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Can near infrared spectroscopy be used to select superior popcorn lines under drought conditions?

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Abstract: This study sought to answer the following questions: i) Is it possible to use the near-infrared (NIR) spectrum for the indirect selection of superior popcorn genotypes based on grain yield (GY), popping expansion (PE), and volume of expanded popcorn per hectare (VP)? ii) Is it possible to detect differences between different water conditions (WC) applied to the soil using NIR? iii) Is it possible to identify the phenological stages (days from male anthesis) of the crop in a given WC using NIR, considering the morphophysiological transformations during plant development? To this end, thirteen popcorn lines, under well-watered and soil water stress imposed at male anthesis, were evaluated for GY, PE, and VP traits and correlated with NIR indices in the 908 to 1680 nm spectrum. Measurements were made at different times in relation to male anthesis. It was impossible to associate the NIR spectra with the GY, PE, and VP traits due to the lack of correlation based on the R^2 values resulting from the analysis of standard normal variate (SNV), first derivative, and SNV + first derivative. In turn, via NIR, it was possible to differentiate the WCs and associate them with the phenological stage the plants were in.

Keywords: near-infrared, popping expansion, water stress, phenological stage.

Introduction

The popcorn seed market presents varieties with high grain yield potential. However, these cultivars only manifest their genetic potential under optimal soil moisture conditions (Lima et al. 2021; Kamphorst et al. 2021a). Abiotic stresses, such as drought, pose a challenge for the full development of the plants (Kamphorst et al. 2022; Viana et al. 2022; Leite et al. 2022; Carvalho et al. 2023; Bispo et al. 2023). The increased frequency of drought events due to climate change is negatively affecting the cultivation of various agricultural crops, including popcorn (Lima et al. 2019; Kamphorst et al. 2019), especial-

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ly in tropical and subtropical regions (Dias et al. 2018), posing a threat to food security (Araus et al. 2010; Adebayo et al. 2014; Daryanto et al. 2016; Fahad et al. 2017). Popcorn is highly susceptible to soil water deficit, which negatively affects important economic traits such as grain yield and popping expansion (Kamphorst et al. 2018, 2019, 2020a; Santos et al. 2021; Carvalho et al. 2023).

Grain yield (GY) is the main target trait for selecting superior popcorn genotypes under drought conditions (Teixeira et al. 2010; Dias et al. 2018). However, the selection success is usually insignificant due to the low heritability often observed for this trait (Daros et al. 2004; Mafra et al. 2019; Santos Junior et al. 2023). However, another important trait of the crop is popping expansion (PE), a variable that relates the volume of popcorn popped to the mass of grains used. Since they are negatively correlated, it is difficult to obtain joint gains in GY and PE (Pereira and Amaral Júnior, 2001; Daros et al. 2004; Freitas et al. 2014; de Lima et al. 2016). In this sense, traits correlated with the main traits (GY and PE) have been sought to overcome this obstacle (Kamphorst et al. 2021b). From the point of view of breeding for drought conditions, what is expected is the use of secondary traits that are correlated, and in high magnitude, to GY and PE, but which are non-destructive, easy, and early to measure, and of high heritability, thus constituting options to assist in the indirect selection of superior genotypes (Monneveux et al. 2008; Liu and Qin, 2021).

At the physiological level, maize plants under soil drought conditions show several changes, such as a decrease in turgor pressure, a decrease in photosynthetic efficiency, a decrease in stomatal conductance, stomatal closure, resulting in low CO_2 assimilation (Kaur et al. 2021). In terms of biochemical and molecular changes, these include pathways involving hormones such as abscisic acid and anthocyanins, Ca^{2+} pathways, the generation of reactive oxygen species (ROS) (H_2O_2), leading to an increase or decrease in protein biosynthesis and degradation pathways (Seleiman et al. 2021; Liu and Qin, 2021; Kaur et al. 2021). In this scenario, in addition to estimating agronomic traits such as GY and PE, popcorn breeding can also evaluate morphophysiological and biochemical traits in the hope that these can serve as tools for the indirect selection of GY and/or PE (Lima et al. 2019; Kamphorst et al. 2019). Several studies show an association between GY and physiological traits related to the leaf green index (Kamphorst et al. 2020a, b, 2021b). However, none have been reported an association with PE, nor with the volume of popcorn expanded per hectare (VP), another trait that has been used in crop breeding, as a possibility of concomitant gains for GY and PE (Amaral Júnior et al. 2016).

Near-infrared (NIR) spectroscopy allows the identification and prediction of C-H, O-H, N-H, and C=O bonds, which are predominant in water and organic compounds such as carbohydrates, oils, alcohols, nucleic acids, and phenolic compounds (Oliveira et al. 2018; Cattaneo and Stellari, 2019; Jiang 2020), and can be a tool for indirect selection of superior plants under drought conditions. Therefore, the quantification of these compounds in plants under different soil water conditions must respect the Lambert-Beer-Bouguer law, in which the amount of light absorbed or refracted is proportional to the concentration of compounds, presenting itself as a fundamental non-destructive tool for the analysis of compounds *in vivo* (Esteve Agelet et al. 2012; Mishra et al. 2020). Given the metabolic differentiation of the different genotypes under drought conditions, it is hoped that their estimates can aid in indirect selection. NIR spectra have already been used to identify different soil moisture conditions (Jiang 2020).

In view of the above, some scientific questions have been raised, such as: can the genetic variability of agronomic traits (GY, CE, and VP) be identified in popcorn in co-association with NIR spectra estimates for indirect selection? Also, is NIR capable of discriminating between contrasting soil water conditions? This last argument stems from the fact that plants subjected to soil water deficit (WS) are expected to show differences in their chemical composition compared

to well-watered plants (WW), since there are successive morphophysiological changes throughout the cycle. Finally, would it be possible to identify the time of male anthesis in plants using NIR spectra? In view of the above, the study aimed to investigate the impact of water stress on agronomic traits (GY, PE, and VP) of thirteen popcorn lines, as well as to use near-infrared spectroscopy as a perspective for the indirect selection of more productive genotypes under drought conditions, differentiating the watering conditions applied and the dates when the spectra were measured.

Material and methods

Genotypes

Thirteen S_7 popcorn lines belonging to the Germplasm Bank of the State University of the North Fluminense Darcy Ribeiro (UENF) were pre-selected from a panel of 50 lines, which were evaluated under conditions of soil water deficit and expressing PE estimates higher than 25 mL g^{-1} (Leite et al. 2022). In general, the lines L217, L220, L221 and L222 are derived from the hybrid IAC 125, from the Agronomic Institute of Campinas; L262 comes from the population URUG 298, from CIMMYT; L328 and L332 are derived from the variety UFV M-2 Barão, from UFV; L480 from SE013, from UEM; L507 and L510 from PA 170 Roxo population, from CIMMYT; and L684, L688, and L691 from the UENF14 population, from UENF (Vittorazzi et al. 2018).

Experimental design and cultivation

The experiment was carried out at the State Agricultural College Antônio Sarlo, in Campos dos Goytacazes, Rio de Janeiro (RJ), in 2021, during a period of low rainfall, corresponding to April to August (fall/winter). The site has an automatic weather station of the National Institute of Meteorology (INMET), which allows a detailed monitoring of the clime. Information on estimates of average temperature, relative humidity and maximum solar radiation can be found in Figure 1.

