High-throughput phenotyping as an auxiliary tool in the selection of corn hybrids for agronomic traits[1]

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ABSTRACT

High-throughput phenotyping (HTP) using vegetation indices (VIs) is an important data source for managing plant breeding programs and can be a promising tool in indirect selection. This study hypothesized that VIs are correlated with agronomic traits in corn, and therefore, HTP can be an auxiliary tool for selecting superior genotypes. The objectives were: i) to analyze the association between agronomic traits and VIs, and ii) to identify superior corn hybrids for the evaluated traits using multivariate techniques. Ten corn hybrids (AGRI 330, AGRI 340, FS575PWU, KTZ006VP3, MG545AW, MG580PW, MG711PW, MZ1780, MZ1952, and TROPI 102) were evaluated for plant height (PH), stem diameter (SD), and grain yield (GY). The VIs studied were NDVI, NDRE, EVI, GNDVI, SAVI, and MSAVI. Pearson's correlation network was constructed to analyze the relationship between the variables, and a canonical analysis was performed to verify the inter-relationship between the variables and hybrids. The VIs evaluated are strongly positively correlated with each other and with PH. The most productive hybrids are MG545AW, FS575PWU and KTZ006VP3. Hybrid MZ1952 has higher correlations with VIs and PH. The findings reveal that VIs can be excellent auxiliary variables for selecting agronomically superior genotypes, being a promising alternative to increase corn breeding efficiency.

Keywords: plant breeding; indirect selection; multivariate analysis; precision agriculture; vegetation indices.

INTRODUCTION

Corn (*Zea mays* L.) is among the most important crops in the economic and social aspects and stands out for being the most grown and consumed grain globally. Given the gradual world population growth (ONU, 2019), the demand for food tends to increase even more. In this scenario, plant breeding can increase crop yield (Borém *et al.*, 2017; Bailey-Serres *et al.*, 2019; Heinz *et al.*, 2020; Taveira *et al.*, 2020). Since the beginning of agriculture, selecting plants for traits desirable to humans has been established. In the 1960s, the Green Revolution relied on genetic improvement in grains, among them corn, for global food security (Bailey-Serres *et al.*, 2019).

In corn breeding programs, one of the ways to obtain high-yielding hybrids is through direct selection on the grain yield trait. However, selecting a particular trait can influence others, which makes crucial the knowledge of the degree of association between traits so that selection achieves the breeder's goal (Cruz *et al.*, 2012). Besides

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searching for the traits of interest, the optimization of the plant evaluation process is also essential in breeding programs to achieve greater efficiency. Thus, when there is a high correlation between a trait that is easy to evaluate and traits of interest that require time and labor with recurrent evaluations in the field, like agronomic traits, the indirect selection is an excellent alternative (Samecima Junior, 2018). Indirect selection aims to optimize costs and labor and advance the selection process by seeking to improve a trait of interest by selecting based on another trait that is more easily analyzed (Ramalho *et al.*, 2012; Borém *et al.*, 2017).

High-throughput phenotyping (HTP) is an important data source for managing breeding programs by providing spatial and temporal information (Shanahan *et al.*, 2001; Da Silva *et al.*, 2020). The use of sensors in agriculture has enabled non-contact and nondestructive evaluation and can be a helpful tool in indirect selection to minimize time, labor, and costs while providing more accurate information. One of the main HTP tools is the use of vegetation indices (VIs). VIs are ratios between the reflected radiation from two or more bands and are efficient algorithms for estimating vegetation canopy, growth, vigor, nutritional status, among other applications (Xue & Su, 2017; Silva Júnior *et al.*, 2018; Prado Osco *et al.*, 2020; Ramos *et al.*, 2020; Santana *et al.*, 2021, Santana *et al.*, 2022).

