DATT2 index.

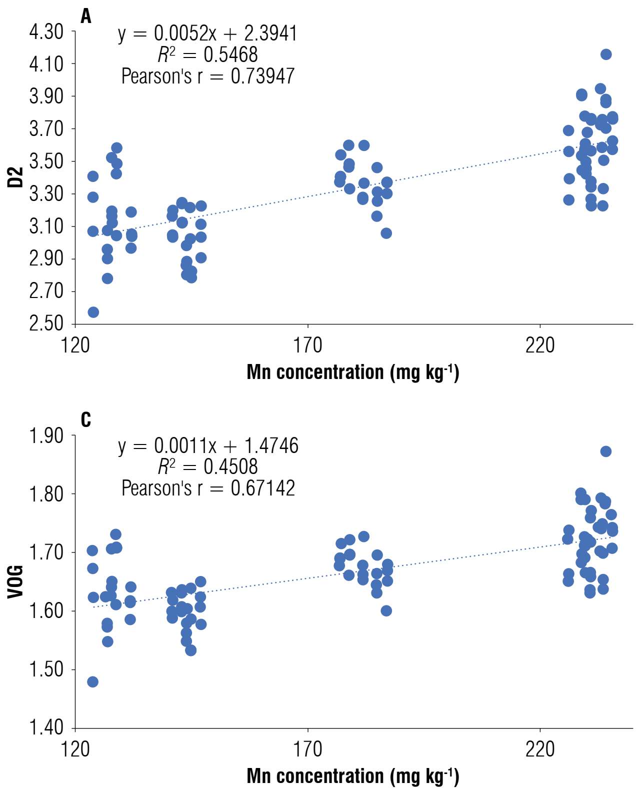

FIGURE 7. Linear regressions between the Mn concentration in rose leaves and spectral indices. A) D2 index, B) MI index, C) VOG index.

D2 index had the highest correlation, reaching 0.739. In Figure 7B, the MI index presented a correlation of 0.738. In Figure 7C, the VOG index showed a correlation of 0.671. These results suggest that all three indices have a high potential for estimating Mn content in leaf tissue during the "straight sepals" phenological stage.

Based on the results presented, it can be inferred that the effect of different Mn doses on rose crop is reflected in the spectral indices, enabling the identification of potential indices for estimating the Mn concentration in leaves using reflectance measurements obtained with the FieldSpec® 4 spectroradiometer. These indices vary depending on the phenological stage of the plants.

For the "palmiche" phenological stage, the DATT4 index stood out, based on the first derivative of reflectance at 706 nm and 730 nm, showing a correlation of 0.6. In the phenological stages of "rice," "chickpea," and "scratch color," the GNDVI index, calculated from reflectance at 560 nm and 840 nm, exhibited correlations of 0.646, 0.664, and 0.664, respectively. In the "straight sepals" phenological stage, the VOG index, calculated using reflectance values at 720 nm and 740 nm, showed a notable correlation of 0.74.

All of these correlations were highly significant, suggesting that these indices have promising potential for estimating Mn concentrations in rose crops, depending on the phenological stage of the plants. The GNDVI index demonstrated outstanding performance, showing strong correlations in the evaluated stages, except for the "palmiche" phase. This index, calculated from reflectance at 560 nm and 840 nm, underscores the importance of this spectral range for estimating Mn concentration in leaves.

Similar findings were reported by Marin *et al.* (2021) in studies on nitrogen estimation in coffee crops, where the GNDVI index allowed the determination of crop areas with nutrient deficiencies (Marin *et al.*, 2021). Additionally, in the case of tomato crop, Padilla *et al.* (2015) found that the GNDVI index proved to be an effective indicator of nitrogen status in plants (Padilla *et al.*, 2015).

The GNDVI (Green Normalized Difference Vegetation Index) showed remarkable performance in advanced phenological stages of rose development, where the pigmentation of leaves is predominantly green. The GNDVI considers reflectance at wavelengths of 560 nm and 840 nm, making it particularly sensitive to the presence of chlorophyll, which has absorption peaks in the red and blue regions of the spectrum (Gamon & Surfus, 1999). In these stages, light absorption by chlorophyll is sufficient to establish significant correlations with the Mn concentration in the leaves. In this study, the GNDVI presented high correlations in the phenological stages of "chickpea" and "scratch color", with correlations of 0.64 and 0.52, respectively.

However, in the "palmiche" and "rice" stages, where the leaves exhibit reddish pigmentation characteristics due to the high presence of anthocyanins and are considered more immature states, the GNDVI showed reduced performance. The presence of anthocyanins may interfere with light absorption at relevant wavelengths, hindering the precise estimation of Mn concentration (Gould & Quinn, 1999). In this context, the DATT4 index proved to be more effective, achieving a significant correlation of 0.6 in both stages

Conclusions

The treatments applied in this study significantly impacted reflectance values. A clear trend was observed in which higher doses of Mn in rose crop resulted in lower reflectance values, while lower dose treatments exhibited notably higher values.

