

High-throughput Phenotyping as an Auxiliary Tool for Watermelon Germplasm Accession Selection

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Abstract

Watermelon cultivation plays an important socioeconomic role in Brazil. Despite all efforts, in genetic improvement programs, selection often involves a limited number of accessions due to complex cultivation requirements for this species, including large evaluation areas. The requirement of large areas results in increased costs and time of evaluations using conventional methodologies. In this context, we assessed the use of imaging to optimize resources and field evaluation time. The study aimed to use image phenotyping with various vegetation indices for watermelon germplasm. In this study, we evaluated 118 watermelon genotypes. In-field manual measurements included the SPAD index of leaves and average Brix and weight of fruits. To determine the potential use of imaging in phenotyping, four vegetation indices –NGRDI, GLI, SAVI, and NDVI – were studied. The germplasm exhibited genetic dissimilarity and the agronomic performance was monitored using images. The NGRDI, NDVI, SAVI and GLI indices remarkably correlated with the BRIX variable and SPAD index, even in germplasms with high dissimilarity. In addition, remote sensing was used to monitor field parameters that were not directly related to the leaf. The NGRDI, NGRDI and SAVI indices were sensitive in capturing this indirect relationship with the BRIX variable.

1 Introduction

Watermelon cultivation (*Citrullus lanatus* L.) represents a significant segment of Brazilian agribusiness, being the third most produced fruit in the country, with a production of 2,240,796 tons on a harvested area of 101,975 hectares [1]. Given this high demand, research has sought alternatives to increase productivity and reduce production costs [2, 3, 4]. Additionally, it is worth noting that watermelon plays an important role in the diet of adults and children, contributing to the supply of potassium, magnesium, vitamin C, vitamin A, folate, and fiber, in addition to exhibiting high bioavailability of antioxidant components, including lycopene and L-citrulline [5].

Despite the crop's potential, progress in obtaining new cultivars is slow due to its complex genetics and the need for extensive production areas during selection. These obstacles may limit the number of accessions evaluated in a germplasm bank. Traditionally, research has prioritized assessing agronomic traits and fruit quality in watermelon, particularly average fruit weight and °Brix, respectively [6, 7]. Additionally, chlorophyll content has been frequently used in field evaluations due to its potential for indirect selection for productivity [8, 9]. For this purpose, field response variables for chlorophyll are traditionally obtained using meters that indicate nitrogen (N) or chlorophyll a and b content in leaves, with the MINOLTA SPAD-502 device being commonly employed.

Several studies have sought to understand and associate crop variability behavior through the relationship between images obtained by remotely piloted aircraft (RPA) and field nutritional data [10, 11, 12]. In recent decades, remote sensing applications have expanded, aiming to use reflectance data to extract agricultural variables and monitor fields [13]. In experiments with vegetables (*Lactuca sativa*, *Cucurbita pepo*, and *Zea mays* subsp. *saccharata*), RPA has been efficiently used to identify superior

genotypes, with consistent results between vegetation indices and field-collected data [14–15, 16, 17, 18]. Among many indicators, leaf area and chlorophyll are directly related to crop productivity [19, 20]. However, few studies correlate these parameters indirectly and non-destructively with spectral responses from vegetation indices in watermelon crops.

In this context, the objective of this study was to evaluate the use of different vegetation indices as a tool for monitoring agronomic performance in watermelon germplasm.

2 Material and methods

The experiment was conducted at the Experimental Vegetable Station of the Federal University of Uberlândia (UFU), in the municipality of Monte Carmelo, Minas Gerais, Brazil, with geolocation at latitude 18° 42' 43" S and longitude 47° 29' 58" W (Fig. 1).

The experiment was arranged in a randomized block design (RBD), with 118 watermelon genotypes evaluated. Each experimental plot consisted of four plants. For each plot, the following parameters were measured: Soluble solids concentration (SS) of the fruits, expressed in °Brix (26°C), using a portable digital refractometer (Atago PAL-1 3810); SPAD index (Soil Plant Analysis Development), measured with a chlorophyll meter (Minolta SPAD-502 CFL1030); Average fruit weight (kg).

The methodological procedures carried out in this study are presented in the flowchart of Fig. 2.

