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**Non-destructive plant phenotyping using a mobile
hyperspectral system to assist breeding research: first results**

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Abstract.

Hybrid plants feature a stronger vigor, an increased yield and a better environmental adaptability than their parents, also known as heterosis effect. Heterosis of winter oilseed rape is not yet fully understood and conclusions on hybrid performance can only be drawn from laborious test crossings. Large scale field phenotyping may alleviate this process in plant breeding.

The aim of this study was to test a low-cost mobile ground-based hyperspectral system for breeding research to easily access important information on crop status and development. Quantitative relationships between vegetation parameters (above ground fresh and dry matter, leaf area index; FM, DM, LAI) and field reflectance measurements were set up using partial least squares regression. At the time, our data set consists of 102 measurements which were acquired during two growing seasons between 2014 and 2016. Models were first set up using the full spectral range as a best case scenario (400-2400nm). Subsequently, performance was evaluated with reduced range (400-800nm) according to the ground-based mobile system. Model validation was performed by means of leave-one-out cross validation (cv).

R_{cv}^2 of the PLSR models for FM and DM based on full spectral range was 0.82. For LAI, R_{cv}^2 was only 0.52. Confining the spectral range increased prediction errors by 15%, 9%, and 5% respectively. Models were successfully applied to three data sets acquired in April 2015 by our mobile ground-based system.

Keywords.

phenotyping, hyperspectral, winter oilseed rape, brassica napus L., mobile ground-based, breeding

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Introduction

Oilseed rape is an important agricultural crop - it is the third largest source of vegetable oil in the world after palm oil and soybean, and the second largest source of protein meal (USDA, 2016). Oilseed rape is further used for the production of biodiesel which was encouraged in the European Union in 2003 by a directive on the promotion of the use of biofuels for transport (2003/30/EG). Germany is the largest producer of rape and turnip rape in the EU-28, with about 26% of the total European production (eurostat, 2015). Almost 12% of the German cropland is grown with this cultivar, exceeded only by wheat and maize (Statistisches Bundesamt 2015).

In view of a growing world population, changing environmental conditions, and land use conflicts, continuous progress in breeding with focus on yield, quality and resistance is of vital importance. Modern plant breeding concentrates on the production of hybrid varieties that feature a stronger vigor, an increased yield and a better environmental adaptability than their parents, also known as heterosis effect. However, genetic mechanisms of heterosis in winter oilseed rape, and their characteristics in changeable environments is not yet fully understood. For this reason, it is still not possible to draw conclusions on hybrid performance of oilseed rape cultivars from parental genotypes. Instead laborious and costly test crossings are required. A system biology approach, which is based on highly complex genomic, phenotypic and environmental data, may represent an alternative solution to solve the problem. However, high-throughput phenotyping under field environmental conditions has been identified as a major bottleneck for future breeding advances (Furbank & Tester, 2011, Cobb et al., 2013) and was titled the new crop breeding frontier (Araus & Cairn, 2014). As a consequence, the interest in field phenotyping increased in recent years, and a wide variety of sensing technologies and sensing platforms were tested to assess complex plant traits (Li et al., 2014a).

Some advantages of mobile ground-based systems are a flexible deployment and payload, an easy operation, a good spatial resolution, and partly relatively low costs. The complexity and level of sophistication of existing approaches greatly varies encompassing special manufactured vehicles with a multitude of sensors (e.g. Andrade-Sanchez et al., 2014, Busemeyer et al., 2014) but also more simple installations on a tractor-boom or rods (e.g. Comar et al., 2012, Montes et al., 2011).

Hyperspectral sensors acquire spectral reflectance in multiple narrow bands. Exploring this information by non-parametric algorithms such as partial least squares regression (PLSR) proved superior to parametric methods based on vegetation indices (e.g. Hansen & Schjoerring, 2003, Pimstein et al., 2007, Thorp et al., 2015). Parametric methods have commonly been applied to multispectral sensor data but the use of a few broad spectral bands features clear limitations compared to multiple narrow bands (Lee et al., 2004). Regardless of the type of sensor and the applied algorithms, the majority of the studies examined the spectral signal of a few crop cultivars particularly wheat, corn, or soybean (e.g. Pimstein et al., 2007, Hansen & Schjoerring, 2003, Siegmann & Jarmer, 2015). Little research focused on winter oilseed rape (e.g. Müller et al., 2008, Thoren & Schmidhalter, 2009).

