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Initiation of Unmanned Aerial System (UAS) Phenotyping of Plant Height and Canopy Width in Guar

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Abstract

The use of the Unmanned Aerial System (UAS) has attracted scientific attention because of its potential to generate high-throughput phenotyping data. The application of UAS to guar phenotyping remains limited. Guar is multi-purpose legume species. India and Pakistan are the world's top guar producers. The U.S. is the world guar largest market with an import value of >$1 billion annually. The objective of this study was to test the feasibility of UAS phenotyping of plant height and canopy width in guar. The UAS data were collected from a field plot of 10 guar accessions on July 7, 2021, and September 27, 2021. The study was organized in a Randomized Complete Block Design (RCBD) with 3 blocks. A total of 23 Vegetation Indices (VIs) were computed. The analysis of variance showed significant genotypic effects on plant weight ($p < 0.05$) and canopy width ($p < 0.05$) during the first flight, and only on plant height ($p < 0.05$) during the second flight. Genotyping effects on most VIs were significant for both flights ($p < 0.05$). Normalized Difference Vegetation Index (NDVI) and Red Edge Normalized Difference Vegetation Index (NDRE) were significantly and highly correlated with plant height ($r = 0.74$) and canopy width ($r = 0.68$). The results will be of interest in developing high throughput phenotyping approach for guar breeding.

Keywords

Guar, Unmanned Aerial System, Vegetation Indices, Phenotyping, Plant Height, Canopy Width

1. Introduction

Guar, [*Cyamopsis tetragonoloba* (L.)], is a diploid legume species ($2n = 2x = 14$) belonging to the family Fabaceae [1]. The world estimate of guar production is 3.4 million metric tons per year, with India and Pakistan accounting for more than 80% of this production (https://www.fas.usda.gov/). The U.S. is the largest guar market in the world and the country imports guar valued at more than $1 billion annually (http://lubbock.tamu.edu/). Guar is a multipurpose legume. Guar seeds have a significant amount of galactomannan known as guar gum content [2]. This product is used in the oil drilling industry, used as a food/feed ingredient, and has several cosmetic and pharmaceutical applications [3]. The immature guar pods can be consumed as vegetables [4]. Guar is also a good rotational crop because it fixes atmospheric nitrogen via symbiosis with soilborne bacteria [5], which contributes to soil fertility. Additionally, guar is also used as green manure or forage. Guar forage yield ranges between 2.9 and 4.7 Mg/ha (dry matter) and had 162 to 225 g crude protein and 606 to 712 g *in vitro* digestible dry matter per kg of dry forage [6].

Guar is a drought- and heat-tolerant crop that is well-adapted in water-limited areas [7]. In the U.S., guar is primarily grown in the southern part of the country with Texas and Oklahoma being the top guar-producing states [8]. In this region, guar is planted between mid-May and mid-July, and the first freeze occurring in early fall defoliates guar leaves, allowing for the mechanical harvest of guar seeds [6]. In the U.S., only 9 guar varieties have been released in the past four decades [9]. As a result, farmers are limited to few guar variety options. Therefore, innovative breeding strategies to accelerate guar breeding are required.

Unmanned Aerial System (UAS) has recently attracted attention because it can be used as a high-throughput phenotyping tool to accelerate plant breeding [10]. UAS phenotyping consists of collecting field plot images using a sensor mounted on a drone. These images are analyzed to extract structural and vegetation indices, which will be used as a selection and prediction tool for crop traits in plant breeding [11]. This technology has been successfully used to estimate agronomic traits such as plant height and grain yield in maize [12], and maturity and grain yield in soybean [13]. This technology has also been used to identify drought and heat stress affecting crops [14].

