RAPID DATA ACQUISITION FOR IN-FIELD PLANT PHENOMICS

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ABSTRACT

High throughput sensing is necessary for the rapid acquisition of plant canopy physical and physiological parameters on field scales. Simultaneous measures of these descriptive parameters will provide a clearer picture of plant response to biotic and abiotic stressors. Information obtained can assist in early identification of desired genetic traits and the degree to which they are expressed. Identifying these traits and their expression can provide higher efficiency in genetic selection for breeding programs and define better management practices for genetics currently on the market. To meet this sensing need, a new multi-parameter sensor system was developed, and accordingly, represents a new integrated approach for measuring radiative transfer and physiological characteristics of plant canopies. The phenomics system was developed and provided by Holland Scientific (Lincoln, NE, USA) and was field tested on winter wheat during the spring growing season of 2013. The system is a combination of active and passive sensors consisting of a three-band active optical sensor (AOS), a multi-parameter data acquisition sensor and geospatial data logger (Holland Scientific GeoSCOUT GLS-400). The AOS (Holland Scientific Crop Circle ACS-430P) provides measurements for red, red-edge (RE) and near infrared (NIR) reflectance, red and red-edge normalized difference vegetation indices (NDVI and NDRE) and estimation models for leaf area index (LAI), plant canopy chlorophyll content (CCC) and optical sensor-to-plant distance. The multi-parameter sensor (Holland Scientific Crop Circle DAS43X) provides measurements for passive upwelling and downwelling photosynthetic active radiation (PAR), passive temperature for both canopy and ambient air, humidity and atmospheric pressure. The Crop Circle DAS43X also includes two 24-bit differential voltage channels with the option of configuring one of the channels as a pulse counter. Canopy data was collected at a rate of 5 samples per second and geo-referenced using a Trimble RTK GPS receiver. From this dual sensor data we were able to derive CCC, LAI, canopy height, canopy temperature departure (ΔT), and fractionally absorbed PAR (fAPAR). Data was collected at three dates on a yield trial study that includes thirteen public varieties adapted for the Great Plains and grown in Eastern Nebraska. We were able to characterize the highest yielding variety as a shorter plant with high LAI, CCC and fAPAR. Potentially, a variety with a higher light use efficiency (LUE) throughout the canopy, most likely a result of an erectophyle structure of the leaves. This particular variety had the highest ΔT in the presence of acute heat stress, indicating increased rates of transpiration as a strategy of heat tolerance. The system has demonstrated that significant discrimination can be obtained for a variety of plant canopy physical and physiological parameters in a high throughput manner using the set of measurements provided by the sensor suite.

Keywords: photosynthesis, chlorophyll, nitrogen, active remote sensing, passive remote sensing, thermal remote sensing, vegetation indices.

Abbreviations: Chl – chlorophyll; N – nitrogen; CCC – canopy chlorophyll content; AOS – active optical sensors; RE – red edge reflectance; NIR – near infrared reflectance; NDVI – normalized difference vegetation index; NDRE – normalized difference red edge; CI – chlorophyll index; LAI – leaf area index; PAR – photosynthetic active radiation; fAPAR – fractionally absorbed photosynthetic active radiation; ΔT – canopy temperature departure; LUE – light use efficiency.

INTRODUCTION

Davis (1949) first proposed the term phenome as the sum total of extragenic, non-autoreproductive portions of the cell, whether cytoplasmic or nuclear. The term may originate from the discipline of biochemistry but has since expanded into a wide variety of biological sciences. It no longer is limited to just describing the cellular environment yet has extended to the description of species and their entire community. Phenomics is now a discipline within plant biology that is focused on the characterization of morphological and physiological traits in response to genetic variation while influenced by environmental factors imposed on the plant. A plant's phenome is the material basis of the phenotype, the sum total of all phenotypic traits expressed at all levels by cell, tissue, and species. Phenotypes are the accumulation of morphological and physiological traits that delineate taxonomic grouping and the extent to which those traits are expressed.

Genomic analysis using single-nucleotide polymorphism (SNP) molecular markers as an assisted selection technique can quickly provide information as to the presence and form of genes associated with specific traits. However, it does not characterize the conditions necessary for expression or the extent of trait expression. Phenomic analysis, when combined with genomic information, can enhance our understanding of a complex genome.

