



THE INTERNATIONAL SOCIETY OF  
PRECISION AGRICULTURE PRESENTS THE  
13th INTERNATIONAL CONFERENCE ON  
**PRECISION AGRICULTURE**

July 31-August 4, 2016 • St. Louis, Missouri USA

## Proximal hyperspectral sensing in plant breeding

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A paper from the Proceedings of the  
**13<sup>th</sup> International Conference on Precision Agriculture**  
**July 31 – August 4, 2016**  
**St. Louis, Missouri, USA**

### **Abstract.**

*The use of remote sensing in plant breeding is challenging due to the large number of small parcels which at least actually cannot be measured with conventional techniques like air- or spaceborne sensors. On the one hand crop monitoring needs to be performed frequently, which demands reliable data availability. On the other hand hyperspectral remote sensing offers new methods for the detection of vegetation parameters in crop production, especially since methods for safe and efficient detection of phenotypic differences are essential to develop adapted varieties by breeding.*

*To address both aspects, a ground-based hyperspectral system called "TriSpek" has been developed to deploy new spectral opportunities and to overcome the problems of data availability and spatial resolution.*

*The TriSpek is capable to cover a spectral an effective spectral range from 400 to 825 nm with 1 nm bandwidth. Using multiple spectrometers allows for correcting the reflectance measurements for incoming radiation on the fly in the field. This option increases data availability since the effects of illumination situations due to different sun angles and clouds can be compensated directly in the field.*

*In an extensive calibration process partial least squares regression models for the determination of several vegetation parameters in rye have been developed. The results show a high prediction quality with coefficients of determination ( $R^2$ ) of 0.85 for fresh matter, 0.90 for dry matter, 0.90 for leaf area index and 0.84 for chlorophyll-a, respectively.*

*Over three growing seasons performance tests with rye were applied at two test-sites in Germany with different candidate strains under drought stress and irrigation. Connecting the spectral/vegetation data to the digital field plans of the experiments allow views of the temporal and spatial dynamics. Applying this concept, heterogenities within plant nurseries caused by elevation or soil differences can be identified indirectly by means of growth variations in the hyperspectral data.*

**Keywords.** *Hyperspectral sensing, plant breeding, vegetation parameters.*

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# 1 Introduction

The investigation of genotypes in plant breeding requires very high personnel costs and a large sample throughput because breeding selection is based on the principle of large sample numbers (Becker 2011). A significant improvement is to be expected, if it is possible to collect various vegetation parameters using non-destructive methods. Spectrometry and imaging remote sensing provide such measurement techniques, but the spatial resolution of air- and satellite-borne sensors is too little for information retrieval in breeding yards. Test plots in the breeding nurseries are often only a few square meters in size. Thus, spectral signatures of the individual plot cannot be resolved with conventional methods, and the so-called mixed pixel problem comes up (Cracknell 1998). Furthermore, optical remote sensing relies on sunny weather. In Central Europe, an operational use in plant breeding is mostly hampered by weather conditions.

Tractor-based measurement methods offer solutions to minimize these problems. There are already a large number of so-called online sensors available which are used in the area of precision farming. Most of the systems have been developed to detect nitrogen in a site specific way. The biggest advantages, and at the same time a disadvantage of these methods, is the use of spectral indices. Using indices, different wavelengths (generally up to four bands) are combined to calculate a synthetic value (index), which should correlate with vegetation properties. The allocation to an index value is very simple and can be implemented quickly directly on the tractor. The disadvantage of the index is that the complexity of a reflection signature is reduced to a single numerical value. On the one hand different absorption and reflexion mechanisms cannot be sufficiently differentiated; on the other hand index values are ambiguous because the same numerical value may occur at different vegetations properties and spectral signatures.

An alternative approach for the indirect determination of vegetation parameters is the usage of complete spectral signatures. At the Julius Kühn Institute (JKI), the TriSpek system has been developed. The TriSpek system is a ground-based online system which is capable to collect high-resolution reflectance data in the field during most weather conditions. The TriSpek system has been tested on the rye breeding nurseries of KWS LOCHOW GmbH at two locations in Germany.