The GNDVI index stands out as a highly effective indicator for estimating Mn concentration in rose leaves across various phenological stages, except in the "palmiche" stage. This index, which considers reflectance at 560 nm and 840 nm, demonstrated significant and strong correlations with Mn levels in rose leaves during the "rice," "chickpea," and "scratch color" phenological stages. Notably, in the "straight sepals" stage, a correlation of 0.739 and an R^2 of 0.546 were achieved. These findings are consistent with previous research on other crops, where GNDVI has been successfully used to assess the nutrient status of plants.

In contrast, the "palmiche" phenological stage exhibited a different dynamic, with the DATT4 index being the only one to show a significant correlation of 0.6 for estimating the Mn concentration. This variation may be related to specific characteristics of the plants at this stage, such as their red coloration, which contrasts with later phenological stages where the stem color turns green. This underscores the need to adapt methodologies according to phenological stages.

The comparison between spectral reflectance and traditional analysis suggests that the spectral reflectance methodology offers several advantages. This methodology allows for *in situ* application, is non-destructive, and provides rapid results. Unlike the traditional approach, which requires a greater amount of labor and samples to obtain results, thus increasing costs, the proposed method optimizes operations and reduces the need for personnel. Each reflectance reading generates data that identifies different nutrient behaviors within the same lot, facilitating the precise evaluation of Mn concentration in leaves. Notably, this procedure does not use chemical reagents, contributing to a more sustainable and efficient practice in the production of cut flowers.

This study establishes an important relationship between spectral reflectance and Mn foliar content in roses, as well as the relevance of the GNDVI index as a promising tool for estimating Mn status in cut flower production. These findings are valuable for monitoring and managing plant nutrition, significantly contributing to precision agriculture and data-driven agronomic decision-making, enhancing sustainability and efficiency in the cut flower industry.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

LJMM designed the conceptual approach and objectives; OFM supervised the experiments, managed data collection and maintenance, performed the statistical analyses and computer codes, coordinated funding; AFT carried out the field and laboratory experiments, and created effective visual representations of the data and findings; LJMM and OFM designed and developed the research methodology, including data collection methods and equipment, verified the accuracy and reliability of the research results through a rigorous validation process; OFM wrote the initial draft. All authors participated in the critical review and approval of the final version of the manuscript.

Literature cited

ASD. (2012). FieldSpec 4 user guide. ASD document 6000979. Malvern Panalytical, Malvern, UK. https://www.malvernpanalytical.com/en/learn/knowledge-center/user-manuals/fieldspec-4-user-guide

Boshkovski, B., Tzerakis, C., Doupis, G., Zapolska, A., Kalaitzidis, C., & Koubouris, G. (2020). Relationships of spectral reflectance with plant tissue mineral elements of common bean (*Phaseolus vulgaris* L.) under drought and salinity stresses.*Communications in Soil Science and Plant Analysis*, *51*(5), 675–686. https://doi.org/10.1080/00103624.2020.1729789

Camo Software SA. (2006). The unscrambler methods. Aspenthec. https://www.camo.com/downloads/U9.6%20pdf%20manual/The%20Unscrambler %20Methods.pdf

Gamon, J. A., & Surfus, J. S. (1999). Assessing leaf pigment content and activity with a reflectometer. *The New Phytologist*, *143*(1), 105–117. https://doi.org/10.1046/j.1469-8137.1999.00424.x

Ghosh, S., Prasanna, V. L., Sowjanya, B., Srivani, P., Alagaraja, M., & Banji, D. (2013). Inductively coupled plasma–optical emission spectroscopy: A review. *Asian Journal of Pharmaceutical Analysis, 3*(1), 24–33. https://ajpaonline.com/HTMLPaper.aspx?Journal=Asian%20Journal%20of%20Pharmaceutical%20Analysis;PID=2013-3-1-6

González Hurtado, M., Hernandez Díaz, M. I., Dupeyrón Martell, D., Rieumont Briones, J., Rodriguez Acosta, C., Cuesta, E., & Sardiña, C. (2007). Síntesis y comportamiento de un material polimérico aplicado como recubrimiento en un fertilizante de liberación controlada. *Revista Iberoamericana de Polímeros*, *8*(4), 275–286. https://reviberpol.files.wordpress.com/2019/08/2007-gonzalez.pdf

Gould, K., Davies, K. M., & Winefield, C. (Eds.). (2008). *Anthocyanins: biosynthesis, functions, and applications*. Springer Science & Business Media.

