



Article

High-Throughput Plant Phenotyping (HTPP) in Resource-Constrained Research Programs: A Working Example in Ghana

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Abstract: In this paper, we present a procedure for implementing field-based high-throughput plant phenotyping (HTPP) that can be used in resource-constrained research programs. The procedure relies on opensource tools with the only expensive item being one-off purchase of a drone. It includes acquiring images of the field of interest, stitching the images to get the entire field in one image, calculating and extracting the vegetation indices of the individual plots, and analyzing the extracted indices according to the experimental design. Two populations of groundnut genotypes with different maturities were evaluated for their reaction to early and late leaf spot (ELS, LLS) diseases under field conditions in 2020 and 2021. Each population was made up of 12 genotypes in 2020 and 18 genotypes in 2021. Evaluation of the genotypes was done in four locations in each year. We observed a strong correlation between the vegetation indices and the area under the disease progress curve (AUDPC) for ELS and LLS. However, the strength and direction of the correlation depended upon the time of disease onset, level of tolerance among the genotypes and the physiological traits the vegetation indices were associated with. In 2020, when the disease was observed to have set in late in medium duration population, at the beginning of the seed stage (R5), normalized green-red difference index (NGRDI) and variable atmospheric resistance index (VARI) derived at the beginning pod stage (R3) had a positive relationship with the AUDPC for ELS, and LLS. On the other hand, NGRDI and VARI derived from images taken at R5, and physiological maturity (R7) had negative relationships with AUDPC for ELS, and LLS. In 2021, when the disease was observed to have set in early (at R3) also in medium duration population, a negative relationship was observed between NGRDI and VARI and AUDPC for ELS and LLS, respectively. We found consistently negative relationships of NGRDI and VARI with AUDPC for ELS and LLS, respectively, within the short duration population in both years. Canopy cover (CaC), green area (GA), and greener area (GGA) only showed negative relationships with AUDPC for ELS and LLS when the disease caused yellowing and defoliation. The rankings of some genotypes changed for NGRDI, VARI, CaC, GA, GGA, and crop senescence index (CSI) when lesions caused by the infections of ELS and LLS became severe, although that did not affect groupings of genotypes when analyzed with principal component analysis. Notwithstanding, genotypes that consistently performed well across various reproductive stages with respect to the vegetation indices constituted the top performers when ELS, LLS, haulm, and pod yields were jointly considered.

Keywords: field-based high-throughput plant phenotyping; resource-constrained; groundnut; early leaf spot; late leaf spot; canopy cover; green area (GA); greener area (GGA); normalized green-red difference index (NGRDI); crop senescence index (CSI)



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1. Introduction

1.1. Background

Research and development (R&D) in developing countries is characterized by low funding. In most of the 15 countries that form the Economic Community of West African States (ECOWAS) sub-region, the 1% gross domestic product (GDP) allocation target set for research funding [1] has not been achieved [2]. As a result, most of the funding for R&D as well as higher education training come from foreign governments and other donor agencies [2]. These developing countries, ironically, have the highest population of resource-poor farmers with limited capability to guard against unfavorable environmental conditions such as drought, pest and diseases. Indeed, the developing countries are predicted to be the most affected by the adverse effects of climate change [3].

To help the resource-poor farmers of Sub-Saharan Africa, plant breeders need to develop cultivars for this region with novel traits that withstand increased drought, pests and disease outbreaks and with yield advantage over the existing cultivars. However, crop breeding in African countries for tolerance to stresses such as drought is largely a ‘numbers game’ that relies on field-based evaluation of thousands of progenies [4]. Currently, there is a pressing need to accurately measure an increasingly large number of plants and plant traits with the goal of providing realistic qualitative and quantitative analyses of plants and plant traits that can help crops better adapt to low-input agriculture and resource-limited environments [5]. Plant breeders of the Sub-Saharan sub-region, therefore, need to adopt innovative strategies to accurately screen large number of progenies, amid low funding and random environmental conditions.

1.2. The Importance of Groundnut and Its Yield Reduction Factors in Ghana

As in many other tropical and semi-arid tropical countries, in Ghana, groundnut is one of the most important leguminous crops, cultivated by smallholder farmers for its edible oil and protein [6,7]. In addition, groundnut can enhance soil fertility by fixing up to 108 kg nitrogen ha⁻¹ [8]. Despite its importance, the yield gap between potential and realized pod yield on farmers’ fields is high. This has been attributed mainly to various abiotic and biotic stresses including drought and temperature extremes [7,9] and leaf spot diseases [10]. These yield-reducing factors have resulted in the ongoing development of improved cultivars by integrating breeding with physiological trait modeling [11], selection of leaf spot tolerance-related traits [10] and aggressive multi-location testing [8,12]. However, progress has been slow due to the length of time required to develop a new groundnut cultivar. For instance, at the Council for Scientific and Industrial Research Institute-Savanna Agricultural Research Institute (CSIR-SARI) in northern Ghana, it takes up to eight years to reach F₈ generation lines because of the unimodal rainfall weather pattern. However, off-season generation advancements can be achieved to allow two cycles per year and reduce the breeding period by half. In this way, two new peanut cultivars were released in 2018 (SARINUT 1 and 2) [13].

1.3. The Scenario of Field Crop Research in Ghana

Traditionally, plant breeders who conduct designed experiments on field crops to measure phenological parameters measure only a small number of plants to represent the entire plot. For instance, a typical groundnut experiment with an objective to evaluate advanced breeding lines uses a plot size of 6.4–8 m². This gives about 160 plants per plot [10,12] out of which about five plants (3% of the expected plant population) are randomly selected each time agronomic data are to be collected. The selection of just five plants out of the lot in a plot has been due to the laborious nature of manual measurement of plant traits which necessitates the careful selection of a predetermined number of plants that must be representative enough in each plot.

Aside from the reduced number of plants, the current measurement protocols are subjective and prone to human error. For instance, the scoring of early and late leaf spot (ELS and LLS) disease severity uses a nine-point scale [10,12,14–16], where one is

complete resistance and nine is complete defoliation/death [17]. The scale assumes that the groundnut leaf spot diseases develop from the base of the plant and progresses towards the terminal bud, with defoliation of basal leaves of infected plants visible at a severity score of four when the topmost leaves are still without lesions. However, groundnut genotypes with lesions from ELS and/or LLS on lower and topmost leaves and yet having all leaves intact have been observed on the field. This usually causes subjectivity in the scoring process making the severity rating of these genotypes dependent upon whom does the assessment rather than the true susceptibility of the genotype. Although this approach has been successfully employed for the development of new groundnut cultivars, it is important that more efficient phenotyping approaches are deployed to improve the precision of data collection in the future.

