High-throughput phenotyping by RGB and multispectral imaging analysis of genotypes in sweet corn

Marina F e Silva 1; Gabriel M Maciel 1; Rodrigo BA Gallis 1; Ricardo Luís Barbosa 1; Vinicius Q Carneiro 2; Wender S Rezende 1; Ana Carolina S Siquieroli 1

1Universidade Federal de Uberlândia (UFU), Monte Carmelo-MG, Brasil; marinafreitas.agro@hotmail.com; gabrielmaciel@ufu.br; rodrigogallis@gmail.com; rluisbarbosa@ufu.br; wendersrezende@gmail.com; carol@ufu.br. 2Universidade Federal de Lavras (UFLA), Lavras-MG, Brasil; vinicius.carneiro@ufla.br

ABSTRACT

Sweet corn (Zea mays subsp. saccharata) is mainly intended for industrial processing. Optimizing time and costs during plant breeding is fundamental. An alternative is the use of high-throughput phenotyping (HTP) indirect associated with agronomic traits and chlorophyll contents. This study aimed to (i) verify whether HTP by digital images is useful for screening sweet corn genotypes and (ii) investigate the correlations between the traits evaluated by conventional methods and those obtained from images. Ten traits were evaluated in seven S3 populations of sweet corn and in two commercial hybrids, three traits by classical phenotyping and the others by HTP based on RGB (red, green, blue) and multispectral imaging analysis. The data were submitted to the analyses of variance and Scott-Knott test. In addition, a phenotypic correlation graph was plotted. The hybrids were more productive than the S3 populations, showing an efficient evaluation. The traits extracted using HTP and classical phenotyping showed a high degree of association. HTP was efficient in identifying sweet corn genotypes with higher and lower yield. The vegetative canopy area (VCA), normalized difference vegetation index (NDVI), and visible atmospherically resistant index (VARI) indices were strongly associated with grain yield.

Keywords: Zea mays, phenotypic data, infrared, plant breeding.

Received on June 25, 2021; accepted on February 3, 2022
phenomics through digital images in the genetic improvement of plants (Zhang et al., 2017; Fernandez-Gallego et al., 2018; Makanza et al., 2018b; Maciel et al., 2019; Wiegmann et al., 2019; Yang et al., 2020; Li et al., 2021).

The use of HTP by digital image, especially in the field, is still incipient (Furbank & Tester, 2011). In addition, most of the studies on phenomics relate to the main crops, such as soybean, yellow corn, and wheat (Zhang et al., 2017; Fernandez-Gallego et al., 2018; Makanza et al., 2018a). There is a lack of studies on phenomics applied to sweet corn, especially on monitor yield. In this context, several studies showed that higher levels of chlorophyll are strongly associated with higher yields in yellow corn due to the higher photosynthetic rate. The traditional method used has been based on the chlorophyll meter (SPAD), capable of obtaining indirect values for chlorophyll content in the leaves (Yang et al., 2012; Xiong et al., 2015). However, breeding programs have many individuals to analyze, making it impossible to use SPAD because it represents a high demand for time during field readings (Maciel et al., 2019).

Thus, this study aimed both to (i) verify whether high-throughput phenotyping by RGB (red, green, blue) and multispectral imaging analysis is useful for screening sweet corn genotypes and (ii) investigate the correlations among the traits evaluated by conventional methods with those obtained from images.

MATERIAL AND METHODS

Experimental setup

The experiment was conducted under sprinkler irrigation at the Plant Experimental Station, in Monte Carmelo, MG, Brazil (18°42′43."S, 47°29′55.5"W, at 873 m altitude) from March 03 to June 20, 2018. The experiment was carried out using a randomized block design, with nine treatments and three replicates (27 plots). The sweet corn plants studied consisted of two commercial hybrids from the companies Seminis (hybrid A) and Syngenta (hybrid B), and seven populations of the S₁ generation (L6P2, L6P15, L7P3, L8P7, L8P10, L8P12, and L8P18) from the Germplasm Bank of the Sweet Corn Breeding Program, Campus Monte Carmelo. All seeds of the S₁ generation used in this study were obtained from three successive, controlled self-pollination of ears collected from street markets, which were initiated in 2016.