For the acquisition of aerial data, a flight was conducted fifty days after planting (DAP) using a Phantom 4 Pro UAV equipped with a 20-megapixel RGB camera and an onboard Mapir Survey3 camera. The autonomous flight was performed using DroneDeploy software at an altitude of twenty meters, with 80% frontal overlap and 75% side overlap, resulting in a GSD of 1.5 cm/pixel. Four vegetation indices (VIs) were used in this study (Table 1).

Table 1
Vegetation indices used in the study

Vegetation indices	Equation	Reference
Normalized Green Red Difference Index (NGRDI)	$\frac{G - R}{G + R}$	[21]
Green Leaf Index (GLI)	$\frac{2 \times G - R - B}{2 \times G + R + B}$	[22]
Soil-adjusted Vegetation Index (SAVI)	$\frac{NIR - R}{NIR + R} \times \frac{1}{1 - \frac{R}{G}}$	[23]
Normalized Difference Vegetation Index (NDVI)	$\frac{NIR - R}{NIR + R}$	[24]

For each experimental plot, radiometric values of the indices were calculated and extracted using ENVI Classic software. These values were then integrated with the vector data of the experimental plots (in

shapefile format) and combined with field data into an .xls spreadsheet using MiniTab 18 software. For spatial analysis, association, and classification of geographic data, ENVI Classic and ArcMap 10.5 software were employed.

The data underwent multivariate analysis to determine dissimilarity among response variables. The dissimilarity matrix was obtained using the Mahalanobis distance matrix (D^2_{ii}) and represented by a dendrogram generated through the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), validated by the cophenetic correlation coefficient. Multivariate analyses were performed using Genes software v.2015.5.0 [25].

3 Results

Vegetation indices showed a significant positive correlation with SPAD and BRIX data, while no significant correlation was observed with mean fruit weight, as assessed by Pearson's correlation coefficient (Fig. 3).

The observed results can be attributed to the low p-values for the response variables SPAD and BRIX, and the high p-value for mean fruit weight data, considering a 5% significance level. The highest correlation values were obtained for the NGRDI and NDVI indices in relation to SPAD (Fig. 3). In contrast, mean fruit weight was not a sensitive variable detectable by vegetation indices. Therefore, to understand the behavior of vegetation indices in response to these variables, statistical regression models were generated (Fig. 4).

Para a variável BRIX foram observados baixos coeficientes de determinação para os índices de vegetação SAVI, NGRDI, NDVI, and GLI (42.3, 31.7, 41.1 e 29.8%).

Regarding the SPAD index variable, the models showed linear behavior for the SAVI, NGRDI, NDVI, and GLI indices (Fig. 5).

The coefficient of determination (R^2) for the response variables showed better performance for the NDVI ($R^2 = 68.1\%$) and NGRDI ($R^2 = 66.4\%$) indices. All models exhibited an increasing linear behavior, indicating that as vegetation index values increase, SPAD index values also increase. Additionally, numerous studies report significant relationships between this variable and digital aerial imagery.

Genetic dissimilarity among experimental treatments was assessed and validated, as shown in the dendrogram (Figs. 6A and 6B).

The cophenetic correlation coefficient was 0.931 (t-test, $p < 0.05$). Thus, the dendrogram effectively represented the matrix data and subsequent clusters with significant results. Group separation was determined by a cutoff line set at 28%, established at the point of abrupt branch change incidence (Fig. 6A).

Consequently, twenty-three distinct groups were formed. The group formation (Fig. 6A) indicates that the evaluated germplasm in the experiment indeed possesses genetic dissimilarity, providing an optimal environment for validating the different vegetation indices.

The variable that contributed most significantly (95%) to the genetic dissimilarity shown in the dendrogram (Fig. 6A) was the SPAD index. This variable is considered an important predictor of various agronomic traits in multiple cultivated species. Given its importance in watermelon germplasm evaluation, the top fifty accessions for SPAD index were selected based on mean values, and a heatmap was generated (Fig. 6B). Notably, even when assessing a reduced number of accessions (50 accessions), the genetic dissimilarity was maintained. The branching patterns for response variables indicate similarity among SPAD, NDVI, SAVI, and GLI variables (Fig. 6B).

The vegetation index values enabled the identification of five distinct detection classes (Fig. 7).

