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Evaluation of *Cedrela fissilis* Vell. seeds with color heteromorphism using near-infrared spectroscopy and their relationship with physiological quality

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ABSTRACT: *Cedrela fissilis* Vell., commonly known as cedro-rosa, is a tree species native to Brazil, with ecological and economic relevance, that exhibits seed heteromorphism associated with seed coat color. In this study, the classification of light- and dark-colored seeds using near-infrared (NIR) spectroscopy and its relationship with physiological quality was evaluated. NIR spectra were obtained, reserve compounds were quantified, and germination and vigor tests were conducted. The NIR spectra, collected from individual seeds, were preprocessed and used to develop classification models based on the Partial Least Squares - Discriminant Analysis (PLS-DA) method. The physiological and biochemical composition data were analyzed using Student's t-test. Dark seeds showed higher thousand-seed weight, total protein content, as well as greater germination and vigor. Light seeds exhibited higher levels of reducing sugars, suggesting a lower degree of maturity or the onset of deterioration. NIR spectroscopy demonstrated high accuracy in distinguishing between light and dark seeds, especially in the spectral band near 1938 nm, whose relevance may be mainly associated with variations in total protein content. Seed coat color proved to be a reliable indicator of the physiological quality of *C. fissilis* seeds.

Index terms: *Cedro-rosa*, chemometrics, NIR spectroscopy, seed germination and vigor.

RESUMO: *Cedrela fissilis* Vell., conhecida como cedro-rosa, é uma espécie arbórea nativa do Brasil, de relevância ecológica e econômica, que apresenta heteromorfismo de sementes associado à cor do tegumento. Neste estudo, uma classificação de sementes claras e escuras por meio da espectroscopia no infravermelho próximo (NIR) e sua relação com a qualidade fisiológica foi avaliada. Foram obtidos espectros NIR, quantificados os compostos de reserva e realizados testes de germinação e vigor. Os espectros NIR, obtidos de sementes individuais, foram pré-processados e utilizados para modelagem com base no método da Análise Discriminante por Mínimos Quadrados Parciais (PLS-DA). Os dados de qualidade fisiológica e composição bioquímica foram analisados pelo teste t de Student. Sementes escuras apresentaram maior peso de mil sementes, teor de proteína total, além de maior potencial de germinação e vigor. Sementes claras apresentaram conteúdo mais elevado de açúcares redutores, sugerindo menor grau de maturidade ou início de deterioração. A espectroscopia NIR mostrou alta precisão na distinção entre sementes claras e escuras, especialmente na região espectral próxima de 1938 nm, cuja relevância pode estar associada às variações no teor de proteína total. A coloração demonstrou ser um bom indicativo da qualidade fisiológica em sementes de *C. fissilis*.

Termos para indexação: Cedro-rosa, quimiometria, espectroscopia NIR, germinação e vigor de sementes.

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INTRODUCTION

Cedrela fissilis Vell. (Meliaceae), commonly known as cedro-rosa, is a tree species native to Brazil, highly valued for its timber and recommended for the restoration of degraded areas. This deciduous species can reach up to 40 meters in height and develop a trunk diameter of up to 300 cm at maturity (Durigan and Nogueira, 1990; Carvalho, 2003; Carvalho, 2005). Its reproduction is predominantly sexual, making seeds essential for species conservation. The seeds, winged at one end, exhibit seed coat coloration ranging from beige to reddish-brown, characterizing a form of seed heteromorphism. This phenomenon, in which a single plant produces seeds with distinct morphological traits or ecological behaviors, can influence germination mechanisms, dispersal strategies, and persistence in the soil seed bank, thereby favoring species survival in ecologically unstable or unpredictable environments (Venable, 1985; Mandák, 1997; Imbert, 2002; Matilla et al., 2005). Seed heteromorphism arises as a natural outcome of interactions among evolutionary, ecological, and physiological factors, as well as the interplay between environmental adaptations and biological constraints. It may also be associated with the seed maturation stage (Venable, 1985; Matilla et al., 2005), a factor that directly affects physiological seed quality, which encompasses germination (viability) and seed vigor (Marcos-Filho, 2015).