Sowing was performed in styrofoam trays, which were then packed in the greenhouse for later transplantation, in order to guarantee the establishment of the desired population of 50 thousand plants per hectare in the field. The seedlings were transplanted to the field when they have reached the V₇ stage, the vegetative stage of sweet corn plants in which two fully expanded leaves were observed (Pereira Filho & Teixeira, 2016). The transplant depth to the field was 4 cm, with spaces between rows and between plants being 0.7 and 0.3 m, respectively. The dimension of each plot consisted of two 5.4-m long rows, with a 0.7-m aisle separating the plots and a useful area of 7.56 m², and 32 plants per plot. Weeds were controlled throughout the crop cycle by means of manual weeding. Finally, crop management with fertilizers and pesticides was carried out using previously recommended methods for sweet corn (Pereira Filho & Teixeira, 2016).

Image capture

Aerial images of the sweet corn plants at the V₇ stage, the vegetative stage of sweet corn plants in which seven fully expanded leaves are observed (Pereira Filho & Teixeira, 2016), were captured using an unmanned aerial vehicle (UAV), the Phantom 4® model, equipped with two optical sensors: an RGB camera (DJI Phantom 4 Pro®) and a multispectral camera (MAPIR Survey 3®).

The RGB camera had a 20-megapixel resolution and included a 5350-mAh battery, a CMOS sensor, and a 9-mm fixed focal length lens. The collected images measured 4864 × 3648 pixels, with longitudinal and lateral overlaps of 80 and 75%, respectively, resulting in a spatial resolution of 0.004 m pixel⁻¹. The multispectral camera (with a 12,000 mAh battery) had a 12-megapixel resolution to capture red (R), green (G), and near-infrared (IR) bands. The spatial resolution was the same as the RGB camera.

Flight plan was previously set to autopilot mode with the DroneDeploy® application. The UAV was configured to cover the experimental area at 3 m s⁻¹ fast and 20 m high. To minimize shadow effects, the images were captured at noon, the period of more uniform solar radiation on the earth’s surface. After the flight, the raw images were stored in the camera memory and later transferred to a computer in JPEG format for processing.

Image processing

The images stored in the computer were loaded into Pix4D software. Then, two orthophotos were generated to represent the set of raw images and georeferenced to represent the whole experimental area. The resulting orthophotos consisted both of an RGB orthophoto with red (band 1), green (band 2), and blue (band 3) channels and a multispectral orthophoto with red (band 1), green (band 2), and near-infrared (band 3) channels. The georeferencing was performed according to the coordinate system EPSG: 32723 – WSG 84/UTM zone 23S.

Besides providing the RGB images with radiometric correction, Pix4D software performed the geometric calibration of the cameras by self-calibration using SFM (Structure from Motion).

Moreover, a radiometric calibration of the Mapir RGN sensor was performed. A calibration target was placed on the ground, and after the flight, the radiometric calibration of the camera was performed, so that the images were in accordance with a known and, therefore, comparable radiometric reference. In practice, the solution was in the calibration plates of the sensor. The camera’s software, Mapir Camera Control, was used in the radiometric calibration.

Data extraction

The traits extracted from the RGB orthophotos were the vegetative canopy area (VCA), the visible atmospherically
resistant index (VARI), and the mean reflectance values of the R, G, and B channels. The mean values of the near-infrared reflectance (NIR) and the normalized difference vegetation index (NDVI) were extracted from the IR orthophotos.

First, using QGIS 3.4.12 software, each plot was delimited and manually cut out in both types of orthophotos, resulting in 30 images per orthophoto (each image represented a single experimental plot; see Figure 1). Next, all images were segmented into two groups, using the k-means clustering algorithm (MacQueen, 1967) with channels “a” and “b” of the scale Lab. Python language was used to segment the images.

From the segmented RGB images, the VCA was calculated by using the numbers of green pixels in each plot, the VCA was calculated by using the images.

Statistical analysis

The data provided the assumptions (homogeneity of variances, normality of residuals, and additivity of block designs) of the analysis of variance (ANOVA) at 0.01 level of significance. Then, the data were fed into ANOVA, and the mean values were grouped using the Scott-Knott test. In addition, the correlations between traits and genotypic coefficient of determination of traits with significant difference by ANOVA were estimated. All the analyses were performed with R software (R Development Core Team, version 3.6.1) using the ExpDes (R package version 1.1.2) and ggplot2 (Package ggplot2 version 3.3.0) packages to test the mean values, estimate the parameters and plot the graphs.

RESULTS AND DISCUSSION

The genotypes evaluated through digital aerial images differed in R, VCA, VARI, and NDVI, but not in G, B, and NIR (Figure 2). There were also differences in the conventionally evaluated traits, that is, the SPAD index, EY, and GY. The results of this study were similar to those of previous studies, indicating that RGB imaging was an useful tool for acquiring phenotypic data and screening sweet corn genotypes (Makanza et al., 2018b).