Among the several VIs available, the *Normalized Difference Vegetation Index* (NDVI) and the *Normalized Difference Red Edge* (NDRE) are the most widely used in plant growth monitoring studies (Bonfil, 2017; Da Silva *et al.*, 2020; Santana *et al.*, 2022). NDVI is correlated with plant height and leaf mass, mainly in absorbing green radiation around 550 nm (Moriwaki *et al.*, 2019). NDRE includes the red band and has higher sensitivity for estimating leaf chlorophyll content and N content than NDVI, especially in C4 plants (Gitelson & Merzlyak, 1994; Portz *et al.*, 2012). Another widely used VI is the Soil Adjusted Vegetation Index (SAVI), which minimizes the soil background effects on spectral reflectance, which is especially important in situations where the soil surface is not entirely covered by vegetation (Zhao *et al.,* 2018)

Spectral reflectance is related to plant yield (Chang *et al.*, 2016). Plant leaf tissues can reflect, absorb, or transmit solar radiation, and the relationship between these factors is variable according to organ surface characteristics and their physiologies. Furthermore, light emission ranges according to wavelength (Silva Júnior *et al.*, 2018). High-yielding phenotypes are related to their respective VIs, which, when observed precisely, are related to their gene constitutions and hence can be subject to selection and improvement (Rutkoski *et al.*, 2016).

Despite being a promising technique, using VIs as auxiliary tool for selecting genotypes in corn breeding programs is still incipient, and studies evaluating the relationship between plant traits and VIs are crucial to improve the efficiency of genotype selection using this approach. Our hypothesis is that the VIs are correlated with agronomic traits in corn, and therefore, HTP can be an auxiliary tool for selecting superior genotypes. In this sense, the objectives of this study were: (i) to analyze the association between agronomic traits and VIs and (ii) to identify superior corn hybrids for the evaluated traits using multivariate techniques.

MATERIAL AND METHODS

Conducting the experiment

The experiment was carried out in the 2019/2020 crop season at the experimental field of the Universidade Federal de Mato Grosso do Sul, located in the municipality of Chapadão do Sul (18°41'33"S, 52°40'45"W, with an altitude of 810 m), Mato Grosso do Sul, Brazil. The region's climate according to the Köppen classification is Aw type (Savanna Tropical). The soil of the experimental field was classified as Red Latosol clayey dystrophic with the following chemical properties in the 0-0.20 m layer: pH (H_2O) = 6.2; exchangeable Al (cmolc dm^{-3}) = 0.0; Ca+Mg (cmolc dm^{-3}) = 4.31; P (mg dm^{-3}) = 41.3; K (cmolc dm^{-3}) = 0.2; organic matter (g dm^{-3}) = 19.74; V (%) = 45.0; Al saturation (%) = 0.0; sum of bases (cmolc dm^{-3}) = 2.3; CEC (cmolc dm^{-3}) = 5.1. Weather conditions throughout the experiment are shown in Figure 1.

Ten treatments were evaluated in a randomized block design with three replicates. The treatments were composed of single hybrids acquired from Agricom Seeds: AGRI 330, AGRI 340, FS575PWU, KTZ006VP3, MG545AW, MG-580PW, MG711PW, MZ1780, MZ1952, and TROPI 102. The plots consisted of five-meters rows, with a spacing of 0.45 m between rows and a density of four plants m^{-1}.

Sowing occurred in December 2019 using conventional soil tillage (plowing and leveling harrow). Fungicide (Pyraclotrobin + Methyl Thiophanate) and insecticide

(Fipronil) were used in the treatment of seeds with at a dose of 200 mL of the commercial product for 100 kg seeds to ensure protection against the pests and soil fungi. Cultural practices were performed according to the needs of the crop.

Assessing agronomic traits and vegetation indices

When the grains were at the R2 stage, usually recommended for the beginning of harvesting (Harrison *et al.*, 1996), the following agronomic traits were evaluated: plant height (PH, m) and stem diameter (SD, cm), sampled from five plants randomly in each plot using a tape measure; and grain yield (GY, kg ha^{-1}), sampled along two meters of the central rows of each plot and, after being weighed and converted to 13% humidity, it was extrapolated to kg ha^{-1}.

