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High-throughput phenotyping early plant vigour of winter wheat

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ABSTRACT

In contrast to high-throughput genotyping which can manage a large number of plants at relatively low cost, phenotyping of many individual genotypes in field trials is still laborious and expensive. Early plant vigour, as an early selection criterion, is a trait that is visually scored due to a lack of suitable phenotyping methods for an accurate detection of this trait in large field trials. A high-throughput phenotyping technique for scoring early plant vigour would enhance the breeding process. This study was conducted to develop a method for scoring phenotypic differences in early plant vigour of 50 winter wheat (*Triticum aestivum* L.) cultivars in a 2-years experiment using a vehicle based multispectral active sensor and two commercially available active sensors, GreenSeeker and CropCircle. Pixel analysis of RGB images revealed to be the most feasible and superior method compared to other possible reference methods. A comparison between the two years 2011 and 2012 confirmed that early plant vigour was affected by genotypic differences. A novel spectral plant vigour index (EPVI) was found to accurately reflect the plant vigour at tillering. Different methods were applied to identify optimal combinations of wavelengths to predict early plant vigour, including multivariate modelling and prediction, contour maps for identifying all possible simple ratios and testing of combined indices. The EPVI and the relative amount of green pixels (RAGP) derived from digital images were significantly related with $r^2 = 0.98$ to each other in both years. A total of 200 plots, 12 m in length, could be measured within 75 min. The EPVI was shown to be an accurate scoring method for the high-throughput screening of large field trials. The rapidity and accuracy of this novel method may contribute to enhanced selection at early growth stages.

1. Introduction

Yield formation of wheat starts in the early growth period when the seedlings' vigour and several environmental factors influence the germination rate and early plant vigour. Seedling vigour is affected by various genetic traits (Richards and Lukacs, 2002; Ellis et al., 2004; Rebetzke et al., 2007) and reflects the plant vigour at early growth stages. Thus, early plant vigour has to be seen as an assessment criterion that substantially affects the final yield as a result of different tillering intensities (Valerio et al., 2009).

Although many laboratory tests for assessing early plant vigour determining factors, such as seedling emergence or seedling vigour (Steiner et al., 1989; Boligon et al., 2011), exist, they are not suitable for selecting breeding lines under field conditions, where germination and early plant vigour are highly dependent on environmental factors (Steiner et al., 1989), such as temperature (Khah et al., 1986), soil moisture (Murungu, 2011), soil type, and other factors including seed storage conditions and seed age (Khah et al., 1989; Ghassemi-Golezani and Dalil, 2011), seed weight, or infection with pathogens (Rajala et al., 2011). Furthermore, the amount of mineral nitrogen, applied as fertilizer has an important influence on early plant vigour in terms of tillering intensity.

However, agronomists and plant breeders rely on the evaluation of early plant vigour in the field where this trait is affected by environmental conditions. The amount of field trials and plots for breeding and research purposes is increasing (White et al., 2012), since modern breeding methods on genotype level (e.g. next-generation sequencing) accelerate the breeding process and more promising genotypes have to be tested in field trials. Thus, high-throughput selection tools for phenotyping such traits are needed. Scoring early plant vigour visually and with several persons in the field is laborious, biased and expensive. Even experienced persons determine early plant vigour subjectively. However, visual scoring of early plant vigour is still conventionally applied because of a lack of alternative methods. Counting the tiller number per plant in each plot would represent a possibly accurate method to detect early plant vigour but relating it to the extent of field trials in breeding programs it has to be seen critical in terms of laboriousness (Scotford and Miller, 2004), accuracy and representativeness (Taylor et al., 2000). A suitable method for detecting early plant vigour or crop coverage is the analysis for the proportion of green pixels in digital images.

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Spectral remote sensing is a widely used tool for agricultural production and crop management with respect to site-specific fertiliser applications (Barker and Sawyer, 2010; Dellinger et al., 2008). Its potential as a vehicle-based high-throughput phenotyping technology under field conditions has recently been demonstrated (Schmidhalter et al., 2001; Thoren and Schmidhalter, 2009; Winterhalter et al., 2011, Erdle et al., 2013). Spectral sensors for this purpose work either actively or passively (Erdle et al., 2011). Whereas active sensors are equipped with their own light sources, passive sensors are dependent on sunlight as the light source. Accordingly, active sensors are not influenced by ambient light conditions (Kipp et al., 2012) and can be used during the day and at night. The operating mode of both sensor systems is equivalent as they are equipped with photodetectors that capture reflected light of specific wavebands in the visible (VIS) and near infrared (NIR) regions of the spectrum. Visible light reflected by plant canopies is strongly affected by the amount of green biomass, which means that chlorophyll influences sensor readings in the visible spectrum. Because nitrogen is involved in chlorophyll formation, spectral indices can be indirectly used to quantify nitrogen supplies in crops and establish cost-saving site-specific application systems for fertilisers (Hatfield et al., 2008; Scharf et al., 2011). A number of optimised indices exist for quantifying parameters such as the biomass (Erdle et al., 2011), N uptake (Mistele and Schmidhalter, 2008), N concentration (Li et al., 2010a,b), water status (Elsayed et al., 2011; Winterhalter et al., 2011) or grain yield (Schmidhalter et al., 2003; Teal et al., 2006; Babar et al., 2006) of common crops. Additionally spectral remote sensing as phenotyping technology has potential to be used as high-throughput phenotyping technique for scoring early plant vigour, if an adequate reference method, such as green pixel analysis on digital images, can be established. Phenotypic differences in early plant vigour or crop coverage could be shown in experiments with increasing nitrogen levels or sowing densities (Lukina et al., 1999; Stevens

