Field based remote sensing models predict radiation use efficiency in wheat.

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Abstract

Wheat yields are stagnating or declining in many regions, requiring efforts to improve the light conversion efficiency, i.e., radiation use efficiency (RUE). RUE is a key trait in plant physiology because it links light capture and primary metabolism with biomass accumulation and yield, but its measurement is time consuming and this has limited its use in fundamental research and large scale physiological breeding. In this study, high-throughput phenotyping (HTPP) approaches were used among a population of field grown wheat with variation in RUE and photosynthetic traits to build predictive models of RUE, biomass and intercepted photosynthetically active radiation (IPAR). Three approaches were used: best combination of sensors, canopy vegetation indices and partial least square regression. The use of remote sensing models predicted RUE with up to 70% accuracy compared to ground truth data. Water indices and NDVI are the better option to predict RUE, biomass and IPAR, and indices related to NPQ (PRI) and senescence (SIP) are better predictors for these traits at the vegetative and grain filling stages respectively. These models will be instrumental to explain canopy processes, improve crop growth, yield modelling, and potentially be used to predict RUE in different crops or ecosystems.

Highlight

Radiation use efficiency can be predicted with ~70% accuracy. Canopy water content, greenness and gas exchange spectral indices are the best predictors for RUE, biomass accumulation and light interception.

Keywords: High-throughput phenotyping, hyperspectral reflectance, partial least square regression, physiological breeding, RUE, vegetation indices, wheat
**Introduction**

Staple crop yields must increase by at least at a rate of 2.4% per year to ensure food security for a growing population, dietary changes and expanding use of biofuels (Foley *et al.*, 2011; Ray *et al.*, 2013). Recent studies suggest that yield gains for staple crops are on average 1.2%-1.3% year\(^{-1}\) therefore it will not be sufficient to meet 2050 food demands (Ray *et al.*, 2012). Moreover climate change predictions of future environmental conditions suggests crops will be subjected to higher temperatures, flooding, drought and shifts in precipitation patterns which will affect development, primary metabolic events, biomass accumulation and yield (Porter *et al.*, 2014; Asseng *et al.*, 2015; Garatuza-Payan *et al.*, 2018). The socio-economic repercussions will be felt worldwide but mostly in low-income countries (Rajaram *et al.*, 1993) and the challenge of raising staple crop yields is one of the main goals for the scientific community in this century (Bailey-Serres *et al.*, 2019).

Wheat (*Triticum aestivum* L.) is one of the most important staple crops and its annual production is estimated to be ~770 million tonnes year\(^{-1}\) (FAOSTAT, 2020). Physiological approaches for wheat improvement have had a pivotal role in reducing the gap between field and theoretical yields. So far, the main physiological traits improved in wheat have been reduction in plant height to minimize lodging, the partitioning of biomass into the grain and optimisation of leaf area index (LAI) (Foulkes *et al.*, 2011; Parry *et al.*, 2011; Reynolds *et al.*, 2012). It has been proposed that to further increase yield it will be necessary to improve photosynthesis and the conversion rate of photosynthetically active radiation (PAR) to biomass by the canopy (Long *et al.*, 2006; Murchie *et al.*, 2009, 2018; Zhu *et al.*, 2010). This conversion rate is known as radiation use efficiency (RUE) and is defined as the biomass (dry weight) generated per unit absorbed radiation, (g MJ\(^{-1}\)) (Monteith, 1977).

Under yield potential conditions, yield has been defined as a function of incident PAR, the fraction of intercepted radiation during the crop cycle (FPAR, \(\varepsilon\)), RUE and the relation between grain dry weight and aboveground dry biomass (HI) (Reynolds *et al.*, 2005). This is expressed in eq. (1):

\[ Yield = \sum_{i=1}^{n} PAR_i \times FPAR_i \times RUE_i \times HI \]

Where \(n\) is the day when a genotype reaches physiological maturity, \(PAR\) is the incident radiation in the \(i^{th}\) day, \(FPAR\) the fraction of incident radiation absorbed of the \(i^{th}\) day, \(RUE\), the radiation use efficiency of the crop cycle and \(HI\) the harvest index.

Theoretically yield could be improved by increasing any of the elements from equation 1 but since traits related to harvest index and light interception are close to optimization, the focus to increase yield should be shifted on improving RUE (Amthor, 2010; Zhu *et al.*, 2010; Parry *et al.*, 2011). Due to the complexity of RUE being the product of many underlying processes that are sensitive to the environment and the fact that measuring it is labour and cost intensive, its potential for increasing
yield it is not currently exploited in wheat breeding programs. Therefore, it is necessary to develop high throughput methods to measure and predict RUE for field research and breeding purposes.

The importance of RUE in plant physiology resides in the association of RUE and yield, as RUE alone can explain ~40% of its variability and it can help us to elucidate the roles of light capture and key plant processes of leaf biochemistry that drives biomass and yield (Hubbart et al., 2018; Molero et al., 2019). Evidence from FACE experiments suggest that there is room for RUE improvement that could be driven by leaf photosynthesis (Ainsworth and Long, 2005, 2020) and it has been suggested that even small increases in these two traits will have a major impact in wheat yield if HI can be maintained to modern levels (Parry et al., 2011). In contrast with the negative correlation existing between aboveground biomass measured at different growth stages and HI (Aisawi et al., 2015; Molero et al., 2019; Sierra-Gonzalez et al., 2021), no negative correlations were observed between HI and RUE measured in the vegetative stages and across the whole crop cycle (Molero et al., 2019). Hence, increasing RUE is a promising strategy to achieve further genetic gains in yield.

In order to measure RUE in a crop canopy it is necessary to harvest aboveground biomass for at least two points in time, which is time consuming and can compromise the accuracy of yield measurements in the remaining plot area. Especially if several harvests throughout the crop cycle are needed this becomes a big issue for breeding programs. However, this may be solved using non-invasive phenotyping techniques.

High-throughput phenotyping (HTPP) refers to the use of novel non-invasive techniques to measure physiological and agronomical traits (e.g., plant growth, biomass accumulation, gas exchange, canopy architecture, organ stoichiometry, grain yield) combining multidisciplinary knowledge that allows plant phenotyping at different spatio-temporal (seconds to years) and hierarchical scales (cells to canopies) (Furbank and Tester, 2011; Fiorani and Schurr, 2013; Tardieu et al., 2017; Araus et al., 2018; Reynolds et al., 2020).

Optical remote sensing techniques are among the most widely used for HTPP. This data usually ranges from 350-2500 nm encompassing areas of the visible (400-700 nm), near infrared (NIR, 700-1350 nm), red edge (680-730 nm) and shortwave infrared (1350-2500 nm) spectrum (Gamon et al., 2019). Hyperspectral data have been used mainly in two ways: spectral indices (also known as vegetation indices, VI) calculated from relations between reflectance at specific wavelengths and physiological traits (Penuelas et al., 1997; Blackburn, 1998; Cabrera-Bosquet et al., 2011; Tattaris et al., 2016) and by using the whole spectra as an individual data point to predict traits of interest (e.g. leaf N and C content, CO₂ assimilation, respiration, maximum velocity of Rubisco carboxylation, electron transport rate, leaf mass and specific leaf areas) using statistical methods such as partial least square regression (PLSR) (Serbin et al., 2014; Yendrek et al., 2017; Silva-Perez et al., 2017; Coast et al., 2019; Fu et al., 2020). The advantages of these two approaches is that hundreds or thousands of
lines can be screened without the need of destructive and time consuming field sampling. Moreover, as HTPP technologies become cheaper, crop physiologists and breeders will be able to study complex traits more cost effectively (Reynolds et al., 2020).

Previous studies have predicted yield in wheat and rye (Montesinos-López et al., 2017; Galán et al., 2020), aboveground biomass in wheat, rice, rye and barley (Babar et al., 2006; Gnyp et al., 2014; Marshall and Thenkabail, 2015; Galán et al., 2020) and RUE in maize (Tewes and Schellberg, 2018) using optical remote sensing approaches, but to date there is not such an effort to predict RUE in the field using a HTPP physiological breeding approach for wheat. The impact of predicting a multicomponent trait like RUE with a HTPP approach in field conditions would be very high for physiological breeding programs while its full implementation would be very feasible in medium term (Furbank et al., 2019; Roitsch et al., 2019).