High-throughput phenomic data collection and analytical interpretation of that data is a rapidly growing field of study. Obtaining detailed measurements, as many parameters as is practical, of a plant's physiological and physical characteristics will better define the true plant phenome. The intent of high-throughput phenotyping is focused on non-destructive techniques to rapidly quantify characteristics ranging from the biochemistry of photosynthesis and its supporting light harvesting plant pigments to components for structural development of plant architecture. Method of measurement approach can vary from close order single point remote sensing devices (proximal sensors) to imaging systems on board aerial or vehicle mounted platforms that may include either nadir or stereoscopic imaging capabilities. Imaging and non-imaging devices both offer their own unique advantages as does airborne to vehicle mounted platforms. Considerations when evaluating and selecting an approach include spatial, spectral, radiometric, and temporal resolution. There exists a trade off when increasing the resolution of one component, the others may decrease.

Stereoscopic imaging can provide a wealth of information describing the morphological characteristics of the plant and the plant community through a direct measure approach. This approach can be employed from multiple images sequentially acquiring overlapping view angles from airborne platforms and from multiple cameras fixed at differing view angles mounted on a field vehicle platform. Aerial platforms have the advantage of regional coverage with instantaneous data acquisition for entire target area of interest. The trade-off is a much diminished spectral resolution with broad spectral bands to ensure sufficient energy for the time of exposure. This requires time for image acquisition and processing of feature recognition algorithms, a limitation of current technology resulting in a stop and go approach to data collection.

Proximal sensing single point approach utilizes well defined radiative transfer functions that require very specific segments of the electromagnetic spectrum for proxy measures of both physical and physiological features. Proximal sensors, by definition, are close to the target of interest and thus, more likely to be deployed on field vehicles. Unmanned aerial vehicles (UAV) may soon be a viable option if flight characteristics can maintain fixed and precise distance from sensor to target at close proximity. With this specificity comes speed of acquisition and processing. Acquisition of multiple data points per second provides a large sample population to better statistically estimate treatment means and variance.

Wanjura and Hatfield (1985) examined the use of both RVI and NDVI for the estimate of cotton (*Gossypium hirsutum* L.), soybeans (*Glycine max* (L.) Merr.), and sunflower (*Helianthus annus* L.) dry matter accumulation and leaf area. The two approaches used were an integration of the temporal index curve and an instantaneous index value for estimation of fractional PAR. Both methods performed very well. Wiegand and Richardson (1984, 1990) utilized both the simple band ratios as well as the data rotation techniques to infer leaf area, evapotranspiration and yield of wheat (*Triticum aestivum* L.) and sorghum (*Sorghum bicolor* L. Moench). They found that using equations that utilize vegetation indices correlate well with the biophysical properties in question and can be used as a tool to quantify stress effects on canopy development and yield performance. Schlemmer et al. (2013) and Gitelson et al. (2003, 2005) examined several spectral indices for the estimation of canopy chl content. Optimization of the $CI_{Red\ Edge}$, utilizing the red edge spectral segment at or near 740nm, resulted in a strong linear response to chl content. Furthermore, the strong relationship established between canopy chl content and canopy N content suggests techniques utilized to estimate canopy chl can also be used to estimate canopy N content.

This study will illustrate the capabilities and ease of use of a newly engineered high throughput proximal phenomic sensor system. This system will estimate proxy's to several physiological and physical variables based on current literature and research.

MATERIALS AND METHODS

The plant canopy measurement system is composed of three primary components (all manufactured by Holland Scientific, Inc., Lincoln, NE, USA): 1) Crop Circle ACS-430P proximal active optical sensor (AOS), 2) Crop Circle DAS43X multi-sensor and 3) a GeoSCOUT 400 data logger for collecting and storing sensor data, see Figure 1. The sensor components were mounted to a height adjustable frame that was subsequently mounted on a John Deere model 5085M tractor, see Figure 2. A RTK GPS receiver (Trimble FMX , Trimble Navigation, Sunnyvale, CA, USA) was utilized as the data source for geolocating data. Functional descriptions for the sensing components are detailed below.

Active Optical Sensor Measurements

Active optical sensing techniques irradiate a plant canopy and measure a portion of the radiation scattered (reflected) from the canopy (Ferte and Balp, 1938; Marihart, 1948; Palmer and Coven, 1971; Beck and Vyse, 1992; Kunimeier et al., 2002; Holland et al., 2004). Instruments such as these have the ability to

Figure 1. Components of the Holland Scientific Phenomic System.

