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Sensors Applied to Plant Breeding: Leaf Reflectance Indices (LRIs) and Resistance to Anthracnose in *Capsicum annuum* L. var. *annuum*

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Abstract

The present work investigated the efficiency of leaf reflectance indices in the identification of *Capsicum annuum* L. var. *annuum* resistant to anthracnose in the fruit. Twenty-five $F_{5:6}$ families originating from contrasting parents were assessed; the parents were accession UENF 2285 (susceptible to anthracnose) and accession UENF 1381, a hot pepper resistant to anthracnose in the fruit. The experiment was carried out in an experimental field in Campos dos Goytacazes, Rio de Janeiro, Brazil, between May and October of 2021. The treatments were arranged in a randomized block design, with three replications and five plants per plot. Fifteen LRIs were estimated using a CI-710 portable mini leaf spectrometer. The assessments covered all plant growth after flowering, and a total of six assessments were performed at 15-days intervals, beginning at 35 and ending 120 days after flowering (DAFs). Analysis of variance in a split-plot scheme was performed, as were tests of mean groupings and principal components analysis (PCA). The best period for evaluating leaf reflectance indices in *C. annuum* var. *annuum* is 120 days after flowering. The leaf reflectance indices PRI, CNDVI and Ctr2 stood out as effective in distinguishing between resistant and susceptible genotypes.

Keywords

Phenotyping, Bell Pepper, CI-710, *Colletotrichum scovillei*

1. Introduction

Climate change, the rise of pathogens and pests, shortages of natural resources, and population growth represent core challenges faced by plant breeders in the 21st century [1]. However, advances in breeding strategies, allied with the use of non-destructive and highly efficient tools, have significantly accelerated the search for more sustainable agriculture able to maintain agricultural yield and promote the food security needed for future generations [2] [3].

High-throughput phenotyping is a tool aimed at quickly and extensively characterizing and measuring traits, which can include the measurement of specific characteristics in plants, animals, microorganisms, or even humans, depending on the field of study. This makes it possible to evaluate many individuals for many traits, in a non-destructive manner, and always striving for high accuracy and precision. It can be used throughout the crop cycle [4]. The criteria for choosing the best approach should take into consideration the characteristic being evaluated and the timing of the assessment. The most successful traits for evaluation integrate, throughout the crop cycle, both in time and space, the performance of the crop in terms of resource capture (e.g., radiation, water, and nutrients) and how these resources are efficiently utilized [5].

Over the past 50 years, a variety of vegetation indices have been proposed and studied for various applications, ranging from identification, quantification to discrimination of water stress, diseases, pests, and nutritional status. Many of these indices are specifically relevant in detecting diseases in plants, as physiological stress manifests through changes in the balance of pigment composition, such as carotenoids, chlorophylls, and xanthophylls [6].

Early detection of stress in plants remains a challenge, but various techniques, such as chlorophyll fluorescence, visible and infrared spectroscopy, and hyperspectral imaging, have been tested. Recent advances in chlorophyll fluorescence retrieval and the use of improved algorithms enable the rapid measurement of these subtle stress parameters [5].

The efficient use of spectral reflectance measurements for disease detection relies on identifying the most significant spectral wavelengths highly correlated with a specific disease. Depending on the application area and objective, only certain regions of the spectrum are of interest. In the visible region (400 to 700 nm), pigment composition has a predominant impact on the spectral signature [7] [8]. On the other hand, the near infrared (700 to 1100 nm) is mainly influenced by leaf structural characteristics and water content [9].

The application of sensors and Leaf Reflectance Indices (LRIs) analyses has allowed breeders to obtain accurate information regarding plant characteristics such as chlorophyll and nutrient levels and responses to abiotic and biotic stress [10] [11]. This data has been crucial for selecting genotypes with enhanced resistance to leaf diseases and sources of environmental stress, as well as agricultural performance, thus aiding in the development of more yield and sustainable cultivars [12] [13].

The development of a bell pepper cultivar resistant to anthracnose caused by *Colletotrichum scovillei* requires a considerable investment of time, resources, and labor, being even more challenging due to the destructive and laborious nature of the evaluation method. To date, is no registered bell pepper cultivar that is resistant and available for agricultural use. The main obstacles to obtaining a resistant cultivar include the complexity of resistant genetic inheritance, the interaction between the plant and pathogen, and the development stage of the fruit [14]. The inheritance of resistance of the genotype UENF 1381 to *C. scovillei* is quantitative, manifesting itself independently in the different ripening stages of the fruit. This resistance is governed by two, possibly different, principal genes with an associated polygenic effect [15].

Due to the complexity of the inheritance of resistance to *C. scovillei*, new methods for analyzing plant-pathogen interaction are necessary to assist breeders in identifying and selecting resistant genotypes more quickly and accurately. In this context, leaf reflectance indices (LRIs) can be used for the indirect selection of traits.