In this study, we present first results with a low-cost hyperspectral system for field phenotyping of winter oilseed rape named PentaSpek mounted on a side-boom of a tractor. First, partial least squares regression (PLSR) models were set up based on field reflectance measurements and in-situ data to access information on above ground biomass fresh matter (FM), above ground biomass dry matter (DM) and leaf area index (LAI) from spectral reflectance data. Model performance was evaluated with respect to parameter and spectral range. Second, models were applied to PentaSpek data acquired during normal operations on a commercial breeding yard at three different growing stages in spring. Results were assessed with regard to individual data acquisition and development over time.

Materials and Methods

Study site

Research of this study has been carried out at two sites located in northern Germany, in the federal state of Lower Saxony. Selective field reflectance measurements of single plots, which were used for model calibration, were conducted on field trials in Brunswick (Fig 1A). They are described in more detail in the next section. Measurements with the ground-based PentaSpek system were acquired near the city of Asendorf on the estate of the Deutsche Saatveredelung AG, a leading German breeding company in agricultural research. Data was acquired on a breeding yard with 1152 single plots, each covering a size of 2 m time 8.50 m (Fig 1A-C). The total area of the breeding yard amounts to 2.1ha which is dominated by sandy silt and sandy loamy silt, and about 45m above sea level. The mean annual temperature of the site is 9.3° C. Annual rainfall sums up to 751 mm.

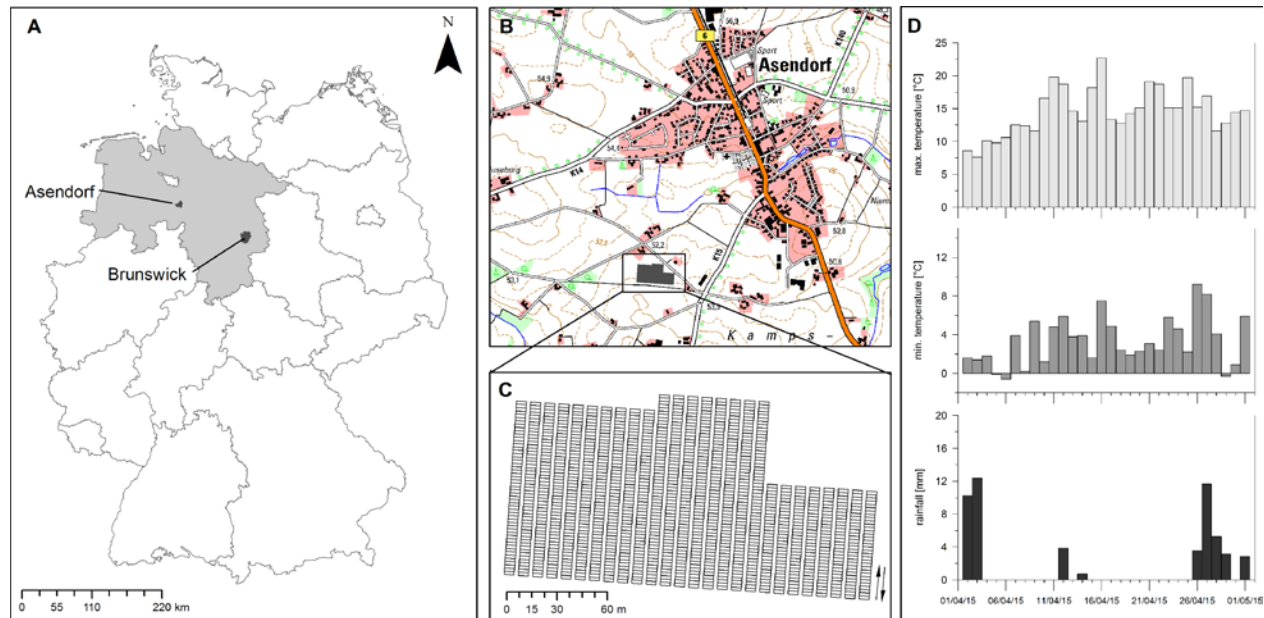


Fig 1. A: Location of field experiments in Brunswick (52°17.3'N, 10°26.1'E) and the breeding yard in Asendorf (52°45.9'N, 8°59.7'E). **B and C:** Close up of the breeding yard near Asendorf comprising 1152 plots. Arrows indicate tractor's driving direction. **D:** Daily temperature (min, max) and rainfall in Asendorf in April 2015.

Field reflectance measurements and sampling of reference data

Reflectance measurements and sampling of winter oilseed rape (*Brassica napus* L.) were conducted on field experiments of the Julius Kühn-Institut in Brunswick, Germany. Experiments were established using ten genotypes, and applying different nitrogen and sulfur fertilization levels to provide a high variability of plant development and growth on a limited area. Field work was undertaken during two growing seasons from 2014 to 2016, before winter (12th October, 2nd November) and after winter (13th March, 1st April, 6th June) resulting in 102 data sets. Canopy reflectance was acquired with the SVC HR1024i field spectrometer from Spectra Vista Corporation ranging from 350nm to 2500nm. Reflectance was recorded from plots of 0.25m². All measurements were acquired relative to a standardized spectralon panel.