Figure 2. Sensor deployment on vehicle.

make canopy reflectance measurements independent of ambient illumination conditions at close ranges typically 0.3m to 2.5m from a canopy. As stated above, the phenomics system utilizes a Crop Circle ACS-430P as the AOS component. The measurements performed by the ACS-430P include: red, red-edge and near infrared (NIR) reflectance, NDVI and NDRE vegetation indices, leaf area index (LAI) and canopy chlorophyll content estimations, and distance from sensor to canopy for height estimation. Descriptions for each measurement are described in the following.

Spectral reflectance and Vegetation index Measurements

The radiometric operational principles governing AOS instrumentation has been reported by Holland et al. (2012). The general equation describing the irradiance on the sensor's photo detector is:

$$H_r = \frac{\rho T_S T_D N_S A \sin^2 \theta}{d^2} \tag{1}$$

where ρ is the target reflectance, T_S is the transmittance of the sensor's source optics, T_D is the transmittance of the detection optics, N_S is the radiance of the source, A is the area of the source objective, θ is the angle by which radiation from a diffuse object arrives at the detector and d^2 is the distance between the object (plant canopy) and the sensor.

Spectral reflectance values for red (670 nm), red-edge (730 nm) and NIR (780nm) are derived from irradiance values for each band using equation 1. Reflectance magnitudes are scaled to result in values ranging from 0 to 100 % reflectance.

Furthermore, use of equation 1 above allows the determination of ratio based vegetation indices that are invariant with respect to distance between the sensor and the object. For example consider the simple ratio vegetation index. Substitution of equation 1 irradiances into the simple ratio formula results in the ratio of actual target reflectance values for two wavelengths, namely $\frac{H_{r\lambda 1}}{H_{r\lambda 2}} = \frac{\rho_{\lambda 1}}{\rho_{\lambda 2}}$. Note, however, the inverse-square law effect has been removed (additional subscripts $\lambda_1$and λ_2 indicated the parameters at two different wavelengths). The resultant measurement is invariant with respect to the distance between the sensor and the object for planar targets. The assumption, with respect to equation 1, is the normalization via calibration and optical geometry for each channel is identical for each waveband of interest.

The ratio described in the prior paragraph is the widely-used simple ratio vegetation index (SRI) in equation 2 below.

$$SRI = \frac{H_{r\lambda 1}}{H_{r\lambda 2}} = \frac{\rho_{\lambda 1}}{\rho_{\lambda 2}} \qquad (2)$$

Similarly, equation 1 can also be applied to the normalized difference vegetation index (NDVI) shown in equation 3 below.

$$NDVI = \frac{H_{r\lambda 2} - H_{r\lambda 1}}{H_{r\lambda 2} + H_{r\lambda 1}} = \frac{\rho_{\lambda 2} - \rho_{\lambda 1}}{\rho_{\lambda 2} + \rho_{\lambda 1}} \qquad (3)$$

where, $\rho_{\lambda 1}$ and $\rho_{\lambda 2}$ are the target reflectance values at wavelengths λ_1 and λ_2.

The Crop Circle ACS430P utilizes functions for equation 3 to calculate both NDVI and NDRE (normalized difference red-edge) indices.

Leaf Area Estimation

LAI estimations performed by the ACS-430P are based on NDVI measurements. The function utilized to estimate LAI is,

$$LAI = k \times ln(1- VI) \qquad (4)$$

where LAI is the leaf area index, k is a canopy specific proportionality coefficient ($-6 < k < -2$) and *VI* is the vegetation index, in this case, NDVI.

It should be noted, equation 4 above was derived from Beer's law for light extinction through a canopy structure (Jones and Vaughan, 2010). Values for coefficient k are highly variety dependent. The coefficient should be carefully calibrated via destructive leaf area testing or cross calibrated with handheld LAI instrumentation. Equation 4 results in reasonably accurate LAI measurements up to LAI's of 4 to 5. For LAI's above 5, other modeling techniques must be utilized

