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Use of Unmanned Aerial Vehicles (UAVs) Imagery in Phenotyping of Bambara Groundnut

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Abstract

In this experiment, proximal measurements and Unmanned Aerial Vehicle (UAV) imagery was used to determine growth stages for bambara groundnut (*Vigna subterranea* (L.) Verdc.). The crop is a high potential crop due to its ability to yield in marginal environments, but neglected and underutilised due to lack of information on its growth in different environments. This study evaluated the correlation between Normalised Difference Vegetation Index (NDVI) derived from the ground as well as airborne sensors to test the ability of remotely sensed data to identify growth stages. NDVI and chlorophyll content of bambara groundnut leaves were measured at ground level at 18, 32, 46 and 88 days after planting (DAP) comprising vegetative, flowering, pod formation and maturity growth stages. The UAV imagery for the experimental plots was acquired with 0.2m resolution at maturity. The result showed a significant ($p < 0.05$) linear relationship between proximal NDVI and chlorophylls content at all growth stages of growth. The R^2 varied from 0.57 in the vegetative stage to 0.78 in the flowering stage. Furthermore, NDVI derived from proximal measurements and UAV data showed a significant ($p < 0.05$) correlation. The observed high correlation between proximal sensors, UAV data and crop parameters suggest that remote sensing technologies can be used for rapid phenotyping to hasten the development of models to assess the performance of underutilised crops for food and nutrition security.

Keywords: chlorophyll content, NDVI, remote sensing, UAV, underutilised crops, vegetation indices

1. Introduction

Plant phenotypes are dynamic and the result of plant interactions with the environment. Understanding these activities in a constantly changing climate is important for the development of plant science, crop management and breeding of new varieties (Pieruschka & Schurr, 2019). The plant research community need to accurately measure the diverse characteristics of plants in order to understand their adaptation to resource-limiting environments. Bambara groundnut (*Vigna subterranea* (L.) Verdc.), is a legume crop that is commonly grown in low-input systems across sub-Saharan Africa and Southeast Asia (Mayes et al., 2019). In addition to having good nutritional characteristics, bambara groundnut is highly tolerant to drought and is able to yield on lands that are not fertile enough for the cultivation of many other crops. However, despite its potential, it remains underutilised due to lack of information on its performance in different environments and in particular, its phenotypic development. This limits the ability to assess its suitability for new locations (Suhairi et al., 2018).

Crop phenotype is the result of interaction between the genotypic and environmental factors. It comprises geometric traits such as height, leaf area index, canopy cover and spectral features and physical traits such as chlorophyll content, biomass and photosynthesis; nutrient contents and yield (Yang et al., 2017). Understanding how these traits change over time is one of the crucial steps in monitoring the crop growth. Contrasting these events with crop management events such as irrigation, fertiliser and pesticide application an essential source for understanding the crop conditions (Prasad et al., 2006).

Various technological approaches based on remotely sensed measurements have been proposed to assess these traits in the field condition (Yang et al., 2017). The commonly used trait for high-throughput screening and phenotyping is the Normalized Difference Vegetation Index (NDVI) derived from canopy reflectance. NDVI is measured using wavelengths within the near infrared (NIR) and visible (VIS) regions of the electromagnetic spectrum. The NDVI is associated to chlorophyll content in the leaf molecules that in turn is related to photosynthetic capacity of the plants. NDVI can be used to estimate the relative crop biomass at different crop developmental stages as well as nitrogen deficiency at crop senescence (Tattaris et al., 2016). There is enough scientific evidence to suggest that NDVI can be successfully used to estimate different crop traits (Jewan et al., 2019; Johnson, 2003; Wall et al., 2008). For example, Leaf Area Index (LAI), which is one of the most important indicators of crop growth has been indirectly estimated using NDVI for soybean, maize (Colombo et al., 2003; Johnson, 2003) and bambara groundnut (Jewan et al., 2019). The NDVI has also been used to forecast the yield of barley, canola, field peas and spring wheat (Mkhabela et al., 2011), bambara groundnut (Jewan et al., 2019), wheat (Wall et al., 2008) and maize (Shanahan et al., 2001). Therefore measurements of NDVI or its estimates can be used in yield assessment models (Prasad et al., 2006).

