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High-throughput phenotyping of barley cultivars under field conditions

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If you’re going through hell; keep going

Winston Churchill

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Zusammenfassung

Während der technische Fortschritt in der Genotypisierung dazu geführt hat, dass eine große Anzahl Getreidesorten schnell und günstig analysiert werden kann, gilt die Feldphänotypisierung noch immer als arbeitsintensiv und teuer. Die Lösung könnten Spektralsensoren sein, mit denen Pflanzenzüchter und Pflanzenwissenschaftler verschiedene Pflanzeneigenschaften messen können, wobei auf arbeitsintensive Methoden (visuelle Bonituren) verzichtet werden kann. Vorangegangene Studien mit Mais und Weizen haben gezeigt, dass die Trockenmasse und die N-Aufnahme mit dieser sensorgestützten „Hochdurchsatz-Phänotypisierung“ erfolgreich detektiert werden können. Informationen über einzelne Pflanzenorgane waren bisher allerdings nicht verfügbar.

Diese Studie wurde durchgeführt, um mit einer Multi-Sensor Plattform phänotypische Unterschiede in Sommer- und Wintergerste zu erfassen. Neben einem passiven Spektrometer und zwei Distanzsensoren, ist das Fahrzeug mit drei 'aktiven' Spektralsensoren ausgestattet. Dreißig Sommer- und sechzig Wintergerstensorten wurden in den Jahren 2013 bis 2016 in der Forschungsstation Dürnast der Technischen Universität München angebaut und die entsprechenden Pflanzenproben in Halme, Blätter, Blattscheiden und Ähren separiert. Die Sensormessungen wurden mit einer partial least squares regression (PLSR) und traditionellen Vegetationsindices ausgewertet, um organspezifische Trockenmassen, N-Aufnahme und Pflanzenhöhen zu ermitteln.

Für die PLS Regressionen wurden die Wellenlängen zwischen 400 und 1000 nm des passiven Spektrometers verwendet. Die Modelle für „Blätter“ und „Halme“ zeigten dabei die besten Ergebnisse. Zudem konnte mit einer Messung zur Blüte der finale Kornertrag prognostiziert werden, wobei es noch immer eine Schwierigkeit darstellt, den Proteingehalt zu bestimmen. Der Ultraschalldistanzsensor konnte durch sein großes Messfeld die reale Pflanzenhöhe am besten nachbilden. Spektralmessungen in Verbindung mit PLS Regressionen stellen ein vielverspre-

chendes Werkzeug für Pflanzenzüchter und -wissenschaftler dar, um die Biomasseentwicklung und N-Aufnahme von Sommer- und Wintergerste zu erfassen. Ein spezialisiertes Sensorfahrzeug bietet zudem größte Flexibilität.

Summary

In contrast to high-throughput genotyping which can handle large numbers of plants at low cost, phenotyping of many individuals in field trials is still laborious and expensive. Spectral proximal sensing may represent a useful tool for plant breeders to phenotype various plant traits by avoiding laborious methods such as visual scoring in the field. Previous work done with wheat and maize indicated the feasibility of advanced high-throughout phenotyping under field conditions for wheat and for maize. Results from these studies as well as previous work has established that aboveground biomass and nitrogen uptake of various plant species can successfully be detected, challenging the option to deliver plant or organ specific information which is not yet in the hand of plant breeders. This study was conducted to assess phenotypic differences in spring and winter barley (*Hordeum vulgare* L.) by using the lightweight vehicle based multi-sensor platform PhenoTrac IV, equipped with several active light sensors, a bidirectional passive sensor making it independent of the ambient incident radiation and two distance sensors. Thirty spring barley cultivars and sixty winter barley cultivars were grown during 2013-16 at the Dürnast Research Station of the Chair of Plant Nutrition. The plants were separated into culms, spikes, leaves and leaf sheaths. Sensor measurements were analyzed by partial least squares regression (PLSR) and 'traditional' vegetation indices to predict organ specific biomass, N-uptake and plant height. PLS regression models were calculated using hyperspectral wavelength information from a bidirectional passive spectrometer from 400 to 1000 nm. Best results were achieved with models of leaves and culms. Additionally, a mid-season prediction of the final grain yield is possible, however, the prediction of the protein content remains challenging. Plant heights were obtained by using commercially available distance sensors. The ultrasonic distance sensor outperformed the laser distance sensor due to a wider measuring field. Spectral proximal sensing may be a useful tool for breeders to screen spring and winter barley. PLS regression represents a promising method for analyzing wavelength spectra and predicting parameters such as biomass development or N-uptake. Bidirectional measurement allowed for measurements independent of the ambient light conditions. The lightweight construction of the carrier vehicle enabled a highly flexible usage.

1. Introduction

1.1. Challenges in Plant Science and Plant Breeding

New technologies in plant breeding, such as ’next-generation sequencing’ or ’marker-assisted selection’, led to an acceleration of breeding processes and an enhanced necessity to test new genotypes in the field (FURBANK & TESTER, 2011; WHITE ET AL., 2012; ARAUS & CAIRNS, 2014). Field trials are necessary to assess specific plant traits, such as the biomass, nitrogen content and plant height in realistic production environments. Plant breeders and agronomists, however, face a bottleneck in phenotyping (WINTERHALTER ET AL., 2011; WHITE ET AL., 2012) due to a lack of efficient high-throughput field phenotyping methods that keep pace with the achievements in high-throughput genomics (WHITE ET AL., 2012). Current field phenotyping approaches, such as visual scoring, are time consuming, labour intensive, costly and biased due to the person’s individual experience (ERDLE ET AL., 2013A; KIPP ET AL., 2014) and emphasise the need for new high-throughput methods. Furthermore, biomass samples need to be taken destructively (Figures 1.1 and 1.2), which can be problematic in small plots.

Figure 1.1.: Biomass sampling at anthesis, in particular, breathing protection and protection goggles are needed to avoid possible allergic reactions

Figure 1.2.: Harvest of biomass samples. Waterproof and rugged clothing is required in high and wet crop stands.

In early selection cycles in plant breeding, large numbers of plants need to be tested, and in agronomic field testing, extensive evaluation of plant performance is also required. Both seed availability and financial constraints frequently necessitate testing of plants in one or several rows, with space limitations also contributing to a need for small plot sizes. Limited resources, therefore, necessitate smaller plots (BROWN & CALIGARI, 2008). In general, plot size depends on the type of experiment, breeding objectives, available resources and equipment, and the stage of breeding (ACQUAAH, 2012). However, plot sizes vary substantially among field trials, ranging from single-plant plots to plots of several hundred square meters (PETERSON, 1994). Small plots with 2-3 rows are usually used in early stages of breeding projects to evaluate varieties quickly and inexpensively (Figure 1.3). In advanced selection cycles, when selection for yield also occurs, larger plots are used, and the data may be collected from middle rows (ACQUAAH, 2012). Such plot trials, thus, aim to predict the performance of the tested varieties by mimicking agricultural field conditions. However, such predictions may be inaccurate since the phenotypic performance of plants grown at different spacing may differ from that of plants grown using conventional agricultural practices (BROWN & CALIGARI, 2008). The small size of plots may be disadvantageous because border row effects are known to influence yield. Depending on the type of plot trial, external rows may show increased yield (ROMANI ET AL., 1993) due to increased tillering (AUSTIN & BLACKWELL, 1980).

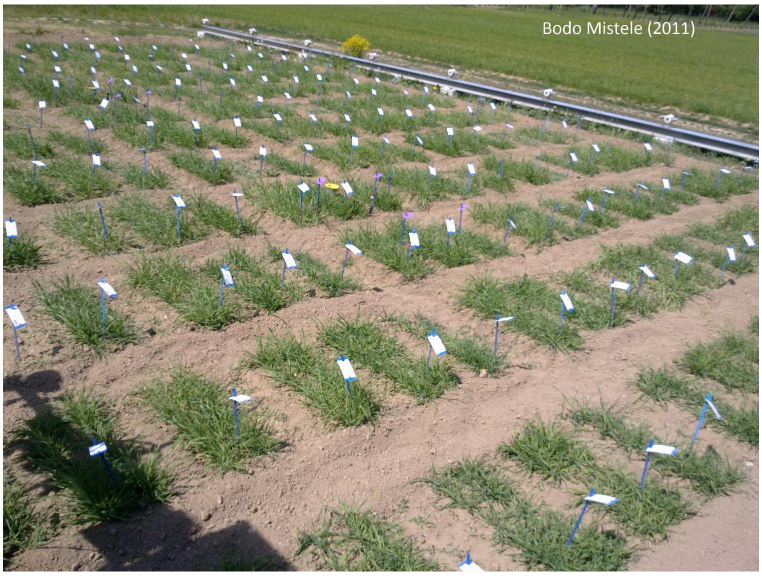

Figure 1.3.: Field trial with winter barley cultivars in two-rowed micro plots

1.2. Section I: Referencing laser and ultrasonic height measurements of barley cultivars by using a herbometre as standard

In addition to tiller number and biomass, plant height represents an important factor for the assessment of crop stands and consequently for fertiliser and pesticide applications (EHLERT ET AL., 2009; LLORENS CALVERAS ET AL., 2011). Plant breeders often select dwarfed cultivars to reduce lodging. In opposition to this, there is a tendency to choose taller plants, especially for the production of energy, due to the shift from fossil-based resources to renewable resources in Europe (HEIERMANN ET AL., 2009; DINUCCIO ET AL., 2010; ZUB ET AL., 2011) or the selection of suitable parental lines for hybrid breeding (LONGIN ET AL., 2012). For agronomists and plant breeders, the most common methods used to measure plant height is by using a meter stick or visual scoring. The German Federal Office of Plant Varieties recommends the use of a ruler to average a plant height by taking a single measurement within each plot and recording the top-most part of the plant, including the ears and awns (BUNDESSORTENAMT, 2000). However, this method is time consuming and not objective due to individual decisions for the highest representative part of a plant. Therefore, the quality might change during the measurements, and it remains challenging to assess the true or representative height of a cultivar by only measuring a few plants within a plot. Measurements of plant height should also reflect a meaningful agronomical or physiological property. Since in cereals such as wheat, barley, rye and oat, but also in rice, particularly after the termination of shooting, photosynthesis predominantly takes place in the top canopy layer, an averaged plant height representing such an activity might be more useful than choosing just the top most position of a plant. This is also reflected in vertical gradients of the nitrogen distribution within plants that are optimised towards the top canopy layer in such cereals. A further challenge represents the leaf angle and the inclination of leaves varying from erectophile to planophile and also being subject to further changes across the development of the plants. Height measurements in barley plants are particularly challenging due to the increased number of tillers per plant differing in height and due to the highly variable length of the flag leaf. In the past few years, several approaches were tested to measure plant height by using distance sensors for cereals or grasses, for example winter wheat (SCOTFORD & MILLER, 2004A; EHLERT & ADAMEK, 2007), rye, grass (EHLERT & ADAMEK, 2007), rice (TILLY ET AL., 2014) and corn (KATAOKA ET AL., 2002; FREEMAN ET AL., 2007; YIN ET AL., 2011). In contrast, no investigations have been made regarding barley (*Hordeum vulgare* L.), and no attempts were made to find out whether it is possible to differentiate between uniformly fertilised varieties at a certain growth stage. Primarily, previous studies focused on the technical feasibility of different concepts, whereas the agronomic aspects were frequently not included in the focus. Most authors used industrial distance sensors that operate either as time-of-flight or as triangulation sensors. The time-of-flight sensors are known for their ability to measure long ranges, whereas triangulation sensors are restricted to short ranges due to their construction (maximum a few meters) by having a higher accuracy. The sensors have to fulfil particular requirements for usage in field trials. For instance, the sensors should be insensitive to dust, vibrations of the carrier platform, direct sunlight and high temperatures. Additionally, the sensor should be able to detect materials such as plant tissues. Detailed measuring principles have been reported by EHLERT ET AL. (2010) and DWORAK ET AL. (2011). Reference measurements represent an important aspect of these trials and have mainly been achieved by using meter sticks (SCOTFORD & MILLER, 2004A; CHATZINIKOS ET AL., 2013); however, in most publications a detailed description of the reference method is missing. In this study, we have adopted as novel reference method for cereals a herbometre, which is often used to record plant height or biomass in pastures and grassland (PAULY ET AL., 2012). This principle confers advantages compared with meter sticks because it allows the measurement of a weighted height, which is considered to be more representative and informative of the average estimated plant height, and further increases the objectivity of the process.

1.3. Section II: High-throughput phenotyping of wheat and barley plants grown in single or few rows in small plots using active and passive spectral proximal sensing

Since the management of field trials comprising a large number of plots is highly labour-intensive, new methods, such as spectral proximal sensing for the estimation of specific plant traits, are becoming increasingly more important (WHITE ET AL., 2012; ERDLE ET AL., 2013A). However, commercially available spectral proximal sensors, such as the GreenSeeker (NTech Industries Inc., Ukiah, CA, USA), as well as hyperspectral passive sensors (ERDLE ET AL., 2011; RISCHBECK ET AL., 2016), were originally designed and tested for field conditions and not specifically for small-plot testing. Therefore, assessment of the sensors in plot trials is of great importance, and particular attention should be paid to the evaluation of the sensed areas. Such evaluation requires the consideration of technical aspects, such as sensor-target distances, and the influences of environmental conditions, such as light intensity and temperature (WINTERHALTER ET AL., 2013; KIPP ET AL., 2014). Sensors should be compatible with various plot designs, which ultimately requires a match between the sensors' field of view and the tested target. Field trials comprising different plot designs (Figure 1.4) and cropped with one or multiple species, are challenging to evaluate, and their potential for assessment by proximal sensing needs to be determined. Non-invasive assessments of small plots must take into account uneven growth due to differences in the light availability or enhanced nutrient and water uptake. It is also important to consider whether middle rows are to be assessed preferentially or an integral assessment of the whole plot is desired. Additionally, reflectance sensors differ in their spectral fields of view, ranging from linear to oval and circular shapes (ERDLE ET AL., 2011; KIPP ET AL., 2014), and are also influenced whether the sensor's orientation is parallel or opposed to the row. Numerous studies have described border row effects (REBETZKE ET AL., 2014) and the advantages and disadvantages of different field trial designs (DEPAUW, 1975; KRAMER ET AL., 1982; MAY & MORRISON, 1986; ROMANI ET AL., 1993), in addition to comparing different spectral sensors (ERDLE ET AL., 2011; KIPP ET AL., 2014; ELSAYED ET AL., 2015). To the best of our knowledge, no studies have compared the performances of active and passive sensors in assessing the single or multiple rows used in breeding or agronomic experiments; such a comparison was the goal of this work.

Figure 1.4.: Field trials within a rain-out shelter platform, illustrating different row designs for spring barley grown in two rows (foreground) and for winter barley grown in six rows (background).

1.4. Section III: Active and passive high-throughput field phenotyping of leaves, leaf sheaths, culms and ears of spring barley cultivars

A fast and non-invasive method to obtain information about the characteristics of cultivars could be spectral proximal sensing (WHITE ET AL., 2012; ERDLE ET AL., 2013B; KIPP ET AL., 2013). Vehicles (e.g., tractors, buggies) are particularly advantageous when a high number of genotypes and/or large plots of field trials need to measured. A further benefit of vehicles is the possibility of combining several sensors on a carrier vehicle to take measurements simultaneously (WINTERHALTER ET AL., 2011; DEERY ET AL., 2014). Studies have been performed for the spectral proximal sensing of cereal plant traits, such as the estimation of aerial biomass or the nitrogen status of spring and winter wheat (ØVERGAARD ET AL., 2013B; ERDLE ET AL., 2013B; LI ET AL., 2013A; XIU-LIANG ET AL., 2014; BAI ET AL., 2016), durum (FERRIO ET AL., 2005), winter barley and rye, corn (HABOUDANE ET AL., 2004; WINTERHALTER ET AL., 2013) and spring barley (YU ET AL., 2012; BENDIG ET AL., 2014, 2015A; XU ET AL., 2014; ELSAYED

ET AL., 2015; LAUSCH ET AL., 2015; TILLY ET AL., 2015; RISCHBECK ET AL., 2016). However, data analysis remains a major challenge. While many authors rely on various vegetation indices (such as the: NDVI, REIP, PRI, WI, SAVI, TCARI) (BEHRENS ET AL., 2006; YU ET AL., 2012; ERDLE ET AL., 2013B; LI ET AL., 2013A; BENDIG ET AL., 2015A; ELSAYED ET AL., 2015; TILLY ET AL., 2015), additional methods, such as 'contour maps' and 'partial least squares regression' (PLSR), have been highlighted as particularly interesting to optimize data analysis. HANSEN & SCHJOERRING (2003) used these methods to detect the biomass and nitrogen status, LI ET AL. (2013A) used these methods to estimate the nitrogen content, and ELSAYED ET AL. (2015) and RISCHBECK ET AL. (2016) used these methods to predict the drought stress and grain yield in barley. However, most authors have investigated biomass parameters that are subjected to increasing nitrogen fertilizer applications with occasionally strong nitrogen deficiencies. By contrast, in plant breeder nurseries, uniform fertilizer treatments are applied, thus lowering the variance due to agronomic treatments. Hence, breeders require spectral sensors and algorithms that allow differences among cultivars to be distinguished from differences resulting from varying agronomic management practices. ACQUAAH (2012) has reported on the various points of view of plant breeders regarding the importance of different plant organs. In addition to grains, there are other important plant organs, such as culms for the production of straw. Furthermore, culms are the most important storage organs of assimilates for translocation processes after anthesis (BIDINGER ET AL., 1977; MIROSAVLJEVIC ET AL., 2015). Unadapted or stressed cultivars particularly rely on the dry matter and nitrogen reserves of culms (PRZULJ & MOMCILOVIC, 2001A,B). Knowledge regarding the characteristics of the leaves of a cultivar or variety is important for plant breeders when optimization of the photosynthetic active area is intended (HABOUDANE ET AL., 2004). ZHU ET AL. (2010) mentioned that an improvement of the leaf area and architecture may avoid saturation effects of individual leaves and support higher grain yields. Additionally, leaves act as a sink for nutrients as well as a source of proteins and are therefore important for grain yield formation (ACQUAAH, 2012). The role of leaf sheaths as a vertical part of leaves has not been widely reported in the literature. SCHNYDER (1993) characterized leaf sheaths as long-term storage for carbohydrates that are influenced by environmental conditions. In this study, cultivars were observed that accumulated up to 20 kg N ha^{-1} in leaf sheaths at anthesis (supplemental Table B.2). The question is, how precisely can these plant organs be detected by spectral sensors? Three active and one passive bidirectional spectrometer were used in this study. While the passive spectrometer depends on sunlight as its source of light, the active sensors use independent light sources, such as LED or Xenon lamps (ERDLE ET AL., 2011). The advantage of active sensors is that they can be applied during changing light conditions or at night without any effect on their readings (HATFIELD ET AL., 2008; KIM ET AL., 2012; KIPP ET AL., 2014). However, the passive spectrometer used in this study is equipped with two detectors. The first one measures global radiation as a reference signal, and the second one measures the reflectance of the plant canopy to avoid effects due to changing light conditions (MISTELE & SCHMIDHALTER, 2010). Technical comparisons among different sensor systems for the prediction of specific plant traits have been performed multiple times. ERDLE ET AL. (2011), WINTERHALTER ET AL. (2013) and ELSAYED ET AL. (2015) evaluated active and passive sensors in winter wheat, corn and spring barley, respectively. The performance of active sensors under changing environmental conditions was evaluated by KIM ET AL. (2012) for the GreenSeeker and KIPP ET AL. (2014) for the GreenSeeker, CropCircle and AFS N-Sensor.

1.5. Section IV: Mid-season prediction of grain yield and protein content of spring barley cultivars using high-throughput spectral sensing

Spring barley is the most important crop for malt and beer production. More than 60 % of global production comes from the European Union, the Balkan countries, Russia and Canada (FAOSTAT, 2015). A reliable forecast of grain yield and protein content before harvest would be useful, especially for the malting and brewing industry. It would simplify the acquisition and management of raw materials (WEISSTEINER & KUEHBAUCH, 2005). A solid prediction of yield parameters is also a major advantage for plant breeders (FERRIO ET AL., 2005). Knowing the performance of different cultivars in the early stages of breeding saves costs and time, since this makes it possible to focus on high-performance cultivars only (ROYO ET AL., 2003). However, a practical method for predicting yield parameters needs to be timesaving, non-destructive and cost-efficient (accounting for both labor and analytic costs). Spectral proximal sensing fulfills these requirements (PRASAD ET AL., 2007; WHITE ET AL., 2012; XIU-LIANG ET AL., 2014). In addition to common vegetation indices such as the NDVI (APARICIO ET AL., 2000) and the REIP (PETTERSSON ET AL., 2006), new methods such as the contour map method and Partial Least Squares Regression (PLSR) have been found to be useful for yield prediction (ELSAYED

ET AL., 2015; RISCHBECK ET AL., 2016). Different approaches have been tested for in-season estimation of yield parameters. For winter wheat, RAUN ET AL. (2001) and MOGES ET AL. (2005) found strong relationships between NDVI and grain yield. However, XUE ET AL. (2007) stated that vegetation indices such as GNDVI or NDVI did not provide a reliable prediction of protein content. The PLSR method was used to predict the yields of twenty-five Durum wheat cultivars by FERRIO ET AL. (2005). They concluded that it worked better for ranking different genotypes than for making accurate predictions of their grain yields. Other studies used the PLSR method to estimate the grain yield and protein content of spring wheat (ØVERGAARD ET AL., 2013B) and winter wheat (XIU-LIANG ET AL., 2014). Their predictions of protein content were more accurate than predictions using vegetation indices. Additionally, ØVERGAARD ET AL. (2013B) highlighted the importance of using several years of data to construct a stable PLSR model. Yield predictions have also been made for spring barley. HANSEN ET AL. (2002) evaluated predictions of grain yield and protein content by comparing ten vegetation indices and PLSR under different nitrogen fertilizer levels and seeding densities. Good relationships were found between grain yield and PLSR; however, only poor results were obtained for protein content. A better prediction was obtained by SÖDERSTRÖM ET AL. (2010), by combining spectral sensing with weather data. WEISSTEINER & KUEHBAUCH (2005) developed a model using satellite spectral remote sensing and ancillary data such as meteorological or pedological data. In different drought stress scenarios, ELSAYED ET AL. (2015) compared vegetation indices against PLSR, and RISCHBECK ET AL. (2016) found that PLSR models were improved when spectral sensing was combined with plant canopy thermal data. Although several authors emphasized the need for a fast and inexpensive method to predict yield and protein content before harvest, most of them used hand-held field spectrometers or satellite data (WEISSTEINER & KUEHBAUCH, 2005; SÖDERSTRÖM ET AL., 2010) with coarse spatial resolution. Only a few authors used vehicle-based spectral proximal sensing to estimate plant traits (MISTELE & SCHMIDHALTER, 2008; ERDLE ET AL., 2013B; KIPP ET AL., 2014). To the best of our knowledge, there have been only a few studies that used independent datasets to validate PLSR models. No studies using ground-based spectral proximal sensing evaluated their models using independent field trials. Most authors were primarily seeking to optimize fertilization strategies, and very few studies were seeking to advance breeding-related phenotyping.

2. Objectives

The main objectives of this Ph.D. thesis are indicated separately for Section I to IV.

The aims of **Section I** were a comparison of the performance of a low-cost ultrasonic sensor and a laser distance sensor implemented in a mobile phenotyping high-throughput platform in field trials to test the possible differentiation of barley cultivars based on sensor measurements.

The purpose of **Section II** was to assess the performance of active and passive spectral sensing in plot designs of one, two, three, and four rows, like those commonly used in breeding trials for wheat and barley. Previous studies have shown no difference in spectral performance when assessing plots with six or more rows. In this work, the influence of different plot designs on biomass and grain yield is illustrated, highlighting the performance of spectral sensors in the non-invasive detection of these traits.

The potential of spectral proximal sensors to detect the characteristics of different plant organs was first shown by ERDLE ET AL. (2013B). In contrast to ERDLE ET AL., who considered later growth stages in winter wheat, **Section III** focuses on spring barley during anthesis and dough ripeness. Sensor measurements were made by using two commercially available and two custom built spectral sensors. The aims of this study were to perform (i) a comparison of different spectral proximal sensors and (ii) a comparison of published vegetation indices, contour maps and PLSR to assess leaves, leaf sheaths, culms and ears in spring barley.

The aims of **Section IV** were (i) to find optimized vegetation indices, (ii) to create PLSR models that could predict the grain yield and protein content of spring barley, (iii) to compare the performance of different vegetation indices and PLSR using independent field trials and (iv) to highlight the advantages of vehicle-based sensing in this context.

3. Materials and Methods

All field experiments were conducted at the Dürnast Research Station of the Technical University of Munich (TUM) in Germany (11°41′60″E, 48°23′60″N, elevation 448 m) between 2013 and 2015. The soil is a mostly homogeneous Cambisol of silty clay loam texture, the annual precipitation is approximately 800 mm and the average temperature is 7.5 °C.

3.1. Field experiments of Section I

The study encompassed three site-years of investigation comprising 1 year of spring barley in 2013 and 2 years of winter barley in 2014 and 2015. The experimental design was a randomized block design with four replications using 30 spring barley cultivars in 2013, three winter barley hybrids and 11 lines in 2014 and 12 hybrids and 48 lines of winter barley in 2015. The plots consisted of 12 rows, 6 m in length. The fungicide and fertilisation treatments followed local recommendations.

3.2. Field experiments of Section II

A randomized block design was used to test both barley (cv. Sandra) and wheat (*Triticum aestivum* L.) (cv. Kerubino), with four planting-row designs and four replicates totaling 40 plots (Fig. 3.1).

Figure 3.1.: UAV image of the field trial. Different plot designs, including one-, two-, three- and four-row designs, were tested using winter wheat and winter barley as crops.

The plots were 10 m in length. The planting-row designs consisted of plots with a single row, plots with two rows with 25-cm row spacing, and plots with three and four rows with 12.5-cm row spacing. The wider 25-cm row spacing is frequently used for testing the performance of barley, whereas the narrower spacing of 12.5 cm is commonly used for testing wheat in breeding nurseries in Germany. Fungicide treatments followed local recommendations. Weeds were removed by hand to remove possible bias in interpreting the results. Nitrogen fertilizer was applied in a single dose at ZS 15 (ZADOKS ET AL., 1974) as ammonium sulphate using the nitrification inhibitor ENTEC (HU ET AL., 2013) with 150 kg N/ha and 60 kg S/ha in amounts corresponding to the different numbers of rows.

3.3. Field experiments of Section III and Section IV

The 3-year study, conducted at the Chair of Plant Nutrition, used 30-34 spring barley cultivars (Table 3.1) in a randomized block design with 4 replicates. The cultivars were chosen to represent different usages. Along with malting and fodder barley cultivars, four hull-less barley cultivars used for human food were cultivated. Due to seed limitations, the cultivar Pirona could be tested in only two of the three years. Plots consisted of 12 rows, 10.9 m in length (16.35 m^2). Fungicide and fertilization treatments followed local recommendations.

Table 3.1.: Overview of spring barley cultivars grown in different years.

Cultivar	Usage	2013	2014	2015
Aspen	Malting	X	X	X
Barke	Malting	X	X	X
Baronesse	Malting	X	X	X
Br8993a3	-	X		
Braemar	Malting	X	X	X
Calcule	Fodder	X	X	X
Carina	Malting	X	X	X
Djamila	Fodder	X	X	X
Eunova	Fodder	X	X	X
Grace	Malting	X	X	X
Hora*	Human food			X
IPZ 24727	Malting	X	X	X
Irina	Malting	X	X	X
Lawina*	Human food	X		
Mackay [AUS]	Malting	X	X	X
Marthe	Malting	X	X	X
Melius	Malting	X	X	X
Paradiesgerste*	Human food			X
Pirona*	Human food		X	X
Power	Malting	X	X	X
Quench	Malting	X	X	X
Salome	Malting	X	X	X
Scarlett	Malting	X	X	X
Shakira	Malting	X	X	X
Sissy	Malting	X	X	X
Solist	Malting	X	X	X
Streif	Fodder	X	X	X
Trumpf/Triumph	Malting	X	X	X
Union	Malting	X	X	X
Ursa	Malting	X	X	X
UTA	Malting			X
Vespa	Fodder	X	X	X
Volla	Malting	X	X	X
Wiebke	Malting	X	X	X

*hull-less barley

3.3.1. Independent validation experiments for the application of PLSR models of Section IV

Field experiments used for evaluating the PLSR models were provided by the Chair of Phytopathology at the Technical University of Munich. These field experiments were located approximately 3 km from the Dürnast Research Station. Methods for sensor measurements, grain harvest and protein-content determinations were similar to those used in the other field experiments. Plots consisted of 12 planting rows, 7.5 m in length (11.25 m^2).

3.3.1.1. Experiment 1 (IV-1)

For experiment IV-1, the cultivars Grace and Scarlett were grown under 3 nitrogen fertilizer levels with 8 and 12 replicates in 2013 and 2014, respectively. In 2015, only Grace was grown with 4 nitrogen fertilizer levels and 12 replicates for each nitrogen level. The nitrogen levels were 0, 40, 80 and 140 kg N ha^{-1}, applied as calcium ammonium nitrate fertilizer. The experimental design was a strip design. Fungicide treatments followed local practices.

3.3.1.2. Experiment 2 (IV-2)

Experiment IV-2 was conducted in 2015 using the cultivars Quench, Grace and Scarlett. The experimental design was a block design with 32 replicates. In this field trial, protein content was not determined. Fungicide and fertilization treatments followed local recommendations.

3.4. Biomass sampling and protein content determination

In Section II, biomass samplings were performed at Zadoks stage 32 (stem elongation), ZS 60 (anthesis) and ZS 85 (soft dough) by cutting plants above the ground along 1 m of each row. The fresh biomass was immediately determined in the field by weighing, and a subsample was oven-dried.