Prominent among the different groups of indices of leaf reflectance are the NPCI (Normalized Pigmented Chlorophyll Index) and the Ctr2 (Carter 2 Index), which are directly related to the proportion of total photosynthetic pigments in relation to chlorophyll (chl). These indices have been used to estimate the status of nitrogen (N) of plants, an essential nutrient that plays a role in both growth and resistance to diseases [16] [17]. Nitrogen plays a fundamental role in the production of pathogenesis-related (PR) proteins involved in defense responses against pathogens [18].

The aim of this work was to investigate the efficiency of leaf reflectance indices in identifying lines of *C. annuum* var. *annuum* resistant to anthracnose in fruits.

2. Materials and Methods

2.1. Location, Vegetable Material, and Cultivation

This experiment was carried out in an experimental field at the Universidade Estadual do Norte Fluminense Darcy Ribeiro, located in the municipality of Campos dos Goytacazes, in northern Rio de Janeiro State, Brazil, at 21˚19'23" southern latitude, 41˚19'40" western longitude, and an average altitude of 14 m. Twenty-five $F_{5:6}$ families derived from crosses of contrasting parents were evaluated. The feminine parent was accession UENF 2285 of the *C. annuum* var. *annuum* species (susceptible to anthracnose), while the male parent was accession UENF 1381 (GBUEL104), a pungent pepper of the species *C. annuum* var. *annuum* that is resistant to anthracnose in fruits of both stages of maturity [19]. These two parents were used as standards for susceptibility and resistance, respectively (**Figure 1**).

The plants were cultivated from May to October of 2021, a period considered within the annual dry season based on the prevailing water regime. The average minimum temperature registered for 2021 was 18°C, and the average maximum was 27°C [20]. With respect to cultivation practices, they were carried out according to the necessities of the crop. A drip irrigation system was employed by

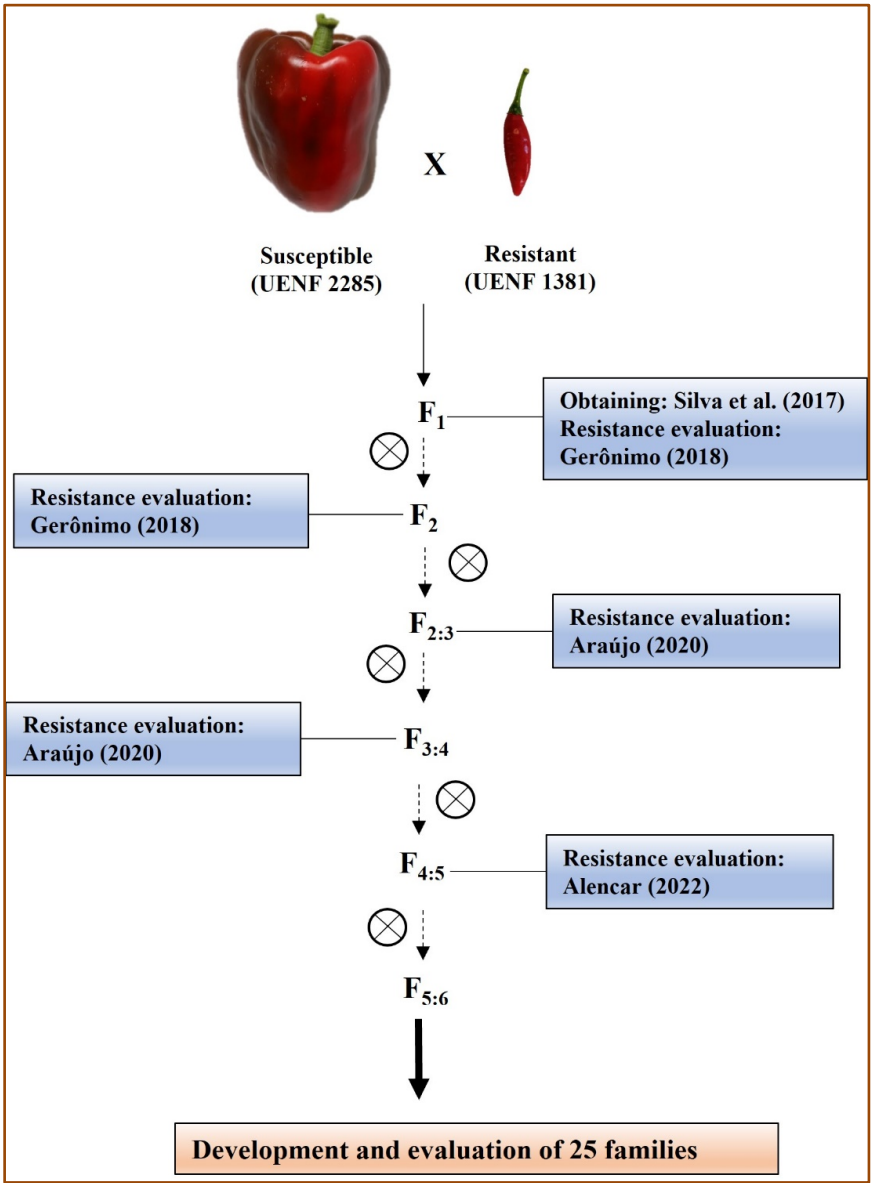

Figure 1. Development and selection of *Capsicum annuum* L. var. *annuum* resistant to anthracnose through the pedigree method [22] [23] [24] [25].

means of a Katif dripper for each plant. Irrigation was performed according to water requirements [21].