Seed quality is traditionally assessed through germination and vigor tests. Although effective, these methods are time-consuming. In addition to these tests, the analysis of the chemical composition of seed reserves is of great interest in seed technology, as vigor and storage potential are influenced by the levels of specific compounds (Carvalho and Nakagawa, 2012). However, the complexity of such analyses restricts their application to specialized laboratories. Given these limitations, rapid and non-destructive methods have gained prominence, such as near-infrared (NIR) spectroscopy, which offers advantages including speed, minimal sample preparation, and the absence of toxic reagents (Agelet and Hurburgh, 2014; Xia et al., 2019). When combined with chemometrics, which consists of the application of statistical methods and mathematical models to chemical data (Ferreira et al., 1999), NIR spectroscopy has proven to be a promising tool for seed classification (Ferreira et al., 2014; Choi et al., 2016; Panero et al., 2022; Soares et al., 2024). These models enable the discrimination of seed lots based on characteristics such as origin, cultivar, deterioration, viability, and vigor, reflecting differences in biochemical composition, particularly in components such as oil, protein, and carbohydrates.

Considering that near-infrared spectroscopy (NIR) can identify variations in the biochemical composition among seed batches, and that seed heteromorphism may reflect different maturation stages, thereby influencing their composition and physiological quality, the present study aimed to evaluate light and dark seeds of *C. fissilis* using NIR spectroscopy and to investigate their relationship with physiological quality.

MATERIAL AND METHODS

Plant Material

The study was conducted with *Cedrela fissilis* Vell. seeds collected in São Paulo state, Brazil, supplied by Arbocenter. According to the supplier, the collection took place in 2023, during fruit ripening on different mother trees with genetic variability, and the seeds had an initial germination rate of 80%. Classified as orthodox (Carvalho, 2003), the seeds were stored in a cold chamber for one year and used in this study due to the unavailability of freshly harvested seeds. Before the evaluations, seeds were visually classified into two color categories: light and dark seeds (Figure 1). This classification was based on the most contrasting tones observed within the original seed lot, and these categories were considered as the experimental treatments.

Physical Seed Quality

Thousand-Seed Weight: the thousand-seed weight was determined according to Rules of Seed Testing - RAS (Brasil, 2009), using eight replications of 100 seeds for each color, weighed on an analytical balance.



Figure 1. Light and dark seeds of *C. fissilis* Vell.

Moisture Content: seed moisture content was determined based on the difference between wet and dry weight after drying at 105 °C for 24 h using the oven-drying method (Brasil, 2009).

Germination and Vigor Analyses

Germination: the germination assay was conducted following the guidelines established by Brasil (2013). Four replications of 25 wingless seeds were used, previously disinfected in a 1% sodium hypochlorite solution for 3 min and then rinsed with deionized water. The seeds were placed on two sheets of Germitest® paper, sprayed with a 1.5% Captan solution, and covered with a third sheet. The paper sheets were pre-moistened with deionized water in a volume equivalent to 2.5 times the dry weight of the paper. The rolls were placed inside plastic bags and kept in a BOD-type germination chamber at a controlled temperature of 25 °C with a 12-hour photoperiod for 21 days. Radicle protrusion was recorded daily, and normal seedling counts were performed at 14 days (first count) and 21 days (final count) after test initiation.

Germination Speed Index (GSI): the GSI was calculated based on normal seedling emergence, according to Maguire (1962).

Electrical Conductivity: the electrical conductivity test was conducted using 25 *C. fissilis* seeds per replication, totaling four replications per treatment. The seeds were immersed in 75 mL of distilled water and kept in a BOD-type germination chamber at 25 °C for 24 h (Vieira et al., 2020). After this period, the electrical conductivity of the solution was measured using a MICRONAL conductivity meter (model B 330). The obtained value was divided by the seed weight, and results were expressed as mean values in $\mu S.cm^{-1}.g^{-1}$ of seeds.

Reserve substances

Oil Content: Total lipid extraction was performed according to Silva (1990) in a Soxhlet apparatus, using hexane as solvent for 24 h. After extraction, the solvent was evaporated in an oven at 40 °C for 24 h. The lipid content was calculated by the difference in mass of the samples before and after extraction.