1.4. Why Practise High-Throughput Plant Phenotyping in Ghana?

High-throughput crop genotyping and other molecular techniques were meant to ensure the accuracy required in the assessment of traits on large numbers of plants within a short time frame. It is facilitated by advances in next-generation sequencing and genotyping technologies enabling the generation of large-scale genomic data and subsequent resources such as molecular markers [18]. As a result, the last two decades have witnessed the deployment of molecular breeding approaches to complement field phenotyping programs. The potential advantages of molecular breeding techniques include the possibility to target multiple traits for improvement, and the ability to tap new alleles from wild related species. After mapping a number of economically important traits, several success stories of high-throughput crop genotyping, including translational genomics, have become available in many crops including groundnut [19–21]. Among the success stories of molecular techniques is the development of high oleate groundnut to enhance health benefits and shelf-life using marker-assisted selection and backcrossing [19], and development of varieties possessing resistance to root-knot nematode [22,23].

However, since results from high-throughput crop genotyping usually require validation through field evaluation, effective and objective phenotyping protocols are needed to ensure that true and promising genotypes are identified during field validation. Hence, for field phenotyping to better augment molecular techniques, high-throughput plant phenotyping (HTPP) has become a promising alternative to the manual procedures during field evaluation. HTPP is the simultaneous production of many data points per observation for a particular trait of interest [24,25] and for multiple plots at the same time. It offers a non-invasive approach to data collection on several genotypes within a short period, and permits repeated measures on the same sample, giving breeders the opportunity to classically track processes (such as disease development) over time [24]. The extreme ends of HTPP are robotic phenotyping platforms (where single plants grow exclusively under artificial conditions) and satellite imagery [26]. Between these two extremes are various gadgets including handheld tools (e.g., SPAD meter, Green Seeker), mobile phenotyping platforms and unmanned aerial vehicles (e.g., light drones) [27–31]. The use of such technologies can improve the precision associated with data collection, leading to high heritability estimates and eventually increased genetic gains or response to selection.

The CSIR-SARI Groundnut Improvement Program currently uses handheld sensors to provide speed and efficiency in data collection. Although their use has led to the discovery of key physiological traits associated with ELS and LLS tolerance [10,32,33] when working with smaller population sizes, the errors associated with their use in larger populations can be remarkable. For instance, an attempt to use a handheld RGB camera during the field phenotyping of the African core collection of 300 genotypes in the 2020 and 2021 growing seasons required nine hours (7 a.m. to 4 p.m.) on one replicate [33]. This, therefore, calls for alternative methods that could augment existing technologies for enhanced field phenotyping. In this paper, we (i) present a procedure for applying drone-based photogrammetry that largely relies on open-source resources as an HTPP method to determine the stage at which the vegetation indices strongly associate with ELS

and LLS diseases' severity in groundnut, and (ii) characterize the reaction of advanced groundnut breeding genotypes to ELS and LLS diseases as well as pod yield as affected by the environment.

2. Materials and Methods

2.1. Study Area, Characteristics of Genotypes and Field Design

The study was conducted at four experimental sites, Nyankpala, Silbele, Wa, and Manga of CSIR-SARI in 2020 and 2021 under rainfed conditions. Three of the sites (Nyankpala, Silbele, and Wa) fall within the Guinea Savanna zone whereas Manga falls within the Sudan Savanna agro-ecological zone. Both agro-ecologies have a unimodal rainfall with annual rains ranging between 900 and 1200 mm. These sites, which are all located in the northern part of Ghana, were recently reported to constitute a complex mega-environment for groundnut [12]. Due to the complex nature of the environment, results from a single site's study in one year may not be representative enough for the entire environment. As a result, testing advanced genotypes ready to undergo on-farm trials in all the locations across several years as part of a multilocation evaluation is necessary to identify good candidates.

Two sets of populations that are distinct with respect to days to maturity totaling 24 genotypes were used in 2020 (Table 1). The populations were part of two breeding populations' evaluation performed at CSIR-SARI. One of the populations has genotypes that mature in 105–120 days after planting (DAP) (medium duration) whereas the other population has genotypes that mature in 90–105 DAP (short duration) in northern Ghana. The medium and short duration populations require at least 1767 and 1521 °C cumulative degree days [11] in northern Ghana to reach physiological maturity, respectively. In 2021, six additional genotypes with similar maturity classification were added to each population tested in 2020, raising the total number of genotypes tested under each set to 18.

Table 1. List of genotypes used and the years they appeared in the experiments.

Medium Duration (MD)			Short Duration (SD)		
No.	Genotype	Years	No.	Genotype	Years
1	ICGV 176044	2020; 2021	1	ICGV 176217	2020; 2021
2	ICGV 176107	2020; 2021	2	ICGV 176166	2020; 2021
3	ICGV 176084	2020; 2021	3	ICGV 176222	2020; 2021
4	ICGV 176067	2020; 2021	4	ICGV 176160	2020; 2021
5	ICGV 176129	2020; 2021	5	ICGV 176214	2020; 2021
6	ICGV 176073	2020; 2021	6	ICGV 176151	2020; 2021
7	ICGV 176033	2020; 2021	7	ICGV 176023	2020; 2021
8	ICGV 176225	2020; 2021	8	ICGV 176156	2020; 2021
9	ICGV 176124	2020; 2021	9	ICGV 176019	2020; 2021
10	ICGV 176203	2020; 2021	10	ICGV 176049	2020; 2021
11	NKATIESARI	2020; 2021	11	CHINESE	2020; 2021
12	GAF 1723	2020; 2021	12	ICGV-IS 08837	2020; 2021
13	GAF 1665	2021	13	ICGV 15403	2021
14	ICGV 176051	2021	14	ICGV 176004	2021
15	ICGV 176112	2021	15	ICGV 176010	2021
16	ICGV 176124b	2021	16	ICGV 176053	2021
17	ICGV 176192	2021	17	ICGV 176154	2021
18	ICGV-SM 10523	2021	18	YENYAWOSO	2021

At each of the study sites in each year, experiments involving the individual populations were laid side-by-side. In 2020 the experiments were laid out in a lattice design with four sub-blocks per replicate. In 2021, a row-column design was used. Three replications per experiment were used in both years. Each plot consisted of eight rows of 2 m length. The intra- and inter-row planting distances were 0.1 and 0.4 m, respectively. Planting was done on relatively flat land after ploughing and harrowing. Fertilizer in the form of NPK with sulfur, zinc and boron (11:22:21 + 5 S + 0.7 Zn + 0.5 B) was applied 14 days after

planting at the rate of 20 N, 40 P, 40 K, 10 S, 1.4 Zn and 1 B all in kilogram per hectare. Weeds were controlled by uprooting at the ground level using a hand hoe. The study sites are known hotspots for ELS and LLS, hence leaf spot infection was allowed to occur naturally.