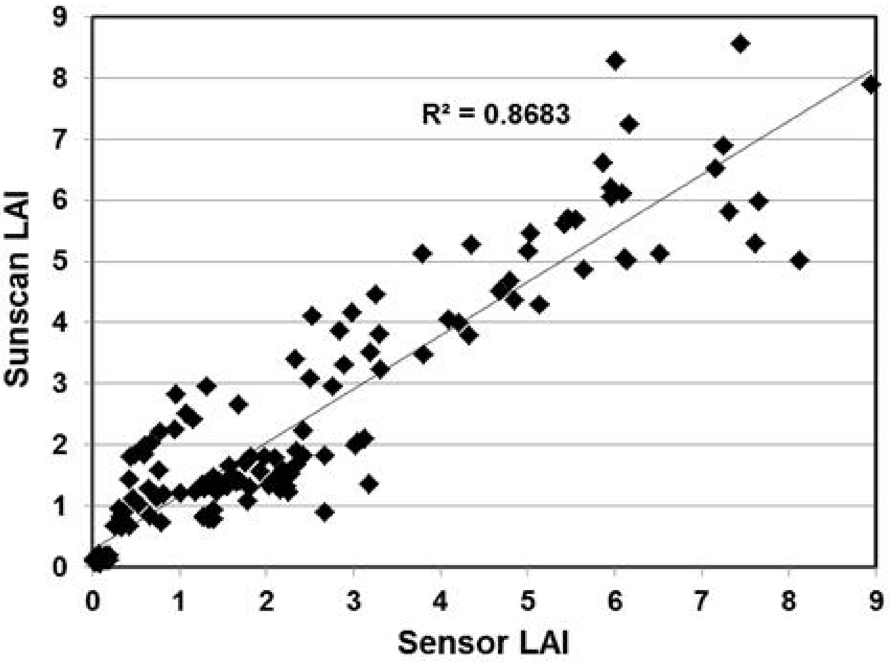

Figure 3. LAI model performance for wheat canopies.

to transform sensor data into reasonable estimates for LAI. Figure 3 demonstrates the ACS-430P's estimation performance with respect to LAI estimation for wheat canopies with LAI's up to 8. The ACS-430P was cross calibrated with a Sunscan LAI meter (Delta-T Devices, Cambridge, UK).

Calibration and evaluation efforts for this measurement are on-going in order to 1) demonstrate the robustness of the model and 2) collect additional calibration for canopies other than wheat.

Canopy Chlorophyll Content Estimation

Canopy chlorophyll content estimation is based on the three waveband background corrected chlorophyll index (BCCI-1) (Holland and Schepers, 2011). The functional form of the vegetation index is

$$BCCI1 = \frac{a \times \rho_{NIR} - b \times \rho_{RE}}{c \times \rho_{RE} - d \times \rho_{E}} \quad (5)$$

where ρ_{NIR}, ρ_{RE}, and ρ_{R} are 780nm, 730nm and 670nm reflectance, respectively, and *a*, *b*, *c* and *d* are scaling coefficients.

Calibration and evaluation efforts for this measurement are on-going in order to 1) demonstrate the robustness of the model and 2) collect additional calibration data for crop canopies other than wheat.

Height Measurement

The Crop Circle ACS-430P computes canopy height by linearizing distance sensitive reflectance data measured by the sensor (Holland et al., 2012). Since the irradiance at sensor's detectors varies inversely with respect to the inverse of the distance squared between the sensor and the plant canopy, a proxy distance between the sensor and the canopy can be calculated using the formula in equation 6 below,

$$d = m \cdot \left(\frac{1}{H_{r\lambda}}\right)^{\beta} + b \tag{6}$$

where β is an irradiance exponent and m and b are the slope and intercept constants, respectively.

When the exponent (β) is set to 0.5 in equation 6, the inverse-square law response of the irradiance measurement for sensor-target distance is transformed into a linear response (Figure 4). Using two known distance values allows determination of the slope and intercept constants, yielding a distance measurement for a target with approximately constant reflectance (ρ). For crop sensing, the average canopy height can be calculated by subtracting distance between the sensor and canopy from the sensor height above ground level.

