**University of São Paulo
“Luíz de Queiroz” College of Agriculture**

Evaluation of vegetation indices from aerial images in soybean breeding

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Dissertation presented to obtain the degree of Master in
Science. Area: Genetics and Plant Breeding

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—George R. R. Martin, A game of thrones.

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RESUMO

Avaliação de índices de vegetação a partir de imagens aéreas no melhoramento de soja

A fenotipagem de alto rendimento (HTP) é uma ferramenta emergente que permite acesso e identificação dos caracteres quantitativos simples e complexos, acelerando descobertas genéticas e seleção. Os índices de vegetação têm sido utilizados para detectar variações no campo, demonstrando correlação com vários caracteres importantes ao desempenho das culturas. Assim, este estudo teve como objetivo principal estimar índices de vegetação e suas correlações com caracteres agronômicos em diferentes populações de soja, utilizando imagens RGB derivadas de veículo aéreo não tripulado (UAV). Foram conduzidos três experimentos na safra 2018/2019: RIL-C (com controle de percevejos), RIL-N (sem controle de percevejos) e LQ (sem fertilização, correção do solo e controle de percevejos) com o objetivo de avaliar a resistência genética ao complexo de percevejos em linhagens de soja. Os genótipos foram avaliados com base nas seguintes características: número de dias para a maturidade (NDM), valor agronômico (AV), acamamento (LOD), altura da planta na maturidade (PHM) e rendimento de grãos (GY). Um sistema UAV com uma câmera RGB acoplada foi usado para adquirir um voo de fotografia aérea sobre o campo durante o estágio R5. Foram estimados o Índice de Vegetação Azul Verde Vermelho (RGBVI), Índice de Folha de Verde (GLI), Índice Resistente à Atmosfera Visível (VARI), Índice de Verdidão Triangular (TGI), Índice de Diferença de Verde Vermelho Normalizado (NGRDI) e dossel (Canopy) do ortomosaico. Modelos lineares mistos foram usados para estimar a variância de cada caractere usando o teste da razão de verossimilhança, e a análise de componentes principais foi realizada usando os BLUPs para verificar o padrão multivariado entre os genótipos. Os resultados mostraram efeitos genotípicos significativos para maioria dos caracteres avaliados. Pode-se observar alta herdabilidade dos caracteres. A análise dos componentes principais revelou que os genótipos apresentaram maior desempenho agronômico no experimento com controle de percevejos, além de mostrar forte correlação entre os caracteres GY e PHM e independência entre os caracteres LOD e NDM. Houve correlações significativas entre os caracteres agronômicos, índices de vegetação e dossel, que podem ser usadas para seleção indireta e seleções conjuntas dos caracteres das melhores linhagens no melhoramento de soja.

Palavras-chave: Imagens RGB, Índices de vegetação, *Glycine max*, Fenotipagem de alto rendimento, Melhoramento de plantas

ABSTRACT

Evaluation of vegetation indices from aerial images in soybean breeding

High throughput phenotyping (HTP) is an emerging tool that allows access and identifies simple and complex quantitative traits, accelerating genetic discoveries, and selection. Vegetation indices have been using to detect variation in the crop field, demonstrating correlation with several traits of crop performance. Thus, this study had the main goal to estimate vegetation indices and their correlation with agronomic traits in different soybean populations using RGB images derived from an unmanned aerial vehicle (UAV). Were conducted three experiments in the 2018/2019 season: RIL-C (stink bugs control), RIL-N (Without stink bugs control), and LQ (Without fertilization, soil correction, and stink bugs control) aiming to evaluate genetic resistance to the stink bug complex in soybean lineages. The genotypes were evaluated based on the following traits: Number the days to maturity (NDM), agronomic value (AV), Lodging (LOD), Plant height maturity (PHM), and grain yield (GY). A UAV system with an RGB camera coupled was used to acquire aerial photography flight over the field during the R5 stage. Was estimated the Red Green Blue Vegetation Index (RGBVI), Gren Leaf Index (GLI), Visible Atmospheric Resistant Index (VARI), Triangular Greenness Index (TGI), Normalized Green Red Difference Index (NGRDI), and canopy from the orthomosaic. Linear mixed models were used to estimate the variance of each trait using the likelihood ratio test, and the principal component analysis (PCA) was performed using the Best Linear Unbiased Predictions (BLUPs) to verify the multivariate pattern among genotypes. The results showed significant genotypic effects for the majority of the traits evaluated. High broad-sense heritability of the traits can be observed. The principal component analysis revealed that the genotypes had more agronomic performance in the experiment with control of stink bugs, also, showed a strong correlation between the traits GY and PHM, and independence between the traits LOD and NDM. There were significant correlations among the agronomic traits, vegetation indices, and canopy, that can be used for indirect selection and joint selections of the traits of the best lineages in the breeding pipeline.

Keywords: RGB images, Vegetation index, *Glicine max*, High throughput phenotyping, Plant breeding

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INTRODUCTION

The plant breeding science is currently in the 4.0 era, which is characterized by technological advances in genetics such as genome resequencing and editing, machine learning, and high throughput phenotyping (WALLACE; RODGERS-MELNICK; BUCKLER, 2018). The advances in biotechnology, such as the next-generation sequencing (NGS) technologies allow scientists to generate genomic data for a large number of samples, rapidly and at a low cost. Also, statistical methods such as genomic-wide association (GWAS) and genomic selections (GS) allow associating the DNA (Deoxyribonucleic acid) polymorphisms data with de phenotypic variation of agronomical traits (WATANABE et al., 2017). All these tools can be combined to improve genetic gains and help breeders to develop new cultivars accordingly to the world evolves and changes.

Soybean [*Glycine max* (L.) Merrill] crop is an economically important worldwide grain, having a total world production of 336.563 million metric tons (MMT) in 2019/2020 (USDA, 2020). Brazil is the producer leader of this grain, with 120.3 MMT in the last season, flowing by the United States, with 96.615 MMT produced (CONAB, 2020). For being rich in protein and lipids, the soybean is present in the human and animal diet, in the form, oil, and feed form, it is also used for biodiesel production.

The productive success of this leguminous is a result of the efforts by companies and research institutions carried out in soybean breeding programs, which offer in the market resistant genotypes to pests, diseases, and abiotic stress, adaptable, and stable to different environments, and also, more productive varieties (NOGUEIRA et al., 2012).

The aim of crop improvement and breeding is correlated with the successful application of methods and technologies applied so far for plant phenotyping. However, field phenotyping is a part of the plant breeding that needs enhancement (PRIYADARSHAN, 2019). Plant phenotyping is defined as the application of methodologies and protocols to measure a specific trait, thanks to manual phenotyping of plants in the field, it is possible to develop and to improve crop varieties (BUSEMEYER et al., 2013).

In soybean breeding, it is visible the challenges faced by conventional phenotyping due to the difficulty of measuring relevant traits. Since the plants grow very closer together it is onerous to do the phenotyping between lines, furthermore, the maturation time among the genotypes is very variable. Thus, faster phenotyping mechanisms could facilitate soybean breeding, being less laborious and expensive (FURBANK; TESTER, 2011).

Plant breeding programs, normally, work with large populations and are phenotyped for several traits using many plots, with replicates, and across multiple environments. This fact results in a large amount of data that needs to be collected to give the breeders the ability to understand the genetics of quantitative traits, especially those related to yield and stress tolerance (WHITE et al., 2012). For this reason, many authors have defined this issue as a “bottleneck” in accelerating the plant breeding process and point the high throughput plant phenotyping as a way out to generate the phenotypic data with fast, automated, and quality technology (BAI et al., 2016; CABRERA-BOSQUET et al., 2012; GOGGIN; LORENCE; TOPP, 2015; SINGH et al., 2016).

High throughput phenotyping (HTP) is an emerging tool that successfully integrates the areas of plant science, engineering, and computer science with the finality to access and identify simple and complex quantitative traits (SHAKOOR; LEE; MOCKLER, 2017). In other words, these tools have the potential to accelerate genetic discoveries and selection (TANGER et al., 2017). Plant height, biomass, grow cycle, flowering time, grain yield, harvest index are some of the traits that HTP image analyses can extract to correlate with yield, stress tolerance, and disease resistance (HARTMANN et al., 2011). Another advantage of HTP in plant breeding is the ability to be adapted to many different species, from plants with small dossels like grass and greeneries to plants with large dossel as sugarcane and eucalyptus (ARAUS et al., 2018).

To accelerate crop breeding and evaluate diseases in the field using high throughput phenotyping, are associated high resolution images datasets, integration of spectrum sensors, and, automated data collection (SANKARAN et al., 2015). Base on these specifications, the dataset obtained can be used to estimate agricultural yield and, therefore, help and accelerate the breeder's decision on selection programs (ARAUS; CAIRNS, 2014; WALTER; LIEBISCH; HUND, 2015).