Hariyadi, B. W., Nizak, F., Nurmalasari, I. R., & Kogoya, Y. (2019). Effect of dose and time of NPK fertilizer application on the growth and yield of tomato plants (*Lycopersicum esculentum* Mill). *Agricultural Science*, *2*(2), 101–111. https://core.ac.uk/download/pdf/230412842.pdf

Humphries, J. M., Stangoulis, J. C. R., & Graham, R. D. (2016). Manganese. In A. V. Barker, & D. J. Pilbeam (Eds.), *Handbook of plant nutrition* (pp. 367–390). CRC Press.

ICA – Instituto Colombiano Agropecuario. (2024). *Con 700 millones de tallos, Colombia aporta variedad, color y belleza a la celebración de San Valentín*. https://www.ica.gov.co/noticias/ica-colombia-exporta-flores-san-valentin-2024

Lehnert, L. W., Meyer, H., & Bendix, J. (2016). Package 'hsdar'. https://www.researchgate.net/profile/Lukas-Lehnert/publication/312599704_Package_'hsdar'_Manage_Analyse_and_Simulate_Hyperspectral_Data/links/5885f9dfaca272b7b44ca3e5/Package-hsdar-Manage-Analyse-and-Simulate-Hyperspectral-Data.pdf

Marin, D. B., Ferraz, G. A. S., Guimarães, P. H. S., Schwerz, F., Santana, L. S., Barbosa, B. D. S., Barata, R. A. P., Faria, R. O., Dias, J. E. L., Conti, L., & Rossi, G. (2021). Remotely piloted aircraft and random forest in the evaluation of the spatial variability of foliar nitrogen in coffee crop. *Remote Sensing*, *13*(8), Article 1471. https://doi.org/10.3390/rs13081471

Mulyadi, T., Hariyandi, Sudradjat., & Kustiyo. (2017). Nitrogen content and carbon stock prediction in oil palm using satellite image analysis. *Asian Journal of Applied Sciences, 5*(4), 677–683. https://www.ajouronline.com/index.php/AJAS/article/view/4899

Padilla, F. M., Peña-Fleitas, M. T., Gallardo, M., & Thompson, R. B. (2015). Threshold values of canopy reflectance indices and chlorophyll meter readings for optimal nitrogen nutrition of tomato. *Annals of Applied Biology*, *166*(2), 271–285. https://doi.org/10.1111/aab.12181

Peng, Y., Nguy-Robertson, A., Arkebauer, T., & Gitelson, A. A. (2017). Assessment of canopy chlorophyll content retrieval in maize and soybean: Implications of hysteresis on the development of generic algorithms. *Remote Sensing*, *9*(3), Article 226. https://doi.org/10.3390/rs9030226

Rashed, M. H., Hoque, T. S., Jahangir, M. M. R., & Hashem, M. A. (2019). Manganese as a micronutrient in agriculture: Crop requirement and management. *Journal of Environmental Science and Natural Resources*, *12*(1–2), 225–242. https://doi.org/10.3329/jesnr.v12i1-2.52040

Ruppenthal, V., & Conte e Castro, A. M. (2005). Efeito do composto de lixo urbano na nutrição e produção de gladíolo. *Revista Brasileira de Ciência do Solo*, *29*(1), 145–150. https://doi.org/10.1590/S0100-06832005000100016

Santoso, H., Tani, H., Wang, X., & Segah, H. (2019). Predicting oil palm leaf nutrient contents in Kalimantan, Indonesia by measuring reflectance with a spectroradiometer. *International Journal of Remote Sensing*, *40*(19), 7581–7602. https://doi.org/10.1080/01431161.2018.1516323

Savitzky, A., & Golay, M. J. (1964). Smoothing and differentiation of data by simplified least squares procedures. *Analytical Chemistry*, *36*(8), 1627–1639. https://pubs.acs.org/doi/pdf/10.1021/ac60214a047

Schepers, J. S., Blackmer, T. M., Wilhelm, W., & Resende, M. (1996). Transmittance and reflectance measurements of corn leaves from plants with different nitrogen and water supply. *Journal of Plant Physiology, 148*(5), 523–529. https://doi.org/10.1016/S0176-1617(96)80071-X

Schmidt, S. B., Jensen, P. E., & Husted, S. (2016). Manganese deficiency in plants: The impact on photosystem II. *Trends in Plant Science*, *21*(7), 622–632. https://doi.org/10.1016/j.tplants.2016.03.001

Valencia, J., Vélez, J., & Arévalo, J. (2018). Evaluation of irrigation in roses in a suitable substrate with three water depths and the percentage of drainage. *Agronomía Colombiana*, *36*(3), 266–273. https://doi.org/10.15446/agron.colomb.v36n3.73912

Yu, E., Zhao, R., Cai, Y., Huang, J., Li, C., Li, C., Mei, L., Bao, L., Chen, J., & Zhu, S. (2019). Determination of manganese content in cottonseed meal using near-infrared spectrometry and multivariate calibration. *Journal of Cotton Research*, *2*, 1–7. https://doi.org/10.1186/s42397-019-0030-5