Total protein: Total protein determination was performed using the Kjeldahl semi-micro method according to Silva (1990). Samples consisting of 0.2 g of crushed seeds were placed in test tubes together with 1 g of digestion mixture composed of sodium sulfate (200 g), copper sulfate (20 g) and selenium (2 g), and 5 mL of sulfuric acid. The samples were digested in a digestion block at 150 °C for 1 h, followed by 300 °C for another 6 h. After digestion, with the cold solution, 10 mL of distilled water were added. Then, 10 mL of sodium hydroxide (50%) were added and distillation was carried out. The distillate was collected in an Erlenmeyer flask (75 mL) containing 10 mL of an indicator solution

composed of boric acid (2%) and a mixed indicator (bromecresol green and 0.1% methyl red in ethyl alcohol). The titration was performed using a 0.02 N hydrochloric acid solution. The total protein content was estimated using a factor of 6.25 (Silva, 1990).

Total Soluble Sugars: Quantification of total sugars was performed using the UV-sulfuric acid method described by Albalasmeh et al. (2013), with modifications. For this, 100 mg of defatted light and dark seed samples were placed in Falcon tubes and homogenized with 4 mL of 80% ethanol. The samples were heated at 70 °C for 1 h, centrifuged, and the supernatant was used for quantification. Absorbance was measured in a spectrophotometer at 315 nm, using 40 µL of the alcoholic extract, 210 µL of deionized water and 1.25 mL of concentrated H_2SO_4. The results were expressed in g.100 g^{-1} of dry mass.

Reducing sugars: The quantification of reducing sugars was performed according to Gonçalves et al. (2010), with modifications. An aliquot of the alcoholic extract, obtained previously, was homogenized with 500 µL of the DNS solution and incubated in a water bath for 5 min. After cooling, 4 mL of deionized water were added, and the reading was performed in a spectrophotometer at 540 nm. The quantification of non-reducing sugars was performed by the difference between the results of total soluble sugars and reducing sugars. The results were expressed in g.100 g^{-1} of dry mass.

Starch: Quantification of total sugars was performed using the UV-sulfuric acid method described by Albalasmeh et al. (2013), with modifications. The pellet was washed three times with 2 ml of 80% alcohol, followed by a water bath for 5 min at 70 °C and centrifugation at 4000 rpm. Then, the supernatant was discarded and the samples were placed in an oven for 24 h at 45 °C. The pellet mass of each tube was weighed and homogenized with 4 ml of concentrated sulfuric acid. The samples were centrifuged at 4000 rpm for 5 min, and an aliquot was used to read the absorbance at 315 nm. To calculate the starch, a correction factor of 0.9 was used to convert free glucose into starch. The results were expressed in g.100 g^{-1} of dry mass.

Anatomy and histochemistry

Sample preparation: seeds from each treatment were fixed in FAA50 for 48 h and then stored in 70% (v/v) ethanol (Johansen, 1940). To obtain histological sections, the seeds were sectioned in transverse planes with the aid of a metal blade and subsequently embedded in methacrylate (Historesin-Leica Biosystems Nussloch, Heidelberg, Germany), according to the manufacturer's instructions. Transverse sections, with a thickness of 8-10 µm, were obtained using an automatically advancing rotary microtome (Leica RM2155, Leica Microsystems Inc., Deerfield, USA). The sections were then stained and mounted in synthetic resin (Permount®) for microscopic analysis.

Histochemical characterization: Xylidine Ponceau dye (Vidal, 1970) was used for identification of protein bodies and acid phloroglucin for detection of lignin (Johansen, 1940). The slides were analyzed and photographed under an optical microscope (AX70 TRF, Olympus Optical, Tokyo, Japan), coupled to a digital camera (Zeiss AxioCam HRc, Göttingen, Germany), using Axio Vision software for image capture.

NIR Data Collection and Preprocessing

Spectral readings were conducted individually on 100 seeds from each color, totaling 200 spectra for analysis. Each spectrum was obtained in the range between 1,000 and 2,500 nm, using an Antaris II spectrometer (Thermo Scientific) in the log reflectance (1/R) mode.