## 2 TriSpek system

The TriSpek system (Lilienthal & Schnug 2010) is made up of three individual spectrometers, which are grouped in modules (Tab. 1).

Table 1. Technical properties of the TriSpek system

Spectral range [nm]	Full resolution	Effective range [nm]	Effective resolution	Max recording speed
340 - 1026	2048 bands @ 0.34 nm	400 - 825	425 bands @ 1 nm	3 msec

The spectrometers cover an effective wavelength range of 400-825 nm and measure thus in 425 discrete channels with 1 nm bandwidth. Two spectrometers are directed downward and measure the reflection of the soil or the plants. The third spectrometer which serves as a light reference is directed upwards and measures the incident light. Since the measured radiation is combined simultaneously with the other two spectrometers, the spectral reflectance can be determined directly in the field (Fig. 1).

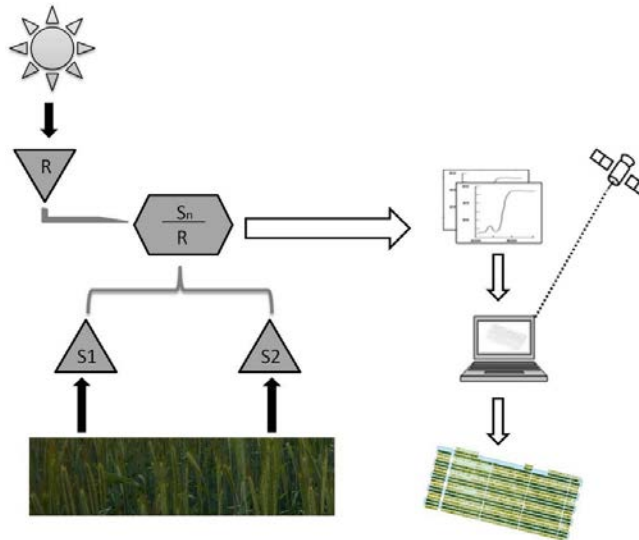


Fig 1. Schematic description of the TriSpek system.

This approach provides a clear advantage over other non-destructive measuring techniques. Due to the permanent correction of exposure conditions illumination differences that may occur during the measuring phase, can be compensated. Passing clouds are no longer a problem and the spectral measurement of the plots is not limited to sunny weather conditions.

Spatial localization of the measuring points is ensured by a Real Time Kinematic (RTK) GPS receiver which is coupled to the spectrometers. Positioning accuracy of each spectral measurement is  $\pm 2.5$  cm. The spatial resolution in the direction of travel is determined by the vehicle speed and the frequency of the RTK-GPS: For example at a speed of about 0.5 km/h, and a received GPS signal at every second a theoretical maximum resolution of 0.15 m in the direction of travel can be achieved. The ground resolution also depends on the field of view of the spectrometer and on the distance of the sensor from the object. Measurements were carried out with a foreoptic of  $14^\circ$  and a distance of 1.8 m to the ground, resulting in a ground coverage of about  $0.4 \text{ m}^2$  (Fig. 2).

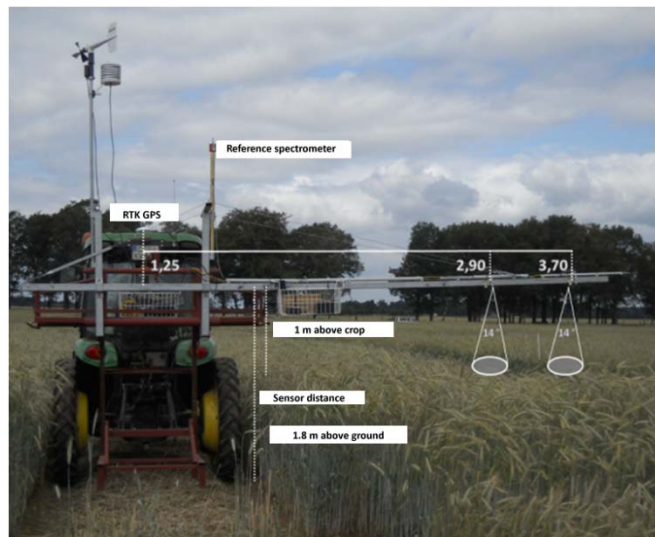


Fig 2. TriSpek system.

