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Determination of the physiological quality of corn seeds by infrared equipment

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ABSTRACT: The use of infrared equipment to evaluate the physiological quality of seeds has become an extremely important technique for the operation, since it is not destructive to the seed and is safe for the operator. The experiment was divided into two stages, in the first, the controlled deterioration curve was carried out, and in the second stage, the validation of the NIR XDS process was carried out, through modeling for classification of the lots. The technique used for controlled deterioration proved to be efficient to show that with a higher band ratio index, the spectrum has a greater amplitude and lower physiological potential, the opposite is also valid, when the band index is smaller, the spectrum amplitude is smaller and higher values of germination and vigor were observed. For the second stage, it was concluded that spectra generated by NIR XDS equipment can be used quickly for decision making on corn seed samples, considering a binary classification for the parameters of germination and vigor according to the approval and disapproval values of lots considered in this study.

Index terms: deterioration, germination, NIR, vigor, *Zea mays* L.

RESUMO: O uso de equipamento de infravermelho para avaliação da qualidade fisiológica de sementes tem se tornado uma técnica de extrema importância, uma vez que, não é destrutiva para a semente e é segura para o operador. O experimento foi dividido em duas etapas, na primeira realizou-se a curva de deterioração controlada, e na segunda etapa, realizou-se a validação do processo NIR XDS, por meio de modelagem para classificação dos lotes. A técnica utilizada para a deterioração controlada mostrou-se eficiente uma vez que: com um índice de razão de banda maior, o espectro tem uma amplitude maior e menor potencial fisiológico, o contrário também é válido, quando o índice de bandas for menor, a amplitude do espectro é menor e notaram-se maiores valores de germinação e vigor. Pelo segundo estudo concluiu-se que, espectros gerados por equipamento NIR XDS podem ser utilizados de forma rápida para tomadas de decisão sobre as amostras de semente de milho, considerando uma classificação binária para os parâmetros de germinação e vigor de acordo com os valores de aprovação e reprovação de lotes considerados neste estudo.

Termos para indexação: deterioração, germinação, NIR, vigor, *Zea mays* L.

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INTRODUCTION

The NIR (Near Infrared by Reflectance) spectrophotometer is a non-destructive technique, with good precision, extremely safe for the operator, and widely used for measuring grain components (Dyer, 2004).

NIR spectroscopy is a technique that measures the detection of near infrared absorbance by a sample. The near infrared is in the range of 800 nm to 2500 nm and has sufficient energy to excite overtones (organic bonds), combinations of molecular vibrations at self-energy levels. NIR spectrophotometers are generally used for quantitative measurements of organic functional groups, especially O-H (fatty acids), N-H (proteins), and C=O (esters). Detection limits are in the order of 0.1% and applications include analyses of pharmaceutical and agricultural products, polymers, and clinical analyses (Alexander et al., 1967).

The NIR technique has been used to replace traditional analyzes to evaluate the potential of near infrared spectrometry to analyze the quality of cotton seeds (Mayrinck et al., 2020), and pepper seeds (*Capsicum annuum* L.) (Seo et al., 2016) depending on different levels of vigor, to identify genetically modified organisms in foods containing soybean (Conceição et al., 2006), and to describe the chemical composition of corn grains subjected to drying and storage (Gutkoski et al., 2009). In seeds, NIR has also been used to select genotypes of commercial interest, such as in oats (Silva et al., 2008).

The physiological processes of germination and vigor are influenced by the chemical composition. Seeds have been intensively studied regarding the chemical composition of their reserves. Modern varieties of soybeans, corn, sorghum, and wheat have been improved and developed for high oil, protein, or carbohydrate content. These modifications generally affect the behavior of the seeds, changing the basic pattern of the species in relation to aspects such as: germination, vigor, storability, and interaction with pathogens (Oliveira, 2015). Seeds with low vigor may be subject to reduced emergence speed, lower production of dry biomass and seedling growth rates can affect the establishment and performance of the crop throughout the cycle and reduce productivity (Gazolla-Neto et al., 2012).

Therefore, this research study was intended to validate infrared equipment (NIR) to assess the physiological quality of wet corn seeds in a safe and fast way that helps to speed up its decision-making forwarding from the industrial unit (drying and processing) to send to the market only high-quality lots.

MATERIAL AND METHODS

The assay was carried out at Bayer Brazil at Uberlândia - MG, in the Production Research Pilot Plant. Corn cobs from the winter 2018, winter 2019, summer 2019, or winter 2020 crops were used, with harvest moisture between 25% and 36%.

FOSS NIR XDS equipment (XDS Rapid Content Analyzer™) was used, this equipment is full spectrum, 400-2500 nm.

The study was divided into two stages: in the first, the controlled deterioration curve was performed, it was necessary to create a “humid chamber” to standardize the initial moisture of the seeds. Standard Germination and RET - primary root protrusion was performed to define lots of genotypes with high and low initial quality. Plastic Gerbox® boxes (230) were assembled with a screen to store the entire volume of seeds necessary for (400 seeds per test), vigor (200 seeds per test), and NIR (1 kg of seeds per run) studies. A microclimate with saturated potassium sulfate solution was created in the boxes. The seeds were placed on the screens to avoid direct contact with the solution and the box was closed with a lid. Three replications of each hybrid were assembled, 38 boxes for each replication (130 at 150 seeds per box).