In Section III and Section IV, biomass sampling was performed at anthesis (ZS 65) and at soft dough ripeness (ZS 85) by harvesting 30 plants from each plot randomly. The plants were separated into ears, leaves, leaf sheaths (2014 and 2015) and culms. Grain was harvested from each plot using a plot harvester. The biomass samples were oven dried at 60 °C for 2 days to achieve a uniform moisture content and then weighed. The N content was detected by mass spectrometry using an Isotope Radio Mass Spectrometer with an ANCA SL 20-20 preparation unit (Europe Scientific, Crewe, UK), and N uptake was calculated by multiplying the plant dry weight by the total N content. The protein content was calculated by multiplying the total N content by 6.25.

3.5. Description of the PhenoTrac 4

The phenotyping platform PhenoTrac 4 is a former yard trac made by Mapro Systems AB, Sweden (Fig. 3.2). The vehicle is equipped with custom made axes that are stepless adjustable for an individual track width. Furthermore, the ground clearance was enhanced up to 0.8 m. The PhenoTrac is all-wheel drive with a hydrostatic drive train. The maximum speed is with regard to German street laws limited to 6 km h^{-1} Vmax. Further technical specifications can be found in Table 3.2.

Figure 3.2.: Phenotyping platform PhenoTrac 4.

Table 3.2.: Technical data of the Phenotrac 4.

Technical Data	
Manufacturer	Mapro Systems AB, Sweden
Car classification (Germany)	Self-propelled monitoring car
Empty weight	950 kg
Maximum permissible weight	1200 kg
Length x Height x Width (without sensor boom)	2980 mm x 2380 mm x 2160 mm
Engine	Daihatsu
Engine displacement	953 ccm
Layout/Number of cylinders	Line 3
Fuel	Diesel
Emission standard	Not classified
Power	19 kW / 1600 rpm
Maximum speed (Germany)	6 km h^{-1}
Ground clearance	80 cm
Adjustable track width	1350 mm - 2000 mm
Other specifications and customizations	Hydraulic front loader (incl. bucket)
	Improved braking system
	Mechanic speed limitation to 6 km h^{-1}
	Removable roll bar
	Removable laptop table
	Improved alternator
	Electric sockets for sensors
	Schuko plug sockets

All sensors are attached to a boom on the front loader. For the power supply of 12 sensors and a laptop, a bigger alternator was implemented. All sensor outputs are co-recorded with RTK GPS, converted and saved to the laptop.

3.5.1. Sensors used on the PhenoTrac 4

3.5.1.1. Thermal sensors

For the detection of heat and drought stress, two Heitronics KT15D (Heitronics GmbH, Wiesbaden, Germany) infrared thermometers are mounted on the PhenoTrac 4. The spectral response is between 8 and 20 μm and the temperature resolution is 0.06 °C. At a distance of about 1 m, the field of view is 3-10 cm which enables point measurements. To reference the ambience temperature, the sensors are equipped with rotating mirrors for the measurement of their own case temperature. This allows reliable measurements under changing environmental conditions. The sensors are mounted from two opposed oblique views at an angle of 45 ° on the

PhenoTrac 4. This angle avoids measurements of the soil and maximizes the plant surface fraction (Elsayed et al. 2015; Rischbeck et al. 2016). The software used on the PhenoTrac 4 is able to distinguish between the sun-lit and the shaded plant canopy by using data of the position of the sun from the reference sensor of the bidirectional passive spectrometer. This method avoids measuring errors when the vehicle is turned around at the end of the field.

3.5.1.2. Distance sensors

The PhenoTrac 4 is equipped with three distance sensors, mounted in a red frame (Fig. 3.3). The sensors selected are a SICK DT20 (black), SICK UM30-14113 (blue, white) (Sick, Waldkirch, Germany) and a Welotec OWTG 4100 PE S1 (orange) (Welotec, Laer, Germany).

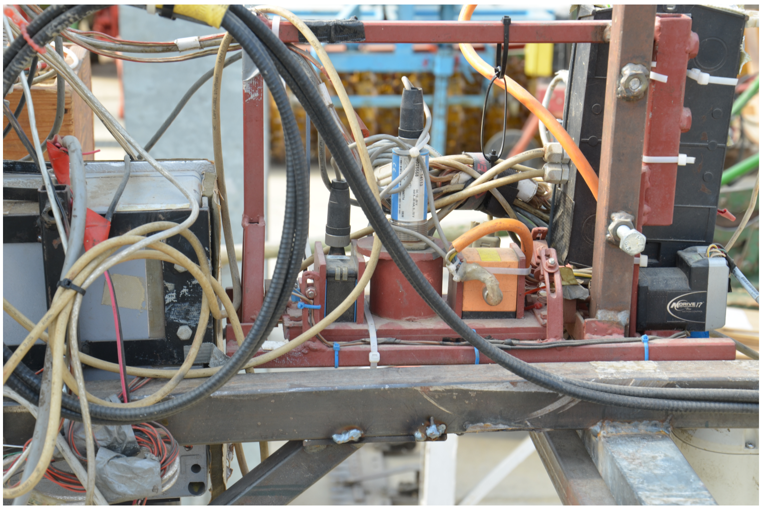

Figure 3.3.: Distance sensors on the PhenoTrac 4.

The sensors used are commercially available and are often used for the positioning of objects (e.g. trains, aircrafts), collision avoidance (robotic arms in production lines), silo fillings or the measurement of object surfaces (e.g. ore on conveyor belts). For the use in field trials, several requirements must be complied. The sensors have to be resistant to dust, vibrations, shocks and the influence of direct sunlight (Ehlert et al. 2009). The measurement principles of each sensor is different. While the OWTG and the DT20 are using laser light sources for the measurement, the UM30 is ultrasonic based. Furthermore, the UM30 and OWTG operate as 'time-of-flight' sensors and the DT20 uses the principle of 'laser triangulation'. The advantage of the 'time-of-flight' sensors is the increased measuring range (up to several hundred meters) compared to the laser triangulation, however, laser triangulation sensors are known for their high accuracy of about a few micrometer (Fig. 3.4). A further description of the sensor performance can be found in Section I.

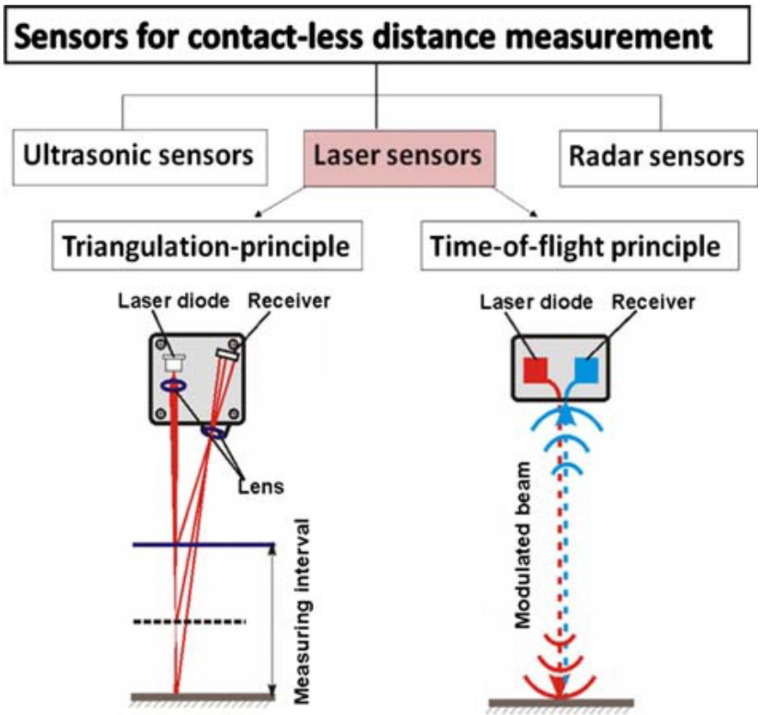

Figure 3.4.: Different principles of height measurement (Ehlert et al. 2009)

3.5.1.3. Spectral proximal sensors

One passive bidirectional spectrometer and three active spectral proximal sensors are mounted on the PhenoTrac 4 (Fig. 3.5).

The passive hyperspectral bidirectional reflectance sensor contains two Zeiss MMS1 silicon diode array spectrometers with a spectral detection range from 300 to 1700 nm at a bandwidth of 3.3 nm (Mistele and Schmidhalter 2010). One spectrometer measures the canopy reflectance of the plants (Fig. 3.5A1), while the second spectrometer is linked to a diffuser using solar radiation as a reference signal (Fig. 3.5A2). It allows stable measurements under changing environmental conditions. The passive spectrometer was calibrated before each measurement using a grey standard. The first active spectral proximal sensor is a CropCircle ACS-470 (Holland

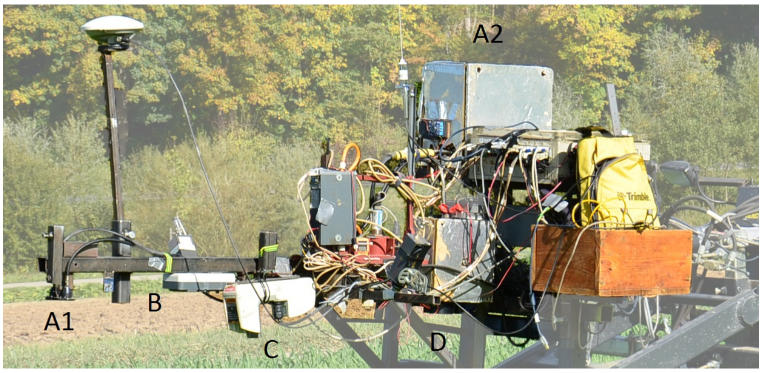

Figure 3.5.: Spectral proximal sensors of the PhenoTrac 4

Scientific, Inc, Lincoln, NE) (Fig. 3.5B), which is a LED based sensor with a selection of filters for wavelengths at 670, 730, and 760 nm. Another active proximal sensor is the GreenSeeker RT100 (NTech Industries, Ukiah, CA, USA) (Fig. 3.5C) that uses two LEDs as a light source and detects the reflection of both in the VIS (656 nm, 25 nm band width) and NIR (774 nm, 25 nm band width) spectral regions. The third active sensor is custom made active flash sensor (AFS) (Fig. 3.5D) that is similar to the N-Sensor ALS (YARA International, ASA). In contrast to the GreenSeeker and CropCircle, the AFS uses a flashing xenon light and filters in the wavelength region of 730, 760, 900, and 970 nm (Erdle et al. 2011). With reference to the manufacturers' information, active sensors were calibrated before delivery and no additional calibration is required. A further description of the sensor performance can be found in Section II, III and IV.

3.6. Section I: Distance sensors for the measurement of plant height

3.6.1. Plant height measurement

Reference measurements of the height were obtained by using a self-constructed low-cost herbometre, similar to a rising plate meter, consisting of a Styrofoam board, 50 cm × 50 cm in size and 4 cm thick, having a weight of 200 g, attached centrally to a conventional folding rule (Figures 3.6 and 3.7). The board was carefully placed on the plant surface, and the ruler was pushed through the hole without exerting any pressure on the board. Depending on the cultivar and the growth stage, the plants were compressed by 0.3-3 cm by the herbometre. The compression was decreasing with progressing maturation of the plants. A barrel roller was used to flatten the soil in early spring to minimise the risk of imprecise measurements due to a rough soil surface. The height measurements were conducted four times within each plot shortly before flowering at ZS 55, as this represents a sensitive growth stage relevant to agronomic and breeder decisions.

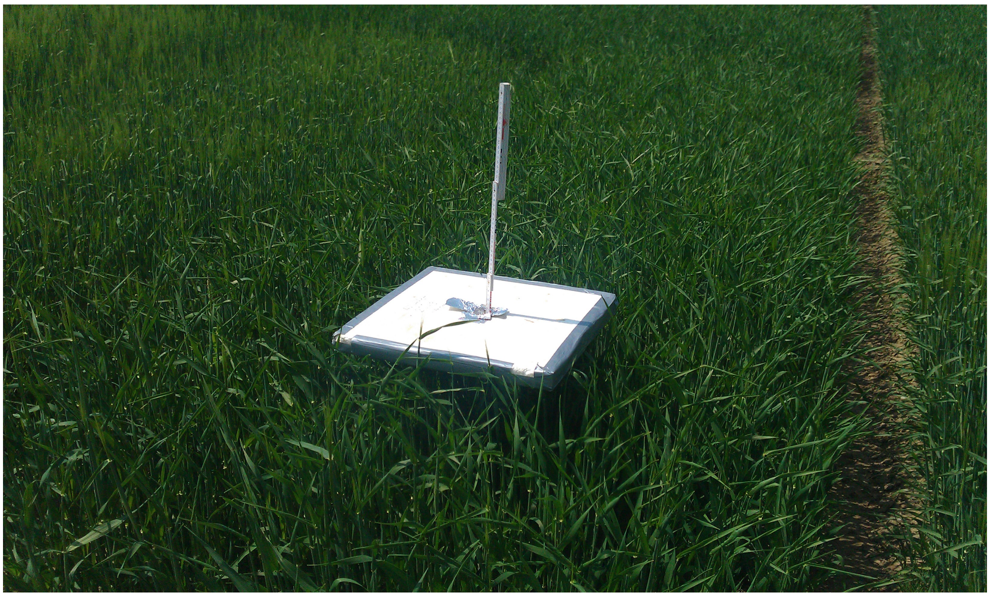

Figure 3.6.: Illustration of the herbometre height measurements serving as the reference method in barley trials at anthesis.

3.6.2. Height measurements with ultrasonic and laser sensor devices

The performances of an ultrasonic sensor and a laser distance sensor were compared under field conditions. The sensors selected were a UM30-14113 ultrasonic sensor (Sick, Waldkirch, Germany) and an OWTG 4100 PE S1 laser distance sensor (Welotec, Laer, Germany) (Table 3.3). The sensors were mounted as closely as possible to each other on a boom, 1.5 m in front of the PhenoTrac IV (RISCHBECK ET AL., 2016), a mobile phenotyping platform of the Chair of Plant Nutrition of the Technical University Munich, in a nadir down-looking position. The sensor outputs were linked and synchronised to the GPS coordinates from a TRIMBLE-RTK-GPS. Calibration of the sensors was conducted on a bare plot. The sensor boom was held at a height of 1

Figure 3.7.: Usage of the self-built herbometer at early growth stages in winter barley

m above the plants, and the driving speed was 3.5 km h^{-1}. The data output comprises 25 measurements across the 6-m plot length. Average values of all of the measurements per plot were calculated, and averaged maximum values, representing the subset of the five highest records, were additionally calculated to gain further information about the highest areas within each plot. The plant height was calculated as:

$$\textit{plant height} = \textit{distance}_{\text{sensor to soil surface}} - \textit{distance}_{\text{sensor to plant surface}}.$$

Furthermore, the ultrasonic sensor is equipped with a 'first-fix' algorithm that analyses the echogram considering the upper part of the plants and the soil. Threshold values were defined to avoid implausible high or low values that may affect the calculation of mean values for the plant height.

Table 3.3.: Technical data of the ultrasonic and distance sensors. Higher frequency records of the distance sensors were averaged to be in line with the GPS records.

Technical data	UM30 ultrasonic distance sensor	OWTG 4100 PE S1 laser distance sensor
Measurement method	Time-of-flight	Time-of-flight
Measuring range	250-3400 mm	200-10 000 mm
Measuring field	0.5 m^2	6 mm
Resolution	1 mm	1 mm
Accuracy	$\leq$ 2% of final value	14-17 mm
Sampling interval (modified)	25 Hz	25 Hz
Transducer frequency	120 kHz	-
Wavelength	-	650 nm
Temperature measurement range	-20 °C - 70 °C	-10 °C - 60 °C
Weight	310 g	295 g
Price (2006)	400 €	280 €

3.6.3. Comparison between the folding ruler and the herbometre

A comparison between the herbometre and a folding ruler was conducted in wheat plots at heading in 2014. For each plot, three measurements were made for the folding ruler and the herbometer, respectively. The uniformity of herbometre measurements within the plots was assessed by means of four replicate measurements in 2014 and 2015. Two operators measured independently of each other winter barley at ZS 55 in 2015 to find out the deviation between twofolding ruler measurements. According to the German Federal Office of Plant Varieties, a single measurement in the centre of each plot was taken.

3.7. Section II, III and IV: Spectral proximal sensors for high-throughput phenotyping of spring barley

All sensor measurements were taken under a clear sky at noon. While collecting information in the field, the sensor outputs were co-recorded along with GPS coordinates from the TRIMBLE RTK-GPS (Trimble, Sunnyvale, CA, USA). The passive hyperspectral bidirectional reflectance sensor contains two Zeiss MMS1 silicon diode array spectrometers with a spectral detection range from 300 to 1700 nm and has a bandwidth of 3.3 nm (Mistele and Schmidhalter, 2008), but it was restricted in this study to 1000 nm. One spectrometer was linked to a diffuser that detected solar radiation as a reference signal. The second spectrometer measured the canopy reflectance with a field of view (FOV) of 12° that was circular in shape, resulting in a scanned area of 0.28 m^2 and covering an area of 5.45 m^2 along the plot. The passive spectrometer was calibrated before each measurement using a grey standard. The active spectral sensor, GreenSeeker RT100 (NTech Industries, Ukiah, CA, USA), uses two LEDs as a light source and detects the reflection of both in the VIS (656 nm, 25 nm band width) and NIR (774 nm, 25 nm band width) spectral regions. The FOV of the GreenSeeker was a strip of approximately 61 by 1.5 cm, resulting in a scanned area of approximately 0.009 m^2 (Fig. 3.9). As a second active spectral sensor, an active flash sensor (AFS) was used that was similar to the N-Sensor ALS (YARA International, ASA) with a flashing xenon light as a light source, producing a spectral range of 650-1100 nm with 10 flashes per second. In this experiment, filters similar to those of the YARA ALS system were chosen: 730, 760, 900, and 970 nm (Erdle et al., 2011). The third active spectral sensor was a CropCircle ACS-470 (Holland Scientific, Inc., Lincoln, NE), which emits white light (light source: 400 to 800 nm), with a selection of filters for wavelengths of 670, 730, and 760 nm. The CropCircle was only used in 2013 and 2015. With reference to the manufacturers' information, the active sensors were calibrated before delivery and no additional calibration was required. Table 3.4 shows the vegetation indices selected for this experiment.

Figure 3.8.: Phenotyping platform PhenoTrac 4 of the Chair of Plant Nutrition from the Technical University of Munich

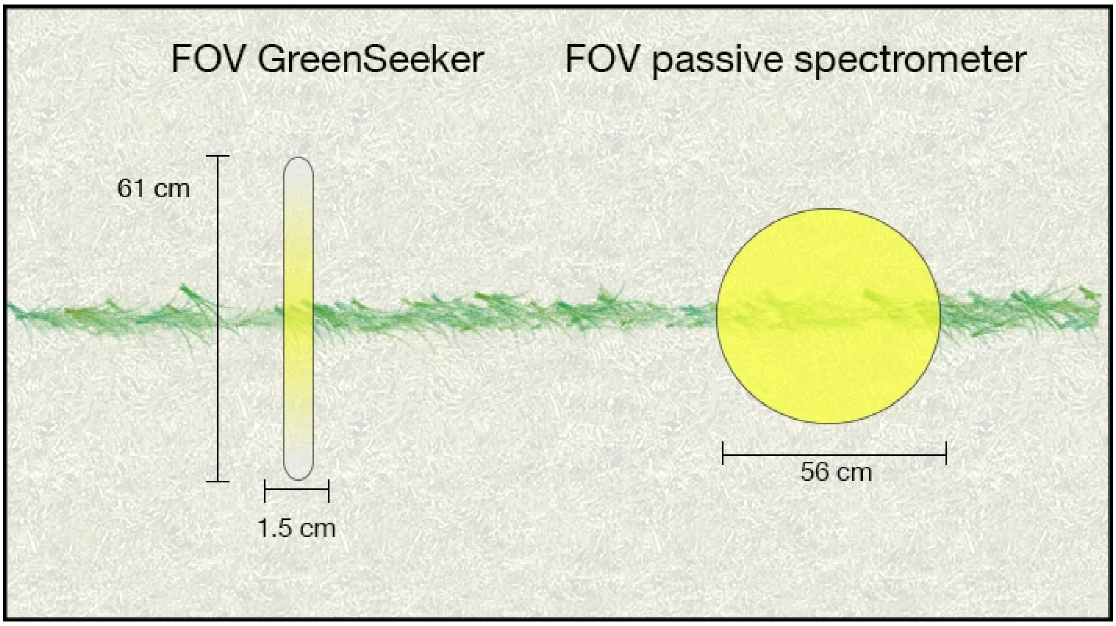

Figure 3.9.: Illustration of different shapes of sensors' fields of view (FOV) in single-row trials. Yellow colour indicates decreasing light intensity in the periphery of the LED-based GreenSeeker (unpublished data).

Table 3.4.: Selected vegetation indices of the four sensor systems used.

Device	Vegetation Index	Reference
GreenSeeker	R_{774}/R_{656}	
	NDVI	(ROUSE ET AL., 1974)
CropCircle	R_{730}/R_{670}	
	R_{760}/R_{730}	(MISTELE & SCHMIDHALTER, 2008)
	R_{760}/R_{670}	(MISTELE & SCHMIDHALTER, 2008)
	NDVI	(ROUSE ET AL., 1974)
AFS	R_{760}/R_{730}	(MISTELE & SCHMIDHALTER, 2010)
	R_{900}/R_{970}	(PEÑUELAS ET AL., 1993)
Passive spectrometer	R_{780}/R_{550}	(MISTELE & SCHMIDHALTER, 2008)
	R_{780}/R_{670}	(PEARSON ET AL., 1972)
	R_{780}/R_{700}	(GUYOT ET AL., 1988)
	R_{760}/R_{670}	(ERDLE ET AL., 2011)
	R_{760}/R_{730}	(MISTELE & SCHMIDHALTER, 2010)
	R_{780}/R_{740}	(MISTELE & SCHMIDHALTER, 2010)
	R_{900}/R_{970}	(PEÑUELAS ET AL., 1993)
	REIP	(GUYOT ET AL., 1988)
	NDVI	(ROUSE ET AL., 1974)

3.8. Statistical analyses

R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria) was used for calculating the coefficients of variation, the standard errors, and linear regressions between the data obtained from the sensors and the destructive measurements. A regression analysis and a one-way ANOVA were used to compare the ultrasonic and distance sensor and the herbometre reference measurements in Section I, the grouping and differentiation between planting-row designs in Section II and the means of the observed and predicted grain yields in Section IV. Tukey's HSD (honest significant difference) multiple comparison test ($p \leq 0.05$) was applied for the grouping of the cultivars and the differentiation between planting-row designs.

3.8.1. Partial least squares regression

Unscrambler® X 10.3 (Camo Software AS, Oslo, Norway) was used to calculate PLS regression models. PLSR is a multivariate statistical method used to find 'latent' structures in the wavelength spectra (X) that best predict the measured parameter (Y). This method is advantageous when dependent (response) variables need to be predicted from large datasets of predictor variables. The dataset is reduced to a few 'principal components' (PC) or 'factors' that are used for prospective predictions of the response variables. A detailed description of PLSR can be found in ESBENSEN ET AL. (2002). In this study, PLSR was used to model the correlation between canopy reflectance spectra (X) between 400 nm and 1000 nm, as measured by the passive spectrometer at anthesis and dough ripeness, and the dry weights and N uptake of the plant organs, grain yield and protein content (Y). All of the spectral data used in Section III to calculate the PLSR models were corrected for light scattering using Standard Normal Variate Transformation (SNV). All spectral data used in Section IV for developing the PLSR models were mean-centered and normalized using unit vector normalization. To develop the PLSR models, all data from the 3 years were used. The dataset was randomly separated into subsets, using ⅔ of the observations for model calibration and ⅓ for model validation.

3.8.2. Section II, III and IV: Selection of optimized vegetation indices via contour maps, NDVI and REIP

The R package "lattice" (R version 3.0.2, R Foundation for Statistical Computing 2013) was used to calculate contour maps. Contour maps are matrices consisting of coefficients of determination for all binary combinations of wavelengths and biomass parameters, grain yield or protein content. NDVI and REIP were chosen since these indices are well known and often used in the literature. NDVI was calculated as follows (ROUSE ET AL., 1974):

$$NDVI = \frac{R_{774} - R_{656}}{R_{774} + R_{656}}$$

REIP was calculated as follows (GUYOT ET AL., 1988):

$$REIP = 700 + 40 \frac{\frac{R_{670}+R_{780}}{2} - R_{700}}{R_{740} - R_{700}}$$

The optimized vegetation index obtained from the contour maps in Section IV was calculated as follows:

$$SR = \frac{R_{820}}{R_{720}}$$

In addition to NDVI and REIP, SR (R_{780}/R_{670}) (PEARSON ET AL., 1972), WI (R_{900}/R_{970}) (PEÑUELAS ET AL., 1993), PRI (R_{531}-R_{570})/(R_{531}+R_{570}) (GAMON ET AL., 1992) and VARI (R_{550}-R_{650})/(R_{550}+R_{650}-R_{470}) (GITELSON ET AL., 2002) were tested. However, results for these indices were low or inconsistent, and are therefore not shown in this study. To assess the quality of the PLSR models, the vegetation indices and the optimized vegetation indices obtained from the contour maps, root mean square errors (RMSE) and the coefficients of determination (R^2) were compared. Finding models with a combination of a low RMSE and a high R^2 was the target objective.

4. Results

4.1. Section I: Referencing laser and ultrasonic height measurements of barley cultivars by using a herbometre as standard

4.1.1. Comparison between the folding ruler and the herbometre

A comparison between the herbometre and a folding ruler is shown in Fig. 4.1a indicating a rather weak relationship with R^2 = 0.29. The relationship between two operators using a folding stick is indicated in Fig. 4.1b. The coefficient of determination was R^2 = 0.83, however, the slopes were statistically different. A comparison of herbometre measurements by different operators was not aimed at in this study, because the handling should deliver comparable values. This is supported by the fact, that very low coefficients of variation were observed for the individual within plot measurements for the herbometre in 2014 and 2015 amounting to 2.8 % and 3.0 %, respectively.

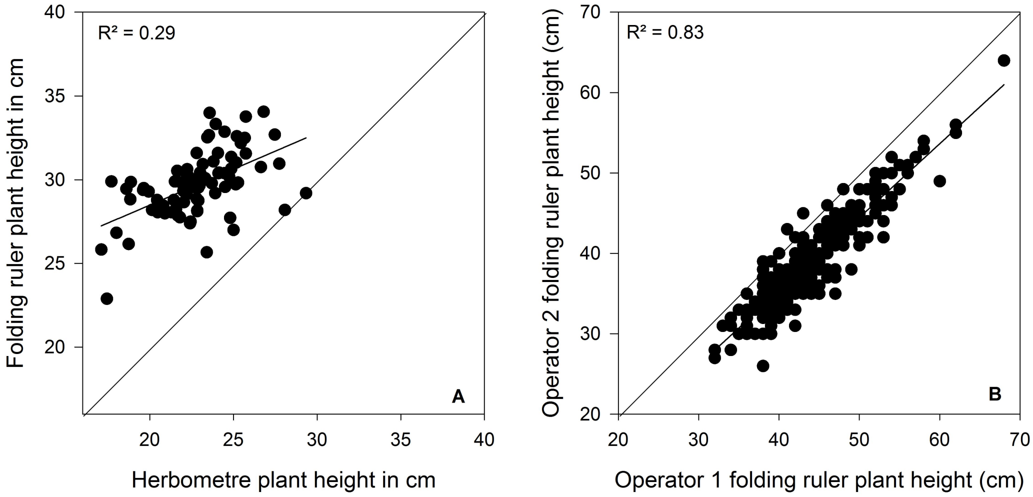

Figure 4.1.: (a) Relationship between a folding ruler and the herbometre in winter wheat 2014. (b) Comparison between the measurements of two different operators in winter barley in 2015. Regression lines and the 1 : 1 lines are indicated.

4.1.2. Relationship between the herbometre reference method and distance sensors

In the year 2013, with spring barley as the crop, the ultrasonic distance sensor was best related to the herbometre reference measurements with R^2 = 0.59** for the average values and with R^2 = 0.64** for the maximum values (Fig. 4.2 and Table 4.1). The laser distance sensor was less closely related to the herbometre measurements with R^2 = 0.30** for the averaged values and R^2 = 0.37** for the maximum values. In the years 2014 and 2015, with winter barley as the crop, improved results, particularly for the ultrasonic distance sensor, were obtained, and the coefficients of determination ranged from R^2 = 0.76** in 2014 to 0.83** in 2015. The best results for the laser distance sensor were obtained in 2014 with R^2 = 0.66**, with the level always lower compared with the ultrasonic sensor (Table 4.1). A deviation between the reference herbometre measurements and the sensors was found, particularly for the laser sensor (Table 4.2), deviating up to 37 cm. Both the laser and the ultrasonic distance sensor underestimated the observed plant heights in 2014 and 2015, whereas in 2013, an overestimation by the ultrasonic sensor was observed.

4.1.3. Discrimination of cultivars by herbometre and distance sensor measurements

The grouping of the cultivars for the herbometre, the laser sensor and the ultrasonic distance sensor for 2014 and 2015 is shown in Table A.1. However, for the laser distance sensor a classification was not possible, due to excessive scattering of the sensor output in 2015. For the spring barley cultivars in 2013, no differentiation between cultivars was found either for the plant heights recorded by the herbometre or for the distance sensors.

