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Differentiation of Plant-Pathogenic Fungi in Soybean Seeds Using Hyperspectral Sensors

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Abstract

Hyperspectral sensors have emerged as a promising approach in the study of plant diseases. The objective was to distinguish between healthy and inoculated seeds, and also to distinguish between genera of plant-pathogenic fungi in soybean seeds, using hyperspectral sensors combined with machine learning. The experimental design was a fully randomized factorial design with six algorithms (Simple Logistic Regression, Support Vector Machine, Artificial Neural Network, Random Forest, REPTree and J48 decision trees) and four phytopathogens (*Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Colletotrichum* sp.) plus the control. Spectral analysis of the seeds was performed using a spectroradiometer (Ocean Optics) consisting of two sensors: NIR and Flame, covering the spectrum from 350 to 2500 nm. It was possible to distinguish between healthy and inoculated seeds, as well as identify the type of phytopathogen, based on each spectral signature. The Simple Logistic Regression and Support Vector Machine algorithms performed best. Hyperspectral sensors combined with machine learning constitute a promising tool for the detection of phytopathogens in seeds, enabling rapid and non-destructive analysis. This promising tool could serve as a complementary alternative to traditional diagnostic methods, which, although accurate, are time-consuming and rely on specialized labor.

Keywords: *Glycine max* (L.) Merrill; spectroradiometer; plant diseases

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1. Introduction

The diseases with the greatest economic impact on soybean cultivation are associated with seed-borne pathogens, which play a key role in their epidemiology. The success of the mechanisms involved in the transmission of pathogens of various etiologies to seeds ensures their introduction into previously disease-free areas, as well as the reinfestation of areas where management measures had previously eradicated the pathogen [1].

An important point is that even seeds containing a small number of propagules can introduce pathogens [2] that are not present in a given geographic region and affect a country's economy. The spread of pathogens via seed leads to the formation of primary disease foci, distributed randomly throughout the crop, which can survive even after harvest through specialized structures and non-host plants. Furthermore, the recurrent introduction of inoculum via seeds contributes significantly to the increased incidence and severity of diseases already established in an area, with serious economic consequences [3].

The microbiota associated with soybean seeds consists of a wide variety of phytopathogenic microorganisms, with fungi being the most frequently detected agents [4]. These pathogens can become associated with the seeds through various routes, such as systemic infection and external contamination during the maturation, harvest, and storage phases [5].

Identification of fungi can reduce seed damage and limit their spread, as it would facilitate the adoption of appropriate physical or chemical treatments [3]. However, traditional methods for detecting fungi associated with seeds, such as incubation-based health tests and microscopic evaluation, have limitations related to processing time and the need for specialized labor. In addition, advanced instrumental techniques such as chromatography, mass spectrometry, X-ray analysis, thermal imaging, and molecular tools [6–9], although highly sensitive, require complex and time-consuming sample preparation steps, high operational costs, and advanced laboratory infrastructure.

In this context, hyperspectral sensors have emerged as a promising approach for the study of plant diseases [10–12]. This technique allows for the acquisition of hundreds of data points across the wavelength spectrum, meaning that the different absorption/reflection characteristics of materials can be accurately represented within a narrow bandwidth of less than 10 nm. In addition, hyperspectral technology offers significant operational advantages over other methods, such as no or minimal need for sample preparation, a non-destructive nature, rapid data acquisition, and the potential for large-scale automation [13,14].

When biological materials are exposed to electromagnetic radiation at different wavelengths, they reflect or absorb energy differently due to variations in their chemical composition and physical structure. Thus, when organic constituents interact with light, a spectral signature is created that can be used to distinguish seed samples. These spectral signatures can reveal the presence of contaminants and their spatial distribution [15]. Therefore, hyperspectral images offer the potential to extract more accurate and detailed information than conventional fungal detection methods.

Thus, the objective of this study was to distinguish between healthy and inoculated seeds, and also to distinguish be-tween genera of plant-pathogenic fungi in soybean seeds, using hyperspectral sensors combined with machine learning.

2. Materials and Methods

The soybeans used were harvested during the 2024/2025 growing season in the Chapadão do Sul region, in the state of Mato Grosso do Sul, Brazil. The region's climate is classified as Aw according to the Köppen climate classification, characterized by rainy summers and dry winters.

Healthy soybean seeds (detected using the "blotter test") were inoculated with different pathogen inocula. To prepare the inoculum, isolates of *Sclerotinia sclerotiorum* (SS), *Macrophomina phaseolina* (MP), *Rhizoctonia solani* (R2), and *Colletotrichum* sp. (Collet) were first cultured on potato–dextrose–agar (PDA) medium. Discs 7 mm in diameter, containing mycelium and viable spores, were transferred to the center of Petri dishes containing PDA and incubated in a growth chamber at 20 °C ± 1 °C under a 12 h photoperiod for 15 days.

