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Use of Unmanned Aerial System (UAS) Phenotyping to Predict Pod and Seed Yield in Organic Peanuts

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Abstract

Peanut (*Arachis hypogaea* L.) is a highly nutritious food that is an excellent source of protein and is associated with increased coronary health, lower risk of type-2 diabetes, lower risk of breast cancer and a healthy profile of inflammatory biomarkers. The domestic demand for organic peanuts has significantly increased, requiring new breeding efforts to develop peanut varieties adapted to the organic farming system. The use of unmanned aerial system (UAS) has gained scientific attention because of the ability to generate high-throughput phenotypic data. However, it has not been fully investigated for phenotyping agronomic traits of organic peanuts. Peanuts are beneficial for cardio system protection and are widely used. Within the U.S., peanuts are grown in 11 states on roughly 600,000 hectares and averaging 4500 kg/ha. This study's objective was to test the accuracy of UAS data in the phenotyping pod and seed yield of organic peanuts. UAS data was collected from a field plot with 20 Spanish peanut breeding lines on July 07, 2021 and September 27, 2021. The study was a randomized complete block design (RCBD) with 3 blocks. Twenty-five vegetation indices (VIs) were calculated. The analysis of variance showed significant genotypic effects on all 25 vegetation indices for both flights ($p < 0.05$). The vegetation index Red edge (RE) from the first flight was the most significantly correlated with both pod ($r = 0.44$) and seed yield ($r = 0.64$). These results can be used to further advance organic peanut breeding efforts with high-throughput data collection.

Keywords

Peanut, Unmanned Aerial System, Vegetation Indices, Phenotyping, Pod Yield, Seed Yield

1. Introduction

Peanuts (*Arachis hypogaea* L.) originated from South America and are now grown in every continent but Antarctica. Within the U.S. this crop is grown in Southeastern US on about 600 thousand hectares, averaging 4500 kg/ha [1]. Peanuts were shown to have health benefits that protect cardio systems and have an overall healthy nutrient profile. They are rich in unsaturated fatty acids in the form of monosaturated fatty acids. These healthy fats found in peanuts and tree nuts contribute to the prevention of coronary heart disease and diabetes when consumed frequently. Consumption is also reported to lower cholesterol. Peanuts and tree nuts are also good sources of vegetable protein and fiber, potassium, calcium, magnesium, tocopherols, phytosterols, phenolic compounds, resveratrol, and arginine [2]. Studies show that peanuts, peanut butter, and peanut oil significantly reduce heart disease risk when consumed daily [2].

As the demand for organic products in the U.S. increased, the same trend occurred for organic peanuts and processed peanut foods [3] [4] [5] [6]. Consumption of organic peanut products is health promoting because it provides high quality nutrients required for human health. This is considered the fastest-growing sector in the peanut industry and there was a supply shortage of nearly 5000 tons of organic peanut in the U.S. [4] [5] [7] [8]. It is predicted that if the demand for organic peanuts increases like that of other organic products, then there will be a need for 125,000 tons in 15 years [6].

Economic incentives exist for organic peanut production, but there are additional costs and lower yields experienced in organic systems due to certification costs, weed management, and diseases with restricted chemical use. During the time of a study conducted by Wann *et al.* [9], certified organic runner-type peanut contract prices were reaching $1100/Mg compared to $390/Mg for conventional runner-type peanuts. Numerous runner-type peanut cultivars with excellent disease resistance and productive capacities in organic production have been released [10] [11] [12].

Manual phenotyping for traits that can predict pod yield, disease resistance, and drought tolerance has been used for many years by breeders. For example, early biomass accumulation and leaf area index are shown to be correlated with pod yield under drought stress in peanuts [13] [14] [15] [16]. In addition, canopy coverage and structure can affect both disease resistance and water use, which in turn affect yield [17] [18]. Previous studies showed that reduced biomass, caused by drought stress, resulted in significant pod yield losses [19] [20] [21].

Direct manual phenotyping of traits for pod yield is used extensively but is time consuming, expensive, and challenging to do on a large scale. However, utilizing unmanned aerial system (UAS) to phenotype traits for predicting peanut yield would be of interest [22]. This is due to UAS utilization allowing the gathering of precise and very detailed imagery for making breeding decisions such as canopy height and canopy volume in a short time with minimal labor involved [23].