Plant height and canopy width are important breeding traits because they dictate plant architecture that will have practical agronomic applications [15]. For example, spreading-type plants can be used as cover crops, whereas more erect-type plants are suitable for mechanical harvest [16] [17]. Plant height and canopy width can be easily phenotyped when a limited number of genotypes are evaluated. However, each year, public plant breeders evaluate thousands of lines, and private plant breeding companies deal with millions of breeding plots. In this context, the development of high-throughput phenotyping technology to evaluate plant height and canopy width will be needed. To the best of our knowledge, there is no study aimed to investigate the use of UAS in phenotyping guar traits. Therefore, the objectives of this study were to assess possible genetic variation in vegetation indices collected using UAS and to identify vegetation indices that best correlate with plant height and canopy width in guar.

2. Materials and Methods

2.1. Materials and Growing Conditions

Ten guar accessions were used for this study. Seven accessions were advanced breeding lines from Texas A&M AgriLife Research. These breeding lines had different plant architectures. Two accessions were released Texas A&M AgriLife Research varieties, which were "Kinman" [18] and "Lewis" [19]. The other accession was "Santa Cruz" which was a released variety from Texas Tech University [20].

The experiment was established at Locket, TX. The research plot is a property managed by the Texas A&M AgriLife & Extension Center, Vernon, TX. The study was conducted between May 15, 2021, and September 30, 2021. Weeds were removed either manually or by running row crop sweeps. The research plot was rainfed.

Prior to planting, seeds were mixed with a certified organic *Bradyrhizobium* powder inoculant. Seeds of each guar accession were sown on a four-row plot on May 15, 2021, using a cone planter. Row spacing was 40 inches and plot length was 12 feet. The seeding rate was 10 seeds/feet. The study was organized in a randomized complete block design with three blocks. An alley of 4 feet was used to separate each block. The experimental unit was defined by the four-row plot seeds of each guar accession were sown.

2.2. Data Collection

The Unmanned Aerial System (UAS) data were collected using a RedEdge-MX sensor (Micasense Inc., Seattle, WA, USA; http://www.micasense.com/) that was mounted on a Matrice 200 Series (SZ DJI Technology Co. Ltd., Shenzhen, China). This sensor resolution is 1280 × 960 pixels. It captured five narrow high resolutions spectral bands: blue (475 × 20 nm width), green (560 × 20 nm width), red (668 × 10 nm width), red-edge (717 × 10 nm width), and near-infrared (NIR) (840 × 40 nm width) [21]. Two flights were conducted for this study. The first flight was conducted on 07/07/2021 corresponding to late vegetative stage, where differences in guar plant architecture can be easily identified. The second flight was performed on 09/27/2021 when senescence began in some plants. Five square wooden plaques served as Ground Control Points (GCPs). Four GCPs were established on each field corner and the remaining one was placed inside the field. GCPs were marked with field flags so that the GCP locations were used for the subsequent flight. Flight parameters were the following: flight height = 30 feet, drone speed = 15 miles/hour, overlap = 85% front 85% back, and angle = 90 degrees. Prior to drone flying, sensor calibration was conducted by taking a picture of the MicaSense calibrated reflectance panel at about 3 feet above this panel. Plant height and canopy width were collected from ten randomly plants from each middle row.

2.3. Image Data Processing

The raw data images were transferred from a Secure Digital (SD) card to computer. The first step was image stitching that was conducted using the Pix4D Mapper software program (Pix4D SA, Prilly, Switzerland). The final output was the creation of orthomosaic images. For each image collected, there were five files corresponding to the five multispectral bands. Each file was processed as a ".tif" file in Pix4D Mapper. The following parameters were used during image orthomosaicing in Pix4D Mapper: Output Coordinate System was Auto Detected, Processing Options Template was Standard/Ag Multispectral. Then, the following steps were performed: processing of initial images, creation of points and mesh creation, and establishment of digital surface model (DSM), orthomosaic, and index.

The orthomosaic images were imported to QGIS 3.22.3 (https://www.qgis.org/en/site/) to extract the mean values of each spectral band at the plot level. The blue, red, green, rede-edge, NIR, and NDVI maps from Pix4D Mapper were used for the data extraction in QGIS 3.22.3. Data were extracted from the two middle rows for each experimental unit. **Table S1** shows the 23 vegetation indices.