The white coloration indicates absence of vegetation and low vegetative vigor, while darker green tones represent higher vegetative vigor. Visual analysis revealed that the NDVI, NGRDI, SAVI, and GLI indices successfully expressed plot dissimilarity at varying magnitudes. The NDVI and NGRDI indices exhibited more significant color contrast variations, corroborating the stronger correlations shown for these indices in Fig. 3. All vegetation indices were capable of detecting planting gaps or complete plant absence (Fig. 8).

4 Discussion

All vegetation indices employed successfully detected plant absence within planting rows across comparable regions. While variable correlations were not universally significant, these indices demonstrate considerable potential for identifying planting gaps. The observed correlations between vegetation indices and SPAD values can be partially explained by the spectral sensitivities of each index.

The green and red spectral bands effectively serve as plant growth and development indicators [26], the SPAD was designed to capture differences in green vegetation vigor, through the red and infra-red spectral bands, making it particularly sensitive to chlorophyll content. Interestingly, the NGRDI, though based solely on visible light, has shown potential for chlorophyll estimation and was strongly correlated with SPAD. This occurs probably because it was designed to capture differences in green vegetation vigor, making it particularly sensitive to chlorophyll content. Therefore, the NGRDI shows particular promise for estimating vegetation fraction, green biomass, leaf chlorophyll variations, and plant phenology [27].

The NDVI, on other hand, utilizes red and near-infrared wavelengths, which are also the basis for SPAD readings, justifying the strong relationship observed. SPAD index values correlate indirectly with plant nitrogen content - itself linked to chlorophyll levels - a parameter that NDVI detects sensitively through vegetation vigor variations [24]. However, NDVI tends to saturate in crops with dense canopies due to

overlapping leaves. In watermelon, whose canopy architecture is less vertically layered, this saturation effect may be reduced, allowing NDVI to maintain correlation even in mature stages.

Like NDVI, the SAVI index is effective in detecting vegetation however, it was specifically developed to minimize the influence of background soil in areas with sparse vegetation by incorporating a soil adjustment factor (L). Although the value of L is often set arbitrarily, when it approaches zero, SAVI behaves similarly to NDVI, which explains the comparable performance observed in this study.

GLI presented the weakest association with the SPAD, probably due the inclusion of the blue band makes it less directly related to the red/NIR range used by SPAD. However, the GLI index produce robust results for leaf chlorophyll variation, serving as an effective vegetation degradation indicator [31]. Supporting studies include [32], who achieved strong SPAD value predictions in corn ($R^2 = 75.0\%$, RMSE = 5.57) using aerial imagery, and [4], who obtained superior results for naked barley ($R^2 = 76.0\%$, RMSE = 3.65) using UAV-based vegetation indices.

Notably, the indices also responded to BRIX values, suggesting that fruit chemical properties (measured indirectly rather than through leaf data) may influence spectral responses [18, 29, 30]. These vegetation indices show strong potential as agronomic performance predictors in watermelon germplasm. High-throughput screening offers particular advantages - being cost-effective, non-destructive, and time-efficient [14–15, 16, 17] - that could significantly enhance watermelon cultivar development.

Most agricultural vegetation indices derive from visible and near-infrared spectral bands that directly correlate with vegetation vigor, facilitating degraded area detection [35, 36]. Our findings establish a foundation for image-based phenotyping in watermelon, with NGRDI, NDVI, SAVI and GLI indices showing particular promise for breeding programs targeting enhanced BRIX and SPAD traits.

5 Conclusion

The agronomic performance of watermelon germplasm can be effectively monitored using imaging techniques. The NGRDI, NDVI, SAVI, and GLI vegetation indices demonstrated significant correlations with both BRIX and SPAD variables, even in environments with high genetic dissimilarity. Remote sensing enables the monitoring of field parameters that, while not directly related to leaf characteristics, indirectly influence vegetation spectral responses. Notably, the NGRDI, NDVI, and SAVI indices proved particularly sensitive in detecting these indirect relationships with the BRIX variable.

Declarations

Funding Declaration in the manuscript

No funding was received for conducting this study.

Ethics, Consent to Participate, and Consent to Publish declarations: not applicable.