Moreover, enforcement of computational algorithms with machine learning to extract and analyze product data (SHAKOOR; LEE; MOCKLER, 2017). In parallel, software: FIELDImageR (MATIAS; CARAZA-HARTER; ENDELMAN, 2020), QGIS (QGIS, 2017), Smart Grain (TANABATA et al., 2012), PHENOME (GOGGIN; LORENCE; TOPP, 2015), Integrated Analysis Platform (IAP) (KLUKAS; CHEN; PAPE, 2014), WEKA (HALL et al., 2009); pipelines (AN et al., 2016; ARTZET et al., 2019; BAWEJA et al., 2018; HARTMANN et al., 2011; LEE et al., 2018; TIRADO et al., 2020), and devices are emerging to facilitate image analysis and making it tangible to beginners.

Direct comparison of remote sensing (RS) approaches, with unmanned aerial vehicles (UAV), for high throughput phenotyping in plant breeding has been studied, demonstrating applicability in measure agronomic traits (HAGHIGHATTALAB et al., 2016; TATTARIS; REYNOLDS; CHAPMAN, 2016; VERGARA-DÍAZ et al., 2016). Moreover, simple conventional cameras, that capture the visible colors red, green and blue, are capable to provide sufficient information for the vegetation index (VI) calculation, meaning that high throughput phenotyping in plant breeding can be done with standard RGB cameras (KHAN et al., 2018).

Thus, several researchers have been using RGB images as an alternative to high throughput phenotyping in plants, once the VIs derivatives can access the crop growth status (FERNANDEZ-GALLEGO et al., 2019; MARCIAL-PABLO et al., 2019; POSSOCH et al., 2016; SANCHES et al., 2018; SONG et al., 2017). A vegetation index represents a combination or a filtering process of multiple spectral data sets to create a single value for each point in an image, generated usually by a mathematical model and used to develop a scale color in the field map (COSTA; NUNES; AMPATZIDIS, 2020; MCKINNON; HOFF, 2017).

The red-green-blue- vegetation index RGBVI, proposed by (BENDIG et al., 2015) is used to monitor biomass in crops like grassland, wheat, and barley, being demonstrated great correlations among the vegetation data (BARETH et al., 2016; LUSSEM et al., 2018; POSSOCH et al., 2016; ROTH; STREIT, 2018). The green leaf index GLI (LOUHAICHI; BORMAN; JOHNSON, 2001) has very accuracy for vegetation extraction since it is correlated with the chlorophyll content (ZHANG et al., 2019b). Also, it has been used in promising studies to predict grain yield in rice (ZHOU et al., 2017), and has a great correlation with leaf area index (LAI) (LIU; WANG, 2018).

Another vegetation index commonly used in high throughput phenotyping is the visible atmosphere resistant index VARI for showing effectively in vegetation cover estimation and for access quality traits (ELSAYED et al., 2016; MORENO et al., 2018). Hunt et al. (2013), develop the triangular green index TGI based on the area of a triangle surrounding the spectral features of chlorophyll, aiming to correlated leaf chlorophyll content to nitrogen leaf content. Thus, the TGI index is used to monitor crop health and growth (BUCHAILLOT et al., 2019; DE OCAMPO; BANDALA; DADIOS, 2019; FUENTES-PEAILILLO et al., 2018a; FUENTES-PENAILILLO et al., 2018b; GIRÃO et al., 2020). The normalized green red difference index NGRDI (HUNT et al., 2005) has been used in remote sensing to predict grain yield, for weed detection, and also, has shown positive and significant correlation with

aboveground biomass (BARRERO; PERDOMO, 2018; ELAZAB et al., 2015, 2016; JANNOURA et al., 2015; MENG MENG et al., 2017).

Numerous and impactful researches have demonstrated the applicability of HTP in soybean breeding (BAEK et al., 2020; MAIMAITIJIAN et al., 2017; MILLER et al., 2018; XING; LIU; GAI, 2017; YU et al., 2016). Regarding works that associate RGB images and vegetation indices, Maimaitijiang et al. (2019), develop a vegetation index weighted canopy volume model for soybean biomass estimation from unmanned aerial system-based at low cost and high accuracy, the results demonstrated the great potential of the model as a single predictor for soybean aboveground biomass estimation. Bai et al. (2016), build a multi-sensor system for high throughput phenotyping in soybean and wheat, the results showed strong correlations among the sensor-based plant traits at the early and late growing season. Yun et al. (2016), observed that multi-temporal images obtained with a UAV and an RGB camera could be applied for quantifying overall vegetation fractions and crop growth status.

Thus, this study had the main goal to estimate vegetation indices and their correlation with agronomic traits in different soybean populations using RGB images derived from an unmanned aerial vehicle.

LITERATURE REVIEW

High throughput plant phenotyping

Plant phenotyping has been used since the early times when farms selected better plants for the next generation based on the traits observed. Posteriorly, with the contributions of Mendel's studies, it was possible to understand that phenotypes were based on genotypes. The genetic laws guided the breeders to improve the genetic gain and, consequently, result in better and new varieties transforming the world food production (BORNHOFEN, 2019; PRIYADARSHAN, 2019).

Although modern agriculture and applied biotechnologies have been changed the way to evaluate plants in the field, in general, the phenotyping process still manual and following the traditional plant breeding system. Traits of interest being measured with a ruler, in other words, it is simply, destructible and passive of human error (ARAUS et al., 2018). Some researchers affirm that the traditional phenotyping is biased by the "breeder eye", and for that reason, it is necessary to modernize the phenotyping, in a way that enables more accurate information, faster and in a non-destructive way (FAHLGREN; GEHAN; BAXTER, 2015; HARTMANN et al., 2011).

Thus, breeders programs are applying high throughput phenotyping of plants with the purpose to observe and study the traits of importance and, through this, withdraw relevant information to accelerate the selection program (GOGGIN; LORENCE; TOPP, 2015; HAGHIGHATTALAB et al., 2016). According to Araus et al. (2014), the traits in the field can be evaluated in different methodological approaches: (i) proximal remote sensing and imaging; (ii) laboratory analyses and samples; (iii) field-based high throughput phenotyping platform.

(i) Proximal remote sensing and imaging

Remote sensing phenotyping approaches are non-destructive methods based on the information provides by near-infrared radiation (NIR) reflected, which are the visible bands and can include hyperspectral, multispectral and convectional imagens; and far-infrared radiation (thermal) emitted by the plants. It can be applied to many breeding purposes, such as yield potential, adaptability to biotic and abiotic conditions, quality traits, and others (ARAUS et al., 2018).

To test the ability to accurately estimate grain yield in maize using spectral reflectance measurements before harvest, Weber et al. (2012), calculated coefficients of determination (R^2) between predicted and actual grain yield were highest for measurements conducted at anthesis and milk-grain stage. The results explain at maximum 23% and 40% of the genotypic variation in grain yield after validation, respectively.

A field-based high throughput phenotyping platform was developed by Andrade-Sanchez et al. (2014), to carrying out four sets of sensors to measure canopy height, sensitive normalized difference vegetation index, and temperature in cotton plants, irrigate under well water and water-limited contend. Is associated with the reduction of soil evaporation (MULLAN; REYNOLDS, 2010; REBETZKE et al., 2004) and weed competition (COLEMAN; GILL; REBETZKE, 2001; LEMERLE et al., 1996). Is primarily used for the assessment of early vigor, thus, is possible to identify plant establishment issues in the field trials. The results show large differentiation among the cultivars and high heritability in the broad between the traits evaluated, besides, was found strong agreement between the systems, the aerial images, and the manual phenotyping approaches. Therefore, the results confirm the accuracy and rapidity of the HTP platforms to measure multiple traits.

To improve pedigree and genomic prediction model accuracies, Rutkiski et al. (2016), measured wheat traits using high throughput phenotyping based on proximal or remote sensing. Was observed that secondary traits increased accuracies for grain yield by 56% in the pedigree, and 70% in genomic prediction models, on average. Therefore, this approach could improve selection in wheat during the early stages if validated in early-generation breeding plots.

(ii) Laboratory analyses and samples

In addition to remote, near-infrared spectrum captures the physical and chemical characteristics of the samples. Thus, laboratory analysis of line isotypes, protein, nitrogen, and oil content, gran texture, and grain weight of intact seeds using Near-infrared reflectance spectroscopy (NIRS) complements phenotyping under field conditions (HACISALIHOGU; LARBI; MARK SETTLES, 2010; MIR et al., 2012; MONTES; MELCHINGER; REIF, 2007; VON MOGEL, 2013).

Thus, Araus et al. (2013), measuring the abundance of the isotypes of carbon, nitrogen and, oxygen in ten durum wheat genotypes subject to different water and nitrogen

availabilities. The results showed that genotypes able to sustain a higher water use and stomatal conductance were the most productive and best adapted, thus, conclude that the physiological basis for the different performances of the three isotopes explaining the genotypic variability in yield.