At 60 days after emergence (DAE), a Sensefly eBee RTK fixed-wing remotely piloted aircraft (RPA) was used as a HTP resource. The eBee has autonomous take-off, flight plan, and landing control and was equipped with the senseFly Parrot Sequoia® multispectral camera, which has a sunshine sensor and an additional 16 MP RGB camera for scouting. The overflight was performed at 100 m altitude, allowing a spatial image resolution of 0.10 m. The overflight was carried out near the zenith due to the minimization of the shadows on the plants at 11 a.m for six minutes. Radiometric calibration was made for the entire scene using a manufacturer-supplied calibrated reflective surface. Multispectral reflectance images were obtained for green (550 nm ± 40 nm), red (660 nm ± 40 nm), red (735 nm ± 10 nm), and near infrared (NIR, 790 nm ± 40 nm) spectral bands. Reflectance values at these wavelengths enabled the calculation of VIs studied. Further details on flight procedures and the acquisition of wavelengths for calculating VIs can be found in Prado Osco *et al.* (2020) and Santana *et al.* (2022).

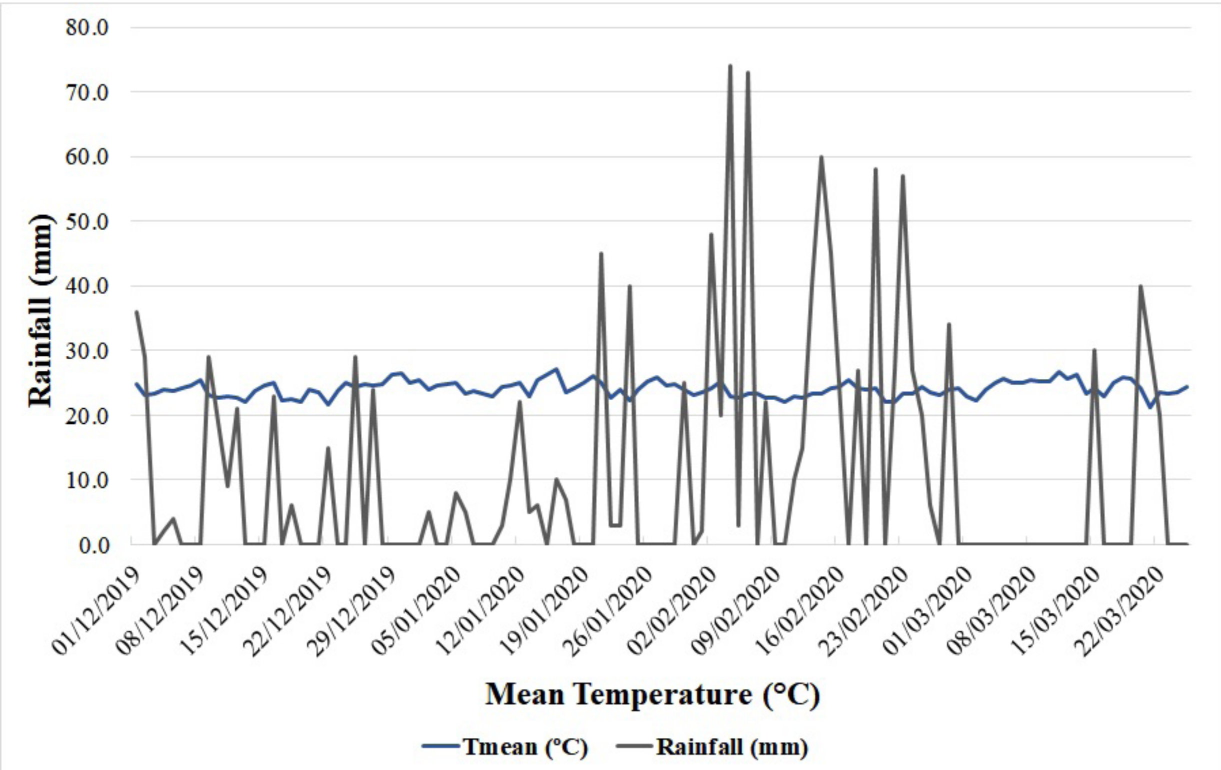

Figure 1: Weather conditions during the 2019/2020 crop season.