Data analysis

The experiment followed a completely randomized design, and statistical analyses were performed using R software (R Core Team, 2025). Data from germination and vigor tests, as well as biochemical analyses, were subjected to Student's t-test at a 5% significance level. Spectral data underwent chemometric pre-processing using Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC), and first- and second-order Savitzky–Golay (SG) derivative

methods. Both raw and pre-processed spectral data were used to train the classification model based on Partial Least Squares Discriminant Analysis (PLS-DA). For this purpose, 70% of the data were used for training (calibration), and the remaining 30% for testing (validation). Model performance was evaluated through the analysis of accuracy and the kappa coefficient, calculated for both training and testing datasets, according to equations 1 and 2.

$$Acuracy = \frac{TP + TN}{TP + TN + FP + FN}, \qquad (1)$$

$$Kappa\ Coeficient = \frac{(Po - Pe)}{(1 - Pe)}, \qquad (2)$$

where *TP* stands for true positives, *TN* for true negatives, *FP* for false positives, *FN* for false negatives, *Po* is the proportion of observed agreement, and *Pe* is the proportion of expected agreement.

RESULTS AND DISCUSSION

Dark seeds have, on average, greater mass than light seeds, as demonstrated by the weight of a thousand seeds (Table 1). Therefore, fewer dark seeds are needed to reach 1 kg of seeds compared to light seeds. Dark seeds showed a higher percentage of radicle emission, normal seedlings and higher GSI, indicating a greater physiological potential in relation to light seeds (Table 2).

The low germination rate presented in Table 3 is consistent with findings reported in previous studies on the storage of *C. fissilis* seeds (Flores et al., 2018; Silva et al., 2020). These studies indicate that both germination and seed vigor of this species undergo a significant decline over storage time, with viability being particularly compromised after storage periods exceeding 380 days. The electrical conductivity test, recognized for its efficiency in assessing membrane permeability that is affected by the seed deterioration process, did not reveal significant differences between light and dark-colored *C. fissilis* seeds in the present study Flores et al. (2018) observed that, compared to the germination speed index, the electrical conductivity test was less effective in indicating the loss of seed quality in *C. fissilis*. The authors suggested that, for this species, the test may be suitable for identifying advanced deterioration levels, but its sensitivity is limited in detecting early or intermediate stages of seed deterioration.

Light- and dark-colored seeds of *C. fissilis* exhibited similar oil content (Figure 2A). In contrast, dark seeds showed

Table 1. Thousand-seed weight and moisture content of light and dark seeds of *C. fissilis*. Means followed by the same letter do not differ from each other by Student's t-test at 5% probability .

Seed	Degree of humidity (%)	Weight of a thousand seeds	Nº seeds.Kg^{-1}
Light	9.30 a	63.94 b	15639
Dark	9.48 a	65.43 a	15284

Table 2. Average results of the physiological parameters of light and dark *C. fissilis* seeds.

Seed color	Radicle emission (%)	FGC (%)	LGC (%)	GSI (%)	CE (µS.cm^{-1}.g^{-1})
Light	31 b	12 b	8 a	1.24 b	136.06 a
Dark	58 a	39 a	11 a	3.31 a	132.22 a

First Germination Count (FGC), Last Germination Count (LGC), Germination Speed Index (GSI), Electrical Conductivity (CE). Means followed by the same letter do not differ from each other by Student's t-test at 5% probability .

higher total protein content (Figure 2B). Silva et al. (2020), in a study with *C. fissilis* seeds, observed that a decrease in total protein content during storage, is detrimental to seed germination and vigor in this species. Studies conducted on seeds of agronomic crops such as wheat and maize have demonstrated that protein content is a key determinant of seedling vigor (Ries and Everson, 1973; Wen et al., 2018). Ries and Everson (1973) showed that, regardless of genotype or environmental conditions, seeds with higher protein content produce more vigorous seedlings.

According to the results obtained in the biochemical analysis of carbohydrates, light and dark seeds of *C. fissilis* presented similar levels of total soluble sugars, non-reducing sugars and starch (Figures 3A, C and D). However, a significant difference was observed in the content of reducing sugars (Figure 3B).