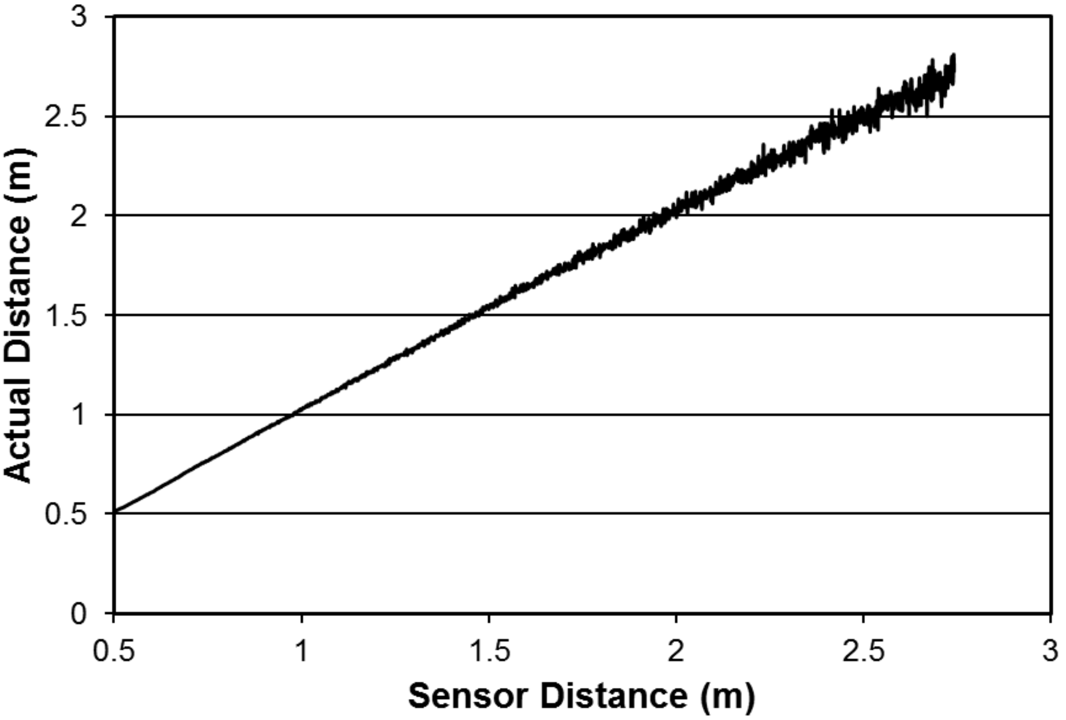

Figure 4. Sensor distance transformation. Here, the linearized response was derived using sensor measurements collected using a diffuse soil surface as target.

Multi-parameter Sensor Measurements

The Crop Circle DAS43X companion sensor is utilized by the phenomic system to collect and process ambient meteorological and passive optical measurements from an array of sensing devices. Measurements performed by the DAS43X include: canopy temperature, ambient air temperature, relative humidity, atmospheric pressure, upwelling/downwelling PAR and voltage. Descriptions for each measurement are described in the following.

Incident and Reflected PAR Measurement

Photosynthetic active radiation (PAR) sensor principles have been well documented in literature (Federer and Tanner, 1966; Biggs et al., 1971). The DAS43X's PAR sensors are comprised of silicon photodiode and a 400 to 700nm hot mirror. The silicon photodiodes in the DAS43X have reasonable spectral responsivity which is well matched to the PAR response and as such these devices require little additional band shaping in order to match this response, albeit, improvement to the response can be made via trimming the hot band filter. It should be noted, since the upwelling and downwelling sensors will typically be compared with respect to each other, over or under spectral estimates of PAR can be minimized to some degree via normalization. The upwelling sensor has a field-of-view of approximately 30 degrees designed to match that of the IRT sensor's field-of-view.

Canopy Temperature Measurement

The thermal infrared sensing component of the DAS43X uses a thermopile detector spectrally configured to measure thermal radiation of an object over the spectral range spanning 6 μm to 14 μm. A secondary thermal sensor is utilized to measure the thermopile die temperature in order to compute the object's actual temperature. Sensor signal conditioning circuitry has been configured to allow high speed temperature measurements every 100 msec. The measurement principle for determining object temperature is defined by equation 7 below

$$R = \varepsilon \times \sigma \times T^4 \tag{7}$$

where R is the energy flux leaving a surface ($W \cdot m^{-2}$), ε is the emissivity of the surface, σ is the Stefan-Boltzmann constant ($5.670373 \cdot 10^{-8}\ W \cdot m^{-2} \cdot K^{-4}$) and T is the temperature (K).

Because sparse canopy structures can have emissivity's as low as 0.93-0.94 whereas dense canopies can have emissivity's up to 0.98, canopy temperature measurements made by the DAS43X use an emissivity of approximately 0.95 which is closely matched to the emissivity of many plant canopies (Bramson, 1968).