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Author Contribution

Pablo Henrique de Souza Assis: Data curation, Formal analysis, Investigation , Methodology, Software and Validation. Rodrigo B Gallis: Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Conceptualization, Visualization, Writing - original draft and Writing - review & editing. Gabriel Mascarenhas Maciel: Conceptualization Formal analysis, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft and Writing - review & editing . Ana Carolina Silva Siquieroli : Visualization, Writing - original draft and Writing - review & editing. Tamer Shamseldin: Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing - original draft and Writing - review & editing .Margareth Evangelista: Validation, Visualization, Writing - original draft and Writing - review & editing .. Reginaldo M de Oliveira: Validation, Visualization, Writing - original draft and Writing - review & editing . Ana Luisa Alves Ribeiro: Formal analysis, Investigation, Methodology, Software, Visualization, Writing - original draft and Writing - review & editing. Camila Soares de Oliveira: ormal analysis, Investigation, Methodology, Software, Visualization, Writing - original draft and Writing - review & editing.

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Figures

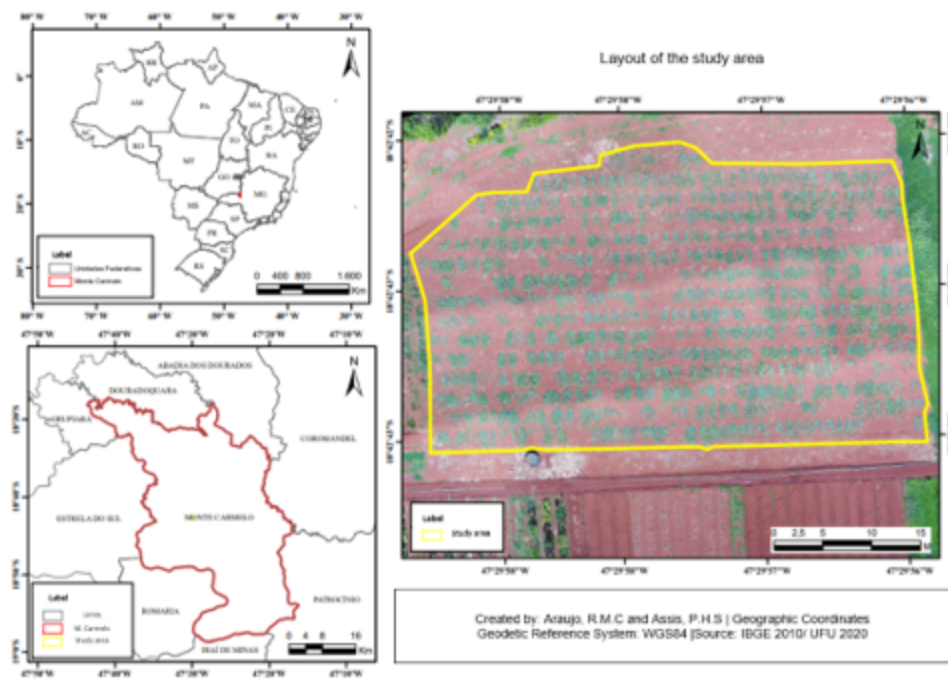


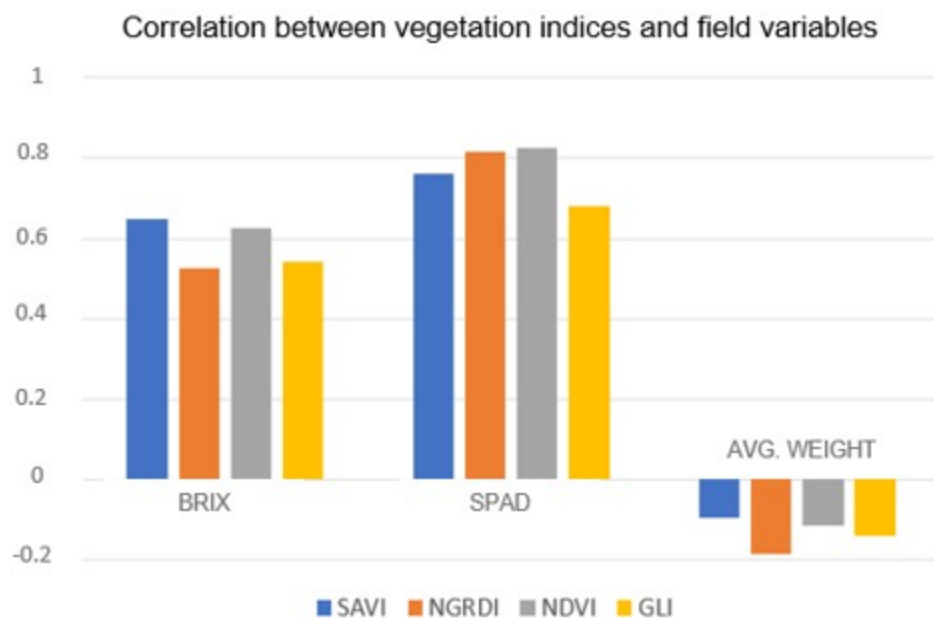
Figure 1

Location of the study area



Figure 2

Flowchart of the methodological procedures



Index	Brix	SPAD	AVG. WEIGHT	STATISTIC
SAVI	0.648	0.762	-0.094	Pearson coefficient
	0.000	0.000	0.390	P. value (<0.05)
NGRDI	0.525	0.815	-0.185	
	0.000	0.000	0.300	
NDVI	0.627	0.825	-0.113	
	0.000	0.000	0.427	
GLI	0.543	0.682	-0.141	
	0.000	0.000	0.194	

Figure 3

Pearson’s correlation coefficient and p-value between vegetation indices and field variables