The hybrids showed higher yields (EY and GY) than the S1 populations. GY of the hybrid A was 25% higher than that of the hybrid B. These results were possibly due to the greater number of loci in heterozygosity, related to heterosis (hybrid vigor), of these hybrids when compared to the S1 populations, which show more loci in homozygosity, which relates to inbreeding depression (Borém et al., 2017).

In general, the hybrids showed higher values of vegetative indices (VARI and NDVI) and VCA than that of the S1 populations. The NDVI was the only index that differed among the S1 populations. The genotypes L6P15 and L8P18 showed the lowest NDVI values. Comparing the highest yielding genotype (hybrid A) with one of the poorest yielding genotype (L8P18), it was possible to see variation in VCA, VARI, and NDVI indices, which were highlighted by color composition in the image (Figure 3). The high number of dark green and blue pixels meant high VARI and NDVI indices, respectively.

VARI and NDVI indices indicate many plant physiological parameters, such as leaf chlorophyll content and leaf nitrogen concentration (Cairns et al., 2012; Vergara-Díaz et al., 2016). High VCA values imply great photosynthetically active area (Blancon et al., 2019). Moreover, this trait provides the crop with an additional benefit related to weed control. As this study evaluated (V, stage), higher VCA at the beginning of the crop cycle promoted faster closure of leaf canopy between the crop rows, leading to less infestation of weeds. All these factors are determinants for a higher EY and GY (Pereira Filho & Teixeira, 2016).

Plants in good physiological condition have high NDVI values, that is, they display high reflectance values in the G and NIR channels and low values predominantly in the R channel (Huang et al., 2012). This pattern was observed in this study, in which the R channel presented the lowest values.
for hybrids A and B (88.63 and 99.96, respectively). Excluding hybrids, the analysis of populations suggests a positive correlation between GY and R (Figure 2).

Most of the traits showed a high correlation with each other (SPAD, VARI, NDVI, VCA, EY, GY), even traits from different methodologies of evaluation (classical phenotyping and high-throughput phenotyping; Figure 4). By contrast, the same traits were negatively correlated with the mean reflectance values of the R channel. Mostly the genotypic coefficient of determination values was high, varying between 0.71 and 0.93, indicative of greater selection efficiency (Figure 4). In this study, R, VCA, VARI, NDVI (traits from high-throughput phenotyping) showed high genotypic coefficient of determination (close to 0.8).

Among the three channels of image (R, G and B) evaluated, the R channel was the only one to show variability regarding genotypes. Moreover, it showed high correlations (close to -0.8) with traits related to plant development (VCA) and yields (EY and GY). These results suggest that, from RGB images, the R channel is the most useful among the other channels of RGB image for selecting superior genotypes of sweet corn.

The correlations among traits are important, as they help to know if it is possible to use traits extracted from RGB and multispectral imaging to better understand or even replace some traits from classical phenotyping. Such traits can work as secondary to those that are important to sweet corn breeding with low heritability or difficult to measure (indirect selection) (Crain et al., 2017). In addition to having a high correlation with important traits, the secondary traits must have high heritability or high genotypic coefficient of determination (Cruz et al., 2012).

The results showed that VARI and NDVI indices can be used in indirect selection for the SPAD index, as the correlation among these traits and the genotypic coefficient of determination was close to 0.8. The SPAD index measures the chlorophyll content of the leaf and, therefore, it relates to starch production and grain yield (Yang et al., 2012; Xiong et al., 2015). However, SPAD is a difficult or unviable index to be measured in sweet corn breeding programs, because it requires a time-consuming evaluation. Then, the VARI and NDVI indices by means of high-throughput phenotyping can replace the SPAD index and benefit sweet corn breeding programs (Miller et al., 2017; Makanza et al., 2018a; Maciel et al., 2019).

These same indices (VARI and NDVI) and other traits from images (R and VCA) were highly correlated to EY and GY. Therefore, these traits can also be used in early selection or pre-selection for predicting the EY and GY, thus reducing costs and time in broad breeding programs (Gracia-Romero et al., 2018; Makanza et al., 2018b; Hinojosa et al., 2019).