The lots chosen were seeds without treatment, from silos or Big Bags, dried (~11%), threshed and sorted. Initial physiological analysis was performed using TPG - standard germination test (Brasil, 2009) and RET - primary root protrusion (ISTA, 2015), and initial moisture with Perten’s Aquamatic 5200 equipment, and then the samples were weighed.

The saturated solution of potassium sulfate in each box allowed the increase of the internal relative moisture to about 95% (Juliano, 1964). The boxes with the solution and with the seeds on screens were sealed and left on benches at a controlled temperature (25 °C).

The initial mass of 6 boxes (2 of each replication for each hybrid) was measured and weighed every 24 h until the mass of the seeds no longer showed any variation.

Once the seeds had uniform initial moisture, the boxes with the solution and the seeds were taken to accelerated aging chambers (Symphony™ model: 3078) with saturated moisture and temperature of 42 °C (±1), type B.O.D. for 96 hours (± 1h), which is equivalent to four days (or until very low germination and vigor are observed) (Krzyzanowski et al., 1999).

Every 24 hours, the boxes were removed from the chamber, the seeds passed through the NIR XDS equipment, at least 1 kg of shelled seeds were enough to complete the conveyor belt for 15 seconds of NIR performance, moisture was measured in a Perten Aquamatic 5200 equipment, and a TPG - standard germination test, according to Rules for Seed Testing (Brasil, 2009), and a vigor test, known as RET - primary root protrusion, were assembled (ISTA, 2015).

In the second stage of the project, where the validation of the NIR XDS process was carried out, through modeling to classify the lots, thus, seed samples, approximately 200, with 10 to 15 cobs in each sample, were taken directly from the trucks that arrived with unprocessed corn from the production field. Several hybrids were harvested at different harvest moistures (25%-36%). They were, immediately husked and threshed by hand. They passed through NIR XDS equipment and then 500 g of each sample were placed in a screened packaging, dried in an experimental dryer, and treated with basic solution treatment.

After treatment, they were packed in multi-leaf labeled paper bags, sent to Laboratory, for TPG (Brasil, 2009) and RET (ISTA, 2015).

Statistical analysis was performed in two stages, the first with controlled deterioration data, a cluster analysis was performed on TPG and RET data. In the cluster analysis, the K-means method was used with the Pearson's correlation coefficient by measure of similarity, and the analyses were implemented using the TIBICO Spotfire and R statistical program. A smoothing method was also applied (Bonat, 2007). In the second step for the dataset for validation the PLS-DA (Barker and Rayens, 2003), the algorithm using the Kennard-Stone method (Kennard and Stone, 1969) and "ordered predictors selection" for discriminant analysis (OPSDA) were used (Roque et al., 2019). Model performance was assessed based on the parameters of sensitivity, specificity, and classification error of the training and test set, which were calculated according to the equations (1), (2) and (3), respectively (Roque et al., 2017).

$$\text{Sensitivity} = VP / (VP + FN) \quad (1)$$

$$\text{Specificity} = VN / (VN + FP) \quad (2)$$

$$\text{Error} = FP + FN / (VP + VN + FP + FN) \quad (3)$$

VP is true positive and VN is true negative, FN is false negative, and FP is false positive. The sensitivity or true positive rate is the percentage of samples belonging to the class that were correctly classified as belonging to the class. The specificity or true negative rate is the percentage of non-class samples that were correctly classified as non-class. Misclassification is the percentage of samples that were misclassified.

RESULTS AND DISCUSSION

In the first phase, results of controlled deterioration were presented. The seeds had 10.4% (hybrid A) and 10.5% (hybrid B) initial moisture and entered in a steady state on the fourth day after test setup, with 22.1% and 23% moisture, respectively.

The water content of the seeds directly influences several aspects of their physiological quality, so its determination is essential in official quality tests carried out in seed lots (Sarmento et al., 2015).

Hybrid A had stable germination in the first 24 h, around 90%, and then gradually reduced the germination percentage to 41% at 96 h. For vigor, hybrid A had a more significant drop reaching 20% at 96 h. Hybrid B showed a stable behavior at 48 h for germination and vigor, after which the percentages were drastically reduced. Hybrid B germination went from 99% to 67%, and RET went from 98% to 53%.

During this phase of controlled deterioration, seed moisture was also measured each day, there was no significant gain in moisture for any of the materials as compared to the beginning of the test. The technique used for controlled deterioration proved to be efficient, as it preserved the moisture content of the seeds during the test between 20 and 25%. An advantage of using saturated salt solutions is that the relative moisture values remain at lower levels, being sufficient to prevent the growth of microorganisms, thus minimizing any concerns about the effects of seed pathogens on the results of germination and vigor tests using deteriorated seeds (Jianhua and MCDonald, 1997).

Once the seeds had uniform initial moisture, the boxes with the solution and the seeds were taken to accelerated aging chambers. It´s possible see a separation of the aging measurement times in deterioration directly in the spectra without any pre-data processing. We have the raw spectra for the assay samples, which passed through the NIR XDS equipment during the controlled deterioration step. Both hybrids (A and B) were observed to show similar spectral behaviors in Figure 2.

As the spectra displayed similar behaviors for both genotypes, the analyses were performed together, hierarchical cluster analysis on TPG data, RET and oil content.

To enable the analysis of the chosen bands, i.e., a and b in Figure 1, P10 and P90 of the data were determined at each time point of the study. P10 corresponds to the lowest absorbance values of the peak band (1100 to 1300 nm) and the valley band (1650 to 1800 nm), whereas P90 corresponds to the highest absorbance values of the peak and valley bands.