After finishing reflectance measurements, leaf area index (LAI) of the same plots was measured using a LAI-2200C plant canopy analyzer (LI-COR, Inc.). Further, plant height and growing stage (BBCH, Meier 2001) were recorded. Plots were harvested after measurements were finished to determine above ground fresh biomass (FM) and dry biomass (DM). DM was weighed after oven drying at 60 °C for at least 24 h.

The PentaSpek system

The PentaSpek system is a ground based proximal sensing system. It consists of five STS-VIS spectrometers (Ocean Optics, Inc.) mounted on a side boom of a tractor. Four spectrometers (S1, S2, S3, S4) are directed downwards recording the reflected radiation. One spectrometer (Sref) is directed upwards, simultaneously measuring incident radiation (Fig 2). The latter can be used to calculate reflectance instantly in the field, and to continuously correct reflected radiation by incident radiation. This way, the system can also work under cloudy conditions. The spatial location of the spectral measurements is determined by a GPS receiver based on top of the tractor cab, taking into account the horizontal and vertical offset of the spectrometer modules.

The STS-VIS spectrometers cover the spectral range from 335 nm to 825 nm with a band width of approximately 1.5nm (FWHM) and a field of view of 4°. The signal-to-noise ratio is greater than 1500:1. Spectrometers were installed at 2 m height above ground that corresponds to a measurement spot size of approximately 0.14 m. Driving speed was set to 1 km/h which is equivalent to 0.27 m/s.

PentaSpek data was acquired at the breeding yard close to Asendorf on April 10th, 16th, and 28th 2015. Selected field plots started flowering at April 23rd but beginning of flowering was assessed for only 5 % of the plots by April 27th. Data was acquired along the short edge (2 m) of the plots. Therefore, measurements were conducted from both sides in order to acquire reflectance of the entire plot.

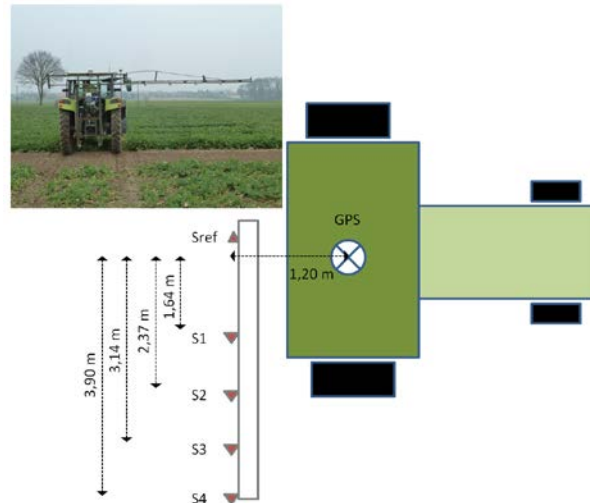


Fig 2. Schematic illustration of the PentaSpek system used on the breeding yard near Asendorf.

Data preprocessing

Field reflectance spectra acquired with the SVC spectrometer were resampled to 1nm. Spectral bands affected by atmospheric absorption between 1340 nm to 1430nm and 1800 nm to 1970 nm were eliminated. Bands less than 400 nm and greater than 2400 nm prone to noise were also removed. Finally, spectral data was normalized using unit vector normalization. For statistical analysis, two data sets were derived. One data set containing the full spectral information, and a second data set with a spectral range according to the PentaSpek system using the wavelength from 400 nm to 800 nm.

Hyperspectral reflectance measurements acquired with the PentaSpek system were first joined with GPS coordinates using the timestamp and taking into account the lateral and horizontal offsets (cf. Fig 2). Spectra were then resampled to 1nm, and confined to a wavelength range from 400 nm to 800 nm. After that, data was normalized by analogy with field reflectance measurements using unit vector normalization, and PLSR models based on the PentaSpek configuration were applied. Subsequently, zonal statistics including median and standard deviation were calculated for each plot

and parameter (LAI, FM, DM). Negative parameter values were excluded from computation.

Statistical analysis

The quantitative relationship between canopy reflectance and crop parameters was set up using partial least squares regression (PLSR). PLSR is a bilinear multivariate modeling approach that projects predictor (X) and response (Y) variables onto a few so-called latent variables or factors by maximizing the covariance between X and Y. This way, problems with multicollinearity and variable selection can be handled effectively. A detailed description of the PLSR algorithm is given by Wold et al. (2001). The analyses were performed with The Unscrambler (CAMO Software AS) applying the PLSR according to Martens & Naes (1989).

