**RESEARCH ARTICLE** 



# Yield predictions of 'Del Cerro' cotton (*Gossypium hirsutum* L.) germplasm by multispectral monitoring in the north coast of Peru

Camila Cruz-Grimaldo<sup>1</sup>, Marite Nieves<sup>1</sup>, Elvis Vera<sup>1</sup>, Moisés Duran<sup>1</sup>, Arturo Morales<sup>1</sup>, Wilian Salazar<sup>2</sup>, and Carlos I. Arbizu<sup>3\*</sup>

<sup>1</sup>Instituto Nacional de Innovación Agraria (INIA), Estación Experimental Agraria Vista Florida, Lambayeque 14301, Perú. <sup>2</sup>Instituto Nacional de Innovación Agraria (INIA), Dirección de Supervisión y Monitoreo en Estaciones Experimentales Agraria, Lambayeque 14301, Perú.

<sup>3</sup>Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas (UNTRM), Facultad de Ingeniería y Ciencias Agrarias, Amazonas 01001, Perú.

\*Corresponding author (carlos.arbizu@untrm.edu.pe).

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# ABSTRACT

Peruvian cotton (Gossypium hirsutum L.) has great acceptance and demand in the national and international textile market due to the excellent quality of its extra-long fiber, durability and resistance. To evaluate cotton cultivar performance, we need to use tools such as drones + sensors. However, these tools have not been widely used in the Peruvian agricultural area. Here we evaluated seven agro-morphological characters of 21 accessions of Del Cerro cotton cultivar from the National Institute of Agrarian Innovation of Peru with highthroughput phenotyping methods. We employed a Matrice 300 RTK unmanned aerial vehicle (UAV) with the MicaSense Dual Red Edge Blue multispectral sensor to assess plant height, yield, and spectral signature during physiological maturity stage; other morphological characters were manually scored. Multispectral monitoring revealed the phytosanitary status of the crop, which begins to enter senescence after 130 d after sowing (DAS) due to the decrease of the vegetation indices (VI). Pearson correlations between yield and VI showed favorable values, exceeding 0.60 at 94 DAS for normalized difference vegetation index (NDVI), relative vigor index (RVI), and normalized difference red edge index (NDRE). Principal component analysis (PCA) was conducted on the same date, a significant correlation was found between NDVI and yield. Additionally, yield prediction equations were generated with the normalized difference water index (NDWI) showing an R value of 0.74 at 130 DAS. The findings of this study suggest that remote sensing evaluation is suitable for estimating 'Del Cerro' cotton yield in infrared (IR) bands, providing a tool for germplasm evaluation that can influence decision-making and better conservation strategies.

Key words: Morphometrics, multivariate analysis, phenomics, UAV, vegetation indices.

# **INTRODUCTION**

The demands of the population in the agricultural, food and textile sectors in the world are increasing, therefore, agricultural production must double; given that by 2050 a world population of 9 billion inhabitants is expected (Xu et al., 2019). Currently, there is a great demand in the textile industry for the quality of fiber used in the production of high-quality garments. In addition, this industry seeks to reduce the use of carcinogenic (artificial) dyes obtained from petroleum through the use of varieties with colored fiber (López Medina et al., 2020). Cotton (*Gossypium* spp.) is cultivated in around 80 countries and occupies about 2.5% of the world's agricultural area (Martínez-Reina and Hernández, 2015), being the species *G. hirsutum* L. the predominant one as it is cultivated in more than 95% of the agricultural area allocated to non-edible crops

(FAOSTAT, 2015; Chinga et al., 2020), and it is used mainly for its fiber for the textile industry and seeds for bovine feed (Chinga et al., 2020).

In Peru, cotton has great significance in sociocultural and economic development; its production is an important source of income for small-scale agriculture (Serquen-Lopez and Iglesias-Osores, 2019). In the geographical region of Lambayeque, located in the northern coast of Peru, *G. hirsutum* traditionally possesses characteristics of early maturity, high productivity and fiber quality. High genetic quality material has been collected, evaluated and selected by the National Institute of Agrarian Innovation (INIA for its Spanish acronym), with the objective of preserving genetic variability and increasing the use of high quality seeds to satisfy the demand for seed in the main cotton areas of the region due to its growing demand (Fernandez-Stark et al., 2016). Cotton (*G. hirsutum*) 'Del Cerro INIA 803' is a multiline cultivar developed in favor of cotton growers from Lambayeque. This cultivar possesses outstanding characteristics of high yield and fiber quality (MINAM, 2020). This cultivar was developed from the Germplasm Bank of the National Program of Regional Crops of INIA and represents an important genetic base for the development of future cultivars.