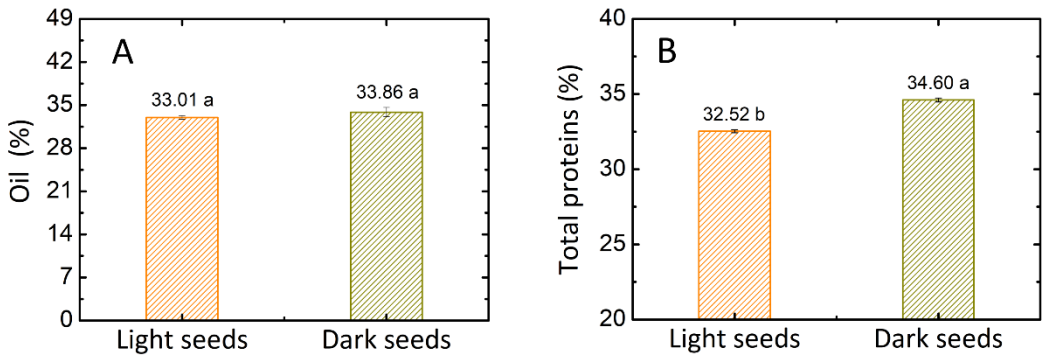

Figure 2. Oil content (A) and total proteins (B) in light and dark seeds of *C. fissilis*. Means followed by the same letter do not differ from each other by Student's t-test at 5% probability . The bars represent the standard error of the mean.

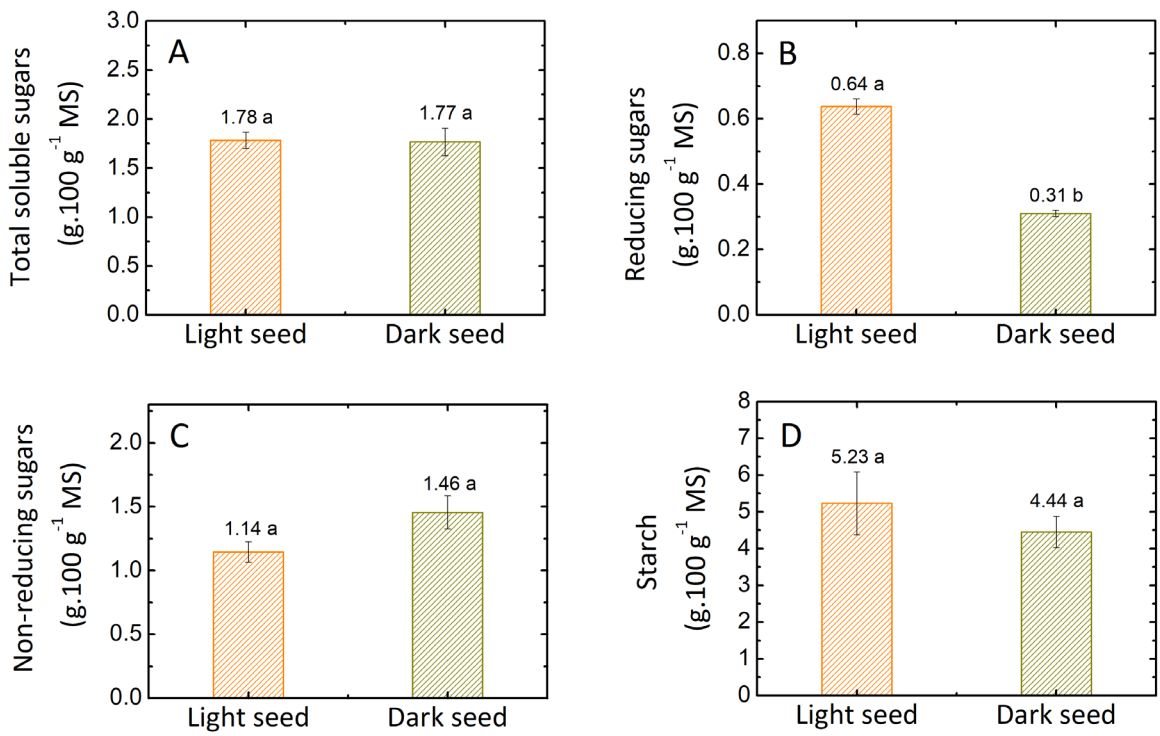

Figure 3. Carbohydrates in light and dark seeds of *C. fissilis*. Total soluble sugars (A); Reducing sugars (B); Non-reducing sugars (C); Starch (D). Means followed by the same letter do not differ from each other by Student's t-test at 5% probability . The bars represent the standard error of the mean.