2.2. Experimental Design

The treatments were arranged in a randomized block design with three repetitions and five plants per plot and distributed in double rows with 1.0 m spacing between rows and 0.5 m between plants.

2.3. Assessment of Leaf Reflectance Indices (LRIs)

The LRIs were estimated using a CI-710 handheld portable leaf spectrometer (CID-BioScience, Inc., Camas, Washington, USA). The device was configured

and calibrated with a signal integration time of 300 ms, Integration Time factor at the highest value, and signal-to-noise ratio of scan average 2 and a boxcar width value of 10 [26]. Two plants per plot were sampled by using the reading carried out in the middle portion of the abaxial side of the leaf.

The assessments covered the plant's growth cycle after flowering. Six assessments were performed with intervals of approximately 15 days, beginning at 35 days and ending 120 days after flowering (DAF) (**Figure 2**).

While the portable leaf spectrometer produced 17 reflectance indices covering different wavelengths, 15 indices were evaluated in this study (**Table 1**).RENDVI and CNDVI were identical, that is, highly collinear, and as CNDVI is widely used, it was decided to discard RENDVI. PRSI is used more to detect leaf senescence, which is not the case in question. Both indices did not show significant differences regarding treatments. After each reading the data was organized on an Excel spreadsheet for statistical analysis.

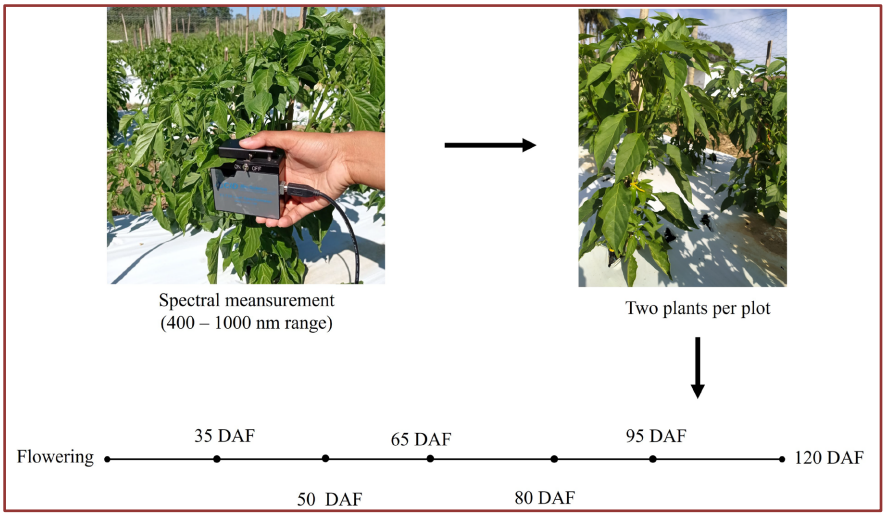

Figure 2. Evaluation of leaf reflectance indices in *Capsicum annuum* L. var. *annuum*. DAF: days after flowering.

Table 1. Classification of Leaf Reflectance Indices and their respective equations and wavelengths, used in the study of the *Capsicum-Colletotrichum* interaction.

Group	LFIs	Equation	Wavelength	Reference
Vegetation Indices	Normalized Difference Vegetation Index (NDVI)	$\frac{(R_{800}-R_{680})}{(R_{800}+R_{680})}$	Red, NIR	[27]
	Transformed Chlorophyll Absorption Ratio Index (TCARI)	$3(R_{700}-R_{670})-0.2.(R_{700}-R_{550})\left(\frac{R700}{R670}\right)$	Green, Red, NIR	[28]
	Cumulative Normalized Difference Vegetation Index (CNDVI)	$\frac{(R_{750}-R_{705})}{(R_{750}+R_{705})}$	Red, NIR	[29]
	Green Index (G)	$\left(\frac{R_{554}}{R_{677}}\right)$	Green, Red	[29]
Light use Efficiency	Photochemical Reflectance Index (PRI)	$\frac{(R_{531}-R_{570})}{(R_{531}+R_{570})}$	Blue, Green	[30]