Therefore, Hacisalihoglu et al. (2010), explored the potential of NIRS to determine individual seed composition in common beans. The traits collected were seed weight, protein, and starch from 91 diverse genotypes, regression models were applied and protein shows the most accurate partial least-squares regression model. Thus, this research demonstrated that NIR is a promising technique for the simultaneous sorting of multiple seed traits in single bean seeds with no sample preparation.

Another study conducted by Hacisalihoglu et al. (2016), tested the accuracy of transferring calibrations between different single seed NIR analyzers of the same design by collecting NIR spectra and analytical trait data for globally diverse soybean germplasm, knowing that single seed NIRS predicts soybean seed quality traits of moisture, oil, and protein. Models build from the data collected on one NIR analyzer had similar accuracy with the spectra collected from another sister device, demonstrating that it is possible to transfer calibrations between similar single seed NIR spectrometers. Therefore, this ability facilitates broader adoption of this high-throughput, nondestructive, seed phenotyping technology.

(iii) Field-based high throughput phenotyping platform

Field-based high throughput phenotyping platforms (HTPPs) are developed combining computational equipment, remote sensing, and high performance. HTPPs are modified vehicles set up with global navigation satellite system (GNSS) devices and sensors to measure canopy height, reflectance, and temperature simultaneously, at the plot level if ground-based or many plots, if an aerial platform. Several HTPPs are being built recently, aiming to adapt the platforms to the cultivar's characteristics and to capture all the important traits, thus, researchers are combining high technology, low cost, rapidly, and lightweight equipment to improve the platforms (ANDRADE-SANCHEZ et al., 2014; MONTES et al., 2011; REBETZKE et al., 2013; WEISS; BIBER, 2011).

A semi-automatic system developed by Comar et al. (2012), to monitor micro plots of wheat cultivars allowed a good description of the dynamics of each micro plots along the cycle and possibilities characterized by two periods, vegetative and senescence. The dynamics of the vegetation indices evaluated were described by bilinear models with its parameters

proving high throughput metric, and the variance analysis showed high heritability for the first period.

Furthermore, Barker et al. (2016), developed a field-based HTPP containing infrared thermometers, ultrasonic sensors, multispectral canopy sensors, and a crop sensing system to measure canopy temperature, crop height, and canopy spectral reflectance of a plant plot demonstrating highly accuracy to log sensors measurements and GNSS position information. This platform has been used to phenotyping wheat and soybean experimental fields and enabling genomic selection on a large scale.

Moreover, Mohd Assari et al. (2018), validated a data set of hyperspectral images of maize plant acquiring in an HTPP for early detection of drought stress. The results showed pre-processing reflectance spectra with the standard variation normalization method effectively reduces the variability due to the expected illumination effects. Besides, the proposed spectral analysis method successfully detected drought stress supporting the adoption of high throughput phenotyping platforms.

Despite all the quality and precision that HTP offers, it is necessary to control environmental conditions to increase the accuracy of performance measurements and the attribution of the phenotypic effects to individual traits and the underlying genetic composition. Some limiting factors that need be improved are minimizing the volume of the phenotypic data generated, hence the common computers have difficulty to manage them, and the correct use of bioinformatics to incorporate genotypic, phenotypic, and environmental data, concomitant, in the modeling analysis, beyond the usual statistics tool applied so far (BLUM, 2011; PIERUSCHKA; POORTER, 2012).

Nevertheless, field phenotyping of the important traits, using low cost, easy to handle tools, may become an integral and key component in the breeding pipeline give the advance and results that this omic science provides not just in the crop field, but also to the academic research (ARAUS; CAIRNS, 2014).

Phenotyping in soybean programs

Soybean breeding is challenging due to the number of traits that must be phenotyped or evaluated in each experimental season. For instance, traits correlated with yield as plant height, flower color, cycle, lodging, agronomic value, maturation, and the main biotic and

abiotic stresses. Adding to that, the experimental fields are composed of thousands of plots implying high demand for time, money, and professionals to phenotype (YANG et al., 2017).

To indicate potential resistant genotypes during the early stages and improved the intensity of selection, Xing et al. (2017), developed a dynamic and efficient high throughput phenotyping method to test antixenosis against common cutworm at the early seedling stage in soybean with damaged leaf percentage (DLP) as an indicator.

Another study due by Maimaitijang et al. (2017), evaluated the power of high spatial resolution RGB, multispectral, and thermal data fusion to estimate soybean biochemical parameters including chlorophyll content and nitrogen concentration, and biophysical parameters including Leaf Area Index (LAI), above-ground fresh and dry biomass. This research indicates that the fusion of low-cost multiple sensor data within a machine learning framework can provide a relatively accurate estimation of plant traits and valuable insight for high spatial precision in agriculture for plant stress assessment.

Furthermore, Yu et al. (2016), developed a dual-camera high throughput phenotyping platform (HTPP) on an unmanned aerial vehicle (UAV) and collected time course multispectral images for large scale soybean. The results showed that the multispectral data collected from the UAV-based HTP platform could improve yield estimation accuracy and maturity recording efficiency in a modern soybean breeding program. Therefore, it is remarkable the importance and necessity of phenotyping in soybean crops and the constant advances of high throughput phenotyping in the breeding programs.

Vegetation Index

A vegetation index (VI) is a spectral transformation metric for measuring the presence and state of vegetation based on the characteristic photosynthetic response of green vegetation to incident light, in other words, are quantitative measurements indicating the vigor of vegetation (BANNARI et al., 1995).

The presence of green vegetation is confirmed by the deviation of red and near-infrared reflectance. According to TUCKER et al. (1979), visible radiation in the red (630-690 nm) is absorbed by chlorophyll while radiation in the near-infrared (760-900 nm) is strongly reflected by leaf cellular structures.

For example, healthy plants exhibit high infrared reflectance and low red reflectance due to the absorption of red light by chlorophyll, resulting in a high index value. Conversely, unhealthy, stressed or dead vegetation, a manifestation of reduced chlorophyll pigment,

displays a low index value. Hence, the combination of these two spectral domains allows to differentiate vegetation from the soil and to determine photosynthetically active biomass through vegetative cover density (KHAN et al., 2018).

Vegetation indices can be used to detect biotic and abiotic stress, soil moisture measurements, quantify the effects of through on vegetations. Moreover, vegetation indices have demonstrated a correlation with several traits of crop performance including biomass, yield potential, and nutrient concentration. However, factors like soil bright, soil color, atmosphere, environment, solar geometry illumination, and sensor viewing conditions can affect the vegetation index effect (GRACIA-ROMERO et al., 2019).

Examples of the applicability can be found in Bajgain et al. (2015), when correlated vegetation indices with soil moisture measurements to assess the sensitivity of tallgrass prairie grasslands to drought. The indices allowed remote identification of drought-affected regions, concluding then, VI can be used to quantify the effects of drought on vegetation. Also, good estimates for dry matter forage yield on a grassland experiment in Germany were obtained by Lussem et al. (2018), when evaluated the robustness of RGB-based vegetation indices. Evidence that VI can be useful for monitoring forage yield throughout the growing season and, consequently, support decisions on intensely managed grasslands.

Moreover, genotypic variation in canopy greenness provides insights into the variation of nitrogen (N_2) and, consequently, the chlorophyll concentration. As explicit by Kaler et al. (2020), when estimated canopy by the Dark Green Color Index (DGCI) identifying significant loci associated with the intensity of greenness in a genome-wide association mapping. The putative loci that were coincident with previously reported genomic regions can be important resources for pyramiding favorable alleles, proving that vegetation indices allow access to genetic information.

According to Khan et al. (2018), RGB images derived from UAV contain sufficient information for vegetation indices estimation using deep neural networks, demonstrating that low-cost VI measurement is possible with standard RGB cameras. Rasmussen et al. (2016), demonstrated that VIs based on UAV imagery has the same ability to quantify crop responses to experimental treatments as ground-based recordings with cameras and advanced sensors. However, angular variation in reflectance (bidirectional reflectance), stitching, and ambient light fluctuations need to be taken into consideration to achieve accuracy.

Fuentes-Peailillo et al. (2018), compared NDVI captured with multispectral câmeras versus Four índices obtained from RGB câmeras to evaluate the capability of identification of

soil and vegetation in images captures with a UAV. The results showed that the TGI - Triangular Greenness Index showed the best behavior, however, the RGB indices present errors in identifying the vegetation in the zone of the rows, thus, is necessary to use more complex clustering techniques. The study of García-Martínez et al. (2020), using spectral information registered through remote sensors mounted on unmanned aerial vehicles, generated high correlation coefficients with TGI, VARI, and canopy cover in the corn grain yield estimation.