In the final stage of seed maturation, there is a decrease in the reducing sugars content and an increase in the concentration of non-reducing sugars, such as sucrose and raffinose family oligosaccharides (RFOs), which contribute to the stabilization of membranes and macromolecules, protecting cellular structures from damage caused by water loss during desiccation at this stage (Koster and Leopold, 1988; Hoekstra et al., 2001; Groot, 2022). Therefore, our results possibly indicate that light-colored seeds did not complete the maturation process properly. On the other hand, the abnormal accumulation of these compounds may also reflect physiological deterioration processes, associated with polysaccharide degradation and cellular oxidative stress. Moreover, these sugars participate in the Amadori reaction, a non-enzymatic process that occurs between the carbonyl groups of reducing sugars and the amino groups of proteins, leading to the formation of stable intermediate compounds, which may compromise protein integrity (Marcos-Filho, 2015). Thus, the higher content of reducing sugars observed in light-colored seeds may be related to an incomplete maturation process or may represent an early indication of seed deterioration.

From the histochemical test performed with the XP reagent, a high amount of proteins was observed in the pink cedar seeds, detected in the tegument, endosperm and cotyledons (Figure 4A). In the light-colored seeds, the protein bodies in the endosperm region appear to be less structured or smaller in size (Figures 4B-D). In contrast, in the dark seeds, well-defined protein bodies are observed, including in the endosperm region (Figures 4E-G). According to the histochemical test performed with the acid phloroglucin reagent, the presence of lignin was detected only in xylem vessels located at one end of the seed coat, in both light and dark seeds (Figures 4H, I). During development, seeds establish a specialized vascular system responsible for the transport of water and nutrients from the maternal plant to the developing embryonic tissues. In species of the Meliaceae family, this system may include well-developed vascular bundles that traverse the seed coat and, even after maturation, may remain within the seed, contributing to its protection against mechanical damage and external stressors (Johri, 1984).

The differences observed between light- and dark-colored *C. fissilis* seeds suggest the influence of distinct maturation stages, as well as possible deterioration effects resulting from storage. Dark seeds exhibited greater mass and higher total protein content. They also demonstrated higher vigor, as indicated by higher radicle emergence rate, a higher number of normal seedlings, and a greater germination speed index. These results are consistent with seeds that have reached

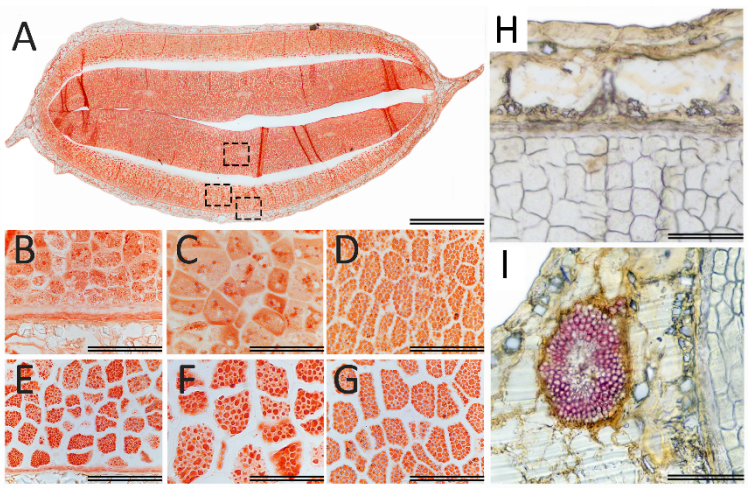

Figure 4. Cross section of *C. fissilis* seeds. Histochemistry with Xylidine Ponceau of a light seed (A); Endosperm region just below the seed coat in the light seed (B) and greater detail (C); Cotyledon region in the light seeds (D); Endosperm region just below the seed coat in the dark seed (E) and greater detail (F); Cotyledon region in the dark seeds (G). Histochemistry with acid phloroglucin for presence of lignin not detected in the seed coat (H). Lignin detected in the xylem vessels present in at the end the seed coat (I). Bars: 1000 µm (A); other images 100 µm.

physiological maturity, which is characterized by higher dry mass, greater accumulation of reserves, and enhanced germination potential and vigor (Rubio et al., 2013; Brito et al., 2015; Groot, 2022). In contrast, light-colored seeds showed lower germination performance, reduced total protein content, and higher levels of reducing sugars, which may indicate incomplete maturation or early signs of deterioration. Therefore, seed coat color is associated with the physiological status of the seeds and may serve as a practical criterion for selecting seeds with superior physiological quality.