After geo-referencing of the spectra, spectral data can be assigned to the individual parcels and a hyperspectral data set is available for analysis.

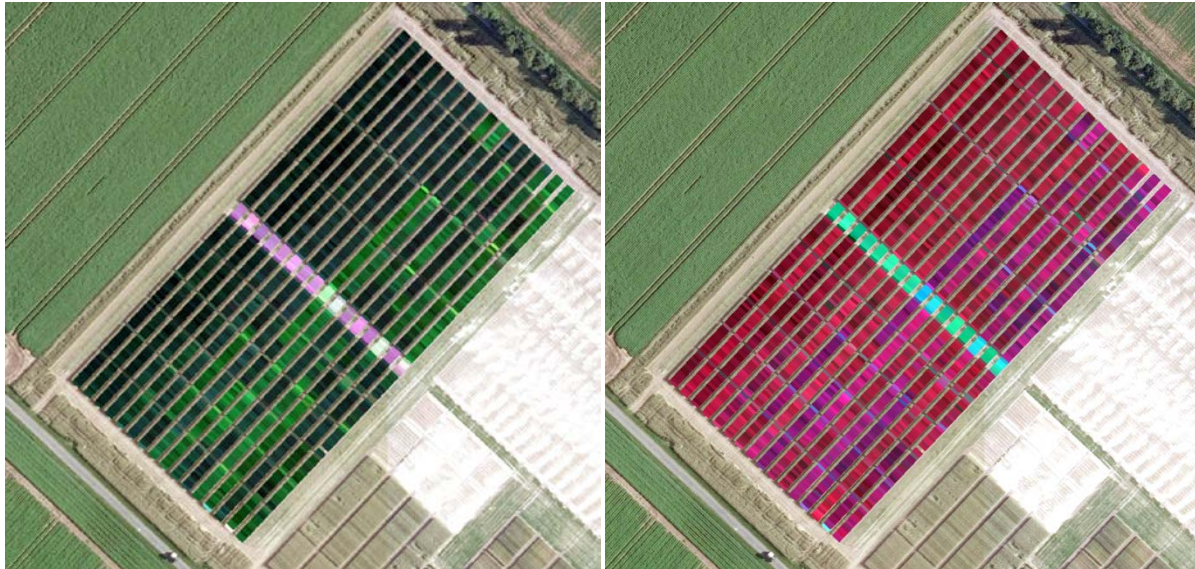


Fig 3. Hyperspectral data set of the breeding nursery in Wohlde from May 8<sup>th</sup>, 2013. Left: true-color (RGB: 680/550/450 nm), right: false-color infrared (RGB: 760/680/550 nm). Background: Aerial photograph from July 1<sup>st</sup>, 2013, Source of background image: [www.geodatenzentrum.de](http://www.geodatenzentrum.de).

### 3 Material and methods

Between the years 2012 to 2014, a total of 488 spectral measurements were carried out in rye with destructive harvesting at the JKI test sites in Braunschweig. Test plots with different varieties and fertilization intensities have been established to produce a large variability in the vegetation parameters and spectral signatures.

Within the growing seasons reference spectra were collected over an area of 0.25 m<sup>2</sup>. Consequently, the phenological development stage and the leaf area index (LAI) were determined. LAI was measured using the LAI-2000 plant canopy analyzer (LI-COR, Inc.). After the plot was completely harvested, fresh and dry matter weight, as well as pigment and nitrogen content have been determined in the laboratory.