Continued

Chlorophyll Indices	Anthocyanin Reflectance Index 1 (ARI1)	$\left(\frac{1}{R_{550}}-\frac{1}{R_{700}}\right)$	Blue, Red	[31]
	Anthocyanin Reflectance Index 2 (ARI2)	$R_{800}\cdot\left(\frac{1}{R_{550}}-\frac{1}{R_{700}}\right)$	Blue, Red, NIR	[31]
	Carotenoid Reflectance Index 1 (CRI1)	$\left(\frac{1}{R_{510}}-\frac{1}{R_{550}}\right)$	Blue, Green	[32]
	Carotenoid Reflectance Index 2 (CRI2)	$\left(\frac{1}{R_{510}}-\frac{1}{R_{700}}\right)$	Blue, Red	[32]
Water Index	Water Band Index (WBI)	$\left(\frac{R_{900}}{R_{970}}\right)$	Blue, NIR	[33]
Carotenoid Indices	Structural Independent Pigment Index (SIPI)	$\frac{\left(R_{800}-R_{445}\right)}{\left(R_{800}-R_{680}\right)}$	Blue, Red, NIR	[34]
	Carter Index 1 (Ctr1)	$\left(\frac{R_{695}}{R_{420}}\right)$	Blue, Red	[35]
	Carter Index 2 (Ctr2)	$\left(\frac{R_{695}}{R_{760}}\right)$	Blue, Red, NIR	[35]
	Flavonoid Reflectance Index (FRI)	$R_{800}\cdot\left(\frac{1}{R_{410}}-\frac{1}{R_{460}}\right)$	Blue, NIR	[36]
	Normalized Chlorophyll Index (NPCI)	$\frac{\left(R_{680}-R_{430}\right)}{\left(R_{680}+R_{430}\right)}$	Blue, Red	[36]

3. Statistical Analyses

3.1. Univariate Analysis

All variables were submitted to a Bartlett homogeneity and Lilliefors normality test to verify compliance with the assumptions of the analysis of variance (ANOVA). The LRIs were analyzed in a split-plot design with the 27 genotypes as the main factor (Factor A) and the six evaluation days (Factor B) as a secondary factor, following the model:

$$Y_{ijkl} = \mu + B_{lk} + A_i + em_{aki} + B_j + AB_{ij} + \xi_{ijk},$$

Where:

μ = effect of the general average.

B_{lk} = effect of the block (k), I = 1, 2, ..., r.

A_i = effect of the genotype factor (A).

B_j = effect of the time factor (B).

AB_{ij} = effect of the interaction of the i-th level of A with the j-th level of B.

ξ_{ijk} = random error.

In the model described the blocks - NID (0, σ^2b)] and the errors are considered random effects [ξ_{ijk} - NID (0, σ^2)], while the genotypes[Gi - NID (0, σ2g)], days, and the genotype x day interaction are considered fixed effects. After analyzing the significance of the variables, the genotype averages were grouped by the Scott-Knott Test (1974) ($p \leq 0.05$). The analyses were carried out by the program Genes [37] and the graphic was created using the biplot package of the

4.1.1. version of the program R [38].

3.2. Principal Component Analysis - PCA

Principal component analysis (PCA) was performed using the matrix of genotype correlation obtained from the 27 genotypes assessed over six distinct periods among the 15 LRIs.

With this method one set of n variables correlated in an x vector is transformed into a new p set of uncorrelated variables, reducing the dimension of p to q. Each new variable is a linear combination of the original variables, a1x, a2x,..., aqx, called principal components that are able to explain the majority of the total variance of the original data. The total variation of the new variables is equal to the total variation of the original variables and the variance of each new variable decreases in order. In other words, of all the possible linear combinations, q1 has the greatest variance, and of all the possible linear combinations uncorrelated with q1, that with the greatest variance is q2, and so on [39]. The graph was elaborated using the package ggplot2 of the program R, version 4.1.1. [40].

4. Results

4.1. Genetic Variability of LRIs

There were statistically significant differences for all of the factors analyzed ($p \leq 0.01$), indicating variability among the genotypes, variation in the leaf reflectance indices (LRIs) through time, and interaction between the genotype and days after flowering (DAF). With respect to the genotype factor, six indices presented significant differences: Ctr2, PRI, NDVI, CNDVI, NPCI, and TCARI. For the DAF factor, only the index Ctr1 failed to show significant changes during the growth cycle. Finally, in the interaction between the genotype and DAF, only the indices NPCI and TCARI were statistically significant, indicating the genotypes responded in distinct manners during the growth cycle (**Table 2**). These results indicate that leaf reflectance indices were sensitive enough to distinguish between different genotypes, as well as changes over the evaluation days.

The experimental variation coefficients (CV%) for the genotype (Factor A) and DAF (Factor B) varied from low to high, exhibiting similar values. In the same way, the coefficients of genotype determination varied from low to high depending on the index analyzed. The six indices that obtained significant differences for the genotype factor had high values for the coefficient of genotype determination (*Vg*) (**Table 2**).

Three LRIs were able to distinguish the resistant parent from the susceptible parent (PRI, CNDVI, and Ctr2), resulting in the formation of two distinct groups (**Figure 3**). Regarding the PRI and CNDVI indices (**Figure 3A and 3B**), most of the lines and the susceptible parent obtained higher means, while the resistant parent (UENF 1381) showed lower means. However, for the Ctr2 index (**Figure 3C**), higher means were observed in the parent UENF 1381, establishing a different pattern compared to the other two indices.

Table 2. Summary of ANOVA, covering mean, experimental coefficient of variation, and genotypic determination coefficient related to the 15 leaf reflectance indices (LRIs) evaluated in 25 families of *Capsicum annuum* L. var. *annuum* and their parents.