Statistical models were set up for LAI, FM, and DM, respectively, using the full spectral range of the SVC HR 1024i field spectrometer, and a reduced spectral range corresponding to the specification of the PentaSpek system. The maximum number of latent variables (LV) was set to 10. The optimal number of LV was determined by using leave-one-out cross-validation and the predicted residual sum of squares statistic. Due to the relatively limited number of samples, validation of the selected model was performed by leave-one-out cross-validation.

The performance of the models was evaluated using the coefficient of determination (R^2) and the root mean square error of cross-validation ($RMSE_{cv}$). Further, the ratio of performance to deviation (RPD) after Chang et al. (2001) was calculated. RPD can be used as an independent measure of model performance to compare models based on different data sets. However, it is affected by the data distribution and may obscure the true model quality if data is highly skewed. Therefore, the ratio of performance of inter-quartile distance (RPIQ) after Bellon-Maurel et al. (2010) was additionally computed.

Results and discussion

Sample descriptive statistics

Winter oilseed rape plots sampled during six field campaigns covered phenological growth stages of leaf development (BBCH 13-17), stem elongation (BBCH 30-32) and inflorescence emergence (BBCH 50 to 55). Corresponding canopy height varied between 0.07 m and 1.52 m. The LAI of the plots was between 0.27 to 5.56 $m^2 m^{-2}$. The mean LAI of 102 samples was 2.03 $m^2 m^{-2}$. The amount of FM ranged from 2.66 to 51.7 $t ha^{-1}$ whereby FM of 50% of the samples was less than or equal to 8.61 $t ha^{-1}$. The DM of the samples varied from 0.12 to 6.95 $t ha^{-1}$ with a mean amount of 1.10 $t ha^{-1}$ correspondingly. All three parameters exhibit a positive skew. One out of 102 plots was not sampled for biomass, and a further measurement of DM was erroneous (Table 1).

Table 1. Descriptive statistics of the model reference data base.

Parameter	No. samples	Median	Minimum	Maximum	Standard deviation
LAI [$m^2 m^{-2}$]	102	2.03	0.27	5.56	1.16
FM [$t ha^{-1}$]	101	8.61	2.66	51.7	11.4
DM [$t ha^{-1}$]	100	1.10	0.12	6.95	1.56

Fig 3 depicts the mean reflectance curve of winter rape of all samples from the field experiments in Brunswick. Spectral curves mirror the typical pattern of green and vital vegetation reflectance with a distinct absorption in the blue (400 to 500 nm) and red (650 to 700 nm) part of the spectrum, a reflectance peak near 550 nm, and a strong increase of reflectance above 700nm (Jones & Vaughan, 2010). This transition zone is called the red-edge region. On average, about 40% of the incident radiation is reflected in the NIR which is due to the internal cellular structure. Accordingly, light is mostly absorbed by plant water in the shortwave part of the spectrum.

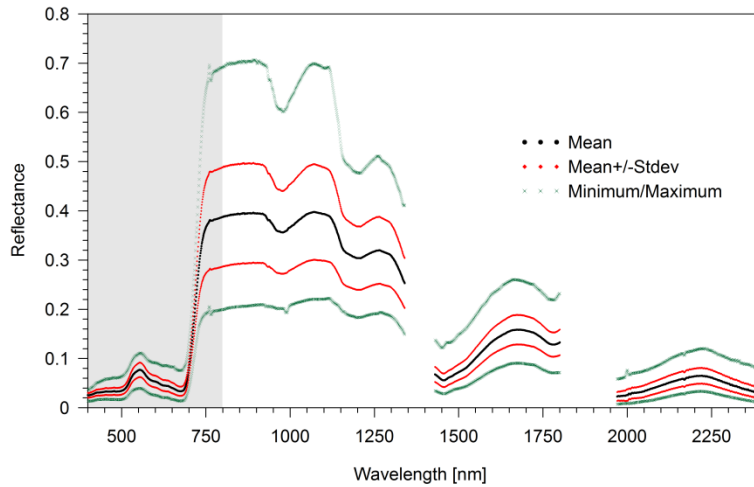


Fig 3. Mean field reflectance spectra (full range) with standard deviation and minimum and maximum reflectance. Reflectance values in the range of the water absorption bands were eliminated. Grey background indicates the spectral range of the PentaSpek system.