MATERIAL AND METHODS

Field experiment

This study was composed by three experiments (RILs-N, RILs-C, and LQ) sown in December, season 2018/2019, at the Experimental Station Farm of Genetics - Anhumas (“Luís de Queiroz” College of Agriculture – ESALQ), Piracicaba, São Paulo, Brazil (Lat.22°45' - 22°50' S Long. 48°00' - 48°05' W). The Anhumas district has Koeppen climatic classification: Cwa, an annual average temperature of 22.8 °C, annual average rainfall of 1290.7 mm, and oxisols and quartzarenic neosols.

The first two experiments were used to evaluate genetic resistance to the stink bug complex in soybean lineages (*Euschistus heros*, *Piezodorus guildinii*, and *Nezara viridula*). RILs – C (Controlled) experiment was applied pesticide (Engeo Pleno®S) in the pod stage formation and RILs-N (Non-controlled) is the experiment without insecticide applications for the control of stink bugs complex. Both experiments (RILs-N and RILs – C) consist of 258 recombinant inbred lines – RILs developed by the Diversity Genetics and Breeding Laboratory (ESALQ - Genetics Department), parents of RIL population (IAC-100 resistant to stink bugs complex, and CD 215 susceptible) and, two checks (AS 3730 e Produza). The alpha lattice design 10x26 with three replicates was used, totalizing 780 plots per experiment. The plot was composed of 4 lines of five meters, spaced with 0.5 m, and the two central lines were evaluated (useful plots). The alpha lattice design allows to capture of the sting bugs dispersion in the experiment and also attenuates the effects of plots not attacked by the insects.

The third experiment, LQ (“Luís de Queiroz” lineages), was sown in a field without soil correction and fertilization, and also, without application of fungicides and insecticides. The population was composed with 299 soybean lineages with variability for resistance to the stink bug complex and Asian rust (*Phakopsora pachyrhizi*), and five checks (IAC 100, CD 250, AS 3730, Produza, and NS 7300). Was used alpha lattice design 16x19 with 3 replicates. In total, LQ had 912 plots with two lines of 5 meters and spaced by 0,5 m, being evaluated the whole plot.

Thus, the aiming was to evaluate the resistance to the stink bug complex of the materials in all experiments. To capture information about the insect infestation was done beat cloths between the plots, from the flowering to the maturity, achieving an average of eighteen stinks bugs per square meter.

The agronomic traits were evaluated in all the experiments and evaluated according to the stages of crop development, as proposed by Fehr and Caviness (1977).

Agronomic traits:

1. Number of days to maturity (NDM): Number of days from sowing to 95% of the mature pods in 90% of the plot;
2. Agronomic value (AV): Evaluated on plant maturity based on a scale of visual notes from 1 to 5, being 1 for plants of low agronomic value and 5 for plants with excellent agronomic traits. (high number of pods, height over 70 cm, high vigor, without lodging, absence of leaf retention and green stems, pods without dehiscence, absence disease symptoms;
3. Lodging (LOD): Evaluated on plant maturity on a scale of visual notes from 1 to 5, being 5 for fully lodging plant and 1 for fully erect plants;
4. Plant height maturity (PHM): Mean of five plants in the useful plot, measure from the ground to the end of the stem in centimeters.
5. Grain yield (GY): Total mass of the seeds yielded in the plot, in kilograms per hectare ($\text{k} \cdot \text{ha}^{-1}$);

Data collect

The aerial image dataset was collected using the HTPP composed by the drone Mavic Pro (DJI Co., Ltd., Shenzhen, China) and its RGB (Red: 560-580 nm; Green: 530-545 nm; Blue: 420-440 nm) camera coupled (resolution 12, 71 M pixels; sensor 1/2.3''(CMOS); lens 78.8° 26 mm). The imaging survey was on 20th February, on a clear and cloudless day, during the R5 crop stage. One flight at 100 meters of height was performed in all experiments, around noon, and according to the flight recommendations. The drone's flight plan contained 60% lateral and 40% frontal overlapping. Fourteen ground control points (GCPs) were evenly distributed in and outside of the field boundaries and measured with a highly accurate RTK-GNSS (Topcon GR-3) position system with a precision of 2.5 cm.

Image processing

The image data was processing using the software Agisoft Metashape Professional (version 1.5.1, Agisoft LLC, St. Petersburg, Russia) to derive an orthomosaic of the

experimental area, with 30 mm spatial resolution. Settings and parameters applied to build the orthomosaic are available in Annex I. Figure 1 shows the field experiments layout placed in the orthomosaic.

Figure 1. Field experiments layout, “Luís de Queiroz” lineages (LQ), Recombinant Inbred Lines without stink bugs control (RIL-N), and Recombinant Inbred Lines with stink bugs control (RIL-C).

The orthomosaic was evaluated according to the pipeline provided by the R package *FIELDimageR* v 0.2.0 (MATIAS; CARAZA-HARTER; ENDELMAN, 2020) to calculate vegetation indices and canopy. The HUE index was used to build the mask to remove the soil effect between and within plots has demonstrated in Figure 2.

Figure 2. Removing soil effect using experiment RIL-C as an example

Then, the image was rotated for better visualization and the construction of the shapefile to delimit each plot in the field, according to the FIELDimager pipeline and exemplified by Figure 3.

Figure 3. Image rotation (left) and shapefile layer addition (right) using the RIL-C experiment as an example.

Next, the RGB based indices were averaged over each plot with the function “fieldIndex” from FIELDimager (v 0.2.0) (MATIAS; CARAZA-HARTER; ENDELMAN, 2020), on a pixels basis from the calibrated orthomosaic. In figure 4 is possible to visualize the variation of the indices values under the experiment. All equations to calculate the VI are available in Table 1.

The Red – Green – Blue Vegetation Index (RGBVI) proposed by Bendig et al. (2015), was used to evaluate chlorophyll alpha (420, 490, and 660 nm) and beta (435, 643 nm) absorption. This is the normalized difference between the squared green reflectance and the product of blue x red reflectance. Green Leaf Index (GLI) determines if the average of the red and blue pixels were greater than the average of green pixels. The resulting image had pixels between +1 and -1, where negative values tend to be soil while positive values tend to be green leaves (LOUHAICHI; BORMAN; JOHNSON, 2001).

Visible Atmospheric Resistant Index (VARI) predicts the vegetation fraction and leaf area index from remote sensing signals in later stage crops. The Triangular Greenness Index (TGI) is defined as the area of the triangle formed by the reflectance signals for red, green, and blue (MCKINNON; HOFF, 2017).

The Normalized Green Red Difference Index (NGRDI) was proposed by (TUCKER, 1979) is defined as the difference in digital numbers between the green and red bands, normalized by dividing by the sum of the bands (HUNT et al., 2005).

Table 1. Applied Vegetation indices. R = reflectance (%).

Index	Equation	Reference	Related Traits
RGBVI - Red – Green – Blue Vegetation Index	$\frac{(R_{green} \cdot R_{green}) - (R_{red} \cdot R_{blue})}{(R_{green} \cdot R_{green}) + (R_{red} \cdot R_{blue})}$	(Bending et al., 2015)	Absorption of chlorophyll Alpha and Beta
GLI - Green Leaf Index	$\frac{(2 \cdot R_{green} - R_{red} - R_{blue})}{(2 \cdot R_{green} + R_{red} + R_{blue})}$	(Louhachi et al., 2001)	Chlorophyll content
VARI - Visible Atmospheric Resistant Index	$\frac{R_{green} - R_{red}}{R_{green} + R_{red} - R_{blue}}$	(McKinnon et al., 2017)	Canopy, biomass, and chlorophyll
TGI - Triangular Greenness Index	$R_{green} - 0.39 \cdot R_{red} - 0.61 \cdot R_{blue}$	(Hunt et al., 2013)	Leaf chlorophyll content
NGRDI - Normalized Green Red Difference Index	$\frac{R_{green} - R_{red}}{R_{green} + R_{red}}$	(Tucker et al., 1975)	Chlorophyll, biomass, and water content

Figure 4. Graphic reports of the variation of the indices and yield values plotted under the RIL-C experiment.

Canopy cover is the area percentage of the area that the plants occupy in the plots, in other words, is the proportion of soil covered by the canopy cover (WALTER et al., 2019). Was calculated with an algorithm from the FIELDImageR package with the function “fieldArea” as exemplified by Figure 5. The same procedure was performed in all experiments.

Figure 5. Output canopy report graphic using the RIL-C experiment as an example.