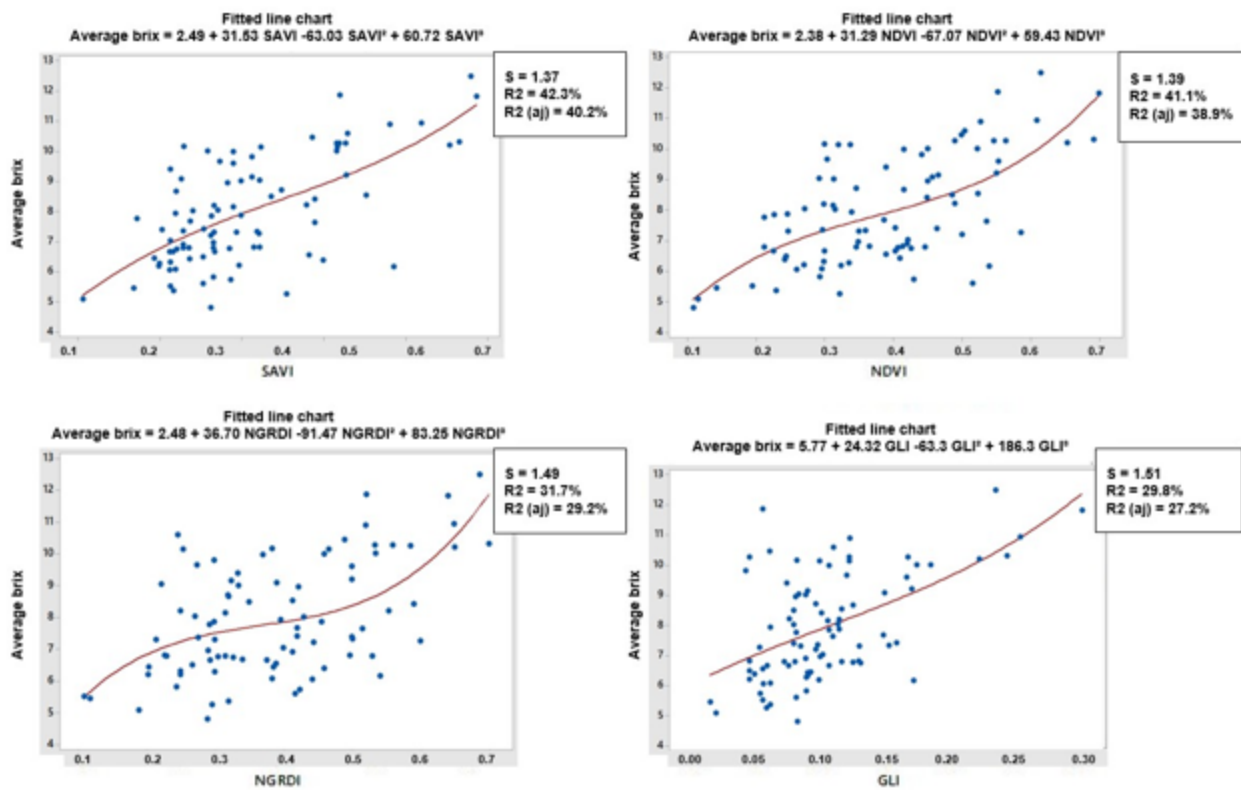


Figure 4

Regression models between vegetation indices and the BRIX Variable