Phenotyping using ground-mounted vehicles can provide information about plant traits on a timescale of many hours for a plot. However, this method is time-consuming and is not practical for large scale and remotely located plots (Han-Ya et al., 2010). Using multiple sensors to take measurements concurrently for many plots may increase the costs (Candiago et al., 2015a; Gevaert et al., 2015). This has recently motivated the use of high-resolution data processing in phenotyping. In addition, field-based phenotyping to monitor the phenology and crop parameters for bambara groundnut landraces has recently been shown to be ineffective (Jewan et al., 2019). Determination of leaf chlorophyll content, which requires sampling from several locations in the leaf to obtain adequate characterisation (Candiago et al., 2015b) usually takes a long time to accomplish. This in turn, hinders the process of calibrating crop models, particularly for less-researched, neglected and underutilised species. Development of rapid NDVI estimation methods for crop parameters using remote sensing approaches can streamline modelling efforts for these crops.

Satellite remote sensing has also proven to be a valuable tool for monitoring crop health, crop modelling, climate change adaptation and mitigation and others (Cobb et al., 2013; Li et al., 2014). However, currently available satellite data are costly, lack sufficient spatial resolution to identify desirable features, cloud cover and have long-term visiting periods (Cobb et al., 2013; Tattaris et al., 2016). Alternatively, unmanned airborne platforms have the ability for monitoring large scale crop parameters using high spatial and spectral resolution images. Remote sensing platforms using low altitude and flexible unmanned airborne platforms provide more affordable tools for crop phenotyping and precision agriculture (Candiago et al., 2015b). Therefore, UAVs can play a crucial role in the high-performance, near real-time phenotyping for large number of plots and field trials to reduce the potential costs. These UAVs provide high spatial and spectral imagery, useful for determining crop vegetation indices (VIs) and plant phenotyping. In this study, proximal measurements and UAV imageries were used to derive NDVI values for bambara groundnut. The objectives of this study were i) to evaluate NDVI obtained from a proximal sensor and its relationship with the selected phenotypic characteristics of bambara groundnut, ii) to compare the data derived from the UAVs with proximal sensors and chlorophyll content, and iii) to evaluate the ability of UAV data in predicting the crop growth stages.

2. Method

2.1 Description of Study Site

The field trial was conducted at the Field Research Centre of Crops for the Future Research Centre, Semenyih, Selangor (2°55′56.96″ N, 101°52′33.59″ E) during July-October 2019. Block 3 which is 0.1 ha in size with a slight slope was designated for this experiment. Randomised sub blocks were created within the field to take measurements of crop phenotypes. The experimental field is shown in Figure 1.