Model calibration and validation

Predictive performance statistics of the PLSR models based on full range field reflectance spectra are summarized in Table 2. For LAI, 57 % of the variability in Y can be explained by the model. There is a broad scatter along the 1:1 line (Fig 4, above) which is illustrated by an $RMSE_{cv}$ of $0.77 \text{ m}^2 \text{ m}^{-2}$. RPD of 1.52 indicates a model with intermediate prediction ability according to a classification by Chang et al. (2001). This is confirmed by an RPIQ of 1.48. Prediction models for FM and DM reach R^2 values of 0.82 with an $RMSE_{cv}$ of 4.85 t ha^{-1} and 0.67 t ha^{-1} respectively. RPD above 2 mark excellent models. However, the number of samples with a great amount of biomass is relatively little which is also reflected by the lower RPIQ values. Further, scatter plots indicate a trend to underestimate higher parameter values, and slightly increasing scatter with increasing biomass (Fig 4, above).

Table 2. Predictive performance statistics of the models based on full range field spectra between 400 to 2400 nm.

Crop	No. LV	R^2_{cv}	$RMSE_{cv}$	SD	RPD	RPIQ
LAI [$\text{m}^2 \text{ m}^{-2}$]	4	0.57	0.77	1.16	1.52	1.48
FM [t ha^{-1}]	8	0.82	4.85	11.4	2.36	1.68
DM [t ha^{-1}]	6	0.82	0.67	1.56	2.33	1.95

The reduction of the full spectral range to match the spectral characteristics of the PentaSpek system (400-800nm) decreased model performance in all three cases (Table 3). $RMSE_{cv}$ increased by 5 %, 15 % and 9 % for LAI, FM and DM respectively. FM and DM models still explain more than 75% of the variability of the response but scatter plots suggest that the underestimation of higher FM and DM values is augmented (Fig 4, below). The red-edge region was found to be the most important region for the characterization of a variety of vegetation parameters such as canopy biomass or LAI (e.g. Hermann et al., 2011, Hansen & Schjoerring, 2003, Müller et al., 2008, Delegido et al., 2011). However, Li et al. (2014b) showed that also a number of wavelengths in the NIR between 800 nm and 1300 nm exhibited high correlations with LAI and improved the accuracy of PLSR models. For biomass, Thenkabail et al. (2013) identified relevant spectral bands near 855 nm, 1045 and 1100nm. Lee et al. (2004) further stated that SWIR region was as important as the red edge for predicting LAI. Darvishzadeh et al. (2009) confirmed that the SWIR region contains relevant information for LAI estimation. The decrease in prediction accuracy can therefore be attributed to the confined spectral range of the PentaSpek system.

Currently, the number of samples with LAI, FM or DM values above $4 \text{ m}^2 \text{ m}^{-2}$, 25 t ha^{-1} and 3 t ha^{-1} , respectively, is comparatively low. Models are thus in a preliminary stage and will be supplemented

with samples during inflorescence emergence and later plant growth stages in the future.

Table 3. Predictive performance statistics of the models based on field spectra ranging from 400 to 800 nm.

Crop	No. LV	R ² cv	RMSE cv	SD	RPD	RPIQ
LAI [m ² /m ²]	4	0.52	0.81	1.16	1.45	1.41
FM [t/ha]	8	0.76	5.58	11.4	2.05	1.46
DM [t/ha]	7	0.78	0.73	1.56	2.13	1.78