The experimental design employed was a completely randomized factorial scheme involving four plant pathogens plus a control, with 20 replicates, totaling 100 samples. Initially, the seeds were superficially disinfected with a 0.5% sodium hypochlorite solution for five minutes and washed in sterilized distilled water. For this reason, the uninoculated seeds were considered healthy.

After the incubation period, plates were selected that contained fungal colonies with mycelial growth covering the entire diameter of the plate (9 cm). Thirty soybeans were placed on each plate, distributed evenly in rows across the surface of the culture medium, and lightly pressed to ensure direct contact with the mycelium. The seeds were removed after 48 h and placed to dry on filter paper at room temperature [16].

Spectral analysis of the seeds was performed using a spectroradiometer (Ocean Optics) equipped with two sensors: NIR and Flame, which together cover the spectrum from 350 to 2500 nm. A white plate made of barium sulfate, which reflects 100% of the light, was used to calibrate the equipment. The sensors were connected to a computer to record each reading using OceanView2.0 software, which logs the readings taken by the equipment.

The data were subjected to machine learning analyses; the spectra obtained by the STS Vis-NIR sensor (Ocean Optics, Orlando, FL, USA) underwent only visual inspection to identify and remove severe noise resulting from acquisition failures or evident interference during data collection. This procedure aimed to only eliminate clearly inconsistent observations, preserving the original spectral information as much as possible. No spectral preprocessing techniques—such as curve smoothing, baseline correction, normalization, derivative calculation, or other filtering methods—were applied. Initially, the data were processed to distinguish healthy seeds from those inoculated with plant pathogens. In the second stage, the data were processed so that the models could identify healthy seeds and the specific pathogens with which they had been inoculated.

Classification was performed using stratified cross-validation with k-fold = 10 and ten repetitions (100 runs for each model). All model parameters were set according to the default configuration of the Weka 3.8.5 software [17], except for the ANN, which used two layers with 10 neurons in each. The algorithms tested were Simple Logistic Regression (SL), Support Vector Machine (SMO), Artificial Neural Network (ANN), Random Forest (RF), REPTree decision trees (DT), and J48.

To assess the accuracy of the models, we used correct classification (CC), Kappa coefficient, and F-score. We then compared the means among the models within each metric using Tukey's test at a 5% significance level, and created bar charts for each variable (CC, Kappa, and F-score).

To select the most informative spectral bands and reduce data dimensionality, wavelengths were ranked using the best-performing algorithms. Prior to model fitting, the spectral reflectance matrix was standardized (Z-score) to avoid bias arising from differences in the scale of variation among the bands. In the SMO model (linear kernel), the algorithm constructed classifiers using the one-vs-one method to differentiate between healthy and inoculated samples, as well as among the five classes (control, R2, collet, MP, and SS). The relevance of each wavelength was determined by the sum of the magnitudes of the coefficients obtained from the equations for each class. Finally, the mean rank position (Mean Rank) derived from both algorithms was calculated to identify and select the subset comprising the 10 most discriminating spectral bands.

3. Results

The data obtained by the hyperspectral sensors revealed distinct variations in light absorption between seeds inoculated with plant pathogens and healthy seeds. Plant pathogens caused changes in reflectance properties compared to healthy seeds, particularly in the visible spectrum (400–700 nm). From 2300 nm onward, the spectral curves of healthy and inoculated seeds behaved similarly (Figure 1).

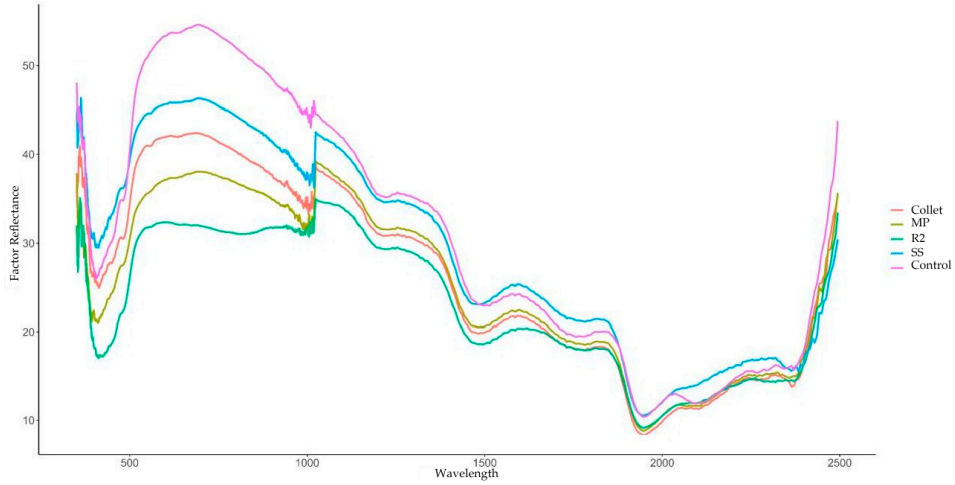

Figure 1. Spectral curves of soybean seeds inoculated with plant pathogens. SS (*Sclerotinia sclerotiorum*), MP (*Macrophomina phaseolina*), R2 (*Rhizoctonia solani*), Collet (*Colletotrichum* sp.), and control (healthy seeds).