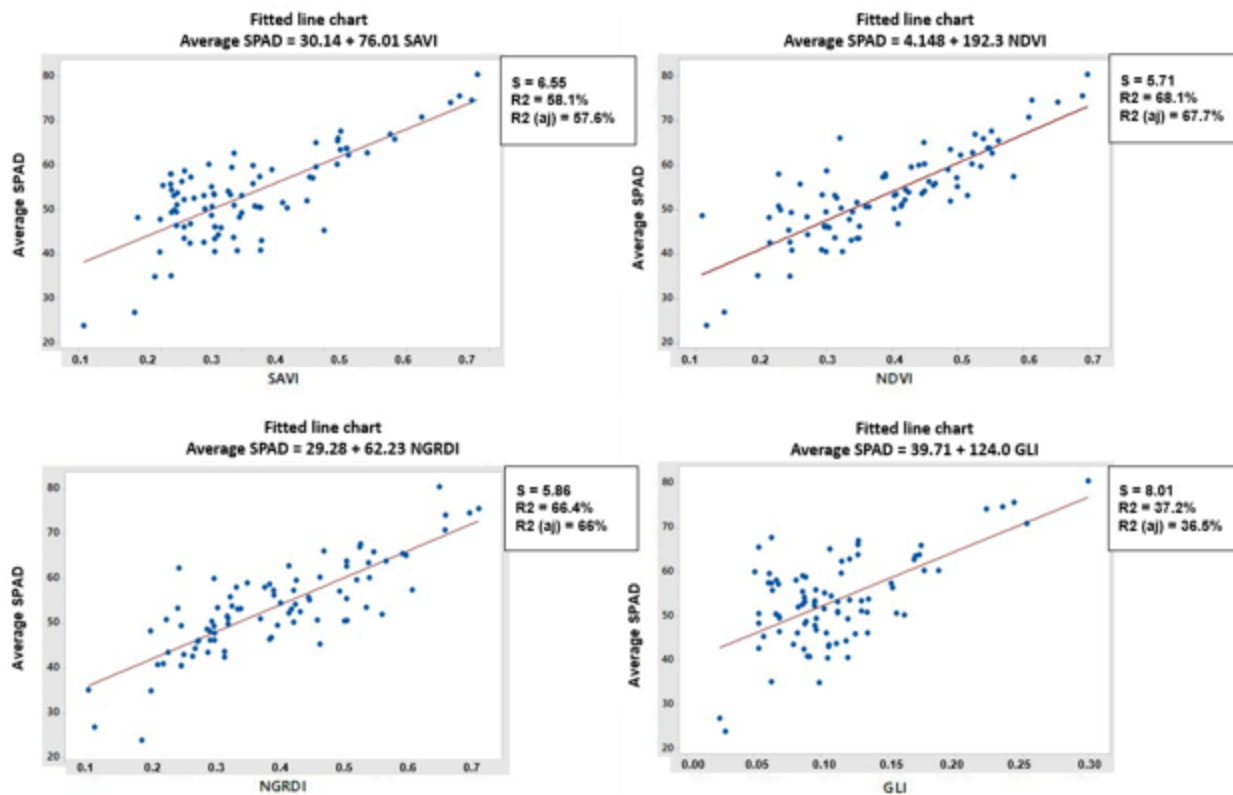


Figure 5

Regression models between vegetation indices and the SPAD index variable.