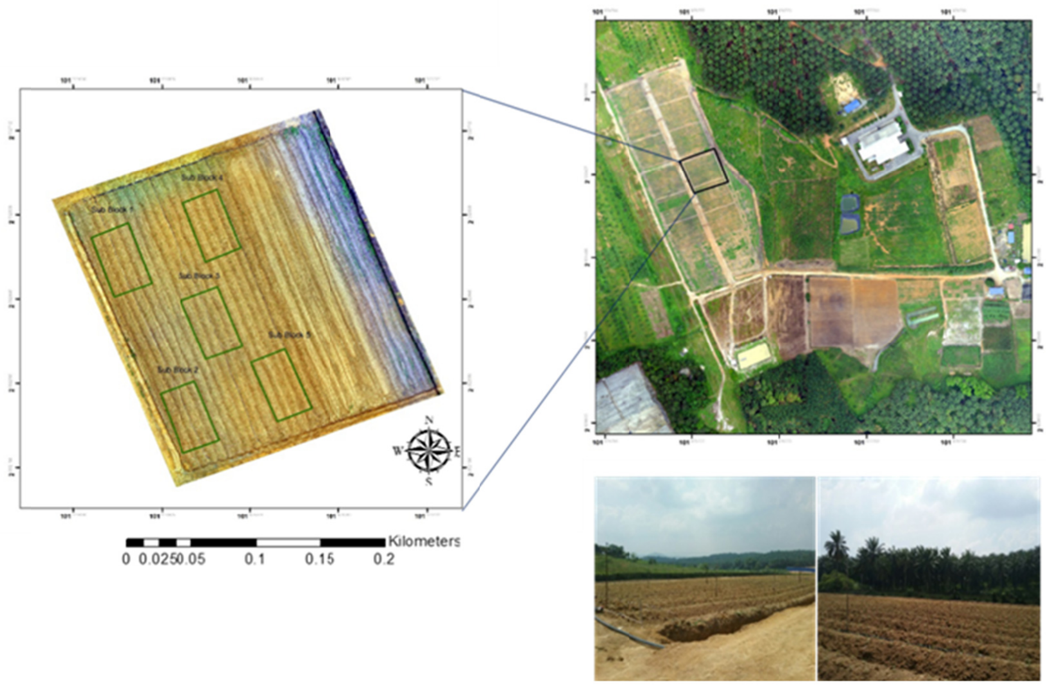

Figure 1. The study site

2.2 Plant Materials and Crop Management

The landrace planted in this experiment, "Songkla", is a fast maturing (90-100 days), red rounded seed obtained from Field Research Centre, Crops for the Future Research Centre. The seeds were sown directly in beds with a spacing of 30 × 40 cm. Fertiliser NPK 15:50:50 was applied during the second week of seed germination and continuously applied within two weeks interval.

2.3 Proximal Data Collection-Data Acquisition and Processing

A Trimble GreenSeeker Handheld NDVI Sensor (Trimble Inc., California) was used to measure NDVI at ground level. The GreenSeeker is a commercially available sensor, capable of measuring canopy reflectance. It is an active system that produces its own light sources: two light emitting diodes (LEDs) illuminate the ground at two specific wavelengths, namely 656 nm (red) and 774 (near infrared). The NDVI is estimated by combining two spectral bands (Prasad et al., 2006). NDVI was estimated as:

$$NDVI = (NIR - RED)/(NIR + RED) \quad (1)$$

In this study, the NDVI measurements were recorded close to noon from 10:00 a.m. to 12:00 p.m. local time. The measurement were taken at about 0.6 m above the canopy cantered over the middle row when the surface of the plant canopy and soil were dry.

A Spectrum Technologies SPAD 502 DL Plus (Spectrum Technology Inc., United States) was used to measure the chlorophyll concentration in bambara groundnut leaves. This crop parameter is crucial for monitoring the plant growth as the chlorophyll concentration is associated with photosynthetic activities (Li et al., 2018). This device utilises two wavelengths, one at 650 nm (red) and one at 940 nm (infrared) through plant leaves. The transmission of both wavelengths passing results in a processed value (SPAD). The measured value provides an indication of the relative amount of chlorophyll content in the leaves with the following Equation (2).

$$SPAD = NIR/RED \quad (2)$$

In this study, the measurements also performed at the same time interval with the GreenSeeker to minimize the potential effect of light intensity on chloroplast movement. The measurements were taken on randomly selected healthy leaves, and the data were collected once in each plant from each plot during the growing season. Both NDVI and SPAD values were recorded at 18, 32, 46, 60, 74 and 88 days after planting (DAP) during different growth stages; vegetative (18 and 32 DAP), flowering (46 DAP), pod formation (60 and 74 DAP) and pod maturity (88 DAP) respectively (Figure 2).

Figure 2. (a) Vegetative (18 and 32 DAP), (b) flowering (46 DAP), (c) podding (60 and 74 DAP) and (d) maturity stage (88 DAP) of bambara groundnut

2.4 UAV Data Collection-UAV Image Acquisition and Processing

Aerial imagery was also used to assess a Visible Atmospheric Resistance Index (VARI) and NDVI values. A UAV DJI Phantom 4 (DJI, China) was used to acquire imagery with 0.2 m resolution during different growth stages and NDVI values was assess at maturity. The flight was conducted directly after the proximal data collection around 12:00 p.m. to 2:00 p.m. local time. However, the multispectral sensor was used to assess NDVI at maturity stage.This UAV has a maximum weight of 1.8 kg and is capable of flying low and mounting lightweight instruments. The flight system consists of an on-board Global Positioning System (GPS). The aerial images were obtained with two cameras installed separately on the UAV; the RGB cameras and the multi-spectral camera for taking photos in the red, green and near-infrared part of the electromagnetic spectrum, enabling the NDVI measurement. The duration of the flight was around 15 to 30 minutes to capture 350-380 images of the whole experimental plot.