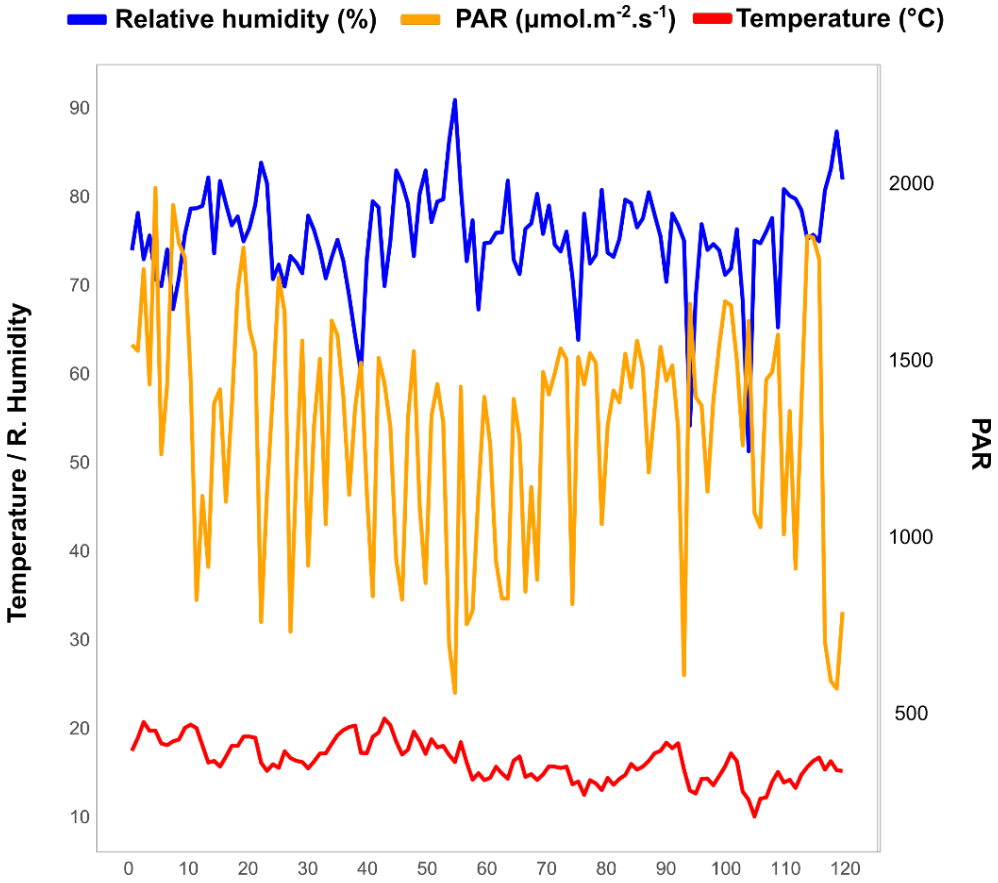


Figure 1. Weather conditions (relative humidity, photosynthetically active radiation, and average temperature) observed during popcorn cultivation.

The experimental area was partially harrowed and furrowed. Each experimental plot consisted of a 4.40 m row, with 0.20 m between plants and 0.80 m between rows, with a total of 23 plants per row. The genotypes were sown with three seeds per hole, along with the sowing fertilization for the experiments, which was 30 kg ha^{-1} of N (urea), 60 kg ha^{-1} of P_2O_5 (triple superphosphate), and 60 kg ha^{-1} of K_2O (potassium chloride). Top dressing fertilization was done 30 days after sowing, with 100 kg ha^{-1} of N (urea).

The experimental design used was a completely randomized block with three replications in two contrasting water conditions (WC), namely well-watered (WW) and water-stressed (WS). The WW condition received irrigation to maintain field capacity (-10 MPa), which was monitored using Decagon MPS-6 tensiometers (Decagon, USA). On the other hand, in the WS condition, irrigation was suspended at the phenological stage of male pre-anthesis, which occurred on day 26th (≅ 68 DAS) and day 23rd (≅ 67 DAS) in the WS and WW conditions, respectively. The date of male anthesis was determined based on previous experiments (Vittorazzi et al. 2018). Figure 2 shows the specifications of the irrigation applied (in mm) throughout the growing season, both in the well-watered (WW) and water-stressed (WS) areas, expressed in days after sowing (DAS). In the WS area, irrigation was suspended from 49 DAS until harvest. However, rainfall occurred at 54, 66, 79, 93, 107, and 120 DAS, totaling 170.20 mm (Figure 2). Rainfall events were recorded at the weather station near the experiment, and the total amount of water applied to the plots was 78.90 mm for the WS and 132.93 mm for WW. Independent of the WC, irrigation was performed by installing one Katif dripper per plant with a flow rate of approximately 2.30 mm h^{-1}.

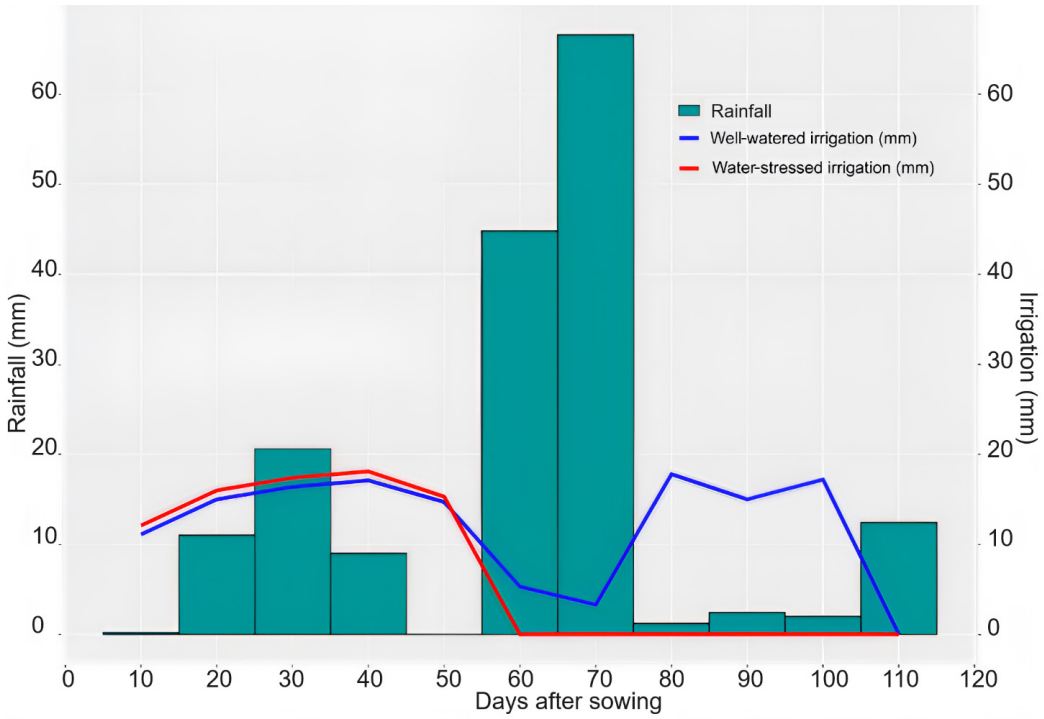


Figure 2. Rainfall and irrigation (mm) applied to early and late genotypes under WW and WS conditions, considering days after sowing.

During the experimental period, soil water potential was monitored using Decagon MPS-6 tensiometers (Decagon, USA). The WS environment had three data collection points, while the WW environment had one data collection point. The tensiometers were installed between the plants at a depth of 0.20 m. The soil in the WW treatment was maintained at field capacity (-0.01 MPa) throughout the growing cycle, while the soil in the WS treatment, the soil reached a permanent wilting point (-1.5 MPa) at 42 days after male anthesis (Figure 3).