Table 3 presents the accuracy and kappa coefficient calculated for the PLS-DA model using NIR spectral data subjected to different preprocessing methods. Due to the broad and frequently overlapping nature of NIR absorption bands, spectral data preprocessing is a critical step for enhancing relevant features, facilitating pattern recognition, and improving classification model performance (Rinnan et al., 2009; Xia et al., 2019). The selection of the most appropriate preprocessing method for calibration model construction was based on the model's ability to accurately predict seed color class. This predictive ability was evaluated using accuracy and the kappa coefficient. Accuracy, a widely used metric in machine learning classification models, represents the proportion of correct predictions relative to actual class values (Manning et al., 2009). As a complementary validation measure, the kappa coefficient was calculated to assess the degree of agreement between predicted and observed classifications. A kappa value of 1 indicates perfect agreement, while values near 0 suggest random agreement (Viera and Garrett, 2005).

The PLS-DA classifier exhibited high discriminative capacity using both raw and preprocessed NIR data. The model achieved an overall test accuracy starting at 98.33%, reaching 100% when preprocessed data were used. A similar trend was observed for the kappa coefficient. According to the interpretation scale proposed by Landis and Koch (1977), the kappa values indicated almost perfect agreement (0.80–0.99), confirming the model's strong ability to discriminate between seed color classes in cedro-rosa. Figure 5a displays the 200 raw NIR spectra of light- and dark-colored *C. fissilis* seeds, recorded within the spectral range of 1,000 to 2,500 nm. Figure 5b shows the mean spectra after preprocessing using the standard normal variate (SNV) method, which was employed as the preprocessing technique for building the calibration model.

In the present study, the most relevant wavelength for the model was identified around 1938 nm (Figure 6). This region is generally associated with the presence of water, lignin, nitrogen, proteins, starch and cellulose (Fourty et al., 1996). The results obtained for moisture, starch, total protein and the histochemical test for lignin indicate that the total protein content played an important role in the differentiation between light and dark seeds by NIR spectroscopy. According to the histochemistry for protein, the difference in protein content would be mainly in the endosperm tissues.

In this study, we show that the seed color is a good indicator of the seed quality and that there were differences in its biochemical composition, but it is important to consider that the seed storage time of approximately one year, as well as the genetic variability, constitute methodological restrictions that must be considered when interpreting the results. Freshly harvested *C. fissilis* seeds may exhibit physiological behavior different from that observed in stored seeds, and genetic diversity may influence the degree of heteromorphism, resulting in variations in the observed physiological and spectral parameters.

Table 3. Accuracy and kappa coefficient results for the training and test datasets for the classification model, using different pre-processing methods, to classify cedar seed based on color.

Model	Preprocessing	Training (n = 70)		Test (n = 30)	
		Accuracy (%)	Kappa Coefficient (%)	Accuracy (%)	Kappa Coefficient (%)
PLS-DA	Raw	99.29	98.57	98.33	96.67
	MSC	99.28	98.57	1	1
	SNV	98.57	97.14	1	1
	SG (d1)	1	1	1	1
	SG (d2)	1	1	1	1