The acquired images were mosaiced and orthorectified by the Pix4Dmapper software. The calculated VIs were: NDVI (*Normalized Difference Vegetation Index*), NDRE (*Normalized Difference Red Edge Index*), EVI (*Enhanced Vegetation Index*), GNDVI (*Green Normalized Difference Vegetation Index*), SAVI (*Soil-Adjusted Vegetation Index*), and MSAVI (*Modified Soil-Adjusted Vegetation Index*). The VIs evaluated here were chosen because of their higher correlation with plant biomass according to Raper &Varco (2015), and are detailed in Table 1.

Table 1: Description of the vegetation indices calculated using the Sequoia multispectral sensor

Abbreviation	Vegetation Index	Equation	References
NDVI	*Normalized Difference Vegetation Index*	$\frac{NIR - red}{NIR + red}$	(Rouse *et al.*, 1974)
NDRE	*Normalized Difference Red Edge Index*	$\frac{NIR - rededge}{NIR + rededge}$	(Gitelson & Merzlyak, 1994)
EVI	*Enhanced Vegetation Index*	$\frac{NIR - red}{(NIR + 6red - 7,5green) + 1}$	(Justice *et al.*, 1998)
GNDVI	*Green Normalized Difference Vegetation Index*	$\frac{NIR - green}{NIR + green}$	(Gitelson *et al.*, 1996)
SAVI	*Soil-Adjusted Vegetation Index*	$(1 + 0,5)\frac{NIR - red}{NIR + red + 0.5}$	(Huete, 1988)
MSAVI	*Modified Soil-Adjusted Vegetation Index*	$\frac{0,5[2NIR + 1 - \sqrt{(2NIR + 1)^2 - 8(NIR - red)}\ ]}{2}$	(Qi *et al.*, 1994)

NIR: near-infrared reflectance; green: green reflectance; red: red reflectance, rededge: red transition reflectance.

Statistical Analysis

To study the existing relationship between the traits and VIs, Pearson's correlations were estimated between the agronomic traits and VIs. A correlation network was constructed to graphically express the functional relationship between the correlations, where the distance between the nodes (variables) is proportional to the absolute value of the correlation between them. The thickness of the lines was controlled by enforcing a cut-off value of 0.60, meaning that only correlations above 0.60 were highlighted. Lastly, positive correlations were plotted by green lines, while negative correlations were highlighted in red. A multivariate canonical analysis was performed to verify the inter-relationship between the variables (agronomic traits and VIs) and treatments (hybrids). All statistical analyses were performed using Rbio software (Bhering, 2017) and followed the procedures recommended by Cruz *et al.* (2012).

RESULTS AND DISCUSSION

Canonical analysis provides a multivariate analysis of variance (MANOVA), in which treatment effect was significant ($p < 0.05$) for all variables analyzed. Thus, it was possible to proceed to Pearson's correlation and canonical analyses to verify the association between variables and the interrelationship between variables and treatments, respectively. Correlation network revealed the existence of high-magnitude positive correlations for most variables evaluated (Figure 2). There was a low-magnitude negative correlation only between PH and SD. Although the correlation between the traits PH and SD was negative of low magnitude, i.e., the taller plants had smaller stem diameters, such result is consistent with the literature. According to Taiz *et al.* (2017), the microfibrils present in most stem cells have an anisotropic behavior, which is growing in a preferential direction, providing a greater expansion in length than in width. Consequently, the cell structure tends to reorganize itself, narrowing on the sides and expanding vertically.

