Title: A multiscale approach to investigate fluorescence and NDVI imaging as proxy of
photosynthetic traits in wheat

Running title: Multi-scale imaging and photosynthesis phenotyping in wheat

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photosynthetic parameters

ABSTRACT

With the development of the digital phenotyping, repeated measurements of agronomic traits over
time are easily accessible, notably for morphological and phenological traits. However high
throughput methods for estimating physiological traits such as photosynthesis are lacking. This study
demonstrates the links of fluorescence and reflectance imaging with photosynthetic traits. Two
wheat cultivars were grown in pots in a controlled environment. Photosynthesis was characterised
by gas-exchange and biochemical analysis at five time points, from booting to 21 days post anthesis.
On the same days imaging was performed on the same pots, at leaf and plant scale, using indoor and
outdoor phenotyping platforms, respectively. Five image variables (F_v/F_m and NDVI at the whole
plant level and F_v/F_m, $\Phi(II)_{532}$ and $\Phi(NPQ)_{1077}$ at the leaf scale) were compared to variables from A-Ci
and A-Par curves, biochemical analysis, and fluorescence instruments. The results suggested that the
image variables are robust estimators of photosynthetic traits, as long as senescence is driving the
variability. Despite contrasting cultivar behaviour, linear regression models which account for the
cultivar and the interaction effects, further improved the modelling of photosynthesis indicators.
Finally, the results highlight the challenge of discriminating functional to cosmetic stay green
genotypes using digital imaging.

Keywords: Rubisco, gas-exchange, chlorophyll fluorescence, phenotyping, crop physiology, leaf to
whole plant, stay-green, *Triticum aestivum*,

Abbreviations:

V_{cmax}: maximum carboxylation rate; J: CO_2-saturated electron transport rate; g_m: mesophyll
conductance, $R_{photoresp}$: photorespiration, k_c^c: carboxylation efficiency; A_{max}: maximum net
assimilation rate; TSP: total soluble protein content; Chl a and b: chlorophyll and b content; Rubisco:
Rubisco content; V_i: initial Rubisco activity; V_t: total Rubisco activity; $\Phi(II)$: PSII operating efficiency or
quantum yield; F_v/F_m: maximum efficiency of the photosystem II; $\Phi(NPQ)$: non-photochemical
quenching; NDVI: normalised difference vegetation index; DPA: Days post anthesis.

INTRODUCTION

Modern agriculture is facing multiple challenges to ensure the food security of a growing population. One of them, and not the least, requires tackling the yield gain stagnation in a context of resource-use sustainability and climate change (FAO, 2017). The release of cultivars tolerant to extreme and highly variable climatic conditions is nowadays required (Korres *et al.*, 2016). Studies of genotype *vs* environment to improve breeding efficiency and allow rapid varietal adaptation are crucial to avoid yield vulnerability globally and at the farm level (Atlin *et al.*, 2017).

In this context, genomics plays a major role, mainly with the identification of genes related to stress tolerance and yield resilience, to inform genomics-assisted breeding for traits related to crop adaptation to climate change (Scheben *et al.*, 2016). However, due to the multigenic and complex nature of yield and responses to the environment, many secondary traits, and consequently underpinning genes, will have differential impacts on adaptation in specific environments (Araus *et al.*, 2008; Dwivedi *et al.*, 2017). In combination with genomics, advances in phenomics are crucial for unravelling the potential of genotypes to adapt in multiple environments (Zhao *et al.*, 2019). Currently, genomics is further advanced compared to phenomics, particularly in terms of the development of high-throughput methods and techniques, creating the so-called phenotype-genotype gap (Furbank & Tester, 2011). While plant phenotyping research has surpassed genomics and proteomics fields in terms of the frequency of publications, more developments are necessary to meet the needs of the research community, breeders, and farmers, especially in developing countries where such facilities are less accessible (Costa et al., 2019; Yang et al., 2020). According to Virlet *et al.* (2022), there is uncertainty as to whether the increasing number of phenotypes measured and the depth of information from the new phenotyping platforms will improve the prediction of quantitative traits. Digital HTP provides a non-destructive and non-invasive method for screening large germplasm panels from plant emergence to maturation. HTP often relies on remote sensing technologies to collect information related to morphological, physiological and phenological traits and provide dynamic information on crop development to understand how the yield is built over time and how it is affected by the environment.