Near infrared spectroscopy is based on the absorption of electromagnetic radiation at wavelengths in the range of 780 – 2500 nm of the light electromagnetic spectrum. Its spectra result from vibration and consequent absorbance, mainly in Carbon-Hydrogen (C-H), Oxygen-Hydrogen (O-H), Sulfur-Hydrogen (S-H), and Nitrogen-Hydrogen (N-H) bonds, which are found in natural compounds, as shown in Figure 2.

To reduce the influence of dataset variations, allowing each observation to be optimally and consistently represented, the spectra data matrix was normalized. Normalization primarily reduces the effects of total intensity response profiles due to variations in sample concentration and light path. The spectrum normalization process is the creation of a rule to extract an index from opposite values, that is, transform the data so that it can be used more easily. In the K-means method, the objective was to group the distances in wavelengths to create indexes, classifications that would separate the materials studied by controlled deterioration treatments to yield a classification by physiological quality. A smoothing method was also applied (Bonat, 2007).

Table 1 shows a higher band ratio index, the spectrum has greater amplitude and lower physiological potential. And the opposite is also true, that is, when the band ratio index is smaller, the spectrum amplitude is smaller, and higher values for germination and vigor are observed.

The ratio between bands mathematical operation allows subtle differences in the spectral behavior of different targets to be identified because only gross differences are observed in original bands (Araujo and Melo, 2010).

Thus, observing the spectral behavior of the targets of interest, for the application of the ratio, the bands are selected based on their maximum and minimum absorbance values to express the gradients of the spectral curve of the objects of interest, providing the enhancement of these targets.

Karn et al. (2017), working with soybean seeds to determine oil and fatty acids by NIR spectroscopy, concluded that the use of NIR is efficient for this purpose. Such studies are similar to the results of this study. The bonds of organic substances absorb part of the incident energy, thus allowing the estimation of the type of molecular bond contained in the samples, which measure the difference between the amount of emitted and reflected light (Fernandes et al., 2010) (Figure 2).