4.1.4. Time and labour requirements of reference and distance sensor measurements

Measurement of the plant height by the herbometre was revealed to be tedious for a single person, although two people could significantly accelerate the work, with one person performing the herbometre measurements and the other recording the height. Altogether, the complete measurements of the field trial required 2 h and 30 min in 2013, 1 h and 10 min for the winter barley trial in 2014 and 4 h for the field trial in 2015, including the subsequent data processing. Thus, the manual measurement of a single plot required 1 min and 20 s, depending on the size of the field trial and the distance between plots. In contrast, the sensor measurements required only one person, and the measurement of 120 plots took 35 min in 2015, 18 min for 56 plots in 2014 and 50 min for 250 plots in 2015. Depending on the design of the field trials (plot number in one row and space required for a turnaround of the vehicle) and the driving speed, the measurement of one 6-m-long plot took 20 s, including subsequent data processing. Thus, the sensor measurements on the mobile sensor platform were four times faster than herbometre measurements. In this experiment, all of the plots had a size of 10.8 m^2. The assessed area covered by the herbometre was 1 m^2 with four measurements taken per each plot. The ultrasonic distance sensor covered 3 m^2 across the whole plot, and the laser distance sensor, with its field of view of 6×6 mm, covered one planting row or 0.036 m^2. According to the driving speed, the sensor output comprised 25 measurements per plot.

Table 4.1.: Coefficients of determination between the sensors and the reference heights as determined by a herbometre ($P \leq 0.01$). Averaged and maximum values are reported for the ultrasonic and the laser distance sensor

Year	Ultrasonic	Laser	Max. ultrasonic	Max. laser
2013	0.59**	0.30**	0.64**	0.37**
2014	0.76**	0.54**	0.76**	0.66**
2015	0.80**	0.31**	0.83**	0.37**

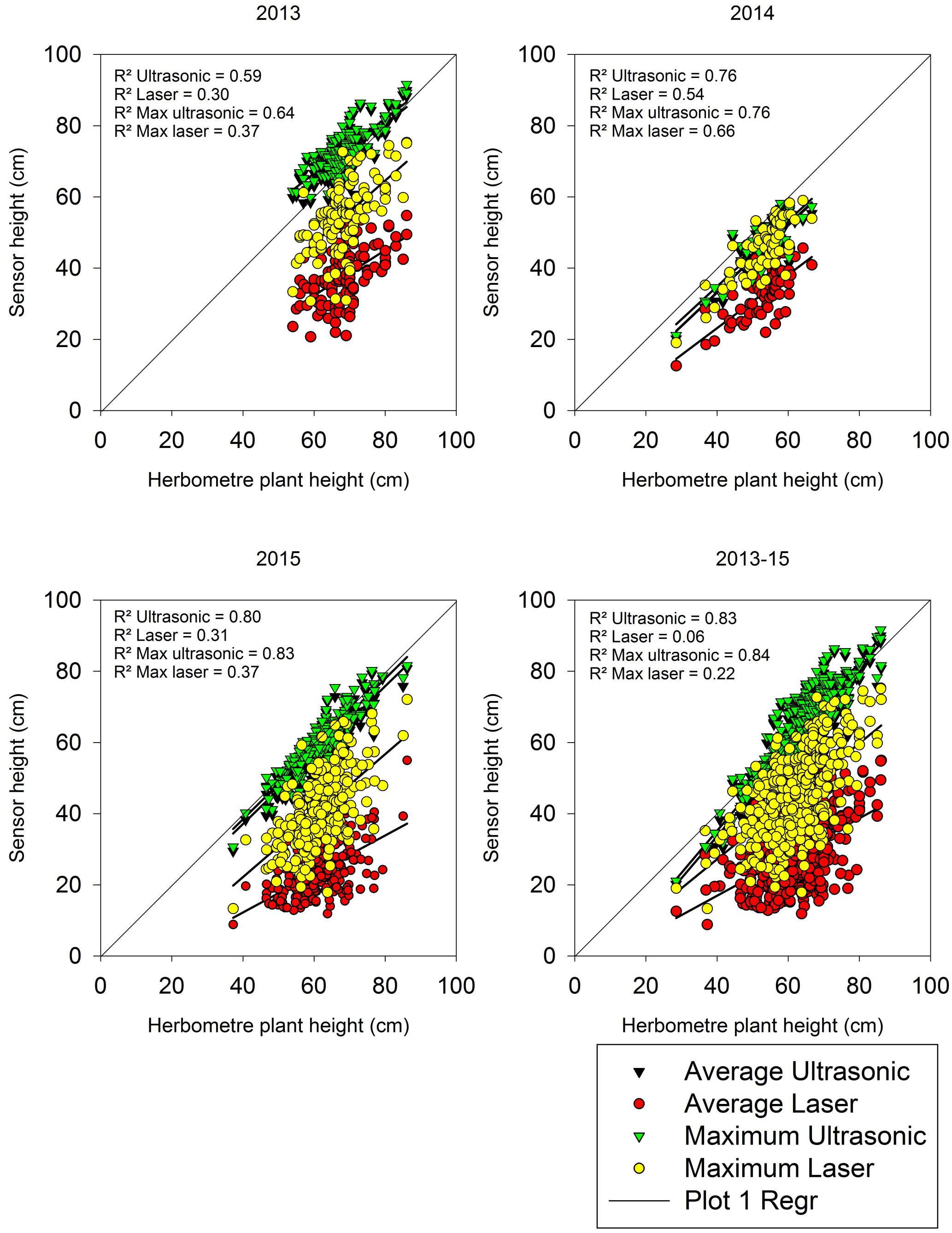

Figure 4.2.: Relationships between manually recorded plant heights by herbometre and sensor measurements in the years 2013, 2014 and 2015. Regression lines and the 1 : 1 lines are indicated.

Table 4.2.: Coefficients of variation (CV), number of samples (n), standard errors of the means (s.e.), heritability (H^2) and deviations (in cm) from the observed plant heights obtained from the herbometre reference measurements. Averaged values and maximum values (Max.) of the height measurements are indicated.

	Year	2013	2014	2015
Measured plant height	Min.	54	28.5	37.3
	Mean	68.4	53.0	61.8
	Max.	86	66.6	86.1
	n	116	66	250
	CV	0.06	0.13	0.12
	s.e.	0.63	0.83	0.45
	H^2	0.24	0.68	0.24
Ultrasonic	Min.	58.4	20.2	29.5
	Mean	71.6	45.4	58.2
	Max.	90.0	56.9	80.8
	RMSE	5.42	8.40	4.94
	Deviation	3.18	-7.59	-3.56
	CV	0.09	0.16	0.13
	s.e.	0.62	0.90	0.49
	H^2	0.42	0.95	0.70
Laser	Min.	20.7	12.6	8.9
	Mean	37.7	32.9	24.0
	Max.	54.8	45.7	55.0
	RMSE	37.4	20.66	38.27
	Deviation	-30.7	-20.05	-37.70
	CV	0.20	0.21	0.29
	s.e.	0.70	0.85	0.44
	H^2	0.27	0.81	0.40
Max. ultrasonic	Min.	59.9	21.1	30.7
	Mean	73.1	46.9	60.0
	Max.	91.7	58.2	81.6
	RMSE	6.3	7.04	3.64
	Deviation	4.6	-6.09	-1.79
	CV	0.09	0.15	0.13
	s.e.	0.61	0.88	0.49
	H^2	0.44	0.95	0.71
Max. laser	Min.	30.8	19.1	13.4
	Mean	54.5	45.6	41.0
	Max.	75.3	59.0	72.1
	RMSE	15.82	8.70	22.25
	Deviation	-13.95	-7.39	-20.75
	CV	0.17	0.17	0.25
	s.e.	0.88	0.97	0.64
	H^2	0.24	0.88	0.38

4.2. Section II: High-throughput phenotyping of wheat and barley plants grown in single or few rows in small plots using active and passive spectral proximal sensing

4.2.1. Effects of different row designs on plant fresh and dry weight, aboveground biomass nitrogen uptake, and grain yield

Even at early stages of development, the different row designs exhibited clear differences. Compared to a reference plot with 10 rows, the single-row plots showed an increase in fresh weight of 124 % for barley and 90 % for wheat at ZS 32. At ZS 65 and ZS 85, this difference grew to an increase of 235 % in wheat biomass in the single-row plot design compared to the 10-row plot. Mean values of the destructively-assessed plant parameters are given in Table 4.3. Significant differences ($p \leq 0.05$) were found between the designs in aboveground fresh and dry weights, as well as in the calculated N uptake; however, no differences in aboveground plant N content were found. Especially for wheat at early stages of growth, no distinction among the one-, two-, and three-row designs, or among the two-, three-, and four-row designs was found for plant fresh weight or dry weight. These designs were characterized by excessive tillering. This trend remained until ZS 85, when the two-, three-, and four-row designs still had comparable biomasses. Even the one-row design showed increases of up to 75 % in plant dry weight compared to the two-row design. Barley showed similar responses, though the four-row design differed significantly from the two-row design at ZS 32 and 65. At ZS 85, however, the two-, three-, and four-row designs all had similar plant fresh weight and dry weight. A statistical grouping of grain yields showed a high compensatory performance, particularly for wheat. No difference was observed among the two-, three-, and four-row designs. Compared to the ten-row plots, grain yields gradually increased with decreasing number of rows.

Table 4.3.: Destructively-assessed values of aboveground plant fresh and dry weight, N content, and aboveground nitrogen uptake of wheat and barley plants as obtained from different plot designs. The plot designs included 1, 2, 3, 4, or 10 rows, and samples were collected from plants at three different stages of development (ZS 32, 65, and 85). Coefficients of variation, standard errors of the means, and plant parameters per plot for the different row designs are indicated, with each value representing the average of four replicates. Rankings are derived from Tukey's HSD-Test, are indicated at $p \leq 0.05$ indicating differences within rows. Different letters (a,b,c,d) denote significant differences.

Variant			One-row				Two-row			
			Fresh-Weight (g)	Dry-weight (g)	N-Content (%)	N-Uptake (g)	Fresh-Weight (g)	Dry-weight (g)	N-Content (%)	N-Uptake (g)
Barley	ZS 32	Means	1113a	243.6a	2.9a	7.1a	1607.1ab	372.4ab	2.5a	9.4ab
		CV	0.14	0.20	0.06	0.21	0.12	0.12	0.08	0.13
		SE	78.58	23.82	0.09	0.75	95.15	21.97	0.11	0.62
	ZS 65	Means	1626.2a	483.3a	1.7a	8.2a	2180.7ab	666.9ab	1.7a	11.9a
		CV	0.14	0.04	0.09	0.10	0.13	0.11	0.12	0.22
		SE	117.60	9.31	0.08	0.43	144.01	35.75	0.11	1.29
	ZS 85	Means	1745.9a	706.7a	1.2a	8.8a	2295.5b	936.9ab	1.1a	10.8a
		CV	0.25	0.22	0.06	0.22	0.19	0.15	0.13	0.25
		SE	218.73	78.50	0.04	0.97	221.52	69.72	0.08	1.35
Wheat	ZS 32	Means	463.2a	109.6a	2.6a	2.8a	842ab	190.9ab	2.5a	4.7ab
		CV	0.10	0.08	0.04	0.09	0.09	0.06	0.05	0.09
		SE	23.50	4.40	0.05	0.13	36.44	5.60	0.06	0.22
	ZS 65	Means	1820a	574.1a	1.8a	10.5a	2584ab	767.9ab	1.6a	12.5a
		CV	0.06	0.13	0.04	0.17	0.10	0.08	0.08	0.12
		SE	58.80	36.74	0.03	0.88	129.08	32.13	0.07	0.75
	ZS 85	Means	2111.7a	971.2a	0.8a	8.4	2994.1ab	1383.9b	0.9ab	13
		CV	0.02	0.06	0.06	0.06	0.04	0.04	0.17	0.17
		SE	21.54	28.85	0.03	0.26	61.88	24.95	0.08	1.14

Table 4.3 continued

Variant			Three-row				Four-row			
			Fresh-Weight (g)	Dry-weight (g)	N-Content (%)	N-Uptake (g)	Fresh-Weight (g)	Dry-weight (g)	N-Content (%)	N-Uptake (g)
Barley	ZS 32	Means	1959.2ab	425.4bc	2.9a	12.5ab	2396.7b	545.7c	2.7a	14.7b
		CV	0.07	0.08	0.09	0.17	0.16	0.14	0.11	0.16
		SE	66.06	17.83	0.13	1.05	189.86	38.82	0.15	1.16
	ZS 65	Means	2595.5b	799.8b	1.8a	15a	2882.7b	857.3b	1.8a	16.2a
		CV	0.11	0.10	0.08	0.19	0.17	0.12	0.23	0.33
		SE	149.18	40.01	0.08	1.43	239.40	51.92	0.22	2.65
	ZS 85	Means	2663.9b	1059.5ab	1.3a	14.3a	2984.6b	1143.7b	1.2a	14.6a
		CV	0.21	0.17	0.12	0.29	0.12	0.07	0.15	0.21
		SE	278.86	91.74	0.08	2.11	177.28	41.60	0.09	1.56
Wheat	ZS 32	Means	949.9bc	200.9ab	2.5a	5.1bc	1163.2c	266.3c	2.6a	7c
		CV	0.17	0.12	0.01	0.12	0.12	0.09	0.08	0.16
		SE	79.89	11.88	0.01	0.30	72.34	12.46	0.11	0.57
	ZS 65	Means	2931.7bc	887.2b	1.8a	16.4a	3406.7c	1004.4b	1.8a	18.5ab
		CV	0.14	0.14	0.10	0.23	0.08	0.10	0.13	0.22
		SE	207.99	60.37	0.09	1.87	139.37	52.70	0.12	2.07
	ZS 85	Means	2822.7b	1338.3b	0.8ab	10.6	3347.8b	1562.5b	0.9ab	14.2
		CV	0.17	0.18	0.08	0.13	0.12	0.11	0.14	0.21
		SE	243.30	121.83	0.03	0.71	195.63	84.89	0.06	1.51

Table 4.3 continued

Variant			Complete plot (10 row)			
			Fresh-Weight (g)	Dry-weight (g)	N-Content (%)	N-Uptake (g)
Barley	ZS 32	Means	4631.8c	1085.5d	2.4a	26.8c
		CV	0.09	0.09	0.11	0.14
		SE	198.34	50.32	0.14	1.92
	ZS 65	Means	5254.5c	1809.9c	1.8a	34b
		CV	0.13	0.16	0.14	0.31
		SE	352.90	145.62	0.13	5.27
	ZS 85	Means	5843.7c	2403.8d	1.3a	31.4b
		CV	0.10	0.12	0.09	0.13
		SE	297.01	139.30	0.06	2.03
Wheat	ZS 32	Means	2773.2d	574.7d	2.7a	15.3d
		CV	0.17	0.15	0.08	0.08
		SE	231.69	42.96	0.11	0.58
	ZS 65	Means	6420.5d	1760.2c	1.7a	30.5b
		CV	0.08	0.09	0.09	0.14
		SE	255.45	75.08	0.08	2.11
	ZS 85	Means	6000c	2896c	0.6b	19.4
		CV	0.04	0.04	0.13	0.11
		SE	132.96	56.94	0.04	1.11

4.2.2. Relationship between plant parameters obtained from the combination of four plot designs and spectral reflectance measurements

Relationships between sensor measurements and four plant parameters for the combined row designs at three sampling dates are given in Table 4.4. In general, the passive spectrometer showed closer linear relationships between selected spectral reflectance indices and plant parameters of both species than did the active sensor. The GreenSeeker showed a closer relationship only for wheat at anthesis. Since neither sensor could detect all biomass parameters from wheat at ZS 85 with the vegetation indices available from the GreenSeeker, a contour map method that allowed testing of all possible dual reflectance indices from the passive spectrometer was further evaluated to find whether enhanced vegetation indices could be obtained. These indices, R_{820}/R_{755} for barley and R_{720}/R_{400} for wheat, resulted in markedly improved relationships in later growth stages.

Table 4.4.: Significant relationships between sensor measurements and plant parameters of wheat and barley, indicated by coefficients of determination (R^2) at * $p \leq 5\%$, ** $p \leq 1\%$. Relationships are indicated for different indices.

	GreenSeeker		Passive spectrometer				
	R_{774}/R_{656}	NDVI	R_{774}/R_{656}	NDVI	R_{800}/R_{770}	R_{820}/R_{755}	R_{720}/R_{400}
Barley ZS 32							
Fresh weight	0.53**	0.48**	0.49**	0.37*	0.86**		
Dry weight	0.41**	0.35*	0.37*	0.28*	0.85**		
N-content							
N-uptake	0.46**	0.46**	0.44**	0.43**	0.84**		
Barley ZS 65							
Fresh weight	0.25*	0.34*	0.85**	0.70**			
Dry weight		0.20*	0.74**	0.61**			
N-content		0.21*					
N-uptake	0.21*	0.29*	0.71**	0.50**			
Barley ZS 85							
Fresh weight	0.50**	0.45**	0.67**	0.64**		0.77**	
Dry weight	0.46**	0.44**	0.60**	0.64**		0.72**	
N-content	0.20*		0.30*			0.27*	
N-uptake	0.43**	0.35**	0.63**	0.53**		0.71**	
Wheat ZS 32							
Fresh weight	0.62**	0.60**	0.86**	0.52**			
Dry weight	0.63**	0.58**	0.88**	0.54**			
N-content							
N-uptake	0.59**	0.55**	0.93**	0.55**			
Wheat ZS 65							
Fresh weight	0.74**	0.69**	0.67**	0.60**			
Dry weight	0.72**	0.70**	0.65**	0.59*			
N-content							
N-uptake	0.51*	0.47*	0.37*	0.33*			
Wheat ZS 85							
Fresh weight							0.66**
Dry weight							0.63**
N-content							
N-uptake							0.40*

Compared to the simple ratio R_{774}/R_{656}, the NDVI showed reduced coefficients of determination caused by saturation effects. In this regard, the simple ratios were less sensitive. For the N content, only weak relationships were obtained. Figures 4.3 and 4.4 depict results from linear regressions for the combined row designs for barley and wheat, respectively. The spread of the regression points for the three sampling dates made it necessary to consider each sampling date separately. For barley, the results from the GreenSeeker demonstrated considerable scatter, and the regressions of both the NDVI and the simple ratio were considerably less similar than were the same indices obtained for wheat.

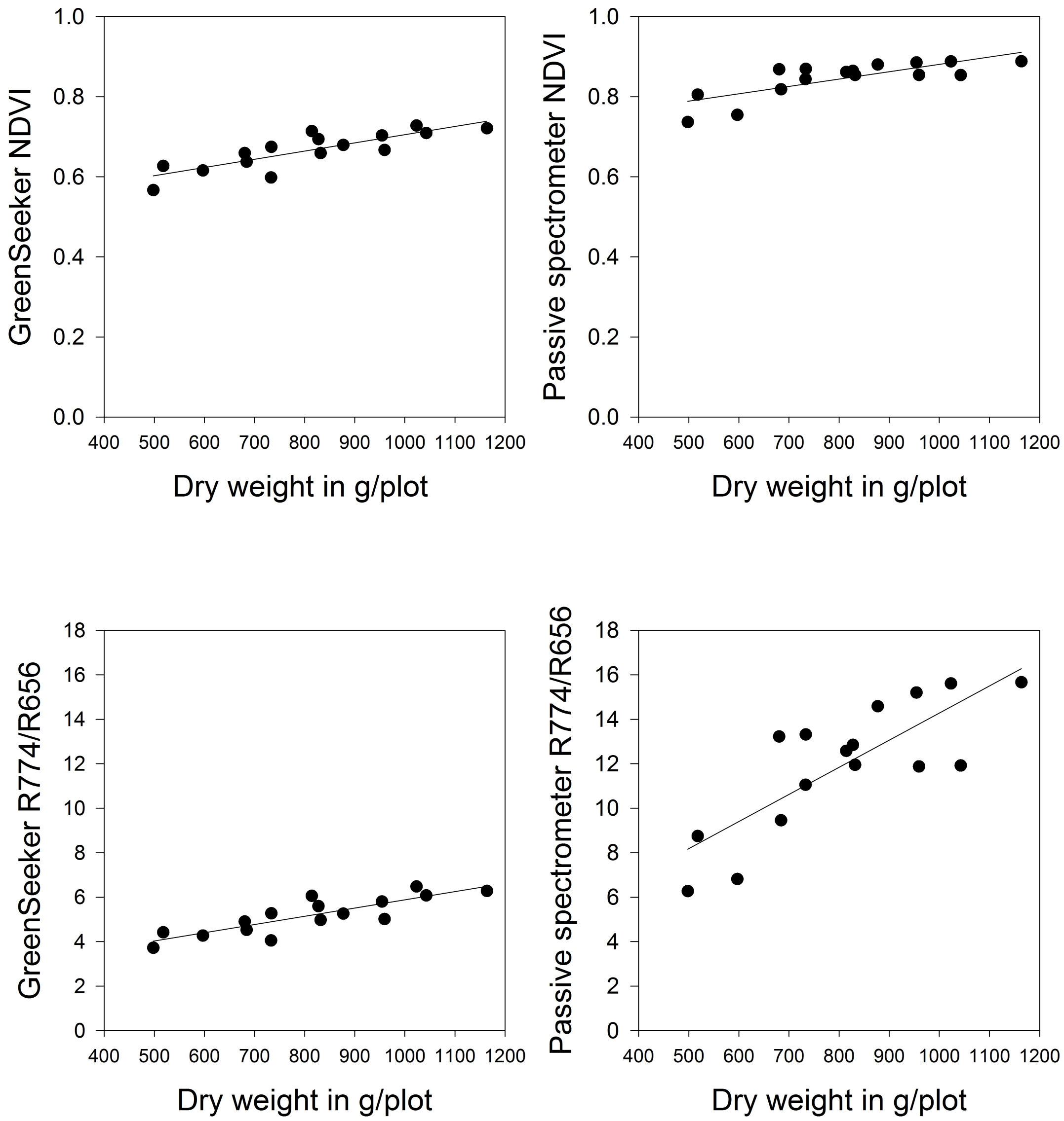

Figure 4.3.: Relationships between spectral indices derived from the two types of sensors and plant dry weight at ZS 65 for wheat, obtained from linear regressions combining the four different row designs

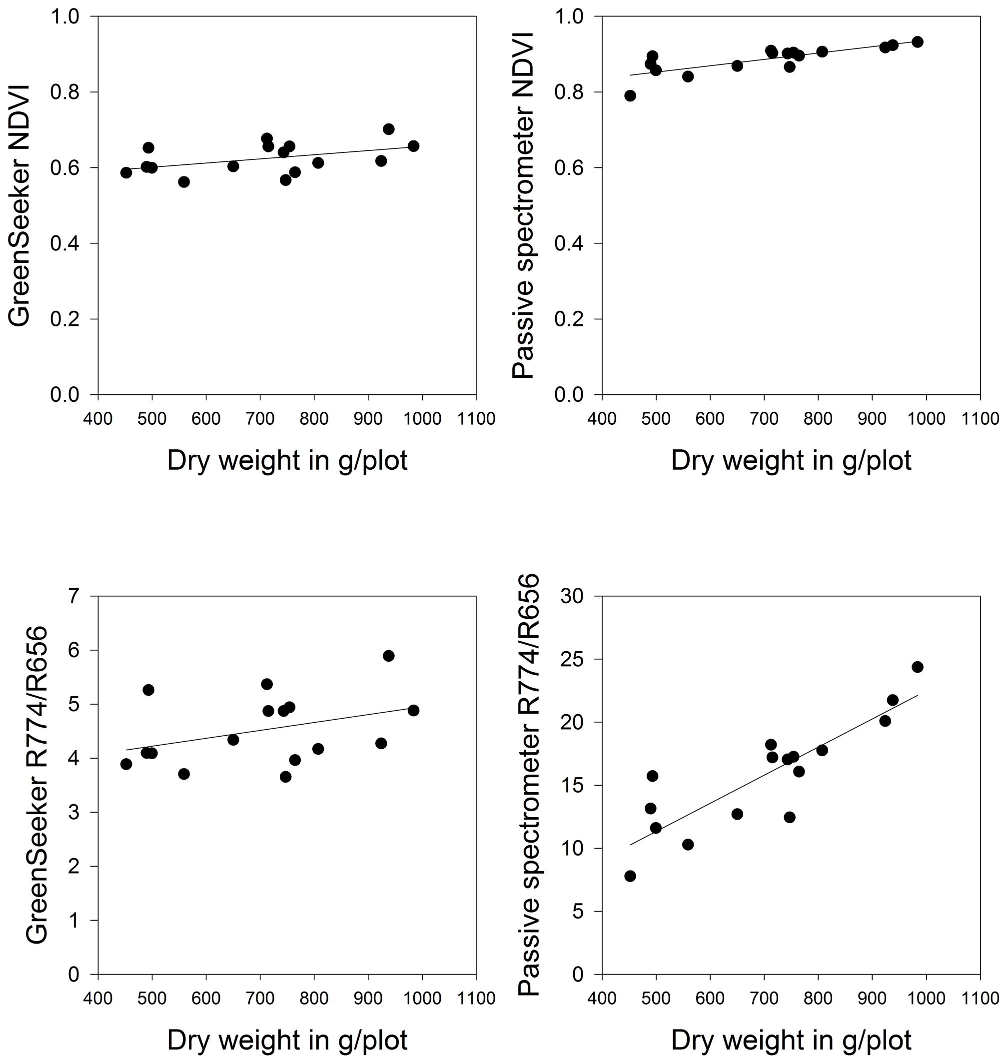

Figure 4.4.: Relationships between spectral indices derived from the two types of sensors and plant dry weight at ZS 65 for barley, obtained from linear regressions combining the four different row designs

4.3. Section III: Active and passive high-throughput field phenotyping of leaves, leaf sheaths, culms and ears of spring barley cultivars

4.3.1. Agronomic parameters and weather conditions

The year 2014 was the most favorable for spring barley due to an average temperature of approximately 18.3 °C during anthesis and evenly distributed precipitation. By contrast, unfavorable weather conditions between germination and anthesis led to a dry weight that was reduced by approximately 42% in 2013. The number of ears per square meter was comparable in all three years, with approximately 635 ears sqm^{-1}. With regard to the total dry weight and total N uptake, significantly lower values were observed in cultivars processed for human nutrition in all years. An exception to this result was the cultivar Pirona, which accumulated the highest total dry weight of all cultivars while having the lowest ear dry weight in 2014.

4.3.2. Detection of the dry weight and N uptake of leaves

An overview of the descriptive statistics of the dry weight and N uptake of leaves is given in supplemental Table B.1. Low dry weight and N uptake were observed due to the unfavorable weather conditions in 2013. The highest leaf biomass values were observed for Pirona and the lowest for Hora. Both cultivars have hull-less grains and are processed for human nutrition; however, Pirona was the tallest cultivar, whereas Hora was one of the smallest. A comparable tendency was observed for culms. Table 4.5 shows the results of the PLSR, and Tables 4.6 and 4.7 shows the linear regressions of the vegetation indices obtained for each sensor.

Table 4.5.: Results of PLSR analysis of the dry weight and N uptake of leaves

Leaves (kg/ha)		Cal					Val			
		PC	Slope	Offset	RMSE	R^2	Slope	Offset	RMSE	R^2
Anthesis	Dry weight	4	0.62	383.9	160.36	0.62	0.60	373.6	181.29	0.65
	N uptake	4	0.74	5.61	5.20	0.74	0.76	4.71	5.23	0.76
Dough ripeness	Dry weight	4	0.56	384	168.04	0.56	0.52	426	181.97	0.52
	N uptake	5	0.66	3.8	2.85	0.66	0.58	4.4	3.52	0.62

Fair relationships were found for the dry weight, whereas for N uptake, slightly better results were found. Compared to the PLSR, the vegetation indices showed much higher RMSEs together with lower coefficients of determination. For the detection of the dry weight and N uptake of leaves, the R_{780}/R_{670} vegetation index was found to be most promising. Slight advantages for the passive spectrometer for detecting leaf dry weight at anthesis were observed; however, the CropCircle performed comparably for measuring N uptake.