2.4. Data Analysis

Analyze of variance (ANOVA) was conducted to assess the genotypic effects on the mean value of each spectral band and the NDVI and chlorophyll data used for ground truthing. ANOVA was performed using JMP Pro 16 ® (SAS Institute Inc., Cary, NC, USA). The statistical model for ANOVA was the following.

$$Y_{ij} = \mu + G_i + B_j + \epsilon_{ij}$$

where $i = 1, 2, \ldots, 10$ and $j = 1, 2, 3$.

In the above equation, Y_{ij} was the mean value of the spectral band/ground truthing data for NDVI and chlorophyll data corresponding to i^{th} genotype (fixed effect) that was located in the j^{th} block (random effect). ϵ_{ij} represented the experimental error associated with the ij^{th} observation. ANOVA was conducted separately for the spectral band data that were extracted from different plot numbers.

Pearson's correlation coefficients between all data were computed using JMP Pro 16® (SAS Institute Inc., Cary, NC, USA). These coefficients were used to assess the accuracy of UAS phenotyping of canopy width, chlorophyll, NDVI, and plant height in guar.

3. Results

3.1. Descriptive Statistics for Plant Height, Canopy Width, and Vegetation Indices

Table 1 shows the descriptive statistics for plant height, canopy width, and

Table 1. LS Means and standard deviation (Std, n = 30) for plant height, canopy width, and vegetation indices.

Parameters	Flight 1		Flight 2	
	LS Means	Std	LS Means	Std
Plant height (cm)	37.82	6.58	49.97	5.59
Canopy width (cm)	33.42	5.44	36.87	4.99
RCC	0.43	0.04	0.53	0.06
GCC	0.57	0.04	0.46	0.06
ExG	299.08	46.61	176.65	81.81
ExG2	0.72	0.11	0.39	0.18
ExR	0.02	0.09	0.28	0.14
ExGR	0.69	0.2	0.11	0.32
GRVI	0.15	0.07	−0.07	0.12
VDVI	0.45	0.06	0.26	0.11
VARI	0.15	0.07	−0.07	0.12
MGRVI	0.29	0.14	−0.13	0.22
CIVE	−112.97	20.54	−59.01	36.04
VEG	7.6	0.87	5.45	1.09
WI	−3.25	5.51	2.55	33.33
NDVI	0.42	0.04	0.96	0.04
NDRE	0.42	0.04	0.96	0.01
GNDVI	−1	0.03	−1	0.03
EVI2	−1.04	0.01	−1.04	0.01
SRRE	2.46	0.21	54.73	2.88
MSR	−1	0.01	−1	0.02
CIG	−1	0.03	−1	0.01
CIRE	1.46	0.21	53.73	2.88
MTCI	0.2	0.01	0.4	0.01
RTVIC	2405.84	165.4	2073.91	353.96

vegetation indices. The average plant height during the first flight was 37.82 cm. The average plant height during the second flight was 49.97 cm. Canopy width increased from 33.42 cm to 36.87 cm from the first flight to the second flight.

Overall, the average values of the 23 vegetation indices from the second flight were numerically higher that the ones from first flight. The most significant change between the two flights was found for SRRE and CIRE where a 50-fold change was identified. During the first flight, the average values of RCC, GCC, ExG, ExG2, ExR, ExGR, GRVI, VDVI, VARI, MGRVI, CIVE, VEG, WI, NDVI, NDRE, GNDVI, EVI2, SRRE, MSR, CIG, CIRE, MTCI, and RTVIC were 0.43, 0.57, 299.08, 0.72, 0.02, 0.69, 0.15, 0.45, 0.15, 0.29, −112.97, 7.60, −3.25, 0.42, 0.42, −1.00, −1.04, 2.46, −1.00, −1.00, 1.46, 0.20, and 2405.84, respectively. During the second flight, the average values of RCC, GCC, ExG, ExG2, ExR, ExGR, GRVI, VDVI, VARI, MGRVI, CIVE, VEG, WI, NDVI, NDRE, GNDVI, EVI2, SRRE, MSR, CIG, CIRE, MTCI, and RTVIC were 0.53, 0.46, 176.65, 0.39, 0.28, 0.11, −0.07, 0.26, −0.07, −0.13, −59.01, 5.45, 2.55, 0.96, 0.96, −1.00, −1.04, 54.73, −1.00, −1.00, 53.73, 0.40, and 2073.91.