Statistical analysis

Statistical analyses were performed using R (version 3.6.1) (R CORE COMPUTING TEAM, 2017). Linear mixed models were used to estimate the variance of the trait using the likelihood ratio test (LRT) with the package lme4 (BATES et al., 2020). RILs experiments were evaluated as two environments (with and without defensives), by the following statistical model:

$$Y_{ilkj} = \mu + G_i + A_j + R_k + B_l(R_k A_j) + \varepsilon_{ijkl} [1]$$

where y_{ilk} is the phenotypic effect/index effect of the genotype i in the block l in the repetition k ; μ is the overall mean; G_i is the i genotype random effect, with $G_i \sim N(0, \sigma_G^2)$; A_j is the j environment fixed effect; R_k is k the repetition fixed effect; $R_k(B_l)$ is the l block effect in the k repetition; ε_{ikl} is the random experimental error, with $\varepsilon_i \sim N(0, \sigma_\varepsilon^2)$. The broad-sense heritability of the genotypes for each trait was calculated based on the following equation, as proposed by Piepho and Möhring (2007).

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{n \text{ Env} \cdot n \text{ Rep}}}$$

where, σ_g^2 is the genetic variance; σ_e^2 is the residual variance; $n \text{ Env}$ is the number of environments that the experiments were evaluated; $n \text{ Rep}$ is the number of repetition in each experiment;

The variance components for LQ experiment were estimated using the following model:

$$y_{ilk} = \mu + G_i + R_k + B_l(R_k) + \varepsilon_{ikl} \quad [2]$$

where, y_{ilk} is the phenotypic effect/ index effect of the genotype i in the block l in the repetition k ; μ is the overall mean; G_i is the i genotype random effect, with $G_i \sim N(0, \sigma_G^2)$; R_k is k the repetition effect; $B_l(R_k)$ is the l block random effect within the k repetition; ε_{ikl} is the random experimental error, with $\varepsilon_i \sim N(0, \sigma_\varepsilon^2)$. The broad-sense heritability of the genotypes for each trait was calculated based on the following equation:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{n \text{ Rep}}}$$

where, σ_g^2 is the genetic variance; σ_e^2 is the residual variance; $n \text{ Rep}$ is the number of repetitions of the experiment.

To verify the multivariate pattern among genotypes the principal component analysis (PCA) was performed using the Best Linear Unbiased Predictions (BLUPs), followed by the construction of a biplot graph with the two first components. The PCA analysis was realized using software R with the support of the “factoextra” package (Kassamba et al. 2017). This plot was used to understand the influence of defensives on soybean RILs population agronomical performance. The correlations between traits were estimated using the BLUPs and Pearson Method, with significance through the Student's t-test.

RESULTS AND DISCUSSION

Recombinant inbred lines analysis

In Tables 2 and 3 are presented the estimates of genetics variance components by the restricted maximum likelihood (REML) test. There were significant effects of genotype and experimental condition for all the traits, as well as, genotype effect nested in each environment for most of the traits. This indicates that at least one genotype had differential performance among the experiment with and without stink bugs control. Significant soybean genotypes differentiation also was found by Matei et al. (2017) evaluating adaptability and stability parameters for modern cultivars in multi-environment trials, and by Kaler et al. (2020) in a genome-wide association study (GWAS) aiming to identify significant loci associated with the intensity of greenness of the soybean canopy determined by the Dark Green Color Index (DGCI).

The broad-sense heritability, considering the agronomic traits, ranged from 0.63 to 0.93 for LOD and NDM, respectively (Table 2). Observing the different environmental conditions, the heritability of the NDM trait was similar. In stress conditions, visual traits like agronomic value and lodging are easier to identify, therefore, presented greater differentiation by the breeder's eye, which justifies the high heritability in the experiments (VELLO; NAZATO, 2017).

Thus, lower results of heritability for these same traits were obtained by Rocha et al. (2015), being 0.6 for PHM, 0.74 for NDM, 0.3 for AV, and 0.2 for grain yield (GY), when evaluated soybean recombinant inbred lines with resistance alleles to stink bugs complex in two environments. Still, Santos et al. (2018), obtained 0.9 for PHM, 0.08 for LOD, 0.63 AV, 0.8 for NDM, and 0.7 for GY values of heritability when correlating agronomic traits with reduced stink bug damage in an $F_{2:3}$ soybean population.

In general, the average values for all traits in the joint analysis were intermediate between those observed in the control and experimental environments. The model [1] was modified to evaluate the genetic effect inside the environment (RILs-C and RILs-N) by removing the parameter A_j .

Concerning the average traits, the number of day to maturity (NDM) range to 105 to 107 days of the cycle in the experiments RIL-C and RIL-N, and with the high heritability is

possible to select early cycle genotypes. The experiment RIL-N obtained the highest average for the agronomic value trait, 3.14, even being a stressful environment. Also, this experiment has taller plants, 72.85 cm on average, which implies the averages of the AV, LOD, and GY traits, since taller plants produce more pods, and tend to lodge.

As expected, the heritability of the trait GY was greater in RILs-C than RILs-N, once the genotype can better explore its genetic potential in controlled environments (FUGANTI-PAGLIARINI et al., 2017). However, the overall average was 1,391.2 kg ha⁻¹ in the RIL-C experiment and 1,670.78 kg ha⁻¹ in the RIL-N experiment. Similar grain yield was found by Aslan (2015) in soybean genotypes experiments for cyst nematode, however, Rocha (2015) obtained superior grain yield evaluating the same lineages for stink bug complex resistance.

Table 2. Genotype fixed effect, broad-sense heritability (h^2), and the general average (μ) of agronomic traits: Number of days to maturity (NDM), agronomic value (AV), lodging (LOD), plant height maturity (PHM), and grain yield (GY).

Experiment	Parameters	NDM	AV	LOD	PHM	GY
Joint Analysis	Genotype	745.57***	262.05***	123.31***	390.87***	134.00***
	h^2	0.89	0.75	0.63	0.81	0.64
	μ	106.58	2.85	1.42	68.50	1,519.23
RIL-C	Genotype	361.54***	118.13***	35.933***	223.32***	102.00***
	h^2	0.93	0.82	0.65	0.89	0.80
	μ	105.22	2.56	1.26	64.14	1,391.20
RIL-N	Genotype	225.93***	100.68***	49.75***	140.62***	38.02***
	h^2	0.89	0.79	0.69	0.83	0.66
	μ	107.92	3.14	1.57	72.85	1,670.78

‘***’, ‘**’, ‘*’ Significant at the 0, 0.001 and 0.01 probability levels, respectively.

The stink bug complex [*Euschistus heros* (F.), *Piezodorus guildinii* (Westwood), *Nezara viridula* (L.), *Dichelops melacanthus* (Dallas), and *Edessa meditabunda* (F.)] is responsible for decrease the grain yield in soybean crops by infesting the field, generating seed damage, and leaf retention, since these insects manifest in the early stage formation of the pods. The stink bugs feed on the seeds that are forming inside the pods, causing unviable grains and, consequently, decreasing the yield production (CORRÊA-FERREIRA; DE AZEVEDO, 2002; CORRÊA-FERREIRA; PANIZZI, 1999; DEPIERI; PANIZZI, 2011). Therefore, it is important

to select genotypes productive, resistant to the stink bugs complex, and also adaptable and stable to different environments.

Thus, table 3 shows the five best genotypes in the joint analysis e for each experiment situation. The performance of the best genotypes for the agronomic traits was obtained from the Best linear Unbiased Prediction (BLUP). Thereby, the genotype T-201 was the more productive lineage and adapted well in both experimental conditions, obtained an average of 1967.43 kg ha⁻¹ yield. Furthermore, this also was the best genotype in the non controlled environment, RIL-C, producing 442.09 kg more than the experimental average. Although, in the controlled experiment, RIL-C, the genotype T-170 obtained the best performance, producing 2060.14 kg ha⁻¹, that is, 555.64 kg more than the local average.

Table 3. Performance of the best genotypes with averages obtained from the Best linear Unbiased Prediction (BLUP) for the traits number of days to maturity (NDM), agronomic value (AV), lodging (LOD), plant height maturity in centimeters (PHM), and grain yield in kg ha⁻¹ (GY).