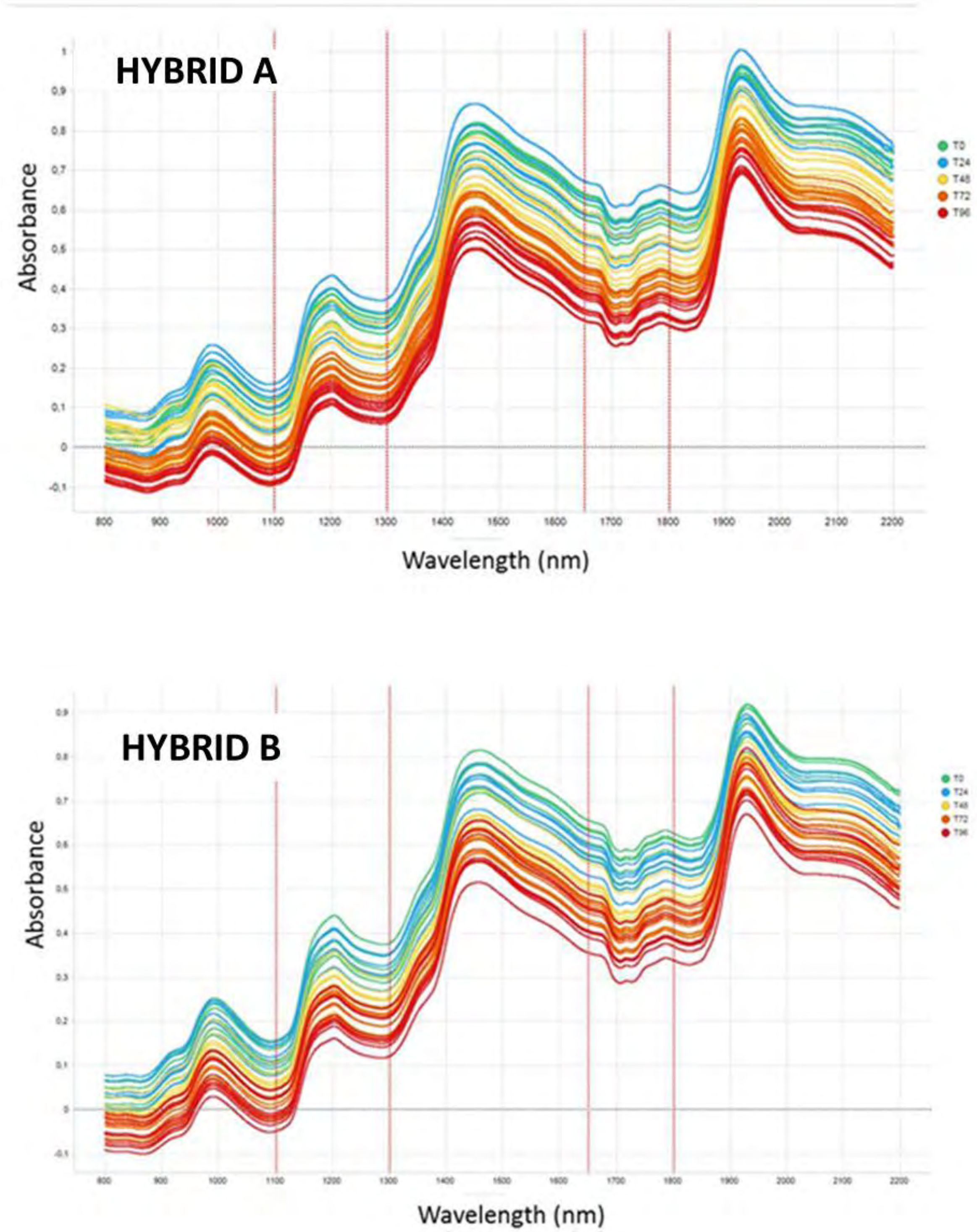
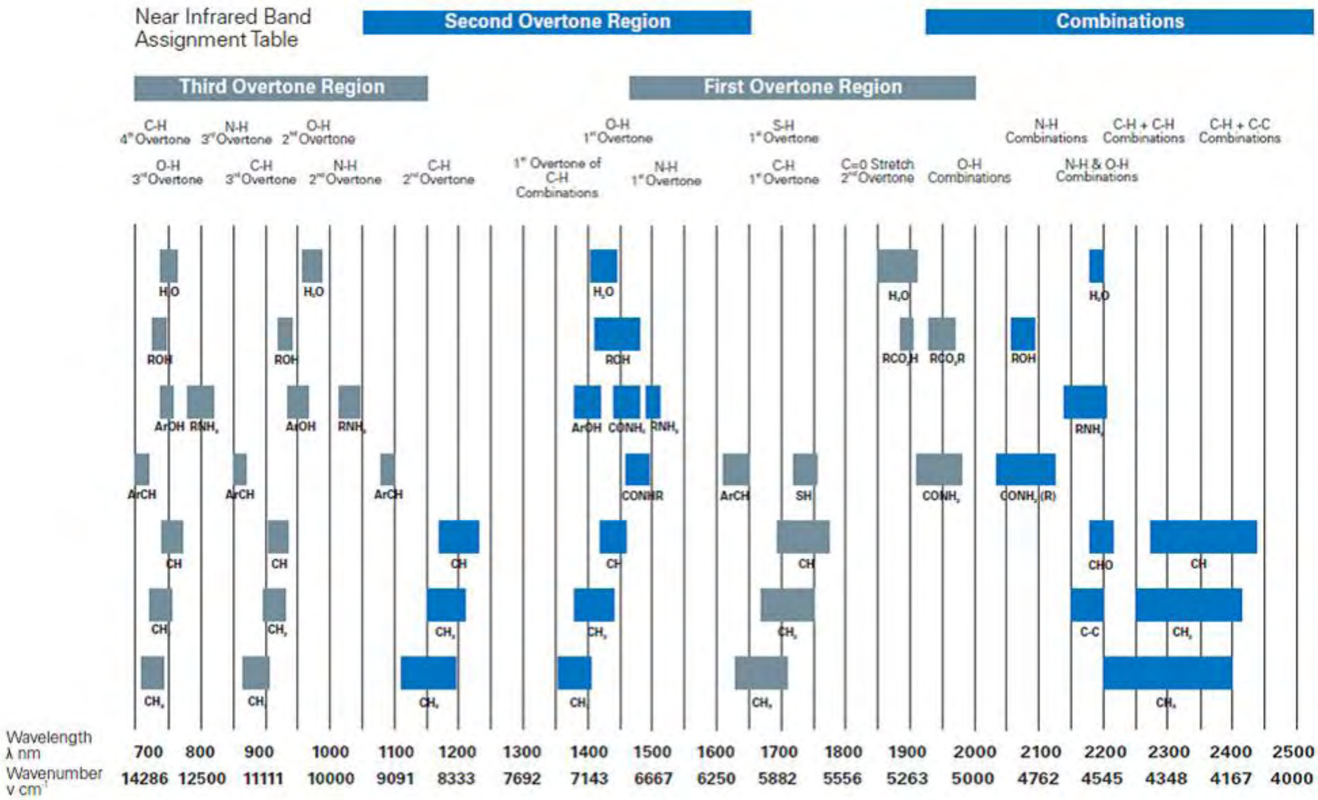


Figure 1. Raw spectra for the assay samples chart. The samples passed through NIR XDS equipment during the controlled deterioration step.

The second dataset contained 452 samples with two replications, all of which were wet, with zero months of storage. This set was obtained to validate the analyses carried out with the previous sets. The germination (9-99%) and vigor (4-97%) contents were verified.



Source: Bruker®.

Figure 2. Functional groups assigned to their respective wavelength/wavenumber (Bruker®).

Table 1. Mean absorbance values for hybrids A and B in the probable oil bands, band ratio, mean TPG (% germination), and RET test mean (% vigor) in each aging period.

Time	P10	P90	P10	P90	P90/P10	P90/P10	Germination	Vigor
	Band 1	Band 1	Band 2	Band 2	Band 1	Band 2	AVG TPG %	AVG RET %
T0	0.13	0.38	0.53	0.63	2.96	1.18	95	97
T24	0.14	0.38	0.50	0.62	2.83	1.24	95	96
T48	0.07	0.30	0.40	0.53	4.44	1.34	88	94
T72	0.02	0.24	0.36	0.45	15.19	1.25	68	73
T96	(0.03)	0.21	0.29	0.43	(8.34)	1.47	41	37

*( ) negative values.

Thus, in this set, a modeling was applied to relate the spectra with the physiological values represented by germination (TPG) and vigor (RET).

Samples with germination greater than or equal to 90% were defined as belonging to one class and samples with germination lower than 90% belonging to another class. Likewise, the samples were divided into two classes in relation to the value of 85% of vigor.