2.4.1 Image Preprocessing and Calibration

The ground control point (GCP) was collected at four different points within the Block 3 by using GPS Trimble (Trimble Inc., California) to achieve the coordinates before the UAV mission was started. The image processing was done using DroneDeploy software (DroneDeploy, Inc., USA) where the images were subsequently mosaicked together. This was done by identifying overlapping region within the images. The images became orthorectified and geometrically corrected, along with lens distrortion and camera angle, to create an entire view of all compiled images in the frame completely where all the points of GCPs were inserted.

2.4.2 Calculation of Vegetation Indices

The orthorectified image was exported and image processing was done using ESRI Arcmap version 10.7 (ESRI, Munich, Germany). Image analysis tool was applied to assess NDVI (using 833nm and 659 nm as the near-infrared (NIR) and Red wavelengths respectively) throughout the geographical extent as shown in Figure 3 (b). The NDVI values was extracted and compare with the NDVI derived from the GreenSeeker and other proximal measurements (SPAD values) at maturity stage.

In addition, a Visible Atmospheric Resistance Index (VARI) index was also calculated using image processing techniques. VARI uses RGB wavelength to perform image classification. The VARI index, minimises reflectance, scattering, and other atmospheric effects to estimate the fraction of healthy vegetation using color correction in an area. The VARI is applied at four different growth stages. This is necessary as to compare and correlate the capability of RGB imagery with NDVI derived from the GreenSeeker and SPAD values. VARI index is calculated as:

$$\text{VARI} = (\text{GREEN} - \text{RED})/(\text{GREEN} + \text{RED-BLUE}) \quad (3)$$

Figure 3 (a, b and c) shows the analysed image to derive NDVI and VARI for the experimental plot during the maturity stage (88 DAP). The specifications of the UAV components are summarised in Table 1.