3.2. Genotypic Effects on Plant Height, Canopy Width, and Vegetation Indices

Table 2 summarizes the analysis of variance showing the effects of the 30 guar genotypes on plant height, canopy width, and vegetation indices. During the first flight, the effect the guar genotypes on plant height, canopy wight, RCC, GCC, ExG, ExG2, ExR, ExGR, GRVI, VDVI, VARI, MGRVI, CIVE, VEG, WI, NDVI, NDRE, GNDVI, EVI2, SRRE, MSR, CIG, CIRE, and MTCI was statistically significant. However, all genotypes were not statistically different in terms of RTIVIC, indicating this parameter might not be suitable to identify genotypic differences in guar during earlier season growth stage. For the second flight, genotypic effects were statistically significant for plant height and all 23 vegetation indices. The 30 guar genotypes were not statically different in terms of canopy width.

3.3. Correlation Analysis

Table 3 shows the Pearson's correlation coefficients between plant height, canopy width, and vegetation indices at each flight. During the first flight, plant height was significantly correlated with RCC (r = −0.47), GCC (r = 0.47), ExG (r = 0.48), ExG2 (r = 0.47), ExR (r = −0.47), ExGR (r = 0.47), GRVI (r = 0.47), VDVI (r = 0.47), VARI (r = 0.47), MGRVI (r = 0.47), CIVE (r = −0.48), VEG (r = 0.47), NDVI (r = 0.74), NDRE (r = 0.74), EVI2 (r = 0.44), SRRE (r = 0.62), MSR (r = 0.44), CIRE (r = 0.62), MTCI (r = 0.47), and RTVIC (r = 0.49). The following vegetation indices, NDVI (r = 0.74), NDRE (r = 0.74), SRRE (r = 0.62) and CIRE (r = 0.62) were best correlated with plant height during the first flight. Canopy width was significantly correlated with WI (r = 0.70), NDVI (r = 0.68), NDRE (r = 0.68), EVI2 (r = 0.39), SRRE (r = 0.38), MSR (r = 0.39), CIRE (r = 0.48), MTCI (r = 0.39), and RTVIC (r = 0.32). WI (r = 0.70) and NDVI (r = 0.68) were best correlated with canopy width during the first flight. However, most vegetation indices that were significantly correlated with plant weight were not significantly correlated with canopy width. This indicates that the choice of vegetation indices should be taken into account when using UAS data for germplasm evaluation.

During the second flight, plant RCC (r = 0.39), GCC (r = −0.39), ExG (r = −0.38), ExG2 (r = −0.39), ExR (r = −0.39), ExGR (r = −0.39), GRVI (r = −0.39),

Table 2. Genotypic effects on plant height, canopy width, and vegetation indices.

Flight 1	Flight 2
Plant height*	Plant height*
Canopy width*	Canopy width
RCC*	RCC*
GCC*	GCC*
ExG	ExG*
ExG2*	ExG2*
ExR*	ExR*
ExGR*	ExGR*
GRVI*	GRVI*
VDVI	VDVI*
VARI*	VARI*
MGRVI*	MGRVI*
CIVE	CIVE*
VEG*	VEG*
WI	WI
NDVI*	NDVI*
NDRE*	NDRE*
GNDVI	GNDVI*
EVI2*	EVI2*
SRRE*	SRRE*
MSR*	MSR*
CIG	CIG*
CIRE*	CIRE*
MTCI*	MTCI*
RTVIC	RTVIC*

*Incidates a significant genotypic effect ($p < 0.05$) on the responses.