Our hypothesis is that prediction models using canopy reflectance data will be more accurate than models using a different combination of sensors (which include leaf reflectance), due to a better representation of canopy processes. The objectives of this study are the prediction of RUE, biomass and intercepted PAR (IPAR) with HTPP techniques based on VI and PLSR models to define which approach will help more to alleviate the phenotyping bottleneck of these traits.

**Materials and methods**

**Wheat population**

Spring bread wheat cultivars were chosen from the ‘Photosynthesis Respiration Tails’ (PS Tails) trial which consisted of 80 genotypes including advanced line material coming from the High Biomass Association Panel (HiBAP) from CIMMYT. This germplasm is characterized by their high aboveground biomass and for containing lines with contrasting RUE expression that varies from the vegetative to the grain filling phase, and has breeding value as it represents material that breeders use for their crosses for yield potential (for further information of HiBAP see (Molero et al., 2019)). For this study a subset of 11 genotypes (Table 1) was selected based on RUE at the vegetative and grain filling stages, yield, HI, flag leaf photosynthesis and plant height to consider different levels of productivity and contrasting canopy architecture. This selection was made using data available from the 2016/2017 field season at CIMMYT’s experimental station (Molero, unpublished data).

The genotypes studied during the 2017/2018 field season were eight, and the complete set of 11 lines were studied during the 2018/2019 and 2019/2020 field seasons (from now on referred to as Y1, Y2 and Y3, respectively). Experiments were carried out at CIMMYT’s Campo Experimental Norman E. Borlaug (CENEB) field station in Ciudad Obregon, Sonora, Mexico (27° 23’ 46” N, 109° 55’ 42” W, 38 mamsl) during the spring wheat growth season that encompasses early December-early May.
Field conditions

Experimental design was a randomised complete block design with three replications in raised beds, two beds per plot (bed width = 0.8 m) and two rows per bed (row width = 0.2 m) in 4 m x 1.6 m plots in Y1 (plot area = 6.4 m$^2$). For Y2, the same experimental design was used but the number of replications was increased to four, and plot length increased to 5 m x 1.6 m, increasing the area (plot area = 8 m$^2$). In Y3, irrigation system was changed to optimize the water use and reduce lodging in the experimental station and a drip irrigation system was put in place. Randomised complete block design was used with the same replications and plot area as Y2 but the plants were sown as six row plots with 15 cm between rows on the flat with drip irrigation.

Sowing dates were December 5th 2017, December 6th 2018 and December 18th 2019 for Y1, Y2 and Y3 respectively. Emergence dates were December 12th 2017, December 12th 2018 and December 26th 2019 (Y1, Y2 and Y3 respectively). Harvest dates were May 8th 2018, April 30th 2019 and May 13th 2020 (Y1, Y2 and Y3 respectively). Seed rate was ~250 g m$^{-2}$ in the three years. Irrigation was applied four times during the crop cycle in approximate 25-day intervals (pre sowing, 25, 50, 75, 100 days after emergence). Plants were grown under optimal conditions in the field with pests, weed control and fertilisation to avoid limitations to yield. In Y1 fertilization was applied in the form of urea (200 kg N ha$^{-1}$) 25 days after emergence (DAE). For Y2 fertilization was divided in 100 kg N ha$^{-1}$ 25 DAE and another 100 kg N ha$^{-1}$ 58 DAE. Finally for Y3, 100 kg N ha$^{-1}$ were applied 30 DAE and 50 kg N ha$^{-1}$ 50 DAE; 50 kg P ha$^{-1}$ were applied in the three cycles when the first application of N was made.

Phenology was scored according to the Zadoks growth scale for cereals (Zadoks et al., 1974). The growth stages recorded were initiation of booting (GS41), anthesis (GS65) and physiological maturity (GS87) when 50% of the shoots in the plot reached each stage. Meteorological data from a nearby station to the experimental site was collected for the whole crop cycle, thermal time and accumulated PAR were calculated for the growth stages where biomass was collected (Table 1).

In order to clarify the acronyms used in this study the reader is referred to Table 4, where all phenological and ground truth traits abbreviations are explained in detail.

Ground truth traits

Light interception

The percentage of light intercepted (LI) was measured using a linear ceptometer (AccuPAR LP-80, Decagon Devices, Pullman, WA, USA) at 40 days after emergence (canopy closure), GS41 and GS65 + 7 days. Incident, reflected and transmitted PAR through the canopy were measured around 11 am-1
pm when clear skies and low wind velocity conditions prevailed following phenotyping protocols (Pask et al., 2013). The following equation was used to calculate the percentage of LI by the canopy:

\[
LI (\%) = \frac{PAR_i - PAR_r - PAR_g}{PAR_i - PAR_T} \times 100
\]

eq. (2)

where LI (\%) is the percentage of light intercepted by the canopy, PAR\(_i\), PAR\(_r\) and PAR\(_g\) are the incident, reflected and transmitted PAR respectively.

LI (\%) was used to estimate the amount of intercepted PAR (IPAR) by the canopy in the same growth stages where aboveground biomass was harvested.

**Aboveground biomass**

Aboveground biomass was harvested at four key developmental growth stages: canopy closure (40 days after emergence, 40DAE), initiation of booting (GS41), initiation of the grain filling period (GS65 + 7 days) and physiological maturity (GS87).

At 40DAE biomass was harvested in 0.4 m\(^2\) (25 cm for each bed in the plot) and at GS41 and GS65 + 7d biomass was harvested in 0.8 m\(^2\) (50 cm for each bed in the plot). Biomass harvests were made leaving 25 cm (40 DAE) and 50 cm (GS41, GS65 + 7 days) at the northern side of the plots to reduce border effects in subsequent harvests. All fresh biomass was weighed and a subsample of 50 shoots was weighted and dried in an oven at 70 °C for 48 h and dry weight was recorded. At GS87, biomass was calculated from the measurement of yield components. For every growth stage the aboveground biomass was calculated as follows:

\[
\text{Aboveground biomass} = \text{Subsample dry weight} \times \frac{\text{Total fresh weight x Harvested Area}}{\text{Subsample fresh weight}}
\]

eq. (3)

**Radiation use efficiency**

RUE was estimated from the slope of the linear regression between aboveground biomass and the corresponding accumulated IPAR during the determined growth period (Monteith, 1977). Incoming radiation from a nearby meteorological station was used to calculate the accumulated PAR by multiplying irradiance x 0.45 to convert it to PAR.

RUE observations in this study are presented for five different growth periods: canopy closure to GS41 (RUE_E40InB), GS41 to GS65 + 7d (RUE_InBA7), pre-grain filling stage (40 DAE to GS65 + 7d, RUE_preGF), grain filling stage (GS65 + 7d to GS87, RUE_GF) and RUE of the crop cycle which comprises the period from canopy closure to physiological maturity (40 DAE to GS87,
RUE_Total). For RUE_GF and RUE_Total a correction factor was used to account for intercepted radiation during the last 25% (in days) of the grain filling period when canopy leaves start to senesce based on a light interception model (Reynolds et al., 2000). Calculations were made as follows:

\[
RUE_{E40lnB} = \frac{(BM\; GS41 - BM\; E40)}{(Acc\; IPAR\; GS41 - Acc\; IPAR\; E40)} \quad \text{eq. (4)}
\]

\[
RUE_{InBA7} = \frac{(BM\; GS65 + 7d - BM\; GS41)}{(Acc\; IPAR\; GS65 + 7d - Acc\; IPAR\; GS41)} \quad \text{eq. (5)}
\]

\[
RUE_{preGF} = \frac{(BM\; GS65 + 7d - BM\; E40)}{(Acc\; IPAR\; GS65 + 7d - Acc\; IPAR\; E40)} \quad \text{eq. (6)}
\]

\[
RUE_{GF} = \frac{(BM\; GS87 - BM\; GS65 + 7d)}{[(M\; acc, 75\%\; grain\; filling) - [(Acc\; IPAR\; GS65 + 7d) + (M\; acc, 25\%\; grain\; filling + 0.5)]]} \quad \text{eq. (7)}
\]

\[
RUE_{Total} = \frac{(BM\; GS87 - BM\; E40)}{Acc\; IPAR\; GS87 + [(M\; acc, 25\%\; grain\; filling + 0.5) - (Acc\; IPAR\; E40)]} \quad \text{eq. (8)}
\]

Remote sensing measurements

Remote sensing data was collected above the canopy using a field spectroradiometer coupled with a pistol grip and throughout the layers of the canopy (flag, second and third leaves) with a field spectroradiometer coupled with a leaf clip. Chlorophyll content (SPAD) was measured in the flag, second and third leaves with a SPAD-502 meter (Konika Minolta, Japan), canopy temperature (CT) was measured using an infrared thermometer (LT 300, Sixth Sense, USA) and Normalized Difference Vegetation Index (NDVI) was measured using a Green Seeker (Trimble, USA) from canopy closure to late grain filling at least once a week described by (Pask et al., 2013).