Air Temperature Measurement

Ambient air temperature measurements were performed using a low thermal mass thermistor. The thermistor was mounted to the DAS43X using long coiled leads to minimize stem effect measurement errors. The sensor was mounted in a polished low emissivity aluminum housing so as to reduce radiative heating by the sensor's enclosure and reflected energy from the canopy. Temperature was calculated by using the Steinhart-Hart equation shown below

$$\frac{1}{T} = A + B \times ln(R) + C \times (ln(R))^3 \tag{8}$$

where T is the temperature (K), A, B, and C are the Steinhart-Hart coefficients and R is the resistance of the thermistor (Ω).

Humidity Measurement

The DAS43X utilizes a relative humidity integrated circuit (RHIC) for sensing water vapor. The sensor consists of a polysilicon dielectric substrate coated with a thin porous layer of platinum, the resulting structure comprising a capacitor, over which is deposited a thin coating of thermoset polymer. The polymer allows the diffusion of water vapor to the porous platinum electrodes while protecting the electrodes from contaminants such as dust and oil as well as exposure to condensation. The diffusion of water vapor into the dielectric causes an incremental change in the structure's capacitance. This capacitance change is typically 0.2 to 1 pF per 1% RH change. Sensitive and stable signal conditioning circuitry within the RHIC quantify the capacitance change due to water vapor and convert this value into a voltage that is readily measurable by the DAS43X's data system. The response time of the RHIC is on the order of 6 to 10 sec for an airflow of 20 l/min. The sensor is capable of measuring RH from 10% to 90% with an accuracy of better than 3% over the temperature range of 0 to 50 °C.

Atmospheric Pressure Measurement

Atmospheric pressure is sensed using a monolithic piezo-resistive transducer comprised of micro-machined sensing element and integrated signal conditioning. The sensor measures absolute pressure over the range spanning from 15 to 115kPa. The sensor die is protected from dust and water moisture using fluorosilicone gel. The sensor has a typical accuracy of 1.5kPa over the temperature span ranging from 0 to 50 °C.

RESULTS AND DISCUSSION

Initial deployment, of the previously described system, was for the spring growing season of winter wheat in 2013 near Lincoln, NE, USA. Three dates of data acquisition were performed on a basic yield trial study. The focus was to simply characterize several public varieties adapted across the Great Plains winter wheat growing region as grown in the Eastern Nebraska environment.

Sensor output data was accumulated and post processed using the Geographical Information System (GIS) ArcMap (ESRI, Redlands, CA, USA). Individual plots were defined using aerial imagery (Cornerstone Mapping, Lincoln, NE, USA) acquired when vegetation could be easily delineated from soil to help guide on-screen digitizing of plot boundaries. Resulting plots were used to generate means from all sample points for each measured variable contained within each individual plot boundary. The temporal data set was accumulated and analyzed using the RStudio (RStudio, Inc., Boston, MA, USA) and InfoStat (InfoStat, Córdoba, Argentina) statistical analysis software. Data from the final acquisition date offered climatic conditions that allowed for greater evaluation and will be the focus.

This yield trial is simply an observational study of 13 public winter wheat varieties from across the Great Plains. Traits examined are CCC, LAI, derived canopy height, fAPAR, and ΔT. Final acquisition date for this study was 14 May 2013 with clear skies and an ambient air temperature near 40^{o}C. The crop was in its early boot stage of growth. Climatic conditions and plant growth stage on this date were nearly ideal for assessing variations in all of the traits being measured.

Figure 5 illustrates how traits like yield and those measured by this system differ across the genetic variation present in this trial grown under the Eastern Nebraska environment. This partial phenome collected under the conditions of this one environment will enable us to characterize these varieties in a more taxonomic way.

From this data set we can characterize the highest yielding variety, TAM303, as a plant that implements a strategy of increasing transpiration during heat events maintaining cooler canopy temperatures. This variety produces high CCC and is the most efficient canopy with respect to PAR absorption. TAM303 is amongst the highest in LAI but does so with the shortest canopy, indicating a much more dense leaf canopy structure. In fact, it does produce a dense erectophyle leaf canopy architecture. It is this structure with its ability to capture the greatest percentage of light energy and its defense against heat that translates into higher yields.

In contrast, the lowest yielding variety, Wesley, can moderately cool itself when experiencing a heat event; it produces a moderate quantity of CCC but is not all that efficient at absorbing PAR, it is a moderate producer of LAI with a mid-range height. Wesley possesses a wider leaf blade that creates a horizontal leaf position in the upper portion of the canopy. This planophyle architecture limits light penetration and relies heavily on the upper leaves to produce a greater amount of photosynthate. Reliance on a smaller portion of the canopy for primary productivity will enhance the impact of abiotic stress events like heat, most likely accounting for its poor performance.