3.1. Classification of Healthy Seeds and Seeds Inoculated with Pathogens

Table 1 presents the mean accuracy values for each fold and each model, along with the comparison between them using Tukey's test. The accuracy obtained in the correct classification (CC) of healthy and inoculated seeds varied depending on the machine learning algorithm used. The DT, J48, RF, and ANN algorithms had the lowest CC values and showed no significant difference among themselves according to the Tukey test at a 5% significance level. The SMO and SL algorithms exhibited CC values exceeding 80% accuracy, although they did not differ from one another (Figure 2a). However, the SMO algorithm showed a higher average number of correct classifications, indicating a more precise ability to distinguish between healthy and inoculated soybean seeds.

Table 1. Comparison of means for model accuracy metrics for the machine learning models in the classification of healthy and inoculated seeds.

ML	CC	Kappa	F-Score
DT	80.2 b	0.12 d	0.43 c
J48	78.3 b	0.20 cd	0.47 c
RF	78 b	0.16 d	0.42 c
RNA	79.8 b	0.32 bc	0.49 bc
SL	86.2 a	0.47 ab	0.67 ab
SMO	89.1 a	0.58 a	0.73 a

Letters in the same column do not differ from each other according to Tukey's test at the 5% level.

The SMO and SL algorithms yielded the highest Kappa values, close to 0.6. Although both outperformed the other algorithms, they did not differ statistically. In contrast, the DT, J48, and RF algorithms showed lower Kappa values (below 0.2), indicating an extremely low level of agreement, while the ANN algorithm showed a value between 0.3 and 0.35.

Therefore, these models exhibit a lower ability to classify healthy and inoculated soybeans, performing worse than the SMO and SL models. Once again, the SMO and SL algorithms achieved the highest mean F-scores (Figure 2c), indicating high classification accuracy, as described by [18]. Despite their good performance, the SMO and SL algorithms did not differ statistically from one another. The other algorithms exhibited lower F-score values, indicating lower classification ability; therefore, they performed worse in distinguishing between healthy and inoculated soybeans.