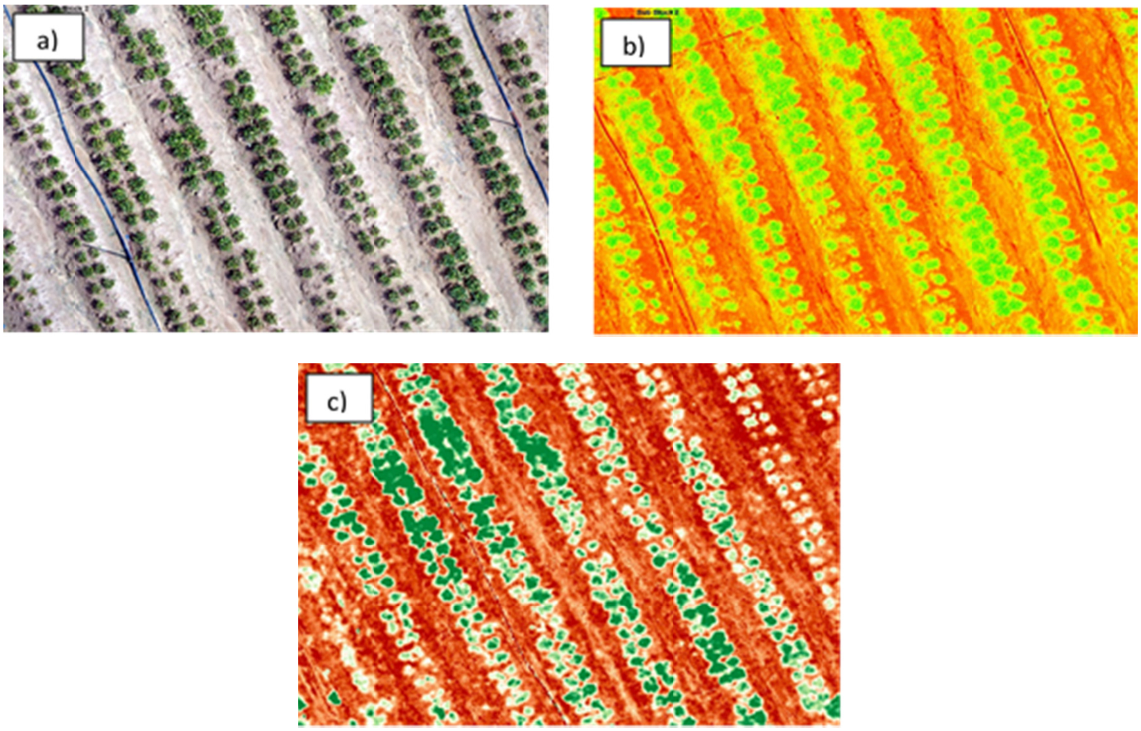

Figure 3 (a). The raw image of UAV-RGB data and (b) the UAV-derived NDVI and (c) the UAV-derived VARI

Table 1. Specification of the UAV components used in the study

UAV	Camera Model	Camera Pixel	Ground Resolution	Focal Length	Flying Altitude
DJI Phantom 4 Advanced	FC6310	20MP	2.14 mm/pix	8.8mm	8.74 m
DJI Phantom 4 Advanced	Canon Power Shot S100	12MP	4.65 mm/pix	5.2 mm	14.8 m

2.5 Statistical Approach

Statistical analysis was carried out using Prism8 (GraphPad Software, USA). Phenotypic correlation coefficients were calculated to study the relationship between proximal and aerial based measurements. The differences between the phenotypic correlations of the proximal and aerial imagery were tested for significance with a Pearson correlation and was applied to the *p*-values of to account for multiple comparisons.

3. Results

3.1 Evaluation of NDVI Obtained From the Proximal Sensor at Different Crop Stages

The relationship between NDVI from Greenseeker and chlorophyll content from SPAD at different developmental stages is shown in Figure 3. The results showed significant ($p < 0.05$) positive linear relationships between proximal NDVI and SPAD values at all growth stages. The highest correlation coefficient of 0.82 (R^2 = 0.78) was observed at 46 DAP, the flowering stage, and the lowest ($R^2 = 0.57$, $r = 0.72$) at 18 DAP during the vegetative stage (Figure 3). The other growth stages showed comparatively higher relationships between both

NDVI and SPAD values. The slope of the positive regression line increased from 18 DAP (vegetative, 27.64) to 46 DAP (flowering, 55.12). The slope of the regression decreased to 45.27 at 88 DAP (maturity stage). The coefficient of determination (R^2) and correlation coeeficient (r) for each growth stage are presented in Table 2.