2.2. Conventional (Manual) Data Collection

In northern Ghana, ELS and LLS diseases occur jointly on the field. Scoring of ELS and LLS disease severity among the genotypes was done using the nine-point scale as described earlier [10,17]. The ELS lesions were distinguished from the LLS lesions using the yellow hallow around the ELS. The scoring was therefore done at R5 and R7 which represent the beginning of kernel development and maturity, respectively [34]. During scoring at each stage, each of the individual plots were assessed and given a single score. Quantitative variables were derived from the severity scores using the area under disease progress curve (AUDPC) as reported in [10] to overcome the limitation posed by the nominal disease scores. Plant productivity in the form of haulm yield (HYLD, kg ha⁻¹) and pod yield (PYLD kg ha⁻¹) were also determined at maturity. The conventional data were recorded electronically using Field Book [35].

2.3. HTPP (Drone-Based) Data Capture

2.3.1. Tools Used

The hardware and software tools used in this study are listed in Table 2. Technically, the highest investment required was the acquisition of a drone. All software tools used were open source. Unlike the conventional data, the HTPP data were recorded only in Nyankpala.

Table 2. Hardware and software tools used in this study.

No.	Tool	Purpose	Commercial/Open Source
Hardware tools			
1	UAV quadcopter with an RGB camera (DJI, California, USA)	Take images	Commercial
2	Smart phone (Apple Inc., Accra, Ghana)	Augment drone	Commercial/personal
3	Laptop (Dell, Accra, Ghana)	Process and analyze images/data	Commercial/personal
Software tools			
1	UAV Forecast (version 2.6.3)	Monitor weather	Open source
2	Pix4Dcapture (version 4.10.0)	Mission planning	Open source
3	WebODM (version 1.9.11)	Generate orthomosaics	Open source
4	QGIS (version 3.16.14)	Georeference orthomosaics	Open source
5	R statistical software (version 4.2.0)	Platform for image analysis packages	Open source
6	UASTools (version 0.4.0)	Generate shapefile for microplots	Open source
7	FieldImageR (version 0.3.3)	Analyze images	Open source

2.3.2. Flight Planning and Image Acquisition

Drone flights were carried out at R1, R3, R5 and R7 in 2020, and at R3, R5 and R7 in 2021. On the day of each flight, the UAV Forecast tool (<https://www.uavforecast.com> (accessed on 3 May 2020)) was used to determine the appropriate time within the day when the drone could be safely flown. It gives recommendation for a particular location based on time of sun rise and set, temperature, wind speed, gust speed, wind direction, precipitation probability, cloud cover and visibility. The iOS version of Pix4Dcapture tool (<https://www.pix4d.com/product/pix4dcapture> (accessed on 3 May 2020)) was used to set flight parameters using an iPhone. On the app, the selected mission was GRID for 2D images, camera angle set at 90°, image overlap for both front and side set at 80% and a flight altitude of 20 m. The in-built GPS of the drone was used to assist in defining the target area of the field which should be captured. In each year, after the first flight, the flight plan and parameters were saved to be used for all subsequent flights. After the flight parameters were set (or loaded in subsequent flights), the smart phone was connected to the

wireless drone controller via a cable and a command was sent to the drone to operate in an autopilot mode. A DJI Mavic Pro was used in 2020 whereas a DJI Phantom 4 Pro was used in 2021. The difference in drone model between years was due to availability. However, we did not expect this to influence the results as they all carry the same camera type. The inbuilt GPS of the drone was used for flight navigation and recording of coordinates of the individual images.

2.3.3. Image Alignment and Orthomosaic Generation

After each flight, images were downloaded from the drone and stitched together to obtain the entire field as one big image, known as an orthomosaic using WebODM [36]. WebODM (<https://github.com/OpenDroneMap/WebODM> (accessed on 8 June 2020)) is a photogrammetry software with advanced features for both UAV- and ground-based image processing based on the structure of motion algorithm. It has about 66 edit task options that can be tweaked to meet different demands. In this study, the default settings were used with slight modifications. The resolution of the orthomosaic image, digital surface and terrain models was changed from 5 cm pixel⁻¹ to 0.55 cm pixel⁻¹ to match the ground sampling distance of the images. Ground sampling distance (GSD) is equivalent distance on the ground of the distance between the centers of two consecutive pixels in an image. The higher the GSD, the lower the spatial resolution of the image. The 0.55 cm pixel⁻¹ was achieved in WebODM by relaxing the capping of maximum output image resolution through GSD. After image stitching, a 'bird's eye view' of the health of plants in the individual plots was obtained using the plant health function. An orthomosaic image was the reconstructed RGB image of the entire field from the individual drone images as shown in Figure 1.

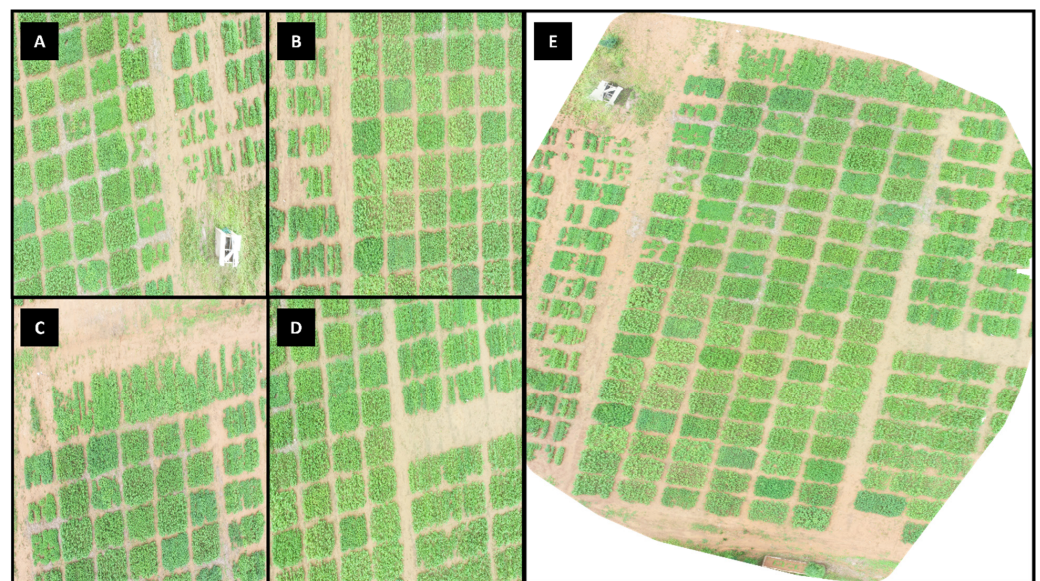


Figure 1. Individual images from the drone (A–D) and the reconstructed field (E).