Table 3. Pearson's correlation coefficients between vegetation indices, plant height, and canopy width.

Vegetation indices	Flight 1		Flight 2	
	Plant height	Canopy width	Plant height	Canopy width
RCC	**−0.47**	−0.35	**0.39**	−0.19
GCC	**0.47**	0.35	**−0.39**	0.19
ExG	**0.48**	0.34	**−0.38**	0.18

Continued

ExG2	**0.47**	0.35	**−0.39**	0.19
ExR	**−0.47**	−0.35	**0.39**	−0.19
ExGR	**0.47**	0.35	**−0.39**	0.19
GRVI	**0.47**	0.35	**−0.39**	0.19
VDVI	**0.47**	0.34	**−0.38**	0.19
VARI	**0.47**	0.35	**−0.39**	0.19
MGRVI	**0.47**	0.35	**−0.39**	0.19
CIVE	**−0.48**	−0.34	**0.38**	−0.18
VEG	**0.47**	0.35	**−0.39**	0.18
WI	0.17	**0.7**	−0.18	0.07
NDVI	**0.74**	**0.68**	−0.34	0.19
NDRE	**0.74**	**0.68**	−0.34	0.19
GNDVI	−0.06	0.19	**0.39**	−0.08
EVI2	**0.44**	**0.39**	−0.31	0.26
SRRE	**0.62**	**0.38**	−0.34	0.2
MSR	**0.44**	**0.39**	−0.21	0.34
CIG	−0.06	0.19	**0.39**	−0.08
CIRE	**0.62**	**0.48**	−0.34	0.2
MTCI	**0.47**	**0.39**	−0.21	0.34
RTVIC	**0.49**	**0.32**	**−0.37**	0.17

Bold indicates a significant correlation at $p < 0.05$.

VDVI ($r = -0.38$), VARI ($r = -0.39$), MGRVI ($r = -0.39$), CIVE ($r = 0.38$), VEG ($r = -0.39$), GNDVI ($r = 0.39$), CIG ($r = 0.39$), and RTVIC ($r = -0.37$). However, none of the vegetation indices (NDVI, NDRE, SRRE, and CIRE) that were best correlated with plant height during the first flight were correlated with plant height during the second flight. This indicates the need for temporal analysis of vegetation indices to model plant height in guar. In addition, none of 23 vegetation indices collected from the second flight were significantly correlated with canopy width. The highest correlation coefficients were identified between canopy and EVI2 ($r = 0.26$), canopy width and MSR ($r = 0.340$), and canopy width and MTCI ($r = 0.34$).

4. Discussion

To the best of our knowledge, this is one of the earliest reports investigating the feasibility of unmanned aerial system (UAS) for the phenotyping of plant height and canopy width in guar. UAS phenotyping consists of collecting filed plot images using a sensor mounted on a drone [10], These images were used to extract mean values of spectral bands (red, green, blue) at the field plot level, and vegetations indices were computed using spectral bands value [10]. For UAS phenotyping, the vegetation indices were used to predict agronomic traits such as plant height, canopy coverage, pod set, biomass, yield, etc. [11]. The UAS data can also be used to identify crop stress drought, heat, disease, etc. [14].

Overall, this study identified guar genotypic differences based on vegetation indices. This result indicates that drone image data can be used to develop UAS-based fingerprinting for guar cultivars. The vegetation indices from the UAS data can be modelled into structural vegetation indices specific to each guar accession. These structural vegetation indices can assist with identifying specific guar genotypes. Maimaitijiang *et al.* (2017) [11] also reported that UAS data can be used to differentiate between various soybean cultivars. These findings suggest that UAS-based phenotyping can help plant breeders identify varieties faster and more reliably. In addition, the results showed that Normalized Difference Vegetation Index (NDVI) and Red Edge Normalized Difference Vegetation Index (NDRE) were best correlated with both plant height and canopy coverage in this study, indicating that these parameters can be used when predicting these agronomic traits in guar. Similar results were also reported in crops such as soybean [14]. In future projects, more guar lines will be evaluated to build machine learning models to better estimate the agronomics of guar. This study will provide a strong foundation for future UAS-related work in establishing high-throughput phenotyping of guar.