Among the parameters required for screening and subsequently selecting the genotypes for yield stability and stress tolerance, physiological traits are of great importance (Großkinsky *et al.*, 2015; Shunmugam *et al.*, 2018; Sreeman *et al.*, 2018). The physiological breeding approach proposes the selection and crossing of parents with different complex, but complementary traits, to achieve cumulative gene action for yield, while selecting progeny using remote sensing, in combination with genomic selection (Reynolds & Langridge, 2016). This is in accordance with the functional phenomics

concept, a multidisciplinary approach that includes plant physiology, genomics, phenomics, and
bioinformatics, aiming to evaluate ideotypes and generate plant models to predict crop behaviour in
different scenarios (York, 2019).

The combination of breeding and high-throughput phenotyping (HTP) of physiological traits in plants
can have a significant impact, especially in C3 crops such as wheat. According to the yield potential
model proposed by Monteith *et al.*, (1977), yield potential is a result of the interaction of the plant
with solar radiation through the efficiencies of intercepting and converting light and allocating
biomass to the organ of interest, with the conversion efficiency, which is strongly linked to the
carbon cycle and photosynthesis, being the variable with highest potential for improvement in C3
plants (Long *et al.*, 2015; Zhu *et al.*, 2010). Current research focuses on finding proxies for
physiological and biochemical parameters in plants, mainly with the advent of remote sensing (Jones
& Vaughan, 2010) and the requirement for faster screening of multiple genetic materials
(Großkinsky et al., 2015), as well as understanding their interaction with the environment (Pauli et
al., 2016). Besides mapping phenological changes and yield-components, the use of these proxies for
measuring the efficiency of carbon fixation through photosynthesis has assumed a prominent role in
crop sciences (van Bezouw *et al.*, 2019). Photosynthesis is generally assessed using gas-exchange
instruments, allowing the measurement of the instantaneous variation of CO_2 assimilation and
transpiration. CO_2 and light response curves (A-C_i and A-PAR, respectively) provide access to
integrative parameters of the functioning of photosynthesis. From those curves, three primary
parameters are generally derived: V_{cmax}, J, and A_{max}. Derived from A-C_i curves, V_{cmax} and J are related
to the assimilation rate limited by the available CO_2 concentration and by the electron transport
required for the regeneration of RuBP, respectively. The A-PAR curve provides information about the
point where light is no longer a limiting factor for CO_2 assimilation, often called A_{max} or A_{sat} (Haworth
et al., 2018). However, collection of CO_2 and light curve responses as well as net CO_2 assimilation, is
time consuming, and often unfeasible at a population scale in field conditions.

Commonly used techniques for indirectly monitoring photosynthesis are based on spectral data
enabling computation of spectral reflectance indices or measuring of sun-induced fluorescence (SIF)
based on chlorophyll fluorescence measurement by an active sensor (Porcar-Castell *et al.*, 2014;
Badgley *et al.*, 2017). In general, spectral indices such as NDVI or its derivatives have been used as an
indirect measure of primary production, as they are almost linearly correlated to the fraction of
absorbed photosynthetically active radiation (Atzberger 2013). However, it has been shown and it is
widely accepted that the photochemical reflectance index (PRI) is the only index able to detect
short-term variations of CO_2 assimilation (Dobrowski *et al.*, 2005, Merlier *et al.*, 2015). Recent
studies showed the possibility of predicting V_{cmax}, J, and A_{max} combining spectral reflectance and

PLSR regression on a small population of wheat in controlled glasshouse conditions and a small set

of cultivars in outdoor conditions (Silva-Perez *et al.*, 2018). Meacham-Hensold *et al.* (2019) reported

that the predictive aspect of the PSLR works across years, but only on the same genetic materials.