Hyperspectral reflectance was measured using a field spectroradiometer with a spectral range from 350-2500 nm with a 3 nm spectral resolution in the visible-near infrared (VNIR) and 10 nm resolution in the shortwave infrared (SWIR) spectrum equipped with an optic fibre with a field of view of 25° (ASD Field Spec ® 3, Boulder, CO, USA). Reflectance was measured at 0.5 m at the nadir of the canopy with a pistol grip (ASD Field Spec ® 3, Boulder, CO, USA) under clear sky conditions and when low wind speeds were predominant to make sure we were collecting the signal from the canopy instead of soil or vegetation/soil mixed signals. Six data points were collected at each plot and then averaged to obtain the reflectance of each plot.

Leaf reflectance was measured using a leaf clip equipped with a halogen bulb as light source (ASD Field Spec ® 3, Boulder, CO, USA). Healthy leaves were clipped in the middle portion and measurements were taken for flag, second and third leaves in one fertile shoot per plot. The first measurement was taken at GS41 and the last at GS75. Both canopy and leaf reflectance data were averaged to get representative values from the vegetative period (40 DAE to GS55) and the grain filling period (GS65 to GS75). Reflectance measurements were made between 10 am-2 pm where the Sun is close to its zenith at this latitude.
For electron transport rate ($J_{\text{max}}$) and maximum velocity of Rubisco carboxylation/N content based on leaf area (Vcmax/Narea) leaf spectral data was used to predict them based on PLSR modelling using Wheat Physiology Predictor, a web tool developed to predict photosynthetic traits derived from light response and ACi photosynthetic curves (www.metabolome-express.org/pheno/) (Silva-Pérez et al., 2017).

Data analysis

Adjusted means from each year were calculated for the ground truth and remote sensing traits as well as the predictions from PLSR using the linear model from package lme4 (R Studio) with the guide user interface META-R v 6.04 (Alvarado et al., 2020) as follows:

$$Y_{ijk} = \mu + \text{Rep}_i + \varepsilon_{ijk}$$  \hspace{1cm} \text{eq. (9)}

Where $Y_{ijk}$ is the ground truth or remote sensing trait, $\mu$ is the mean effect, Rep$i$ is the effect of the $i$th replicate, and $\varepsilon_{ijk}$ is the error associated with the $i$th replication.

If statistically significant differences were not found between genotypes, vegetation indices were adjusted with phenology from GS41 for vegetative period averages and phenology from GS65 for grain filling period averages as covariates. Phenotypic correlations between RUE and remote sensing traits (SPAD, CT, NDVI Green Seeker and vegetation indices) were calculated using Pearson product-moment correlations and a threshold was established to select only VI with statistically significant phenotypic correlations ($p<0.05$).

Vegetation Indices

After field sampling, average reflectance collected above the canopy and the leaves from each plot was processed using View Spec Pro software (Analytical Spectral Devices Inc., Boulder, CO, USA). These values were later used to calculate different VI available from the literature (Li et al., 2010; Garbulsky et al., 2011; Ollinger, 2011; Pask et al., 2013) and Index Database (https://www.indexdatabase.de/) using R Studio (R Core Team, 2016). In table 2 are shown the VI which correlated significantly with RUE, biomass and IPAR, and that were used for building the predictive models.

Partial Least Square Regression

Averaged reflectance spectral data of each plot collected above the canopy was post-processed to remove spurious data in areas of the spectra where negative or higher than 1 value were present. Spectral reflectance from 350-1800 nm and 1951-2450 nm were then used to predict RUE, biomass
and IPAR using the Principal Component and Partial Least Squares Regression package (pls) in R (Mevik and Wehrens, 2007) following the method proposed in (Serbin et al., 2014).

While building the models 80% of the dataset was used as training data and 20% was used as test data to validate the PLSR models. The number of components used in the models was based on the smallest root mean square error in the cross-validation stage (RMSEP-CV) and smallest prediction of the residual sum of squares (PRESS) from the training dataset. After these steps, PLSR modelling generates loadings and scores which are used to generate regression coefficients and intercepts for each individual wavelength and thus the model can be build multiplying those values against each wavelength reflectance value. The regression coefficient ($R^2$), the model bias and the relative error of prediction were considered to evaluate and compare the models.

**Linear models**

To build the linear models using the best combination of sensors (bcs) and vegetation indices measured above the canopy (cVI), best subset regression was used with RUE, biomass and IPAR of the different growth stages as dependent variables and the remote sensing traits as independent variables using the software Sigma Plot 13.0 (Systat Software Inc., San Jose, CA, USA). These linear models assume an association between the dependent and independent variables as follows:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + \cdots b_lx_l$$  \hspace{1cm} \text{eq. (10)}

Where $y$ is the dependent variable, $x$ the independent variable and $b$ the regression coefficients.

To compare the predictive ability of the models presented in this study a set of criteria was considered such as the regression coefficient ($R^2$), the variance inflation factor (VIF) to avoid multicollinearity between the variables used to predict RUE or biomass and the root mean square error (RMSE), calculated as follows:

$$RMSE = \sqrt{\frac{\sum(X_i-Y_i)^2}{n}}$$  \hspace{1cm} \text{eq. (11)}

Where $X_i$ are the predicted values, $Y_i$ the observed values and $n$ the total number of observations.
Results

Accumulated IPAR

IPAR_E40_bcs was predicted using a linear combination of CTvg and NDVIGS vg. This model had the best performance of all methods and growth stages for this trait with $R^2 = 0.91$ and RMSE of 4.78 MJ m$^{-2}$. In contrast, IPAR_E40_cVI was best predicted using NDWI-3 from the vegetative period and model performance was $R^2 = 0.75$ and RMSE of 7.72 MJ m$^{-2}$. With PLSR modelling the lowest $R^2 = 0.5$ and highest prediction error RMSE = 14.49 MJ m$^{-2}$ were found for IPAR_E40 (Figure 1).

IPAR_InB_bcs was predicted using NDVIGS vg and PRIvg measured at the canopy level. Model performance was the lowest for this trait with $R^2 = 0.61$ and RMSE = 17.93 MJ m$^{-2}$ (Table 3). IPAR_InB_cVI predictions were worse than IPAR_InB_bcs, but they were made only using the Optimised Soil Adjusted Vegetation Index (OSAVI) from the vegetative period with $R^2 = 0.33$ and RMSE = 23.22 MJ m$^{-2}$. PLSR predictions were poor when the canopy was not fully closed, and we hypothesise that this could be due to mixed reflectance from leaves and soil affecting IPAR predictions with this method (Figure 1).

IPAR_A7_bcs predictions were made using CTgf, PRIvg, GNDVI-1gf, and NDWI-4gf with $R^2 = 0.86$ and RMSE = 17.66 MJ m$^{-2}$. Two of these remote sensing traits are related to canopy water content, transpiration and plant water uptake (canopy temperature and NDWI-4) and one is related to LAI and canopy greenness (GNDVI-1) (Table 3, Figure 1). Predictions with cVI were made using PRI from the vegetative period and rNDVI from the grain filling period, model statistics were $R^2 = 0.66$ and RMSE = 26.31 MJ m$^{-2}$. PLSR predictions were better than cVI with RMSE = 24.99 MJ m$^{-2}$ and $R^2 = 0.7$ (Figure 1).