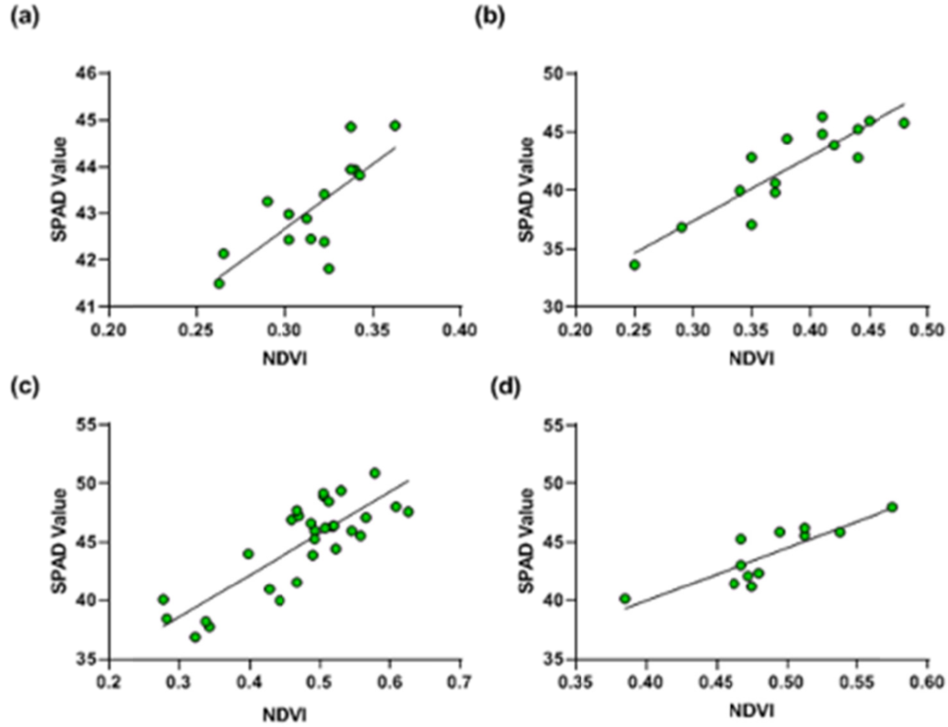

Figure 3. The relationship between NDVI from the Greenseeker and SPAD values at (a) vegetative (n = 15), (b) flowering (n = 15), (c) pod formation (n = 30) and (d) maturity (n = 12) stage of bambara groundnut

Table 2. Statistical relationship between NDVI derived from the Greenseeker and SPAD at different stages

Growth stage	Day After Planting (DAP)	R^2	Correlation Coefficient	Equation
Vegetative	18-32	0.57	0.72**	Y = 27.64X + 34.39
Flowering	46	0.78	0.82**	Y = 55.12X + 20.87
Pod formation	60-74	0.68	0.69**	Y = 35.84X + 27.83
Pod maturity	88	0.71	0.81**	Y = 45.27X + 21.87

Note. Y = SPAD Value; X = NDVI Value; ** Significant at 0.05.

3.2 Evaluation of Proximal Sensors and UAVs Imagery Derived Data Relationship

The proximal measurements and UAV data were significantly related ($p < 0.05$ and r = 0.45, $R^2 = 0.12$) (Figure 4). At the maturity stage of the crop, UAV -derived NDVI showed a significant ($p < 0.05$) positive relationship (r = 0.73, $R^2 = 0.55$) with SPAD values (Figure 5).

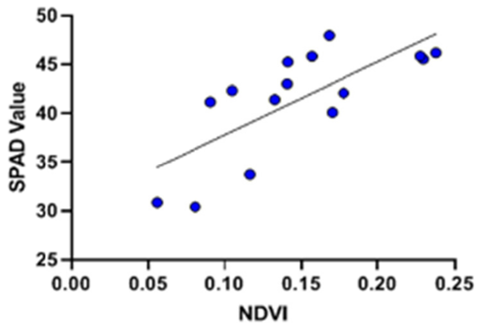

Figure 4. The correlation between NDVI derived from the Greenseeker and UAV at the maturity stage

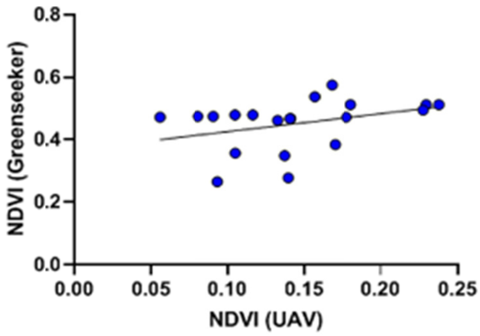

Figure 5. The correlation between NDVI derived from UAV and SPAD

3.3 Evaluation of the Reliability of UAV Imagery Derived Data

The average NDVI along with the confidence interval (CI) and growth stages were plotted against the days after planting (Figure 6). Differences in NDVI were observed throughout the lifespan of the crop, parallel to the growth stages. Phenology of bambara groundnut correlated well (significant at $p < 0.05$) with proximal NDVI. The NDVI increased from vegetative to the podding stage in which the highest value was observed at 60 DAP. Then the NDVI decreased during the maturity stage.