Currently, remote sensing is used in multiple areas, including agricultural monitoring with unmanned aerial vehicles (UAV) (Fan and Lu, 2021), providing high-precision and resolution information. Shi et al. (2016) indicated that UAVs can be easily adapted to different crop types and phenological stages. Additionally, UAVs require less human intervention for information collection. The use of multispectral cameras allows obtaining vegetation indices (VI) for the evaluation of different aspects of the crop, such as vigor through the normalized vegetation index (NDVI) (Zarco-Tejada et al., 2012), while the normalized difference red edge (NDRE) index evidence leaf wilting (Patrick et al., 2017). In addition, Rampazzo et al. (2022) used VI information to determine plant injury by pre-emergence herbicides. On the other hand, with UAVs large areas can be monitored quickly and repetitively (Sankaran et al., 2015), which significantly benefits high-throughput phenotyping research (Bendig et al., 2014), saving time and economic resources.

According to Campuzano-Duque and Buenaventura-Baron (2020), to achieve optimal phenotyping, it is necessary to significantly speed up the breeding processes to select genotypes with high yields, as well as tolerance to climate change to select those genotypes with the best production and fiber quality traits (Xu et al., 2019). Therefore, Araus and Cairns (2014) recognizes the importance of improvement in phenotyping characterization to obtain new high-yielding cultivars. However, factors that can affect data quality and make the results difficult to interpret are heterogeneous agroclimatic conditions and uncontrolled environments (Chinga et al., 2020).

This study focuses on evaluating agro-morphological traits with multispectral images acquired through a UAV to generate information for estimating the yield of 'Del Cerro' cotton. This will contribute to both phenotypic and genotypic research, aiming to develop new technology that excels not only in terms of production but also in fiber quality. This approach aims to provide a competitive and profitable option benefiting small and medium-scale producers in the Lambayeque region.

# MATERIALS AND METHODS

#### Study area

The study was conducted in an area of 0.2 ha at the Vista Florida Agricultural Experiment Station (EEA Vista Florida) of National Institute of Agrarian Innovation (INIA), in the district of Picsi, Chiclayo province, Lambayeque geographical region, Peru (Figure 1). The cotton (*Gossypium hirsutum* L.) season lasted 6 mo, from November 2021 to May 2022, presenting a minimum and maximum temperature of 15.7 and 28.1 °C respectively, relative humidity of 81% and precipitation of 4.4 mm, obtained from the Lambayeque meteorological station (Servicio Nacional de Meteorología e Hidrología del Perú - SENAMHI). The soil analysis was carried out in the Soil, Water, and Fertilizer Laboratory (LABSAF for its Spanish acronym) of the EEA Vista Florida. Results indicated this soil presents a loam soil (47% sand, 32% silt, 21% clay), electrical conductivity 4.15 mS cm<sup>-1</sup>, pH 7.30, organic matter 1.15%, 285 mg kg<sup>-1</sup> K, 6.18 mg kg<sup>-1</sup> P and active limestone (CaCO<sub>3</sub>) 2.30%.

A randomized complete block design was employed with 21 accessions with three random replicates, and bed dimensions of 1.5 m wide and 5 m long. In addition, 4 to 5 seeds were planted per plot with a separation of 0.3 m. The genotypes were provided by the National Program for Regional Cotton Crops of INIA. These genetic materials were collected in 2007.



**Figure 1.** Location of study area at the Vista Florida Agricultural Experimental Center in Lambayeque (Peru), EPSG:32171 zone 17S.

Five gravity fed irrigations were carried out distributed throughout the vegetative period of the crop, with critical phases for the use of water resources: The beginning of budding (33 d after sowing, DAS) and maximum flowering (61 DAS), corresponding to the second and third irrigation in which the first and second fertilization of the crop was conducted, respectively. The NPK edaphic fertilization dosages were 180-100-150, and foliar applications that complemented soil fertilization, especially in the reproductive phase with the application of Ca, B and K, to provide weight and quality of the harvestable organ.