IPAR_PM_bcs and IPAR_PM_cVI predictions were made using the same remote sensing traits, PRIvg, SAVIvg and SIPI-1gf, $R^2 = 0.8$ and RMSE = 35.33 MJ m$^{-2}$ (Table 3). PLSR predictions at physiological maturity performed the best in comparison to the other growth stages for this method with $R^2 = 0.8$ and RMSE = 32.94 MJ m$^{-2}$. IPAR_PM can be certainly be predicted by either of the three methods proposed here and obtain similar results (Figure 1).

Biomass

The best estimation of BM_E40_bcs resulted from the linear model using $J_{\text{max}}$ measured in the third leaf during the vegetative period (JTLvg) with $R^2 = 0.2$ and RMSE = 24.53 g m$^{-2}$, whereas the linear combination of WI, NDVI and SAVI from the vegetative growth period resulted in the best estimations for and BM_E40_cVI with $R^2 = 0.17$ and RMSE = 25.83 g m$^{-2}$ (Table 3). PLSR predictions at this growth stage performed worse compared to the other methods with $R^2 = 0.02$ and...
The use of leaf reflectance measurements to predict biomass at this growth stage performed better than predictions using canopy reflectance.

**BM_InB_bcs** was predicted using a combination of VIs measured above the canopy (NDWI-4canvg), flag leaf (GIFLvg) and third leaf (TCARITLvg). The model performance was $R^2 = 0.42$ and RMSE = 53.35 g m$^{-2}$ (Table 3, Figure 2). **BM_InB_cVI** predictions were made with NDWI-4 and TCARI measured at the vegetative period ($R^2 = 0.34$, RMSE = 55.8 g m$^{-2}$). PLSR predictions were the worst of the three methods at GS41 having less accuracy and the highest error with $R^2 = 0.19$ and RMSE = 82.12 g m$^{-2}$ (Figure 2).

**BM_A7_bcs** was predicted using PRI measured above the canopy during the vegetative period and in the flag leaf during the grain filling period (PRIcanvg, PRIFLgf). Model performance was $R^2 = 0.32$ and RMSE = 76.92 g m$^{-2}$ (Figure 2). **BM_A7_cVI** was predicted using PRI from the vegetative period (PRIcanvg). Predictions were less accurate ($R^2 = 0.18$, RMSE = 83.18 g m$^{-2}$) compared to bcs but it was noteworthy that for both linear methods PRI was the common index used (Figure 2, Table 3) and PLSR predictions performed worst compared to the other methods ($R^2 = 0$, RMSE = 98.38 g m$^{-2}$) (Figure 2).

**BM_PM_bcs** predictions were the most accurate of the three methods at physiological maturity with $R^2 = 0.67$ and RMSE = 74.39 g m$^{-2}$ (Table 3). **BM_PM_cVI** was predicted using pigment indices, these predictions were the least accurate of this growth stage for any method with $R^2 = 0.28$ and RMSE = 107.96 g m$^{-2}$. PLSR predictions were more accurate than cVI with $R^2 = 0.47$ and RMSE = 100.41 g m$^{-2}$ (Figure 2).

**Radiation use efficiency**

From 40 days after emergence to initiation of booting (RUE_E40InB_bcs) was predicted using water and chlorophyll indices (Table 3), predictions with this method at this growth stage were less accurate in comparison to the other methods $R^2 = 0.29$ and RMSE = 0.46 g MJ$^{-1}$ (Figure 3). **RUE_E40InB_cVI** predictions were the best at this growth stage ($R^2 = 0.53$ and RMSE 0.27 g MJ$^{-1}$). Vegetation indices used for this method were related to chlorophyll (PSSRbvg) and water content (WIVg). PLSR model performed better than bcs at this growth stage ($R^2 = 0.34$, RMSE = 0.31 g MJ$^{-1}$) (Figure 3), but in general in all the traits predicted in this study PLSR modelling produced less accurate results compared to bcs or cVI models (Figure 3, Table 3).

**RUE_InBA7_bcs** was predicted using NDWI-3 measured above the canopy, EVI and TCARI$_{505}$ at the third leaf (NDWI-3canvg, EVITLvg, TCARI$_{505}$TLvg). **RUE_InBA7_cVI** was predicted using NDWI-3 and EVI measured at the vegetative period. Both models performed the same with $R^2 = 0.27$ and
RMSE = 0.37 g MJ\(^{-1}\) (Table 3) and were better compared to PLSR estimations \(R^2 = 0\) and RMSE = 0.55 g MJ\(^{-1}\) (Figure 3).

RUE\(_{\text{preGF\_bcs}}\) was predicted using the chlorophyll content of the third leaf measured with a SPAD meter (Table 3). The model estimations with this method resulted in poor estimations with \(R^2 = 0.21\) and RMSE = 0.21 g MJ\(^{-1}\) (Figure 3) but in return this model is the easiest to build as only uses measurements from a sensor very easy to deploy in the field. RUE\(_{\text{preGF\_cVI}}\) model performance was similar to \(\text{bcs}\) (Table 3) with \(R^2 = 0.19\) and RMSE = 0.22 g MJ\(^{-1}\). All RUE predictions with \(\text{bcs}\) method at the vegetative period (RUE\(_{\text{E40InB}}, \text{RUE\_InBA7}, \text{RUE\_preGF}\)) were predicted with traits related to chlorophyll content in the bottom of the canopy. PLSR estimations were the worst of the three methods with \(R^2 = 0.02\) and RMSE = 0.33 g MJ\(^{-1}\) (Figure 3).

RUE\(_{\text{GF\_bcs}}\) estimations were the best with \(R^2 = 0.61\) and RMSE = 0.23 g MJ\(^{-1}\) but also was the model that used most variables which can reduce the applicability on field conditions (Table 3). RUE\(_{\text{GF\_cVI}}\) estimations were outperformed by the \(\text{bcs}\) model but we found a trend at grain filling where VI related to chlorophyll content and gas exchange were used to predict IPAR and biomass (Table 3). PLSR predictions at grain filling were the worst for any model at any given growth stage with \(R^2 = 0\) and RMSE = 0.52 g MJ\(^{-1}\) (Figure 3).

RUE\(_{\text{Total\_bcs}}\) predictions were made with NDWI-2 measured above the canopy, CUR from the second leaf measured on the vegetative stage, and NPCI from the third leaf measured during the grain filling period (NDWI\(_{-2}\text{canvg}, \text{CURSLvg}, \text{NPCITLgf}\)) (Table 3). Our results show the predictions with \(\text{bcs}\) model at physiological maturity were the most accurate of any growth stages/methods used (\(R^2 = 0.69\), RMSE = 0.11 g MJ\(^{-1}\)) for RUE, in comparison RUE\(_{\text{Total\_cVI}}\) had lower accuracy in the predictions but similar RMSE (\(R^2 = 0.53\), RMSE = 0.13 g MJ\(^{-1}\)), which indicates that RUE predictions could be done faster just by using VI at the canopy scale and results will not differ much from the \(\text{bcs}\) method.

**Discussion**

RUE is a key trait that underpins crop productivity due to its close relation with photosynthesis, biomass accumulation and yield and it is of great interest in breeding for higher yield potential (Murchie et al., 2009; Reynolds et al., 2012; Hubbart et al., 2018; Molero et al., 2019; Joynson et al., 2020). However, its complex nature caused by the interaction of several physiological processes affecting it at different growth stages and the difficulty to screen it in large field trials has not allowed physiologists and breeders to fully implement HTPP approaches to predict it (Furbank et al., 2019). In this study a HTPP approach is proposed and validated with ground truth data collected during three field growth cycles by combining different remote sensing techniques using hyperspectral reflectance to calculate vegetation indices and PLSR to develop statistical models that provide the flexibility to be
tested in large wheat populations in yield potential conditions. Eventually this can be extended to populations grown under different environmental conditions (e.g. heat, drought and nutrient deficiency stresses) or in other important crops such as rice, barley, or rye.