	Mean Squared									
Indices	Block (DF = 2)	Genotype (A) (DF = 26)	Residual a (DF = 52)	DAF (B) (DF = 5)	AB (DF = 130)	Residual b (DF = 270)	Mean	CV (%) A	CV (%) B	*Vg*
ARI1	0.000004	0.000001^{ns}	0.000001	0.000017**	0.000001^{ns}	0.000001	0.002	46.59	46.56	26.23
ARI2	0.009757	0.005717^{ns}	0.00366	0.070815**	0.003104^{ns}	0.003567	0.142	42.53	41.99	35.98
CRI1	0.000144	0.000052^{ns}	0.000043	0.000232**	0.000033^{ns}	0.000031	0.028	23.22	19.63	16.91
CRI2	0.000187	0.000059^{ns}	0.000053	0.00013**	0.000041^{ns}	0.000039	0.030	23.80	20.29	10.51
Ctr1	0.083987	0.240501^{ns}	0.15807	0.181829^{ns}	0.104036^{ns}	0.081631	2.685	14.80	10.63	34.27
Ctr2	0.000661	0.003546**	0.000852	0.009312**	0.000808^{ns}	0.000786	0.342	8.51	8.17	75.96
FRI	6.050208	1.277193^{ns}	1.326554	4.919119**	0.723834^{ns}	0.000621	2.195	52.46	34.63	0.00
PRI	0.000021	0.0001**	0.000039	0.004041**	0.00004^{ns}	0.000043	0.031	19.87	20.92	61.03
G	0.176495	0.065511^{ns}	0.052071	0.253536**	0.027087^{ns}	0.028351	2.401	9.50	7.01	20.51
NDVI	0.003212	0.001786**	0.000966	0.009217**	0.000621^{ns}	0.000667	0.702	4.42	3.67	45.91
CNDVI	0.000259	0.003284**	0.00081	0.008339**	0.000637^{ns}	0.000621	0.308	9.21	0.06	75.33
NPCI	0.00465	0.003211**	0.001579	0.010625**	0.00155**	0.00093	0.140	28.23	21.66	50.83
TCARI	63.50483	62.46071**	20.151114	114.422**	27.71579**	21.251408	47.764	9.39	9.65	67.73
WBI	0.055475	0.003077^{ns}	0.004042	0.02277**	0.001045^{ns}	0.00117	1.031	6.16	3.31	0.00
SIPI	0.00112	0.001215^{ns}	0.000881	0.003897**	0.000548^{ns}	0.000618	0.730	4.05	3.40	27.49

^{ns} not significant and ** significant at $p \leq 0.01$ based on the F-test probability. DF = degrees of freedom; CV (%) A = experimental coefficient of variation of the plot; CV (%) B = experimental coefficient of variation of the subplot; *Vg* = genotypic determination coefficient.

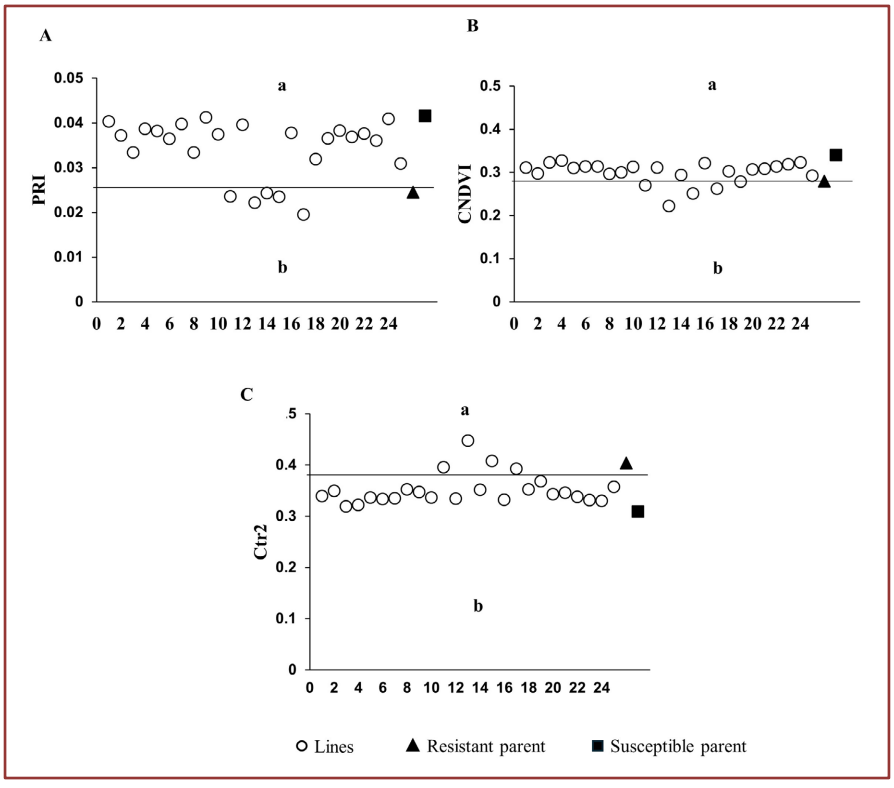

Figure 3. Scatter plot of means. A) PRI: photochemical reflectance index; B) CNDVI: cumulative normalized difference vegetation index, and C) Ctr2: Carter 2 index of 25 lines of *Capsicum annuum* L. var. *annuum* and their respective parents.