2.3.4. Data Extraction

Since we did not use ground control points (GCPs) and the drones used did not have real-time kinematic (RTK), the orthomosaics of the same experiment did not exactly align when overlaid. Technically, this was not a problem. However, it meant that separate shapefiles had to be created for each flight's image. To avoid this so that a single pipeline worked for all images, orthomosaics from later flights were georeferenced to the first flight using Raster Georeferencer tool in QGIS (<https://www.qgis.org/en/site/> (accessed on 8 June 2020)). It is, however, strongly recommended to use either RTK or GCPs or perhaps both, if the researcher is interested in estimating plant height from drone photogrammetry.

Downstream data extraction from the orthomosaic images were performed after WebODM processing using UASTools, QGIS and FIELDimager [37,38]. UASTools was used to create a shapefile for each experiment comprising of individual plots. Each plot in the shapefile had an area of 4 m². Out of the ground area of 6.4 m², all plants in an area of 4 m² excluding the plot border rows was sampled for HTPP data capture. The alignment of plots in the shapefile with that of the orthomosaics was checked in QGIS and corrected where necessary to ensure that only inner portions of the plots were sampled. Shapefile alignment for each flight mission within a year was performed from georeferenced orthomosaics.

Plot data were extracted from the orthomosaic images in conjunction with the shapefile using FIELDimager. Since estimation of vegetation indices is on a pixel basis with which background soil could interfere, soil in the background was removed using the overall hue index (HUE) [39]. The original HUE has been modified in FIELDimager to better capture soil color. After soil removal, the proportion of ground covered by plant canopy (canopy cover) was estimated from the true plant canopy remaining. Then the individual pixels of red, green and blue color channels were divided by 255 to convert them into a 0–1 scale. After scaling, the pixelated values were used to estimate the normalized green red difference index (NGRDI, Equation (1)) and visible atmospherically resistant index (VARI, Equation (2)). NGRDI has been found to be highly associated with leaf chlorophyll content, biomass and water content of the plants [40], whereas VARI was associated with canopy, biomass and chlorophyll content [41]. Green area (GA, proportion of pixels with hue value of between 60 and 180°), greener area (GGA, proportion of pixels with hue value of between 80 and 180°) and crop senescence index (CSI, Equation (3)) were estimated using BreedPix [42,43]. BreedPix is a JRE-based plugin that analyses individual plot images. As a result, the reconstructed image, as shown in Figure 1E, cannot be directly analyzed to obtain individual plot data. Hence, a custom function was written in R statistical software (which can be obtained from the corresponding author) to crop out the individual plots from the preprocessed orthomosaics using the shapefile.

$$NGRDI = \frac{G - R}{G + R} \quad (1)$$

$$VARI = \frac{G - R}{G + R - B} \quad (2)$$

$$CSI = \frac{GA - GGA}{GA} * 100 \quad (3)$$

where R , G and B are the red, green and blue reflectance of the individual pixels.

2.4. Statistical Analysis

Location, growth stage and trait (conventional or drone-based) specific analysis was done using a linear model with R statistical software Version 4.1.2 [44] taking into account the design features of the experiment. Unbiased estimates of genotypic means were estimated using the emmeans package [45]. Correlation analysis was done between the drone- and ground-based means using agricolae package [46] to understand if the stages at which HTPP data were taken had influenced the relationship between the vegetation indices and AUDPC for ELS and LLS severity and the other traits.

Beyond the quantitative relationship analysis, the temporal changes in the genotypic vegetation indices were modeled to understand how the genotype's reaction to leaf spot diseases affected the temporal dynamics of the indices over time. Since the modeling process was to describe the temporal dynamics and not for prediction, equations were only assumed to accurately describe the process when modeling had an adjusted R² of at least 0.70 when they met all the statistical assumptions. Visualization of the modeling results was done using ggplot2, cowplot and ggpubr packages [47–49].

Since the populations used were composed of advanced breeding lines ready to be sent for on-farm testing, the stability of the genotypes across locations was assessed based

on the additive main effects and multiplicative interaction (AMMI) model. The number of principal components (PCs) to retain was determined using F_R -test [50,51].

3. Results

3.1. Early and Late Leaf Spot Disease Tracking Using Conventional and HTPP

Based on the median scores, the medium duration population had lower AUDPC scores for ELS and LLS than the short duration population except for the ELS in 2021 (Table 3). Similar observation was for the maximum AUDPC scores for ELS and LLS. This suggests that the medium duration population was more tolerant to ELS and LLS diseases than the short duration. However, since the maximum AUDPC scores for ELS and LLS of the medium duration population were always higher than the minimum AUDPC scores of the short duration population, there were some genotypes from both populations with similar tolerance levels to the two leaf spot diseases. Nevertheless, the most tolerant genotype(s) among the two populations as depicted by the minimum AUDPC scores was from the medium duration (Table 3).

Table 3. Summary of AUDPC scores among the studied groundnut genotype.

	ELS			LLS		
	Median	Min	Max	Median	Min	Max
2020						
Medium duration	35.88	12.83	56.19	46.13	15.39	56.12
Short duration	61.48	41.39	72.60	62.09	41.84	70.53
2021						
Medium duration	67.78	34.68	90.86	67.05	30.85	88.29
Short duration	61.66	41.50	78.75	69.20	42.94	85.60

ELS = early leaf spot; LLS = late leaf spot; Min = minimum; Max = maximum.