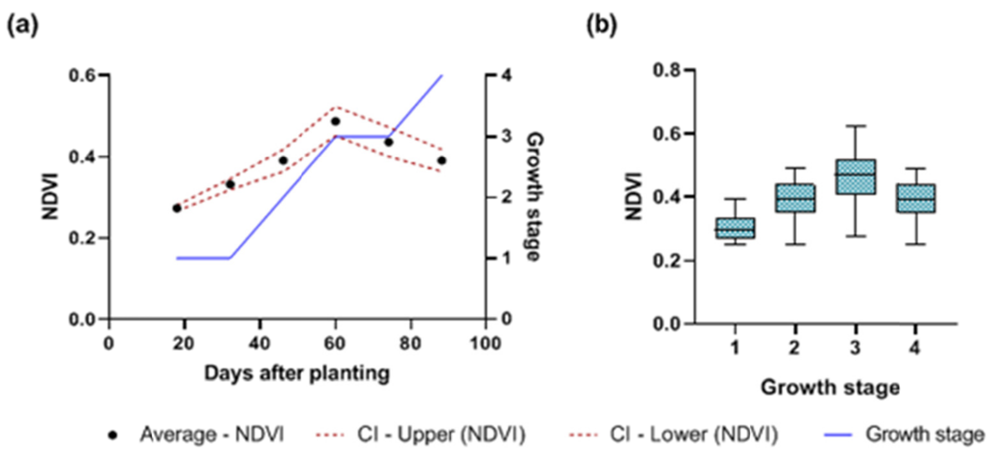

Figure 6 (a) Variation of NDVI and crop growth stages throughout the lifespan and (b) variation of NDVI in different stages

3.4 Evaluation of SPAD Values, GreenSeeker and VARI

There was no significant ($p > 0.05$) correlation between SPAD values and VARI during any growth stage. Also, VARI did not show significant ($p > 0.05$) relationships between Greenseeker and UAV derived NDVI values suggesting that it cannot be used in the determination of phenology of bambara groundnut.

4. Discussion

4.1 Correlation between Proximal Sensors in Different Crop Stages.

Figure 3 shows that SPAD values and NDVI derived from the GreenSeeker were correlated at all growth stages although during vegetative growth (18 DAP) there was a relatively weak correlation. During this stage, the canopy has a low number of leaves and the small leaf area affects the signal received by the sensor. This is due to the reflectance of plant canopy in visible and near infrared regions that is effected by the amount of green tissue present. The higher the absorption, the higher the value of NDVI. Both biomass and chlorophyll content affect the proximal sensing measurements above the crop (Amaral et al., 2015). This result confirms other results obtained for maize and wheat (Eitel et al., 2008; Solari et al., 2008).

The slope of the regression decreased to 45.27 at 88 DAP (maturity stage, Figure 3). This can be associated with the leaf senescence. During the senescence, at least 10% of leaves are senesced without new leaves being formed to replace them, this phenomenon indicates the beginning of canopy decline. Thus reddening of canopy leaves (Mabhaudhi & Modi, 2013). Similarly, other studies show that temporal profiles of NDVI vary every fortnight, especially at the beginning and at the end of each cycle (Junges et al., 2019). These results show that NDVI has the potential to reflect the vegetation change and canopy development corresponding to canopy photosynthetic capacity (CPC) at different crop growth stages for Bambara groundnut.

4.2 Applicability of Both Methods in Deriving the Vegetation Indices

Data from both proximal and remote sensing methods were positively correlated. The proximal measurements shown the capability to predict yield and biomass in maize, wheat and for plant breeding in field condition (Eitel et al., 2008; Solari et al., 2008; Tattaris et al., 2016). However, both systems have their own advantages and drawbacks. The present study is based on spatial and spectral relationship between GreenSeeker and UAV imagery. GreenSeeker provides low resolution data, but with higher focus on the crop and therefore little influence of the space between rows that in turn leads to limited covered area. UAV imagery provides high spatial resolution spectrometry that can be used to generate data for large numbers of plots, in a fraction of time that is required to make ground-based measurements (Gnädinger & Schmidhalter, 2017). In addition, the use of high resolution and low altitude UAVs can address other drawbacks of proximal sensing systems, such as non-simultaneous measurement of various plots, trafficability, small row spaces, plot geometries requiring specific sensor configurations, and vibrations resulting from uneven field slope (Tattaris et al., 2016). However, GreenSeeker is an active system, it is less influenced by lighting conditions. Although highly correlated, the NDVI derived from the GreenSeeker did not exhibit the same frequency distributions, which means caution should be exercised when using this data for site specific crop management.