### Agro-morphological characterization

The agro-morphological characterization of height and length between nodes were carried out manually using a ruler. Likewise, the number of nodes, fruit branches, vegetative branches and axillary stem branches was counted in five random plants per accession. However, for performance, 10 plants per accession were evaluated, with the following equation:

Number of plants = 
$$\frac{10000}{\text{row spacing} \times \text{plant spacing}} \times 2$$
  
Yield = Number of plants  $\times \text{N}^{\circ} \frac{\text{organ}}{\text{plant}} \times \text{lint weight}$ 

Agro-morphological traits (plant height, nodal position, internode length, nodes, fruiting branches, vegetative branches, axillary buds) were evaluated following the protocol described by the Institute of Agrarian Development of Lambayeque (IDAL for its Spanish acronym) established in the manual of agrophysical management of extra-long fiber varieties of cotton from the central and northern coast of the National Cotton Institute (IPA for its Spanish acronym) (IDAL, 2010). These traits were assessed in order to correlate them with vegetation indices for performance estimation.

## Acquisition and processing of multispectral information

**Multispectral monitoring using remote sensors.** Aerial multispectral images of the fields were acquired using an unmanned aerial vehicle (UAV) Matrice 300 RTK quadcopter (DJI, Shenzhen, China) carrying as payload a Dual RedEdge+Blue multispectral camera (MicaSense, Seattle, Washington, USA) calibrated prior to flight with the panel AIRNOV reflectance. In addition, it has 10 global shutter camera modules that provide images in 10 bands: Blue (444 and 475 nm), green (560 and 650 nm), red (650 and 668 nm), near infrared (NIR, 842 nm) and red edge (RE, 705, 717 and 740 nm) and a low precision GPS (2.5 m) that can record the geographic coordinates of the image. The band image size was 1280×800 pixels.

To collect the multispectral aerial image data, an automated flight of UAVs was programmed with a flight path preset at a speed of 2.5 m s<sup>-1</sup> and at a height of 35 m above ground level (AGL), achieving a sample resolution from the ground 2.5 cm for multispectral images. The lateral and frontal overlap of the collected images was 80%, during the nine monitoring periods, with flight hours ranging from 11:00 to 13:00 h.

**Multispectral processing.** Multispectral images were employed to calculate vegetation indices in each monitoring. The data processing flow consists of the following three steps: (i) Creation of the reflectance maps, for which the photogrammetry software Pix4Dmapper (V4.8.0, Pix4D S.A., Prilly, Switzerland) was used; (ii) alignment of the map's reflectance generated using ArcGIS software (ArcMap 15.1; Esri, Redlands, California, USA); for each band image of the reflectance maps, confirming that they coincide. Additionally, fixed projective transformation of maps based on common point locations, to finally align pixel by pixel. (iii) Segmentation of the accessions, to obtain accurate information, the K-Means Clustering for Grids module was used in QGIS (3.22.3) (QGIS Geographic Information System, London, UK) to group the pixels into two classes (soil and vegetation) using the combined method minimum distance and simple scaling as indicated by García-Martínez et al. (2020).

## Calculation of vegetation indices

In the ArcGIS software (ArcMap 15.1), the calculation of vegetation indices was carried out for each accession by monitoring; these indices are derived from the bands of the multispectral images. In the present study, the vegetative indices calculated are described in Table 1. In the calculation, for the SAVI index, a factor L = 0.20 was used based on the evaluations reported by Candiago et al. (2015), Xun et al. (2021) and Dhakal et al. (2022), where ranges between 0.10 to 0.25 are recommended for cotton and sorghum crops, among others.

Indices	Equation	Source					
Normalized difference vegetation index (NDVI)	$NDVI = \frac{NIR - Red}{NIR + Red}$	Peñuelas et al., 1993					
Normalized difference red edge index (NDRE)	$NDRE = \frac{NIR - RedEdge}{NIR + RedEdge}$	Gitelson and Merzlyak, 1994					
Soil adjusted vegetation index (SAVI)	$SAVI = \frac{(NIR - Red)(1 + L)}{NIR + Red + L}$	Huete, 1988					
Normalized difference water index (NDWI)	$NDWI = \frac{Green - NIR}{Green + NIR}$	Xu et al., 2019					
Ratio vegetation index (RVI)	$RVI = \frac{NIR}{Red}$	Feng et al., 2020					

## Table 1. Vegetation indices applied for cotton evaluation.

## Characterization of spectral signatures

Spectral signatures are unique to each plant species and are influenced by the composition and concentration of leaf pigments, as well as other characteristics of plant cells (Falcioni et al., 2020). The spectral signatures were obtained from the geographic information systems tools, using the open-source software QGIS v.3.22.3 in its Semi-Automatic Classification Plugin. Polygons of the 21 accessions of cotton were prepared with a cut in the 10 bands of the electromagnetic spectrum available in the Micasense RedEdge-MX Dual camera, from which the spectral signatures defined as "the differential behavior presented by reflected radiation – reflectance or emitted from some type of surface or terrestrial object" (Falcioni et al., 2020).