The implementation of this methodology can reduce drastically the time and manual labour needed to measure RUE and its components. Field aboveground biomass harvests and ceptometer measurements take time and cost more resources than implementing a HTPP method to assess RUE components and there is an opportunity to reduce the experimental error caused by different people sampling in the same experiment. If the data produced with these models coupled with UAV imaging plus a pipeline for data extraction and upscaling can shift the narrative in physiological breeding as genetic gains for this trait are not often seen due to its phenotyping bottleneck.

Physiological mechanisms underlying the vegetation indices models

Our models indicate that at the vegetative period, which encompasses the phenological stages from canopy closure to anthesis, two water indices (WI, NDWI) and two greenness indices (EVI, NDVI) were used to build models to predict RUE, biomass or IPAR. Water indices have been associated with biomass accumulation in wheat with very strong phenotypic correlations at the vegetative stages of booting and heading (Babar et al., 2006) which is within the period of our measurements for the vegetative stage. Water indices and EVI are more sensitive to variations in LAI than NDVI, this means that during the vegetative period where LAI is larger in comparison to the grain filling stage in wheat (Calderini et al., 1997), WI, NDWI and EVI can be a better option than to predict RUE, biomass and IPAR than NDVI. Therefore, we suggest to predict RUE, biomass and light interception using the abovementioned VI especially once the canopy closes and NDVI values are close to saturation (0.9) and in a physiological-breeding context this becomes a problem because during the vegetative stages there are not big differences between the phenological development of different wheat genotypes and the genotypic differences in NDVI might be negligible due to higher LAI at this growth period, while evidence indicate water indices correlate well with biomass and most importantly are able to capture genotypic differences at GS41 (Babar et al., 2006; Prasad et al., 2009; Gutierrez et al., 2010).

During the grain filling period (GS65 to GS79) the common VIs to predict RUE and its components were PRI and SIPI. Correlations between PRI and RUE indicate that if PRI increases RUE will increase as well (higher PRI lower non photochemical quenching, NPQ), this implies that there could be a source limitation or source-sink co-limitation in these genotypes at grain filling (Acreche et al., 2009). PRI has been related to photosynthetic processes such as the xanthophyll cycle, NPQ, chlorophyll fluorescence, carotenoids/chlorophyll ratio and RUE measured at leaf and ecosystem scales (Garbulsky et al., 2011). NPQ plays a key role for fast annual growth plants such as rice and wheat as it can increase productivity through biomass accumulation and photosynthetic rates by
enhancing photoprotection in high-light environments by limiting photoinhibition (Hubbart et al., 2018), preventing the over-reduction of PSII and regulating the electron transport factors that can help optimise field CO₂ assimilation (Murchie and Ruban, 2020).

Correlations of PRI with RUE have been found to be consistent across leaves, canopies and ecosystems with R² ranging from 0.4-0.75 (Garbulsky et al., 2011). In wheat, using PRI alone is not enough to predict RUE/light use efficiency (LUE) due to drastic reduction in canopy chlorophyll content when the senescence period starts (Wu et al., 2010), but our results show that using PRI combined with VI related to chlorophyll content (VARI, RARSa, GI) and canopy senescence (SIPI) can improve the model predictions as shown in RUE_GF_bcs compared to RUE_GF_cVI (Table 3).

The activation of NPQ causes the reduction of long-term photosynthetic capacity particularly in top and middle parts of the canopy in erect genotypes where light availability can exceed the needs of photosynthesis. In addition, leaves in the lower part of the canopy should have rapid responses to changes in light caused by Sun position through the day and wind movement (Murchie and Niyogi, 2011). Efficiently disengaging photoprotective NPQ during changes from high to low light is a mechanism that has been demonstrated to increase plant biomass up to 20% in tobacco compared to plants without this ability (Kromdijk et al., 2016). Additionally, slow responses of photosynthesis to increasing light could cost up to 21% of CO₂ assimilation in wheat (Taylor and Long, 2017).

Then, it will be possible to increase RUE by designing a new wheat ideotype with a “smart canopy” for wheat with erect flag leaves to allow light penetration to lower (and usually shaded) parts of the canopy and to avoid light saturation, similarly to what has been proposed for sorghum canopies (Mantilla-Perez et al., 2020). Evidence found in wheat canopies indicate that erectophile genotypes can have up to 11% higher biomass and 24% higher yields compared to planophile genotypes (Richards et al., 2019) therefore, the addition of erectophile genotypes and the use of remote sensing models that correlate NPQ and PRI can become important in wheat physiological breeding to increase RUE, biomass and yield, especially because wheat is grown under contrasting light environments across different latitudes which leaves the door open to further increase the genetic gains of these traits.

The structural insensitive pigment index (SIPI) is correlated with the chlorophyll content and rate of senescence of the canopy. The use of this index in our models imply that canopies that can stay greener for longer periods of time will benefit from higher biomass and IPAR accumulation and increase RUE rates in the later stages of the crop cycle, where remobilisation of nutrients to the grains, optimal N distribution through the canopy and yield formation are critical (Foulkes and Murchie, 2011; Sinclair and Rufty, 2012). It has been suggested that developing canopies which can stay greener for longer periods of time will be one of the keystones for yield improvement in future warmer climates (Lopes and Reynolds, 2012). Although in this study models fitted better using SIPI
instead of NDVI or SPAD measurements, which are usually the traits used for stay-green, this could suggest that VI related to chlorophyll or other pigment content could potentially be used interchangeably to score senescence which is closely correlated to IPAR.

**Partial least square regression models**

To our knowledge this is the first study where predictions of RUE, biomass and IPAR in field grown wheat are made with PLSR modelling. Previous attempts to predict genetic variation in physiological traits with this method have been made mostly at leaf scale considering only top of the canopy leaves. Traits such as \( A_{\text{max}} \), \( g_s \), \( V_{\text{e, max}} \), \( J_{\text{max}} \) have been predicted successfully with \( R^2 \) of 0.49, 0.34, 0.74, 0.7, respectively in spring wheat (Silva-Perez et al., 2017), \( V_{\text{e, max}} (R^2 = 0.89) \), \( J_{\text{max}} (R^2 = 0.93) \) and N leaf content per mass basis (\( R^2 = 0.89 \)) on aspen and cotton (Serbin et al., 2012); \( V_{\text{e, max}} (R^2 = 0.65) \), N leaf content (\( R^2 = 0.96 \)) and chlorophyll content (\( R^2 = 0.85 \)) in maize (Yendrek et al., 2017), leaf dark respiration (\( R^2 = 0.5-0.63 \)), leaf N content (\( R^2 = 0.91 \)) and LMA (\( R^2 = 0.75 \)) (Coast et al., 2019).

Predictions of traits mentioned above at leaf level were more accurate in comparison to our predictions of RUE or biomass where in some cases no correlation between predictions and observations were found, especially during the grain filling period (Figure 3, \( R^2 = 0 \)). Our hypothesis for this poor performance of PLSR models is that RUE and biomass accumulation are more complex physiological processes in the hierarchical scale of yield than gas exchange in single leaf layers or organ stoichiometry, as these two might be affected by more physiological traits happening within the canopy, plus the effects of root physiology and biomass accumulation at different growth stages during the crop cycle. Most of the studies have used sunlit leaf measurements from the top of the canopy to upscale whole canopy physiological processes assuming that top of the canopy leaves are representative of the whole canopy (Gara et al., 2019). This is not true, especially in crop canopies where there is a very dynamic light environment caused by wind, gaps due to planting methods, poor stand establishment, lodging, pest and disease effects or even biomass harvests, which can influence photosynthetic rates from leaves lower in the canopy and this could reduce or boost biomass accumulation and therefore RUE (Murchie et al., 2018).