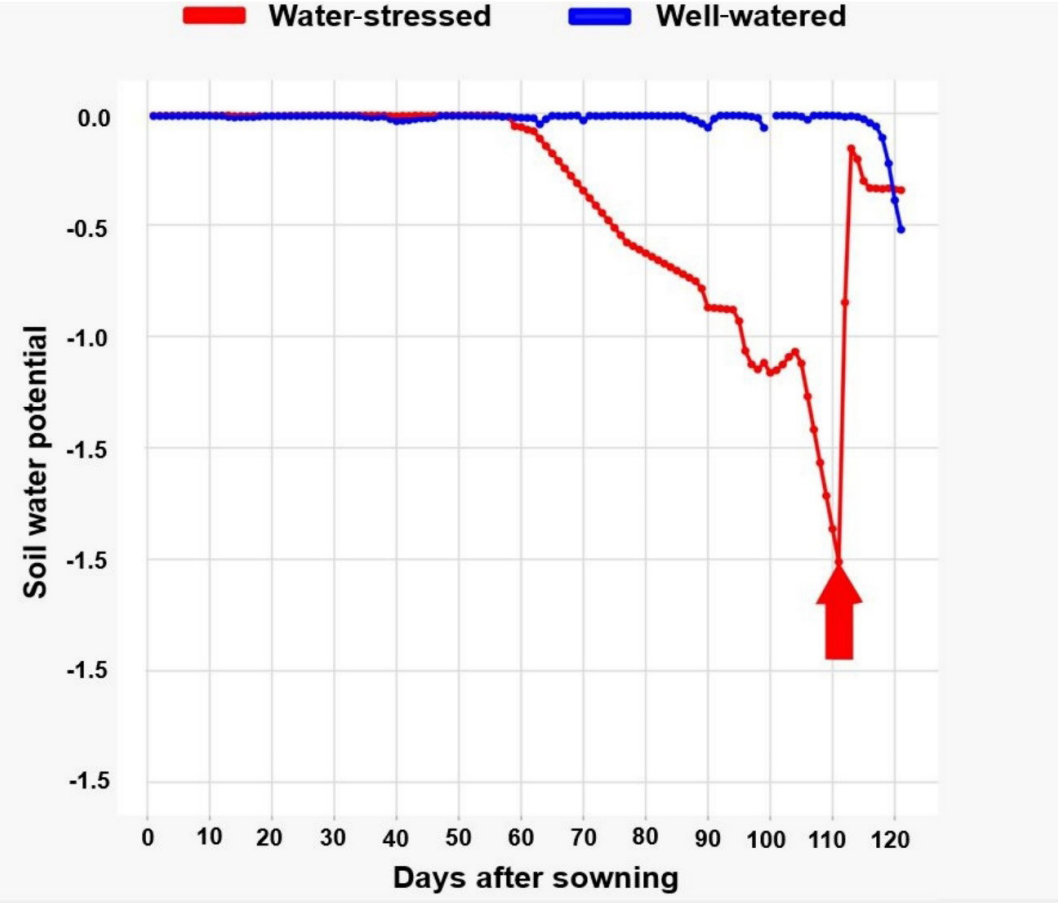


Figure 3. Soil water potential during popcorn cultivation under contrasting water conditions (WS and WW), dotted line delineates male flowering.

Agronomic traits

Grains were harvested at physiological maturity. At this time, the following traits were evaluated: grain yield (GY), popping expansion (PE), and volume of expanded popcorn per hectare (VP). These traits were measured based on all the plants in the useful area of the plot. GY was obtained in grams per plot, corrected to 13% moisture, and expressed in kg ha^{-1}. PE was obtained by microwaving 30 g of grain in a special paper bag for popping, at a power of 1,000 W, for 1 min and 45 s in three replicates of each genotype. The volume of expanded popcorn was quantified in a graduated cylinder in mL, and the PE was determined by dividing the popcorn volume by the grain mass (30 g) and expressed in mL g^{-1}. VP was obtained by multiplying GY by PE and expressed in m^3 ha^{-1}.

Statistical analysis

Individual analyses of variance were performed for each WC (WW and WS), and a joint analysis of the experiments was performed to estimate the significance of the interaction effect between genotypes (G) and water conditions. The following statistical model was used in the individual analysis of variance: $\boldsymbol{Y_{ij} = \mu + G_i + B_j + \varepsilon_{ij}}$, where: Y_{ij} is the observation of the i-th genotype in the j-th block; μ is the general constant; G_i is the effect of the i-th treatment; B_j is the effect of the j-th block; and $\boldsymbol{\varepsilon_{ij}}$ is the experimental error. The following statistical model was used in the joint analysis of variance: $\boldsymbol{Y_{ijk} = \mu + Gi + B/A_{jk} + A_j + GA_{ij} + \varepsilon_{ijk}}$, where: $\boldsymbol{Y_{ijk}}$ is the observation of the i-th genotype in the j-th WC in the k-th block; $\boldsymbol{\mu}$ is the general constant; Gi is the random effect of the i-th genotype; $\boldsymbol{B/A_{jk}}$ is the effect of the k-th block within WC j; $\boldsymbol{A_j}$ is the fixed effect of the j-th environment with NID; $\boldsymbol{GA_{ij}}$ is the random effect of the interaction between the i-th genotype and the j-th WC; and $\boldsymbol{\varepsilon_{ijk}}$ is the average experimental random error associated with the observation $\boldsymbol{Y_{ijk}}$, with NID (0, σ^2).

When statistical differences were observed for the source of variation G, the means were

grouped by the Scott-Knott test at the 5% probability level using the *Rstudio* software, and the graphs were generated using the ggplot2 package (Cruz 2016).

NIR spectrum

Acquisition of biological samples

In their different WCs, NIR spectral measurements were taken from the stems of popcorn plants in each plot in two positions, one near to the ground and the other near the main ear, at five different related to male anthesis (MA), namely: before this event, referring to negative values (-19 and -6); and at male anthesis, referred to as "milestone-0", and after anthesis, referring to positive values (3, 11, and 23). A total of ten biological samples were considered, measured in thirteen genotypes, including repetitions of the different positions in two WCs (WW and WS) and on five different measurement dates.

The biological samples were named WW -19 and WS -19, corresponding to the detection of the spectrum of the stems 19 days before MA, under WW and WS conditions, respectively; WW -6 and WS -6, corresponding to the detection of the spectrum of the stems six days before MA under WW and WS conditions, respectively; WW 3 and WS 3, corresponding to the detection of the spectrum of the stems at three days after MA under WW and WS conditions, respectively; WW 11 and WS 11, corresponding to the detection of the spectrum of the stems at 11 days after AM under WW and WS conditions, respectively; and WW 23 and WS 23, corresponding to the detection of the spectrum of the stalks at 23 days after AM under WW and WS conditions, respectively.

Near infrared (NIR) spectral detection

To analyze the near-infrared (NIR) spectrum of the biological samples, spectral readings were acquired ranging from 908.10 to 1674.19 nm with a resolution of 6.14 nm, employing the portable MicroNIR 16OnSite-W (CA, USA) device. The data was acquired using *SpectralSoft mobile software*, version 50.1 (*Bluetooth Low Energy*, BLE). The data were exported in csv format, and chemometric processing was performed using Python.