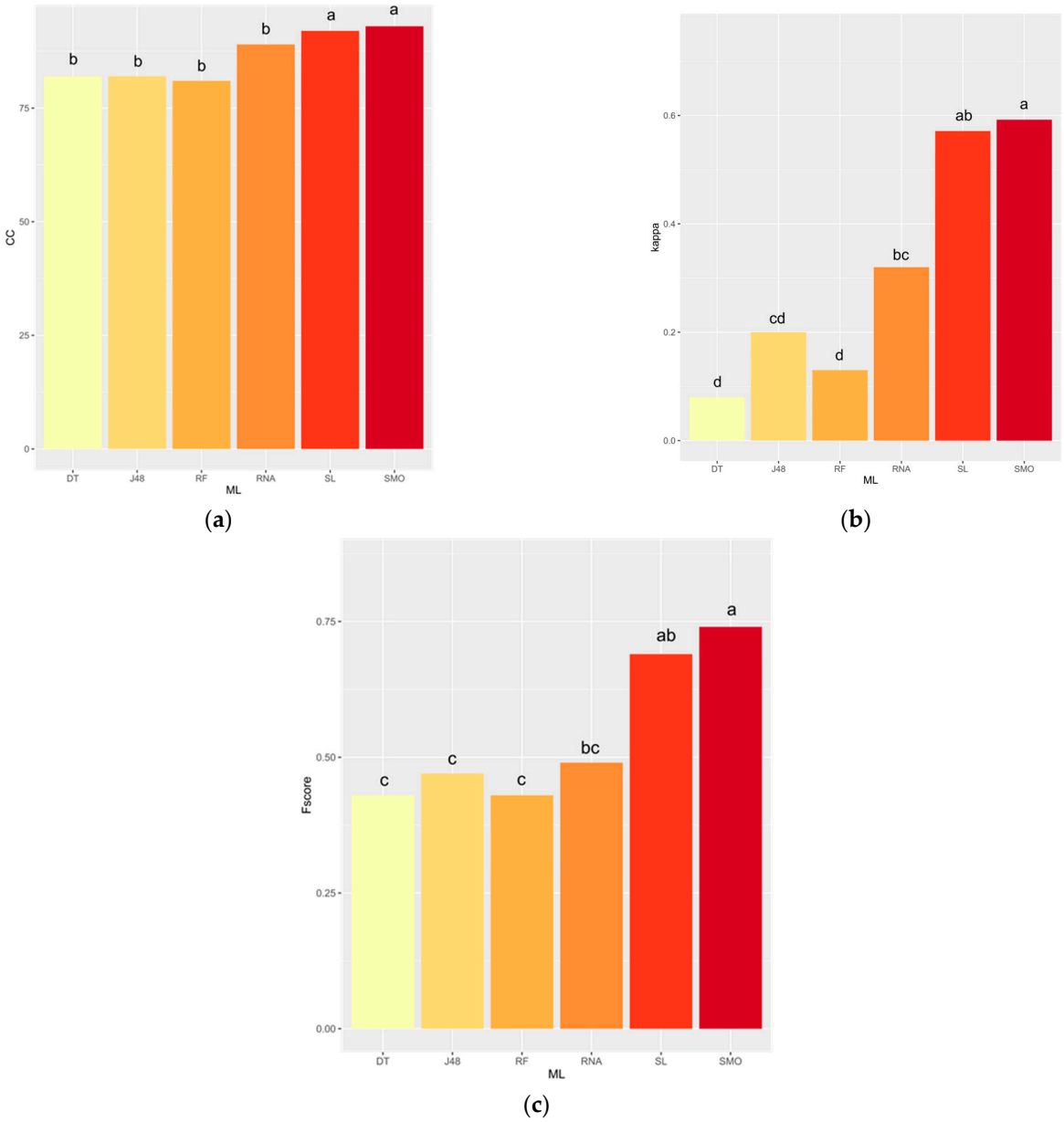

Figure 2. Correct classification rate (CC) (**a**), Kappa coefficient (**b**), and F-score (**c**) for the machine learning models in the classification of healthy and inoculated seeds. Means followed by the same letter do not differ from one another according to Tukey's test at the 5% significance level.

In Figure 3, The confusion matrix revealed that the model performed better for inoculated seeds, with 78 correct classifications for the SL and SMO models. These results suggest that the features used by the model were sufficient to completely discriminate between the treatments, corroborating the previous comparison of means.

Based on this evidence—namely, that each plant pathogen generates distinct spectral signatures due to the structural and biochemical changes caused by the infection process in soybean seeds—the data were subjected to a new classification stage using the same machine learning algorithms, this time to classify healthy seeds and each inoculated phytopathogen, in order to verify whether the spectral signatures are sufficient for accurate classification, allowing the pathogens to be distinguished from one another.

Figure 4 shows the 10 spectral bands from the best-performing models (SL and SMO); the visible region—specifically within these bands—contributed most to distinguishing

between healthy and pathogen-infected seeds. However, the bands contributing the most were 2402 (SVM) and 2115 (SL).

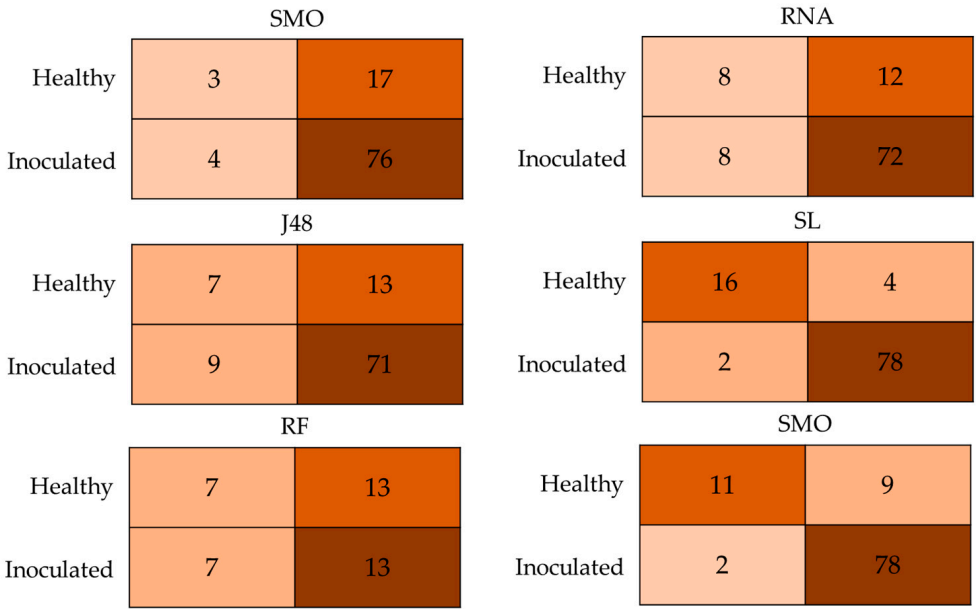

Figure 3. Confusion matrices of all models for the classification for the machine learning models in the classification of healthy seeds and seeds inoculated.