The PLS-DA is a method based on PLS regression (Miaw et al., 2018), in which the dependent variable is categorical. This is a supervised pattern recognition method that uses the X matrix of independent variables, i.e. the NIR spectra with the categorical variable Y for classification model building. To build PLS-DA models, a training set with well-defined classes is necessary, as well as a validation set, so that the model can be used to classify new samples.

The complete sets of germination and vigor data were divided into a training and testing set using the Kennard-Stone algorithm (Kennard and Stone, 1969) separately for each class in order to maintain the proportionality of the classes. The separation of training and test sets was performed for each class following the proportion of 70 and 30%, respectively. Table 2 shows the distribution of samples from the germination and vigor sets, for each class in the training and testing sets.

The training set was used to build the classification model. The PLS-DA with random cross-validation was applied with 10% divisions of the training set. The models for both germination and vigor were built with NIR spectra centered on the mean.

In addition to building the PLS-DA models with all wavelengths of the NIR spectrum, that is, with all variables, a variable selection method called “ordered predictor selection” was also used for discriminant analysis (OPSDA) (Roque et al., 2019). Variable selection is used to improve the predictive capacity of a model, in addition to making the model interpretive with the selection of wavelengths related to a modeled property or characteristic. OPSDA is a method based on obtaining an information vector that contains information about the location of the variables to improve classification. Thus, this method tests several subsets of variables and finds the one with the best classification parameters. After selecting the variables, OPSDA builds a PLS-DA model using the selected variables only.

The classification models obtained with the variables selected by OPSDA showed a better predictive capacity than those built with all wavelengths. Thus, the results presented were obtained with OPSDA and the selected variables for germination and vigor, as shown in Figure 3 (A and B).

For germination, 900 variables were selected (wavelengths), which were spread over much of the entire spectrum, being more concentrated between 800 and 1500 nm, with some small selection bands at longer wavelengths, as shown in Figure 3A.

As for vigor, Figure 3B, 880 variables were selected in the ranges between 800 – 1132 nm and 1571 – 1681 nm. Models built using the selected variables for germination and vigor had better predictive capacity, that is, less classification error compared to the models built with all variables. Similar wavelength ranges were found in the work of Shrestha et al., 2017 when studying tomato seed viability.

Table 3 shows the parameters used to assess classification model performance, for germination and vigor. Parameters are expressed as values between 0 and 1, where an ideal classification has sensitivity and specificity of 1 and error of 0.

Result analysis showed that the errors of both the training and the test sets are smaller for vigor when compared to errors for germination. In the germination model, sensitivity and specificity were less than 1, indicating the occurrence of false negatives and false positives.

Considering the class with germination less than or equal to 90%, the occurrence of false negatives is not a worrying scenario, since incorrectly classified samples with low germination will be segregated and processed separately and more rigorously, in terms of physiological protection, as well as samples that actually have germination lower than 90%. False positives are a worrying scenario, as these will be treated as samples with high germination, which is not true. For the vigor model, the training set had no errors, and the test set had some errors with the worst scenario, that is, false positives considering the vigor class greater than or equal to 85%.

Wang et al. (2020) when studying vigor detection for sweet corn seeds using near infrared spectroscopy (NIRS), managed to classify the seeds into aged seeds (germination 3%-6%) and non-aged seeds, with 98% technique accuracy. Likewise, Kandpal et al. (2016) managed to separate viable and non-viable melon seeds using the non-destructive NIR technique. Kusumaningrum et al. (2018) stated that it is possible to assess the viability of soybean seeds quickly and non-destructively by NIR. This reiterates the fact that the method proposed in this study is more discriminatory. In contrast, Agelet et al. (2012) were unable to separate viable and non-viable seeds for soybean and corn crops using the NIR.

Table 2. Number of samples in each class in the training and testing sets for germination and vigor.

	Total	Classes	Total/Class	Training	Test
Germination	225	≥ 90%	142	99	43
		< 90%	83	58	25
Vigor	221	≥ 85%	104	73	31
		< 85%	117	82	35