Figure 6 illustrates the wide distribution of varietal means while providing the principle component eigenvectors for each of the measured traits. These vectors indicate the contribution of each trait and the relationship with respect to the statistical variation present. For this collection event we see that CCC and LAI have very similar contributions to the potential differences of these varieties. In this specific situation it may not be necessary to include both traits to gain separability.

With larger population panels targeting specific genes, one could easily characterize how genetic structure will express itself under similar conditions for the traits measured. Larger panels may provide statistical separation that would show greater divergence between CCC and LAI. Most likely, larger panels will require a greater number of traits measured, thereby, producing a more complete phenome.

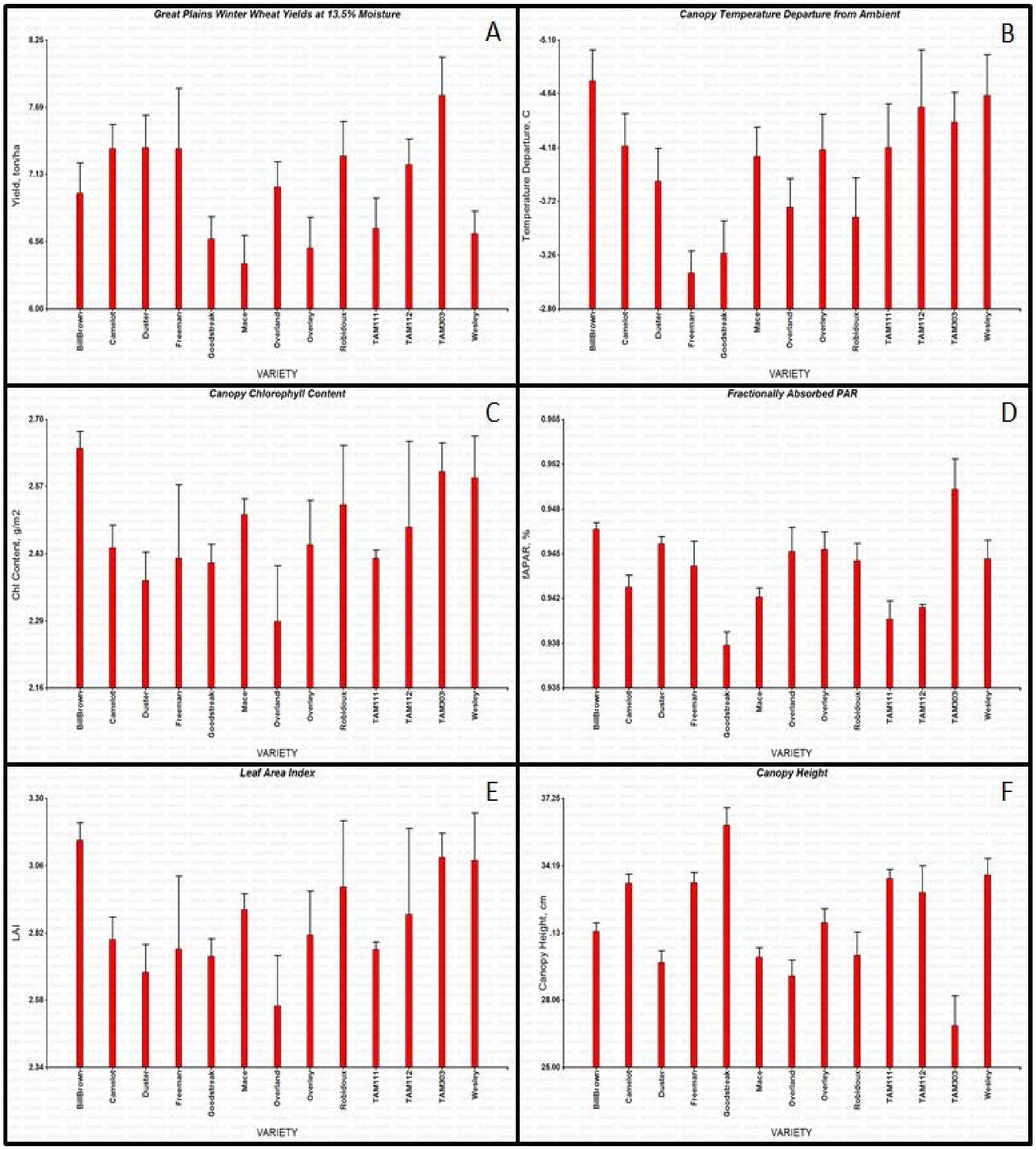

Figure 5. Results for thirteen Great Plains winter wheat varieties for final yield and five sensor measured traits on 14 May 2013 (Panel A – Yield, B – ΔT, C – CCC, D – fAPAR, E – LAI, F – Canopy Height).