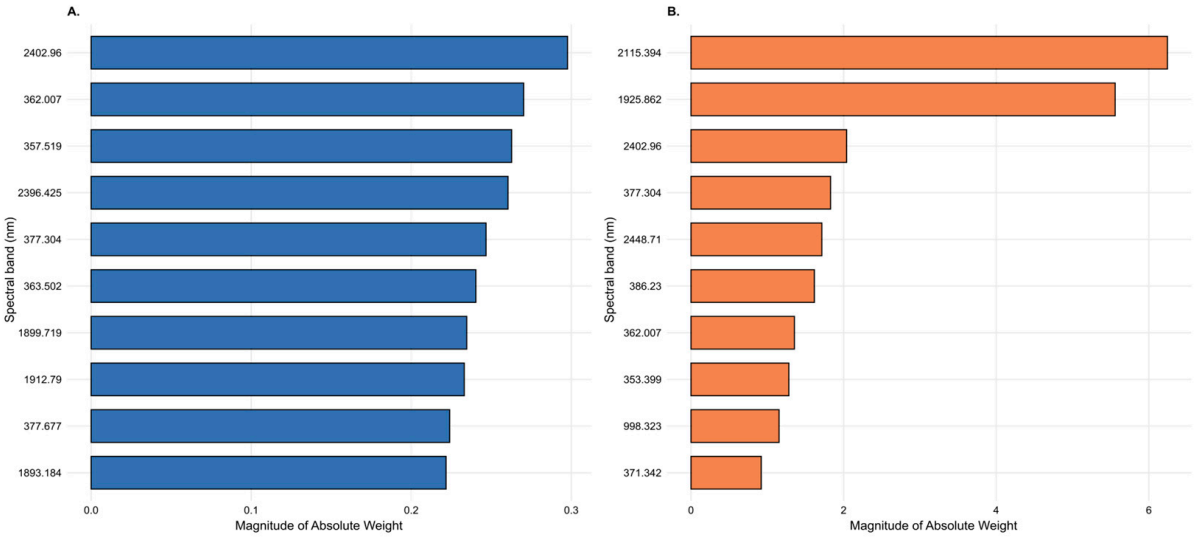

Figure 4. Top 10 spectral bands selected by (**A**) Support Vector Machine (SVM) and (**B**) Logistic Regression (SL), comparison of the most relevant bands for discriminating healthy seeds and seeds inoculated.

3.2. Identification of Diseased and Healthy Soybean Seeds and Fungal Pathogen Differentiation

Table 2 presents a comparison of the mean accuracy metrics for the models classifying pathogen inoculation types on the seeds and in Figure 5a shows that the SL algorithm achieved the highest average accuracy, close to 70%, indicating good classification performance. SMO, on the other hand, performed worse than SL in terms of accuracy. On the other hand, the DT, J48, RF, and ANN algorithms showed significantly lower accuracy values, with averages close to or below 40%, and no statistically significant differences among them. This demonstrates that these models have a lower ability to identify different pathogens based on spectral curves.

Table 2. Comparison of means for model accuracy metrics for the machine learning models in the classification of healthy seeds and seeds inoculated with each plant pathogen.

ML	CC	Kappa	F-Score
DT	26.3 c	0.08 c	0.44 b
J48	27.2 c	0.10 c	0.35 b
RF	25.2 c	0.07 c	0.35 b
RNA	27.1 c	0.09 c	0.40 b
SL	65.2 a	0.56 a	0.70 a
SMO	55.3 b	0.44 b	0.70 a

Letters in the same column do not differ from each other according to Tukey's test at the 5% level.