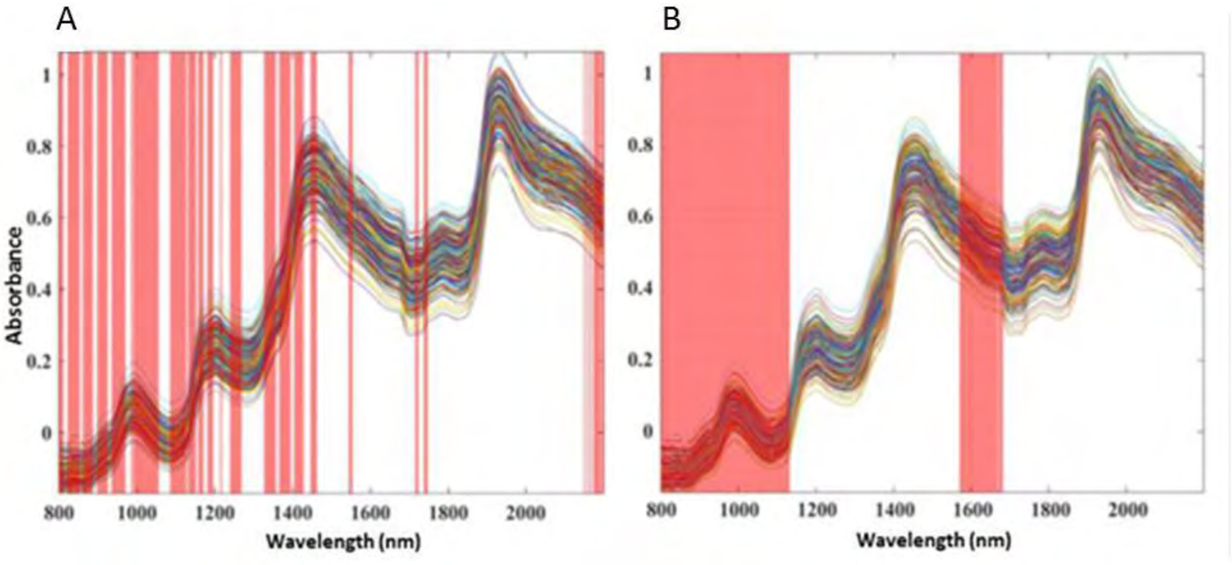


Figure 3. Variables selected by the OPSDA method for Germination (A) and Vigor (B).

Table 3. Assessment parameters of OPSDA models for germination and vigor in corn seed NIR spectra.

OPSDA models		Germination (%) 15 nvls*		Vigor (%) 6 nvls*	
Classes		≥ 90%	< 90%	≥ 85%	< 85%
Training	Sensitivity	0.939	0.983	1.000	1.000
	Specificity	0.983	0.939	1.000	1.000
	Error	0.038		0.000	
Test	Sensitivity	0.907	0.840	1.000	0.943
	Specificity	0.840	0.907	0.943	1.000
	Error	0.118		0.030	

*nvls: number of PLS-DA latent variables.

Figure 4 shows charts with the Y values predicted by the OPSDA models for germination and vigor. When building the OPSDA models, the discrimination threshold between classes is determined through a normal probability density function, in which Y values above the discrimination threshold are considered to belong to the class under analysis, and values below that do not belong to that class.

Figures 4A and 4B referring to germination show false negatives and false positives in the training and test sets, respectively, as mentioned above. In Figure 4C, referring to vigor, the classification result is optimal with 100% accuracy. However, in Figure 4D, referring to the test set of the vigor model, there are some false positives.

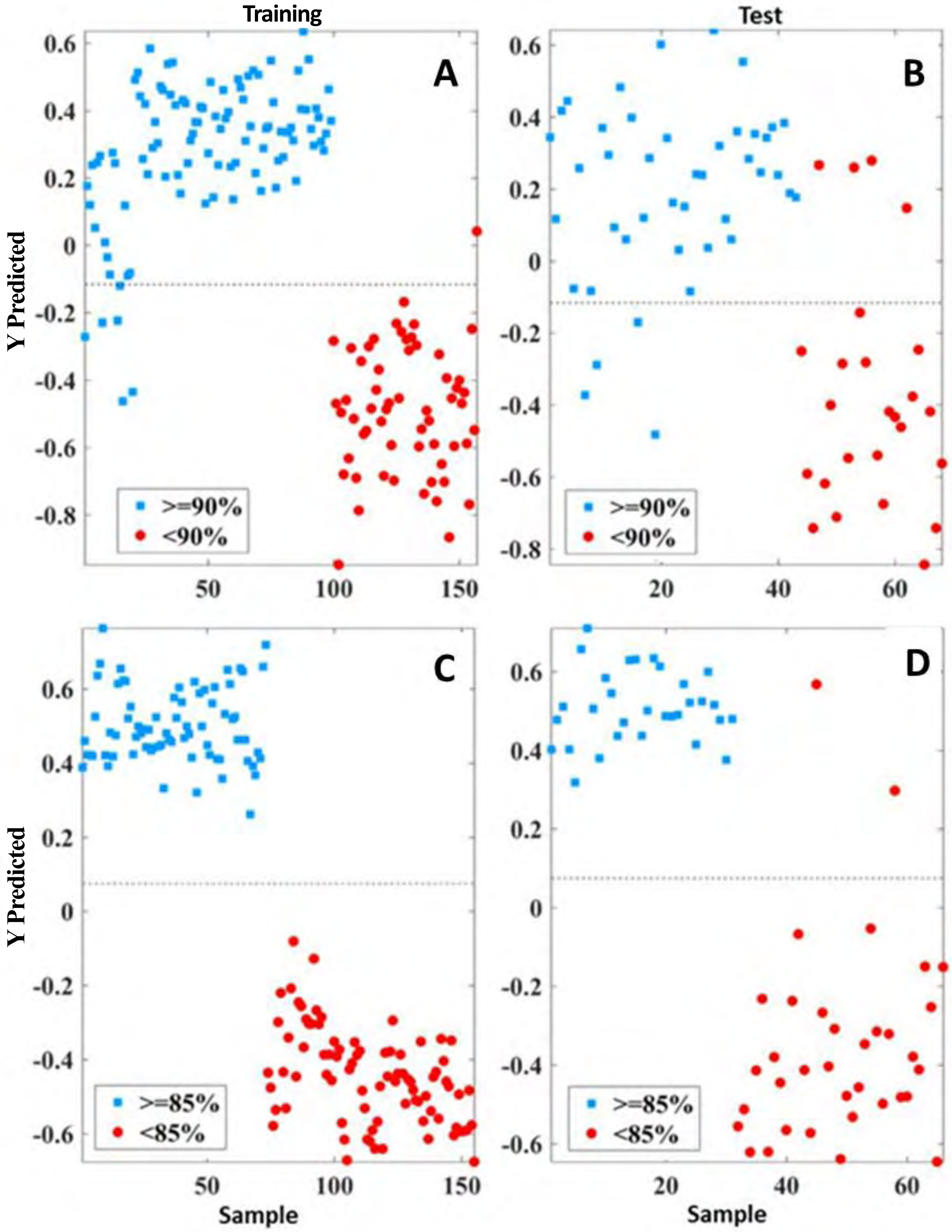


Figure 4. Predicted Y values for the training (A and C) and test (B and D) sets of the OPSDA models for Germination (A and B) and Vigor (C and D). The dashed horizontal line is the discrimination threshold for the classes.