The VIs showed a strong positive correlation between them, which is expected because, although there are differences between them, their formulas are similar, favoring smaller differences between their values. This leads to the fact that plants with high NDVI values also obtained high values for the other indices. Moreover, PH also showed a high positive correlation with all the VIs. The correlation between VIs and PH supports that plant height can be estimated from vegetation indices, as reported in a previous study carried out by Prado Osco *et al.* (2020) with corn crop in Brazil. This is mainly due to the fact that VIs are able to measure plant attributes such as chlorophyll content and biomass, which in turn are highly related with plant height.

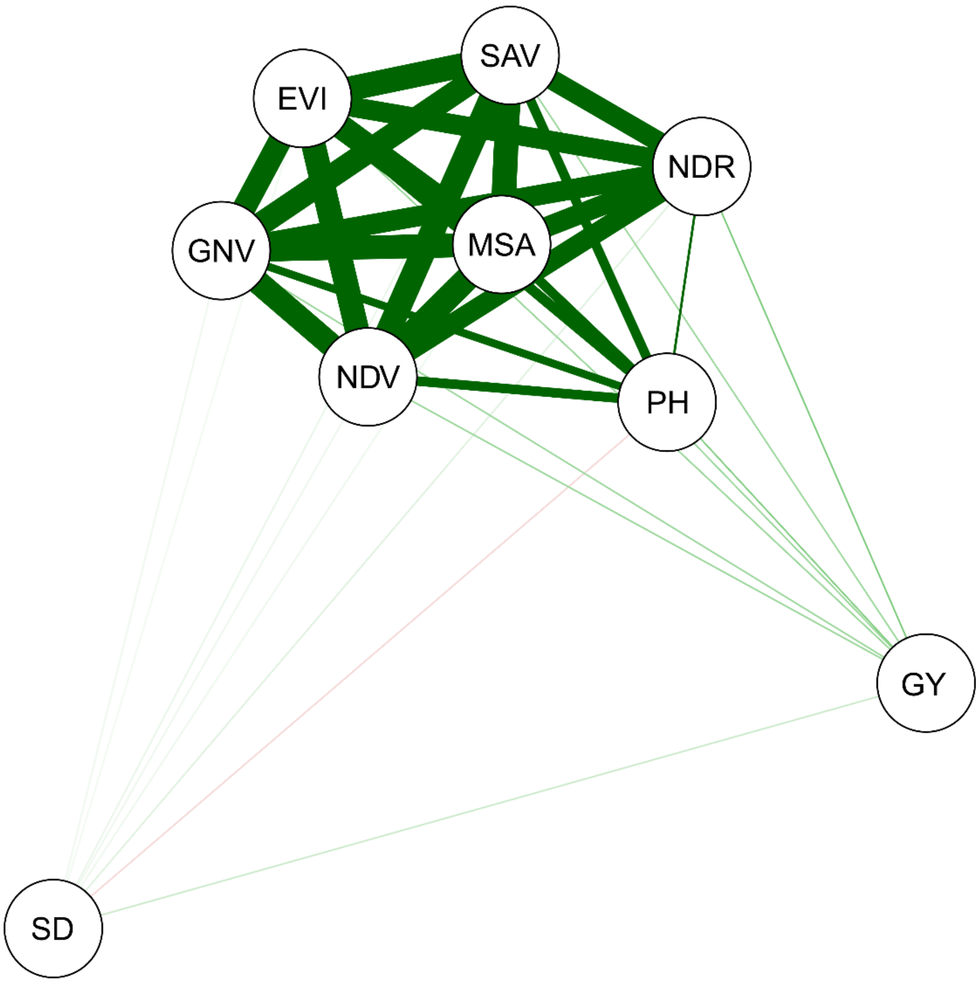

Figure 2: Correlation network between agronomic traits plant height (PH), stem diameter (SD) and grain yield (GY), and vegetation indices NDVI, NDRE, EVI, GNDVI, SAVI and MSAVI, evaluated in ten corn hybrids.