## Data analysis

The r-Pearson correlation was employed to measure the linear correlation between cotton yield and vegetation indices. We also used the corrplot package in R (R Foundation for Statistical Computing, Vienna, Austria) to visualize the correlation matrix. In addition, a principal component analysis (PCA) was conducted with the R libraries factoMineR (Lê et al., 2008) and factoextra (Kassambara and Mundt, 2020) in order to determine the most relevant index.

# **RESULTS AND DISCUSSION**

#### Agro-morphological parameters

The collection of agro-morphological trait data was obtained during the beginning of the opening of reproductive organs at 128 DAS, obtaining an average height of 1.25 m and number of 16 fruit branches; However, the accessions that stand out the most in this parameter were: 47, 82, 86, 89 and 100 (Table 2), while in performance, accession 33 was the most promising. However, the earliest was accession 111.

Table 2. Agro-morphological characteristics of cotton obtained *in situ* at 128 d after sowing (DAS).

	Plant	Nodal	Internode		Fruiting	Vegetative	Axillary
Cotton accessions	height	position	length	Nodes	branches	branches	buds
	cm		cm	Nr	Nr	Nr	Nr
35	1.19	5.00	7.00	17.00	13.00	3.00	7.00
36	1.23	6.00	6.00	20.00	15.00	2.00	2.00
37	1.29	5.00	6.60	22.00	17.00	2.00	1.00
47	1.24	5.00	5.60	22.00	18.00	2.00	1.00
48	1.19	5.00	6.60	20.00	15.00	2.00	8.00
49	1.19	6.00	6.40	20.00	16.00	2.00	10.00
59	1.23	5.00	6.00	20.00	15.00	2.00	8.00
63	1.20	6.00	7.20	22.00	16.00	2.00	8.00
69	1.22	5.00	7.20	19.00	14.00	1.00	9.00
70	1.21	5.00	6.00	20.00	16.00	2.00	3.00
73	1.23	6.00	6.20	20.00	15.00	3.00	7.00
82	1.35	5.00	6.40	22.00	18.00	2.00	1.00
83	1.24	5.00	6.80	21.00	16.00	1.00	9.00
86	1.35	5.00	5.80	22.00	18.00	1.00	2.00
89	1.26	6.00	5.80	22.00	18.00	2.00	3.00
90	1.27	6.00	7.00	19.00	14.00	1.00	9.00
94	1.13	6.00	6.00	20.00	16.00	1.00	5.00
97	1.31	6.00	5.80	23.00	18.00	1.00	1.00
98	1.20	5.00	6.80	21.00	17.00	1.00	2.00
100	1.34	5.00	6.60	22.00	18.00	2.00	4.00
111	1.28	6.00	8.20	21.00	16.00	2.00	8.00
Average	1.25	5.00	6.48	21.00	16.00	2.00	5.00
Standard dev.	0.06	0.26	0.63	1.37	1.48	0.54	3.37

#### **Evaluation of vegetation indices**

Figure 2 shows the NDVI, SAVI, NDRE indices that fluctuate in the following ranges, the first presents values above 0.8, SAVI varies between 0.6 to 0.7 and NDRE in the range of 0.4 to 0.5. In the first case, the NDVI seems to overestimate the health values and presents values above 0.8, which are indicators of good plant health despite the presence of pests (aphids) and phytotoxicities observed during the fifth monitoring (107 DAS). The SAVI and NDRE indices present the lowest values; the first one has an adjustment value of L = 0.2, suggesting to differentiate the effect of the soil on the crop, and the second index uses reflectance values from the RED EDGE band, which would be more susceptible in this crop for health analysis. Likewise, with respect to the adjustment value L = 0.2 of SAVI, correlations greater than 0.6 were obtained, which improves the results obtained by Candiago et al. (2015) and Dhakal et al. (2022), in which Pearson r correlations lower than 0.5 were obtained, complementing the results of Xu et al. (2019) who indicate that the NIR band would be sufficient for

the differentiation of progenies, but not for health monitoring. This technology contemplates contributions to improve the prediction of growth and development parameters. Feng et al. (2020) mentioned that the NDVI and the RVI index do include considerations of health status and vigor in their estimation, which makes it necessary to carry out an isolated study of water stress and its effect using remote monitoring analysis techniques. In addition, Ashapure et al. (2020) obtained yield prediction correlations greater than 0.7 using vegetative indices at 70 DAS, which is consistent with our results obtained for correlations of RVI, NDVI and SAVI index for stages between 70 and 150 DAS.