Data pre-processing

The spectral data collected and obtained for the entire evaluation cycle were then pre-processed using *standard normal variate* (SNV), first derivative smoothing, and SNV + first derivative to adjust the baseline (Barros et al. 2021).

Construction of the calibration curve

The pre-processed data were subjected to a calibration set and was selected using the Kennard-Stone algorithm (Sousa et al. 2011). The selected calibration set contained 70% of the spectral samples for model building, and 30% of the spectral samples formed the prediction set and did not participate in model building, as they were used for external validation (Barros et al. 2021). The number of latent variables was determined by cross validation, defining the optimal number of factors as those represented by the instrumental variables. The optimal number of latent variables was calculated for each spectral sample obtained, representing the model with the lowest cross validation error and, consequently, the highest predictive capacity.

The linearity of the model was assessed by the coefficient of determination for calibration (R^2c) and prediction (R^2p) using the expression: ; in which: is the reference value; is the value of the quantity calculated by the model; and is the average of the reference values (Barros et al. 2021).

In addition to the linearity of the data, the prediction and calibration errors were used as accuracy metrics to verify model performance. The square root of the prediction error - Root Mean Square Error of Prediction (RMSEP) - and the square root of the average calibration error - Root Mean Square Error of Calibration (RMSEC) - were represented by the following equations: and ; where: is the number of degrees of freedom for the calibration set; and is the number of prediction samples used to calculate the quantities (Barros et al. 2021). The root mean square error (RMSE) metric was calculated for both the calibration set (RMSEC) and the prediction set to assess the accuracy of the quantification models. The linearity of

the model was assessed by the coefficient of determination for calibration (R^2c) and prediction (R^2p).

Application of the partial least squares (PLS) method

Multivariate statistical analysis was performed on two different segments of the data. First, all measurements were used to build and validate the model. In a second step, the data were divided into two large blocks to better predict the quantities under study, obtaining a well-watered WW model and another model with water-stressed WS.

The PSL consisted of modeling each parameter separately using the k-fold method (k=5), suitable for small sample sets, in which n sets are generated for n samples, with a different sample left for testing and the others used for training; then test predicted values are then generated for each sample, and the coefficient of determination (R^2) between the predicted and reference values is calculated to estimate the correlation.

Classifier via linear discriminant analysis (LDA)

The data already processed in SNV + Derivative was used in the Fisher Linear Discriminant Analysis (LDA) classifier, a technique used in statistics for machine learning to find linear combinations of traits. The data was divided into 70% of the spectral samples to build the model (training class) and 30% to evaluate its predictive ability. To select the NIR spectral samples, two *sets* were randomly compared, with the only restriction being that all three measurements had to be in the same *set* (calibration or prediction), to ensure that the model was not biased. The accuracy of the data was also obtained based on the samples classified and weighted by the total number of spectral samples, according to the following equation: ; where: VP: consists of the data that are true positives, NN: consists of the data that are true negatives, FP: consists of the data that are false positives, and FN: consists of the data that are false negatives.

In addition to accuracy, sensitivity was estimated, which consists of the ability of the analysis to present individuals who are truly positive, i.e., able to correctly identify the spectrum for each WC. Specificity is the ability of the analysis to identify true negatives, i.e., to correctly identify phenological stages about the corresponding spectrum. The mathematical equations used were: and ; where: VP: consists of the data that are true positives; NN: consists of the data that are not true negatives; FP: consists of the data that are false positives; and FN: consists of the data that are false negatives.

In addition, a confusion matrix was created to accurately predict the correlation among the spectra, the WCs, and the phenological stage.

Results

Phenotypic expression of popcorn lines under different water conditions

In both WW and WS conditions, there was genetic variability between the genotypes evaluated for the agronomic traits studied (GY, PE, and VP) (Table 1), statistically proven by the significance of the F test ($p < 0.01$). An exception was observed in the case of PE in WW. The overall average for GY was 1,249.88 Kg ha^{-1} in WW conditions and 830.47 Kg ha^{-1} in WS conditions, representing a reduction of 33.56% for this trait. For PE, the average was 22.16 mL g^{-1} in WW conditions and 19.94 mL g^{-1} in WS conditions, representing a reduction of 10.02%. The trait VP showed a reduction of 41.34% since the total average reached 28.11 m^3 ha^{-1} in WW conditions and 16.49 m^3 ha^{-1} in WS. In the individual analyses by WC, the CVe values ranged from 9.92% (for PE in WS) to 31.94% (for VP in WS). In the joint analysis, regardless of the source of variation, i.e., genotypes (G), WCs, and the G*WC interaction, the traits GY, PE, and VP showed statistical significance (Table 1).

Table 1 - Summary of the analysis of variance of the environments evaluated, the joint analysis of variance, and general averages of agronomic traits of popcorn lines under well-watered (WW) and water-stressed (WS) conditions.

SOURCES OF VARIATION		DF	GY (Kg ha^{-1})	PE (mL g^{-1})	VP (m^3 ha^{-1})
			Mean Square		
WC – WW	Genotype	12	562,263.03**	30.26^{ns}	375.81**
	Block	2	36,662.14	6.41	65.73
	Error	24	99,130.92	17.00	78.68
	CVe (%)		25,19	18.60	31.56
	Mean		1.249,88	22.16	28.11
WC – WS	Genotype	12	467,355.44**	29.21**	232.61**
	Block	2	4,740.69	5.10	2.48
	Error	24	31,877.02	3.91	26.70
	CVe (%)		21,50	9.92	31.94
	Mean		830,47	19.94	16.49
COMBINED ANALYSIS	Genotype	12	464,460.45**	25.01*	214.38**
	WC	1	3,430,230.66**	96.59*	2,632.04**
	Genotype x WC	12	5,651,58.01**	34.46**	394.03**
	Error	48	65,503.97	10.95	52.68
	CVe (%)		24.60	15.36	32.55
	Mean		1,040.17	21.05	22.30

DF: degree of freedom; GY: grain yield; PE: popping expansion; VP: volume of popcorn expanded per hectare; WC: water condition; WS: water-stressed; WW: well-watered; CVe (%): coefficients of environmental variation; ns: not significant; **: significant at 0.01% and *: significant at 0.05%.

Agronomic potential of popcorn lines under the water conditions evaluated

The average GY ranged from 624.00 kg ha^{-1} to 2,020.17 kg ha^{-1} in WW and from 265.00 kg ha^{-1} to 1,223.83 kg ha^{-1} in WS (Table 2). In WW, the group of lines with the highest GY estimates included L222, L328, L332, and L507; in WS, it included L217 and L691. The lines with the lowest GY estimates were L220, L292, and L691 in WW and L220, L292, L332, L507, L510, and L684 in WS.

The PE averages ranged from 18.67 mL g^{-1} to 27.70 mL g^{-1} in WW; and from 16.03 to 22.67 mL g^{-1} in WS (Table 2). Lines L220, L510, L684, and L691 had the highest averages in WS conditions. The VP averages ranged from 10.93 m^3 ha^{-1} to 46.80 m^3 ha^{-1} and from 6.10 m^3 ha^{-1} to 27.82 m^3 ha^{-1} in WW and WS, respectively. In WW conditions, lines L221, L222, L328, L332, L507, and L691 were the ones with the highest averages, and in WS, this was the case for lines L217 and L691 (Table 2).

Table 2 – Means and mean test for agronomic traits of popcorn lines evaluated under different water conditions.