Among the medium duration population, the strongest (in terms of magnitude) and stable (with respect to direction) relationships between the vegetation indices and AUDPC for ELS and LLS were found at beginning maturity in each of the two years (R7, Table 4). At R7, NGRDI, VARI, canopy cover, GA and GGA all had negative correlations with AUDPC for ELS in 2020 and 2021, and LLS in 2021. On the other hand, the relationship between CSI and AUDPC for ELS and LLS at R7 was positive ($0.05 > p < 0.05$). The correlation coefficient between GGA and AUDPC for ELS at R7 was above 0.5 every year and was consistently negative. Canopy cover and GA had weaker relationships with AUDPC for ELS in both years with values ranging between 0.30 and 0.40 ($p > 0.05$). The relationship between the vegetation indices and AUDPC for LLS were generally weak in 2020. However, in 2021, strong relationships were observed as early R5 (beginning of kernel development) between the conventional and vegetation indices, with NGRDI and VARI consistently having a negative correlation with AUDPC for both diseases starting at R3.

With respect to the short duration population, NGRDI and VARI consistently had negative relationships with AUDPC for ELS and LLS from R3 through to R7 in both years, 2020 and 2021 (Table 4). However, such consistent relationship between the AUDPC for the leaf spot diseases and canopy cover, GA and GGA was only observed in the year 2021. Correlation coefficients were generally higher in 2021 than in 2020 for the short duration population although the reverse was true for ELS at R7 (Table 4).

Table 4. Correlation between vegetation indices and AUDPC.

Reproductive Stage	MD				SD			
	ELS AUDPC		LLS AUDPC		ELS AUDPC		LLS AUDPC	
	2020	2021	2020	2021	2020	2021	2020	2021
R3								
NGRDI	0.67 *	−0.22 ns	0.78 **	−0.50 *	−0.18 ns	−0.43 ns	−0.32 ns	−0.81 ***
VARI	0.56 ns	−0.28 ns	0.64 *	−0.54 *	−0.25 ns	−0.42 ns	−0.33 ns	−0.82 ***
Canopy cover	0.73 **	0.28 ns	0.91 ***	0.51 *	0.20 ns	−0.54 *	0.01 ns	−0.74 ***
GA	0.73 **	0.27 ns	0.90 ***	0.53 *	0.25 ns	−0.49 *	−0.02 ns	−0.70 **
GGA	0.72 **	0.22 ns	0.90 ***	0.50 *	0.20 ns	−0.38 ns	−0.02 ns	−0.70 **
CSI	−0.64 *	−0.06 ns	−0.83 ***	−0.29 ns	−0.03 ns	0.37 ns	0.00 ns	0.69 **
R5								
NGRDI	0.03 ns	−0.60 **	0.32 ns	−0.80 ***	−0.65 *	−0.62 **	−0.80 **	−0.87 ***
VARI	0.10 ns	−0.61 **	0.36 ns	−0.79 ***	−0.58 ns	−0.63 **	−0.62 *	−0.88 ***
Canopy cover	0.46 ns	0.10 ns	0.75 **	0.27 ns	0.05 ns	−0.43 ns	0.15 ns	−0.70 **
GA	0.40 ns	0.08 ns	0.77 **	0.30 ns	−0.42 ns	−0.46 ns	−0.43 ns	−0.71 ***
GGA	0.40 ns	−0.41 ns	0.77 **	−0.42 ns	−0.44 ns	−0.55 *	−0.45 ns	−0.83 ***
CSI	−0.37 ns	0.56 *	−0.71 **	0.72 ***	0.35 ns	0.55 *	0.34 ns	0.84 ***
R7								
NGRDI	−0.74 **	−0.63 **	−0.38 ns	−0.86 ***	−0.88 ***	−0.62 **	−0.64 *	−0.86 ***
VARI	−0.66 *	−0.64 **	−0.28 ns	−0.86 ***	−0.88 ***	−0.62 **	−0.63 *	−0.86 ***
Canopy cover	−0.33 ns	−0.37 ns	0.12 ns	−0.37 ns	−0.69 *	−0.54 *	−0.69 *	−0.86 ***
GA	−0.35 ns	−0.33 ns	0.15 ns	−0.28 ns	−0.77 **	−0.55 *	−0.71 *	−0.86 ***
GGA	−0.51 ns	−0.54 *	0.00 ns	−0.68 **	−0.83 ***	−0.61 **	−0.56 ns	−0.88 ***
CSI	0.67 *	0.58 *	0.21 ns	0.81 ***	0.77 **	0.63 **	0.38 ns	0.86 ***

MD = medium duration; SD = short duration; ELS = early leaf spot; LLS = late leaf spot; AUDPC = area under disease progress curve; R3 = beginning pod; R5 = beginning seed; R7 = beginning maturity; NGRDI = normalized green-red difference index; VARI = variable atmospheric resistance index; GA = green area; GGA = greener area; CSI = crop senescence index; * = significant at 0.05; ** = significant at 0.01; *** = significant at 0.001; ns: not significant.

3.2. Temporal Dynamics of the Vegetation Indices among the Genotypes

The changes in the genotypic scores with respect to the vegetation indices over time followed a unimodal distribution which were well fitted by a quadratic function (adjusted $R^2 = 0.77 \leq x \leq 1$, SS1. Table S1). In 2021, there were not enough data points to estimate the various coefficients of the curve. As a result, the curves were not fitted. The rankings of the genotypes changed over time as the genotypes grew and developed towards maturity, although some of the genotypes maintained their ranking position. For instance, among the medium duration, genotype ICGV 176225 showed high NGRDI and VARI at R1 and R3, but dropped steadily in the rank and magnitude after R3 (SS2. Figure S1). On the other hand, ICGV 176073 consistently had the highest scores for NGRDI whereas ICGV 176073 and ICGV 176044 both had high scores for VARI at all reproductive growth stages (Figures 2 and S1). There was a complete crossover in genotypic rankings for canopy cover, GA and GGA after R3 (SS2. Figure S2). In the short duration population, crossovers in genotypic rankings for NGRDI and VARI largely occurred after R5 whereas that for CSI occurred after R3 (Figures 2 and S3). However, genotype ICGV 176214 was consistently the highest followed by ICGV 176023 for NGRDI and VARI after R1 (Figures 2 and S3). There was equally a complete crossover in genotypic rankings for canopy cover, GA and GGA after R1 (Figures 2, 3 and S4).