Unmanned aerial vehicles (UAVs) have been used in peanuts to monitor seedling emergence rate, variety selection, maturity variability on irrigated and rainfed fields, early detection of bacterial wilt, drought tolerance phenotyping, and canopy height measurements [23]-[29]. However, this has not been done for a certified organic field to predict peanut yield, which would be a valuable tool for organic peanut breeders. Therefore, the objective of this study was to assess the accuracy of UAS data to phenotype pod and seed yield using organic Spanish-type peanuts which can be identified and released in the future.

2. Materials and Methods

2.1. Plant Materials and Growing Conditions

A total of 16 Spanish peanut breeding lines and 4 commercial checks from Texas A&M AgriLife-Research, TX, Stephenville, provided by Dr. John Cason, were used for this study. The 20 lines were planted in a certified organic plot with MfA soil at Locket, TX. No synthetic chemical fertilizers or pesticides were applied as per utilizing an organic management system. Weeds were controlled manually or with row crop sweeps. The plot was rainfed and irrigation (2 inches/10 - 14 days) was conducted when rainfall was not sufficient. Before planting, Exceed Superior Legume Inoculant was mixed with the seeds. An Almaco four row planter with cone planters was used to plant the study. The test was planted in a Compete Randomized Block Design with 3 replications. Seeds were planted on May 15, 2021, on 1.016 meters spacing in 2 row 3.048 meter plots at a rate of 100 seeds per plot. At the end of the growing season, peanuts were dug with a 2 row KMC digger and allowed to dry in the field. Once dry, peanuts were threshed with a Kincaid small plot thrasher to maintain purity between samples.

After threshing, samples were weighed to get total weight, then a random 250 g sample weight was taken to Texas A&M Foundation Seed. Here pods were put through a pre-sizer to separate them into small, medium, and large. Following this each size was placed in a compartment of a peanut sheller and the samples were graded following the USDA grading guidelines for Spanish peanuts. The ELK, medium, and #1 peanut seeds were put together and weighed to get a total seed weight for each line as well.

2.2. Data Collection

UAS data was conducted by using a RedEdge-MX sensor (Micasense, Inc., Seattle, WA, USA; http://www.micasense.com/) mounted to a Matrice M200 Series (SZ DJI Technology Co. Ltd., Shenzhen, China) drone. The sensor has a resolution of 1280 × 960 pixels and captures five narrow high resolution spectral bands: blue (475 × 20 nm width, red (668 × 10 nm width), red-edge (717 × 10 nm width), green (560 × 20 nm width), and near-infrared [NIR (840 × 40 nm width). Two flights were conducted for this study. The first flight was on July 07, 2021, and the second flight was on September 27. 2021. Five ground control points (GCPs), 30.48 cm × 30.48 cm square wooden plaques, were used with one in the middle of the field and the remaining four on each corner of the organic field. Each flight was done at a height of 30 meters above ground level, at a speed of 3 meters/second, with pictures taken one per second with a 90-degree camera angle, and pictures overlapping 85% in the front and back. Sensor calibration occurred at takeoff via taking a picture of the MicaSense calibrated reflectance panel from about 1 meter away without any shadows.

2.3. Image Data Processing

Flight images were transferred from a Secure Digital (SD) card to a computer. Image stitching was then conducted using Pix4D Mapper software (Pix4D SA, Prilly Switzerland) which resulted in the creation of orthomosaic images to be used for analysis. Each image collected had five files corresponding to each of the five spectral bands. Within Pix4D Mapper, each image was processed as a ".tif" file with the following parameters used: output coordinate system was auto detected, and processing options template was Standard/Ag Multispectral. Following this, initial images were processed; creating points and mesh creation, as well as establishing a digital surface model (DSM), orthomosaic, and index.

Finalized orthomosaic images were loaded into QGIS 3.22.3 (https://www.qgis.org/en/site/) to extract the mean values of each spectral band (green, red, and blue) at the plot level [30]. The following maps were used for data extraction in QGIS from Pix4D Mapper: NIR, NDVI, blue, red, green, and red-edge. Data was extracted from each peanut and **Table S1** shows the 25 vegetation indices constructed in this study using the mean values which were then used to predict agronomic traits such as canopy height and width in guar or pod and seed yield in this study [31] [32].