Lines	WW						WS					
	GY (Kg ha^{-1})		PE (mL g^{-1})		VP (m^3 ha^{-1})		GY (Kg ha^{-1})		PE (mL g^{-1})		VP (m^3 ha^{-1})	
L217	1,445.83	b	25.63	a	37.06	a	1,186.10	a	18.67	b	22.14	a
L220	624.00	c	24.23	a	15.12	b	546.77	d	22.67	a	12.37	b
L221	1,242.00	b	27.70	a	34.53	a	903.40	c	20.37	b	18.50	b
L222	1,628.90	a	22.43	a	36.67	a	887.53	c	17.37	b	15.33	b
L292	797.97	c	26.13	a	20.50	b	580.23	d	20.03	b	11.57	b
L328	1,863.23	a	21.30	a	40.13	a	993.80	b	16.77	b	16.73	b
L332	2,020.17	a	23.50	a	46.80	a	585.67	d	17.00	b	9.93	b
L480	1,144.67	b	20.20	a	22.80	b	997.67	b	17.73	b	19.20	b
L507	1,586.57	a	26.87	a	42.33	a	519.93	d	19.50	b	10.30	b
L510	1,186.37	b	20.60	a	26.03	b	265.00	d	23.00	a	6.10	b
L684	1,109.47	b	19.80	a	21.80	b	582.63	d	24.30	a	13.77	b
L688	1,170.13	b	18.67	a	21.87	b	742.50	c	16.03	b	11.80	b
L691	1,629.17	a	24.03	a	39.15	a	1,223.83	a	22.73	a	27.82	a

WS: water-stressed; WW: well-watered; GY: grain yield; PE: popping expansion; VP: volume of popcorn expanded per hectare. Means followed by the same letter belong to the same group by the Scott-Knott method at 5% probability.

Calibration of the PLS model for the correlation of agronomic traits

Initially, the curves and spectral patterns of the data collected from each sample were analyzed, as illustrated in (Figure 4).

After maintaining similar vibrational patterns between the samples, the SNV, first derivative, and SNV + first derivative spectral treatments were performed, which showed that the spectral pattern remained the same, as illustrated in Figure 5.

Regression procedures were applied to these spectra on the data pre-treated with SNV, derivative, and SNV + derivative; however, they did not show high values indicating linearity, resulting in low predictive power (Table 3). In addition to correlation coefficients below the generally acceptable level (greater than 0.70), the metrics used to evaluate prediction errors, using both the data intended for calibration and prediction (RMSEC and RMSEP, respectively), showed unreliable prediction values.

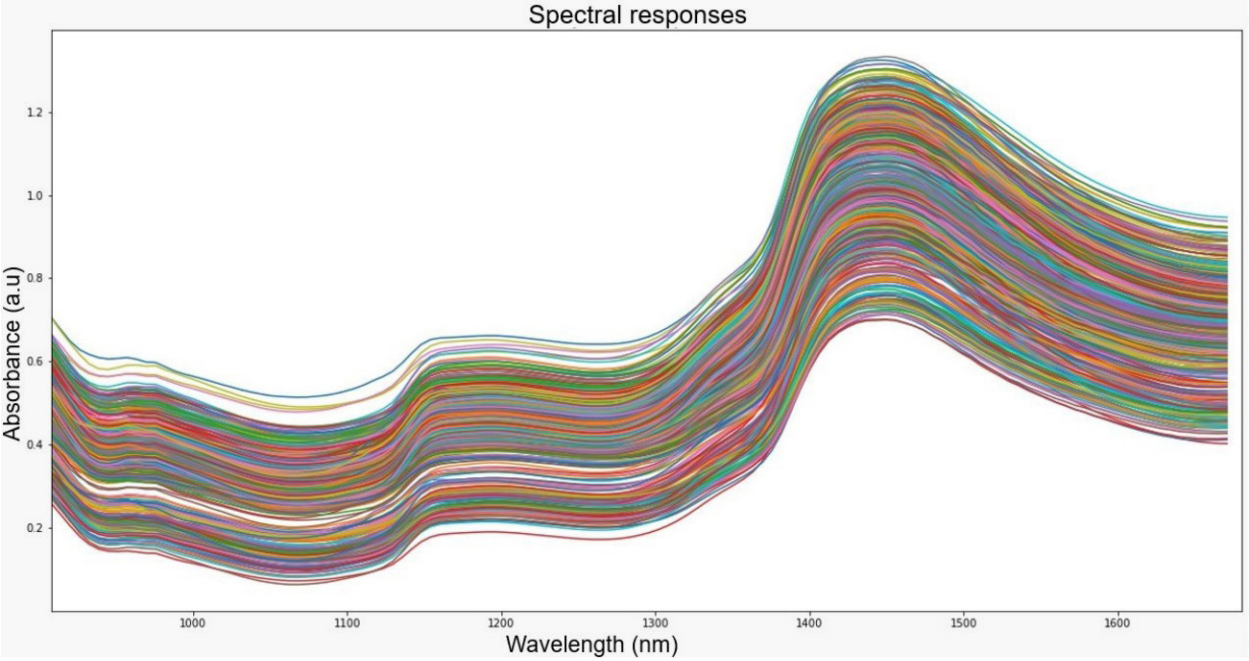


Figure 4. Vibrational pattern of samples collected from popcorn stems under WW and WW conditions, grown in 2021.

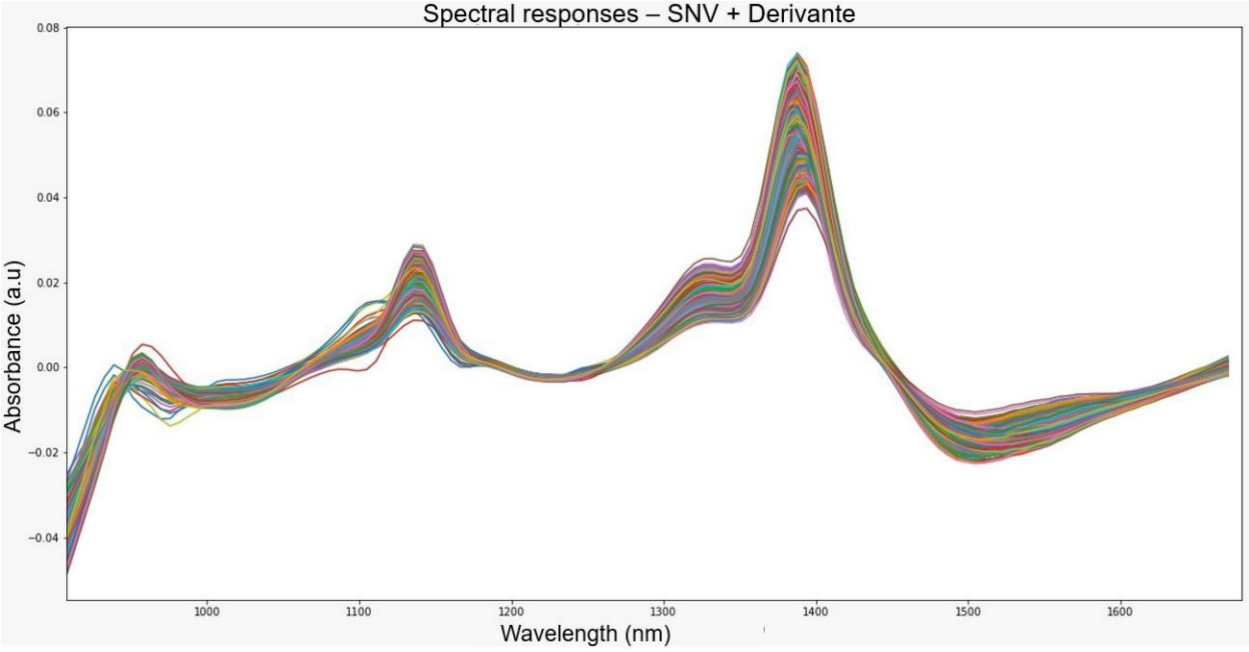


Figure 5. Spectral curve after smoothing the data treated by SNV and first derivative.