The variance of the bandwith between both methods in deriving the vegetation indices also limits the extraction of vegetation information. The proximal sensor has broad bandwith compare to the multispectral sensor deploy on the UAV which having narrow bandwith. The proximal sensor (Greenseeker and SPAD) is using a group of bands which leads to a lack of sensitivity especially when applying the vegetation indices on the heterogeneous canopies which consists of cover crop, weed and soil in the interows. This hetegeneneous canopies will lead to the difficulties in discriminating area of interest particularly when vegetation indices respond to other vegetation such as weeds rather than area of interest (Xue & Su, 2017). However, multispectral sensor by using the UAV has the advantages of discriminating heterogenous canopies and sensitivity towards detection of spectral properties on green vegetation. Previous studies had been conducted using UAV imagery for different application such as vegetation and soil segmentation (Hassanein et al., 2018) and crop row detection (Hassanein et al., 2019). The segmentation of vegetation and soil fraction can be implemented by vegetation indices (VIs) using different spectral bands and their combinations (Mesas-Carrascosa et al., 2020). Color vegetation indices (CVIs) are used to emphasize plant greenness using common red, green and blue (RGB) sensors on UAV platforms (Torres-Sánchez et al., 2014). Similar in Jiangsu, China, the study was conducted for estimation of nitrogen where it stated that the indices from multispectral sensor images derived from UAV performed better in the most cases compared to other indices from proximal sensor (Zheng et al., 2018).

Although the other vegetation index, VARI, was not correlated well with the proximal sensing data at all growth stages, it demonstrated that UAV-based RGB imaging with visible wavebands for assessing vegetation indices was consistent with the results shown in Figure 3 (c). Previous studies have demonstrated that UAV-based RGB indices can be used for crop health monitoring and estimating growth traits in oilseed rape (McKinnon & Hoff, 2017; Wan et al., 2018). RGB images only provide limited crop physiological information. The canopy reflectance reacts strongly to the blue and green light (Amaral et al., 2015; Sulik & Long, 2015). Thus, although VARI is not a replacement for NDVI, the VARI algorithm applied to an RGB sensor can provide valuable information and also be a useful tool to assist farmers in identifying crop stress, monitoring field for crop phenotyping it is shown for sugarcane and oilseed rape (Amaral et al., 2015; McKinnon & Hoff, 2017; Wan et al., 2018).

4.3 Advantages, Limitations and Future Work

Aerial based imagery for crop phenotyping is an efficient, cost-effective and suitable technique for complex environments. It can help with rapid identification of growth information with high resolution data. Similar studies were conducted for crop phenotyping (Liebisch et al., 2015; Tattaris et al., 2016) and precision agriculture (Candiago et al., 2015b) which they provide a low-cost approach in order to obtain accurate results for phenotyping in the field environment. However, it will be more useful if hyperspectral sensors could also be used with the aerial-based sensors. This is the key limitation in current crop phenotyping, which limits the amount of information that can be derived from these platforms. In fact, by having hyperspectral data which combine properties of imaging and spectroscopy (Kumar et al., 2016) high resolution spectral vegetation indices such as soil adjusted vegetation indices (SAVI), enhance vegetation indices (EVI) etc. along with crop parameters such as leaf area index (LAI), soil fertility, soil moisture, level of crop stress, yield prediction, biomass can be estimated.

Further study is required to evaluate the capability of data fusion between proximal sensors (SPAD, GreenSeeker) with canopy temperature or any other related data with aerial-based sensors (UAV imagery) to improve monitoring of other crop growth-related traits in field observations. Fusion of data from multiple sensors could provide more information for crop phenotyping which may be especially helpful for underutilised crop studies.

Finally, collecting low cost UAV data (Wang et al., 2018) and linking this data to the global satellite remote sensing databases, in a consistent format that can be shared with other stakeholders working on neglected and underutilised crops will help with the inclusion of these crops in the global crop monitoring projects such as GEOGLAM (Becker-Reshef et al., 2018) for yield forecasting and crop monitoring.

5. Conclusion

Developing rapid crop phenotyping methods for neglected and underutilised crops is an important step towards ensuring food and nutrition security in a warming world. In this experiment, we found positive relationship between different sensors used for phenotyping and determining developmental stages for bambara groundnut, a neglected and underutilised crop. The results show that there is a potential application for UAV based crop phenotyping in the field. However, there is still a need for validation of results in different environments using different genotypic varieties of bambara groundnut before it can successfully be used for predicting growth stages.

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