**Figure 2**. Comparison of vegetation indices according to days after sowing (DAS): Normalized difference vegetation index (NDVI) (a), soil adjusted vegetation index (SAVI) (b), normalized difference red edge index (NDRE) (c), normalized difference water index (NDWI) (d) and ratio vegetation index (RVI)(e).

## Crop yield prediction model

Figure 3 shows the r-Pearson correlation between vegetation indices and crop yield by monitoring date. The greatest correlation between crop yield and vegetation indices occurs at 73 and 87 DAS with 0.70 and 0.66 respectively with the NDRE index, while the SAVI (0.41) and RVI (0.20) indices present a correlation of medium importance for the fourth monitoring (94 DAS); Likewise, a low correlation is observed in the last monitoring (162 DAS). On the other hand, correlations inversely proportional to performance are shown in NDWI (-0.62) and SAVI (-0.56) at 73 and 130 DAS.



**Figure 3.** r-Pearson between cotton yield and vegetation indices: 37 (a), 73 (b), 87 (c), 94 (d), 107 (e), 114 (f), 121 (g), 130 (h) and 162 d after sowing (DAS) (i). NDWI: Normalized difference water index; RVI: ratio vegetation index; SAVI: soil adjusted vegetation index; NDVI: normalized difference vegetation index; NDRE: normalized difference red edge index.

The results of the PCA analysis are presented in Figure 4 corresponding to the seventh monitoring (121 DAS), because on that date the agro-morphological parameters were obtained for each accession. Figure 4 shows the relationship of the evaluated traits with the indices NDVI, SAVI, NDRE, RVI and NDWI, reporting significant variations between the agro-morphological traits and the NDWI index, however, it is inversely proportional to the NDVI and RIV indices.



**Figure 4.** Principal component analysis for vegetation indices and agro-morphological traits of cotton at 121 d after sowing (DAS). NDWI: Normalized difference water index; RVI: ratio vegetation index; SAVI: soil adjusted vegetation index; NDVI: normalized difference vegetation index; NDRE: normalized difference red edge index.

This study correlated agro-morphological parameters of cotton with vegetation indices obtained from multispectral UAV images throughout the vegetative period of this crop. One of the main advantages of using UAVs is the adaptability of these remote sensors to different stages of the vegetative period of crops. In addition, they can be controlled remotely (Shi et al., 2016) covering large areas (Xu et al., 2019). Basinger et al. (2020) suggested that indices based on NIR and VIS bands allow differentiation of cover type and, to a lesser extent, plant phenotypes of the same species at a multispectral monitoring scale. Here, we obtained very significant values for the vegetative indices (SAVI, NDVI, RVI, NDRE and NDWI) and yield of cotton, with an r-Pearson correlation value of 0.98 for the case of the RVI in mature stages of the crop, and values greater than 0.90 during all monitoring dates. Similarly, we obtained values greater than 0.90 for NDVI index, which is consistent with a speck count prediction study developed by Feng et al. (2020) where correlation values of 0.84 were obtained with respect to the morphological variables and correlations of up to 0.83 were reached for plant height estimation.

Haghverdi et al. (2018) used similar vegetative indices with satellite images, obtaining correlations of 0.60 to 0.84 for the estimation of yields and crop heights using artificial neural network (ANN) techniques. In a more recent work, Siegfried et al. (2023) obtained correlations of 0.70 using multiple regression models. It is necessary to continue with the development of accurate models for predicting yields and variables that will amplify the scope and impact of studies on this type of techniques for the development and selection of promising progenies, as is the case in Brazil where remote techniques were used for plant height and biomass predictive frameworks, reaching correlations of 0.87 (Silva et al., 2023).