Table 3 - Regression models without treatment, SNV pre-processing, a first derivative with order and seven point window, and SNV + first derivative for the agronomic traits GY, PE, and VP correlated to NIR spectra.

		Modelos de Regressão			
		Without treatment	**SNV**	**Derivative**	**SNV + Derivative**
GY	R^2 calibration	0.17	0.09	0.11	0.17
	R^2 prediction	0.01	0.07	0.00	0.02
	RMSEC	443.63	452.66	461.76	450.24
	RMSEP	543.01	481.97	504.56	495.78
	VL	5	7	7	8
PE	R^2 calibration	0.00	0.01	0.05	0.02
	R^2 prediction	0.00	0.00	0.00	0.01
	RMSEC	40.99	41.78	40.99	38.88
	RMSEP	44.36	42.75	42.07	42.68
	VL	2	2	5	3
VP	R^2 calibration	0.00	0.14	0.10	0.09
	R^2 prediction	0.00	0.13	0.08	0.09
	RMSEC	12.61	11.74	121.38	11.94
	RMSEP	13.24	12.34	124.23	122.80
	VL	2	11	4	5

GY: grain yield, PE: popping expansion, VP: volume of popcorn expanded per hectare, SNV: standard normal variable, R^2 calibration: calibration coefficient of determination, R^2 prediction: prediction coefficient of determination, RMSEC: average calibration error, RMSEP: average prediction error, and LV: latent variables.

NIR spectra to differentiate water conditions

The 669 samples obtained were divided into 70% for training and 30% for testing, consisting of 468 and 201 samples, respectively. The accuracy and sensitivity results showed that in the WS condition, the accuracy was 0.91 and the sensitivity was 0.97, while in the WW condition, the accuracy was 0.97 and the sensitivity was 0.92 (Table 4).

Table 4 - Prediction and sensitivity indexes for assessing the water conditions used to grow popcorn.

Class	Precision	Sensitivity	Analyzed readings	Analyzed samples
WS	0.91	0.97	102	34
WW	0.97	0.92	99	33

WS - water-stressed; and WW – well-watered.

The confusion matrix between the WCs accurately predicted the classes (Table 5). It is worth noting that in this matrix, it is possible to see the error between the classes, so the model was 97% accurate in predicting the WS class, with 0.03% of the data misclassified as WW; and 92% accurate for the WW class, with 0.08% misclassified as WS.

Table 5 - Confusion matrix with predictive models of error and accuracy between WS and WW classes (data in %) using NIR spectra obtained from different water conditions used to grow popcorn.

	Prediction	
Class	**Water-stressed**	**Well-watered**
Water-stressed	0.97	0.03
Well-watered	0.08	0.92

The dark blue color shows the correlation of the class with itself and its prediction percentage.

The classification process was repeated 30 times. For each stage, different sets of samples were determined, 30% of which comprised the test, and the data were processed by derivation followed by SNV, together with the use of the LDA classifier (Figure 6). The average accuracy of the 30 classification rounds was 91.50%, reproducing the predictive ability of the model. There was also a slight variation in accuracy between predictions, demonstrating the robustness of the data for classifying WC.

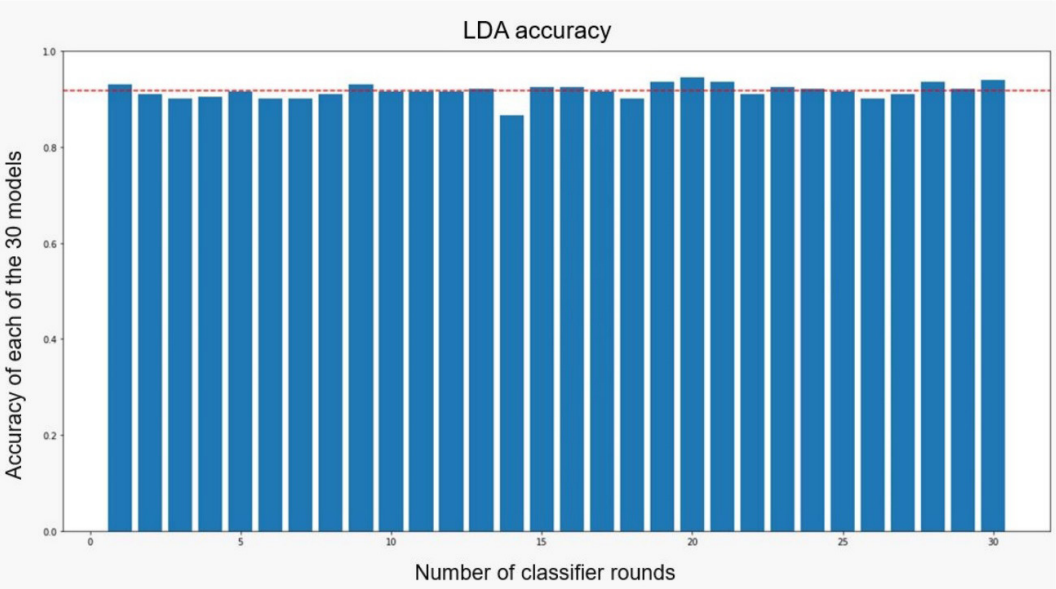


Figure 6. Spectral curve after smoothing the data treated by SNV and first derivative.

NIR spectra to differentiate between measurement dates from male anthesis onwards

For the "days to anthesis" classes, the -19 class with 21 NIR readings and seven samples, showed an accuracy of 1.00 and a sensitivity of 1.00. For the -6 class, 42 readings were analyzed and 14 samples gave an average estimate of 0.98 precision and 1.00 sensitivity. In class 3, 42 readings were taken on 14 samples, with an estimated precision and sensitivity of 1.00. For class 11, 48 readings were taken on 16 samples, giving an accuracy and sensitivity of 1.00. For class 23, 48 readings were also taken on 16 samples with an accuracy and sensitivity of 1.00 (Table 6).

Table 6 - Accuracy and sensitivity indexes for testing classes (days) resulting from the evaluations before and after male anthesis in classifying different evaluation dates for two water conditions for popcorn cultivation.

Class	Precision	Sensitivity	Analyzed readings	Analyzed samples
-19	1.00	1.00	21	7
-6	0.98	1.00	42	14
3	1.00	0.97	42	14
11	1.00	1.00	48	16
23	1.00	1.00	48	16

-19: means 19 days before male anthesis, -6: refers to 6 days before male anthesis, 3: refers to 3 days after male anthesis, 11: refers to 11 days after male anthesis, and 23: refers to 23 days after male anthesis.

Based on the confusion matrix of the "days to anthesis" classes, it can be seen that class -19 was 100% accurate in associating data from the same class, with no prediction error for the others. The same was true for classes -6, 3, and 23. Class 11, on the other hand, was 97% accurate when correlated with itself; however, there was a 0.02% error when class 11 data was misclassified with class -6 (Table 7).

Table 7 – Confusion matrix with predictive models of error and correctness between classes (days) resulting from the evaluations before and after male anthesis (data in %) using NIR spectral data obtained from different evaluation dates in popcorn.