Table 4.6.: Results of linear regression of the dry weight and N uptake of leaves showing the tested vegetation indices from the active sensors at anthesis and dough ripeness. Significant R^2-values are indicated at $p \leq 0.05$ (*) and $p \leq 0.01$ (**)

Leaves (kg/ha)			CropCircle				GreenSeeker		AFS	
			R_{730}/R_{670}	R_{760}/R_{730}	R_{760}/R_{670}	NDVI	R_{774}/R_{656}	NDVI	R_{760}/R_{730}	R_{900}/R_{970}
Dry weight	Anthesis	Slope	0.0007	0.0005	0.0024	0.0003	0.0023	0.0003	0.0003	0.0000
		Offset	0.81	1.22	-0.26	0.11	0.93	0.22	1.20	0.97
		RMSE	1125.63	1125.24	1126.65	1126.30	1049.48	1050.16	1049.23	1049.45
		R^2	0.39**	0.26**	0.26**	0.36**	0.49**	0.44**	0.44**	0.14**
	Dough ripeness	Slope	0.0008	0.0003	0.0021	0.0003	0.0015	0.0002	0.0003	0.0000
		Offset	0.74	1.26	-0.01	0.08	1.40	0.22	1.21	1.00
		RMSE	907.84	907.34	908.55	908.46	914.19	915.33	914.37	914.58
		R^2	0.19**	0.08	0.10	0.13*	0.19**	0.16*	0.31**	0.01
N uptake	Anthesis	Slope	0.0175	0.0188	0.1002	0.0095	0.0762	0.0090	0.0097	0.0026
		Offset	1.07	1.32	0.07	0.21	1.56	0.28	1.32	0.96
		RMSE	27.22	26.99	28.06	28.05	22.78	24.17	23.23	23.56
		R^2	0.47**	0.66**	0.76**	0.6**	0.63**	0.63**	0.43**	0.5**
	Dough ripeness	Slope	0.0563	0.0362	0.2065	0.0266	0.1027	0.0162	0.0145	0.0034
		Offset	0.78	1.18	-0.39	0.06	1.48	0.22	1.27	0.98
		RMSE	27.22	26.99	28.06	28.05	22.78	24.17	23.23	23.56
		R^2	0.59**	0.52**	0.54**	0.56**	0.45**	0.43**	0.48**	0.14*

Table 4.7.: Results of linear regression of the dry weight and N uptake of leaves showing the tested vegetation indices from the passive sensor at anthesis and dough ripeness. Significant R^2-values are indicated at $p \leq 0.05$ (*) and $p \leq 0.01$ (**)

Leaves (kg/ha)			Passive spectrometer							
			R_{780}/R_{550}	R_{780}/R_{670}	R_{780}/R_{700}	R_{760}/R_{730}	R_{780}/R_{740}	R_{900}/R_{970}	REIP	NDVI
Dry weight	Anthesis	Slope	0.0028	0.0186	0.0029	0.0002	0.0001	0.0001	0.0037	0.0002
		Offset	2.51	-5.75	1.04	1.13	1.13	1.12	715.69	0.57
		RMSE	1047.97	1055.61	1049.37	1049.30	1049.30	1049.31	420.36	1049.83
		R^2	0.52**	0.56**	0.55**	0.54**	0.28**	0.47**	0.4**	0.49**
	Dough ripeness	Slope	0.0025	0.0113	0.0023	0.0002	0.0001	0.0002	0.0045	0.0003
		Offset	2.30	-1.68	1.09	1.10	1.12	1.06	713.79	0.42
		RMSE	913.32	917.05	914.48	914.49	914.46	914.52	305.32	915.13
		R^2	0.33**	0.4**	0.33**	0.32**	0.25**	0.37**	0.17*	0.29**
N uptake	Anthesis	Slope	0.0844	0.5583	0.0927	0.0077	0.0013	0.0027	0.1131	0.0076
		Offset	3.49	0.65	1.88	1.20	1.16	1.15	716.90	0.64
		RMSE	20.90	16.15	22.38	23.34	23.38	23.39	776.29	23.85
		R^2	0.53**	0.59**	0.67**	0.63**	0.16*	0.46**	0.44**	0.6**
	Dough ripeness	Slope	0.1467	0.6337	0.1390	0.0151	0.0036	0.0093	0.3164	0.0191
		Offset	2.76	0.88	1.50	1.13	1.13	1.10	714.06	0.48
		RMSE	20.90	16.15	22.38	23.34	23.38	23.39	776.29	23.85
		R^2	0.54**	0.6**	0.58**	0.58**	0.4**	0.51**	0.4**	0.51**

4.3.3. Detection of the dry weight and N uptake of leaf sheaths

The descriptive statistics of the leaf sheaths can be found in supplemental Table B.2. In 2015, an approximately 35 % higher dry weight and 66 % higher N uptake at anthesis were found compared to those in 2014. The cultivars Shakira and Pirona showed the lowest dry weight and N uptake in both years, and IPZ 24727 showed the highest. The results of the PLSR are presented in Table 4.8. Good relationships were found for the N uptake of leaf sheaths; however, the dry weight of this plant organ was barely detectable by sensing at dough ripeness.

Table 4.8.: Results of PLSR analysis of the dry weight and N uptake of leaf sheaths

Leaf sheaths (kg/ha)			Cal				Val			
		PC	Slope	Offset	RMSE	R^2	Slope	Offset	RMSE	R^2
Anthesis	Dry weight	4	0.62	383.9	160.36	0.62	0.6	373.6	181.29	0.65
	N uptake	4	0.76	1.97	2.32	0.76	0.79	1.30	2.64	0.69
Dough ripeness	Dry weight	3	0.25	562	182.71	0.25	0.20	617	218.22	0.21
	N uptake	5	0.49	4.3	2.42	0.49	0.54	4.0	2.16	0.50

Linear regressions between the leaf sheaths and vegetation indices are shown in Tables 4.9 and 4.10. For the vegetation indices, only weak relationships with high RMSEs were observed. The best coefficient of determination (R^2 = 0.38) was delivered by the AFS sensor for N uptake at anthesis.

Table 4.9.: Results of linear regression of the dry weight and N uptake of leaf sheaths showing the tested vegetation indices from the active sensors at anthesis and dough ripeness. Significant R^2-values are indicated at $p \leq 0.05$ (*) and $p \leq 0.01$ (**)

Leaf sheaths (kg/ha)			CropCircle				GreenSeeker		AFS	
			R_{730}/R_{670}	R_{760}/R_{730}	R_{760}/R_{670}	NDVI	R_{774}/R_{656}	NDVI	R_{760}/R_{730}	R_{900}/R_{970}
Dry weight	Anthesis	Slope	0.0006	0.0005	0.0018	0.0003	-0.0013	-0.0001	-0.0003	0.0000
		Offset	1.04	1.48	1.41	0.25	4.45	0.61	1.75	1.02
		RMSE	576.57	576.16	576.22	577.29	424.44	427.04	426.27	426.76
		R^2	0.17*	0.25**	0.20**	0.22**	0.11	0.11	0.16*	0.23**
	Dough ripeness	Slope	0.0382	0.0108	0.0812	0.0125	0.0941	0.0121	-0.0033	0.0071
		Offset	1.28	1.64	2.12	0.35	2.48	0.41	1.50	0.99
		RMSE	547.36	547.06	546.68	548.10	474.97	476.42	475.65	476.01
		R^2	0.16*	0.07	0.14*	0.16*	0.14*	0.16*	0.00	0.24**
N uptake	Anthesis	Slope	0.0345	0.0245	0.1003	0.0144	-0.0879	-0.0087	-0.0201	0.0017
		Offset	1.06	1.50	1.49	0.27	4.44	0.61	1.75	1.03
		RMSE	7.33	6.99	6.88	8.00	4.84	5.73	5.20	5.51
		R^2	0.16*	0.23**	0.18*	0.20**	0.24**	0.27**	0.37**	0.29**
	Dough ripeness	Slope	0.0004	0.0001	0.0008	0.0001	0.0009	0.0001	0.0000	0.0001
		Offset	1.20	1.62	1.95	0.33	2.34	0.39	1.53	0.98
		RMSE	5.00	4.71	4.48	5.66	3.76	4.86	4.22	4.50
		R^2	0.23**	0.12	0.20**	0.25**	0.25**	0.28**	0.01	0.41**

Table 4.10.: Results of linear regression of the dry weight and N uptake of leaf sheaths showing the tested vegetation indices from the passive sensor at anthesis and dough ripeness. Significant R^2-values are indicated at $p \leq 0.05$ (*) and $p \leq 0.01$ (**)

Leaf sheaths (kg/ha)			Passive spectrometer							
			R_{780}/R_{550}	R_{780}/R_{670}	R_{780}/R_{700}	R_{760}/R_{730}	R_{780}/R_{740}	R_{900}/R_{970}	REIP	NDVI
Dry weight	Anthesis	Slope	-0.0010	-0.0132	-0.0010	0.0000	0.0000	0.0000	0.0002	-0.0001
		Offset	6.31	23.55	5.03	1.44	1.19	1.25	719.72	0.91
		RMSE	423.19	411.94	424.05	426.48	426.65	426.61	531.00	426.84
		R^2	0.03	0.15	0.05	0.01	0.00	0.05	0.00	0.08
	Dough ripeness	Slope	0.0003	-0.0610	0.0010	0.0025	0.0027	-0.0036	0.0855	-0.0004
		Offset	4.97	10.83	3.63	1.35	1.16	1.26	718.57	0.78
		RMSE	473.25	469.23	474.18	475.76	475.89	475.82	516.79	476.16
		R^2	0.00	0.00	0.00	0.02	0.07	0.01	0.04	0.00
N uptake	Anthesis	Slope	-0.0928	-0.9203	-0.0913	-0.0059	-0.0012	-0.0031	-0.0447	-0.0079
		Offset	6.51	23.60	5.19	1.46	1.20	1.25	720.21	0.92
		RMSE	5.32	12.67	4.95	5.31	5.43	5.41	684.30	5.57
		R^2	0.13*	0.35**	0.16*	0.09	0.02	0.15*	0.02	0.23**
	Dough ripeness	Slope	0.0001	-0.0002	0.0001	0.0000	0.0000	0.0000	0.0010	0.0000
		Offset	5.10	11.26	3.71	1.35	1.16	1.28	718.58	0.80
		RMSE	4.00	8.24	3.69	4.30	4.40	4.34	776.37	4.61
		R^2	0.00	0.00	0.00	0.02	0.11	0.05	0.05	0.00

4.3.4. Detection of the dry weight and N uptake of culms

The descriptive statistics of the culms are given in supplemental Table B.3. The culm N uptake of 2013 and 2015 was on a comparable level; however, in 2014, 38 % less nitrogen was accumulated at anthesis. In contrast to 2013 and 2015, in 2014, the culms reached the highest dry weight at dough ripeness. In the other years, decreasing dry weight in later growth stages was observed. For the PLSR (Table 4.11), good relationships with R^2 = 0.53-0.66 were found for both the dry weight and N uptake of culms. However, the N uptake at anthesis showed a high number of principal components and a marked difference between the calibration and validation results.

Table 4.11.: Results of PLSR analysis of the dry weight and N uptake of culms

Culms (kg/ha)			Cal				Val			
		PC	Slope	Offset	RMSE	R^2	Slope	Offset	RMSE	R^2
Anthesis	Dry weight	5	0.59	1627	721.76	0.59	0.63	1470	728.36	0.54
	N uptake	7	0.66	23.64	12.41	0.66	0.73	19.77	12.31	0.53
Dough ripeness	Dry weight	4	0.65	1317	606.73	0.65	0.54	1635	763.03	0.61
	N uptake	6	0.61	6.1	4.45	0.61	0.55	7.0	4.72	0.60

The results of the linear regressions are shown in Tables 4.12 and 4.13. The passive spectrometer showed improved performance with regard to the R_{900}/R_{970} and R_{780}/R_{670} indexes. The coefficients of determination of the VIs and PLSR were comparable; however, the RMSEs for the VIs were almost four times higher considering the dry weight. The best performance of the passive spectrometer was obtained at dough ripeness, whereas CropCircle showed enhanced performance at anthesis.

Table 4.12.: Results of linear regression of the dry weight and N uptake of culms showing the tested vegetation indices from the active sensors at anthesis and dough ripeness. Significant R^2-values are indicated at $p \leq 0.05$ (*) and $p \leq 0.01$ (**)

Culms (kg/ha)			CropCircle				GreenSeeker		AFS	
			R_{730}/R_{670}	R_{760}/R_{730}	R_{760}/R_{670}	NDVI	R_{774}/R_{656}	NDVI	R_{760}/R_{730}	R_{900}/R_{970}
Dry weight	Anthesis	Slope	0.0001	0.0001	0.0007	0.0001	0.0003	0.0000	0.0000	0.0000
		Offset	0.83	1.12	-0.98	0.09	1.99	0.32	1.45	0.94
		RMSE	4476.54	4476.26	4478.30	4477.26	4179.85	4181.47	4180.37	4180.87
		R^2	0.49**	0.56**	0.64**	0.59**	0.11	0.14*	0.02	0.33**
	Dough ripeness	Slope	0.0003	0.0001	0.0009	0.0001	0.0004	0.0001	0.0001	0.0000
		Offset	0.50	1.08	-1.23	-0.06	1.08	0.15	1.19	0.97
		RMSE	3953.33	3952.78	3954.98	3953.86	3872.40	3873.29	3872.30	3872.51
		R^2	0.46**	0.29**	0.37**	0.39**	0.34**	0.34**	0.43**	0.08
N uptake	Anthesis	Slope	0.0051	0.0023	0.0092	0.0019	-0.0196	-0.0023	-0.0033	-0.0003
		Offset	0.99	1.47	1.14	0.23	4.59	0.64	1.76	1.04
		RMSE	66.23	65.77	66.07	66.95	68.07	71.78	70.72	71.40
		R^2	0.13	0.03	0.02	0.08	0.15*	0.16*	0.18*	0.03
	Dough ripeness	Slope	0.0531	0.0294	0.1783	0.0244	0.0579	0.0094	0.0099	0.0009
		Offset	0.66	1.16	-0.64	0.01	1.75	0.26	1.28	1.00
		RMSE	17.74	17.76	17.57	17.78	17.47	17.62	17.61	17.62
		R^2	0.37**	0.25**	0.29**	0.34**	0.24**	0.24**	0.38**	0.02

Table 4.13.: Results of linear regression of the dry weight and N uptake of culms showing the tested vegetation indices from the passive sensor at anthesis and dough ripeness. Significant R^2-values are indicated at $p \leq 0.05$ (*) and $p \leq 0.01$ (**)

Culms (kg/ha)			Passive spectrometer							
			R_{780}/R_{550}	R_{780}/R_{670}	R_{780}/R_{700}	R_{760}/R_{730}	R_{780}/R_{740}	R_{900}/R_{970}	REIP	NDVI
Dry weight	Anthesis	Slope	0.0004	0.0016	0.0004	0.0000	0.0000	0.0000	0.0006	0.0000
		Offset	3.92	6.59	2.31	1.22	1.16	1.16	716.82	0.67
		RMSE	4177.99	4175.40	4179.54	4180.60	4180.66	4180.66	3495.44	4181.13
		R^2	0.10	0.05	0.14	0.16*	0.05	0.13	0.15*	0.14
	Dough ripeness	Slope	0.0006	0.0029	0.0006	0.0001	0.0000	0.0000	0.0012	0.0001
		Offset	2.07	-2.56	0.81	1.07	1.13	1.04	713.18	0.37
		RMSE	3871.46	3875.85	3872.66	3872.41	3872.36	3872.44	3200.45	3873.08
		R^2	0.46**	0.56**	0.50**	0.47**	0.21**	0.56**	0.27**	0.49**
N uptake	Anthesis	Slope	-0.0126	-0.1624	-0.0179	-0.0009	0.0003	-0.0004	-0.0006	-0.0016
		Offset	6.21	24.14	5.15	1.43	1.17	1.23	719.41	0.91
		RMSE	66.53	51.47	67.54	71.03	71.28	71.22	649.93	71.52
		R^2	0.04	0.18*	0.09	0.03	0.03	0.03	0.00	0.10
	Dough ripeness	Slope	0.0940	0.4410	0.0923	0.0095	0.0017	0.0070	0.1788	0.0132
		Offset	2.96	1.16	1.63	1.16	1.14	1.10	714.86	0.49
		RMSE	17.22	13.82	17.34	17.61	17.62	17.62	112.55	17.61
		R^2	0.38**	0.49**	0.43**	0.39**	0.16*	0.50**	0.22**	0.42**

4.3.5. Detection of the dry weight and N uptake of ears

The lowest dry weights and N uptake were observed in 2014 (Supplemental Table B.4). The results of the PLSR analysis are shown in Table 4.14. Medium correlations between the biomass parameters and wavelengths were found. Additionally, a high number of principal components was found to be optimal.

Table 4.14.: Results of PLSR analysis of the dry weight and N uptake of ears

Ears (kg/ha)		Cal					Val			
		PC	Slope	Offset	RMSE	R^2	Slope	Offset	RMSE	R^2
Dough ripeness	Dry weight	7	0.57	2299	924.41	0.57	0.64	2158	951.59	0.49
	N uptake	7	0.57	29.7	12.19	0.57	0.63	27.8	12.46	0.50

Considering the linear regressions of the vegetation indices shown in Table 4.15, no correlations were observed. The RMSEs were as high as the mean values observed for the biomass parameters.

Table 4.15.: Results of linear regression of the dry weight and N uptake of ears showing the tested vegetation indices from the active sensors at anthesis and dough ripeness. Significant R^2-values are indicated at $p \leq 0.05$ (*) and $p \leq 0.01$ (**)

Ears (kg/ha)			CropCircle				GreenSeeker		AFS	
			R_{730}/R_{670}	R_{760}/R_{730}	R_{760}/R_{670}	NDVI	R_{774}/R_{656}	NDVI	R_{760}/R_{730}	R_{900}/R_{970}
Dry weight	Dough ripeness	Slope	0.0001	0.0001	0.0003	0.0000	0.0001	0.0000	0.0000	0.0000
		Offset	0.79	1.23	0.15	0.09	2.41	0.40	1.50	0.98
		RMSE	5475.74	5475.32	5476.36	5476.41	5478.77	5480.70	5479.64	5480.14
		R^2	0.10	0.07	0.05	0.08	0.01	0.00	0.02	0.03
N uptake	Dough ripeness	Slope	0.0072	0.0034	0.0189	0.0028	0.0045	0.0002	-0.0011	0.0004
		Offset	0.79	1.27	0.18	0.10	2.36	0.40	1.51	0.98
		RMSE	70.58	70.13	71.14	71.24	69.19	71.09	70.02	70.53
		R^2	0.11	0.05	0.05	0.07	0.01	0.00	0.03	0.03

Table 4.16.: Results of linear regression of the dry weight and N uptake of ears showing the tested vegetation indices from the passive sensor at anthesis and dough ripeness. Significant R^2-values are indicated at $p \leq 0.05$ (*) and $p \leq 0.01$ (**)

Ears (kg/ha)			Passive spectrometer							
			R_{780}/R_{550}	R_{780}/R_{670}	R_{780}/R_{700}	R_{760}/R_{730}	R_{780}/R_{740}	R_{900}/R_{970}	REIP	NDVI
Dry weight	Dough ripeness	Slope	0.0000	-0.0004	-0.0001	0.0000	0.0000	0.0000	0.0000	0.0000
		Offset	4.72	10.23	3.52	1.33	1.14	1.27	717.92	0.78
		RMSE	5476.54	5471.22	5477.69	5479.81	5479.99	5479.87	4791.26	5480.34
		R^2	0.00	0.01	0.01	0.00	0.07	0.04	0.00	0.02
N uptake	Dough ripeness	Slope	-0.0051	-0.0296	-0.0064	-0.0004	0.0004	-0.0009	-0.0079	-0.0013
		Offset	4.81	10.26	3.55	1.33	1.14	1.27	718.26	0.79
		RMSE	66.88	61.88	68.08	70.19	70.37	70.25	644.12	70.72
		R^2	0.01	0.01	0.01	0.00	0.06	0.05	0.00	0.03

4.4. Section IV: Mid-season prediction of grain yield and protein content of spring barley cultivars using high-throughput spectral sensing

4.4.1. Calculating PLSR models

Descriptive statistics for grain yield and protein content are given in Table 4.17. Unfavorable weather and soil conditions in spring and early summer 2013 contributed to low grain yields and protein contents that year. The hull-less barley cultivars had the lowest grain yields in all three years. However, these cultivars had the highest protein contents, as high as 16.6 %.

Table 4.17.: Descriptive statistics of grain yield and protein content in field trials used for developing the PLSR models (2013-2015).

		Grain yield in kg ha^{-1}	Protein content in %
2013	N	114	112
	Min	1782.9	7.9
	Max	6852.8	11.7
	Mean	5510.6	9.1
	CV (%)	12.8	7.7
	h^2	0.22	0
2014	N	116	115
	Min	4079.5	6.7
	Max	9079.0	14.3
	Mean	6488.6	8.5
	CV (%)	11.9	7.3
	h^2	0.79	0.46
2015	N	132	129
	Min	1732.7	7.7
	Max	8543.3	16.6
	Mean	5729.9	10.7
	CV (%)	14.6	5.0
	h^2	0.72	0.80

Using the contour map analysis (Figure 4.5), good relationships were obtained between grain yield and the R_{820}/R_{720} spectral index at anthesis. For protein content, by contrast, an optimized spectral index could not be derived and neither NDVI or REIP was able to explain the observed variation. For vegetation indices such as VARI, PRI or WI, only low coefficients of determination with high RMSEs were obtained. Table 4.18 compares the PLSR models and the vegetation indices. For NDVI and REIP, differences were found between the calibration and validation subsets. Regression plots of PLSR and the optimized index R_{820}/R_{720} are shown in Figure 4.6. As an alternative method to predict protein content, a PLSR model of leaf N uptake at anthesis was tested.

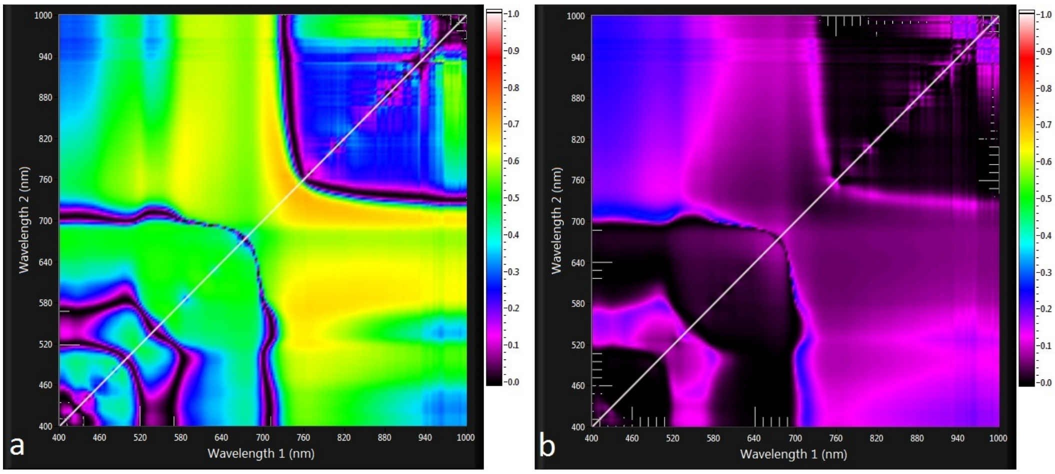

Figure 4.5.: Correlation matrices (contour maps) showing coefficients of determination (R^2) with grain yield (a) and protein content (b) for all wavelength combinations in a range of 400 - 1000 nm from the passive spectral sensor.

Table 4.18.: Description of the PLSR models for grain yield and protein content, and the linear regression of the contour-map-based spectral index.

PLS regression		Cal				Val			
	PC	Slope	Offset	RMSE	R^2	Slope	Offset	RMSE	R^2
Grain yield in kg ha^{-1}	4	0.77	1131.3	646.1	0.78	0.89	546.4	543.7	0.80
Protein content in %	5	0.57	3.98	0.77	0.57	0.55	4.14	0.80	0.54
Leaf N uptake in kg ha^{-1}	4	0.74	5.61	5.20	0.74	0.76	4.71	5.23	0.76
Linear regression									
R_{820}/R_{720}	-	0.00019	1.10	5313.2	0.73	0.00022	0.98	5292.2	0.74
NDVI	-	0.00006	0.45	5312.5	0.62	0.00006	0.57	5282.2	0.57
REIP	-	0.00090	715.9	4621.6	0.65	0.00094	715.83	4584.9	0.58

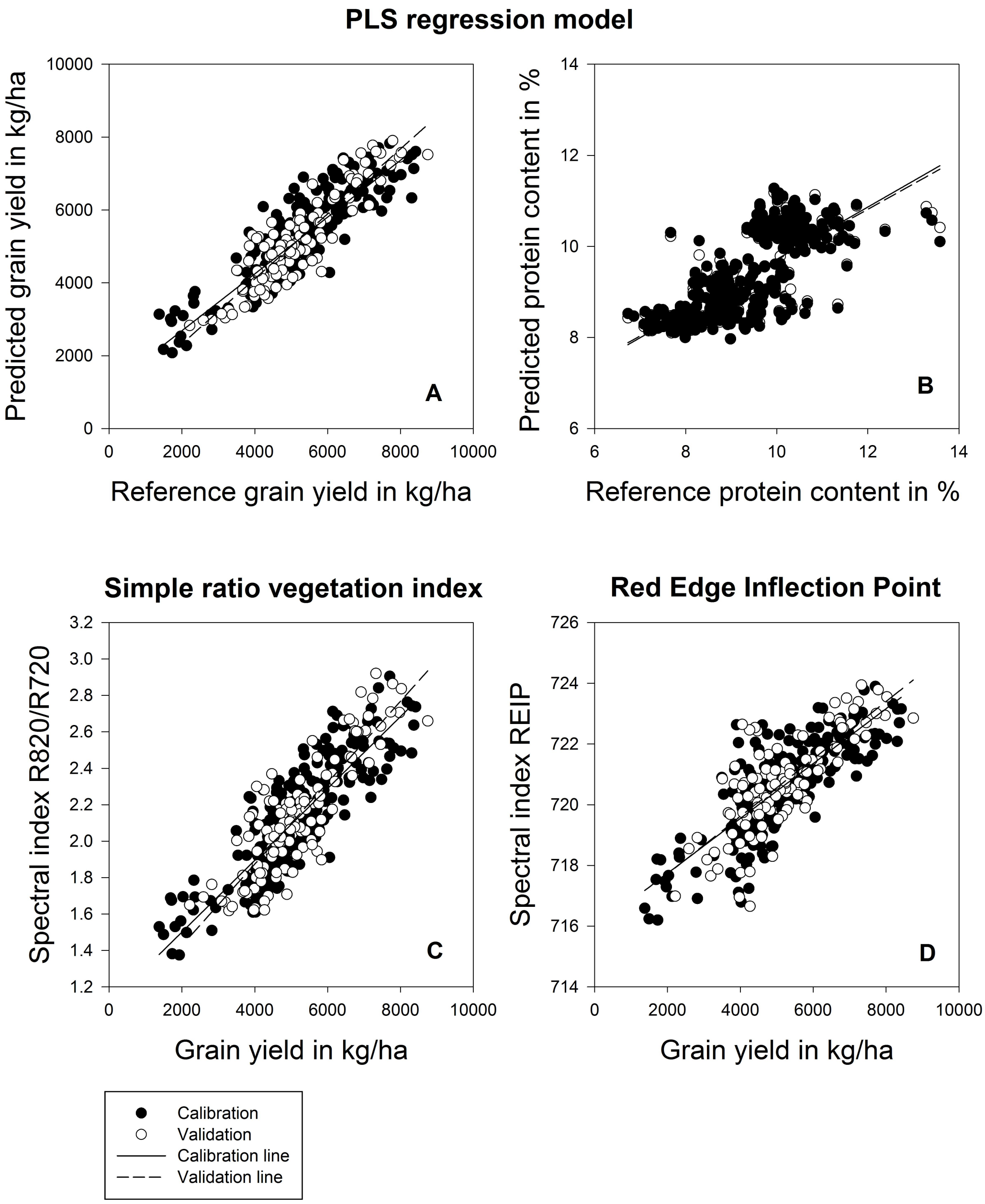

Figure 4.6.: Regression plots of the PLSR models and the optimized spectral index for grain yield and protein content predictions.

4.4.2. Application of PLSR in independent field trials.

A description of yield parameters from the independent field trials is given in Table 4.19.

Table 4.19.: Descriptive statistics of grain yield and protein content in the field trials used for evaluating the PLSR models.

Year		Grain yield in kg ha^{-1}	Protein content in %	PLSR grain yield in kg ha^{-1}	PLSR Protein content in %	PLSR Leaf N content %
2013 IV-1	N	48	48	48	48	48
	Min	1534.5	8.6	1902.2	8	13.7
	Max	9873.1	11.5	5732.5	9.7	24.7
	Mean	6047.4	9.8	3693.1	8.9	17.1
	CV (%)	41.3	7.7	26.9	7.7	15.0
	h^2	0.93	0.89	0.9	0.41	0.43
2014 IV-1	N	72	72	72	72	72
	Min	4685.2	7.8	2674	8	10.9
	Max	12949.2	12.3	9073.7	8.9	46.2
	Mean	9608.4	10.4	6774.9	8.5	34.5
	CV (%)	24.1	7.9	25.5	7.9	28.3
	h^2	0.96	0.9	0.98	0.21	0.98
2015 IV-1	N	47	48	47	48	48
	Min	1495.5	10.7	2284.4	9.4	10.4
	Max	8446.5	13.6	8475.7	10.2	39.7
	Mean	4646.7	11.6	5138.1	9.8	23.5
	CV (%)	37.3	6.4	38.4	1.8	41.5
	h^2	-	-	-	-	-
2015 IV-2	N	96	-	96	-	-
	Min	2810.6	-	3433.7	-	-
	Max	6406.8	-	5396.8	-	-
	Mean	4829.9	-	4380.1	-	-
	CV (%)	18	-	78.4	-	-
	h^2	0.08	-	0.19	-	-

Table 4.19 continued.

Year		R_{820}/R_{720} grain yield in kg ha^{-1}	NDVI grain yield in kg ha^{-1}	REIP grain yield in kg ha^{-1}	Measurement duration plot^{-1} (Data processing incl.)
2013 IV-1	N	48	48	48	58 sec
	Min	1245.3	-2701.1	2694.8	
	Max	8097.9	5899.3	10910.1	
	Mean	4144.8	1925.2	6919.5	
	CV (%)	45.6	127.4	35.8	
	h^2	0.88	0.9	0.93	
2014 IV-1	N	72	72	72	55 sec
	Min	3234.8	2855.7	1368.6	
	Max	12602.5	7384	11347.4	
	Mean	8458.4	6430	7869.2	
	CV (%)	33.6	17.8	33.3	
	h^2	0.98	0.95	0.98	
2015 IV-1	N	47	47	47	48.2 sec
	Min	1390.1	-1411.1	-488.7	
	Max	12267.4	7203.2	11052.9	
	Mean	6056.9	3998.9	5882.6	
	CV (%)	56.4	67.4	53.6	
	h^2	-	-	-	
2015 IV-2	N	96	96	96	35 sec
	Min	2892.7	853.5	3875.6	
	Max	6292.6	4970.1	7051.9	
	Mean	4301.6	3164.2	5201.5	
	CV (%)	67.2	31.8	12.2	
	h^2	0.27	0.29	0.17	

For predicting yield parameters using PLSR and linear regression models, each field experiment was predicted separately. Coefficients of determination between the predicted and observed yield parameters are shown in Table 4.20. Fair results were obtained for grain yield in 2014 and 2015. However, for predictions of protein content, only weak correlations (R^2 = 0.28) were obtained in 2014. Predictions of protein content by the PLSR model for leaf N uptake provided good correlations for 2014 and 2015 while having high RMSEs; however, high overestimations of the true protein contents were observed.

Figures 4.7 and 4.8 show regression plots between the observed and predicted values. Prediction results were shifted relative to observed results. Compared to the observed grain yields in experiment IV-1, the predicted yields were 39 % and 29 % lower in 2013 and 2014, respectively. An opposite shift was seen in 2015, when the PLSR model predicted an average grain yield of 5138.0 kg ha^{-1}, which represents an overestimation of approximately 12 %. For experiment IV-2, an underestimation of 9 % was observed. Results for REIP and PLSR were comparable, they but differed from results for the linear regression of the optimized vegetation index. While the coefficients of determination and the over- or under-estimation were of similar magnitude among these three methods, the RMSEs for linear regression were several times higher than those for REIP and PLSR. The PLSR prediction was able to differentiate among the cultivars of experiment IV-2 (Figure 4.9), but the REIP results were less precise.