5. Conclusion

Overall, genotypic effects on plant height were significant during both flights. However, genotypic effects on canopy width were significant only during the first flight. During the first flight, genotypic effects on 22 vegetation indices, except Core Red Edge Triangular Vegetation Index (RTVIC), were significant. During the second flight, genotypic effects on all 23 vegetation indices were significant. Normalized Difference Vegetation Index (NDVI) and Red Edge Normalized Difference Vegetation Index (NDRE) were best correlated with both plant height and canopy width during the first flight.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplement

Table S1. List of vegetation indices.

Vegetation Index	Abbreviation	Equation	References
Red Chromatic Coordinate Index	RCC	R/(R + G + B)	Woebbecke *et al.* (1995)
Green Chromatic Coordinate Index	GCC	G/(R + G + B)	Woebbecke *et al.* (1995)
Excess Green Index	ExG	(2G – B – R)	Woebbecke *et al.* (1995)
Excess Green Index 2	ExG2	(2G – B – R)/(R + G + B)	Woebbecke *et al.* (1995)
Excess Red Index	ExR	(1.4R – G)/(R + G + B)	Meyer *et al.* (1999)
Excess Green minus Excess Red Index	ExGR	(ExG2 – ExR)	Meyer *et al.* (2006)
Green Red Vegetation Index	GRVI	(G – R)/(G + R)	Hunt *et al.* (2005)
Visible Band Difference Vegetation Index	VDVI	(2G – R – B)/(2G + R + B)	Xiaoqin *et al.* (2015)
Visible Atmospherically Resistant Index	VARI	(G – R)/(G + R – B)	Gitelson *et al.* (2002)
Modified Green Red Vegetation Index	MGRVI	(G^2 – R^2)/(G^2 + R^2)	Bendig *et al.* (2015)
Color Index of Vegetation	CIVE	(0.441R – 0.881G + 0.385B + 18.747)	Kataoka *et al.* (2003)
Vegetative Index	VEG	G/(R^0.667*B^0.334)	Hague *et al.* (2006)
Woebbecke Index	WI	(G – B)/(R – G)	Woebbecke *et al.* (1995)
Normalized Difference Vegetation Index	NDVI	(NIR – R)/(NIR + R)	Rouse *et al.* (1974)
Red Edge Normalized Difference Vegetation Index	NDRE	(NIR – RE)/(NIR + RE)	Gitelson and Merzlyak (1994)
Green Normalized Difference Vegetation Inxed	GNDVI	(NIR – G)/(NIR + G)	Gitelson and Merzlyak (1994)
Enhanced Vegetation Index 2	EVI2	2.5 × (NIR – R)/(NIR + 2.4 × R + 1)	Huete *et al.* (2002)
Red Edge Simple Ratio	SRRE	(NIR)/(RE)	Gitelson *et al.* (2005)
Modified Simple Ratio	MSR	(NIR/R – 1)/[((NIR/R) + 1))^(1/2)]	Wu *et al.* (2008)
Green Chlorophyll Index	CIG	(NIR/G) – 1	Gitelson *et al.* (2003)
Red Edge Chlorophyll Index	CIRE	(NIR/RE) – 1	Gitelson *et al.* (2003)
Medium Resolution Imaging Spectrometer Terrestrial Chlorophyll Index	MTCI	(NIR – RE)/(RE + R)	Dash and Curran (2004)
Core Red Edge Triangular Vegetation Index	RTVIC	100(NIR – RE) – 10(NIR – G)	Nicolas *et al.* (2010)