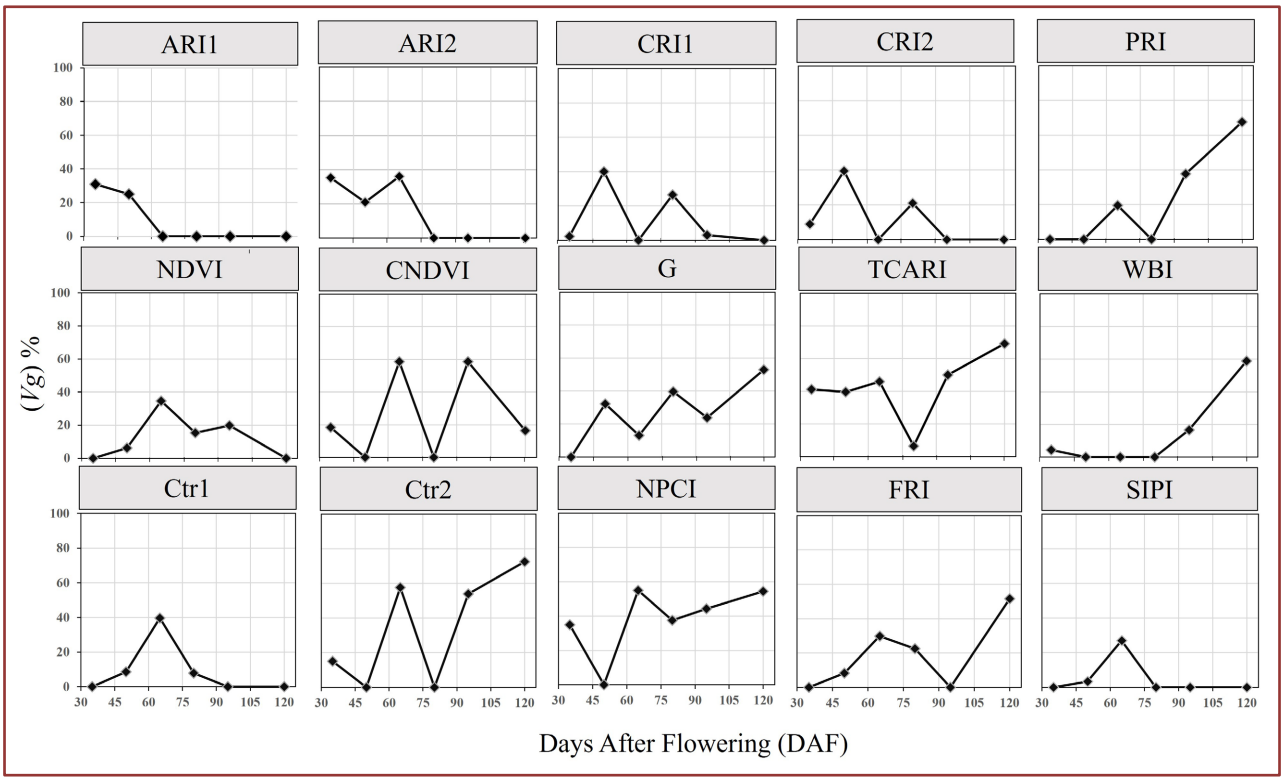

Figure 4. Genotypic determination coefficient (*Vg*) of the LRIs for data collected between 35 and 120 days after flowering *in Capsicum annuum* L. var. *annuum.*

From the individual analysis of the Leaf Reflectance Indices, low values were observed for the genotypic determination coefficient in the first two evaluations (35 and 50 days after flowering - DAF) and at 80 DAF. The highest Vg values were recorded at 120 DAF, indicating that the greatest genetic variability is better explained in the final stages of plant development. This suggests that the LRIs can be effectively studied in just a single evaluation (**Figure 4**).

4.2. Principal Component Analysis

Principal component analysis (PCA) was performed using data from all leaf reflectance indices of the genotypes and days after flowering evaluated. The first and second components explained 69.81% and 19.44% of the total variability, respectively, or 89.25% of the total variation (**Figure 5**).

On the PC1 axis, eight LRIs (NPCI, CTR1, FRI, CRI1, CRI2, TCARI, CTR2, and WBI) were grouped forming angles smaller than 90 degrees, indicating positive correlation among them and negative correlation with the other indices. The same interpretation applies to the PC2 axis.