Table 4.20.: Comparisons among the prediction results using PLSR and vegetation indices for grain yield and protein content ($p \leq 0.01$).

Grain yield in kg ha^{-1}	PLSR			Linear regression (R_{820}/R_{720})		
	RMSE	R^2	Over-/Underestimation (%)	RMSE	R^2	Over-/Underestimation (%)
2013 IV-1	2883.97	0.82***	-39.1	3883.69	0.84***	-31.5
2014 IV-1	2967.96	0.90***	-28.7	4593.08	0.89***	-12
2015 IV-1	673.15	0.95***	11.8	7247.29	0.94***	30.3
2015 IV-2 All	712.40	0.74***	-9.3	5124.07	0.74***	-10.9
Quench	495.15	0.74***	-6.5	5356.01	0.72***	-5.9
Scarlett	576.85	0.83***	-2.7	5202.14	0.75***	-10.8
Grace	917.91	0.71***	-16.8	4797.84	0.73***	-15.5
Protein content in %	**PLSR**			**PLSR Leaf N content to protein content**		
	RMSE	R^2	Over-/Underestimation (%)	RMSE	R^2	Over-/Underestimation (%)
2013 IV-1	1.2	0.07	-8.7	7.6	0.08	74.3
2014 IV-1	2.0	0.28	-18.0	25.7	0.61	231.8
2015 IV-1	1.8	0.03	-15.0	15.1	0.60	103.3

Table 4.20 continued

	Linear regression (NDVI)			Linear regression (REIP)		
	RMSE	R^2	Over-/Underestimation (%)	RMSE	R^2	Over-/Underestimation (%)
2013 IV-1	4256.20	0.82***	-68.2	1146.20	0.91***	14.4
2014 IV-1	3489.70	0.75***	-33.1	1947.50	0.89***	-18.1
2015 IV-1	1363.90	0.9***	-13.9	1938.50	0.95***	26.6
2015 IV-2 All	1762.00	0.67***	-34.5	653.00	0.62***	7.7
Quench	1414.70	0.49***	-26.9	615.00	0.80***	10.7
Scarlett	2087.90	0.6***	-48.2	889.40	0.77***	18.5
Grace	1718.70	0.64***	-30.8	331.66	0.72***	-3.3

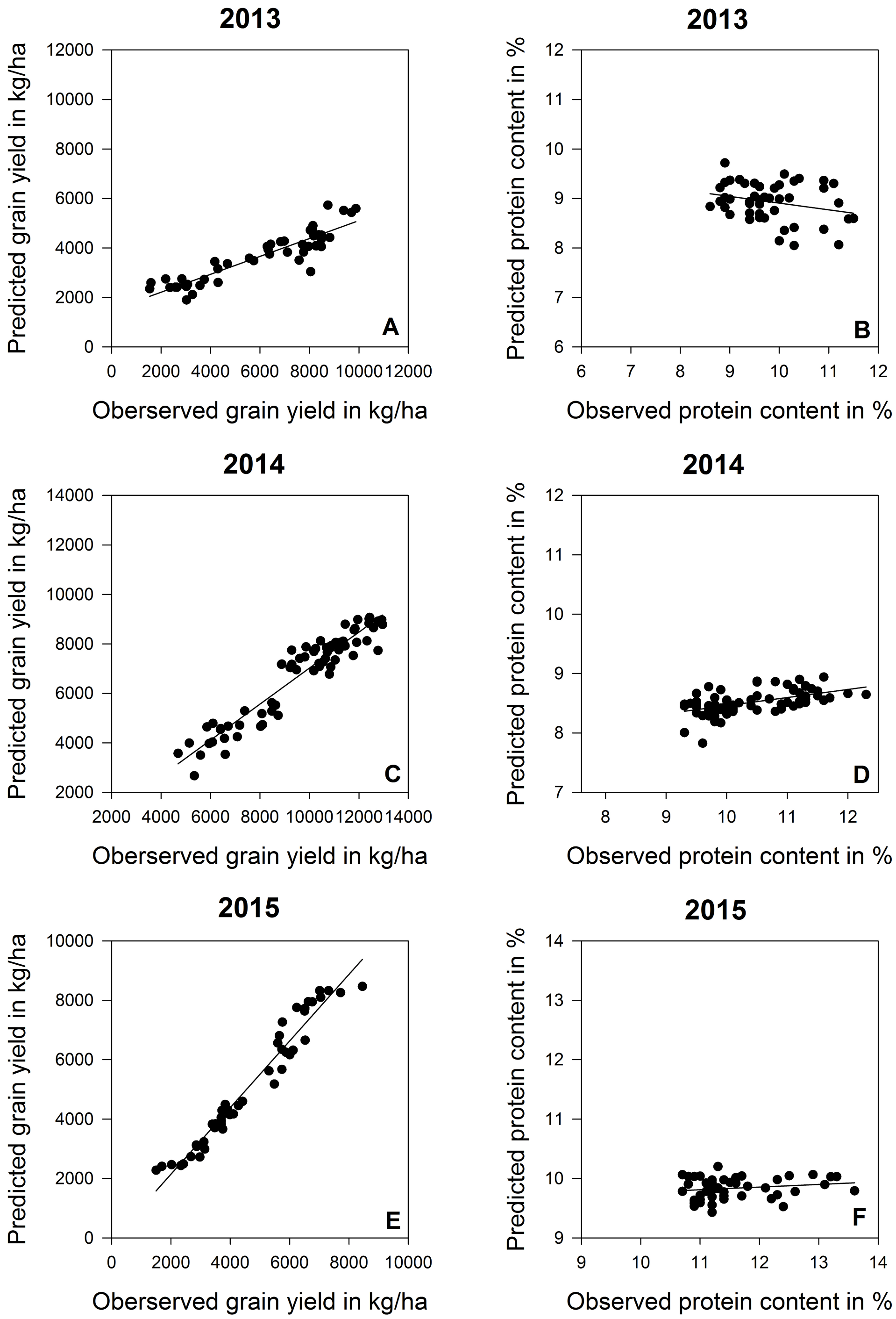

Figure 4.7.: Regressions between observed and predicted yield parameters from 2013 to 2015.

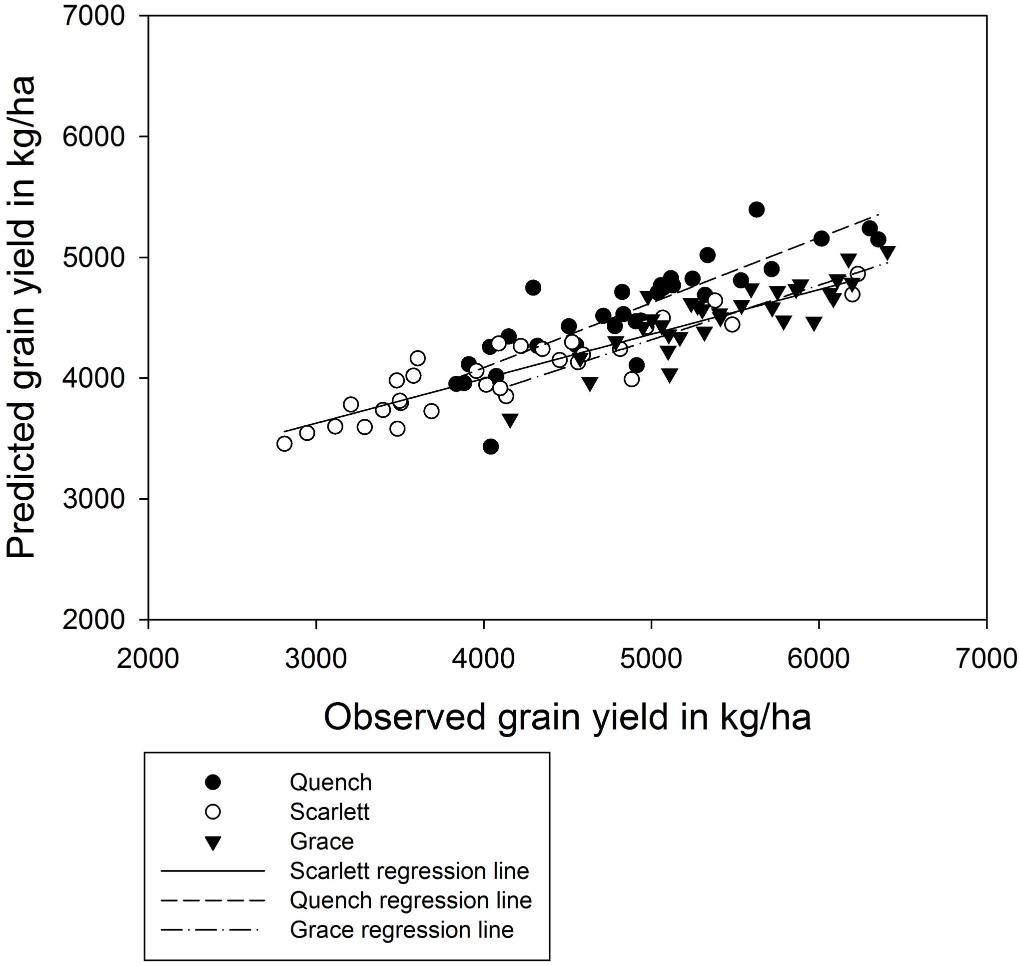

Figure 4.8.: Predictions of grain yields in experiment IV-2.

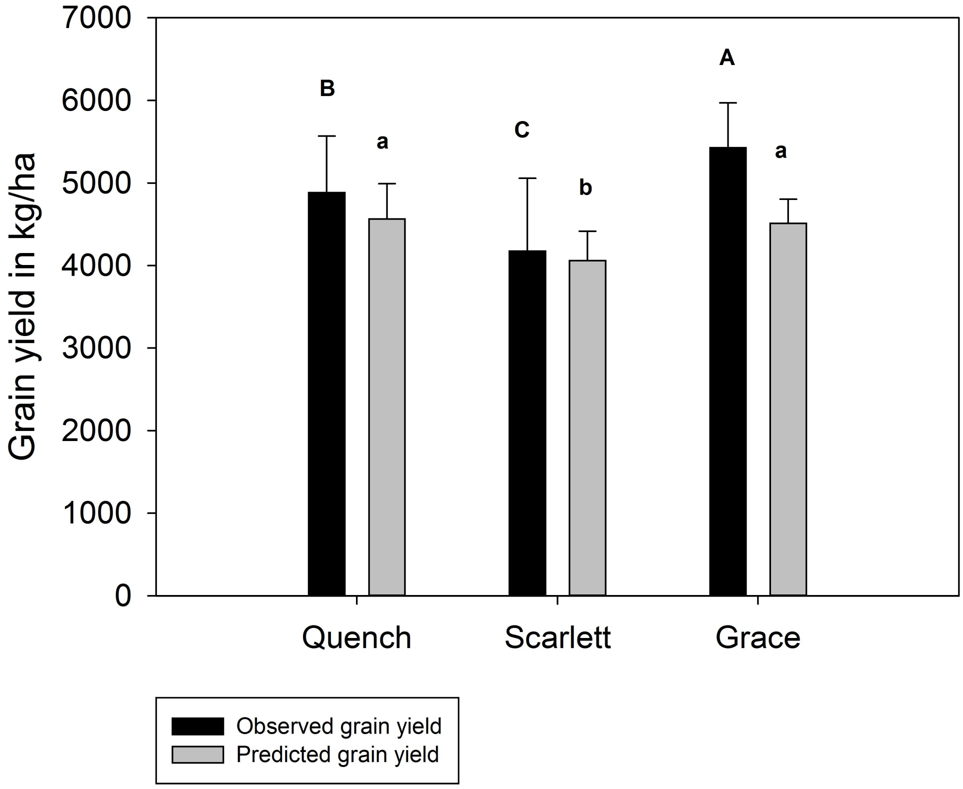

Figure 4.9.: Comparisons between observed and predicted grain yields using PLSR. Different letters indicate statistically significant differences at p≤0.05.

5. Discussion

5.1. Section I: Referencing laser and ultrasonic height measurements of barley cultivars by using a herbometre as standard

Even though the meter or yard stick is considered as reference method for assessing the height of plants in breeding plots, there is no commonly adopted procedure for assessing the plant height in performing such measurements. Depending on the individual observer height assessments in breeding plots can be based on the inspection of the top most leaf (leaves) or are based on the visual inspection of an estimated averaged canopy height, which is indeed subjective. Depending on the plant development, particularly measurements at earlier growth stages tend to be tedious, requiring the observer to bend towards the observation point, and are indicating also some degree of variance and subjectivity as evidenced in Fig. 4.1b. It follows, that an exact definition of putatively assumed reference height measurements does not exist or is individually adopted. There is also little agronomic value in the detection of the top most position of an individual leaf (leaves), which varies considerably among cultivars with different growth habits, represented for example, by erectophile or planophile leaves, and being further affected by environmental conditions such as the wind. Therefore, the assessment of a standardised height measurement such as delivered by the herbometre approach is clearly preferred to a yardstick measurement, as the herbometre is less susceptible to variation between individual observers and reflects physiologically more relevant information indicative of enhanced photosynthetic activity within canopies where gradients in the distribution of the nitrogen content and light interception occur. Previous efforts to assess the height of plants have primarily emphasised technical aspects of the methodology. In contrast, this report also focuses on the feasibility of distance sensors being used for agronomic decisions, particularly for plant breeders. The results over the 2 years indicate that the sensors can obviously differentiate barley cultivars in height, stressing, however, the need for multi-annual assessments due to the different plant heights observed in 2014 and 2015 (Fig. 5.1 and supplemental Table A.1). The accuracy of the sensors was revealed to be sufficient to distinguish cultivars in the uniformly fertilised field trials typical for breeder nurseries. The results of other investigations frequently included non-uniform treatments and were based on different seeding rates (SCOTFORD & MILLER, 2004B), different biomass densities (EHLERT ET AL., 2010), different nitrogen fertiliser treatments (YIN ET AL., 2011) or compiled measurements across different growth stages. Although such assessments are common in Plant Sciences, plant breeders require measurements under uniform management conditions to be able to differentiate cultivars. The use of a herbometre as a reference method compared with a regular folding rule proved to be advantageous. This method incorporates several advantages, making the measurement of the maximum height probably more objective, which is of particular relevance if several operators are involved in visual inspections of the plant height. It is argued, that the herbometre provides a weighted height, allowing for a better representation of the average plant height by smoothly bending the leaves and awns and by exerting the same force on a given area. Additionally, the measurement comprises an area of 0.25 m^2, which is advantageous compared with single plant estimates with a ruler as reported by SCOTFORD & MILLER (2004B) and YIN ET AL. (2011). The low values of the heritability in 2013 and 2015 (Table 4.2) are caused by heterogeneous soils. Soil analysis showed significantly differing and partly low pH values in two of the four replications in 2013, which likely affected plant growth site-specifically. The field trial in 2015 was conducted on a field site characterised by varying topography attributes. The upper part of the field had a lower soil water-holding capacity than the lower part, which again influenced the uniformness of plant growth in the individual replications. The research station Dürnast, where the experiments were performed, is located in the tertiary hill sites including rather heterogeneous field sites. It is plausible that replicate measurements of plant growth will show a lower variance on more homogeneous field sites used for breeding purposes leading also to a higher heritability. Commercial distance sensors must comply with several requirements to allow for successful use in field trials, such as resistance to dust, vibrations, shocks and the influence of direct sunlight (EHLERT ET AL., 2009). The distance sensors used in this work used the time-of-flight measuring principle and were chosen for their higher measuring range, up to 10 m, which makes them suitable for measurements of tall plants such as maize crops (compared with triangulation sensors), and for their low acquisition costs. The results indicate that these sensors, mounted on a vehicle and exposed to a rugged drive characteristic for tramlines in field experiments, were able to distinguish different heights of contrasting winter barley cultivars at anthesis in 2014 and 2015, whereas no differentiation could be made with either measurement principle or with the herbometre reference in 2013. In part, this may have been caused by the wet weather conditions during the early summer, resulting in rather uneven crop stands, in addition to deep and uneven (bumpy) wheel ruts. Vibrations and swinging of the front loader appeared to be the main source of errors and inaccuracies, especially at higher driving speeds. A further improvement could be achieved with a stabilising wheel (CHATZINIKOS ET AL., 2013) or a feeler rod as described by EHLERT ET AL. (2009). The sensor performance depends further on the leaf angle, the size of the leaves and the covered area (KATAOKA ET AL., 2002). This might be a further reason for decreased accuracies in plant height as obtained by the laser distance sensor in barley, due to the risk of measuring interspaces between planting rows caused by the small measuring area of 6 mm $\times$ 6 mm. In contrast, the ultrasonic distance sensor, measuring an area of 0.5 m^2, is apparently less affected by the variable crop stand density of barley. EHLERT ET AL. (2010) suggested that the laser sensor readings not only reflect the highest but also the lower parts of a plant. Therefore, in addition to the average plant height, we have recorded the maximum plant height averaged from the five most increased measurement values, and this is most likely the reason for the negative deviations from the referenced plant height shown in Table 4.2. The ultrasonic distance sensor performed better than the laser distance sensor due to the increased measuring area of 0.5 m^2 and consequently higher accuracies obtained within the barley crops stands (Fig. 4.2). Nevertheless, industrial distance sensors are evidently beneficial in saving costs and workloads by assuring a constant data quality. Furthermore, a single person can do such measurements during regular fertiliser and pesticide applications and during paralleled other measurements, allowing the person to phenotype the plants with high-throughput. ERDLE ET AL. (2013B) and KIPP ET AL. (2014) have previously reported examples of concomitant measurements done with the PhenoTrac IV sensor platform, including assessments of the biomass and nitrogen uptake at anthesis. Data fusion of spectral, thermal and canopy height parameters allowed for improved yield prediction of drought stressed spring barley (RISCHBECK ET AL., 2016). Available and cost-effective industrial distance sensors represent a powerful high-throughput phenotyping tool for breeders and plant scientists to estimate plant height and identify a distinction among cultivars for specific breeding goals. If the sensors were attached to a tractor used for fertiliser or pesticide applications, measurements can be done simultaneously and no further costs for transport, deployment or maintenance will occur. The view has been expressed that smaller cultivars contribute to the goal of preventing lodging (STANCA ET AL., 1979; MATUŠINSKY ET AL., 2015), fostering the need for detailed detection of the plant height. Sensing the height in a fast and economical way may allow enhanced selection along these lines.

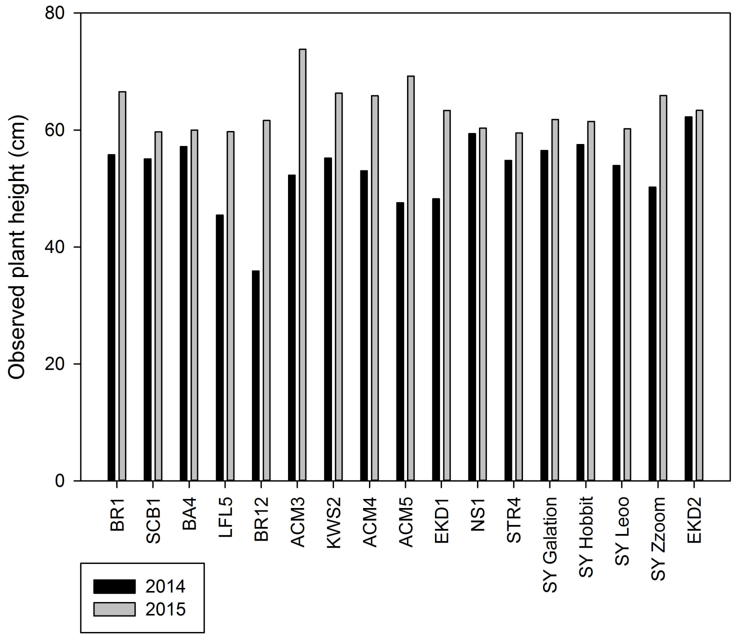

Figure 5.1.: Plant heights of different barley cultivars assessed in 2014 and 2015 by herbometre measurements.

5.2. Section II: High-throughput phenotyping of wheat and barley plants grown in single or few rows in small plots using active and passive spectral proximal sensing

Border row effects, which cause enhanced growth of plants in border rows, have long been well-known, and recommendations for their avoidance, such as harvesting border rows and front sides separately (PETERSON, 1994), have been reported. However, due to the small numbers of seeds and limited resources available in early selection cycles, plots sharing one to three rows are common and yield estimates are, therefore, biased (ROMANI ET AL., 1993; ACQUAAH, 2012). This is of lesser importance in early selection cycles that focus on the overall performance of varieties, but it should be avoided in later cycles due to competitive effects of neighbouring plants. Still REBETZKE ET AL. (2014) mentioned that the results of small plots are not representative and that there is a need for multi-row plots to simulate field conditions. This agrees with the findings of our study. At all three growth stages, both wheat and barley showed relatively higher fresh and dry weights, as well as greater nitrogen uptake, in single and multiple-row plots than in a plot comprising 10 rows. However, these results cannot be generalized since only single varieties of each species were tested. Further research of the performance of multiple cultivars in small plots needs to be done. Several authors (WINTERHALTER ET AL., 2011; WHITE ET AL., 2012; KIPP ET AL., 2013) have demonstrated that spectral proximal sensing is a suitable tool for breeders and plant scientists to evaluate plant parameters in a non-destructive and high-throughput manner. Studies performed with wide plots of 10 rows demonstrated comparable or, frequently, superior performance of passive sensors compared to active sensors, including the one tested in this study, for wheat (ERDLE ET AL., 2011), maize (WINTERHALTER ET AL., 2013), and barley (ELSAYED ET AL., 2015). These sensors were also tested in different environments by considering the effects of temperatures, light intensity, and surface conditions (KIM ET AL., 2012; KIPP ET AL., 2014). However, no previous study had tested the performance of spectral proximal sensors in different plot designs. The results showed decreasing spectral reflectance in the one- and two-row plots, indicating an interfering signal received by the sensor. This was most likely due to the higher fraction of soil in the sensor's FOV. Chemical analysis of the harvested plant material and visual scoring of the plots indicated that neither nutrient deficiencies nor plant diseases occurred in the different plots, and weeds and other objects were manually removed before each measurement. Therefore, it can be concluded that spectral information from bare soil interfered with the spectral sensing of plants, particularly at early growth stages. In later growth stages, distances between sensors and soil increase resulting in a reduced influence of the soil. The GreenSeeker, in particular, with its extended FOV of 1.5 $\times$ 61 cm (Figure 3.9), may be more susceptible to spectral information from the soil in the one- or two-row plots, in which the planted rows were 15 and 35 cm apart, respectively, especially at early stages of growth. The one-row design covered approximately 25 % of the measurement field of the GreenSeeker, whereas this value was approximately 34 % for the passive spectrometer. For the two-row design, these values were approximately 57 % for the GreenSeeker and

80 % for the passive spectrometer. In addition, the light intensity decreases on the periphery of the GreenSeeker, which leads to lower reflection values. KIM ET AL. (2012) showed that the best performance was obtained in central positions within 30 cm of the light strip. Previous research has indicated that the intensity of LED light emitted decreases with increasing distance (WINTERHALTER ET AL., 2013; KIPP ET AL., 2014). As a result, the crop stand is not entirely perceived. This is in contrast to passive sensing, which uses the sun as a light source, the intensity of which does not appreciably decrease within the crop stand. However, this may increase the likelihood that passive sensing will detect information from the soil surface in less dense crop stands. The results from this study also show that fresh and dry weights do not increase linearly in plots with different numbers of rows, with the largest values observed in the one-row design. It is likely that optimized light conditions, together with improved nutrient and water supply, enhanced growth in border rows or in designs with fewer rows. Since only one cultivar of each species was investigated, different performances of other cultivars cannot be excluded. A comparison of the performance of the sensors and evaluation of the best performing indices revealed that the best results were obtained from the passive sensor with the indices R_{774}/R_{656} and R_{800}/R_{770}. In agreement with previous results (ERDLE ET AL., 2011), saturation effects became apparent for the index NDVI independent of the sensor. The passive hyperspectral sensor generally outperformed the active sensor, with superior performance of the active sensor found only for wheat at anthesis. Active sensors have the advantage of being independent of light conditions, enabling their use at night, though the bi-directional passive sensor used in this study does allow compensation for changes in light conditions in the day. Overall, the results show that spectral sensing can be carried out quite successfully in plot designs with few rows; however, some further optimization is still needed, particularly for single rows. The sensors' FOV did not optimally match such a design, offering one avenue for improvement. For example, the GreenSeeker could be aligned along single rows, while the passive sensor could be positioned closer to the plants, thus covering a higher fraction of the plants' area because the measurements are not distance dependent. Still, superior performance of the passive sensor has been demonstrated for plot designs with two, three, or four rows. Taken together, these results suggest that enhanced high-throughput spectral sensing can be used in plot designs with few rows, thereby allowing the evaluation of the performance of varieties or cultivars in early selection cycles. Since early selection cycles, in particular, evaluate many hundreds or thousands of varieties, a highly interesting potential for enhanced breeding is indicated. However, neighboring effects due to different varieties being in close contact with each other should be

considered or avoided. Follow-up work should address the feasibility to extend these findings to an extended set of cultivars or varieties representing different species.

5.3. Section III: Active and passive high-throughput field phenotyping of leaves, leaf sheaths, culms and ears of spring barley cultivars

The performance of three active spectrometers and one passive spectrometer was evaluated to detect differences in the measured dry weight and nitrogen uptake of leaves, leaf sheaths, culms and ears of a set of 30-34 spring barley cultivars at anthesis and dough ripeness. Furthermore, contour maps and PLSR were compared with various published vegetation indices.

5.3.1. Contour maps

The contour map method, testing all possible dual indices, did not provide improved results compared to the selected indices. While LI ET AL. (2013A,B), ELSAYED ET AL. (2015), RISCHBECK ET AL. (2016) and YU ET AL. (2012) indicated improvements of contour map based vegetation indices compared to published VIs, no improved wavelength combination was found in this study. Although ELSAYED ET AL. (2015) and RISCHBECK ET AL. (2016) used a similar set of cultivars and the same sensors, their results differ from those found in this study. This discrepancy might be due to the increased variance in their studies induced by different nitrogen fertilizer levels or drought stress levels.

5.3.2. PLSR

Without exception, PLSR analysis outperformed the simple vegetation indices as well as the indices derived from contour map analysis. Markedly reduced RMSEs and higher coefficients of determination were achieved by PLSR, in agreement with the results from other studies on winter wheat (HANSEN & SCHJOERRING, 2003; LI ET AL., 2013A), spring wheat (ØVERGAARD ET AL., 2013B), durum wheat (FERRIO ET AL., 2005) and spring barley (ELSAYED ET AL., 2015).

ØVERGAARD ET AL. (2013B) reported that at least 2 years of data are necessary to obtain stable PLSR models. The results from this study are in line with this recommendation since the PLSR models showed increased precision when further data were added (results not shown). The best results were obtained for leaves, culms and leaf sheaths at anthesis. However, for culms at anthesis and ears, a marked difference between the calibration and validation models was obtained. A large number of principle components points to a rather unstable model.

5.3.3. Published vegetation indices

Although ERDLE ET AL. (2013B) reported that the R_{760}/R_{730} index is suitable for the detection of the dry weight of ears in winter wheat, neither a published VI nor the PLSR was able to provide satisfactory relationships for spring barley. The perpendicular positioning of the sensors could be a possible reason. Since the ears were still in a vertical posture at dough ripeness, the sensors may not have been able to detect these organs. Considering the performance of the published VIs, R_{780}/R_{670} was found to be the most closely related to the biomass parameters of leaves and culms. Saturation effects of the NDVI were observed, especially for the passive spectrometer and GreenSeeker at anthesis. The same problem was reported by HABOUDANE ET AL. (2004). In general, moderate coefficients of determination were observed between the published VIs and dry weight and nitrogen uptake of culms and leaves. Other studies on spring barley (e.g., BEHRENS ET AL. (2006); BENDIG ET AL. (2015B); ELSAYED ET AL. (2015); TILLY ET AL. (2015)) presented better or at least similar results; however, those previous findings were based on different fertilizer levels or drought and heat stress.

5.3.4. Comparison of sensors

Several comparisons between spectral proximal sensors have been previously performed. ERDLE ET AL. (2011), WINTERHALTER ET AL. (2013) and ELSAYED ET AL. (2015) found a slight advantage of the passive spectrometer, in particular, when nitrogen parameters were detected. These findings were confirmed by this study. The R_{780}/R_{670} index and NDVI were more precise when measured with the passive spectrometer. The performance of the active sensors depends on their light source, which is weaker than sunlight (WINTERHALTER ET AL., 2013). Furthermore, their performance depends on the target distance. The emitted light follows the inverse square law. A doubled measuring distance leads to a four times lower light intensity (KIPP ET AL., 2014). Since the sensor carrier was positioned 1 m above the plant canopy (in line with the recommendations of the manufacturers), differences in the canopy density, plant architecture and penetration depth may contribute to the slightly decreased performance of the active sensors (WINTERHALTER ET AL., 2013; KIPP ET AL., 2014).