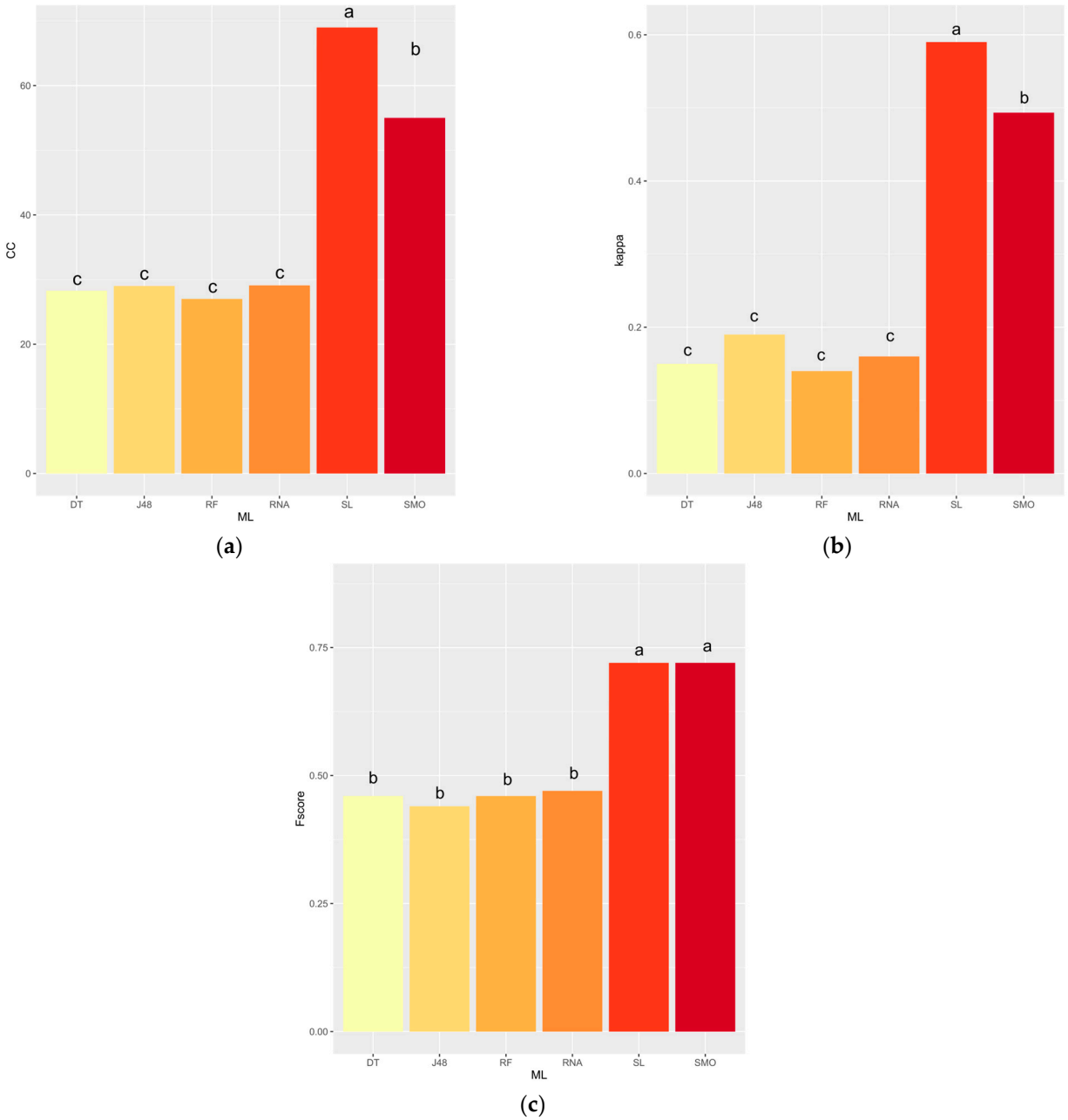

Figure 5. Correct classification rate (CC) (**a**), Kappa coefficient (**b**), and F-score (**c**) for the machine learning models in the classification of healthy seeds and seeds inoculated with each plant pathogen. Means followed by the same letter do not differ from one another according to Tukey's test at the 5% significance level.

The SL and SMO algorithms stood out with the highest Kappa values (Figure 5b), outperforming the others; however, SL achieved a higher value than SMO. In contrast, the DT, J48, RF, and ANN algorithms had Kappa values below 0.2, indicating inconsistent classification. These data reinforce the limitations of these models and highlight the advantages of others in classifying healthy seeds and seeds inoculated with different phytopathogens based on spectral curves.

For the F-score (Figure 5c), the SL and SMO algorithms again stood out, yielding the highest values, thereby reinforcing their efficiency in classifying phytopathogens in soybean seeds; however, they did not differ from one another. In contrast, the DT, J48, RF, and ANN algorithms exhibited significantly lower F-scores, but did not differ from one another according to the Tukey test at the 5% level.

To further substantiate the findings, confusion matrices (Figure 6) were constructed for all models regarding the classification of the different infections, confirming the higher accuracy rate of the SL model and aligning with the previously reported accuracy figures.