The upscaling of those approaches to populations in field conditions is then limited by the need for

ground-truth data to build solid and more universal models. The deployment of active sensors for

large scale field phenotyping is still complex and so far, few platforms to our knowledge, such as the

Field Scanalyzers in the UK and the US, are equipped with chlorophyll fluorescence cameras (Virlet *et*

al., 2017; Herritt *et al.*, 2020). Laser induced fluorescence transient (LIFT) is another approach which

seems more easily usable on field phenotyping trials, but still requires development for large field

trials (Wyber *et al.*, 2018).

In this study, we compared remote sensing-based reflectance and fluorescence imaging with

traditional approaches to measure and evaluate photosynthesis and fluorescence parameters. At the

whole plant scale, NDVI and maximum potential quantum yield were imaged under field conditions

(Virlet *et al.*, 2017). Leaf scale fluorescence imaging was realized in controlled environment

conditions to collect maximum potential quantum yield, effective quantum yield of PSII, and non-

photochemical quenching parameters (Demidchik *et al.*, 2020). Prior to imaging, the same leaves

were used for gas exchange and handheld fluorescence measurements, and biochemical analysis

were conducted for Rubisco related traits. The biological variability relied on two different wheat

cultivars and repeated measurements throughout plant development. Comparison between image

variables and traditional approaches was investigated through linear regression models.

MATERIAL & METHODS

Plant material and experimental conditions

The plant material was grown in a controlled environment room at Rothamsted Research,

Harpenden, UK, from July to October 2018. The plants were grown under a day length of 16 hours,

at temperatures of 18/15°C (day/night), relative humidity of 65/75% (day/night) and irradiance of

700 µmols photons m^{-2} s^{-1} (approximately 500 µmols photons m^{-2} s^{-1} at leaf level). Two wheat

cultivars (cv. Cadenza and Bobwhite) with contrasting growth and maturation patterns were used.

Five wheat seeds were sown in 5 L pots, no thinning was performed, with each pot considered an

experimental unit. The experiment was organized into three blocks of five pots of each cultivar, for a

total of 30 pots. Zadoks' scale was used for the evaluation of plant development. Plants were

phenotyped at the following developmental stages: booting (Z4.5), anthesis (Z6.5) and 7, 14 and 21

days after anthesis (Z6.5+7, Z6.5+14 and Z6.5+21, respectively). At each time point, one pot per
cultivar and per block was used.

Phenotyping methods

169 Multiple phenotyping methods were used to evaluate physiological parameters of the plants at
170 whole plant, leaf and biochemical levels. The order of phenotyping was as follows: gas-exchange (A-
171 C_i and A-PAR curves), chlorophyll index, light and dark-adapted chlorophyll a fluorescence through
172 handheld devices and the Phenocenter, fluorescence and reflectance through the Field Scanalyzer,
173 and finally, the biochemical analysis.

Gas-exchange experiments

The response of net photosynthesis (A) to intercellular CO_2 concentration (C_i) and photosynthetic
active radiation (PAR) was evaluated through A-C_i and A-PAR curves, performed on the central part
of flag leaves, using an infra-red gas analyser model LI6400-XT, with an attached leaf chamber
LI6400-40 for combined gas-exchange and fluorescence measurements (LI-COR Biosciences). A-C_i
curves conditions were: flow of 200 µmols s^{-1}, block temperature of 20°C, photosynthetic photon
flux density (PPFD) of 1800 µmols m^{-2} s^{-1} and relative humidity in the chamber controlled between
55 and 65%. The steps of the A-C_i curve were 400, 300, 200, 100, 400, 450, 550, 700, 1000, and 1200
µmols mol^{-1} for the reference CO_2 concentration. The A-PAR curves' conditions for flow, block
temperature, and relative humidity were the same for A-C_i curves and CO_2 reference concentration
was set at 400 µmols mol^{-1}. The steps of the A-PAR curves were 1800, 1400, 1000, 500, 250, 100, 50,
and 0 µmols m^{-2} s^{-1} photons.