The spectral data were normalized before modelling using unit vector normalization. With this normalization differences that may arise between individual spectrometers or different measurement dates can be compensated without a loss of information content (Fig. 4).

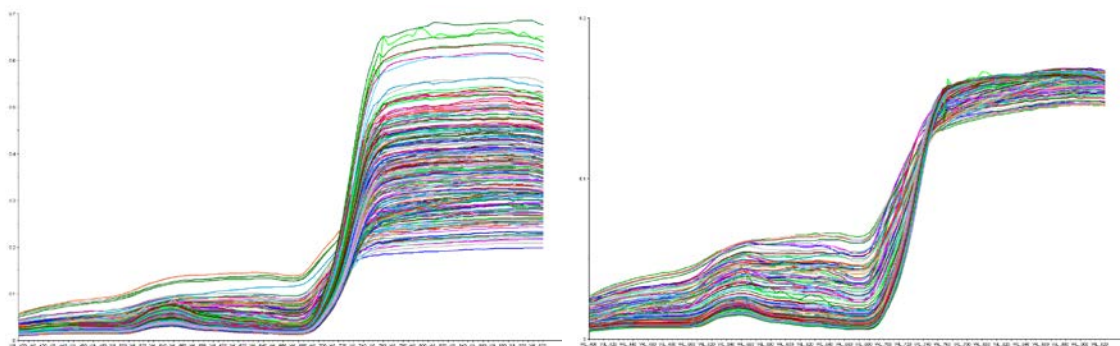


Fig 4. Original reflectance spectra of rye (left) and unit vector normalized spectra (right).

To model the vegetation parameters from spectral reflectance data partial least squares regressions (PLSR) have been used. PLSR transforms the original spectra to new orthogonal factors (latent variables). These latent variables are chosen in a way that they have a maximum correlation with the dependent variable (e.g., LAI). PLSR is a method which is widely used in the near-infrared spectroscopy. The overall dataset was split into a calibration (cal) and a validation data set (val) so that the quality of the models could be validated independently. The coefficient of determination ( $R^2$ ), the square error for the calibration and validation data (RMSEC, RMSEP) and the ratio of standard deviation to the standard error of prediction (RPD) (Malley et al. 2004) were used as a measure of the estimation accuracy for the calibration and validation data sets.

## 4 Results and discussion

PLSR models have been developed for fresh and dry matter, leaf area index, pigments and nitrogen content. The results show a high prediction quality with coefficients of determination ( $R^2$ ) above 0.8. Table 2 presents the quality parameters for the prediction models.

Table 2. Overview of model qualities for the prediction of vegetation parameters from reflectance spectra.

Parameter	Unit	No. of latent variables	N	Min	Max	STD	$R^2_{cal}$	$R^2_{val}$	RMSEC	RMSEP	RPD	
											$RPD_{cal}$	$RPD_{val}$
Fresh matter	[t/ha]	4	149	1.7	51.1	15.1	0.85	0.85	5.90	5.83	2.55	2.59
Dry matter	[t/ha]	5	66	0.2	12.2	3.7	0.90	0.92	1.15	1.00	3.22	3.70
LAI	[m <sup>2</sup> /m <sup>2</sup> ]	6	140	0.6	5.7	1.4	0.90	0.91	0.46	0.43	3.26	3.04
Chlorophyll-a	[µg/cm <sup>2</sup> ]	6	188	5.0	53.1	10.8	0.84	0.80	4.33	4.87	2.51	2.23
Chlorophyll-b	[µg/cm <sup>2</sup> ]	5	188	1.2	31.7	6.2	0.84	0.87	2.46	2.28	2.52	2.72
Carotenoide	[µg/cm <sup>2</sup> ]	7	186	2.3	19.7	3.2	0.64	0.65	1.92	1.93	1.69	1.67
Nitrogen content	[%]	7	65	0.7	5.2	1.5	0.98	0.97	0.24	0.24	6.25	6.25

The models have been applied to the spectral data of the breeding nurseries acquired with the TriSpek system, resulting in maps of the different vegetation parameters, e.g. LAI (Fig. 5).