It was observed that the susceptible parent UENF 2285, the only susceptible genotype, was isolated from the other genotypes by the clustering of the indices G, SIPI, PRI, CNDVI, and NDVI. A second group was formed by the resistant parent UENF 1381 and 13 more lines close to the center of the component axis. The third group, with 12 lines, was further away from the resistant parent, and

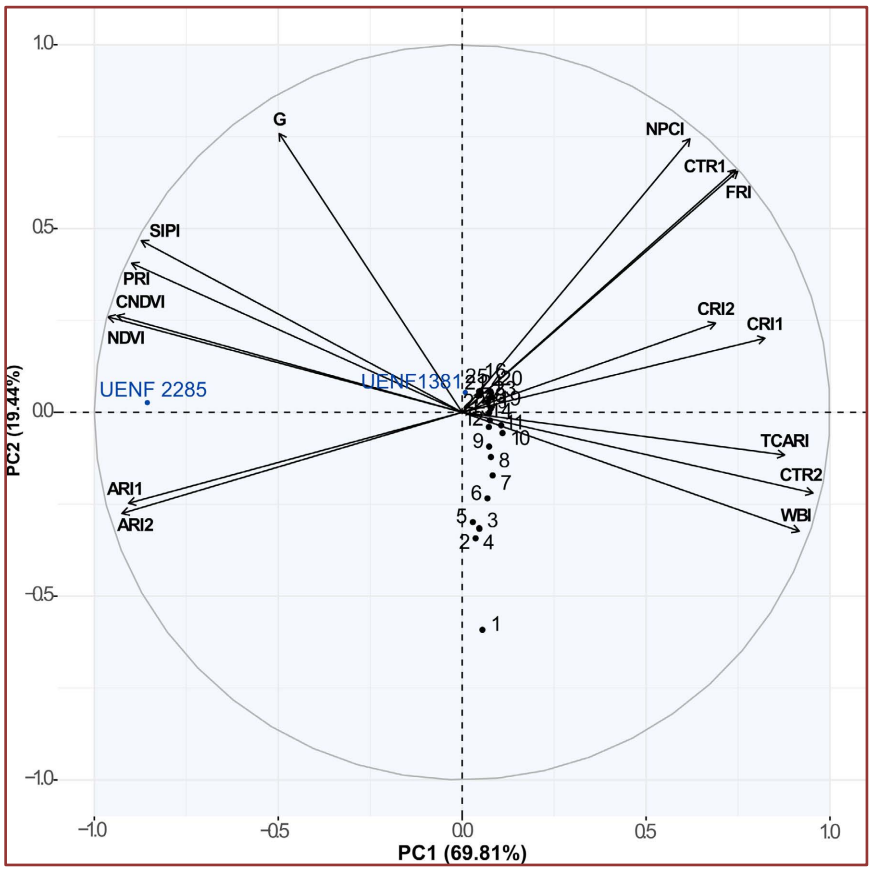

Figure 5. Biplot graph of principal component analysis (PCA) using 15 leaf reflectance indices (LRIs) obtained from six evaluations of 25 lines of *Capsicum annuum* L. var. *annuum*, along with their resistant parent (UENF 1381) and susceptible parent (UENF 2285).

the LRIs that contributed most to this divergence were TCARI, Ctr2, and WBI. The order of importance of the traits showed that the LRIs with the greatest contribution to the differentiation of genotypes were: NDVI, FRI, Ctr1, SIPI, PRI, Ctr2, CNDVI, WBI, NPCI, and ARI2 (**Figure 5**).

5. Discussion

5.1. Genetic Variability of LRIs

One of the most important estimates in plant breeding programs is that of the genotypic determination coefficient or heritability (h^2). This component results from the interaction of genetic and environmental effects and their interaction. However, it is important to emphasize that various factors can affect its estimate, such as the very feature under investigation, the estimation method utilized, the sample size being assessed, the number and type of environment, the experimental unit considered, and the accuracy with which the experiment is conducted [4].

The h^2 represents the reliability of the phenotypic value as an indication of the genetic value of a trait. The greater the heritability, the greater the expected ge-

netic progress. This allows breeders to perform selection based on phenotypic values, maximizing genetic gains. In addition, h^2 helps in choosing the most suitable breeding method to be used. This information is valuable for guiding efforts aimed at the genetic improvement of plants [41].

In the individual analysis, higher *Vg* values for the 15 LRIs were obtained at the fruiting stage of the plant (120 DAF). We observed a trend of increasing *Vg* values throughout the growth cycle. This observation highlights the importance of time as a significant factor in the expression of genetic variability associated with LRIs. Genetic variation seems to manifest more prominently in later stages of the plant's life cycle, specifically at 120 DAF. This pattern may have important implications for selection strategies, suggesting that a single evaluation at a later stage of development can provide representative insights into genetic variability related to LRIs.

Based on the results of the analysis of variance, six indices of leaf reflectance were identified that had the potential to detect significant differences between genotypes. However, using the Scott-Knott mean clustering test, only four of these indices were able to clearly distinguish the resistant parent (UENF 1381) from the susceptible parent (UENF 2285).

The leaf reflectance indices PRI, CNDVI, and Ctr2 were able to discriminate between the resistant and susceptible parent. In addition to distinguishing the parents, these three indices enabled the clustering of the lines L11, L13, L14, and L16 along with the resistant parent. These results suggest that these LRIs have the potential to be used as tools for selecting genotypes more resistant to anthracnose, but further studies are needed for confirmation. Furthermore, it is worth noting that all these three indices showed high values of the genotypic determination coefficient (*Vg*), indicating that they can be considered reliable indicators in the characterization and selection of the studied genotypes.