The data from the A-C_i and A-PAR curves were used to calculate additional parameters using the
Response Curve Fitting 10.0 and Light Response Curve Fitting 1.0 tools, respectively (available at
http://landflux.org/tools). The tool for A-C_i curve is based on the parameter calculation method
proposed by Ethier and Livingston (2004) while the A-PAR curve toll uses the method developed by
Marshall and Biscoe (1980) and Thornley and Johnson (1990). From the A-C_i curves, the following
parameters were modelled/calculated: maximum carboxylation rate (V_{cmax}), CO_2-saturated electron
transport rate (J), chloroplastic CO_2 concentration (C_c), inter-cellular CO_2 concentration (C_i),
intercellular CO_2 photo-compensation point (C_i^*), chloroplastic CO_2 photocompensation point (Γ^*),
CO_2 compensation point (Γ), CO_2 transfer conductance (g_i), mesophyll conductance (g_m),
mitochondrial respiration in the light (R_d), photorespiration ($R_{photoresp}$), carboxylation efficiency (k_c^c).
From the A-PAR curves, the following parameters were modelled/calculated: dark respiration (R_{dark}),

apparent quantum yield (Φ), light compensation point (LCP) and maximum net assimilation rate
(A_{max}).

Fluorescence and SPAD measurements

In addition to the above-mentioned measurements, chlorophyll a content and fluorescence were
measured in the same areas of the flag leaves analysed for gas-exchange. The chlorophyll index
(SPAD) was measured using the SPAD-502 chlorophyll meter (Konica Minolta). The light-adapted
fluorescence parameter, quantum efficiency of PSII ($\Phi(II) = (F_m' - F_s) / F_m'$) was assessed using the
following equipment: Fluorpen FP100 (PSI, $\Phi(II)FP$), FMS2 (Hansatech, $\Phi(II)FM$) and Handy Pea
(Hansatech, $\Phi(II)HP$). The dark-adapted fluorescence (F_v/F_mHP) was accessed using the Handy Pea
after dark adaptation of 30 minutes. $\Phi(II)$ was measured with the Fluorpen using the quantum yield,
QY function. The saturating light was set up at 3,000 µmols $m^{-2} s^{-1}$ photons. The FMS2 was used in its
default setup (Gain = 50, Mod = 3) and measurement was performed over a 2.5 s period, averaging
the equivalent F_s signal for 1.8 s before the application of 100-unit saturating pulse over a 0.7 s
duration to determine F_m'. The Handy Pea was used in its default configuration. The OJIP curve was
measured under light and dark-adapted conditions with only F_v/F_m considered in both conditions.
For the light-adapted leaves, the measured F_v/F_m was assumed to be equivalent to $\Phi(II)$.

Phenocenter

The same flag leaves that were used for the previous measurements were also used for fluorescence
imaging at the Phenocenter (Demidchik *et al.*, 2020). The Phenocenter (LemnaTec) is a laboratory-
scale, semi-automated system to record and analyse images of plants, through the IMAGING-Pulse-
Amplitude-Modulation (PAM) MINI (Waltz). This facility is located in Crop Health and Protection
(CHAP), Harpenden, UK. The flag leaves were cut under water using a blade (adapted from Driever *et*
al., 2014) and then placed in a water reservoir attached to a black surface using double-sided tape.
The tips of the flag leaves were also cut to fit the device and inserted into the Phenocenter
(Supplementary Fig. S1). Three flag leaves were analysed at a time. For each leaf sample, three
circular areas were defined at 25%, 50%, and 75% of the length of the sample. The three values
obtained from each area were averaged to calculate the final sample value for all the measured
parameters.

The leaves were left to adapt to darkness for 30 minutes, inside the Phenocenter. Following this,
they were exposed to a saturating light pulse, and F_v/F_m was measured (F_v/F_mPc). This measurement
was followed by a light curve, with points at each 20 seconds, at the following PAR levels: 1252,
1077, 702, 532, 462, 282, 232, 112, 57, and 2 µmols $m^{-2} s^{-1}$ photons. At these points the efficiency of

the photosystem II (Φ(II)) and the non-photochemical quenching (Φ(NPQ)) were measured. An

example of the leaf Φ(II) can be seen on Figure 1.