Table 1
Results of the relative amount of green pixels derived from digital images and EPVI values for 2011 and 2012, with each value representing the average of four replications. Cultivar rankings of RAGP and EPVI for 2011 and 2012 derived by the Student–Newman–Keuls test are indicated at $p \leq 0.05$.

Cultivar	2011		2012	
	Relative amount of green pixels	EPVI	Relative amount of green pixels	EPVI
W00984.2	64.2^{a,b,c,d,e,f,g,h}	0.63^{a,b,c,d,e,f,g}	26.6^{a}	0.41^{a,b,c,d,e}
Alcazar	60.9^{a,b,c,d,e,f,g,h}	0.59^{a,b,c,d,e,f}	18.3^{a}	0.33^{a,b,c}
Magnifik	65.1^{a,b,c,d,e,f,g,h}	0.70^{c,d,e,f,g}	24.7^{a}	0.39^{a,b,c,d,e}
Figura	62.3^{a,b,c,d,e,f,g,h}	0.61^{a,b,c,d,e,f,g}	26.2^{a}	0.39^{a,b,c,d,e}
Oakley	61.1^{a,b,c,d,e,f,g,h}	0.65^{a,b,c,d,e,f,g}	21.0^{a}	0.35^{a,b,c,d,e}
Timber	53.6^{a,b,c,d}	0.57^{a,b,c}	18.1^{a}	0.33^{a}
Liman R	53.4^{a,b,c}	0.58^{a,b,c,d,e}	19.6^{a}	0.33^{a,b,c}
Arina	63.3^{a,b,c,d,e,f,g,h}	0.60^{a,b,c,d,e,f}	21.7^{a}	0.36^{a,b,c,d,e}
CH-111.13716	59.3^{a,b,c,d,e,f,g}	0.63^{a,b,c,d,e,f,g}	24.8^{a}	0.39^{a,b,c,d,e}
CH-111.13930	58.4^{a,b,c,d,e,f}	0.59^{a,b,c,d,e,f}	18.8^{a}	0.31^{a,b,c}
CH-111.13521	64.9^{a,b,c,d,e,f,g,h}	0.63^{a,b,c,d,e,f,g}	29.4^{a}	0.40^{a,b,c,d,e}
Mirage	57.5^{a,b,c,d,e,f}	0.61^{a,b,c,d,e,f,g}	22.8^{a}	0.35^{a,b,c,d,e}
Piotta	60.3^{a,b,c,d,e,f,g,h}	0.65^{a,b,c,d,e,f,g}	25.4^{a}	0.36^{a,b,c,d,e}
Alchemy	63.7^{a,b,c,d,e,f,g,h}	0.67^{b,c,d,e,f,g}	21.7^{a}	0.37^{a,b,c,d,e}
CH-194.10518	63.2^{a,b,c,d,e,f,g,h}	0.60^{a,b,c,d,e,f}	23.0^{a}	0.36^{a,b,c,d,e}
Cubus	72.8^{f,g,h}	0.72^{c,d,e,f,g}	32.0^{a}	0.46^{d,e}
Türkis	71.0^{d,e,f,g,h}	0.71^{c,d,e,f,g}	30.0^{a}	0.40^{a,b,c,d,e}
Akteur	62.4^{a,b,c,d,e,f,g,h}	0.61^{a,b,c,d,e,f,g}	27.9^{a}	0.43^{a,b,c,d,e}
Hermann	68.9^{b,c,d,e,f,g,h}	0.68^{b,c,d,e,f,g}	27.7^{a}	0.41^{a,b,c,d,e}
Impression	58.2^{a,b,c,d,e,f}	0.63^{a,b,c,d,e,f,g}	28.9^{a}	0.42^{a,b,c,d,e}
Schamane	71.6^{e,f,g,h}	0.68^{b,c,d,e,f,g}	31.0^{a}	0.41^{c,d,e}
Manager	62.1^{a,b,c,d,e,f,g,h}	0.69^{b,c,d,e,f,g}	24.1^{a}	0.37^{a,b,c,d,e}