2.4. Data Analysis

Analysis of variance (ANOVA) was calculated to determine genotypic effects on the mean values of each spectral band, pod yield, and seed yield. JMP Pro 16 (SAS Institute Inc., Cary, NC, USA) was used to conduct ANOVA. The below model was used for ANOVA.

$$Y_{ij} = u + G_i + B_j + E_{ij} \text{ where } i = 1, 2, \cdots, 10 \text{ and } j = 1, 2, 3$$

where Y_{ij} is the mean value of the spectral band/pod yield/seed yield which corresponds to the i^{th} genotype (fixed effect) which was in the j^{th} block as a random effect. E_{ij} represented experimental error associated with the ij^{th} observation.

Pearson's correlation coefficients were also calculated between all data using JMP Pro 16® (SAS Institute Inc., Cary, NC, USA) and subsequently used to assess the accuracy of UAS phenotyping compared to manually collected data in peanuts.

3. Results

3.1. Descriptive Statistics for Pod Yield, Seed Yield, and Vegetation Indices

Descriptive statistics for vegetation indices, pod yield, and seed yield are in Table 1. The average pod yield was 1976.66 pound per acre (lb/ac), with a standard

Table 1. LS Means and standard deviation (std, n = 60) for pod yield, seed yield, and vegetation indices for organic peanuts.

Agronomic traits	LS Means		Std	
Pod yield (lb/ac)	1976.66		1475.5	
Seed yield (lb/ac)	860.35		773.66	
Vegetation indices	Flight 1		Flight 2	
	LS Means	Std	LS Means	Std
RE	0.158	0.01	0.988	0.002
NIR	0.421	0.025	0.988	0.002
NDVI	0.855	0.046	0.988	0.002
RCC	0.4	0.023	0.4	0.022
GCC	0.6	0.023	0.6	0.022
ExG	331.318	28.45	334.154	24.329
ExG2	0.801	0.069	0.801	0.065
ExR	−0.041	0.056	−0.041	0.052
ExGR	0.841	0.125	0.842	0.117
GRVI	0.201	0.046	0.201	0.043
VDVI	0.5	0.037	0.5	0.035
VARI	0.201	0.046	0.201	0.043
MGRVI	0.384	0.086	0.384	0.08
CIVE	−127.116	12.537	−128.364	10.722
WI	−2.651	3.307	−3.301	1.688
NDVI2	0.455	0.026	0	0
NDRE	0.455	0.026	0	0
GNDVI	−0.997	0	−0.992	0
EVI2	−1.035	0.001	−1.03	0.001
SRRE	2.681	0.175	1	0
MSR	−0.996	0	−0.991	0.001
CIG	−0.998	0	−0.996	0
CIRE	1.681	0.175	0	0
MTCI	0.002	0	0	0
RTVIC	2505.761	97.764	2495.568	70.023

deviation of 1475.50 lb/ac. The average seed yield was 860.35 lb/ac, with a standard deviation of 773.66 lb/ac. In this study, a total of 25 vegetation indices were computed. The NDVI2, NDRE, SRRE, and CIRE averages were higher in flight 1 than flight 2, while RE and NIR averages in flight 2 were higher than flight 1.

3.2. Genotypic Effects on Pod Yield, Seed Yield, and Vegetation Indices

The analysis of variance is summarized in Table 2 for the 20 lines listing the

Table 2. Genotypic effects on pod yield, seed yield, and vegetation indices of organic peanuts.

Agronomic traits	
Pod yield*	
Seed yield*	
Vegetation indices	
Flight 1	**Flight 2**
RE*	RE*
NIR*	NIR*
NDVI*	NDVI*
RCC*	RCC*
GCC*	GCC*
ExG*	ExG*
ExG2*	ExG2*
ExR*	ExR*
ExGR*	ExGR*
GRVI*	GRVI*
VDVI*	VDVI*
VARI*	VARI*
MGRVI*	MGRVI*
CIVE*	CIVE*
WI*	WI*
NDVI2*	NDVI2*
NDRE*	NDRE*
GNDVI*	GNDVI*
EVI2*	EVI2*
SRRE*	SRRE*
MSR*	MSR*
CIG*	CIG*
CIRE*	CIRE*
MTCI*	MTCI*
RTVIC*	RTVIC*

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

significant effects of genotype on pod yield, seed yield, and vegetation indices. Peanut genotypes had a statistically significant effect on pod and seed yield. For both flights, genotypic effects were significant for RE, NIR, NDVI, RCC, GCC, ExG, ExG2, ExR, ExGR, GRVI, VDVI, VARI, MGRVI, CIVE, WI, NDVI2, NDRE, GNDVI, EVI2, SRRE, MSR, CIG, CIRE, MTCI, and RTVIC. These indicate that vegetation indices can be used to develop phenotypic fingerprinting for peanuts.