5.3.5. Biomass parameter

The year 2013 was characterized by remarkably low heritability (h^2 = 0.18-0.49) due to severe weather conditions and a flood in certain areas of the field trial. This also led to an inconsistent dry weight and N uptake in the cultivars. The highest and most consistent heritability was observed for leaves (h^2 = 0.75-0.85), whereas culms showed low heritability (h^2 = 0.31-0.38), particularly at dough ripeness. The dry weight and N uptake of leaves are important factors that plant breeders use to assess the photosynthetic potential of a plant (ZHU ET AL., 2010; ACQUAAH, 2012). In this study, the dry weight of leaves amounted to 25 % of the total aboveground biomass and accumulated up to 30 % of the total N uptake at anthesis. The dry weight of culms was approximately 75 % of the total aboveground biomass and stored approximately 70 kg N ha^{-1} at anthesis. At dough ripeness, only 16 kg N ha^{-1} remained within the culm biomass. These findings are in line with the studies of BIDINGER ET AL. (1977) and MIROSAVLJEVIC ET AL. (2015), which described the culm as the most important storage organ. The leaf sheaths showed inconsistent behavior. While culms and leaves translocated dry weight and nitrogen during grain filling, leaf sheaths accumulated dry weight and nitrogen. The assumption of SCHNYDER (1993), who identified wheat leaf sheaths as a type of storage organ, were be supported in this study for barley. Furthermore, the spectral sensors showed limitations considering the detection of leaf sheaths, especially at dough ripeness. In this growth stage, only a weak relationship (R^2 = 0.27) between the total aboveground biomass and leaf sheaths was found, and no relationships were observed between the biomass parameters of leaf sheaths and leaves or culms. The same results were obtained for the relationships of the biomass parameter of ears with the other plant organs. However, a highly significant relationship (R^2 = 0.73) was found for the dry weight of ears at dough ripeness and total biomass at anthesis. It is assumed that the detectability of different plant organs is mainly influenced by their contribution to the total aboveground biomass. Spectral proximal sensing combined with suitable PLSR models is a convenient method for obtaining information about leaves and culms at anthesis and dough ripeness. A suitable phenotyping platform enhances the performance of phenotyping. By driving at an average speed of approximately 5.5 km h^{-1}, the measurement of a single plot takes approximately 0.8 to 1.8 seconds, while destructive measurements with subsequent laboratory analysis is tedious and time consuming. Spectral sensors are non-invasive and objective and therefore offer an enhanced tool that can keep pace with high-throughput genotyping techniques and thereby widen the phenotyping bottleneck (WINTERHALTER ET AL., 2011; WHITE ET AL., 2012; KIPP ET AL., 2014).

5.4. Section IV: Mid-season prediction of grain yield and protein content of spring barley cultivars using high-throughput spectral sensing

5.4.1. Performance of contour maps and vegetation indices

The contour maps in Figure 4.5(a) show a narrow (orange) band of wavelengths in the near-infrared area, indicating vegetation indices potentially suited for predicting grain yield. This result is in agreement with ELSAYED ET AL. (2015) and RISCHBECK ET AL. (2016), who found strong correlations in the same wavelength band. In this work, the R_{820}/R_{720} index was found to be the optimal vegetation index. Although coefficients of determination explained 94 % of the variation in experiment IV-1 in 2015, the index showed the highest RMSEs and the highest over- or under-estimations compared to the measured yields. For protein content (Figure 4.5b), no wavelength combination could be found to explain the observed variation. Good relationships were obtained between NDVI and grain yield, whereas yield predictions for the uniformly fertilized cultivars in experiment IV-2 showed only moderate correlations. This agrees with the findings of ELSAYED ET AL. (2015) for spring barley and ØVERGAARD ET AL. (2013B) for spring wheat. NDVI is prone to saturation, particularly in highly fertilized treatments. In contrast with PETTERSSON ET AL. (2006), who conducted measurements at stem elongation, in this study better results were obtained from the REIP index at anthesis. REIP outperformed PLSR in 2013 and 2014.

5.4.2. Performance of the PLSR analysis

In this work, the PLSR models were calculated using data from field trials with approximately 30 cultivars representing a range of different uses. The specific objective was to develop a model comparing the spectral signatures of different cultivars, rather than differentiating among nitrogen management practices. PLSR models developed to compare cultivars performed better at predicting the grain yield and protein content of spring barley under different nitrogen fertilizer levels than vice versa. ØVERGAARD ET AL. (2013B) recommended using at least two years of data to obtain reliable models. Results from this study are in line with this recommendation, since the PLSR models showed an increased precision when further data were added. Compared to the results of HANSEN ET AL. (2002), slightly better results were obtained in this study for the PLSR model for protein content prediction. HANSEN ET AL. (2002) did not find any effect of nitrogen fertilizer level on protein content, which agrees with our findings. They further stated that protein content is mainly related to events after anthesis. This might explain the low prediction results (R^2 = 0.28) in this study. Protein content predictions from a PLSR model of leaf N uptake gave better results; however, the values were largely overestimated and had high RMSEs. SÖDERSTRÖM ET AL. (2010) found improved models for protein content when combining spectral data and weather data. For predicting grain yields via PLSR, most authors used a combination of ancillary data (such as: weather, soil conditions, phenology) and spectral data (WEISSTEINER & KUEHBAUCH, 2005; PETTERSSON ET AL., 2006; SÖDERSTRÖM ET AL., 2010; RISCHBECK ET AL., 2016), or a combination of different vegetation indices (HANSEN ET AL., 2002; ELSAYED ET AL., 2015). In this study, only the wavelengths between 400 and 1000 nm were used for PLSR. Another novel aspect of this study is the use of independent field trials to test grain yield predictions. As seen in Table 4.20, the performance of these predictive tools is promising. Although FERRIO ET AL. (2005) suggested that an accurate quantification is difficult, and the best use of PLSR is a simple ranking, we found close relationships between observed and predicted grain yields. Additionally, it was possible to distinguish among cultivars. However, for experiment IV-1 in 2013 and 2014, a strong underestimation of the yield was seen. This could be due to differences in crop management practices between the independent field trials and the field trials used for developing the PLSR models. Although management followed local recommendations, the dates of sowing and the dates of pesticide and nitrogen applications differed between the two sets of field trials. Almost all authors using PLSR for the prediction of grain yield in wheat (FERRIO ET AL., 2005; ØVERGAARD ET AL., 2013A; XIU-LIANG ET AL.,

2014) and barley (PETTERSSON ET AL., 2005; ELSAYED ET AL., 2015; RISCHBECK ET AL., 2016) concluded that PLSR is a superior method to vegetation indices. However, in this study, REIP was found to be comparably accurate. The advantage of PLSR is the simultaneous analysis of several predictor variables, which can improve the stability of the model. In contrast, REIP is easily calculated and needs only four different wavelengths, which can be measured using a less-expensive spectrometer.

5.4.3. Duration of plot measurements

As seen in Table 4.19, different durations were recorded for the measurements done on each plot. The duration depended on the soil conditions and the design of the field trial. The wheel ruts were bumpy in 2013, which led to an average driving speed of about 3.8 km h^{-1}, whereas flat soil conditions in 2015 made it possible to take measurements at 5.5 km h^{-1}. Higher speeds would be possible, but German laws limit custom vehicles to 6 km h^{-1} Vmax. Additionally, turning around at the end of each plot row can take a substantial amount of time, depending on the available space. In contrast with 2014, which used a quadratic field trial design, experiment IV-2 in 2015 had more plots in one row, which resulted in a long and narrow field trial design with fewer turn-arounds. The main advantage of vehicle-based spectral proximal sensing is the fast, non-invasive assessment of plant traits, which also decreased labor and analytic expenses (MONTES ET AL., 2007; ELSAYED ET AL., 2015).

A. Supplemental Tables Section I

Table A.1.: Plant heights obtained from herbometre reference measurements and maximum and average height values recorded by the laser and the ultrasonic distance sensor, with each value representing the average of four replicated plot measurements in 2014 and 2015 for the investigated barley varieties.

	2014			2015	
Variety	Average plant height from herbometre (cm)	Average maximum height from ultrasonic sensor (cm)	Average maximum height from laser distance sensor (cm)	Average plant height from herbometre (cm)	Average maximum height from ultrasonic sensor (cm)
BA5				42.8g	42.1d
STR2				51.7fg	46.6cd
SCB5				53.1fg	50.3bcd
IGP1				54.3fg	54.2bcd
BA1				54.6efg	50.8bcd
LFL3				55.1defg	51.6bcd
STR3				55.7cdefg	54.3abcd
ACM1				55.7bcdefg	54.3abcd
LFL2				57.7bcdefg	57.1abcd
STR7				57.9bcdefg	56.9abcd
SY Pabloo				58.4bcdef	56.9abcd
BR10				58.5abcdef	57.8abcd
BR3				59.4abcdef	58.4abcd
STR4	54.8abcd	43.7def	44.4abc	59.5abcdef	55.4abcd
SCB1	55.1abcd	45.6cde	44.5abc	59.7abcdef	55.9abcd
LFL5	45.4e	37.0f	35.9cd	59.7abcdef	57.6abcd
STR6				59.8abcdef	59.0abcd
BA4	57.2abcd	52.2abc	52.4ab	60.0abcdef	60.6abcd
SCB3				60.0abcdef	57.7abcd
BR7				60.2abcdef	58.6abcd
STR5				60.2abcdef	55.6abcd
SY Leoo	53.9abcde	49.3abcd	44.7abc	60.2abcdef	58.8abcd
NS1	59.4ab	54.1ab	51.5ab	60.3abcdef	58.42abcd
SCB2				60.9abcdef	57.9abcd
SCB4				61.4abcdef	59.2abcd
SY Hobbit	57.5abc	50.3abcd	48.5ab	61.5abcdef	60.4abcd
BR12	35.9f	28.5g	28.6d	61.6abcdef	61.8abc
SY Tatoo				61.7abcdef	59.8abcd
SY Galation	56.5abcd	48.7bcd	50.1ab	61.8abcdef	60.9abcd
BA3				62.2abcdef	60.0abcd
BA2				62.2abcdef	60.9abcd

Supplemental Table A.1 continued

	2014			2015	
Variety	Average plant height from herbometre (cm)	Average maximum height from ultrasonic sensor (cm)	Average maximum height from laser distance sensor (cm)	Average plant height from herbometre (cm)	Average maximum height from ultrasonic sensor (cm)
EKD1	48.3cde	39.5ef	35.4cd	63.3abcdef	60.2abcd
EKD2	62.3a	56.6a	55.9ab	63.4abcdef	59.5abcd
SCB7				63.8abcdef	60.8abcd
SY Trooper				63.9abcdef	62.9abc
SY Volume				64.0abcdef	60.0abcd
SY Jallon				64.2abcdef	61.7abc
BR11				64.7abcdef	66.0ab
BR8				65.1abcdef	61.4abc
BR4				65.2abcdef	62.8abc
SY Wootan				65.2abcdef	64.0abc
ACM4	53.0abcde	48.2bcd	42.5bc	65.9abcdef	63.7abc
SY Zzoom	50.2bcde	48.3bcd	47.81abc	65.9abcdef	62.2abc
SY Troophy				66.3abcdef	62.9abc
STR1				66.3abcdef	67.4ab
KWS2	55.2abcd	51.6abc	51.28ab	66.3abcdef	65.6ab
BR1	55.7abcd	49.8abcd	48.87ab	66.6abcdef	68.3a
BR5				67.1abcdef	60.9abcd
SY Quadra				67.1abcde	67.4ab
BR2				68.2abcde	64.5ab
BR6				68.8abcde	69.0a
ACM5	47.6de	42.6def	43.46abc	69.2abcde	69.1a
SCB6				69.3abcde	70.4a
SY Tektoo				70.4abcd	68.2a
ACM2				70.9abc	70.4a
BR9				71.8ab	67.4ab
ACM3	52.3bcde	50.3abcd	47.78abc	73.8a	69.3a
LFL4				62.2abcdef	60.8abcd
SY Celoona				62.5abcdef	60.0abcd
LFL1				63.1abcdef	60.3abcd

B. Supplemental Tables Section III

Table B.1.: Descriptive statistics of barley leaves

	Leaves (kg/ha)	Anthesis		Dough ripeness	
Year		Dry weight	N uptake	Dry weight	N uptake
2015	CV (%)	22.41	25.06	23.95	30.52
	Heritability (h^2)	0.75	0.84	0.74	0.84
	Std. error	21.86	0.65	20.64	0.44
	Std. Dev.	245.36	7.33	228.95	4.79
	N	126	126	123	121
	Min	397.93	8.11	327.82	3.07
	Max	1680.26	44.80	1548.66	27.81
	Mean	918.65	24.57	818.98	12.40
2014	CV (%)	14.90	20.35	18.53	26.74
	Heritability (h^2)	0.69	0.66	0.71	0.71
	Std. error	28.08	0.79	27.43	0.55
	Std. Dev.	297.20	8.33	289.03	5.83
	N	112	111	111	111
	Min	629.18	15.50	515.40	5.89
	Max	2451.52	55.77	1904.66	29.79
	Mean	1271.08	30.27	1052.43	15.39
2013	CV (%)	18.42	26.74	17.63	23.46
	Heritability (h^2)	0.38	0.49	0.30	0.24
	Std. error	15.65	0.31	12.93	0.17
	Std. Dev.	167.82	3.31	138.10	1.76
	N	115	115	114	113
	Min	334.49	4.05	306.62	2.09
	Max	1277.08	22.01	1160.99	12.41
	Mean	815.20	10.56	772.71	7.09

Table B.2.: Descriptive statistics of barley leaf sheaths

	Leaf sheaths (kg/ha)	Anthesis		Dough ripeness	
Year		Dry weight	N uptake	Dry weight	N uptake
2015	CV (%)	21.14	21.24	34.39	27.68
	Heritability (h^2)	0.39	0.78	0.53	0.67
	Std. error	15.43	0.27	28.10	0.31
	Std. Dev.	173.23	3.06	311.65	3.36
	N	126	126	123	120
	Min	315.29	4.82	286.62	3.97
	Max	1189.40	22.30	1943.35	23.65
	Mean	698.55	11.99	866.71	10.63
2014	CV (%)	35.35	45.97	16.86	20.10
	Heritability (h^2)	0.27	0.30	0.46	0.53
	Std. error	15.43	0.19	13.13	0.15
	Std. Dev.	163.28	1.97	138.34	1.60
	N	112	111	111	111
	Min	240.23	1.96	344.96	3.58
	Max	1698.50	19.70	984.95	11.07
	Mean	425.65	4.02	640.87	6.77

Table B.3.: Descriptive statistics of barley culms

	Culms (kg/ha)	Anthesis		Dough ripeness	
Year		Dry weight	N uptake	Dry weight	N uptake
2015	CV (%)	17.87	19.12	21.36	24.74
	Heritability (h^2)	0.57	0.73	0.31	0.38
	Std. error	94.00	1.61	81.82	0.36
	Std. Dev.	1055.11	18.08	907.38	4.02
	N	126	126	123	122
	Min	2463.03	29.34	1701.83	5.59
	Max	7622.66	135.50	6533.70	29.70
	Mean	4842.49	77.07	3631.65	14.31
2014	CV (%)	15.49	17.25	15.96	25.71
	Heritability (h^2)	0.77	0.68	0.68	0.55
	Std. error	82.64	1.07	102.39	0.77
	Std. Dev.	874.56	11.23	1078.79	8.12
	N	112	111	111	111
	Min	1855.09	26.84	2513.00	11.40
	Max	6896.53	86.21	8365.79	51.42
	Mean	3820.60	49.8	7 4710.70	22.94
2013	CV (%)	20.94	17.74	19.37	21.54
	Heritability (h^2)	0.37	0.14	0.38	0.38
	Std. error	65.70	1.38	50.50	0.26
	Std. Dev.	704.51	14.80	539.19	2.77
	N	115	115	114	114
	Min	1393.72	30.87	1435.54	5.57
	Max	5522.94	119.34	4396.35	18.42
	Mean	3259.61	80.92	2790.98	11.21

Table B.4.: Descriptive statistics of barley ears

	Ears (kg/ha)	Dough ripeness	
Year		Dry weight	N uptake
2015	CV (%)	16.95	18.71
	Heritability (h^2)	0.67	0.78
	Std. error	111.12	1.53
	Std. Dev.	1227.37	16.81
	N	122 120	
	Min	2632.56	35.36
	Max	9479.07	123.13
	Mean	5936.00	77.83
2014	CV (%)	20.36	21.27
	Heritability (h^2)	0.63	0.66
	Std. error	108.79	1.39
	Std. Dev.	1146.17	14.65
	N	111	111
	Min	1877.72	27.07
	Max	7379.50	107.15
	Mean	4200.37	54.56
2013	CV (%)	16.63	17.45
	Heritability (h^2)	0.18	0.28
	Std. error	94.17	1.22
	Std. Dev.	1005.45	13.03
	N	114	114
	Min	2132.40	30.66
	Max	8179.26	103.44
	Mean	5816.68	76.11

C. Author contributions and Abstracts

C.1. Section I: Referencing laser and ultrasonic height measurements of barley cultivars by using a herbometre as standard

G.B.[1] and U.S.[2] conceived and designed the experiments; B.M.[3] built and adjusted the sensor system; G.B. performed the experiments; G.B analyzed the data; G.B. (¾) and U.S. (¼) wrote the paper.

Assessment of plant height is an important factor for agronomic and breeder decisions; however, current field phenotyping, such as visual scoring or using a ruler, is time consuming, labour intensive, costly and subjective. For agronomists and plant breeders, the most common method used to measure plant height is still a meter stick. In a 3-year study, we have adopted a herbometre similar to a rising plate meter as a reference method to obtain the weighted plant height of barley cultivars and to evaluate vehicle-based ultrasonic and laser distance sensors. Sets of 30 spring barley cultivars and 14 and 60 winter barley cultivars were tested in 2013, 2014 and 2015, respectively. The herbometre was well suited as a reference method allowing for an increased area and was easy to handle. The herbometre measurements within a plot showed very low coefficients of variation. Good and close relationships (R^2 = 0.59, 0.76, 0.80) between the herbometre and the ultrasonic distance sensor measurements were observed in the years 2013, 2014 and 2015, respectively, demonstrating also increased values of heritability. Hence, both sensors were able to differentiate among barley cultivars in standard breeding trials. For the sensors, we observed a 4-fold faster operating time and 6-fold increase of measurement points compared with the herbometre measurement. Based on these results, we conclude that

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distance sensors represent a powerful and economical high-throughput phenotyping tool for breeders and plant scientists to estimate plant height and to differentiate cultivars for agronomic decisions and breeding activities potentially being also applicable in other small grain cereals with dense crop stands. Particularly, ultrasonic distance sensors may reflect an agronomically and physiologically relevant plant height information.

C.2. Section II: High-throughput phenotyping of wheat and barley plants grown in single or few rows in small plots using active and passive spectral proximal sensing

G.B. and U.S. conceived and designed the experiments; G.B. performed the experiments; G.B analyzed the data; G.B. (¾) and U.S. (¼) wrote the paper.

In the early stages of plant breeding, breeders evaluate a large number of varieties. Due to limited availability of seeds and space, plot sizes may range from one to four rows. Spectral proximal sensors can be used in place of labour-intensive methods to estimate specific plant traits. The aim of this study was to test the performance of active and passive sensing to assess single and multiple rows in a breeding nursery. A field trial with single cultivars of winter barley and winter wheat with four plot designs (single-row, wide double-row, three rows, and four rows) was conducted. A GreenSeeker RT100 and a passive bi-directional spectrometer were used to assess biomass fresh and dry weight, as well as aboveground nitrogen content and uptake. Generally, spectral passive sensing and active sensing performed comparably in both crops. Spectral passive sensing was enhanced by the availability of optimized ratio vegetation indices, as well as by an optimized field of view and by reduced distance dependence. Further improvements of both sensors in detecting the performance of plants in single rows can likely be obtained by optimization of sensor positioning or orientation. The results suggest that even in early selection cycles, enhanced high-throughput phenotyping might be able to assess plant performance within plots comprising single or multiple rows. This method has significant potential for advanced breeding.

C.3. Section III: Active and passive high-throughput field phenotyping of leaves, leaf sheaths, culms and ears of spring barley cultivars

G.B. and U.S. conceived and designed the experiments; G.B. performed the experiments; G.B analyzed the data; G.B. (¾) and U.S. (¼) wrote the paper.

To optimize plant architecture (e.g., photosynthetic active leaf area, leaf-stem ratio), plant physiologists and plant breeders rely on destructively and tediously harvested biomass samples. A fast and non-destructive method for obtaining information about different plant organs could be vehicle-based spectral proximal sensing. In this 3-year study, the mobile phenotyping platform PhenoTrac 4 was used to compare the measurements from active and passive spectral proximal sensors of leaves, leaf sheaths, culms and ears of 34 spring barley cultivars. Published vegetation indices (VI), partial least square regression (PLSR) models and contour map analysis were compared to assess these traits. The PLSR models of leaves, leaf sheaths and culms showed strong correlations (R^2 = 0.61-0.76). Published vegetation indices depicted similar coefficients of determination; however, their RMSEs were substantially higher. No wavelength combination could be found by the contour map method to improve the results of the PLSR or published VIs. The best results were obtained for the dry weight and N uptake of leaves and culms. The PLSR models yielded satisfactory relationships for leaf sheaths at anthesis (R^2 = 0.69), only a low performance for all of the sensors and methods was observed at dough ripeness. No relationships with ears were observed. Active and passive sensors performed comparably, with slight advantages observed for the passive spectrometer. The results indicate that tractor-based proximal sensing in combination with PLSR models may represent a suitable tool for plant breeders to assess relevant morphological traits, allowing for a better understanding of plant architecture, which is closely linked to physiological performance.

C.4. Section IV: Mid-season prediction of grain yield and protein content of spring barley cultivars using high-throughput spectral sensing

G.B. and U.S. conceived and designed the experiments; G.B. and K.H.[4] performed the experiments; G.B analyzed the data; G.B. (¾) and U.S. (¼) wrote the paper.

The ability to forecast grain yields and protein contents of spring barley is of particular interest for the malting and brewing industry, as well as for plant breeding. However, methods for early predictions of grain yield and protein content should ideally be timesaving, non-destructive and inexpensive. In this 3-year study using the mobile phenotyping platform PhenoTrac 4, proximally sensed reflectance data of 34 cultivars were used to develop vegetation indices and to calibrate PLSR models, followed by subsequent validation in independent field trials. A comparison among PLSR, the NDVI and REIP indices and an optimized vegetation index indicated that PLSR and REIP (R^2 = 0.71-0.95) gave superior predictions of grain yield. In contrast, protein content could not be predicted reliably. As an alternative, a PLSR model of leaf N uptake at anthesis was tested to predict grain protein content. Satisfactory correlations were obtained with R^2 = 0.61, but protein content was considerably overestimated. The results show that tractor-based proximal sensing is a high-throughput, non-destructive and precise method to predict the grain yield of spring barley and could be a suitable tool to deliver information for the brewing industry and plant breeders.

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D. Publication I

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Referencing laser and ultrasonic height measurements of barley cultivars by using a herbometre as standard

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Abstract. Assessment of plant height is an important factor for agronomic and breeder decisions; however, current field phenotyping, such as visual scoring or using a ruler, is time consuming, labour intensive, costly and subjective. For agronomists and plant breeders, the most common method used to measure plant height is still a meter stick. In a 3-year study, we have adopted a herbometre similar to a rising plate meter as a reference method to obtain the weighted plant height of barley cultivars and to evaluate vehicle-based ultrasonic and laser distance sensors. Sets of 30 spring barley cultivars and 14 and 60 winter barley cultivars were tested in 2013, 2014 and 2015, respectively. The herbometre was well suited as a reference method allowing for an increased area and was easy to handle. The herbometre measurements within a plot showed very low coefficients of variation. Good and close relationships ($R^2 = 0.59, 0.76, 0.80$) between the herbometre and the ultrasonic distance sensor measurements were observed in the years 2013, 2014 and 2015, respectively, demonstrating also increased values of heritability. Hence, both sensors were able to differentiate among barley cultivars in standard breeding trials. For the sensors, we observed a 4-fold faster operating time and 6-fold increase of measurement points compared with the herbometre measurement. Based on these results, we conclude that distance sensors represent a powerful and economical high-throughput phenotyping tool for breeders and plant scientists to estimate plant height and to differentiate cultivars for agronomic decisions and breeding activities potentially being also applicable in other small grain cereals with dense crop stands. Particularly, ultrasonic distance sensors may reflect an agronomically and physiologically relevant plant height information.

Additional keywords: breeding, distance sensor, high-throughput, phenomics, plant height, precision phenotyping, rising plate meter.

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Introduction

New technologies in plant breeding, such as 'next-generation sequencing' or 'marker-assisted selection', led to an acceleration of breeding processes and an enhanced necessity to test new genotypes in the field (Furbank and Tester 2011; White *et al.* 2012; Araus and Cairns 2014). Field trials are necessary to assess specific plant traits, such as the biomass, nitrogen content and plant height in realistic production environments. Plant breeders and agronomists, however, face a bottleneck in phenotyping (Winterhalter *et al.* 2011; White *et al.* 2012) due to a lack of efficient high-throughput field phenotyping methods that keep pace with the achievements in high-throughput genomics (White *et al.* 2012). Current field phenotyping approaches, such as visual scoring, are time consuming, labour intensive, costly and biased due to the person's individual experience (Erdle *et al.* 2013; Kipp *et al.* 2014) and emphasise the need for new high-throughput methods.

In addition to tiller number and biomass, plant height represents an important factor for the assessment of crop stands and consequently for fertiliser and pesticide applications (Ehlert *et al.* 2009; Llorens *et al.* 2011). Plant breeders often select dwarfed cultivars to reduce lodging. In opposition to this, there is a tendency to choose taller plants, especially for the production of energy, due to the shift from fossil-based resources to renewable resources in Europe (Heiermann *et al.* 2009; Dinuccio *et al.* 2010; Zub *et al.* 2011) or the selection of suitable parental lines for hybrid breeding (Longin *et al.* 2012).

For agronomists and plant breeders, the most common methods used to measure plant height is by using a meter stick or visual scoring. The German Federal Office of Plant Varieties recommends the use of a ruler to average a plant height by taking a single measurement within each plot and recording the topmost part of the plant, including the ears and awns (Bundessortenamt 2000). However, this method is time consuming and not objective due to individual decisions for the highest representative part of a plant. Therefore, the quality might change during the measurements, and it remains challenging to assess the true or representative height of a cultivar by only measuring a few plants within a plot. Measurements of plant height should also reflect a meaningful agronomical or physiological property. Since in cereals such as wheat, barley, rye and oat, but also in rice, particularly after

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the termination of shooting, photosynthesis predominantly takes place in the top canopy layer, an averaged plant height representing such an activity might be more useful than choosing just the top most position of a plant. This is also reflected in vertical gradients of the nitrogen distribution within plants that are optimised towards the top canopy layer in such cereals. A further challenge represents the leaf angle and the inclination of leaves varying from erectophile to planophile and also being subject to further changes across the development of the plants. Height measurements in barley plants are particularly challenging due to the increased number of tillers per plant differing in height and due to the highly variable length of the flag leaf.

In the past few years, several approaches were tested to measure plant height by using distance sensors for cereals or grasses, for example winter wheat (Scotford and Miller 2004; Ehlert *et al*. 2007), rye, grass (Ehlert *et al*. 2007), rice (Tilly *et al*. 2014) and corn (Kataoka *et al*. 2002; Freeman *et al*. 2007; Yin *et al*. 2011). In contrast, no investigations have been made regarding barley, and no attempts were made to find out whether it is possible to differentiate between uniformly fertilised varieties at a certain growth stage. Primarily, previous studies focused on the technical feasibility of different concepts, whereas the agronomic aspects were frequently not included in the focus.

Most authors used industrial distance sensors that operate either as time-of-flight or as triangulation sensors. The time-of-flight sensors are known for their ability to measure long ranges, whereas triangulation sensors are restricted to short ranges due to their construction (maximum a few meters) by having a higher accuracy. The sensors have to fulfil particular requirements for usage in field trials. For instance, the sensors should be insensitive to dust, vibrations of the carrier platform, direct sunlight and high temperatures. Additionally, the sensor should be able to detect materials such as plant tissues. Detailed measuring principles have been reported by Ehlert *et al*. (2010) and Dworak *et al*. (2011).

Reference measurements represent an important aspect of these trials and have mainly been achieved by using meter sticks (Scotford and Miller 2004; Chatzinikos *et al*. 2013); however, in most publications a detailed description of the reference method is missing. In this study, we have adopted as novel reference method for cereals a herbometre, which is often used to record plant height or biomass in pastures and grassland (Pauly *et al*. 2012). This principle confers advantages compared with meter sticks because it allows the measurement of a weighted height, which is considered to be more representative and informative of the average estimated plant height, and further increases the objectivity of the process.

The aims of this 3-year study were a comparison of the performance of a low-cost ultrasonic sensor and a laser distance sensor implemented in a mobile phenotyping high-throughput platform in field trials with 30 spring barley and 60 winter barley cultivars and to test the possible differentiation of barley cultivars based on sensor measurements.

Materials and methods

Field experiments and height measurement

The field experiments were conducted at the Dürnast research station of the Technical University of Munich in Germany (11°41′60″E, 48°23′60″N, elevation 448 m). The soil is mostly Cambisol with silty clay loam texture. The annual precipitation is ~800 mm, and the average temperature is 7.5°C.

The study encompassed three site-years of investigation comprising 1 year of spring barley in 2013 and 2 years of winter barley in 2014 and 2015. The experimental design was a randomised block design with four replications using 30 spring barley cultivars in 2013, three winter barley hybrids and 11 lines in 2014 and 12 hybrids and 48 lines of winter barley in 2015. The plots consisted of 12 rows, 6 m in length. The fungicide and fertilisation treatments followed local recommendations.

Reference measurements of the height were obtained by using a self-constructed low-cost herbometre, similar to a rising plate meter, consisting of a Styrofoam board, 50 cm × 50 cm in size and 4 cm thick, having a weight of 200 g, attached centrally to a conventional folding rule (Fig. 1). The board was carefully placed on the plant surface, and the ruler was pushed through the hole without exerting any pressure on the board. Depending on the cultivar and the growth stage, the plants were compressed by ~0.3–3 cm by the herbometre. The compression was decreasing with progressing maturation of the plants. A barrel roller was used to flatten the soil in early spring to minimise the risk of imprecise measurements due to a rough soil surface. The height measurements were conducted four times within each plot shortly before flowering at ZS 55 (Zadoks *et al*. 1974), as this represents a sensitive growth stage relevant to agronomic and breeder decisions.