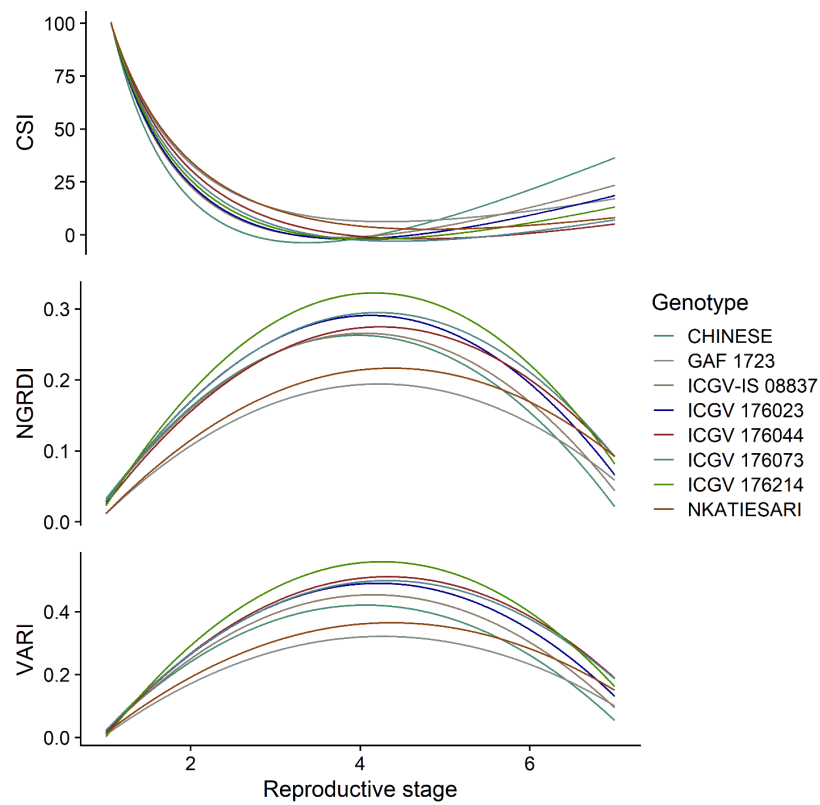


Figure 2. Crop senescence index (CSI), normalized green red difference index (NGRDI) and variable atmospheric resistance index (VARI) of selected medium duration (MD) and short duration (SD) genotypes.

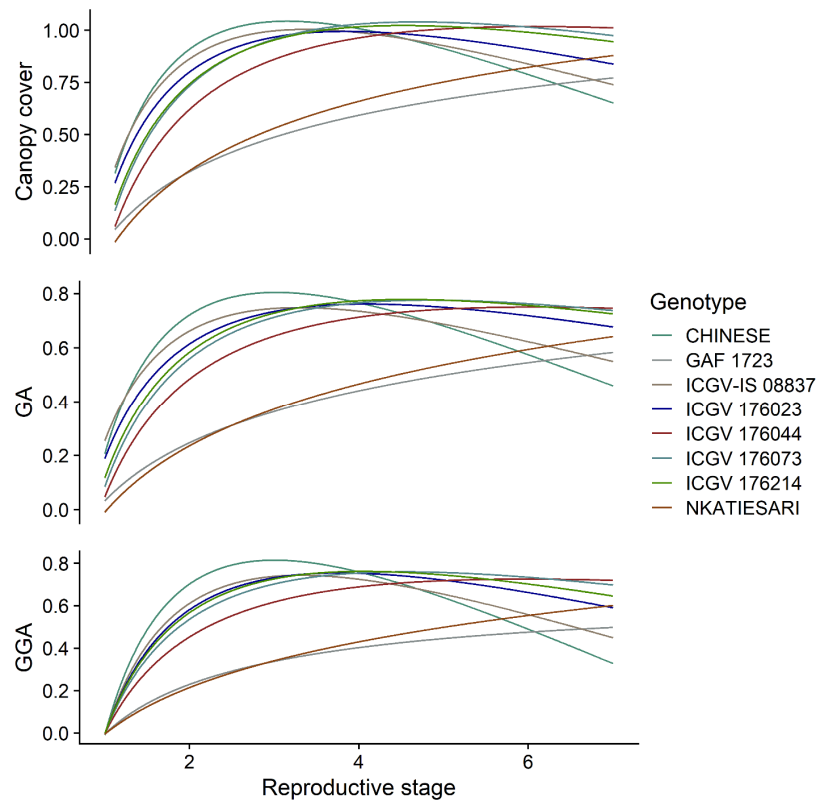


Figure 3. Canopy cover, green area (GA) and greener area (GGA) of selected medium duration (MD) and short duration (SD) genotypes.

Despite the crossovers in genotypic rankings for the vegetation indices across the reproductive growth stages, the pattern of genotypic clustering appeared to follow growth stage. This observation was consistent across populations and years (Figure 4). Although the scores at R1 and R7 were completely different, there was some similarities in performance at R3 and R5, as shown by the proximity of the clusters. In 2021, a few genotypes (in black circles) did not cluster according to the growth stages. These included NKATIESARI, GAF 1723, ICGV-IS 08837 and CHINESE which are known as tolerant, moderately tolerant, moderately susceptible and susceptible, respectively, and used here as [10,12]. The genotypes whose clustering defied growth stage in the PCA and those that consistently had high score for vegetation indices were selected for detailed data study. It was observed that genotypes that combine leaf spot diseases tolerance with high yield had the highest scores with regards to the vegetation indices (Figures 2 and 3).