	Prediction				
Class	-19	-6	3	11	23
-19	1.00	0.00	0.00	0.00	0.00
-6	0.00	1.00	0.00	0.00	0.00
3	0.00	0.00	1.00	0.00	0.00
11	0.00	0.02	0.00	0.97	0.00
23	0.00	0.00	0.00	0.00	1.00

The dark blue color shows the correlation of the class with itself and its prediction percentage. The light blue color shows the prediction error of the class.

The high accuracy of the classification model for days to male anthesis was observed by selecting 30 different samples to form the model training and test classes using the LDA classifier. The average accuracy of the 30 classifications was 99%, demonstrating that it is possible to diagnose the measurement date of male anthesis using NIR spectroscopy (Figure 7).

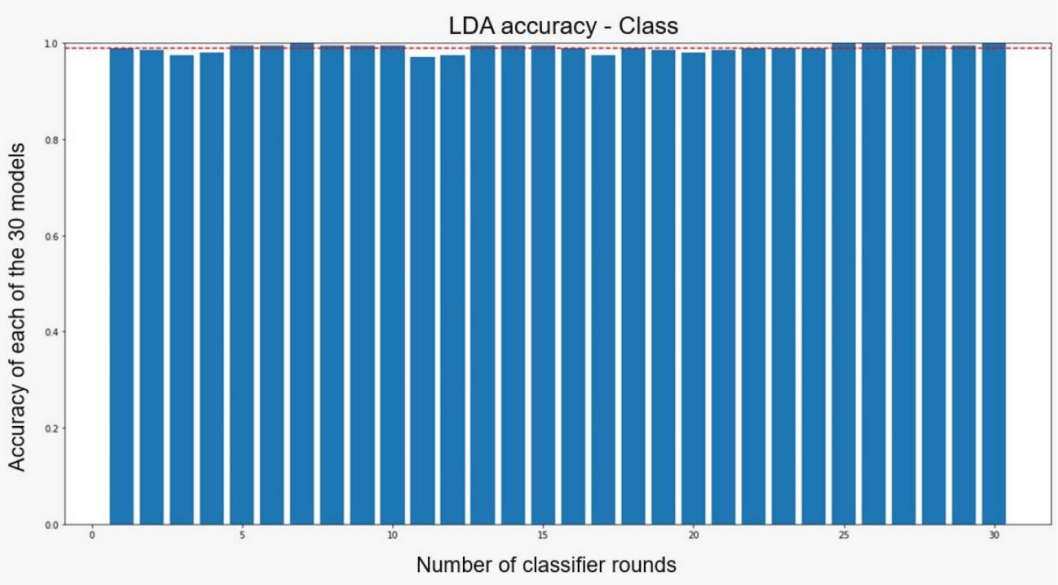


Figure 7. Average accuracy of the 30 runs of the LDA model for classifying the evaluations before and after male anthesis based on different dates for two water conditions applied to popcorn cultivation.

NIR spectra to jointly differentiate the WCs with measurement dates starting from male anthesis

The results for accuracy and sensitivity showed that in the WS condition, regardless of the measurement date of male anthesis, the values were higher than 0.78 (Table 8). In the WW condition, regardless of the measurement date of male anthesis, the accuracy and sensitivity results were higher than 0.77 (Table 8).

Table 8 - Prediction and sensitivity indexes for assessing the classification of the WC applied and the classes (days) resulting from before or *posteriori* male anthesis with classification on different dates and in two WC used for growing popcorn.

Class	Precision	Sensitivity	Analyzed readings	Analyzed samples
WS -19	1.00	1.00	39	13
WS -6	0.78	1.00	21	7
WS 11	0.95	0.95	21	7
WS 23	0.78	1.00	18	6
WW -19	0.94	0.94	9	3
WW -6	0.95	0.77	27	9
WW 3	1.00	1.00	21	7
WW 11	0.94	0.94	18	6
WW 23	1.00	0.81	33	11

WS: water-stressed condition, WW: well-watered condition. Negative values refer to the days the samples were evaluated before male anthesis, and positive values refer to the days after male anthesis.

The confusion matrix of the proposed model (anthesis dates + WC) shows that the samples readings that were misclassified were predicted in classes with the same anthesis date but with misclassified WC. In other words, the classifier correctly detected all the evaluation dates with anthesis, only missing the WC of a few readings (Table 9). The class WS -19 class was found to have an accuracy of 1.00 and did not express any prediction error, as well as the classes WS -6, WS 23, WW -19, and WW 3. The WS 11 class showed a correlation of 0.95 with itself but an estimated error of 0.05 with WW 11. The WW -6 class showed a correlation accuracy of 0.77 with itself, but an error of 0.23 with the WS -6 class. The WW 11 class showed a correlation accuracy of 0.94 with itself but an error of 0.06 with the WS 11 class. Finally, the WW 23 class showed an accuracy of 0.81 with itself but an error of 0.03 with the WW -6 class and 0.16 with the WS 23 class (Table 9).

The LDA classifier was run on 30 sets of samples for training/testing to perform a joint classification of the WS condition in terms of male anthesis dates. High accuracy was also obtained, with an averaging of 92.93% for the different scenarios. For this characterization, considering the red dotted line representing the average accuracy of the model, there was a low variability in the accuracy, demonstrating the capacity and robustness of the classification, with a high accuracy rate (Figure 8).

Table 9 - Confusion matrix with predictive error and correctness models for classifying the water condition applied and the classes (days) resulting from the evaluations before and after male anthesis for the popcorn lines.

	Prediction								
Class	**WS-19**	**WS-6**	**WS11**	**WS23**	**WW-19**	**WW-6**	**WW3**	**WW11**	**WW23**
WS-19	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WS-6	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WS11	0.00	0.00	0.95	0.00	0.00	0.00	0.00	0.05	0.00
WS23	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
WW-19	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
WW-6	0.00	0.23	0.00	0.00	0.00	0.77	0.00	0.00	0.00
WW3	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
WW11	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.94	0.00
WW23	0.00	0.00	0.00	0.16	0.00	0.03	0.00	0.00	0.81

The dark blue color shows the correlation of the class with itself and its prediction percentage. The light blue color shows where the class had a prediction error.

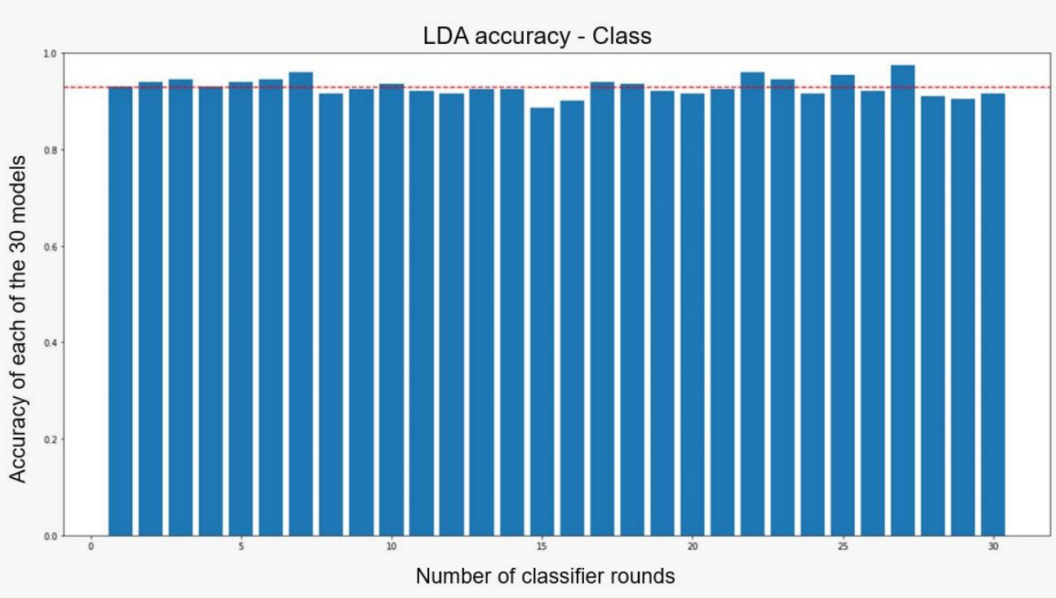


Figure 8. Average accuracy of 30 runs of the model for classifying the applied water condition resulting from evaluations before and after male anthesis for popcorn grown under two water conditions.