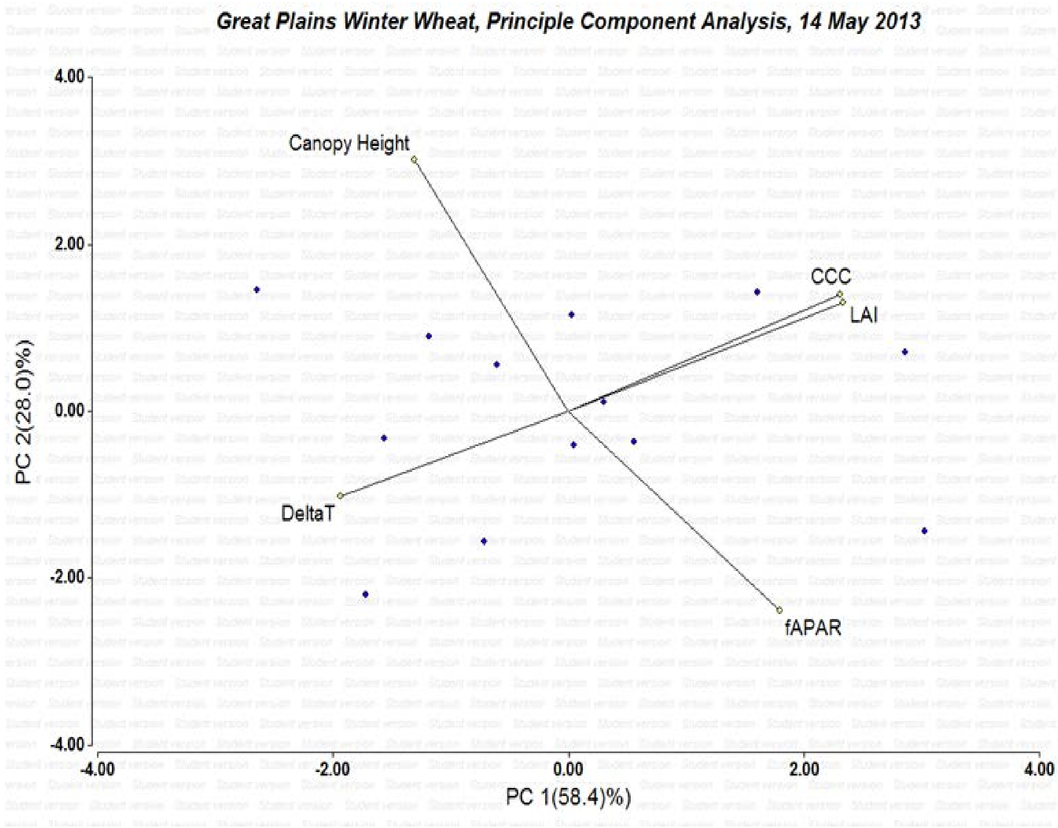

Figure 6. Principle Component Analysis for thirteen Great Plains winter wheat varieties using the five sensor measured traits on 14 May 2013.

CONCLUSION

Results indicate this system is a practical method for acquiring a rapid characterization of the plant community's biophysical properties. From this data we were able to derive canopy chlorophyll content (CCC), leaf area index (LAI), canopy height, canopy temperature departure (ΔT), and fractionally absorbed PAR (fAPAR) creating a partial phenome. When comparing phenomes, we were able to characterize the highest yielding variety in one study as a shorter plant with high LAI, CCC and fAPAR. This variety was also able to combat heat stress through increased transpiration resulting in a larger absolute ΔT. Potentially, this variety suggests the capability of higher light use efficiency (LUE) throughout the canopy, most likely a result of the erectophyle structure of the leaves.

The phenomics system has demonstrated that significant discrimination can be obtained for a variety of plant canopy physical and physiological parameters in a high throughput manner using the set of measurements provided by the sensor suite. These measurements constitute a partial phenome that can describe the degree of expression for genes that impact the measured traits. The result is a quantitative assessment that can provide breeders accuracy and efficiency to their field selection process.

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