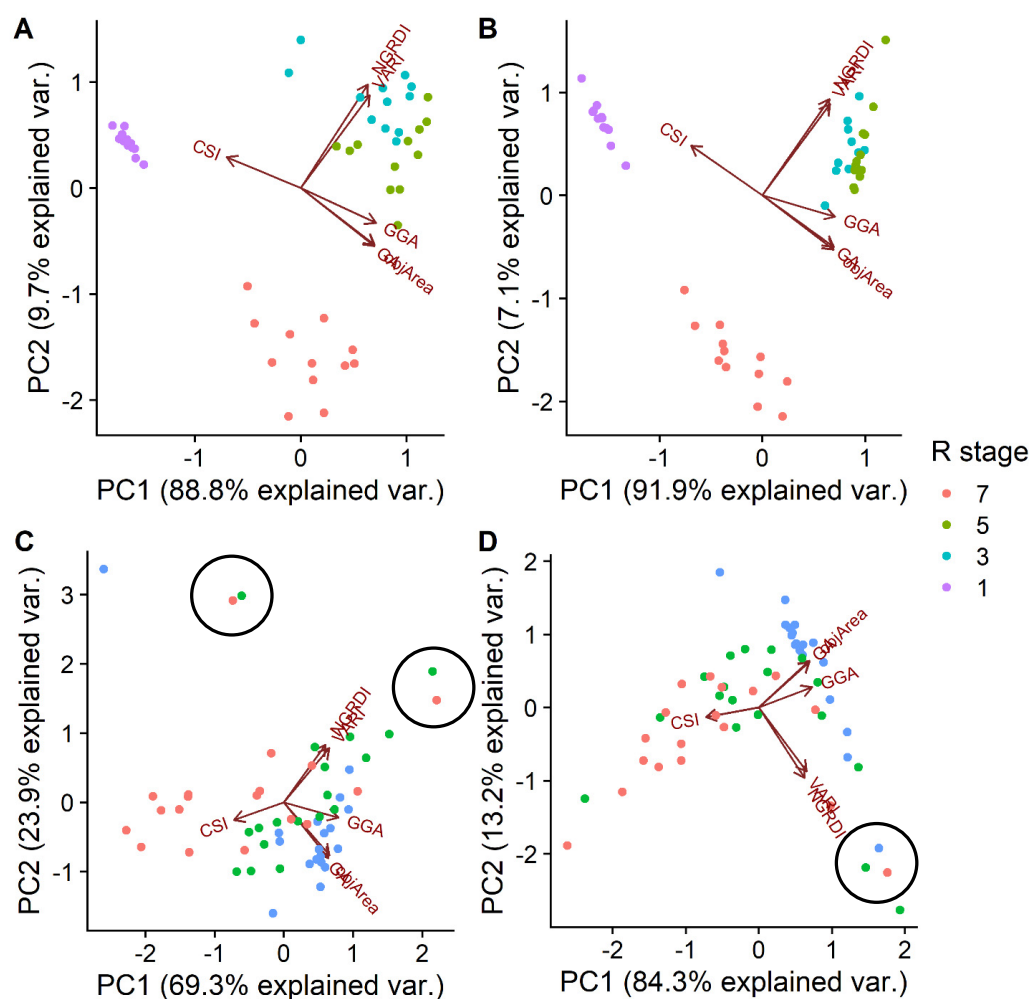


Figure 4. Biplot of first and second principal components showing genotype clusters across growth stages. (A,C) Medium duration population in 2020 and 2021. (B,D) Short duration population in 2020 and 2021.

3.3. The Effect of Genotype-By-Environment Interaction

There was a significant interaction between the genotypes and the environment ($p < 0.05$) for all the manually measured traits in both years, except for haulm yield of the medium duration population in 2020 (Table 5). The signal in the genotype by environment sum of squares [12,50] was high (up to 93% in some of the traits) although that for haulm yield was quite low in 2020. The high $G \times E$ interaction suggests that genotype and location were the main drivers of the observed interaction relative to random

error. Location and genotype main effects were also significant. Generally, locations with below-average scores for leaf spot diseases had higher scores for yield traits.

Table 5. AMMI model summary showing contribution of the various sources of variation.

Variation Source	df	Sum of Squares			
		ELS AUDPC	LLS AUDPC	Haulm Yield (kg ha^{-1})	Pod Yield (kg ha^{-1})
MD in 2020					
Environment Ghana	3	9905.4 ***	14,938.8 ***	441,412,003 ***	32,875,500 ***
Genotype (G)	11	3923.1 ***	11,262.3 ***	16,931,646 **	23,250,254 ***
Genotype x Environment	33	4699.6 ***	5372.9 *	23,674,776 ns	12,776,519 ***
G \times E signal		0.7581	0.4496	0.2733	0.7691
SD in 2020					
Environment Ghana (E)	3	35,052 ***	14,005.6 ***	171,352,883 ***	16,265,746 ***
Genotype (G)	11	2799 ***	7702.7 ***	33,553,632 ***	19,915,906 ***
Genotype x Environment	33	1911 **	3095.6 ***	10,956,981 *	15,445,714 ***
G \times E signal		0.5238	0.7165	0.3965	0.7738
MD in 2021					
Environment Ghana (E)	3	178,981 ***	154,147 ***	156,608,615 ***	264,852,830 ***
Genotype (G)	17	21,916 ***	17,784 ***	49,675,436 ***	77,008,509 ***
Genotype x Environment	51	33,535 ***	32,443 ***	115,776,686 ***	38,860,002 ***
G \times E signal		0.9302	0.8380	0.8175	0.8258
SD in 2021					
Environment Ghana (E)	3	146,356 ***	195,735 ***	664,657,480 ***	284,254,679 ***
Genotype (G)	17	11,577 ***	15,269 ***	69,544,867 **	47,883,935 ***
Genotype x Environment	51	14,675 ***	11,339 ***	116,259,584 **	26,920,315 ***
G \times E signal		0.8592	0.6924	0.5232	0.8008

ELS = early leaf spot; LLS = late leaf spot; AUDPC = area under disease progress curve; * = significant at 0.05; ** = significant at 0.01; *** = significant at 0.001.

In 2020, among the medium-duration population, genotypes with ELS AUDPC scores above and below average score were more stable (close to the plot origin) than those in between (SS3. Figure S5). This scenario was not too obvious with regards to the AUDPC for LLS. Genotypes ICGV 176073 combined high pod yield with high yield stability. With regards to the short duration population, each of the traits had a different genotype that was best (SS3. Figure S6).

In 2021 and among the medium duration population, genotype GAF 1665, which was not part of the tested genotypes in 2020, was more stable and tolerant to leaf spot diseases (SS3. Figure S7). Its haulm and pod yields were, however, below the average score despite the high stability for these two traits, respectively. Among the short duration, genotype CHINESE had the highest ELS AUDPC score, which was consistent across the locations, leading to high stability for ELS AUDPC (SS3. Figure S8). This was, however, not the case for LLS AUDPC.

4. Discussion

The HTPP based on image analysis using open-source tools can offer researchers in low income countries the ability to use indices that are related to target trait(s) to assess superior individuals within breeding populations. It allows data extraction from every visible component of the plant in the image. It also enables the extraction of data from each pixel in the image, which give information on several independent subunits of the plot. For instance, an image taken on a 5 \times 5 m plot from the height allowing 1 cm GSD can provide 250,000 datapoints when the data are extracted on pixel basis. The mean from the 250,000 datapoints may therefore better approximate the plot than the point data obtained by manual measurements. As a result, the ability to apply this technique in low-income countries is therefore expected to enhance the quality of research in four ways; (i) objectively

collect data as opposed to the subjective way, e.g., of scoring leaf spot disease severity in groundnut, (ii) every visible plant part in a plot can be separately and collectively analyzed, (iii) automate data recording thereby eliminating human error, and (iv) relatively cheap cost for the investment. It is, however, important to determine which vegetation indices are best associated with the primary trait of interest and at what stage of growth is such association strongest and most meaningful.