5.2. Principal Component Analysis - PCA

Through PCA, it was possible to identify the indices that had the greatest contribution to distinguish the resistant lines from the susceptible parent (UENF 2285). This parent was isolated from all other genotypes by the clustering of the LRIs G, SIPI, PRI, NDVI, and CNDVI. Of these, PRI and CNDVI were also relevant for distinguishing the parents by the Scott-Knott mean clustering test, with higher means observed for the susceptible parent UENF 2285.

PRI (Photochemical Reflectance Index) is directly related to the efficiency of light use in the plant's xanthophyll cycle and can be used to monitor different aspects of the plant's physiological response to biotic and abiotic stresses [42]. This index can range from −1 to 1, with higher values indicating greater efficiency of photosystem II [43]. On the other hand, CNDVI (Cumulative Normalized Difference Vegetation Index) is associated with the photosynthetic activity of vegetation and can vary from −1 to 1 depending on environmental factors such as temperature and humidity. This index is extremely sensitive to climatic changes [44].

The indices NPCI, Ctr1, FRI, CRI1, and CRI2 grouped the resistant parent UENF 1381 and 13 more lines close to the center of the component axes, indicating that part of the variation observed in the genotypes resides in a few LRIs or a combination of these. The lines evaluated have homozygosity rates above 90% for many traits, so PCA analysis detected low variation among the LRIs. The susceptible parent UENF 2285 was isolated due to genetic divergence between the resistant parent and the lines. Therefore, the breeder may choose to discard traits (LRIs) with little variation, as their study does not significantly contribute to the differentiation of genotypes.

The study of LRIs for the identification of genotypes resistant to biotic and abiotic stress is based on the wavelength of leaf reflectance [45]. The indices Ctr1, Ctr2, and NPCI are directly related to the proportion of total photosynthetic pigments relative to chlorophyll and are part of the carotenoid indices group. These are used to estimate plant nitrogen (N) status, as under limited N conditions, chlorophyll loss occurs, resulting in changes in leaf reflectance spectrum [36]. Nitrogen plays an important role in plant growth and is essential for disease resistance [17]. It is worth highlighting that among the defense genes activated in non-host resistance (NHR) and hypersensitivity response (HR), there are some associated with the production of pathogenesis-related proteins (PRs), including nitrogen-containing compound biosynthesis pathways [18]. Therefore, these indices can be used to select *C. annuum* genotypes with a higher capacity to absorb and utilize nitrogen efficiently, resulting in more vigorous plants with greater disease resistance.

On the other hand, TCARI (Transformed Chlorophyll Absorption Ratio Index) is a highly sensitive index to chlorophyll variation and very efficient in its estimation, as it does not capture the reflectance of non-photosynthetic materials. Studies in the bean crop demonstrate that TCARI is a good index for estimating leaf diseases, as it minimizes the effects of background or soil reflectance in aerial images [46]. Regarding the nutritional aspects of the plant, especially regarding nitrogen, TCARI has better accuracy compared to other indices, mainly due to the use of red edge to increase chlorophyll estimation. This is because the increase in near-infrared reflection is due to the increase in nitrogen concentration in the leaves [47].

The flavonoid reflectance index (FRI) is related to the content of chlorophyll, carotenoid, and flavonoid. Flavonoids are part of the secondary metabolism of plants and can perform various functions, including those related to plant defense [48]. As a defense compound, they can be divided into two groups: pre-formed and induced compounds [49]. The involvement of pre-formed flavonoids plays an important role in the host-pathogen interaction. These compounds are stored in strategically important locations, where they can play a direct signaling role in defense. Induced compounds are synthesized by the plant in response to physical injuries, infection, or stress [50]. They can also be constitutively synthesized, but their biosynthesis is enhanced under the effect of various types of stress or can occur as phytoalexins only after infection or vari-

ous types of stress [49].

6. Conclusion and Recommendations

Regarding the phenological stage of *C. annuum* var. *annuum* genotypes, the evaluation of reflectance indices at 120 days was more reliable, as heritability was higher during this period. The leaf reflectance indices PRI, CNDVI, and Ctr2 stood out as effective in distinguishing between resistant and susceptible genotypes. On the other hand, ARI1 and Ctr1 indices may be excluded due to high correlation with other indices. The indices with the greatest contribution to the differentiation of resistant and susceptible genotypes were NDVI, FRI, SIPI, and PRI.

The next steps of this research involve exploring the application of predictive models, such as machine learning models, to predict anthracnose resistance in *C. annuum* var. *annuum* based on leaf reflectance indices. Furthermore, additional studies are needed to validate the effectiveness of these indices under different environmental conditions, including variations in soil, climate, and agricultural practices.

It is essential to deepen our understanding of leaf reflectance indices (PRI, CNDVI, Ctr2, NDVI, FRI, SIPI) to better elucidate the underlying mechanisms of anthracnose resistance and its relationship with phenotypic traits. Investigating other leaf reflectance indices may also provide additional or complementary insights to those already identified, thus expanding our knowledge of the relationship between leaf reflectance indices and disease resistance in *C. annuum*.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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