Experiment	Genotypes	NDM	AV	LOD	PHM	GY
Joint Analysis	T-201	116.17	3.42	1.43	76.749	1967.43
	T-123	110.73	3.56	2.2	81.419	1940.48
	T-209	114.52	3	1.52	74.999	1922.38
	T-018	114.83	2.71	2.4	80.889	1873.29
	T-254	109.43	3.21	1.35	74.669	1868.61
RIL-C	T-170	109.94	3.41	1.44	81.16	2060.14
	T-172	105.6	3.21	1.32	73.57	2050.32
	T-130	111.36	3.86	1.51	84.6	2026.55
	T-244	106.18	3.63	1.55	79.48	2017.67
	T-201	116.83	3.65	1.32	81.05	2002.78
RIL-P	T-201	114.81	3.46	1.58	74.48	1967.43
	T-254	106.31	3.55	1.45	73.5	1940.48
	T-113	107.56	3.08	1.41	72.23	1922.38
	T-165	105.5	3.32	1.42	72.26	1873.29
	T-161	109.68	3.74	2.08	76.78	1868.61

Considering the vegetation indices, the broad-sense heritability, ranged from 0.16 to 0.70 for TGI and RGBVI indices, respectively, in the two environments (Table 4). Overall, the VIs showed high broad-sense heritability and the significant effects of genotype allow

concluding that exists genetic variation. Also, the average values of the indices were similar, since they vary to -1 and 1, except for the TGI index, whose value is a result product of the red, green, and blue bands. Thus, The RGBVI index showed a better average, indicating that is possible to capture information about chlorophyll absorption based on this vegetation index. In general, a higher average of the index values was expected, however, it is believed that the flight time of the UAV, R5, did not allow capturing a large variation between reflectance due to the intense and uniform green in the experiments (ZHANG et al., 2019b). The canopy cover is calculated by the sum of the green pixels in the plot and the average can be justified by the plant failures within the plots.

As the RIL-N experiment was a stressful environmental condition, the plants in that plots exhibited lower green band value, since the canopy percentage is measured by the green area of the plot, this trait showed low heritability in the broad sense, 0.38. On the other hand, the canopy in the RIL-C experiment had high and significant genetic variance, showing differences among the genotype lineages and reduced environmental effect.

Moderated to high broad-sense heritability was also found by Fernandez-Gallego et al. (2019) in vegetation indices from RGB images, under three durum wheat growth conditions: rainfed, support irrigation, and late planting. Furthermore, moderately high canopy cover and senescence broad-sense heritability ($h^2 < 0.5$) in maize hybrid evaluated under low soil nitrogen was obtained by Makanza et al. (2018). This demonstrates that capture images during the plant cycle stage, where the color difference is more visible, that is, making more flights throughout the cycle, can significantly increase the accuracy of the results.

Table 4. Genotype fixed effect, broad-sense heritability (h^2), and the general average (μ) of the vegetation indices and canopy.

Experiment	Parameters	RGBVI	GLI	VARI	TGI	NGRDI	Canopy
Joint Analysis	Genotype	39.33***	40.55***	60.54***	3.14*	55.00***	36.48***
	h^2	0.44	0.45	0.51	0.16	0.50	0.43
	μ	0.30	0.15	0.19	2234156.2	0.12	0.21
RIL-C	Genotype	47.94***	45.03***	31.63***	20.14***	32.66***	46.10***
	h^2	0.70	0.69	0.64	0.57	0.64	0.69
	μ	0.28	0.14	0.16	2659244	0.10	0.26
RIL-N	Genotype	22.62***	22.79***	20.87***	7.49**	20.72***	5.92*
	h^2	0.58	0.58	0.57	0.42	0.57	0.38
	μ	0.33	0.17	0.21	1809068.48	0.13	0.17

‘***’, ‘**’, ‘*’ Significant at the 0, 0.01, and 0,05 probability level.

In the principal Components Analysis – PCA (Figure 6) is possible to observe that some traits had greater influence than others to divide groups of experiments RIL-C and RIL-N. The direction of the traits arrows LOD, PHM, and GY indicated that the genotypes had greater agronomic performance in the controlled experiment. As the arrows of the AV and NDM traits are perpendiculars between the two experiments, it can be found good genotypes for these traits in both experiments. Nevertheless, by the directions of the arrows, some genotypes in the experimental condition demonstrated better performance for these traits.

The principal component analysis of the agronomic traits also showed that the trait yield is strongly correlated with plant height. Several authors have confirmed the correlation of these traits since, higher plants producing more pods, thus being more productive (MACHADO, 2017; NOGUEIRA, 2011; SOUSA, 2013; VIANNA et al., 2019). On the other hand, the PCA showed an angle greater 90° between NDM and LOD, proving independence among the traits. A lower correlation between the number of days to maturity and lodging was found in soybean lineages resistant to Asiatic rust (*Phakopsora pachyrhizi*) and to stink bug complex (MOREIRA, 2015; OLIVEIRA, 2019).

Figure 6. Biplot of the principal components analysis of recombinant inbred lines for the agronomic traits: lodging (LOG), grain yield (GY), plant height maturity (PHM), agronomic value (AV), and the number of days to maturity (NDM).

On the other hand, the principal component analysis for the vegetation indices and canopy (Figure 7) showed that the VARI, NGRDI, RGBVI, and GLI are positively correlated. The arrow position indicates independence and a negative correlation between TGI with the other indices this may be explained by the data of this study was collected. A low and negative coefficient correlation between the TGI index and remote sensing UAV RGB vegetation indices was also found by Buchaillot et al. (2019). Moreover, the PCA analyses showed that the canopy trait allowed differentiation among the experiments RIL-C and RIL-N.

Figure 7. Biplot of the principal components analysis of the recombinant inbred lines for the vegetation indices: Visible Atmosferich Resistencec Index (VARI), Normal Green Red Differentiation Index (NGRDI), Red Green Blue Vegetation Index (RGBVI), Green Leaf Index (GLI), Triangular Greenness index (TGI); and Canopy.

LQ experiment analysis

There are significant effects of genotype for the experiment in the unfertilized area for the majority of the agronomic traits evaluated, except for lodging (Table 5). The understanding of the relation between the traits is of valuable interest by the genetic breeding, mostly in traits difficult to measure and with low heritability, because the heritability is a parameter that indicates the proportion of phenotypic variability that is attributed to genetic causes, allowing to predict the possibility of gains with the selection (CRUZ; REGAZZI; CARNEIRO, 2012).

Thus, The broad-sense heritability ranged from 0.19 to 0.81 for the traits LOD and PHM, respectively. The high heritability value for the trait PHM allows select genotypes based on this phenotype. Although, lower heritability in the broad sense was found in two segregant populations of soybean by Shigihara (2018) for the same trait. The heritability for the trait NDM (68%) is important in breeding programs with the goal of early cultivars, thus, the average of days to plant maturity in this experiment was of 114 days. Similar results of heritability for this same trait was found in an F₂ population by Giaretta (2019).

Concerning the average of the agronomic traits, in this experiment the genotypes had 114.81 days average for the number of days to maturity, indicating that are sort cycle genotypes. The LQ lineages also presented low agronomic value comparing with the RILs experiments, this can be justified by the soil without fertilization and correction of the experiment field. In addition, the average plant height maturity trait was 50.23 cm, which also explains the lodging trait value, 1.04 on a 1 to 5 scale, once lower plants do not tend to lodge.

The grain yield production was 1,380.89 Kg ha⁻¹ in this experiment, however, the higher yield was obtained by Oliveira (2019) studying the same population. The results could be related to the field experimental condition in which the genotypes were submitted, in an area without soil correction, fertilization, and pest and disease control.

Table 5. Genotype fixed effect, broad-sense heritability (h^2), and the general average (μ) of agronomic traits: Numer of days to maturity (NDM), agronomic value (AV), lodging (LOD), plant height maturity (PHM), and grain yield (GY).

Experiment	Parameters	NDM	AV	LOD	PHM	GY
LQ	Genotype	52.534***	42.414***	1.26	130.16***	22.355***
	h^2	0.68	0.66	0.19	0.81	0.56
	μ	114.81	2.06	1.04	50.23	1,380.89

‘***’ Significant at the 0 probability level.

Table 6 shows the performance of the best genotypes of the LQ experiment with averages obtained with the Best Unbiased Linear Predictor (BLUP) for the agronomic traits. Thus, the lineage LQ-200 the more productive, with a grain yield of 1389.66 kg ha⁻¹, 199.76 kg more than the overall average.

Table 6. Performance of the best genotypes with averages obtained from the Best linear Unbiased Prediction (BLUP) for the traits number of days to maturity (NDM), agronomic value (AV), lodging (LOD), plant height maturity in centimeters (PHM), and grain yield in kg ha⁻¹ (GY).

Experiment	Genotypes	NDM	AV	LOD	PHM	GY
LQ-A	LQ-200	131.18	1.61	1.02	51.06	1389.66
	LQ-226	116.02	1.96	1.04	54.94	1369.21
	LQ-141	114.22	1.96	1.04	49.98	1364.84
	LQ-147	117.59	1.96	1.02	56.13	1360.36
	LQ-204	115.27	1.87	1.02	53.55	1351.85

The vegetation indices studied had significant genetic effects indicating genetic variation among the genotypes (Table 7). The (VI) broad sense heritability ranged from 0.30 to 0.59 for the VARI and GLI indices, respectively. Lower heritability values were found by Jenkins (2020) for the RGB bands in soybean genotypes at the R5 stage, however, better results are visible in the vegetative stage. According to Gitelson (2004), the green vegetation has strong absorption in the red band of the spectrum, thus, the reflectance is low (3 – 5%). Therefore, the epoch of the flight in the crop cycle is an important factor to consider when wishing to estimate vegetation indices.