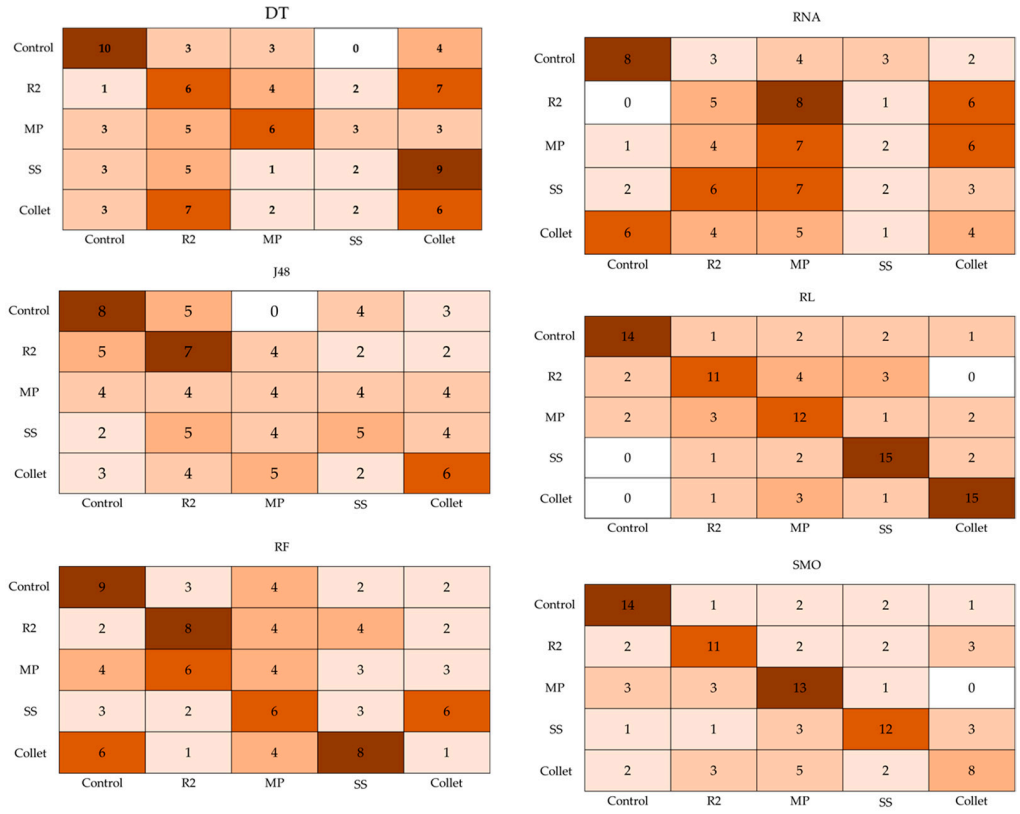

Figure 6. Confusion matrices of all models for the classification for the machine learning models in the classification of healthy seeds and seeds inoculated with each plant pathogen.

The accuracy in classifying between inoculated and non-inoculated seeds was highest for the SMO algorithm, which showed the best overall performance across the three metrics used, indicating a high level of accuracy and good consistency among the models. However, in the classification of phytopathogens, the best algorithm was SL, indicating high agreement in the classification. These results indicate greater relevance for classification, demonstrating that the SL and SMO algorithms are the most suitable based on the spectral curves generated by each treatment. Among the top 10 bands distinguishing the various soybean Figure 7 seed inoculation treatments, the visible range—specifically the 300 nm band—stood out.

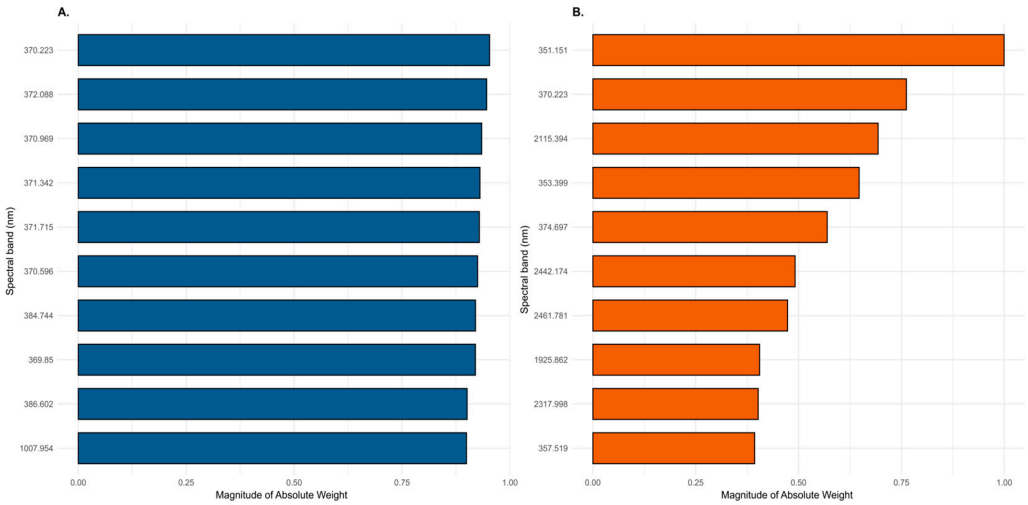

Figure 7. The top 10 spectral bands selected by (**A**) Support Vector Machine (SVM) and (**B**) Logistic Regression (LR); comparison of the most relevant bands for discriminating healthy seeds and seeds inoculated with each plant pathogen.