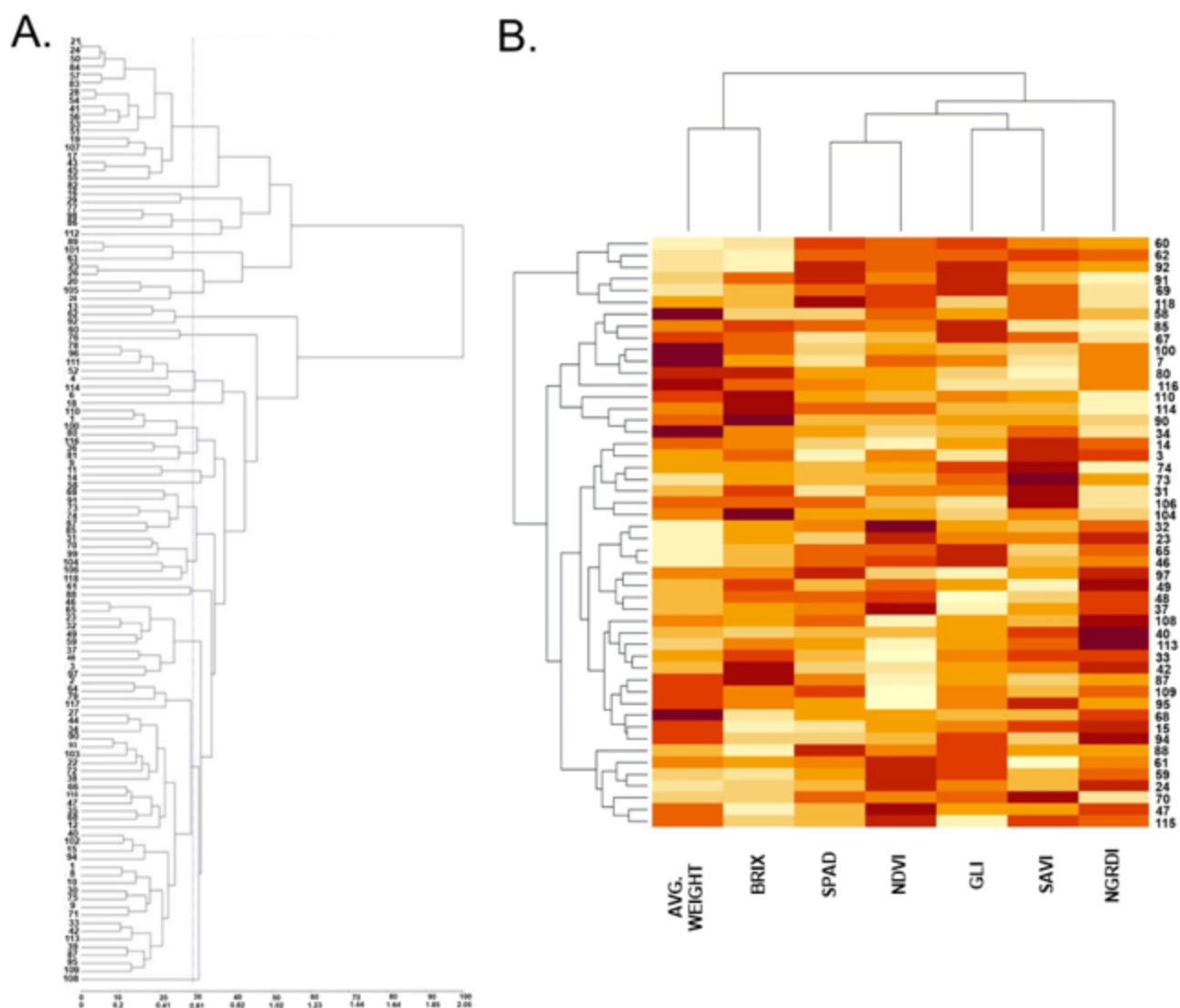


Figure 6

A) Dendrogram representing genetic dissimilarity among 118 watermelon genotypes, obtained by the UPGMA method using the generalized Mahalanobis matrix. B) Heatmap containing the top 50 genotypes for chlorophyll/SPAD content, BRX, mean weight, and vegetation indices