Considering the canopy cover, the low heritability observed can be explained by the field experimental condition, resulting in equal levels of genetic and residual variation, however, still supports the potential to achieve genetic gain through selection on this trait (WALTER et al., 2019). Average canopy cover is a promissory parameter that enables indirect selection in the early stages of the breeding pipeline for breeding programs focused on yield improvement (XAVIER et al., 2017).

Table 7. Genotype fixed effect, broad-sense heritability (h^2), and the general average (μ) of the vegetation indices and canopy.

Experiment	Parameters	RGBVI	GLI	VARI	TGI	NGRDI	Canopy
	Genotype	24.298****	26.368****	3.35*	4.43**	7.788***	9.103***
LQ	h^2	0.58	0.59	0.30	0.33	0.41	0.43
	μ	0.3854	0.2023	0.3507	1358179	0.2094	0.6843

‘****’, ‘***’, ‘**’, ‘*’ Significant at the 0, 0.001, 0.01, and 0.05 probability level, respectively.

Traits correlation

In tables 8 and 9 are present the genetic correlation of the agronomic traits, vegetation indices, and canopy evaluated in the three experiments. In general, the estimates of genetic correlations followed a similar standard of magnitude and significance between the joint analysis, the RIL-C, RIL-N, and LQ experiments. Once the genetic correlations are inheritable, it can be used as the indirect selection in breeding programs (CRUZ; REGAZZI; CARNEIRO, 2012). In figure 7 is possible to visualize dispersion graphics of the traits correlation for all experiments.

Moderate to low significant correlations can be observed between the agronomic traits and the vegetation indices, according to Zhang et al. (2019a), the results accuracy can increase when the data is collected on the same day as the UAV flight. Moreover, the radiometric calibration of the UAV flight associated with many flights over the crop cycle increase the correlation among the traits and reduce the experimental error (MAIMAITIJIANG et al., 2020; YUN et al., 2016).

A high magnitude correlation was observed between the NGRDI and VARI indices and GY in the joint analysis indicating that can use the indirect selection in these traits for grain yield gain or be used to predict soybean yield. Similar results were found by Fernandez-Gallego et al. (2019), in an alternative to low-cost assessments to predict grain yield in durum wheat using RGB images at early crop stages. Thus, conventional cameras are a cost-efficient easy-to-use alternative to breeding programs and research institutions to use HTP with high accuracy.

In general, the TGI was less correlated with grain yield than the other RGB indices, which may be due to TGI’s lower sensitivity to green plant cover at the canopy scale in comparison to other

RGB vegetation, even though it is more sensitive than other vegetation indices to chlorophyll content at the leaf level (HUNT et al., 2014). Similar correlation results between the TGI index and GY were found by Fernandez-Gallego et al. (2019) when evaluated yield in durum wheat using RGB images. Also, the negative and significant correlation between the TGI index and the other parâmetros evaluated could be related to the plant's cycle stage, R5, even though various studies have been using TGI as an indicator of plant status due to its correlation with chlorophyll or N content (GITELSON; KAUFMAN; MERZLYAK, 1996; HUNT et al., 2013), this index could be related with other mineral nutrients (GARZA et al., 2020).

A good genetic correlation between traits is over that 0.7 (FALCONER; MACKAY, 1996), thus was found a high correlation between plant height maturity and agronomic value in all experiments. The agronomic value is by a note scalar based on the agronomic traits of the plot when the plants are in senescence, thus, taller and productive genotypes increase the AV note. A factor that negatively contributes to the note AV is the period that the plants stay in the field, that is, the number of days the plant cycle lasts, since plants with long cycles are more subject to biotic and abiotic stress. The correlation between PHM and AV was 0.87, 0.87, 0.75, and 0.85 in the joint analysis, RIL-C, RIL-N, and LQ experiment, respectively. However, a lower correlation between AV and PHM, 0.14, was found by Oliveira (2019) evaluating the same lineages of the LQ experiment.

Concerning the trait grain yield, was found a significant correlation for the agronomic traits evaluated, except for LOD in the LQ experiment. Because it is a complex trait, which is influenced by many genes, it was not found correlations in great magnitude, indicating the joint selection for all the traits for the selection of the best lineages in these populations. The positive and significant correlation between PHM and GY is because taller plants tend to produce more pods, increasing, thus, the yield (LEITE et al., 2015; TARTAGLIA et al., 2015). Also, plants with short cycles staying less time in the field, being less exposed to abiotic and biotic factors that increase the grain quality and, consequently, the yield (RODRIGUES et al., 2015).

Was found significant correlations between yield and GLI index, 0.51, 0.55, 0.26, and 0.57 for the joint analysis, RIL-C, RIL-N, and LQ experiments, respectively. Similar correlations between the GLI vegetation index and yield were obtained by Zhou et al. (2017) at different growth stages in rice.

The correlations among the vegetation indices and with canopy are high because it is used the same bands, red, green, blue, and also by being correlated to the same traits, like chlorophyll and biomass.

Strong and significant correlations between the canopy and the vegetation indices can be observed in the experiments, differing from the results of Travlos et al. (2017) in weedy vetch with data collected 126 days after sowing. However, a significant relationship between NGRDI and aboveground biomass was observed by Jannoura et al. (2015) although, the correlation was not strong, which indicated that NGRDI vegetation index may be sensitive to biomass before canopy closure. Similar results for correlation of canopy and VARI and GLI indices were also found by Maimaitijian et al. (2019) in soybean aboveground biomass. In this work, it was realized tree flights during the crop cycle with a UAV system based in RGB images, which increases the accuracy of the results, thus, it is suggested, many flights during the season.

Moderate to high significant correlations between canopy cover and grain yield was found in the experiment demonstrating that the secondary trait average canopy coverage measuring using the HTP platform can be used for selection in early stages of soybean breeding pipelines, alone or in combination with grain yield (MOREIRA et al., 2019). Similar results were found by Makanza et al. (2018), a significative genetic correlation of 0.6 between canopy features and grain yield in maize senescence.

Table 8. Genetic correlation estimates between agronomic traits, vegetation indices, and canopy of the RILs experiment. Number of days to maturity (NDM), agronomic value (AV), lodging (LOD), plant height maturity (PHM), grain yield (GY), red-green-blue vegetation index (RGBVI), green leaf index (GLI), visible atmospheric resistant index (VARI), triangular greenness index (TGI), normalized green-red difference index (NGRDI), and Canopy.

Joint Analysis RIL-C and RIL-N										
	AV	LOD	PHM	GY	RGBVI	GLI	VARI	TGI	NGRDI	Canopy
NDM	0.57***	0.17*	0.54***	0.39***	0.40***	0.41***	0.42***	-0.25***	0.43***	0.25***
AV		0.22**	0.87***	0.59***	0.52***	0.53***	0.59***	-0.44***	0.58***	0.47***
LOD			0.54***	0.17*	0.19*	0.19*	0.17*	-0.18*	0.18*	0.08
PHM				0.54***	0.51***	0.51***	0.55***	-0.43***	0.54***	0.41***
GY					0.57***	0.58***	0.72***	-0.51***	0.70***	0.63***
RGBVI						1***	0.88***	-0.84***	0.93***	0.65***
GLI							0.90***	-0.84***	0.94***	0.65***
VARI								-0.83***	0.99***	0.72***
TGI									-0.84***	-0.54***
NGRDI										0.72***
RIL-C										
	AV	LOD	PHM	GY	RGBVI	GLI	VARI	TGI	NGRDI	Canopy
NDM	0.44***	0.24***	0.44***	0.42***	0.33***	0.32***	0.29***	-0.20***	0.30***	0.18*
AV		0.38***	0.87***	0.64***	0.55***	0.55***	0.57***	-0.45***	0.57***	0.46***
LOD			0.60***	0.29***	0.30***	0.30***	0.34***	-0.25***	0.33***	0.15*
PHM				0.64***	0.55***	0.55***	0.57***	-0.44***	0.57***	0.42***
GY					0.68***	0.69***	0.78***	-0.61***	0.77***	0.68***
RGBVI						1***	0.89***	-0.95***	0.93***	0.76***
GLI							0.91***	-0.87***	0.94***	0.76***
VARI								-0.85***	1***	0.77***
TGI									-0.86***	-0.71***
NGRDI										0.78***
RIL-N										
	AV	LOD	PHM	GY	RGBVI	GLI	VARI	TGI	NGRDI	Canopy
NDM	0.56***	0.05	0.50***	0.13*	0.34***	0.35***	0.36***	-0.21**	0.37***	0.16*
AV		-0.02	0.75***	0.37***	0.36***	0.37***	0.45***	-0.28***	0.44***	0.32***
LOD			0.44*	0.12**	0.12*	0.12*	0.09	-0.19*	0.10	0.14*
PHM				0.34***	0.34***	0.35***	0.40***	-0.31***	0.39***	0.33***
GY					0.25***	0.26***	0.42***	-0.23**	0.39***	0.33***
RGBVI						1***	0.87***	-0.80***	0.92***	0.54***
GLI							0.88***	-0.80***	0.93***	0.55***
VARI								-0.78***	0.99***	0.58***
TGI									-0.79***	-0.50***
NGRDI										0.58***

‘***’, ‘**’, ‘*’ Significant at the 0, 0.01, and 0.05 probability levels, respectively.