3.3. Correlation Analysis

Pearson's correlation coefficients between pod yield, seed yield, and vegetation indices for each flight are in Table 3. During the first flight, pod yield was significantly correlated with RE ($r = 0.44$), NDVI 2 ($r = -0.37$), NDRE ($r = -0.37$),

Table 3. Pearson's correlation coefficients between vegetation indices, pod yield, and seed yield.

Vegetation indices	Flight 1		Flight 2	
	Pod yield	Seed yield	Pod yield	Seed yield
RE	**0.44***	**0.64**	0.1	−0.15
NIR	0.03	0.2	0.1	−0.15
NDVI	−0.03	−0.03	0.1	−0.15
RCC	0.03	0.06	0.06	0.23
GCC	−0.03	−0.06	−0.06	−0.23
ExG	−0.02	−0.06	−0.08	−0.23
ExG2	−0.03	−0.06	−0.06	−0.23
ExR	0.03	0.06	0.06	0.23
ExGR	−0.03	−0.06	−0.06	−0.23
GRVI	−0.03	−0.06	−0.06	−0.23
VDVI	−0.02	−0.06	−0.06	−0.23
VARI	−0.03	−0.06	−0.06	−0.23
MGRVI	−0.02	−0.06	−0.06	−0.22
CIVE	0.02	0.06	0.08	0.23
WI	−0.08	0	−0.02	−0.06
NDVI2	**−0.37**	**−0.4**	0	0
NDRE	**−0.37**	**−0.4**	0	0
GNDVI	0.05	0.35	0.13	0.22
EVI2	−0.02	0.04	−0.04	−0.24
SRRE	**−0.38**	**−0.41**	0	0
MSR	−0.02	0.08	−0.04	−0.24
CIG	0.05	0.35	0.13	0.22
CIRE	**−0.38**	**−0.41**	0	0
MTCI	−0.14	−0.07	0	0
RTVIC	−0.02	−0.07	−0.12	−0.23

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

SRRE ($r = -0.38$), and CIRE ($r = -0.38$), and seed yield was significantly correlated with RE ($r = 0.64$), NDVI 2 ($r = -0.40$), NDRE ($r = -0.40$), SRRE ($r = -0.41$), and CIRE ($r = -0.41$). A scatterplot of the correlations, during flight one, can be seen in **Figure 1**. Unfortunately, during the second flight, no significant correlations occurred between pod yield and seed yield. These results indicate that UAS data collected earlier in the season are more useful to predict pod and seed yield in peanuts.

4. Discussion

Pod and seed yield data were taken at the end of the season. The seed yield was 56.5% less than the pod yield. This could be due to having pods that weren't completely full and some which had small seeds. The seed weight collected for each line was that of the ELK, medium, and #1 seeds during grading. During grading, anything that falls through all three screens (for ELK, medium, and #1) is then subjected to further grading as being splits, ok, damaged/diseased, and foreign matter. Hence, not every seed was included in the seed weight. A lot of variation was observed among the different lines for pod and seed yield as indicated