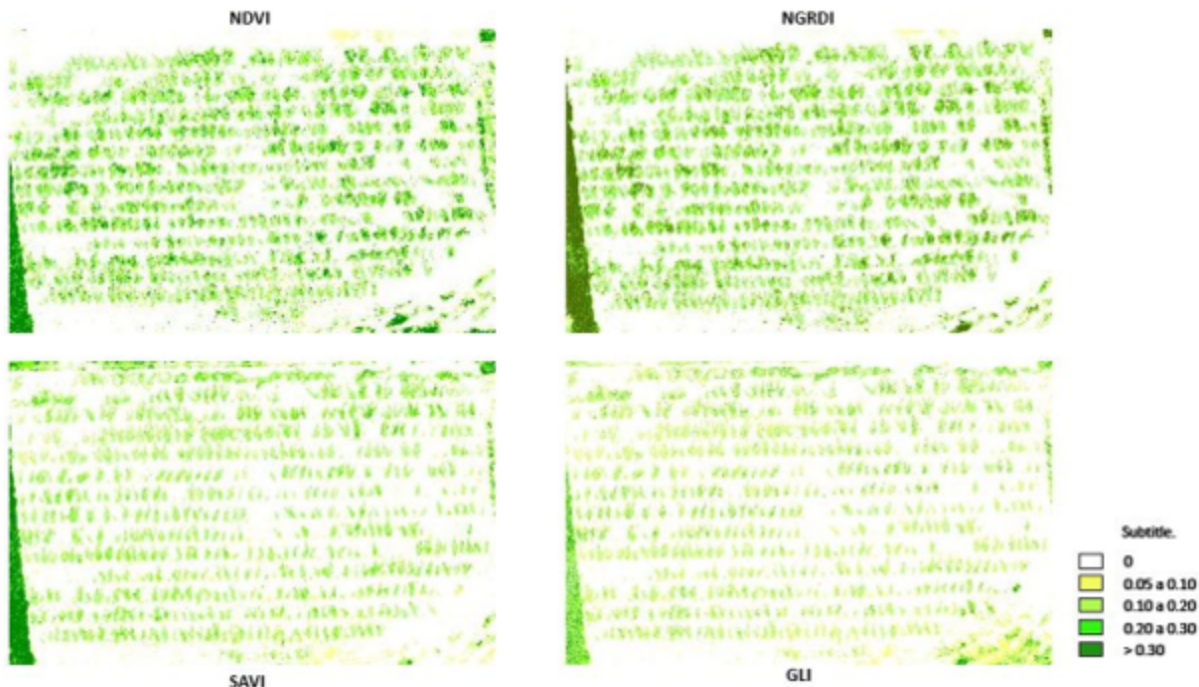


Figure 7

Vegetation indices in the experimental area.

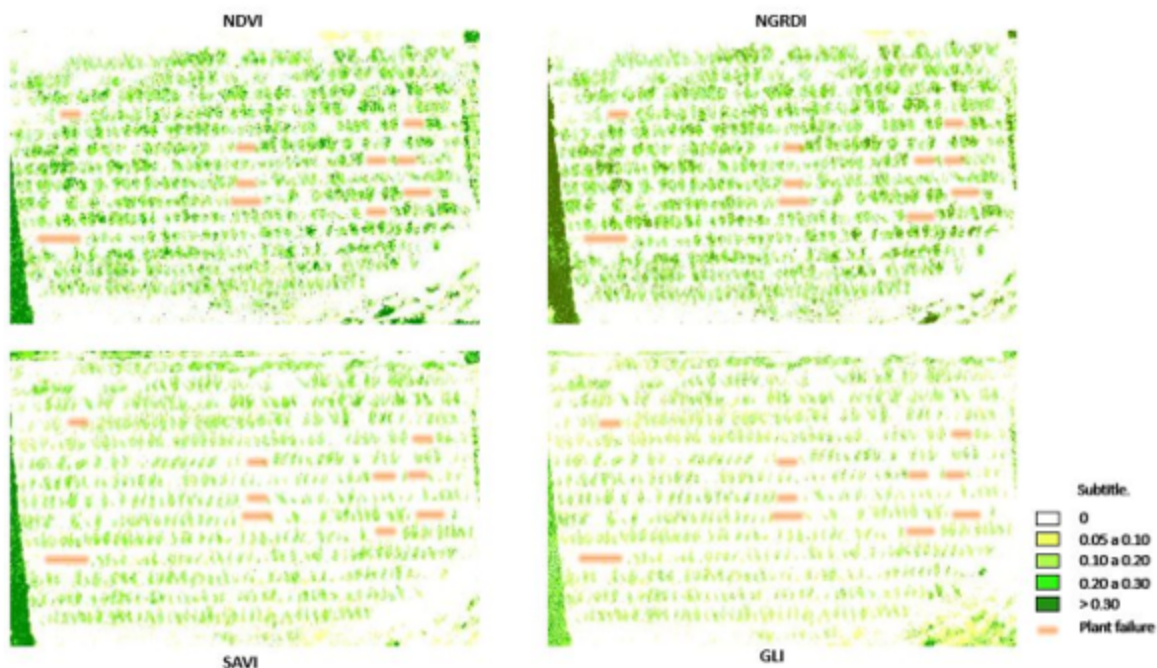


Figure 8

Planting gaps detected in the experimental area through vegetation indices.