4. Discussion

The phytopathogens can cause changes in the physiology and biochemistry of the seed, including discoloration [19], by feeding on the reserve tissue and causing biotic stress, thereby reducing cellular respiration. These biochemical variations can generate distinct spectral signatures, manifested as changes in reflectance due to the action of the phytopathogen, caused by deficiencies in chemical composition, the succession of chlorotic and necrotic tissue, or the appearance of typical fungal structures, such as hyphae and conidia [20].

These highly complex and unique fungal disease patterns allow us to identify the causative agent based on spectral properties [21]. Thus, the spectral curves are influenced by the material's chemical composition, allowing the spectroradiometer to detect significant changes for pathogen classification.

These changes are related to light absorption by fungal structures, resulting in distinct spectral curves for each plant pathogen. Spectral variations occur due to the structures produced by these pathogens, such as sclerotia (*Sclerotinia sclerotiorum*), microsclerotia (*Macrophomina phaseolina*), atheres, and conidia (*Colletotrichum* spp.), as well as the pigmented mycelium and sclerotia of *Rhizoctonia solani*, which interfere with light absorption, generating distinct spectral signatures.

In the electromagnetic spectrum range between 2000 and 2500 nm (short-wave infrared), bands associated with water absorption are present [22]. This region stood out in the classification of healthy and inoculated seeds, wavelengths located within the short-wave infrared (SWIR—1300–2500 nm) range, a region strongly associated with water content and combination bands involving C–O and O–H bonds. The dry matter of plants consists of various organic compounds such as cellulose, lignin, proteins, and amino acids, which cause combinational and harmonic vibrational bands in this region of the spectrum [23]. Therefore, the increase in light absorption by *Sclerotinia sclerotiorum* may be related to changes in seed tissues.

Hyperspectral images revealed significant variations in the spectral patterns of soybeans, allowing for the differentiation between seeds considered healthy and those inoculated with different pathogens. The biochemical and structural changes caused by the pathogens generated distinct spectral signatures, especially in the visible spectrum (400 to 700 nm) and short-wave infrared (2000 to 2500 nm) bands. This region in visible, is

strongly associated with seed energy reserves, explaining its significant contribution to the classification results.

It is important to note that multiclass classification, in which the classes are the treatments, imposes a high level of complexity on the models due to subtle variations in spectral signatures, requiring greater discriminatory power related to the different variations caused by pathogens. A combined analysis of Figures 2 and 3 highlights the performance of the machine learning algorithms in classifying healthy and inoculated seeds, as well as in classifying the plant pathogens used. The metrics of Correct Classification (CC), Kappa Coefficient, and F-score indicate that the SL and SMO algorithms perform better than the others.

The results obtained in this study represent recent advances in the use of optical techniques combined with artificial intelligence for the detection of plant-pathogenic fungi in seeds. In this context, ref. [24] demonstrated that the laser biospeckle technique, combined with machine learning algorithms, was able to identify *Colletotrichum truncatum* infections in soybean seeds with high accuracy. The authors also noted that the biological activity associated with fungal growth altered detectable optical patterns, enabling the rapid and non-destructive discrimination between healthy and infected seeds. Similarly, the results obtained in this study are consistent with those of [25], who used RGB images of fungal colonies and machine learning algorithms to identify plant-pathogenic fungi, as well as others associated with storage in bean seeds.

Given that the ability to detect fungal colonization in seeds highlights the importance of these approaches for quality control programs, further studies will be carried out to validate the method using naturally infected seeds collected from seed production fields, and to increase the number of samples.

5. Conclusions

The colonization of seeds by plant-pathogenic fungi causes physiological and biochemical changes that result in significant variations in light absorption, as evidenced by spectral curves, particularly in the visible spectrum (400–700 nm) and the short-wave infrared spectrum (2000–2500 nm).

Hyperspectral sensors combined with machine learning algorithms were able to distinguish between healthy and inoculated seeds, as well as identify the type of plant pathogen present, based on each spectral signature. The Simple Logistic Regression (SL) and Support Vector Machine (SMO) algorithms performed best in both binary and multiclass classification.

This is a promising tool for detecting plant pathogens in seeds, enabling rapid, non-destructive analysis, and could serve as an efficient complementary alternative to traditional laboratory diagnostic methods, which, although accurate, are more time-consuming and rely on specialized personnel.

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Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

SS	*Sclerotinia sclerotiorum*
MP	*Macrophomina phaseolina*
R2	*Rhizoctonia solani*
Collet	*Colletotrichum* sp.
PDAP	Potato–Dextrose–Agar
NIR	Near-Infrared
ANN	Artificial Neural Network
SL	Simple Logistic Regression
SMO	Support Vector Machine
RF	Random Forest
DT	REPTree Decision Trees
CC	Correct Classification

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