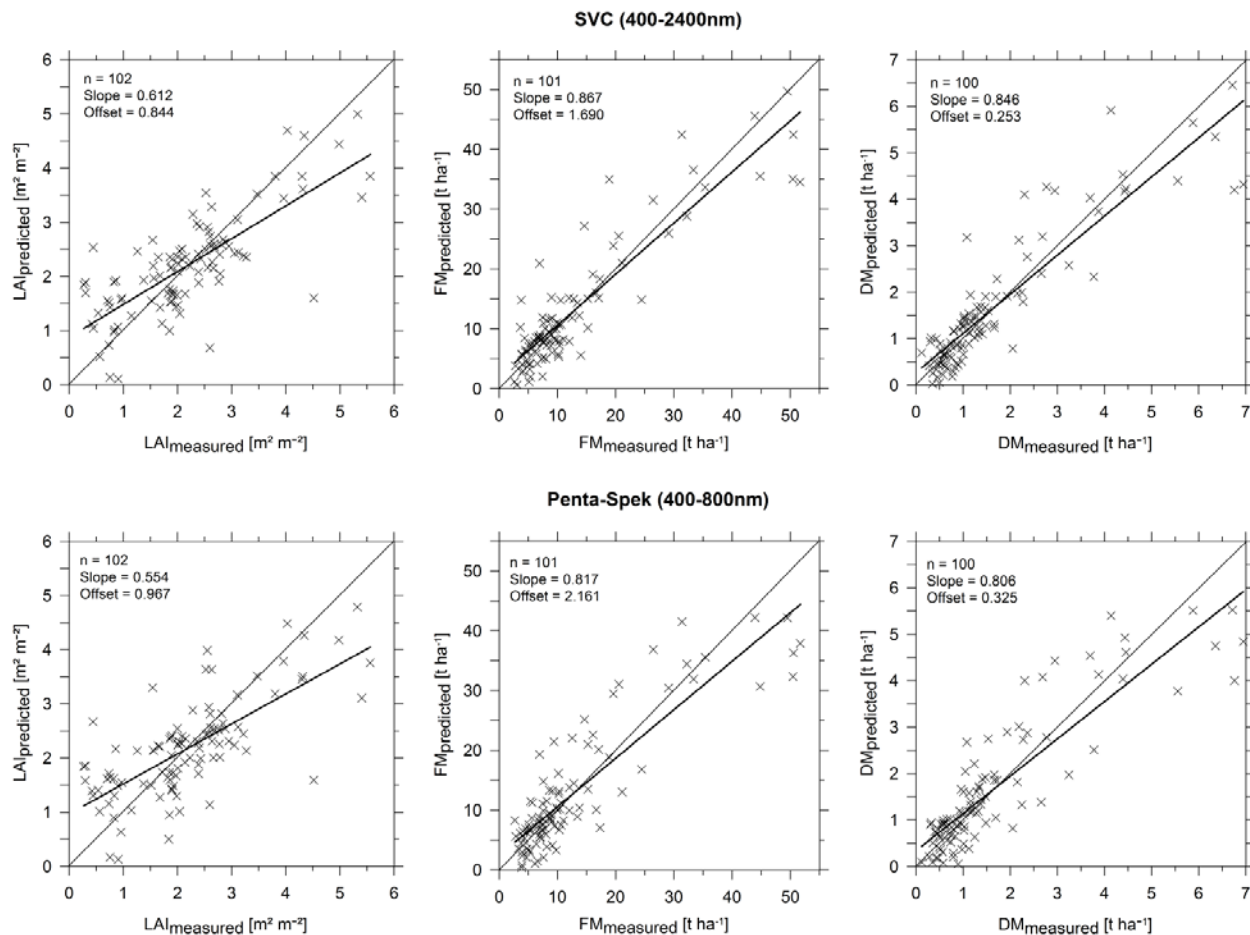


Fig 4. Plots of measured versus predicted values for LAI, FM, and DM based on cross-validated PLSR models using the full spectral range (SVC, above) and a reduced spectral range (PentaSpek, below).

Spatial explicit parameter estimation using PentaSpek data

Processing raw data and subsequent screening of the data quality disclosed a defect of the second spectrometer (S2). Thus, data acquired by this device was removed from all three data sets before parameter estimation. The remaining number of spectral measurements averaged to 24, 25 and 22 records per plot for the first, second and third acquisition date. According to GPS time, acquisition of data from all 1152 plots lasted about 6 hours (Table 4).

Table 4. PentaSpek data acquisition dates and times (UTC).

Acquisition date	10 th April	16 th April	28 th April
Start time (t _s)-End time (t _E)	8.30AM-2.40PM	9.30AM-3.00PM	6.30AM-12.50AM
Total time	6h 30min	5h 30min	6h 20min
Solar elevation (t _s)- (t _E)	32.6-30.4	41.4-29.4	21.3-47.6

Results based on median values are displayed in Fig 5. LAI maps show a continuous increase of values over the sensing period as expected with an overall mean LAI of 3.9, 4.5, and 4.8 $\text{m}^2 \text{m}^{-2}$ on April 10th, 16th, and 28th, respectively. This is also clearly shown by the boxplot statistics in Fig 6. Maps on FM specify a distinct increase of fresh biomass by 15.2 t ha^{-1} on average between April 16th and April 10th. That corresponds to average FM values of 24.8 t ha^{-1} and 40.0 t ha^{-1} . However, estimated FM values on April 28th are in turn lower than in mid-April and account for 30.4 t ha^{-1} only. Boxplots show that similar results were obtained for DM which amounts to 3.9, 5.9 and 5.7 t ha^{-1} on average on April 10th, 16th and 28th, respectively.