Because of the high costs of new sensors, this study could only estimate a few indices. However, sensors that capture more bands of the electromagnetic spectrum, such as thermal and fluorescence cameras, help extract several other indices [i.e. Soil Adjusted Vegetation Index (SAVI) and Green Normalized Difference Vegetation Index (GNDVI)] and relevant information (i.e. leaf temperature, leaf chlorophyll fluorescence and diseases) (Araus et al., 2012).

![Figure 1. Experimental setup. A. Aerial image mosaic of the experiment with plots of sweet corn hybrids, captured with an unmanned aerial vehicle platform; B. the experimental setup with single plot details; and C. plot image segmentation by the k-means clustering algorithm. Monte Carmelo, UFU, 2021.](image-url)
The traits obtained from images in field depend on the cultivation management used during the crop cycle and on the phenological stage at the time of image capture (Vergara-Díaz et al., 2016; Fernandez-Gallego et al., 2018; Gracia-Romero et al., 2018). Therefore, in order to recommend a broad use of traits from high-throughput phenotyping in sweet corn breeding, further studies under different conditions are essential.

Therefore, the high-throughput phenotyping based on digital image analysis was efficient in identifying sweet corn genotypes with higher and lower yield among populations and between hybrids and populations. The VCA, NDVI, and VARI indices were strongly associated with grain yield, allowing the efficient and quickly selection of more productive genotypes in an indirect and non-destructible way. The hybrids were more productive than S₃ populations, showing that the proposal of this research is an efficient tool to help the selection of superior genotypes on breeding programs.

**Figure 2.** Mean values of nine genotypes of sweet corn. *Means followed by the same letter and color belong to the same group according to Scott-Knott test at 0.05 of significance.; R, G, B, and NIR = mean reflectance values in the red, green, blue, and near-infrared bands, respectively; VCA = vegetative canopy area (m²); VARI = visible atmospherically resistant index; NDVI = normalized difference vegetation index; SPAD = soil plant analysis development index; EY = ear yield (kg ha⁻¹); GY = grain yield (kg ha⁻¹). Monte Carmelo, UFU, 2021.
ACKNOWLEDGMENTS

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Universidade Federal de Uberlândia (UFU).

REFERENCES


CRAIN, J; REYNOLDS, M; POLAND, J. 2017. Utilizing high-throughput phenotypic data

Figure 3. Vegetative canopy color scales. Canopy traits (VCA, VARI, and NDVI) from the plot image. Shades of blue represent more vigorous plants (NDVI values tending to be closer to 1). Shades of red correspond to stressed or not persisting sweet corn (NDVI values tending to be closer to 0). VCA = vegetative canopy area; VARI: visible atmospherically resistant index; NDVI = normalized difference vegetation index. Monte Carmelo, UFU, 2021.

Figure 4. Phenotypic correlation among traits estimated by both classical and high-throughput phenotyping. Correlations among traits (off-diagonal) and genotypic coefficient of determination (diagonal). Positive and negative correlations are colored in green and red, respectively. The diameter of circle is proportional to the magnitude of the correlation. R = mean reflectance values in the red; VCA = vegetative canopy area; VARI = visible atmospherically resistant index; NDVI = normalized difference vegetation index; SPAD = soil plant analysis development index; EY = ear yield (kg ha⁻¹); GY = grain yield (kg ha⁻¹). Monte Carmelo, UFU, 2021.


HUANG, J; LIAO, H; ZHU, Y; SUN, J; SUN, Q; LIU, X. 2012. Hyperspectral detection of rice damaged by rice leaf folder (*Cnaphalocrocis medinalis*). *Computers and Electronics in Agriculture* 82: 100-107.

LI, D; QUAN, C; SONG, Z; LI, X; YU, G; LI, C; MUHAMMAD, A. 2021. High-throughput plant phenotyping platform (HT3P) as a novel tool for estimating agronomic traits from the lab to the field. *Frontiers in Bioengineering and Biotechnology* 8: 1533.


XIONG, D; CHEN, J; YU, T; GAO, W; LING, X; LI, Y; PENG, S; HUANG, J. 2015. SPAD-based leaf nitrogen estimation is impacted by environmental factors and crop leaf characteristics. *Scientific Reports* 5: 13389.

YANG, Y; TIMLIN, D; FLEISHER, DH; LOKHANDE, S; CHUN, JA; KIM, SH; STAVER, K; REDDY, VR. 2012. Nitrogen concentration and dry-matter accumulation in maize crop: assessing maize nitrogen status with an allometric function and a chlorophyll meter. *Communications in Soil Science and Plant Analysis* 43: 1563-1575.