From the results presented, the OPSDA models have high accuracy (Accuracy = 1 - Error), that is, percentage of correct answers for the model, being 0.962 (96.2%) and 0.882 (88.2%) in the training and test sets, respectively, for germination. For vigor, the accuracy was even greater, being 100% for the training set and 97% for the test set. Therefore, these OPSDA models based on NIR spectra can be easily used for decision making on corn seed samples, considering a binary classification for the germination and vigor parameters according to the pass and fail values for lots used by the company.

CONCLUSION

Spectra generated by NIR XDS equipment can be easily used for decision making on corn seed samples, considering a binary classification with 88.2% accuracy for the Germination (uper or lower 90% germination threshold) and 97% accuracy for the vigor (uper or lower 85% primary root protrusion threshold) parameters according to the pass and fail values of lots considered in this study.

REFERENCES

AGELET, L.E.; ELLIS, D.D.; DUVICK, S.; GOGGI, A.S.; HURBURGH, C.R.; GARDNER, C.A. Feasibility of near infrared spectroscopy for analyzing corn kernel damage and viability of soybean and corn kernels. *Journal of Cereal Science*, v.55, p.160-165, 2012. https://doi.org/10.1016/j.jcs.2011.11.002

ALEXANDER, D.E.; COLLINS, F.I.; RODGERS, R.C. Analysis of oil content of maize by wide-line NMR. *Journal of the American Oil Chemists' Society*, v.44, p.555-558, 1967. https://doi.org/10.1007/BF02901248

ARAUJO, T.P.; MELLO, F.M. Processamento digital de Imagens digitais – razão entre bandas. *Geociências*, v.29, n.1, p.121-131, 2010. https://www.revistageociencias.com.br/geociencias-arquivos/29_1/Art%2009_Araujo.pdf

BARKER, M.; RAYENS, W. Partial least squares for discrimination. *Journal of Chemometrics*, n.17, p.166–173, 2003. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/epdf/10.1002/cem.785

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para Análise de Sementes*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília, DF: MAPA/ACS, 2009. 399p. https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946_regras_analise__sementes.pdf

BONAT, W.H. *Regressão Local (LOESS)*. 2007. 5p. https://docplayer.com.br/128043619-Regressao-local-loess.html

CONCEIÇÃO, F.R.; MOREIRA, A.N.; BINSFELD, P.C. Detecção e quantificação de organismos geneticamente modificados em alimentos e ingredientes alimentares. *Ciência Rural*, v.36, p.315-324, 2006. https://doi.org/10.1590/S0103-84782006000100053

DYER, D.J. Analysis of oilseeds and coarse grains. In: ROBERTS, C.A.; WORKMAN, J.; REEVES III, J.B. (Eds.). *Near-Infrared spectroscopy in agriculture*. Madison: IM Publishing, 2004. https://doi.org/10.2134/agronmonogr44.c12

FERNANDES, F.; FERNANDES, A.; BUENO SOBRINHO, A.A.; MONTEIRO, H.D.C.; SILVA, A. Uso de espectrometria de refletância no infravermelho próximo (NIRS) na análise de carbono de Neossolos do Pantanal. *Embrapa Pantanal,* 2010. https://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/883578

GAZOLLA-NETO, A.; AUMONDE, T.Z.; PEDÓ, T.; OLSEN, D.; VILLELA, F.A. Níveis de umidade do solo de várzea e seus efeitos sobre a emergência e crescimento inicial de plântulas de soja. *Informativo Abrates*, v.22, p.28-31, 2012. https://scholar.google.com.br/citations?view_op=view_citation&hl=pt-BR&user=tNDDiyUAAAAJ&citation_for_view=tNDDiyUAAAAJ:GnPB-g6toBAC

GUTKOSKI, L.C.; EICHELBERGER, L.; SANTIN, J.A.; PORTELLA, J.A.; SPIER, F.; COLUSSI, R. Avaliação da composição química de milho seco e armazenado em silo tipo alambrado com ar natural forçado. *Food Science and Technology*, v.29, p.879-885, 2009. https://doi.org/10.1590/S0101-20612009000400028

ISTA. International Seed Testing Association. *International Rules for Seed Testing*. Zürich: ISTA, 2015. www.seedtest.org/en/international-rules-for-seed-testing-rubric-3.htm

JIANHUA, Z.; MCDONALD, M.B. The saturated salt accelerated aging test for small-seeded crops. *Seed Science and Technology*, v.25, n.1, p.123-131, 1997. https://agris.fao.org/agris-search/search.do?recordID=CH9700211

JULIANO, B.O. Hygroscopic equilibria of rough rice. *Cereal Chemistry*, v.41, p.191-197, 1964.https://www.researchgate.net/publication/275823222_Hygroscopic_Equilibria_of_Rough_Rice

KANDPAL, L.M.; LOHUMI, S.; KIM, M.S.; KANG, J.S.; CHO, B.K. Near-infrared hyperspectral imaging system coupled with multivariate methods to predict viability and vigor in muskmelon seeds. *Sensors and Actuators b: Chemical*, v.229, p.534-544, 2016. https://doi.org/10.1016/j.snb.2016.02.015

KARN, A.; HEIM, C.; FLINT-GARCIA, S.; BILYEU, K.; GILLMAN, J. Development of rigorous fatty acid near-infrared spectroscopy quantitation methods in support of soybean oil improvement. *Journal of the American Oil Chemists' Society,* v.94, p.69-76, 2017.