The correlation analysis between cotton yield and the vegetation indices was conducted at 130 DAS as a greater correlation was achieved. Indices NDVI, RVI, NDRE and NDWI possess a positive correlation of 0.69,

0.72, 0.72 and 0.74, respectively (Figure 5.) Likewise, these indices presented a better coefficient of determination (r<sup>2</sup>) for crop yield and the following indices: NDVI (0.47), RVI (0.52), NDRE (0.52) and NDWI (0.54). Alcântara et al. (2023) mentioned that quantitative values in crop yield are influenced by the environment, which explains the moderate r<sup>2</sup> obtained in this work. These authors also indicated that maize cultivation has a greater linear correlation with yield when it has higher values in height and vegetation indices. Additionally, potentially commercial genotypes can be identified when characters with high heritability present a linear correlation with performance (Ribeiro et al., 2010), thus reducing economic expenses such as labor, equipment purchase, and selection time in breeding programs (Samecima Junior, 2018; Osco et al., 2020; Alcántara et al., 2023). On the other hand, Osco et al. (2020) employed a random forest algorithm and UAV-based multispectral imagery to predict leaf N concentration and plant height of maize and concluded that this approach is appropriate to predict both agronomic variables in maize.



**Figure 5**. Linear regression equation between five vegetation indices and yield of cotton assessed at 130 d after sowing (DAS). NDVI: Normalized difference vegetation index; SAVI: soil adjusted vegetation index; RVI: ratio vegetation index; RVI: relative vigor index; NDRE: normalized difference red edge index; NDWI: normalized difference water index.

#### Spectral signatures

The spectral signatures showed the reflectance values in 10 multispectral bands, where the historical performance was analyzed in six moments of the growth period of the 'Del Cerro' cotton crop. Figure 6 depicts the values of the electromagnetic spectrum, observing homogeneity in the coastal aerosol and blue bands which present minimum reflectance values. In the green band we see a greater reflectance at 42 d and it decreases as the development of the crop progresses, which reflects a greater concentration of chlorophyll in the leaves. In the red band (668 nm) we can see a homogeneity in the evaluations carried out after 78 DAS, which is related to the previously evaluated indices that present low variation. Finally, in the NIR band, we can see at 42 DAS that it presents lower reflectance values and subsequently increases until day 99 and 119, which show its highest values and then gradually decreases until day 180, which is where the cotton plantation enters a period of boll filling and maturation.



Figure 6. Spectral sign of del Cerro cotton cultivar. DAS: Days after sowing.

Cotton breeding work heavily depends on the availability of good quality of phenotypic data (Pabuayon et al., 2019). According to Dhakal et al. (2022), cotton phenotypic studies are based on broadband- and narrowband-based vegetation indices using satellite imagery or near-surface sensing platforms (Thenkabail et al., 2000; Broge and Mortensen, 2002; Zhao et al., 2007). However, information on the prediction of the canopy area and plant density of cotton is still limited (Pabuayon et al., 2019: Dhakal et al., 2022). The characterization of the spectral signature by cotton cultivar may allow correlate the results obtained in this work in detecting morphological variables according to cultivar and cotton crop family. The confusion matrix indicated a low variability in the spectral bands evaluated on different dates for the 'Del Cerro' cotton progenies, and together with additional morphological characterization conducted in laboratories, this matrix can complement the information from remote monitoring.

# CONCLUSIONS

The application of unmanned aerial vehicles (UAVs) implies an increase in efficiency in the agro-morphological characterization process, reducing time in obtaining parameters in the field. The UAVs in conjunction with multispectral cameras are very useful tools for calculating vegetation indices, which allow monitoring of crop health. In addition, a significant correlation was found between the normalized difference water index (NDWI) and yield. The performance showed significant correlations with the normalized difference red edge (NDRE) index (0.7) during the first multispectral monitoring. The difference in the spectral response between the soil,

leaves and flowers shows a potential for the detection of flowers through multispectral images, which can be used for multi-temporal monitoring in the flowering stage, allowing the measurement of traits of great importance for 'Del Cerro' cotton crop.

#### Author contribution

Conceptualization: C.C-G., M.D., M.N. Methodology: M.N., M.D. Software: C.C-G, E.V, M.D. Validation: C.C-G., M.D., E.V. Formal analysis: E.V., C.C-G. Investigation: C.C-G., M.N., E.V. Resources: C.A., W.S. Data curation: C.C-G, E.V., A.M. Writing-original draft: C.C-G., M.D. Writing-review & editing: A.M., C.A., C.C-G. Visualization: C.C-G. Supervision: C.A. Project administration: W.S. Funding acquisition: C.A., W.S. All co-authors reviewed the final version and approved the manuscript before submission.

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