Table 9. Genetic correlation estimates between agronomic traits, vegetation indices, and canopy in soybean lineages: Number of days to maturity (NDM), agronomic value (AV), lodging (LOD), plant height maturity (PHM), grain yield (GY), red-green-blue vegetation index (RGBVI), green leaf index (GLI), visible atmospheric resistant index (VARI), triangular greenness index (TGI), normalized green-red difference index (NGRDI), and Canopy.

LQ										
	AV	LOD	PHM	GY	RGBVI	GLI	VARI	TGI	NGRDI	Canopy
NDM	0.3***	0.17*	0.27***	0.11*	0.22*	0.22*	0.15*	-0.07	0.18*	0.19*
AV		0.33***	0.85***	0.33***	0.45***	0.45***	0.31***	-0.21*	0.38***	0.37***
LOD			0.39*	0.01	0.17*	0.18*	0.13*	-0.03	0.16*	0.07
PHM				0.29***	0.49***	0.5***	0.36***	-0.28***	0.43***	0.42***
GY					0.56***	0.57***	0.59***	-0.55***	0.6***	0.6***
RGBVI						1	0.89***	-0.83***	0.96***	0.80***
GLI							0.89***	-0.82***	0.96***	0.8***
VARI								-0.87***	0.98***	0.79***
TGI									-0.87***	-0.79***
NGRDI										0.81***

***, * Significant at the 0 and 0.05 probability levels, respectively.

Figure 8. Dispersion graphics of the traits correlation for the RIL-C, RIL-N, and LQ experiment, respectively.

CONCLUSION

This research allowed evaluated vegetation indices in the soybean crop. There were significant correlations among the agronomic traits, vegetation indices, and canopy, that can be used in the selection of the best lineages in the breeding pipeline. However, these are preliminary studies to build competence and understand the high throughput phenotyping tools available to apply in plant breeding programs. Furthermore, it is suggested to apply more flights during the crop cycle and collect traits associated with the time of flight to increase the accuracy of the results. For future studies, can be used the canopy trait as a covariable in the yield prediction model.

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Annex

Agisoft Metashape

Processing

Report 13

January

2020

Survey Data

Fig. 1. Camera locations and image overlap.

Number of images:	107	Camera stations:	107
Flying altitude:	106 m	Tie points:	102,753
Ground resolution:	3.24 cm/pix	Projections:	258,287
Coverage area:	0.15 km ²	Reprojection error:	0.586 pix

Camera Model	Resolution	Focal Length	Pixel Size	Precalibrated
FC220 (4.73mm)	4000 x 3000	4.73 mm	1.57 x 1.57 μm	No

Table 1. Cameras.

Camera Calibration

FC220 (4.73mm)

107 images

Type	Resolution	Focal Length	Pixel Size
Frame	4000 x 3000	4.73 mm	1.57 x 1.57 μm

	Value	Error	F	Cx	Cy	K1	K2	K3	P1	P2
B1	-4.65207									
B2	-4.88722									
K4	-0.691542									
F	2983.23	2.1	1.00	-0.16	-0.25	-0.76	0.79	-0.81	-0.02	0.02
Cx	-21.3789	0.67		1.00	0.16	0.06	-0.04	0.02	0.17	0.01
Cy	-27.8426	0.51			1.00	0.18	-0.19	0.19	0.04	0.22
K1	0.0892626	0.00023				1.00	-0.98	0.96	0.05	-0.02
K2	-0.425462	0.00089					1.00	-0.99	-0.01	0.02
K3	0.915968	0.001						1.00	-0.01	-0.02
P1	0.000455705	7.7e-006							1.00	-0.17
P2	0.000187681	1.4e-005								1.00

Table 2. Calibration coefficients and correlation matrix.

Ground Control Points

Fig. 3. GCP locations and error estimates.

Z error is represented by ellipse color. X,Y errors are represented by ellipse shape.

Estimated GCP locations are marked with a dot or crossing.

Count	X error (cm)	Y error (cm)	Z error (cm)	XY error (cm)	Total (cm)
14	5.27362	6.31478	8.93612	8.22724	12.1467

Table 3. Control points RMSE.

X - Easting, Y - Northing, Z - Altitude.

Count	X error (cm)	Y error (cm)	Z error (cm)	XY error (cm)	Total (cm)
2	2.90914	2.71605	2.72827	3.97995	4.82529

Table 4. Check points RMSE.

X - Easting, Y - Northing, Z - Altitude.

Label	X error (cm)	Y error (cm)	Z error (cm)	Total (cm)	Image (pix)
1	-1.36109	-4.6979	0.160106	4.89371	0.330 (7)
2A	3.70087	2.95401	-0.836131	4.80851	0.203 (9)
HD	-2.09039	-7.65374	-12.2372	14.5842	0.853 (9)
HD1	7.99017	-2.95769	0.0448347	8.52014	0.919 (9)
HD2	-3.77049	-1.40609	-3.45702	5.30516	0.835 (10)
HD3	3.12074	3.91855	-2.49554	5.59659	0.999 (8)
HD4	5.26492	-1.31496	-0.0780571	5.42721	0.952 (9)
9A	4.09426	-14.6031	8.83371	17.5513	0.600 (7)
10A	2.82274	9.07654	-14.3068	17.1766	0.607 (6)
11	-0.59964	6.02489	6.3848	8.79913	0.511 (9)
12	-2.54375	8.91632	9.72755	13.4386	0.553 (8)
13A	4.25407	3.81659	-14.9299	15.9864	0.548 (6)
14	-6.08458	-2.52927	16.208	17.4962	0.382 (7)
15	-12.932	-2.94749	6.7519	14.8833	0.810 (9)
Total	5.27362	6.31478	8.93612	12.1467	0.708

Table 5. Control points.

X - Easting, Y - Northing, Z - Altitude.

Label	X error (cm)	Y error (cm)	Z error (cm)	Total (cm)	Image (pix)
3	-0.279812	3.32287	0.14044	3.33759	0.117 (4)
4	-4.10462	-1.92675	3.8558	5.95209	0.065 (2)
Total	2.90914	2.71605	2.72827	4.82529	0.102

Table 6. Check points.

X - Easting, Y - Northing, Z - Altitude.

Digital Elevation Model

Fig. 4. Reconstructed digital elevation model.

Resolution: 6.41 cm/pix
Point density: 244 points/m²

Processing Parameters

General

Cameras	107
Aligned cameras	107
Markers	16
Coordinate system	WGS 84 / UTM zone 22S (EPSG::32722)
Rotation angles	Yaw, Pitch, Roll

Point Cloud

Points	102,753 of 389,702
RMS reprojection error	0.233316 (0.58605 pix)
Max reprojection error	4.98622 (10.6991 pix)
Mean key point size	2.58946 pix
Point colors	3 bands, uint8
Key points	No
Average tie point multiplicity	2.82777

Alignment parameters

Accuracy	Highest
Generic preselection	Yes
Reference preselection	Yes
Key point limit	100,000
Tie point limit	10,000
Adaptive camera model fitting	Yes
Matching time	7 minutes 3 seconds
Alignment time	56 seconds

Optimization parameters

Parameters	f, cx, cy, k1-k3, p1, p2
Adaptive camera model fitting	No
Optimization time	1 seconds

Depth Maps

Count	107
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Reconstruction parameters

Quality	High
Filtering mode	Moderate
Processing time	30 minutes 45 seconds

Dense Point Cloud

Points	59,398,586
Point colors	3 bands, uint8

Reconstruction parameters

Quality	High
Depth filtering	Moderate
Depth maps generation time	30 minutes 45 seconds
Dense cloud generation time	12 minutes 7 seconds

DEM

Size	20,221 x 26,708
Coordinate system	WGS 84 (EPSG::4326)

Reconstruction parameters

Source data	Dense cloud
Interpolation	Enabled
Processing time	58 seconds

Orthomosaic

Size	27,485 x 35,271
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General

Coordinate system	WGS 84 (EPSG::4326)
Colors	3 bands, uint8

Reconstruction parameters

Blending mode	Mosaic
Surface	DEM
Enable hole filling	Yes
Processing time	2 minutes 34 seconds

Software

Version	1.5.1 build 7618
Platform	Windows 64