Height measurements with ultrasonic and laser sensor devices

The performances of an ultrasonic sensor and a laser distance sensor were compared under field conditions. The sensors selected were a UM30–14113 ultrasonic sensor (Sick, Waldkirch, Germany) and an OWTG 4100 PE S1 laser distance sensor (Welotec, Laer, Germany) (Table 1). The sensors were mounted as closely as possible to each other on a boom, 1.5 m in front of the PhenoTrac IV (Rischbeck *et al*. 2016), a mobile phenotyping platform of the Chair of Plant Nutrition of the Technical University Munich, in a nadir down-looking position. The sensor outputs were linked and synchronised to the GPS coordinates from a TRIMBLE-RTK-GPS. Calibration of the sensors was conducted on a bare plot. The sensor boom was held at a height of ~1 m above the plants, and the driving speed was 3.5 km h^{-1}. The data output comprises ~25 measurements across the 6-m plot length. Average values of all of the measurements per plot were calculated, and averaged maximum values, representing the subset of the five highest records, were additionally calculated to gain further information about the highest areas within each plot. The plant height was calculated as:

$$\text{plant height} = \text{distance}_{\text{sensor to soil surface}} - \text{distance}_{\text{sensor to plant surface}}.$$

Furthermore, the ultrasonic sensor is equipped with a ‘first-fix’ algorithm that analyses the echogram considering the upper part of the plants and the soil. Threshold values were defined to avoid

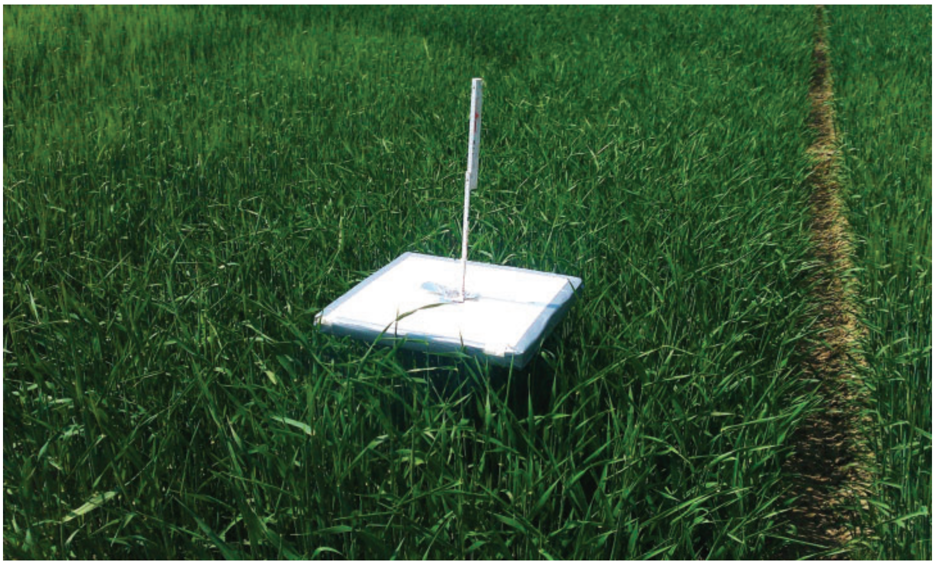

Fig. 1. Illustration of the herbometre height measurements serving as the reference method in barley trials at anthesis.

Table 1. Technical data of the ultrasonic and distance sensors. Higher frequency records of the distance sensors were averaged to be in line with the GPS records

Technical data	UM30 ultrasonic distance sensor	OWTG 4100 PE S1 laser distance sensor
Measurement method	Time-of-flight	Time-of-flight
Measuring range	250–3400 mm	200–10 000 mm
Measuring field	0.5 m^2	6 mm
Resolution	1 mm	1 mm
Accuracy	≤2% of final value	14–17 mm
Sampling interval (modified)	25 Hz	25 Hz
Transducer frequency	120 kHz	–
Wavelength	–	650 nm
Temperature measurement range	−20°C−70°C	−10°C−60°C
Weight	310 g	295 g
Price (2006)	400 €	280 €

implausible high or low values that may affect the calculation of mean values for the plant height.

Comparison between the folding ruler and the herbometre

A comparison between the herbometre and a folding ruler was conducted in wheat plots at heading in 2014. For each plot, three measurements were made for the folding ruler and the herbometer, respectively.

The uniformity of herbometre measurements within the plots was assessed by means of four replicate measurements in 2014 and 2015.

Two operators measured independently of each other winter barley at ZS 55 in 2015 to find out the deviation between two-folding ruler measurements. According to the German Federal Office of Plant Varieties, a single measurement in the centre of each plot was taken.

Statistical analyses

R version 3.1.2 was used for statistical analysis. A regression analysis and a one-way ANOVA were used to compare the ultrasonic and distance sensor and the herbometre reference measurements. Tukey's HSD multiple comparison test was applied for the grouping of the cultivars, with a *P*-value of 5%.

Results

Comparison between the folding ruler and the herbometre

A comparison between the herbometre and a folding ruler is shown in Fig. 2*a* indicating a rather weak relationship with $R^2 = 0.29$. The relationship between two operators using a folding stick is indicated in Fig. 2*b*. The coefficient of determination was $R^2 = 0.83$, however, the slopes were statistically different.

A comparison of herbometre measurements by different operators was not aimed at in this study, because the handling should deliver comparable values. This is supported by the fact, that very low coefficients of variation were observed for the individual within plot measurements for the herbometre in 2014 and 2015 amounting to 2.8% and 3.0%, respectively.

Relationship between herbometre reference method and distance sensors

In the year 2013, with spring barley as the crop, the ultrasonic distance sensor was best related to the herbometre reference measurements with $R^2 = 0.59$** for the average values and with $R^2 = 0.64$** for the maximum values (Fig. 3 and Table 2). The laser distance sensor was less closely related to the herbometre measurements with $R^2 = 0.30$** for the averaged values and $R^2 = 0.37$** for the maximum values.

In the years 2014 and 2015, with winter barley as the crop, improved results, particularly for the ultrasonic distance sensor, were obtained, and the coefficients of determination ranged from $R^2 = 0.76$** in 2014 to 0.83** in 2015. The best

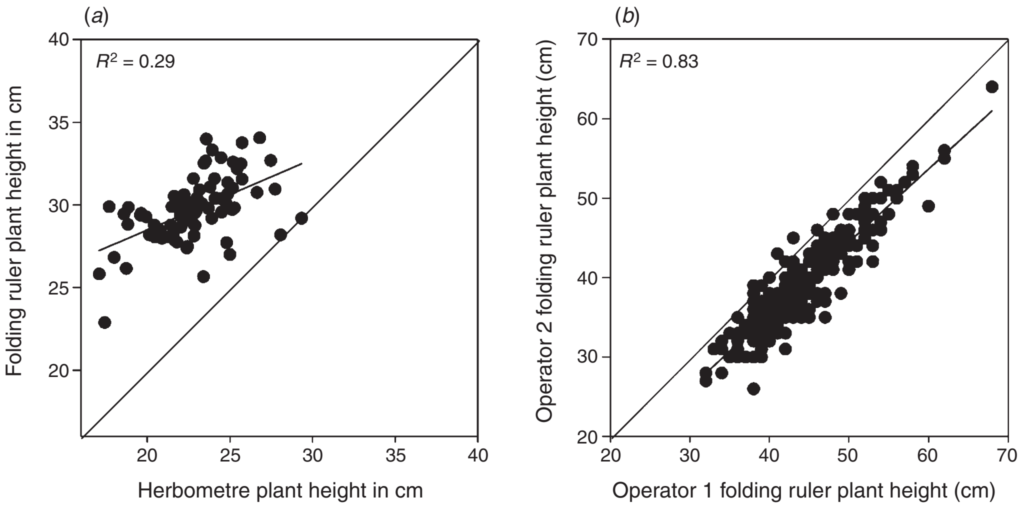

Fig. 2. (*a*) Relationship between a folding ruler and the herbometre in winter wheat 2014. (*b*) Comparison between the measurements of two different operators in winter barley in 2015. Regression lines and the 1 : 1 lines are indicated.

results for the laser distance sensor were obtained in 2014 with $R^2 = 0.66$**, with the level always lower compared with the ultrasonic sensor (Table 2). A deviation between the reference herbometre measurements and the sensors was found, particularly for the laser sensor (Table 3), deviating up to 37 cm. Both the laser and the ultrasonic distance sensor underestimated the observed plant heights in 2014 and 2015, whereas in 2013, an overestimation by the ultrasonic sensor was observed.

Discrimination of cultivars by herbometre and distance sensor measurements

The grouping of the cultivars for the herbometre, the laser sensor and the ultrasonic distance sensor for 2014 and 2015 is shown in table S1, available as Supplementary material at journal's website. However, for the laser distance sensor a classification was not possible, due to excessive scattering of the sensor output in 2015. For the spring barley cultivars in 2013, no differentiation between cultivars was found either for the plant heights recorded by the herbometre or for the distance sensors.

Time and labour requirements of reference and distance sensor measurements

Measurement of the plant height by the herbometre was revealed to be tedious for a single person, although two people could significantly accelerate the work, with one person performing the herbometre measurements and the other recording the height. Altogether, the complete measurements of the field trial required 2 h and 30 min in 2013, 1 h and 10 min for the winter barley trial in 2014 and 4 h for the field trial in 2015, including the subsequent data processing. Thus, the manual measurement of a single plot required ~1 min and 20 s, depending on the size of the field trial and the distance between plots. In contrast, the sensor measurements required only one person, and the measurement of 120 plots took ~35 min in 2015, 18 min for 56 plots in 2014 and 50 min for 250 plots in 2015. Depending on the design of the field trials (plot number in one row and space required for a turnaround of the vehicle) and the driving speed, the measurement of one 6-m-long plot took ~20 s, including subsequent data processing. Thus, the sensor measurements on the mobile sensor platform were four times faster than herbometre measurements. In this experiment, all of the plots had a size of 10.8 m^2. The assessed area covered by the herbometre was 1 m^2 with four measurements taken per each plot. The ultrasonic distance sensor covered 3 m^2 across the whole plot, and the laser distance sensor, with its field of view of 6 × 6 mm, covered one planting row or ~0.036 m^2. According to the driving speed, the sensor output comprised ~25 measurements per plot.

Discussion

Even though the meter or yard stick is considered as reference method for assessing the height of plants in breeding plots, there is no commonly adopted procedure for assessing the plant height in performing such measurements. Depending on the individual observer height assessments in breeding plots can be based on the inspection of the top most leaf (leaves) or are based on the visual inspection of an estimated averaged canopy height, which is indeed subjective. Depending on the plant development, particularly measurements at earlier growth stages tend to be tedious, requiring the observer to bend towards the observation point, and are indicating also some degree of variance and subjectivity as evidenced in Fig. 2*b*. It follows, that an exact definition of putatively assumed reference height measurements does not exist or is individually adopted. There is also little agronomic value in the detection of the top most position of an individual leaf (leaves), which varies considerably among cultivars with different growth habits, represented for example, by erectophile or planophile leaves, and being further affected by environmental conditions such as the wind.

Therefore, the assessment of a standardised height measurement such as delivered by the herbometre approach is clearly preferred to a yard stick measurement, as the herbometre is less susceptible to variation between individual observers and

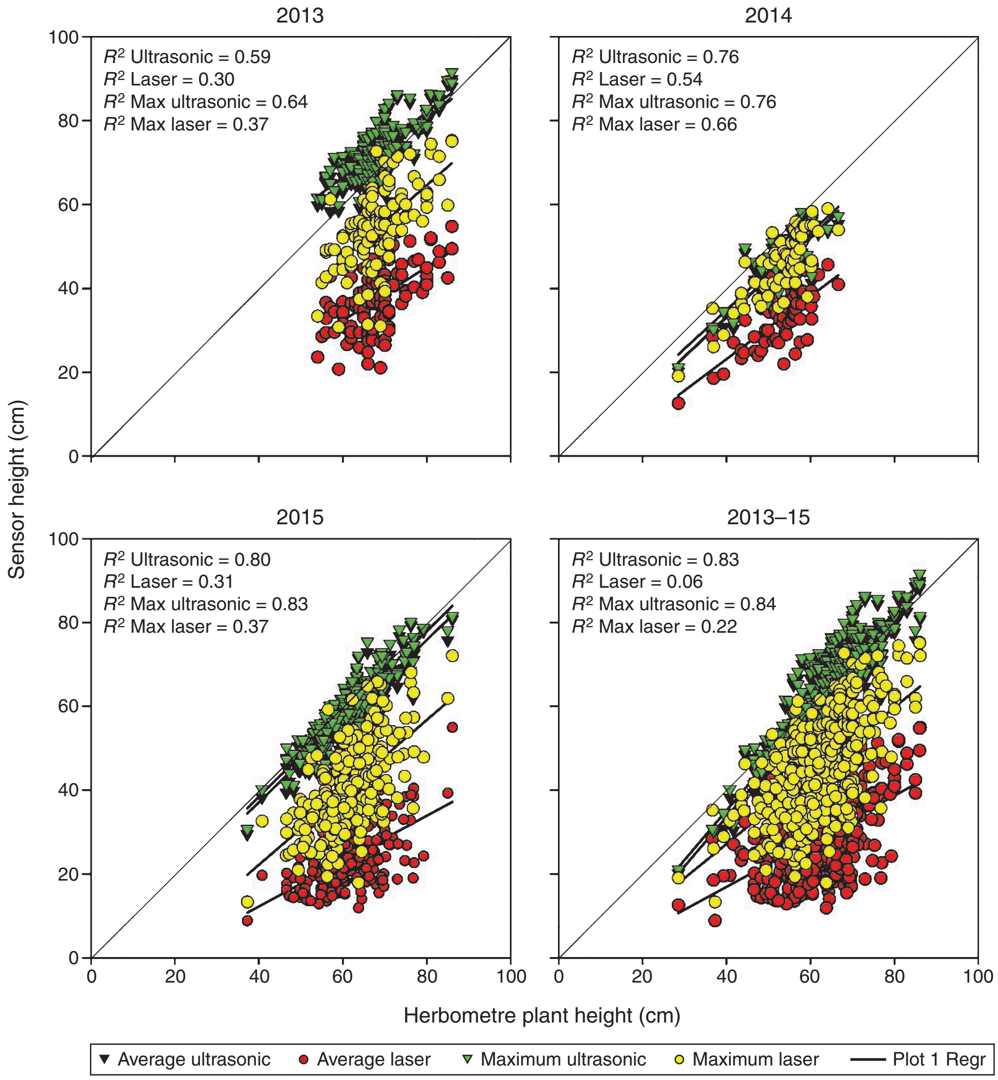

Fig. 3. Relationships between manually recorded plant heights by herbometre and sensor measurements in the years 2013, 2014 and 2015. Regression lines and the 1 : 1 lines are indicated.

Table 2. Coefficients of determination between the sensors and the reference heights as determined by a herbometre ($P < 0.01$). Averaged and the maximum values are reported for the ultrasonic and the laser distance sensor

Year	Ultrasonic	Laser	Max. ultrasonic	Max. laser
2013	0.59**	0.30**	0.64**	0.37**
2014	0.76**	0.54**	0.76**	0.66**
2015	0.80**	0.31**	0.83**	0.37**

reflects physiologically more relevant information indicative of enhanced photosynthetic activity within canopies where gradients in the distribution of the nitrogen content and light interception occur.

Previous efforts to assess the height of plants have primarily emphasised technical aspects of the methodology. In contrast, this report also focuses on the feasibility of distance sensors being used for agronomic decisions, particularly for plant breeders. The results over the 2 years indicate that the sensors can obviously differentiate barley cultivars in height, stressing, however, the need for multi-annual assessments due to the different plant heights observed in 2014 and 2015 (Fig. 4 and table S1). The accuracy of the sensors was revealed to be sufficient to distinguish cultivars in the uniformly fertilised field trials typical for breeder nurseries. The results of other investigations frequently included non-uniform treatments and were based on different seeding rates (Scotford and Miller 2004), different biomass densities (Ehlert *et al.* 2010), different nitrogen fertiliser treatments (Yin *et al.* 2011) or compiled measurements across different growth stages. Although such assessments are common in Plant Sciences, plant breeders require measurements under uniform management conditions to be able to differentiate cultivars.

The use of a herbometre as a reference method compared with a regular folding rule proved to be advantageous. This method incorporates several advantages, making the measurement of the maximum height probably more objective, which is of

Table 3. Coefficients of variation (CV), number of samples (*n*), standard errors of the means (s.e.), heritability (H^2) and deviations (in cm) from the observed plant heights obtained from the herbometre reference measurements. Averaged values and maximum values (Max.) of the height measurements are indicated

	Year	2013	2014	2015
Measured plant height	Min.	54	28.5	37.3
	Mean	68.4	53.0	61.8
	Max.	86	66.6	86.1
	n	116	66	250
	CV	0.06	0.13	0.12
	s.e.	0.63	0.83	0.45
	H^2	0.24	0.68	0.24
Ultrasonic	Min.	58.4	20.2	29.5
	Mean	71.6	45.4	58.2
	Max.	90.0	56.9	80.8
	RMSE	5.42	8.40	4.94
	Deviation	3.18	–7.59	–3.56
	CV	0.09	0.16	0.13
	s.e.	0.62	0.90	0.49
	H^2	0.42	0.95	0.70
Laser	Min.	20.7	12.6	8.9
	Mean	37.7	32.9	24.0
	Max.	54.8	45.7	55.0
	RMSE	37.4	20.66	38.27
	Deviation	–30.7	–20.05	–37.70
	CV	0.20	0.21	0.29
	s.e.	0.70	0.85	0.44
	H^2	0.27	0.81	0.40
Max. ultrasonic	Min.	59.9	21.1	30.7
	Mean	73.1	46.9	60.0
	Max.	91.7	58.2	81.6
	RMSE	6.3	7.04	3.64
	Deviation	4.6	–6.09	–1.79
	CV	0.09	0.15	0.13
	s.e.	0.61	0.88	0.49
	H^2	0.44	0.95	0.71
Max. laser	Min.	30.8	19.1	13.4
	Mean	54.5	45.6	41.0
	Max.	75.3	59.0	72.1
	RMSE	15.82	8.70	22.25
	Deviation	–13.95	–7.39	–20.75
	CV	0.17	0.17	0.25
	s.e.	0.88	0.97	0.64
	H^2	0.24	0.88	0.38

particular relevance if several operators are involved in visual inspections of the plant height. It is argued, that the herbometre provides a weighted height, allowing for a better representation of the average plant height by smoothly bending the leaves and awns and by exerting the same force on a given area. Additionally, the measurement comprises an area of 0.25 m^2, which is advantageous compared with single plant estimates with a ruler as reported by Scotford and Miller (2004) and Yin *et al*. (2011). The low values of the heritability in 2013 and 2015 (Table 3) are caused by heterogeneous soils. Soil analysis showed significantly differing and partly low pH values in two of the four replications in 2013, which likely affected plant growth site-specifically. The field trial in 2015 was conducted on a field site characterised by varying topography attributes. The upper part of the field had a lower soil water-holding capacity than the lower part, which again influenced the uniformness of plant growth in the individual replications. The research station Dürnast, where the experiments were performed, is located in the tertiary hill sites including rather heterogeneous field sites. It is plausible that replicate measurements of plant growth will show a lower variance on more homogeneous field sites used for breeding purposes leading also to a higher heritability.

Commercial distance sensors must comply with several requirements to allow for successful use in field trials, such as resistance to dust, vibrations, shocks and the influence of direct sunlight (Ehlert *et al*. 2009). The distance sensors used in this work used the time-of-flight measuring principle and were chosen for their higher measuring range, up to 10 m, which makes them suitable for measurements of tall plants such as maize crops (compared with triangulation sensors), and for their low acquisition costs. The results indicate that these sensors, mounted on a vehicle and exposed to a rugged drive characteristic for tramlines in field experiments, were able to distinguish different heights of contrasting winter barley cultivars at anthesis in 2014 and 2015, whereas no differentiation could be made with either measurement principle or with the herbometre reference in 2013. In part, this may have been caused by the wet weather conditions during the early summer, resulting in rather uneven crop stands, in addition to deep and uneven (bumpy) wheel ruts. Vibrations and swinging of the front loader appeared to be the main source of errors and inaccuracies, especially at higher driving speeds. A further improvement could be achieved with a stabilising wheel (Chatzinikos *et al*. 2013) or a feeler rod as described by Ehlert *et al*. (2009). The sensor performance depends further on the leaf angle, the size of the leaves and the covered area (Kataoka *et al*. 2002). This might be a further reason for decreased accuracies in plant height as obtained by the laser distance sensor in barley, due to the risk of measuring interspaces between planting rows caused by the small measuring area of 6 mm × 6 mm. In contrast, the ultrasonic distance sensor, measuring an area of 0.5 m^2, is apparently less affected by the variable crop stand density of barley. Ehlert *et al*. (2010) suggested that the laser sensor readings not only reflect the highest but also the lower parts of a plant. Therefore, in addition to the average plant height, we have recorded the maximum plant height averaged from the five most increased measurement values, and this is most likely the reason for the negative deviations from the referenced plant height shown in Table 3.

The ultrasonic distance sensor performed better than the laser distance sensor due to the increased measuring area of 0.5 m^2 and consequently higher accuracies obtained within the barley crops stands (Fig. 3). Nevertheless, industrial distance sensors are evidently beneficial in saving costs and workloads by assuring a constant data quality. Furthermore, a single person can do such measurements during regular fertiliser and pesticide applications and during paralleled other measurements, allowing the person to phenotype the plants with high-throughput. Erdle *et al*. (2013) and Kipp *et al*. (2014) have previously reported examples of concomitant measurements done with the PhenoTrac IV sensor platform, including assessments of the biomass and nitrogen uptake at

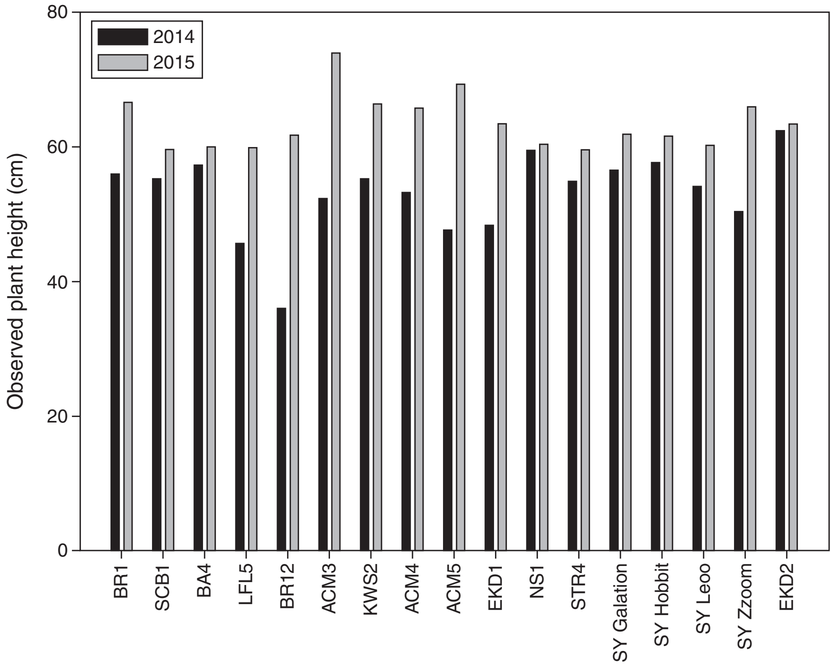

Fig. 4. Plant heights of different barley cultivars assessed in 2014 and 2015 by herbometre measurements.

anthesis. Data fusion of spectral, thermal and canopy height parameters allowed for improved yield prediction of drought-stressed spring barley (Rischbeck *et al.* 2016). Available and cost-effective industrial distance sensors represent a powerful high-throughput phenotyping tool for breeders and plant scientists to estimate plant height and identify a distinction among cultivars for specific breeding goals. If the sensors were attached to a tractor used for fertiliser or pesticide applications, measurements can be done simultaneously and no further costs for transport, deployment or maintenance will occur.

The view has been expressed that smaller cultivars contribute to the goal of preventing lodging (Stanca *et al.* 1979; Matušinsky *et al.* 2015), fostering the need for detailed detection of the plant height. Sensing the height in a fast and economical way may allow enhanced selection along these lines.

Conclusions

In this study, an ultrasonic and a laser distance sensor were evaluated for the assessment of barley plant heights as part of breeding activities. A herbometre was used to reference the plant heights as objectively as possible. The ultrasonic distance sensor was best related to the herbometre reference measurements. This may be attributed to the increased measuring field of 0.5 m^2, whereas the laser distance sensor only had a measuring field of 6 by 6 mm and was more prone to failures resulting from measurements of the interspaces between the planting rows. For winter barley, the sensors were able to differentiate different cultivars in uniformly managed field trials typical in breeder nurseries. We found a 4-fold faster operating time and 6-fold increase of the measurement density compared with the herbometre reference assessments. Possible inaccuracies of tractor-based sensors may occur due to vibrations resulting from uneven wheel ruts. Industrial distance sensors proved to be advantageous in terms of saving costs and workloads and by providing a consistent and objective quality of data. We further suggest that the averaged plant height as estimated by the herbometre measurements, and best mimicked by ultrasonic measurements, represents the most agronomical and physiologically meaningful plant height record.

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E. Publication II

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High-Throughput Phenotyping of Wheat and Barley Plants Grown in Single or Few Rows in Small Plots Using Active and Passive Spectral Proximal Sensing

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Abstract: In the early stages of plant breeding, breeders evaluate a large number of varieties. Due to limited availability of seeds and space, plot sizes may range from one to four rows. Spectral proximal sensors can be used in place of labour-intensive methods to estimate specific plant traits. The aim of this study was to test the performance of active and passive sensing to assess single and multiple rows in a breeding nursery. A field trial with single cultivars of winter barley and winter wheat with four plot designs (single-row, wide double-row, three rows, and four rows) was conducted. A GreenSeeker RT100 and a passive bi-directional spectrometer were used to assess biomass fresh and dry weight, as well as aboveground nitrogen content and uptake. Generally, spectral passive sensing and active sensing performed comparably in both crops. Spectral passive sensing was enhanced by the availability of optimized ratio vegetation indices, as well as by an optimized field of view and by reduced distance dependence. Further improvements of both sensors in detecting the performance of plants in single rows can likely be obtained by optimization of sensor positioning or orientation. The results suggest that even in early selection cycles, enhanced high-throughput phenotyping might be able to assess plant performance within plots comprising single or multiple rows. This method has significant potential for advanced breeding.

Keywords: border-row effect; high-throughput; phenomics; phenotyping; plant breeding; plot design; precision; spectral proximal sensing

1. Introduction

In early selection cycles in plant breeding, large numbers of plants need to be tested, and in agronomic field testing, extensive evaluation of plant performance is also required. Both seed availability and financial constraints frequently necessitate testing of plants in one or several rows, with space limitations also contributing to a need for small plot sizes. Limited resources, therefore, necessitate smaller plots [1]. In general, plot size depends on the type of experiment, breeding objectives, available resources and equipment, and the stage of breeding [2]. However, plot sizes vary substantially among field trials, ranging from single-plant plots to plots of several hundred square meters [3]. Small plots with 2–3 rows are usually used in early stages of breeding projects to evaluate varieties quickly and inexpensively. In advanced selection cycles, when selection for yield also occurs, larger plots are used, and the data may be collected from middle rows [2]. Such plot trials, thus, aim to predict the performance of the tested varieties by mimicking agricultural field conditions. However, such predictions may be inaccurate since the phenotypic performance of plants grown at different spacing may differ from that of plants grown using conventional agricultural practices [1]. The small size of plots may be disadvantageous because border row effects are known to influence yield. Depending on the type of plot trial, external rows may show increased yield [4] due to increased tillering [5].

Since the management of field trials comprising a large number of plots is highly labour-intensive, new methods, such as spectral proximal sensing for the estimation of specific plant traits, are becoming increasingly more important [6,7]. However, commercially available spectral proximal sensors, such as the GreenSeeker (NTech Industries Inc., Ukiah, CA, USA), as well as hyperspectral passive sensors [8,9], were originally designed and tested for field conditions and not specifically for small-plot testing. Therefore, assessment of the sensors in plot trials is of great importance, and particular attention should be paid to the evaluation of the sensed areas. Such evaluation requires the consideration of technical aspects, such as sensor-target distances, and the influences of environmental conditions, such as light intensity and temperature [10,11]. Sensors should be compatible with various plot designs, which ultimately requires a match between the sensors' field of view and the tested target.

Field trials comprising different plot designs (Figure 1) and cropped with one or multiple species, are challenging to evaluate, and their potential for assessment by proximal sensing needs to be determined.

Non-invasive assessments of small plots must take into account uneven growth due to differences in the light availability or enhanced nutrient and water uptake. It is also important to consider whether middle rows are to be assessed preferentially or an integral assessment of the whole plot is desired. Additionally, reflectance sensors differ in their spectral fields of view, ranging from linear to oval and circular shapes [8,11], and are also influenced whether the sensor's orientation is parallel or opposed to the row.

Numerous studies have described border row effects [12] and the advantages and disadvantages of different field trial designs [4,13–15], in addition to comparing different spectral sensors [8,11,16]. To the best of our knowledge, no studies have compared the performances of active and passive sensors in assessing the single or multiple rows used in breeding or agronomic experiments; such a comparison was the goal of this work. Since wide plots with many rows have already been tested repeatedly [7,10,16,17], the purpose of this work is to assess the performance of active and passive spectral sensing in plot designs of one, two, three, and four rows, like those commonly used in breeding trials for wheat and barley. Previous studies have shown no difference in spectral performance when assessing plots with six or more rows. In this work, the influence of different plot designs on biomass and grain yield is illustrated, highlighting the performance of spectral sensors in non-invasive detection of these traits.

Figure 1. Field trials within a rain-out shelter platform, illustrating different row designs for spring barley grown in two rows (**foreground**) and for winter barley grown in six rows (**background**).