NDVI, created by Rouse *et al.* (1974), uses the wavelengths of the red (660 ± 12 nm) and near-infrared (770 ± 12 nm) bands. Plants that have higher reflectance in the green and near-infrared tend to absorb more red (Shiratsuchi, 2014). The chlorophyll molecule reflects the visible range, while the near-infrared is reflected by the leaf mesophyll (Oliveira, 2017), pointing to suitable conditions for photosynthesis. Therefore, NDVIs close to 1 (maximum value) refer to healthier and more yielding plants.

However, the red and near-infrared bands may be influenced by the biomass, impairing the accuracy of yields. An alternative is to employ VIs that use wavelengths in the green range (Oliveira, 2017). In this sense, we calculated NDRE, covering wavelengths in the rededge and near-infrared (NIR) bands; EVI, allowing the relation between green, red and NIR; GNDVI, relating green to NIR; SAVI and MSAVI, which, although they also use only NIR and red, are adjusted according to the parameters of the soil and the vegetation itself, respectively.

Regarding GY, the interaction with the other evaluated traits was moderate, allowing its indirect selection. Quantitative traits, such as yield, are difficult to select because they are highly influenced by the environment. One strategy in this situation is indirect selection, which also reduces time and labor (Samecima Junior, 2018). Thus, traits with high heritability and presenting a linear correlation with yield identify superior genotypes in this aspect (Ribeiro *et al.*, 2010). The plants that presented higher heights and VIs were the highest yielding ones, and hence the linear correlation was positive.

Figure 3 shows the canonical analysis, which allows knowing the interrelationship between the treatments and the evaluated variables. The accumulated variance in the first two canonical variables was 99.6%, indicating high accuracy in interpreting the constructed biplot. Cruz *et al.* (2011) and Mingoti (2007) recommend employing this procedure when the first two canonical variables retain at least 80% of the total variation. This analysis made it possible to verify that hybrids MG545AW, FS575PWU, and KTZ006VP3 stood out for their grain yield since these hybrids are on the same axis of the GY trait.

A high-magnitude positive relationship between VIs and PH provided by Pearson's correlation analysis was also observed in the canonical analysis, as they fall on the same axis. The hybrid MZ1952 was the most correlated with such variables according to its graphical positioning, also occupying the same axis. Although GY is not inserted in this quadrant, its proximity is significant. Similar results were obtained by Taveira *et al.* (2020), who evaluated the association between spectral data and agronomic traits in soybean using a multivariate approach. The authors verified a strong association between the studied VIs (NDVI and NDRE) but found no association between VIs and agronomic traits.