Fig 5. Estimated leaf area index for the measurement of June 3<sup>rd</sup> 2013 in Wohlde.

In addition to the spatial representation of the vegetation parameters the temporal variation of each parameter can be considered: The rye genotypes were grown in an irrigated and non-irrigated environment as drought stress experiment and spectral measurements have been performed weekly. In 2013 slight drought stress occurred at the research station in Wohlde until May 12<sup>th</sup>, after that sufficient precipitation was available. The early drought stress period can be characterized with the temporal behavior of the chlorophyll-a concentrations (Fig. 6). In particular, the non-irrigated variants showed significantly lower chlorophyll-a level in early May, but compensate according to the rainfall at May 14<sup>th</sup>.

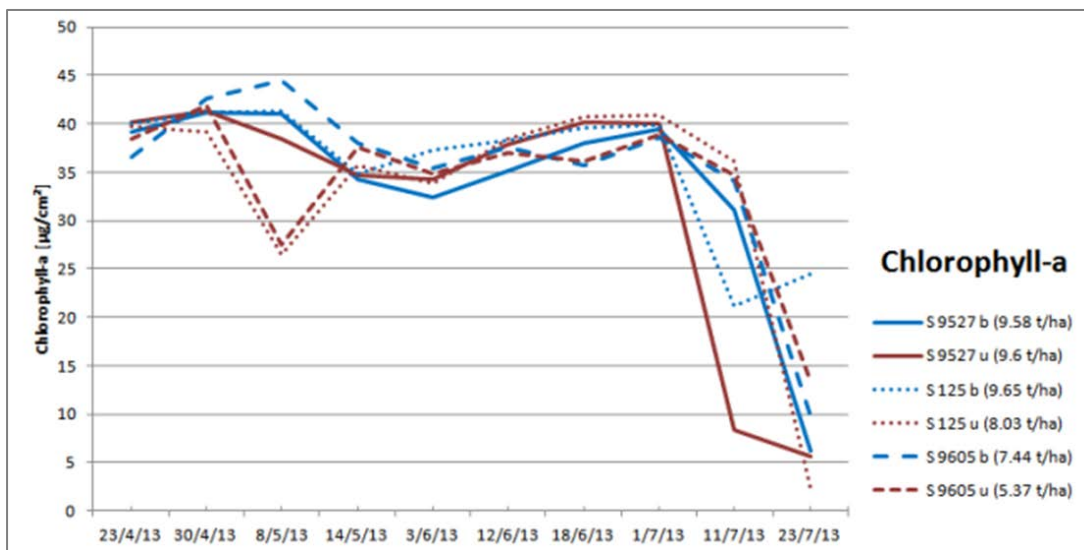


Fig 6. Temporal development of the chlorophyll-a content for selected genotypes in 2013 (red: non irrigated, blue irrigated variants).

## 5 Conclusion

The use of hyperspectral measurement technology in plant breeding can help to develop predictive models for the non-destructive detection of several crop parameters like biomass (fresh/dry), leaf area index, pigment contents (chlorophyll a and b, and carotenoids) and the total nitrogen content. By connecting the spectral data with the digital field maps, monitoring can be dynamically performed, also in a spatial context. It becomes possible to detect heterogeneities within breeding nurseries indirectly by identifying growth differences in the hyperspectral data and take the affected parcel into account when evaluating the breeding experiments. Using high temporal resolution, monitoring of the development of the vegetation parameters of different genotypes becomes possible, and offers valuable information for the final selection process in plant breeding.

## Acknowledgements

The authors wish to thank the Ministry of Agriculture which funded the “ESOB” project, by the innovation programme under the number FKZ 511-06.01-28-1-54.057-10.

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