Potenzial	67.3^{a,b,c,d,e,f,g,h}	0.68^{b,c,d,e,f,g}	26.0^{a}	0.40^{a,b,c,d,e}
Julius	66.2^{a,b,c,d,e,f,g,h}	0.64^{a,b,c,d,e,f,g}	29.3^{a}	0.39^{a,b,c,d,e}
Pamier	51.8^{a,b}	0.52^{a}	22.4^{a}	0.34^{a,b,c,d}
JB Asano	72.9^{f,g,h}	0.74^{e,f,g}	29.0^{a}	0.44^{b,c,d,e}
Kredo	56.1^{a,b,c,d,e,f}	0.58^{a,b,c,d}	20.9^{a}	0.32^{a,b}
Famulus	64.1^{a,b,c,d,e,f,g,h}	0.67^{b,c,d,e,f,g}	22.9^{a}	0.36^{a,b,c,d,e}
Genius	58.8^{a,b,c,d,e,f,g}	0.59^{a,b,c,d,e,f}	23.3^{a}	0.35^{a,b,c,d,e}
Linus	51.6^{a}	0.55^{a,b}	22.9^{a}	0.33^{a,b,c}
Meister	54.8^{a,b,c,d,e}	0.58^{a,b,c,d}	27.2^{a}	0.40^{a,b,c,d,e}
Orcas	54.8^{a,b,c,d,e}	0.58^{a,b,c,d,e,f}	23.2^{a}	0.34^{a,b,c,d,e}
Muskat	73.5^{f,g,h}	0.71^{c,d,e,f,g}	30.9^{a}	0.39^{a,b,c,d,e}
Kerubino	75.9^{g,h}	0.74^{f,g}	39.2^{a}	0.48^{e}
KWS Erasmus	63.8^{a,b,c,d,e,f,g,h}	0.63^{a,b,c,d,e,f,g}	29.4^{a}	0.41^{a,b,c,d,e}
Matrix	70.3^{c,d,e,f,g,h}	0.73^{d,e,f,g}	34.3^{a}	0.42^{a,b,c,d,e}
Florian	60.9^{a,b,c,d,e,f,g,h}	0.64^{a,b,c,d,e,f,g}	27.8^{a}	0.38^{a,b,c,d,e}
Sailor	60.8^{a,b,c,d,e,f,g,h}	0.64^{a,b,c,d,e,f,g}	30.9^{a}	0.42^{a,b,c,d,e}
Norin	58.3^{a,b,c,d,e,f}	0.64^{a,b,c,d,e,f,g}	27.1^{a}	0.37^{a,b,c,d,e}
Komet	72.9^{f,g,h}	0.72^{c,d,e,f,g}	34.0^{a}	0.45^{a,b,c,d,e}
Colonia	76.9^{h}	0.75^{g}	37.7^{a}	0.45^{b,c,d,e}
Tabasco	70.2^{c,d,e,f,g,h}	0.71^{c,d,e,f,g}	33.1^{a}	0.45^{c,d,e}
Sophytra	63.9^{a,b,c,d,e,f,g,h}	0.66^{a,b,c,d,e,f,g}	26.8^{a}	0.38^{a,b,c,d,e}
Akratos	64.8^{a,b,c,d,e,f,g,h}	0.64^{a,b,c,d,e,f,g}	28.8^{a}	0.39^{a,b,c,d,e}
Sokrates	60.6^{a,b,c,d,e,f,g,h}	0.63^{a,b,c,d,e,f,g}	28.5^{a}	0.40^{a,b,c,d,e}
Winnetou	66.2^{a,b,c,d,e,f,g,h}	0.72^{c,d,e,f,g}	32.5^{a}	0.44^{c,d,e}
KWS Bogus	62.2^{a,b,c,d,e,f,g,h}	0.67^{b,c,d,e,f,g}	32.9^{a}	0.44^{b,c,d,e}
Wilson	70.4^{c,d,e,f,g,h}	0.73^{d,e,f,g}	29.4^{a}	0.42^{a,b,c,d,e}
Glaucus	69.1^{c,d,e,f,g,h}	0.71^{c,d,e,f,g}	30.4^{a}	0.44^{b,c,d,e}
SH 401	68.7^{a,b,c,d,e,f,g,h}	0.68^{b,c,d,e,f,g}	26.9^{a}	0.39^{a,b,c,d,e}
Mean	63.7	0.65	27.0	0.39