Discussion

Phenotypic expression of popcorn lines under different water conditions

It was observed that there was genetic variability for the most economically important traits of popcorn, i.e., GY, PE, and VP, and that the suspension of irrigation in the pre-anthesis phase was sufficient to differentiate the WCs. The suspension of irrigation from the pre-anthesis stage is a widely used practice for genotypic evaluation under drought conditions in maize (Cairns et al. 2012; Zia et al. 2013; Adebayo et al. 2014; Kamphorst et al. 2019, 2020b). Through this action, plants can express possible temporal adaptations to water limitation, which can be measured in the final yield (Romano et al. 2011; Cairns et al. 2012). At this phenological stage, the application of water stress can affect pollen viability, zygote formation, and grain filling, which are biological processes that are highly sensitive to soil water limitation (Zinselmeier et al. 1995), thereby reducing GY and its components.

In this study, the GY trait was more affected by soil water limitation. In Kamphorst et al. (2021 a), who evaluated popcorn lines and hybrids under drought conditions at differ-

ent times, soil water deficit applied between pre-anthesis, and grain filling reduced the yield covariates to a lesser extent; however, the sum of these effects also drastically reduced the main variable, in this case, GY. In their research, Kamphorst et al. (2021) (Kamphorst et al. 2021a) found that the yield component most affected in one growing season was the number of grains per row, while in another, it was the 100-grain weight. According to the authors, this effect was influenced by the time when the permanent wilting point was reached in the soil, when the reduction in the number of grains per row was due to water-stressed shortly after flowering; and the reduction in grain mass, close to the R3 stage. In this study, permanent wilting occurred 42 days after male flowering, which tends to reduce the estimates of the GY and PE traits.

Compared to GY and VP, PE was less affected by soil water limitation. Kamphorst et al. (2020 a), evaluating popcorn lines in two crop seasons, recorded reductions of 29.31% and 9.66% when comparing WS and WW conditions; and Lima et al. (2019), evaluating popcorn lines and hybrids, found a reduction of 9.08%. The expansion process is related to the moisture contained in the grain starch granules of the kernel, which exert pressure on the pericarp when heated (≈180 ºC), the rupture of which exposes the endosperm (Silva et al. 1993). In this sense, the lack of water during grain formation can affect the physical and chemical properties and interfere with the grain's ability to expand.

Due to the significant effect of the genotype*water condition interaction, a differential response of popcorn lines is expected. In the plant selection process, these interactions interfere with the recommendation of cultivars for specific environments and with selection gains (Hallauer et al. 2010). An alternative for obtaining genetic gains is identifying variables for indirect selection that are decisive in expressing key traits. Interestingly, these variables do not show significant G*WC interaction but are significantly and positively associated with the main traits (Kamphorst et al. 2019). In this sense, Kamphorst et al. (2021a) described that due to the lack of significance for the interactions G*Crop season, G*WC, and G* Crop season*WC, the traits number of grains per row and 100-grain weight are of interest for indirect selection in popcorn, both in WW and WS conditions. The traits mentioned here have been highlighted as important in research with popcorn under suitable water conditions (Amaral Júnior et al. 2016; Cabral et al. 2016; de Lima et al. 2016) and in research with the crop under drought conditions (Kamphorst et al. 2019, 2020a).

GY and PE are the most important traits in popcorn. Under drought conditions, studies show that additive genetic action is predominant for PE, while non-additive action is most important for the expression of GY and its components (Lima et al. 2019, 2021; Kamphorst et al. 2021a). Since it is possible to exploit dominance deviations to increase GY expression, PE becomes the most important selective trait *per se*. For this reason, lines L220, L510, L684, and L691 which have the highest PE averages regardless of WC, are promising parents for use in drought breeding programs. Although these lines were selected for having a PE $\geq$ 25 mL g^{-1}, during this cultivation period, no line showed a PE equal to or greater than this value, which can be interpreted as the occurrence of significance for the G*WC interaction, since PE is considered oligogenic and is also influenced by the action of the environment (Pereira and Amaral Júnior 2001).

Regardless of the WC, we can generally highlight the L217 and L691 lines, which, in addition to having higher PE estimates, also expressed higher GY and VP values when compared to the other lines. These lines can be recommended as parents for obtaining superior hybrids.

Can NIR spectra identify the presence of water limitation in the soil and the different phenological stages?

It was possible to observe the near infrared (NIR) spectra of the different samples, corresponding to the measurements made on the stems of the popcorn lines at different times of male anthesis in two water conditions (WCs). In general, the samples showed

a similar spectral profile in terms of chemical composition in the region of the electromagnetic spectrum studied, even after the pre-processing applied, i.e., SNV and first derivative, aimed at smoothing the spectrum and varying its overall shape (Figure 4).

It was impossible to detect a direct correlation between the NIR spectra and the most economically important traits for the popcorn crop, i.e., GY, PE, and VP. One explanation for the lack of correlations may be related to the short spectral range of the equipment used, from 908 to 1,676 nm, which does not cover the entire NIR spectrum, since the equipment that was able to better predict the parameters had ranges below or above the one used (Ferreira de Oliveira et al. 2020; Jiang 2020). In this study, several factors may have affected the identification of spectra in the popcorn crop, such as the number of samples evaluated, which requires a time window that may have resulted in a loss of standardization of the temperature and humidity of the samples. In addition, the presence of popcorn stem residue may have directly affected the spectral reading.

Given the lack of a relationship between the main traits of the popcorn, an attempt was made to find a relationship between the NIR spectra to find out whether they can classify the water conditions applied and whether they can record the chemical changes that occur in the plants due to the phenological stages. In the separate analyses of the confusion matrix of the LDA classifier for water conditions, phenological stages, and the two associates, there was a slight variation in accuracy between the predictions, demonstrating the robustness of the data for classifying water conditions.

Conclusions

Lines L691 - superior for GY, PE, and VP in WS - and L217 - superior for GY and VP in WS - and L220, L510, and L684 - superior for PE in WS - are of interest to obtain superior segregants in terms of expansion and grain yield under drought conditions. NIR spectroscopy did not show any association that would allow indirect selection of GY, PE, or VP, which may be related to the short spectrum of the equipment used, the non-standardization of the temperature, and, or the presence of residues and wax in the stalk of the samples. NIR spectroscopy made it possible to discriminate the water condition concerning the phenological stage of the plant, and it is recommended that further studies be conducted with a greater number of genotypes and better standardization in obtaining the data to reliably predict the spectral indexes that allow us to see the possibility of discriminating genotypes with adaptation to water deficit.

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