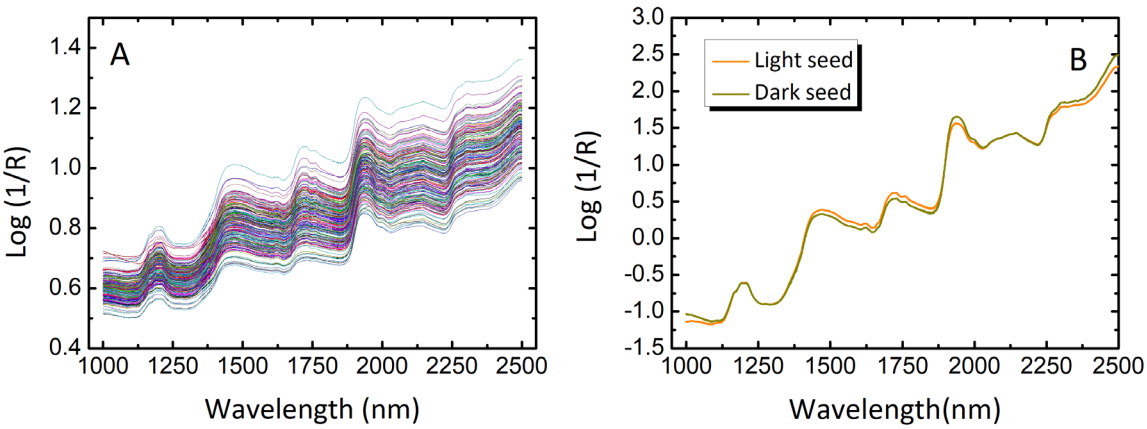

Figure 5. Raw NIR spectra (A), average of the raw spectra preprocessed using the SNV method (B).

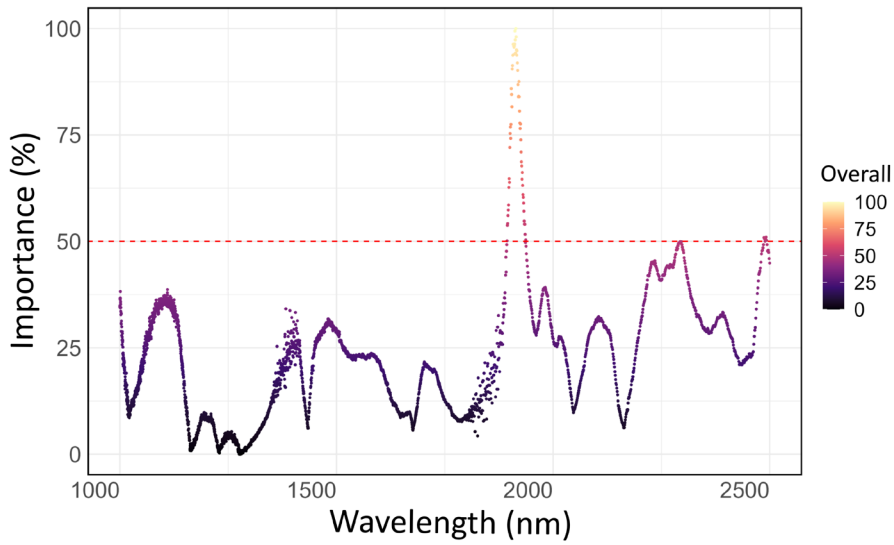

Figure 6. Variable importance indicating the main wavelengths of the spectrum for the development of the PLS-DA model.

For future work, we recommend adopting more objective and standardized methods for categorizing seeds by color, such as instrumental colorimetry or visible spectrum spectrophotometry, which enable accurate and comparable classification across studies.

CONCLUSIONS

The color of *C. fissilis* Vell. seeds is associated with their physiological potential and suggests the influence of different maturation stages, as well as possible effects of deterioration resulting from storage. Near-infrared spectroscopy (NIR) demonstrated high precision in distinguishing between light and dark seeds, with emphasis on the spectral region near 1938 nm, whose relevance may be related to variations in total protein content. Therefore, NIR spectroscopy stands out as a promising tool for the qualitative monitoring of maturation stages and the post-harvest physiological conservation status of *C. fissilis* seeds.

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AUTHORS' CONTRIBUTION

Karoline Geralda Mendes contributed with: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing - original draft; Laércio Junio da Silva contributed with: Methodology, formal analysis, Data curation, Visualization, Writing—review & editing; Edgard Augusto de Toledo Picoli contributed with: Methodology, Data curation, Visualization, Writing—review & editing; Vinícius Pinto Aguiar contributed with: Data curation; Lausanne Soraya de Almeida: Conceptualization, Methodology, Project administration, Resources, Supervision, Writing - review & editing; Jean Marcel Sousa Lira: Conceptualization, Methodology, Project administration, Supervision, Writing - review & editing.

DATA AVAILABILITY

Additional data will be made available by the authors upon reasonable request.

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