2. Materials and Methods

2.1. Plot Experiments and Biomass Sampling

Field experiments were conducted at the Dürnast Research Station of the Technical University of Munich (TUM) in Germany (11°41′60″ E, 48°23′60″ N) in 2014. The soil is a mostly homogeneous Cambisol of silty clay loam texture, the annual precipitation is approximately 800 mm, and the average temperature is 7.5 °C.

A randomized block design was used to test both barley (*Hordeum vulgare* L. cv. Sandra) and wheat (*Triticum aestivum* L. cv. Kerubino), with four planting-row designs and four replicates, totalling 40 plots (Figure 2).

Figure 2. UAV image of the field trial. Different plot designs, including one-, two-, three- and four-row designs, were tested using winter wheat and winter barley as crops.

The plots were 10 m in length. The planting-row designs consisted of plots with a single row, plots with two rows with 25-cm row spacing, and plots with three and four rows with 12.5-cm row spacing.

The wider 25-cm row spacing is frequently used for testing the performance of barley, whereas the narrower spacing of 12.5 cm is commonly used for testing wheat in breeding nurseries in Germany.

Fungicide treatments followed local recommendations. Weeds were removed by hand to remove possible bias in interpreting the results. Nitrogen fertilizer was applied in a single dose at ZS 15 [18] as ammonium sulphate using the nitrification inhibitor ENTEC [19] with 150 kg·N/ha and 60 kg·S/ha in amounts corresponding to the different numbers of rows.

Biomass samplings were performed at Zadoks stage 32 [18] (stem elongation), ZS 60 (anthesis) and ZS 85 (soft dough) by cutting plants above the ground along 1 m of each row. The fresh biomass was immediately determined in the field by weighing, and a subsample was oven-dried at 60 °C for three days until a constant dry weight was reached. The nitrogen content was determined by mass spectrometry using an isotope ratio mass spectrometer with an ANCA SL 20-20 preparation unit (Europe Scientific, Crewe, UK) and nitrogen uptake was calculated by multiplying plant dry weight by N concentration.

2.2. Spectral Reflectance Measurements

Two different sensors were used in this study: a passive bi-directional reflectance sensor customized by the Chair of Plant Nutrition from the TUM and a GreenSeeker RT100 (NTech Industries Inc., Ukiah, CA, USA). The passive bidirectional reflectance sensor contained two Zeiss MMS1 silicon diode array spectrometers with a spectral detection range from 300 to 1000 nm and a bandwidth of 3.3 nm [20]. One spectrometer was linked to a diffuser detecting the solar radiation as a reference signal. The second spectrometer measured the canopy reflectance with a field of view (FOV) of 12° within a circular shape, resulting in a sensor-target distance of approximately 1 m with a FOV of approximately 0.28 m^2. The GreenSeeker used two LEDs as a light source and detected the reflection of visible (656 nm, ~25 nm band width) and near-infrared (774 nm, ~25 nm band width) spectral regions. The FOV of the GreenSeeker was a strip of approximately 61 by 1.5 cm, resulting in a scanned area of approximately 0.009 m^2 (Figure 3) [8]. Both sensors were mounted on a frame in front of the PhenoTrac IV [9], a sensor-vehicle platform customized by TUM (Figure 4), at a height of approximately 1 m above the plant canopy. The measurements were conducted under clear sky conditions at noon, one hour after biomass sampling. Afterwards, the normalized difference vegetation index (NDVI) [21] was calculated as follows:

$$NDVI = \frac{R774\text{ nm} - R656\text{ nm}}{R774\text{ nm} + R656\text{ nm}}$$

In addition, the simple ratio (SR) was determined as follows:

$$SR = \frac{R774\text{ nm}}{R656\text{ nm}}$$

and three simple ratios were further selected based on a contour map analysis depicting all dual wavelength combinations:

$$SR = \frac{R800\text{ nm}}{R770\text{ nm}}$$

$$SR = \frac{R820\text{ nm}}{R755\text{ nm}}$$

$$SR = \frac{R720\text{ nm}}{R400\text{ nm}}$$

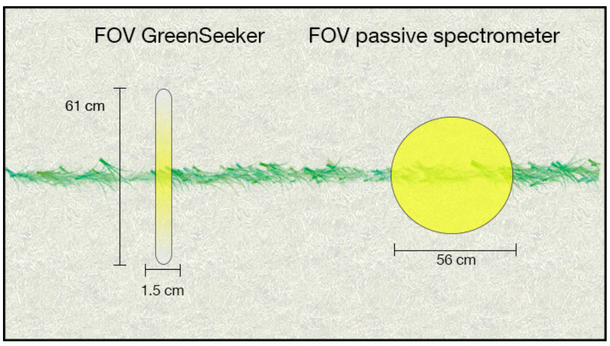

Figure 3. Illustration of different shapes of the sensors' fields of view (FOV) in single-row trials. Yellow colour indicates decreasing light intensity in the periphery of the LED-based GreenSeeker (unpublished data).

Figure 4. Sensor carrier PhenoTrac IV, customized by the Chair of Plant Nutrition from the Technical University of Munich.

2.3. Statistical Analysis

R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria) was used for calculating the coefficients of variation, the standard errors, and linear regressions between the data obtained from the sensors and the destructive measurements. An analysis of variance (ANOVA) with Tukey's HSD (honest significant difference) test ($p \leq 0.05$) was used to group and differentiate between planting-row designs. For an enhanced analysis of optimized wavelength combinations, a contour map analysis was used.

3. Results

3.1. Effects of Different Row Designs on Plant Fresh and Dry Weight, Aboveground Biomass Nitrogen Uptake, and Grain Yield

Even at early stages of development, the different row designs exhibited clear differences. Compared to a reference plot with 10 rows, the single-row plots showed an increase in fresh weight of 124% for barley and 90% for wheat at ZS 32. At ZS 65 and ZS 85, this difference grew to an increase of 235% in wheat biomass in the single-row plot design compared to the 10-row plot. Mean values of the destructively-assessed plant parameters are given in Table 1. Significant differences ($p \leq 0.05$) were found between the designs in aboveground fresh and dry weights, as well as in the calculated N uptake; however, no differences in aboveground plant N content were found. Especially for wheat at early stages of growth, no distinction among the one-, two-, and three-row designs, or among the two-, three-, and four-row designs was found for plant fresh weight or dry weight. These designs were characterized by excessive tillering. This trend remained until ZS 85, when the two-, three-, and four-row designs still had comparable biomasses. Even the one-row design showed increases of up to 75% in plant dry weight compared to the two-row design.

Barley showed similar responses, though the four-row design differed significantly from the two-row design at ZS 32 and 65. At ZS 85, however, the two-, three-, and four-row designs all had similar plant fresh weight and dry weight.

A statistical grouping of grain yields showed a high compensatory performance, particularly for wheat. No difference was observed among the two-, three-, and four-row designs. Compared to the ten-row plots, grain yields gradually increased with decreasing number of rows.

Table 1. Destructively-assessed values of aboveground plant fresh and dry weight, N content, and aboveground nitrogen uptake of wheat and barley plants as obtained from different plot designs. The plot designs included 1, 2, 3, 4, or 10 rows, and samples were collected from plants at three different stages of development (ZS 32, 65, and 85). Coefficients of variation, standard errors of the means, and plant parameters per plot for the different row designs are indicated, with each value representing the average of four replicates. Rankings are derived from Tukey's HSD-Test, are indicated at $p \leq 0.05$ indicating differences within rows. Different letters (a,b,c,d) denote significant differences.

Variant			One-Row				Two-Row			
			Fresh-Weight (g)	Dry-Weight (g)	N-Content (%)	N-Uptake (g)	Fresh-Weight (g)	Dry-Weight (g)	N-Content (%)	N-Uptake (g)
Barley	ZS 32	Means	1113 ^{a}	243.6 ^{a}	2.9 ^{a}	7.1 ^{a}	1607.1 ^{ab}	372.4 ^{ab}	2.5 ^{a}	9.4 ^{ab}
		CV	0.14	0.20	0.06	0.21	0.12	0.12	0.08	0.13
		SE	78.58	23.82	0.09	0.75	95.15	21.97	0.11	0.62
	ZS 65	Means	1626.2 ^{a}	483.3 ^{a}	1.7 ^{a}	8.2 ^{a}	2180.7 ^{ab}	666.9 ^{ab}	1.7 ^{a}	11.9 ^{a}
		CV	0.14	0.04	0.09	0.10	0.13	0.11	0.12	0.22
		SE	117.60	9.31	0.08	0.43	144.01	35.75	0.11	1.29
	ZS 85	Means	1745.9 ^{a}	706.7 ^{a}	1.2 ^{a}	8.8 ^{a}	2295.5 ^{b}	936.9 ^{ab}	1.1 ^{a}	10.8 ^{a}
		CV	0.25	0.22	0.06	0.22	0.19	0.15	0.13	0.25
		SE	218.73	78.50	0.04	0.97	221.52	69.72	0.08	1.35
Wheat	ZS 32	Means	463.2 ^{a}	109.6 ^{a}	2.6 ^{a}	2.8 ^{a}	842 ^{ab}	190.9 ^{ab}	2.5 ^{a}	4.7 ^{ab}
		CV	0.10	0.08	0.04	0.09	0.09	0.06	0.05	0.09
		SE	23.50	4.40	0.05	0.13	36.44	5.60	0.06	0.22
	ZS 65	Means	1820 ^{a}	574.1 ^{a}	1.8 ^{a}	10.5 ^{a}	2584 ^{ab}	767.9 ^{ab}	1.6 ^{a}	12.5 ^{a}
		CV	0.06	0.13	0.04	0.17	0.10	0.08	0.08	0.12
		SE	58.80	36.74	0.03	0.88	129.08	32.13	0.07	0.75
	ZS 85	Means	2111.7 ^{a}	971.2 ^{a}	0.8 ^{a}	8.4	2994.1 ^{ab}	1383.9 ^{b}	0.9 ^{ab}	13
		CV	0.02	0.06	0.06	0.06	0.04	0.04	0.17	0.17
		SE	21.54	28.85	0.03	0.26	61.88	24.95	0.08	1.14

Table 1. *Cont.*

Variant			Three-Row				Four-Row			
			Fresh-Weight (g)	**Dry-Weight (g)**	**N-Content (%)**	**N-Uptake (g)**	**Fresh-Weight (g)**	**Dry-Weight (g)**	**N-Content (%)**	**N-Uptake (g)**
Barley	ZS 32	Means	1959.2 ^{ab}	425.4 ^{bc}	2.9 ^{a}	12.5 ^{ab}	2396.7 ^{b}	545.7 ^{c}	2.7 ^{a}	14.7 ^{b}
		CV	0.07	0.08	0.09	0.17	0.16	0.14	0.11	0.16
		SE	66.06	17.83	0.13	1.05	189.86	38.82	0.15	1.16
	ZS 65	Means	2595.5 ^{b}	799.8 ^{b}	1.8 ^{a}	15 ^{a}	2882.7 ^{b}	857.3 ^{b}	1.8 ^{a}	16.2 ^{a}
		CV	0.11	0.10	0.08	0.19	0.17	0.12	0.23	0.33
		SE	149.18	40.01	0.08	1.43	239.40	51.92	0.22	2.65
	ZS 85	Means	2663.9 ^{b}	1059.5 ^{ab}	1.3 ^{a}	14.3 ^{a}	2984.6 ^{b}	1143.7 ^{b}	1.2 ^{a}	14.6 ^{a}
		CV	0.21	0.17	0.12	0.29	0.12	0.07	0.15	0.21
		SE	278.86	91.74	0.08	2.11	177.28	41.60	0.09	1.56
Wheat	ZS 32	Means	949.9 ^{bc}	200.9 ^{ab}	2.5 ^{a}	5.1 ^{bc}	1163.2 ^{c}	266.3 ^{c}	2.6 ^{a}	7 ^{c}
		CV	0.17	0.12	0.01	0.12	0.12	0.09	0.08	0.16
		SE	79.89	11.88	0.01	0.30	72.34	12.46	0.11	0.57
	ZS 65	Means	2931.7 ^{bc}	887.2 ^{b}	1.8 ^{a}	16.4 ^{a}	3406.7 ^{c}	1004.4 ^{b}	1.8 ^{a}	18.5 ^{ab}
		CV	0.14	0.14	0.10	0.23	0.08	0.10	0.13	0.22
		SE	207.99	60.37	0.09	1.87	139.37	52.70	0.12	2.07
	ZS 85	Means	2822.7 ^{b}	1338.3 ^{b}	0.8 ^{ab}	10.6	3347.8 ^{b}	1562.5 ^{b}	0.9 ^{ab}	14.2
		CV	0.17	0.18	0.08	0.13	0.12	0.11	0.14	0.21
		SE	243.30	121.83	0.03	0.71	195.63	84.89	0.06	1.51

Table 1. *Cont.*

Variant			Complete Plot (10 Row)			
			Fresh-Weight (g)	**Dry-Weight (g)**	**N-Content (%)**	**N-Uptake (g)**
Barley	ZS 32	Means	4631.8 [c]	1085.5 [d]	2.4 [a]	26.8 [c]
		CV	0.09	0.09	0.11	0.14
		SE	198.34	50.32	0.14	1.92
	ZS 65	Means	5254.5 [c]	1809.9 [c]	1.8 [a]	34 [b]
		CV	0.13	0.16	0.14	0.31
		SE	352.90	145.62	0.13	5.27
	ZS 85	Means	5843.7 [c]	2403.8 [d]	1.3 [a]	31.4 [b]
		CV	0.10	0.12	0.09	0.13
		SE	297.01	139.30	0.06	2.03
Wheat	ZS 32	Means	2773.2 [d]	574.7 [d]	2.7 [a]	15.3 [d]
		CV	0.17	0.15	0.08	0.08
		SE	231.69	42.96	0.11	0.58
	ZS 65	Means	6420.5 [d]	1760.2 [c]	1.7 [a]	30.5 [b]
		CV	0.08	0.09	0.09	0.14
		SE	255.45	75.08	0.08	2.11
	ZS 85	Means	6000 [c]	2896 [c]	0.6 [b]	19.4
		CV	0.04	0.04	0.13	0.11
		SE	132.96	56.94	0.04	1.11

3.2. Relationship between Plant Parameters Obtained from Combination of the Four Plot Designs and Spectral Reflectance Measurements

Relationships between sensor measurements and four plant parameters for the combined row designs at three sampling dates are given in Table 2. In general, the passive spectrometer showed closer linear relationships between selected spectral reflectance indices and plant parameters of both species than did the active sensor. The GreenSeeker showed a closer relationship only for wheat at anthesis. Since neither sensor could detect all biomass parameters from wheat at ZS 85 with the vegetation indices available from the GreenSeeker, a contour map method that allowed testing of all possible dual reflectance indices from the passive spectrometer was further evaluated to find whether enhanced vegetation indices could be obtained. These indices, R820/R755 for barley and R720/R400 for wheat, resulted in markedly improved relationships in later growth stages.

Table 2. Significant relationships between sensor measurements and plant parameters of wheat and barley, indicated by coefficients of determination (R^2) at * $p \leq 5\%$, ** $p \leq 1\%$. Relationships are indicated for different indices. The closest relationships are indicated in bold.

	GreenSeeker		**Passive Spectrometer**				
Barley ZS 32	774/656	NDVI	774/656	NDVI	800/770	820/755	720/400
Fresh weight	0.53 **	0.48 **	0.49 **	0.37*	**0.86 ****		
Dry weight	0.41 **	0.35 *	0.37 *	0.28 *	**0.85 ****		
N-content							
N-uptake	0.46 **	0.46 **	0.44 **	0.43 **	**0.84 ****		
Barley ZS 65							
Fresh weight	0.25 *	0.34 *	**0.85 ****	0.70 **			
Dry weight		0.20 *	**0.74 ****	0.61 **			
N-content		0.21 *					
N-uptake	0.21 *	0.29 *	**0.71 ****	0.50 **			
Barley ZS 85							
Fresh weight	0.50 **	0.45 **	0.67 **	0.64 **		**0.77 ****	
Dry weight	0.46 **	0.44 **	0.60 **	0.64 **		**0.72 ****	
N-content	0.20 *		**0.30 ***			0.27 *	
N-uptake	0.43 **	0.35 **	0.63 **	0.53 **		**0.71 ****	
Wheat ZS 32							
Fresh weight	0.62 **	0.60 **	**0.86 ****	0.52 **			
Dry weight	0.63 **	0.58 **	**0.88 ****	0.54 **			
N-content							
N-uptake	0.59 **	0.55 **	**0.93 ****	0.55 **			
Wheat ZS 65							
Fresh weight	**0.74 ****	0.69 **	0.67 **	0.60 **			
Dry weight	**0.72 ****	0.70 **	0.65 **	0.59 *			
N-content							
N-uptake	**0.51 ***	0.47 *	0.37 *	0.33 *			
Wheat ZS 85							
Fresh weight							**0.66 ****
Dry weight							**0.63 ****
N-content							
N-uptake							**0.40 ***

Compared to the simple ratio R774/R656, the NDVI showed reduced coefficients of determination caused by saturation effects. In this regard, the simple ratios were less sensitive. For the N content, only weak relationships were obtained.

Figures 5 and 6 depict results from linear regressions for the combined row designs for barley and wheat, respectively. The spread of the regression points for the three sampling dates made it necessary to consider each sampling date separately. For barley, the results from the GreenSeeker demonstrated considerable scatter, and the regressions of both the NDVI and the simple ratio were considerably less similar than were the same indices obtained for wheat.

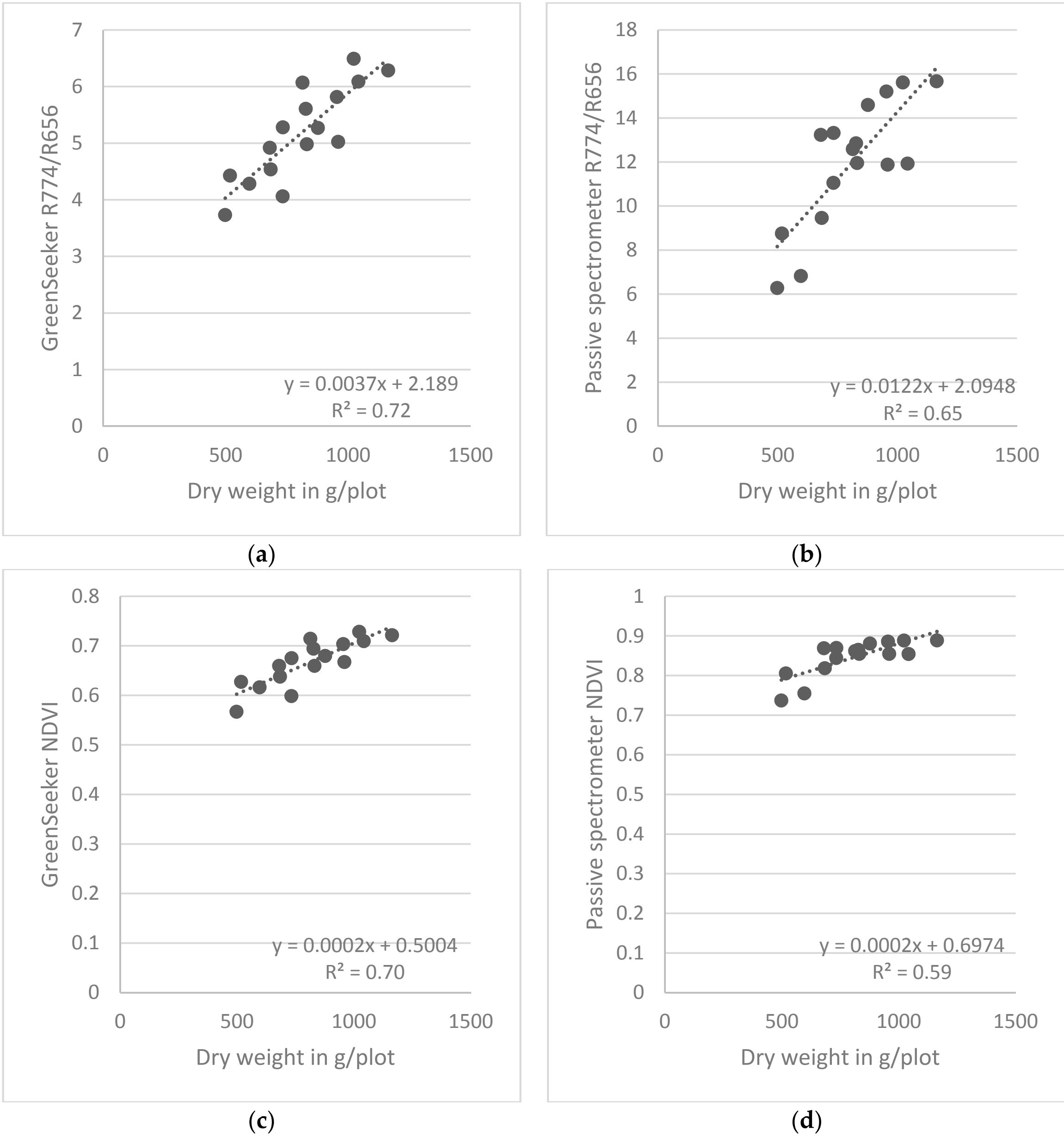

Figure 5. Relationships between spectral indices derived from the two types of sensors and plant dry weight at ZS 65 for wheat, obtained from linear regressions combining the four different row designs.

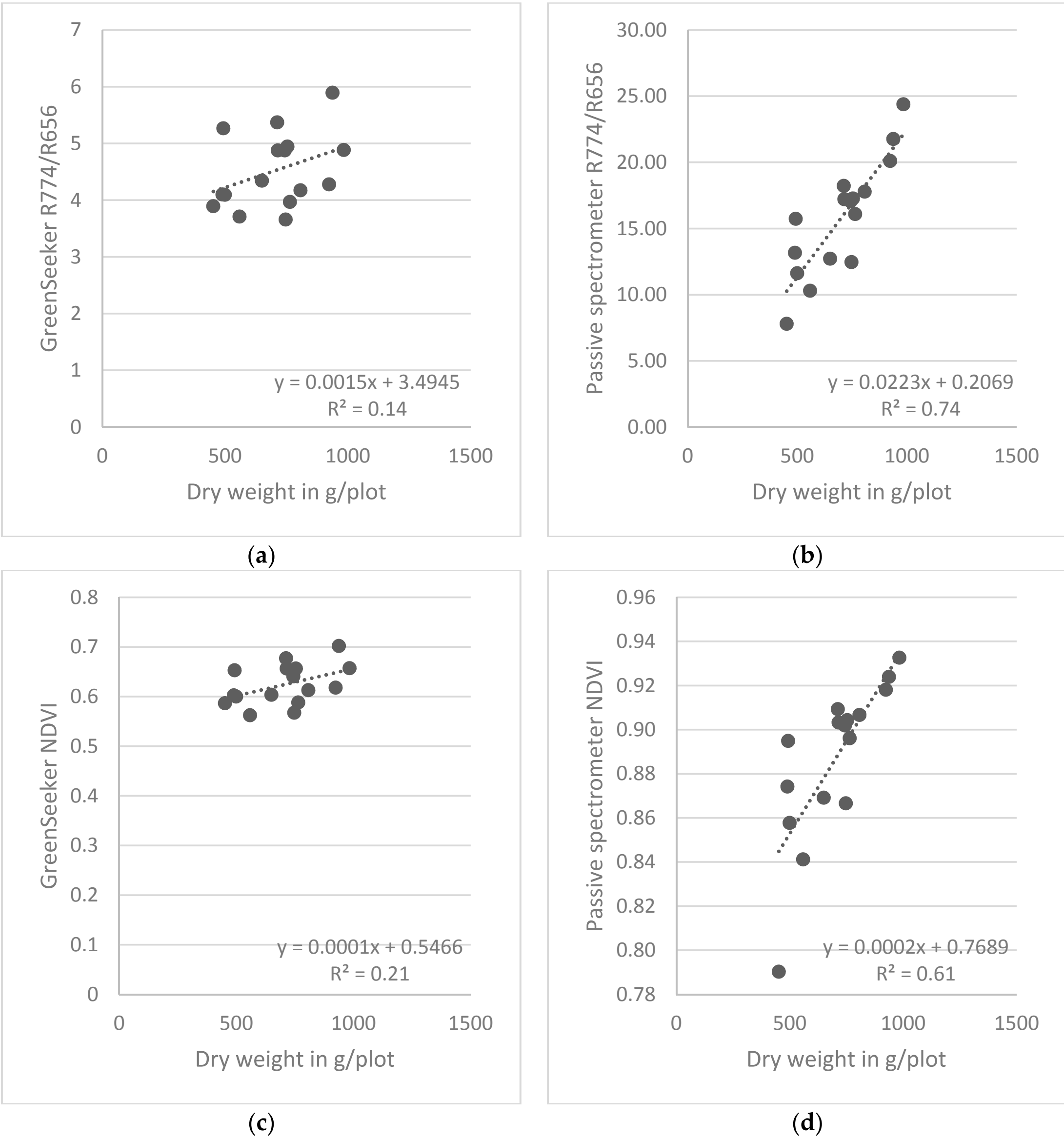

Figure 6. Relationships between spectral indices derived from the two types of sensors and plant dry weight at ZS 65 for barley, obtained from linear regressions combining the four different row designs.

4. Discussion

Border row effects, which cause enhanced growth of plants in border rows, have long been well-known, and recommendations for their avoidance, such as harvesting border rows and front sides separately [3], have been reported. However, due to the small numbers of seeds and limited resources available in early selection cycles, plots sharing one to three rows are common and yield estimates are, therefore, biased [2,4]. This is of lesser importance in early selection cycles that focus on the overall performance of varieties, but it should be avoided in later cycles due to competitive effects of neighbouring plants. Still [12] mentioned that the results of small plots are not representative and that there is a need for multi-row plots to simulate field conditions. This agrees with the findings of our study. At all three growth stages, both wheat and barley showed relatively higher fresh and dry weights, as well as greater nitrogen uptake, in single and multiple-row plots than in a plot comprising 10 rows. However, these results cannot be generalized since only single varieties of each species were tested. Further research of the performance of multiple cultivars in small plots needs to be done.

Several authors [6,17,22] have demonstrated that spectral proximal sensing is a suitable tool for breeders and plant scientists to evaluate plant parameters in a non-destructive and high-throughput manner. Studies performed with wide plots of 10 rows demonstrated comparable or, frequently, superior performance of passive sensors compared to active sensors, including the one tested in this study, for wheat [8], maize [10], and barley [16]. These sensors were also tested in different environments by considering the effects of temperatures, light intensity, and surface conditions [11,23]. However, no previous study had tested the performance of spectral proximal sensors in different plot designs. The results showed decreasing spectral reflectance in the one- and two-row plots, indicating an interfering signal received by the sensor. This was most likely due to the higher fraction of soil in the sensor's FOV. Chemical analysis of the harvested plant material and visual scoring of the plots indicated that neither nutrient deficiencies nor plant diseases occurred in the different plots, and weeds and other objects were manually removed before each measurement. Therefore, it can be concluded that spectral information from bare soil interfered with the spectral sensing of plants, particularly at early growth stages. In later growth stages, distances between sensors and soil increase resulting in a reduced influence of the soil. The GreenSeeker, in particular, with its extended FOV of 1.5 $\times$ 61 cm (Figure 3), may be more susceptible to spectral information from the soil in the one- or two-row plots, in which the planted rows were 15 and 35 cm apart, respectively, especially at early stages of growth.

The one-row design covered approximately 25% of the measurement field of the GreenSeeker, whereas this value was approximately 34% for the passive spectrometer. For the two-row design, these values were approximately 57% for the GreenSeeker and 80% for the passive spectrometer. In addition, the light intensity decreases on the periphery of the GreenSeeker, which leads to lower reflection values. Kim et al. [23] showed that the best performance was obtained in central positions within 30 cm of the light strip. Previous research has indicated that the intensity of LED light emitted decreases with increasing distance [10,11]. As a result, the crop stand is not entirely perceived. This is in contrast to passive sensing, which uses the sun as a light source, the intensity of which does not appreciably decrease within the crop stand. However, this may increase the likelihood that passive sensing will detect information from the soil surface in less dense crop stands.

The results from this study also show that fresh and dry weights do not increase linearly in plots with different numbers of rows, with the largest values observed in the one-row design. It is likely that optimized light conditions, together with improved nutrient and water supply, enhanced growth in border rows or in designs with fewer rows. Since only one cultivar of each species was investigated, different performances of other cultivars cannot be excluded.

A comparison of the performance of the sensors and evaluation of the best performing indices revealed that the best results were obtained from the passive sensor with the indices R774/R656 and R800/R770. In agreement with previous results [8], saturation effects became apparent for the index NDVI independent of the sensor. The passive hyperspectral sensor generally outperformed the active sensor, with superior performance of the active sensor found only for wheat at anthesis. Active sensors have the advantage of being independent of light conditions, enabling their use at night, though the bi-directional passive sensor used in this study does allow compensation for changes in light conditions in the day.

Overall, the results show that spectral sensing can be carried out quite successfully in plot designs with few rows; however, some further optimization is still needed, particularly for single rows. The sensors' FOV did not optimally match such a design, offering one avenue for improvement. For example, the GreenSeeker could be aligned along single rows, while the passive sensor could be positioned closer to the plants, thus covering a higher fraction of the plants' area because the measurements are not distance dependent. Still, superior performance of the passive sensor has been demonstrated for plot designs with two, three, or four rows.

Taken together, these results suggest that enhanced high-throughput spectral sensing can be used in plot designs with few rows, thereby allowing the evaluation of the performance of varieties or cultivars in early selection cycles. Since early selection cycles, in particular, evaluate many hundreds or thousands of varieties, a highly interesting potential for enhanced breeding is indicated. However, neighbouring effects due to different varieties' being in close contact with each other should be considered or avoided. Follow-up work should address the feasibility to extend these findings to an extended set of cultivars or varieties representing different species.

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