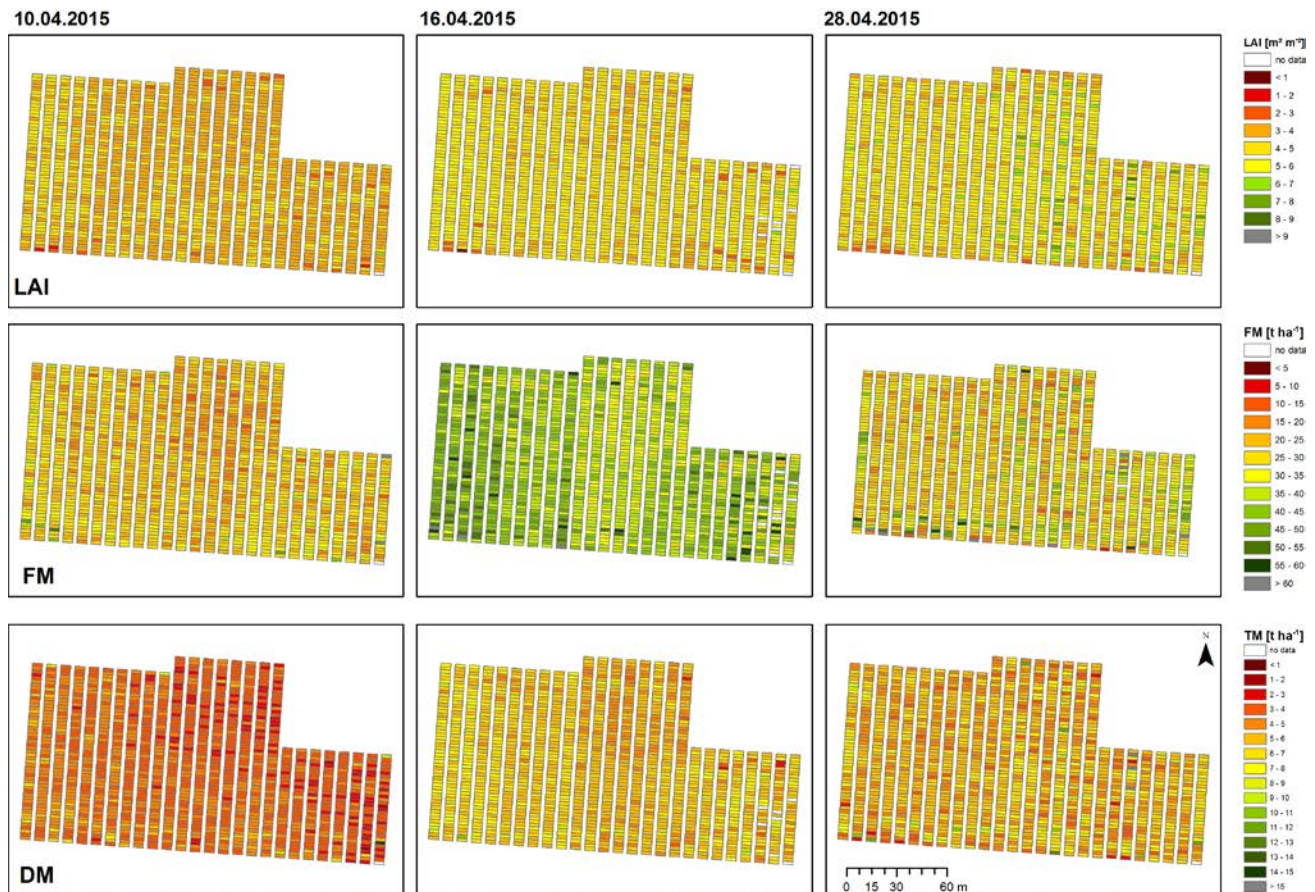


Fig 5. Estimated LAI, FM and DM of a breeding yard near Asendorf on three days in April 2015 based on hyperspectral data acquired with the PentaSpek system.

As no ground truth data was recorded during data acquisition results on parameter development over time as derived from spectral reflectance of the PentaSpek system can only be verified for plausibility. LAI development is in accordance with Behrens & Diepenbrock (2006a) who reported an LAI increase of winter oilseed rape (*Brassica napus* L.) of approximately 1 $\text{m}^2 \text{m}^{-2}$ between beginning of shooting to beginning of flowering within 21 days. In our experiment, LAI development is similar. LAI increased by 0.9 $\text{m}^2 \text{m}^{-2}$ in 18 days. Mean absolute values did, however, not agree and were on average one and a half times higher at the beginning of flowering than in other studies (Behrens & Diepenbrock, 2006a; Grosse et al., 1992). Grosse et al. (1992) studied yield formation of winter oilseed rape in a field experiment with four to eight replications during three years. They reported maximum growth rates of 0.283 $\text{t DM ha}^{-1} \text{d}^{-1}$ for most of the genotypes which occurred 10 to 13 days after beginning of flowering. In earlier development stages (BBCH 31 to 61), increase in biomass was

much lower (cf. Behrens & Diepenbrock, 2006b). A mean increase by 2 t DM ha⁻¹ and 15.2 t FM ha⁻¹ during 6 days as detected by the PentaSpek system seems therefore unrealistic. An average gain by 1.8 t DM ha⁻¹ and 5.6 t FM ha⁻¹ during 18 days may be feasible. Assuming a linear growth rate, this would correspond to 0.1 t DM ha⁻¹ and 0.31 t FM ha⁻¹ per day and is in accordance with experimental data published by Diepenbrock et al. (2000). Dry matter accumulation varies with genotype and environment and is affected by the previous crop, the type of N fertilizer and N supply (Diepenbrock et al. 2000, Grosse et al., 1992). Hence it is difficult to judge whether experimental data from other sites is reliable enough to draw conclusions. The weather conditions in April 2015 provide, however, no reasonable grounds for a distinctly higher increase in LAI, FM and DM between the first and second day of acquisition compared to the second and third day of acquisition (Fig 1).