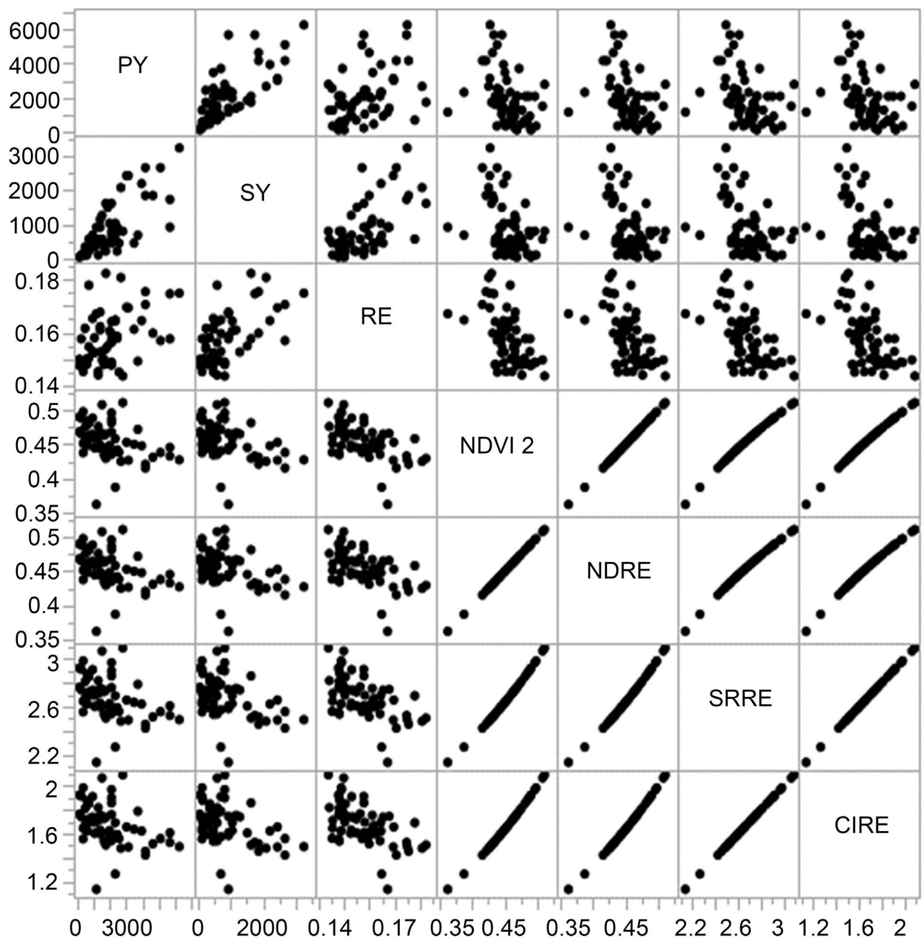

Figure 1. Scatterplots of pod yield (PY), seed yield (SY), and vegetation indices significantly correlated with PY and SY during the first flight on organic peanuts.

by the large standard deviation value. This could be due to genetic differences. NDRE, SRRE, and CIRE use RE and NIR values to be calculated. During Flight 1, even though RE and NIR are smaller than Flight 2 they are also further apart from each other instead of being equal. This difference accounts for NDRE, SRRE, and CIRE being higher during Flight 1 than Flight 2.

Genotypes had a statistically significant effect on pod and seed yield as well as all twenty-five vegetation indices calculated. This is due to the variation seen among the 16 breeding lines and 4 commercial cultivars involved and not due to the environment.

Overall, the red edge (RE) vegetation index correlated the best with both pod and seed yield based on data from the first flight on July 07, 2021, and a correlation was not present between pod and seed yield from Flight 2 data taken September 27th, 2021. The two flights were 2 months and 20 days apart so there may not have been a lot of change in plant growth during that time and even with the average RE and NIR values, in **Table 1**, these values were equal during Flight 2 unlike Flight 1. Hence, the peanut plants may have been in the reproductive phase during Flight 2 and not growing as much as they were already established.

The study done by Sarkar *et al.* [29], measuring canopy height in peanuts found that the red-green-blue (RGB) aerial images which derived canopy height were highly correlated to manually measured height data (R2 = 0.953). We gathered canopy information quickly using UAS as red edge (RE) looks at the vegetation reflectance and with more vegetation present, the image becomes redder. In this study, genotypic differences were observed based on the twenty-five vegetation indices which can be used to develop a way to distinguish one variety from another as suggested for guar and soybean cultivars [31] [32]. This will give breeders the ability to identify varieties faster as previously reported by Balota and Oakes [25] who used a UAS platform for variety selection in peanuts. Further studies involving more breeding lines will aid in building learning models which can improve the estimates obtained for seed and pod yield.

5. Conclusion

Overall, genotypic effects on pod and seed yield were statistically significant. Also, genotypic effects on all twenty-five vegetation indices were significant during both flights. Red edge (RE) is a good indicator of pod and seed yield for the 20 lines tested, based on UAS data collected earlier during the growing season. Since there was no correlation during the second flight, temporal analysis of vegetation indices to model both pod and seed yield in organic peanuts are needed.

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Conflicts of Interest

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

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Appendix

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	NDVI2	(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 Index	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)