This highlights the importance of using measurements which integrate the whole canopy instead of just the sunlit part of the canopy and in future studies the use of punctual reflectance measurements instead of averages might result in better PLSR predictions for the traits presented in this study. Arguments can be made that measuring leaf reflectance from the different layers of the canopy could be used instead of measuring reflectance above the canopy to represent the canopy optical properties, but in a HTPP physiological breeding context this would take much more time in the field than collecting ground truth data, negating the benefits of the methods and might not be worth doing as our results show that \( cVI \) models perform similarly to \( bcs \) models in most of the growth stages (Figure 5).
Models built using VIs from the literature were the most accurate predictors of RUE, biomass and IPAR in most of the growth stages (Figure 5). We suggest predicting RUE directly instead of estimating it from its components since predictions of biomass, IPAR carry their own source of error and then predicting RUE from those increases the error prediction further (Table 3). Using the models built with canopy VIs allowed us to capture the highest accuracy predicted values of RUE, biomass and IPAR indicating genotypes that could perform the best without increasing measurement time in the field, as measuring all the leaves from the canopy could have entailed, underlining the applicability of these models in physiological breeding programs.

Should we rely on remote sensing for studies of growth analysis?

This is the first effort to predict RUE in a HTPP field based physiological breeding context in wheat with data collected across three different crop cycles. The approaches to predict RUE and its components showed acceptable level of accuracy (53% in the vegetative growth stage, 61% during grain filling and 69% considering the whole crop cycle) but we recognize that models can be improved by increasing the number of genotypes or including data from different environments. The models presented in this study have major implications for physiological breeding as improving C fixation through RUE represents the baseline to increase crop yields. We agree that using remote sensing models cannot fully replace the collection of ground truth data but it can reduce considerably the amount of time (i.e. from 3 days of field work and lab sample processing to 45 minutes measuring hyperspectral reflectance in the field) and resources spent especially on big trials where hundreds of lines could be screened in a matter of hours and be used in QTL or GWAS studies to bridge the gap between phenomics and genomics. In addition, the present approach could help to predict RUE and biomass in experiments where biomass sampling is not possible due to plot size (<1 m) typically used to select plant genetic resources in pre-breeding programs. Finally, the models built with data collected at leaf and canopy scale in this study can be used to refine C cycle models built with satellite imagery data and increase the link between remote sensing platforms to increase our understanding of C cycle dynamics at the regional scale.
Data availability

The data supporting the findings in this study are available from the corresponding author (Erik H. Murchie) upon request.

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Author contribution

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Gemma Molero: Conceptualization, Funding acquisition, Methodology, Writing – review & editing.

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Erik H. Murchie: Conceptualization, Funding acquisition, Project administration, Methodology, Writing – original draft, Writing – review & editing.
Table 1. Reference ID, cross name, average days to initiation of booting (DTInB), days to anthesis (DTA), days to physiological maturity (DTPM), intercepted PAR, aboveground biomass and radiation use efficiency measured at different growth stages for the wheat genotypes studied. ~Genotypes studied only in Y2 and Y3.

| D | Cross name          | DTInB | DT | DTA | IPAR | IPARI | IPAR | BME | BM | BME | RUE_E40 | RUE_In | RUE_pr | RUE_GF | RUE_Total |
|---|---------------------|-------|----|-----|------|-------|------|-----|----|-----|---------|---------|--------|--------|-----------|
| 1 | KRICHAUFF           | 60    | 77 | 119 | 224.39 | 365.82 | 543.2 | 7 | 833.56 | 188.4 | 466.3 | 978     | 905.5   | 1210   |
| 2 | W15.92/4/PASTOR/HXL7573/2*BAU/3/WBL L1 | 59    | 74 | 113 | 230.22 | 359.2 | 524.4 | 4 | 762.38 | 217.1 | 515.1 | 9    | 905.5   | 1210   |
| 3 | KUKRI               | 64    | 79 | 117 | 232.44 | 405.17 | 575.9 | 8 | 836.45 | 196.1 | 569.5 | 1075    | 975     | 1319   |
| 4 | MUNAL #1            | 65    | 80 | 116 | 226.94 | 406.3 | 565.1 | 7 | 804.02 | 199.2 | 558.6 | 998.7   | 1319    | 1235   |
| 5 | JANZ                | 60    | 73 | 116 | 229    | 371.49 | 534.4 | 1 | 822.75 | 190.0 | 497.9 | 1493    | 1235    | 1260   |
| 6 | CHEWINK #1          | 62    | 80 | 118 | 233.53 | 385.28 | 567.8 | 1 | 843.43 | 186.3 | 517.5 | 943.6   | 1390    | 217    |
| 7 | SOKOLL/PUB94.15.1.12/WBL1 | 60    | 75 | 116 | 232.73 | 375.19 | 551.8 | 8 | 833.83 | 211.9 | 495.6 | 1127.9  | 1445    | 192    |
| 8 | PUB94.15.1.12/FRET2/CR0C_1/AE.SQUARR OSA/53/4 | 59    | 74 | 116 | 230.92 | 368.04 | 538.4 | 5 | 824.21 | 214.3 | 551.0 | 1027.9  | 1445    | 22     |
| 9 | CHEWINK #1          | 62    | 80 | 118 | 233.53 | 385.28 | 567.8 | 1 | 843.43 | 186.3 | 517.5 | 943.6   | 1390    | 217    |
|   | Mean                | 61    | 76 | 116 | 229.31 | 377.26 | 547.7 | 6 | 816.59 | 205.1 | 607.0 | 1127.2  | 1416    | 211    |
|   | H²                  | 0.91  | 0.92 | 0.88 | 0.92 | 0.76 | 0.48 | 0.57 | 0.7 | 0.23 | 0.25  | 0.46    | 0.6     |
BM_E40: biomass 40 days after emergence, BM_InB: biomass at initiation of booting, BM_A7: biomass 7 days after anthesis, BM_PM: biomass at physiological maturity, IPAR_E40: accumulated intercepted PAR 40 days after emergence, IPAR_InB: accumulated intercepted PAR at initiation of booting, IPAR_A7: accumulated intercepted PAR 7 days after anthesis, IPAR_PM: accumulated intercepted PAR at physiological maturity.

RUE_E40InB: RUE from the period of 40 days after emergence to initiation of booting calculated with APAR, RUE_InBA7: RUE from the period of initiation of booting to 7 days after anthesis calculated with APAR, RUE_preGF: RUE pre grain filling calculated with APAR, RUE_GF: RUE grain filling calculated with APAR, RUE_Total: RUE of the whole crop cycle calculated with APAR.

ms: marginally significant (0.1>p>0.05), * = significant at p<0.05, ** = significant at p<0.01, *** = significant at p<0.001, ns: not significant. H² = Heritability, G = Genotype, Y = Environment, GxY = Interaction genotype by environment.
Table 2. Common remote sensing physiological traits found to correlate with radiation use efficiency, biomass and intercepted PAR during the three field seasons measured in this study. Vegetation indices were calculated with data collected with an ASD Field Spec hyperspectral radiometer and when stated Green Seeker sensors, infrared thermometer and SPAD meter were also used to collect data.