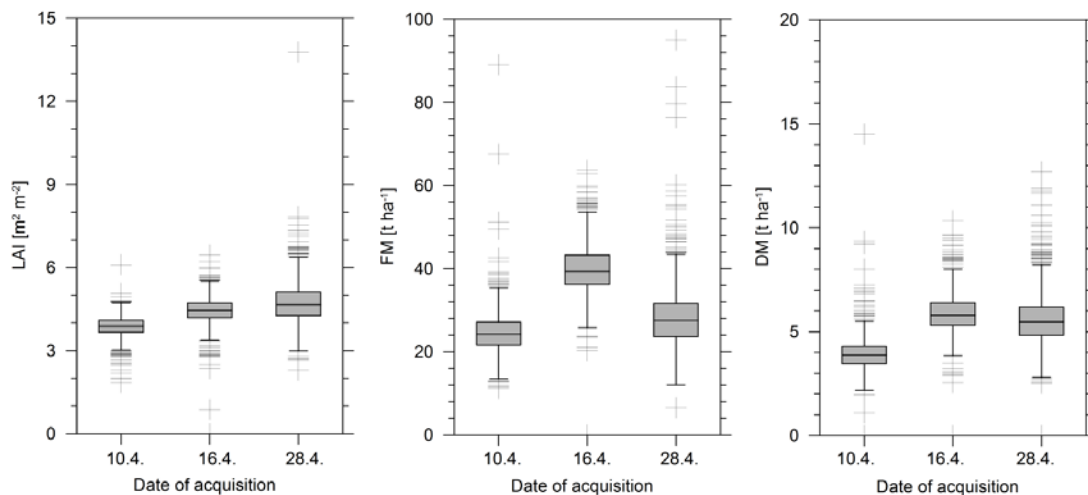


Fig 6. Boxplot statistics of LAI, FM and DM estimations per acquisition date.

Estimations of LAI, FM and DM are in a first instance subject to the accuracy of the PLSR models (cf. Table 2). Beyond that they may be affected by the time and duration of acquisition due to variations in sun elevation and illumination conditions. Because data acquisition took place during normal work operations on the breeding yard, start and end time varied considerably between the days of data acquisition. Particularly striking is April 28th. At that day, data acquisition started much earlier in the morning than on April 10th and 16th (Table 4). Accordingly, mean reflectance observed at 780 nm at that day was only 32 % compared to 51 % and 58 % on the first and second acquisition date respectively (data not shown). Problems may further arise from the long period of data acquisition (6 hours) which favors undesirable changes in ambient light conditions due to a mix of sun and clouds. Therefore, some of the irregularities discussed earlier on may be attributed to the time of day and the duration of acquisition. Similar problems were encountered by other authors (Comar et al., 2012). These problems could be overcome by measurements of all plots in a short period of time using a hyperspectral imaging camera mounted on an aerial platform.

Summary

In this study, we presented a low-cost ground-based hyperspectral system (PentaSpek) for high-throughput plant phenotyping in breeding research of winter oilseed rape. In a first step, PLSR models based on hyperspectral field reflectance measurements and in-situ data of vegetation parameters were set up for the prediction of three important biophysical variables. These models were applied to PentaSpek data acquired in a breeding yard at the beginning, middle and end of April 2015.

First results demonstrate that the system enables the acquisition of information on properties such as LAI, FM and DM of winter oilseed rape in large breeding yards in less than one day. Frequent operations deliver information on plant development. Presently a lack of reference data hampered a more comprehensive data analysis. However, results showed that the adherence to standard protocols defining the start and end time of acquisition is a crucial point to ensure data quality. Further, minimum requirements for weather conditions seem indispensable to downsize negative effects due to varying illumination conditions.

Next steps comprise the extension of the model data base in terms of number of measurements and phenological development stages of the samples. Further, a profound analysis of the impact of changing sun position and elevation during data acquisition will be undertaken. As an alternative to the ground-based system, which is likely to overcome problems to varying illumination conditions, a hyperspectral camera mounted on an unmanned aerial platform will be tested.

Acknowledgements

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