The vegetation indices used in this study were associated with traits previously related to the ELS and LLS diseases. For example, [40] found that NGRDI was associated with chlorophyll content, biomass and water content. Similarly, [41] found that VARI was a good estimator for plant canopy, biomass and chlorophyll content. Leaf spot diseases cause brown lesions and defoliation in groundnut [10]. As a result, the area covered by the plant canopy and plant discoloration in the presence of disease are expected to provide an indication of genotypic tolerance. Therefore, genotypes with high vigor and tolerant to leaf spot diseases are expected to have higher values for canopy cover, NGRDI, VARI, GA and GGA and lower values for CSI. At the same time, genotypes with lower AUDPC values appear to be tolerant. As a result, a negative relationship between the vegetation indices except CSI and ELS and LLS AUDPC values are expected.

Although it has been shown that leaf spot diseases tolerance in groundnut is associated with longer maturity duration [52,53], that scenario did not clearly stand out in this study when the medium and short duration populations were compared (Table 3; Figures 2 and 3). One reason could be that genotypes that constituted the populations were not only selected based on their reaction to leaf spot diseases but also on their performance for other traits. For instance, single seed descent based on index selection is usually used at CSIR-SARI's Groundnut Improvement Program. The genotypes that are superior in other traits but with low tolerance to ELS and LLS can get selected. Nevertheless, the minimum AUDPC score among the short duration is always higher than that of the medium duration. This suggests the most tolerant genotype(s) among the two populations used in this study were part of the medium-duration population.

The vegetation indices that describe the area of soil covered by plants, namely canopy cover, GA and GGA, exhibited two kinds of relationship with the AUDPC for ELS and LLS under the medium-duration population. That is, it was positive at the initial stages, and negative towards the end of the growing cycle (Table 4). This means that the susceptible genotypes within this population ranked higher for canopy cover, GA and GGA at the beginning (R3 and R5) and lower at the end. However, at R3, lesions from ELS and LLS had not formed although some were visible at R5. As a result, values of canopy cover, GA and GGA for the medium duration population at R1 and R3 estimated plant vigor rather than leaf spot diseases tolerance. This observation confirms the basis of an earlier study [10] where genotypes with high seedling vigor were more susceptible to ELS and LLS at later stages of growth. It was therefore not surprising that at the latter stages, when leaf spot was causing defoliation, the direction of the relationship changed from positive to negative (Table 4). The delay in change of direction from positive to negative with regards to the medium-duration population could be due to the ability of genotypes to maintain their leaves with appreciable quantity of lesions caused by the ELS and LLS. With the short duration population, however, leaf defoliation started almost at the same time as when lesions begin to show. This therefore explains why the canopy cover, GA and GGA had negative relationships with ELS and LLS as early as R3 in that population (Table 4).

NGRDI and VARI had negative relationships with the leaf spot diseases even at R3 ($p > 0.05$; $p < 0.001$) when lesions were not visible to enable human scoring for the diseases, respectively. However, ELS and LLS diseases appear as microscopic lesions which later enlarge into bigger and more visible lesions. This means that the physiological damage to the plant takes place before the lesions become visible. As a result, vegetation indices that are associated with the plants' physiological traits are expected to give early signal of the presence of the disease. This might therefore be the reason why NGRDI and VARI had

consistently negative associations with ELS and LLS under the two populations starting from R3 (Table 4). The relationship, however, became stronger as the disease progressed.

Although there were complex crossovers in genotypic rankings across the various reproductive growth stages in 2020 for the vegetation indices, genotypes that had consistently high scores for NGRDI, VARI, GA, GGA and canopy cover had the best average performance for ELS, LLS AUDPC, and haulm yield. For example, genotypes ICGV 176073, ICGV 176044, ICGV 176214, and ICGV 176023 had the highest NGRDI and VARI at all reproductive growth stages during the season (Figure 2). At the end of the season, they also had the best performance for ELS, LLS AUDPC, and haulm yield not only in Nyankpala, but across all locations (SS3. Figures S5–S8). This indicates that integrating HTPP tools could provide effective acceleration of cultivar development, non-invasive assessment, and objective field phenotyping.

The AMMI model waGhana effective in partitioning the overall variation into the various sources and further decomposing the interaction term to reveal the signal (true genotype-by-environment interaction) and random noise. Genotypes, locations and their interaction were all important sources of variation for ELS, LLS, haulm and pod yield (Table 5) highlighting the importance of optimizing production practices to complement the genetic aspect of the phenotype [12].

5. Conclusions

Funding for R&D in developing countries such as Ghana is very low. Yet the need to increase genetic gain requires the use of tools such as high-throughput phenotyping which may require high initial investment for the hardware, but free software. This study sought to determine the feasibility of implementing HTPP with open-source tools to set a roadmap for its adoption by the research community. However, care should be taken in the choice of drone as not all mapping software are open-source and the open-source mapping software does not accept every drone.

A strong relationship existed between vegetation indices used in this study and leaf spot diseases' severity scores. Better results were obtained at R7 stage, although early stages could also prove effective. However, if other traits such as yield are also of interest in addition to tolerance to leaf spot diseases, then the whole reproductive growth curve should be studied.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy12112733/s1>, SS1. Table S1: Regression equations representing the changes in the vegetation indices over time; SS2. Figure S1: Genotypic performance in CSI, NGRDI and VARI over time among the medium duration population; SS2. Figure S2: Genotypic performance in canopy cover, GA and GGA over time among the medium duration population; SS2. Figure S3: Genotypic performance in CSI, NGRDI and VARI over time among the short duration population; SS2. Figure S4: Genotypic performance in canopy cover, GA and GGA over time among the short duration population; SS3. Figure S5: AMMI biplot for the medium-duration population in 2020; SS3. Figure S6: AMMI biplot for the short-duration population in 2020; SS3. Figure S7: AMMI biplot for the medium-duration population in 2021; SS3. Figure S8: AMMI biplot for the short-duration population in 2021.

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