| Trait | Meaning | Equation | Physiological relevance | Reference |
|-------|---------|----------|-------------------------|-----------|
| CT    | Canopy Temperature | N/A | Stomatal conductance, transpiration, root water uptake | Reynolds et al., 1994 |
| CRI   | Carotenoid Reflectance Index | $(1/R510)-(1/R550)$ | Carotenoid content | Steddom et al., 2003 |
| CUR   | Curvature Index | $(R675*690)/R683^2$ | Diurnal variation of chlorophyll fluorescence, Fv/Fm | Zarco-Tejada et al., 2000 |
| EVI   | Enhanced Vegetation Index | $2.5[(R900-R680)/(R900+6*R680-7.5*R475+1)]$ | Photosynthetic capacity, canopy greenness without saturation problems | Huete et al., 2002 |
| GI    | Green Index | $R554/R677$ | Canopy greenness, yield | Smith et al., 1995 |
| GNDV  | Green Normalized Differenced Vegetation Index-1 | $R810-[(R510+R561)/2]/R810+[(R510+R561)/2]$ | Canopy greenness, photosynthetic capacity, N status | Gitelson and Merzlyak, 1997 |
| Jmax  | Maximum electron transport rate | Partial least square regression modelling | Leaf e⁻ transport rate | Silva-Perez et al., 2017 |
| NDVI  | Normalized Differenced Vegetation Index | $(R800-R680)/(R800+R680)$ | Chlorophyll content, canopy greenness, photosynthetic capacity, energy absorption | Tucker, 1979 |
| NDVI GS | Normalized Differenced Vegetation Index measured with a Green Seeker sensor | $(R800-R680)/(R800+R680)$ | Chlorophyll content, canopy greenness, photosynthetic capacity, energy absorption | Tucker, 1979 |
| NDWI  | Normalized Differenced Water Index | $(R860-R1240)/(R860+R1240)$ | Canopy water content | Gao, 1996 |
| NDWI-2 | Normalized Differenced Water Index-2 | $(R970-R850)/(R970+R850)$ | Canopy water content | Babar et al., 2006 |
| NDWI-3 | Normalized Differenced Water Index-3 | $(R970-R920)/(R970+R920)$ | Canopy water content | Babar et al., 2006 |
| Index Code | Name                                      | Formula                                      | Description                                                                 | Reference               |
|-----------|-------------------------------------------|----------------------------------------------|------------------------------------------------------------------------------|-------------------------|
| NDWI-4    | Normalized Differenced Water Index-4      | (R970-R880)/(R970+R880)                      | Canopy water content                                                        | Babar et al., 2006      |
| NPCI      | Normalized Pigments Chlorophyll ratio Index | (R680-R430)/(R680+R430)                      | Canopy water and N status                                                   | Penuelas et al., 1994   |
| OSAVI     | Optimized Soil Adjusted Vegetation Index  | (1+0.16)(R800-R670)/(R800+R670+0.16)        | Chlorophyll content and canopy greenness reducing the effect of soil interference | Daughtry et al., 2000   |
| PRI       | Photochemical Reflectance Index           | (R531-R570)/(R531+R570)                     | Carotenoid content, xanthophyll cycle, gas exchange, non-photochemical quenching | Penuelas et al., 1995   |
| PSSRa     | Pigment Specific Simple Ratio of chlorophyll a | R800/R675                                | Chlorophyll a content                                                       | Blackburn, 1998         |
| PSSRb     | Pigment Specific Simple Ratio of chlorophyll b | R800/R650                                | Chlorophyll b content                                                       |                        |
| RARSa     | Ratio Analysis of Reflectance Spectra of chlorophyll a | R675/R700                              | Chlorophyll a content                                                       | Chapelle et al., 1992   |
| RARSb     | Ratio Analysis of Reflectance Spectra of chlorophyll b | R675/(R650*R700)                  | Chlorophyll b content                                                       | Blackburn, 1998         |
| RGR       | Red Green Ratio                           | (R612+R660)/(R510+R560)                    | Red pigments and chlorophyll content                                        | Steddom et al., 2003    |
| rNDVI     | Red edge Normalized Difference Vegetation Index | (R750-R705)/(R750+R705)                      | Chlorophyll content, canopy greenness, photosynthetic capacity, energy absorption | Sims and Gamon, 2002    |
| SAVI      | Soil Adjusted Vegetation Index            | [(R800-R680)/(R800+R680+0.75)](1+0.75)     | Chlorophyll content and canopy greenness without soil interference           | Huete, 1988             |
| SIPI-1    | Structure Insensitive Pigment Index-1      | (R800-R445)/(R800-R680)                    | Carotenoid and chlorophyll content                                          | Penuelas et al., 1995   |
| SIPI-2    | Structure Insensitive Pigment Index-2      | (R800-R435)/(R415-R435)                    | Plant senescence related to stress                                          | Pask et al., 2013       |
| SPAD      | N/A                                       | N/A                                          | Plant chlorophyll content                                                   |                        |
| SR-1      | Simple Ratio-1                            | R800/R680                                   | Canopy greenness and chlorophyll content                                    | Sims and Gamon, 2002    |
| TCARI  | Transformed Chlorophyll Absorption Reflectance Index | 3[(R700-R670)-0.2(R700-R550)](R700/R670) | Canopy greenness, chlorophyll content, gas exchange reducing the effect of soil and non-photosynthetic components | Haboudane et al., 2002 |
|--------|------------------------------------------------|------------------------------------------|-------------------------------------------------------------------------------------------------|----------------------|
| TCARI  | Transformed Chlorophyll Absorption Reflectance Index calculated with reflectance from 705 and 750 nm | 3[(R750-R705)-0.2(R750-R550)](R750/R705) | Canopy greenness, chlorophyll content, gas exchange reducing the effect of soil and non-photosynthetic components | Wu et al., 2008 |
| VARI   | Visible Atmospherically Resistant Index | (R560-R660)/(R560+R660-R459) | Canopy coverage | Steddom et al., 2003 |
| V_{\text{max}}/N_{\text{area}} | Maximum velocity of Rubisco carboxilation/N content based on leaf area | Partial least square regression modelling | Photosynthetic N use efficiency | Silva-Perez et al., 2017 |
| WI     | Water Index | R900/R970 | Canopy water content | Penuelas et al., 1997 |
Table 3. Models used to predict radiation use efficiency, biomass and PAR interception at the different growth stages measured in this study. Two models are presented for each trait, the first is the best combination of sensors (bcs) and the second the vegetation indices derived from hyperspectral measurements at the canopy level (cVI). bv = 10 highest values for each trait.