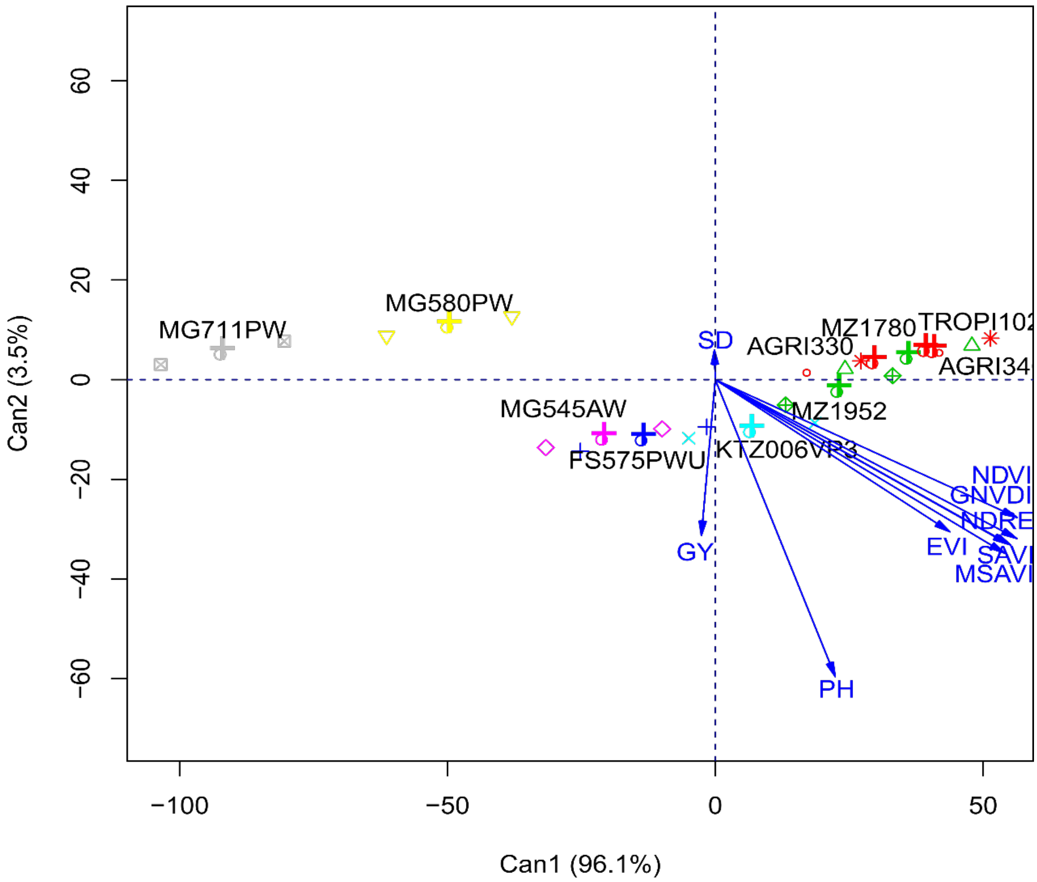

Figure 3: Canonical analysis applied to the agronomic traits plant height (PH), stem diameter (SD) and grain yield (GY), and vegetation indices NDVI, NDRE, EVI, GNDVI, SAVI and MSAVI, evaluated in ten corn hybrids.

The other hybrids showed no correlation with agronomic traits and VIs. AGRI330, AGRI340, MZ1780, and TROPI102, which occupy the upper right axis, are close to the VIs and hybrid MZ1952 but not close enough to be correlated, due to the separation by axis. The hybrids most distant from the evaluated variables and the other hybrids were MG711PW and MG580PW, which occupied the upper left axis. The trait SD did not fit on the axes and, consequently, did not obtain a relationship with the other variables and hybrids by the canonical analysis.

The findings reported in this study reveal that VIs can be excellent auxiliary variables for selecting superior genotypes in breeding programs. In a corn breeding program where hundreds of lines are assessed annually, the measurement of agronomic traits is costly, as it is a labor and time-consuming task. Furthermore, long periods of field evaluations cause exhaustion and human fatigue, which may result in lower accuracy of the assessments (Prado Osco *et al.*, 2020; Ramos *et al.*, 2020; Samecima Junior, 2018). In this sense, VIs can be used as auxiliary traits in the indirect selection, where genotypes with higher values for the VIs studied here could be selected for better agronomic performance, reducing the number of in-field measurements. Therefore, HTP using VIs arises as a promising alternative to increase the efficiency of crop breeding programs, providing accurate results in a shorter time.

CONCLUSIONS

The vegetation indices NDVI, NDRE, EVI, GNDVI, SAVI and MSAVI are strongly positively correlated with each other and with plant height, which, in turn, show moderate positive correlation with grain yield. The stem diameter has low negative correlation with plant height.

The hybrids with higher yields were MG545AW, FS575PWU and KTZ006VP3. Hybrid MZ1952 has higher correlations with vegetation indices and plant height compared to the other varieties. AGRI330, AGRI340, MZ1780 and TROPI102 are closer to each other but have no significant correlation with the variables evaluated by canonical analysis; the same occurred with the hybrids MG711PW and MG580PW.

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