KENNARD, R.W.; STONE, L.A. Computer aided design of experiments. *Technometrics,* v.11, p.137–148, 1969. https://doi/abs/10.1080/00401706.1969.10490666

KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇA-NETO, J.B. Vigor de sementes: conceitos e testes. Londrina: *ABRATES,* 1999. 2018p. https://www.alice.cnptia.embrapa.br/bitstream/doc/446594/1/Vigordesementes.pdf

KUSUMANINGRUM, D.; LEE, H.; LOHUMI, S.; MO, C.; KIM, M.S.; CHO, B.K. Non-destructive technique for determining the viability of soybean (*Glycine max*) seeds using FT-NIR spectroscopy. *Journal of the Science of Food and Agriculture*, v.98, n.5, p.1734-1742, 2018.

MIAW, C.S.W.; ASSIS, C.; SILVA, A.R.C.S.; CUNHA, M.L.; SENA, M.M.; SOUZA, S.V.C. Determination of main fruits in adulterated nectars by ATR-FTIR spectroscopy combined with multivariate calibration and variable selection methods. *Food Chemistry*, v.254, p.272–280, 2018. https://doi.org/10.1016/j.foodchem.2018.02.015

MAYRINCK, L.G.; LIMA, J.M.E.; GUIMARÃES, G.C.; NUNES, C.A.; OLIVEIRA, J.A. Use of near infrared spectroscopy in cotton seeds physiological quality evaluation. *Journal of Seed Science*, v.42, e202042016, 2020. https://doi.org/10.1590/2317-1545v42227169

OLIVEIRA, L.E.M. Fatores que afetam a composição química das sementes. 2015. http://www.ledson.ufla.br/metabolismo-da-germinacao/mobilizacao-de-reservas/fatores-que-afetam-a-composicao-quimica-das-sementes/.

ROQUE, J.V.; DIAS, L.A.S.; TEÓFILO, R.F. Multivariate calibration to determine phorbol esters in seeds of *Jatropha curcas* L. using near infrared and ultraviolet spectroscopies. *Journal of the Brazilian Chemical Society*, v.28, p.1506–1516, 2017. https://doi.org/10.21577/0103-5053.20160332

ROQUE, J.V.; CARDOSO, W.; PETERNELLI, L.A.; TEÓFILO, R.F. Comprehensive new approaches for variable selection using ordered predictors selection. *Analytica Chimica Acta*, v.1075, p.57-70, 2019. https://doi.org/10.1016/j.aca.2019.05.039

SARMENTO, H.G.S.; DAVID, A.M.S.S.; BARBOSA, M.G.; NOBRE, D.A.C.; AMARO, H.T.R. Determinação do teor de água em sementes de milho, feijão e pinhão-manso por métodos alternativos. *Revista Energia na Agricultura*, v.30, n.3, p.249-256, 2015. https://doi.org/10.17224/EnergAgric.2015v30n3p250-256

SEO, Y.W.; AHN, C.K.; LEE, H.; PARK, E.; MO, C.; CHO, B.K. Non-destructive sorting techniques for viable pepper (*Capsicum annuum* L.) seeds using Fourier transform near-infrared and raman spectroscopy. *Journal of Biosystems Engineering*, v.*41*, p.51-59. https://doi.org/10.5307/JBE.2016.41.1.051

SILVA, C.F.L.; MILACH, S.C.K.; SILVA, S.D.A.; MONTERO, C.R. Near infrared reflectance spectroscopy (NIRS) to assess protein and lipid contents in *Avenal sativa* L. *Crop Breeding and Applied Biotechnology*, [S.l.], v.8, p.127-133, 2008. https://www.researchgate.net/profile/Sandra-Milach-2/publication/26634724_Near_infrared_reflectance_spectroscopy_NIRS_to_assess_protein_and_lipcontents_in_Avena_sativa_L/links/00b7d53a0af7f12001000000/Near-infrared-reflectancespectroscopy-NIRS-to-assess-protein-and-lipid-contents-in-Avena-sativa L.pdf?_sg%5B0%5D=started_experiment_milestone&origin=journalDetail

SHRESTHA, S.; DELEURAN, L.C.; GISLUM, R. Separation of viable and non-viable tomato (*Solanum lycopersicum* L.) seeds using single seed near-infrared spectroscopy. *Computers and Electronics in Agriculture*, v.142, p.348-355, 2017. https://doi.org/10.1016/j.compag.2017.09.004

WANG, Y.; PENG, Y.; ZHUANG, Q.; ZHAO, X. Feasibility analysis of NIR for detecting sweet corn seeds vigor. *Journal of Cereal Science*, n.93, p.102977, 2020. https://doi.org/10.1016/j.jcs.2020.102977