| Trait      | Model                                                                 | $R^2$ | Adj. $R^2$ | RMSE | $R^2_{bv}$ | RMSE_bv |
|-----------|------------------------------------------------------------------------|-------|------------|------|------------|---------|
| RUE_E40InB| -9.347 + 12.906WIcanvg – 4.004NDVITLvg – 0.795TCARITLvg                | 0.46  | 0.4        | 0.29 | 0.02       | 0.26    |
|           | -15.443 – 0.0674PSSRb_vg + 16.469WI_vg                                 | 0.53  | 0.5        | 0.27 | 0.31       | 0.28    |
| RUE_InBA7 | -1.791 + 13.247NDWI-3canvg + 4.721EVTTLvg + 6.656TCARITLvg             | 0.27  | 0.19       | 0.37 | 0.45       | 0.28    |
|           | 7.543 + 28.717NDWI-3_vg – 3.123EVI_vg                                  | 0.27  | 0.22       | 0.36 | 0.17       | 0.35    |
| RUE_preGF | 0.47 + 0.0446CRI_vg                                                    | 0.21  | 0.18       | 0.21 | 0.53       | 0.16    |
|           | 19.762 + 0.0389CRI_vg – 22.547NDVI_vg + 10.455NDWI_vg + 53.698PRI_vg   | 0.19  | 0.06       | 0.22 | 0.01       | 0.25    |
| RUE_GF    | 2.523 – 10.05VARcanvg – 4.661RARSacangf + 16.258SIPI-1TLvg + 1.17GI_TLvg + 0.0112JFLgf – 0.00401Vcmax/Narea_SLvg | 0.61  | 0.51       | 0.23 | 0.55       | 0.18    |
|           | 3.886 – 79.296PRI_vg – 0.675GI_gf                                      | 0.27  | 0.22       | 0.29 | 0.01       | 0.36    |
| RUE_Total | 5.972 – 15.681NDWI-2canvg – 5.458CURSLvg + 2.21NPCITLgf                | 0.69  | 0.65       | 0.11 | 0.85       | 0.05    |
|           | 0.845 + 0.992RGR_gf                                                   | 0.53  | 0.51       | 0.13 | 0.23       | 0.15    |
| BM_E40    | 294.202 – 0.394JmaxTLvg                                                | 0.2   | 0.17       | 24.53| 0.01       | 31.07   |
|           | 56.67 + 610.986WI_vg – 844.888NDVI_vg + 308.836SAVI_vg                 | 0.17  | 0.07       | 25.83| 0.09       | 31.4    |
| BM_InB    | 89.423 – 220.49NDWI-4canvg + 213.15GIFLvg – 344.448TCARITLvg            | 0.42  | 0.35       | 53.35| 0.09       | 56.14   |
|           | -206.393 – 7575.28NDWI-4_vg + 737.072TCARI_vg                         | 0.34  | 0.29       | 55.8 | 0.03       | 64.26   |
| BM_A7     | 435.468 + 14412.02PRIcanvg + 9039.94PRIFLgf                            | 0.32  | 0.27       | 76.92| 0.31       | 85.28   |
|           | 696.304 + 15902.35PRI_vg                                              | 0.18  | 0.15       | 83.18| 0.38       | 88.51   |
|        | BM_PM                        | IPAR_E40                       | IPAR_InB                      | IPAR_A7                        | IPAR_PM                      |
|--------|------------------------------|-------------------------------|------------------------------|-------------------------------|------------------------------|
|        | 361.694 + 98.526PSSRaFLvg + 106.66RARShSLvg – 1.52SIPI-2SLvg – 135.394SR-1TLvg | 674.582 – 44.419CRI_vg + 43.295PSSRa_vg – 2.543SIPI2_vg | 289.723 – 9.158CTvg + 168.407NDVIGS vg | 80.287 – 2056.97NDWI-3_vg | 26.039 + 306.267NDVIGS vg + 6808.693PRIcan vg |
|        |                              |                               |                              |                               | -500.416 + 1051.142OSAVI_vg | 289.723 + 306.267NDVIGS vg + 6808.693PRIcan vg |
|        |                              |                               | -875.05 + 36.048CTgf + 6718.306PRIcan vg + 509.163GNDVI-1cangf – 2997.16NDWI-4cangf | 618.021 + 6935.272PRI_vg – 33.644rNDVI_gf | 40.181 + 12435.71PRIcan vg + 1050.561SAVIcan vg – 201.546SIPI-1cangf |
|        |                              |                               | 0.86                         | 0.66                          | 0.18                         |
|        |                              |                               | 0.84                         | 0.63                          | 0.78                         |
|        |                              |                               | 17.66                        | 26.31                         | 35.33                        |
|        |                              |                               | 0.63                         | 0.24                          | 0.11                         |
|        |                              |                               | 15.57                        | 29.22                         | 28.02                        |
|        |                              |                               |                              |                               |                              |
|        |                              |                               |                              |                               |                              |
|        |                              |                               |                              |                               |                              |
|        |                              |                               |                              |                               |                              |
| Abbreviations: E40InB = 40 days after emergence to initiation of booting period, InBA7 = Initiation of booting to 7 days after anthesis period, preGF = pre grain filling period (40 days after emergence to 7 days after anthesis), GF = Grain filling period (7 days after anthesis to physiological maturity), Total = crop cycle, E40 = 40 days after emergence, InB = Initiation of booting, A7 = 7 days after anthesis, PM = Physiological maturity, RMSE = Root Mean Square Error, can = measurement at canopy level, FL = measurement at the flag leaf, SL = measurement at the second leaf, TL = measurement at the third leaf. |
Table 4. List of acronyms used in this study.

| Acronym       | Meaning                                                                 |
|---------------|-------------------------------------------------------------------------|
| BM_E40       | Biomass harvested 40 days after emergence                              |
| BM_InB       | Biomass harvested at initiation of booting (GS41)                      |
| BM_A7        | Biomass harvested 7 days after anthesis (GS65 + 7 days)                |
| BM_PM        | Biomass harvested at physiological maturity (GS87)                    |
| IPAR_E40     | Accumulated intercepted PAR 40 days after emergence                   |
| IPAR_InB     | Accumulated intercepted PAR at initiation of booting (GS41)           |
| IPAR_A7      | Accumulated intercepted PAR 7 days after anthesis (GS65 + 7 days)      |
| IPAR_PM      | Accumulated intercepted PAR at physiological maturity (GS87)          |
| RUE_E40InB   | RUE from the period of 40 days after emergence to initiation of booting |
| RUE_InBA7    | RUE from the period of initiation of booting to 7 days after anthesis  |
| RUE_preGF    | RUE from the period of 40 days after emergence to 7 days after anthesis |
| RUE_GF       | RUE from the period of 7 days after anthesis to physiological maturity  |
| RUE_Total    | RUE from the period of 40 days after emergence to physiological maturity |
| FL           | Measurement taken at the flag leaf                                    |
| SL           | Measurement taken at the second leaf                                   |
| TL           | Measurement taken at the third leaf                                    |
| can          | Measurement taken above the canopy                                    |
| vg           | Data averaged from the vegetative period (from canopy closure to 7 days after anthesis) |
| gf           | Data averaged from the grain filling period (from 7 days after anthesis to late grain filling) |
**Figure legends**

Figure 1. Intercepted accumulated PAR predictions with the different approaches used. Left panels represent predictions using the best combination of sensors ($bcs$), middle panel are predictions using vegetation indices derived from canopy reflectance ($cVI$) and the right panels represent predictions made with partial least square regression (PLSR). Data points represent the genotype adjusted means of the 8 genotypes studied in Y1 and 11 genotypes studied in Y2 and Y3 ($n = 30$).

Figure 2. Aboveground biomass predictions with the different approaches used. Left panels represent predictions using the best combination of sensors ($bcs$), middle panel are predictions using vegetation indices derived from canopy reflectance ($cVI$) and the right panels represent predictions made with partial least square regression (PLSR). Data points represent the genotype adjusted means of the 8 genotypes studied in Y1 and 11 genotypes studied in Y2 and Y3 ($n = 30$).

Figure 3. Radiation use efficiency predictions with the different approaches used. Right panels represent predictions using the best combination of sensors ($bcs$), middle panel are predictions using vegetation indices with canopy reflectance ($cVI$) and the left panels represents predictions made with partial least square regression (PLSR). Data points represent the genotype adjusted means of the 8 genotypes studied in Y1 and 11 genotypes studied in Y2 and Y3 ($n = 30$).

Figure 4. Venn diagram highlighting the correlation between remote sensing traits with aboveground biomass (green circle), light interception (yellow circle) and radiation use efficiency (red circle) during the vegetative (canopy closure to 7 days after anthesis) and grain filling period (7 days after anthesis to physiological maturity). Indices in the middle of the diagram indicate that can be used to predict the three traits.

Figure 5. Comparison of the approaches to build the models used to predict radiation use efficiency in the different growth stages measured in the crop cycle. X axis labels from left to right represent observed values, predictions using the best combination of sensors (Predicted_combination), predictions through the estimation of RUE components (biomass and IPAR) (Predicted_components_combination), predictions using canopy vegetation indices (Predicted_canopy VI), predictions through the estimation of RUE components using canopy VI (Predicted_components_canopy VI), predictions of RUE using canopy reflectance models derived from partial least square regression (Predicted_PLSR) and predictions of RUE through its components using PLSR (Predicted_components_PLSR).
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Figures 1. Intercepted accumulated PAR predictions with the different approaches used. Left panels represent predictions using the best combination of sensors (h2o), middle panel are predictions using vegetation indices derived from canopy reflectance (h39) and the right panels represent predictions made with partial least square regression (PLSR). Data points represent the genotype adjusted means of the 8 genotypes studied in Y1 and 11 genotypes studied in Y2 and Y3 (n = 36).
Figure 2. Aboveground biomass predictions with the different approaches used. Left panels represent predictions using the best combination of sensors (bcs), middle panel are predictions using vegetation indices derived from canopy reflectance (cvi) and the right panels represent predictions made with partial least square regression (PLSR). Data points represent the genotype adjusted means of the 8 genotypes studied in Y1 and 11 genotypes studied in Y2 and Y3 (n = 30).
Figure 3. Radiation use efficiency predictions with the different approaches used. Right panels represent predictions using the best combination of sensors (fsc), middle panel are predictions using vegetation indices with canopy reflectance (vsc) and the left panels represents predictions made with partial least square regression (PLSR). Data points represent the genotype-adjusted means of the 8 genotypes studied in Y1 and 11 genotypes studied in Y2 and Y3 (n = 30).
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