Field Scanalyzer

The pots of the analysed plants were taken to the field and placed on a platform underneath the

Field Scanalyzer (LemnaTec) (Supplementary Fig. S2). The Field Scanalyzer is a phenotyping platform

(Virlet *et al.*, 2017) installed in a field area at Rothamsted Farm, Harpenden, UK. The CropReporter®

(Phenovation) (Jalink & van der Schoor, 2011) chlorophyll fluorescence imager and the Hyperspec®

Inspector™ VNIR camera (Headwall Photonic) were used to capture images of the entire plants.

The fluorescence was measured at night to collect the F_v/F_m parameter. The camera was positioned

at 1m above the top of the plant, and a serie of flashes of red light (620nm) was used to induce the

chlorophyll fluorescence (CF) response for 1400 ms (4400 µmols m^{-2} s^{-1} at 70 cm from the bottom of

the camera) saturating the electron transfer between the two photosystems (PSII and PSI), and

records 24 images within that time. The exposure time of the first image was initiated using the

trigger at the rising edge of LED illumination, and this image was called ground fluorescence (F_0). The

image with the maximum fluorescence (F_m) was then processed to create a mask to remove the

background using the Otsu thresholding approach, which was then applied to the F_0 image. A

composite image is then created by combining F_m and F_0 images to obtain the F_v/F_m image to extract

the F_v/F_m average values from the plant. Figure 2 shows the final F_v/F_mSc image obtained for

Bobwhite and Cadenza at each time point for one of the repetitions.

The hyperspectral images were captured on the same day or the following day of the fluorescence

measurement, depending on the weather conditions. A white reference panel, Ultra-lightweight

Reflectance Target (Zenith Lite™ Ultralight Targets 95%R, Sphereoptics®), mounted on a tripod was

also scanned during the acquisition. This panel was used later to convert the raw data into

reflectance data using the method describe by Sadeghi-Tehran *et al.* (2021). After segmenting the

plant from the background, NDVI was computed from the 680 and 800 nm wavelengths.

Biochemical analysis

The same cut leaves used for the Phenocenter analysis were kept in a tube with water and were

transferred to a cabinet to activate photosynthesis and Rubisco for 40 mins before sampling. Cabinet

conditions were maintained at 25°C, 70% relative humidity and an irradiance of 900 µmols photons

m^{-2} s^{-1} at the leaf level. The samples were taken from the centre of the area using a freeze clamp

with 2 cm head diameter, and sample area calculated by measuring the sample width. The samples

were immediately transferred to foil packets, snap frozen in liquid nitrogen, and stored at -80°C until

analysis.

Proteins were extracted from leaf samples as described by Carmo-Silva *et al.* (2017). This protein

extract was used to determine Rubisco activity via incorporation of $^{14}CO_2$ in acid-stable products

(Carmo-Silva *et al.*, 2017), and activation state was calculated from the ratio of initial/total Rubisco

activity (V_i, V_t). An aliquot of the homogenate was used to determine chlorophyll a and b content

(Chl a and Chl b) using ethanol (Wintermans & de Mots, 1965) and measuring absorbance in a

microplate reader SPECTROstar Nano (BMG LabTech). The supernatant prepared for Rubisco activity

was also used to quantify total soluble protein (TSP) via the Bradford assay (Bradford, 1976), and the

Rubisco content (Rubisco) was determined by [^{14}C] carboxyarabinitol-1,5-bisphosphate (^{14}C-CABP)

binding assay (Whitney *et al.*, 1999).

Data analysis

In order to conduct gas-exchange, fluorescence and SPAD measurements, as well as Phenocenter

and biochemical analysis, two plants of each pot were used as technical replicates, and their values

were averaged to form one biological replication for each block. However, for the Scanalyzer

analysis, only one value was determined for the whole pot.

The data was organized in two factors: two cultivars (cv. Bobwhite and Cadenza) and five

developmental stages (Z4.5, Z6.5, Z6.5+7, Z6.5+14 and Z6.5+21), with three blocks, resulting in a

total of 30 points. The parameters were split into two groups: image-based parameters obtained

from the Field Scanalyzer and the Phenocenter, and the more "traditional" parameters collected by

gas-exchange, biochemical analysis and handheld devices.

The relationship between the image-based variables and each of the traditional parameters was

investigated using linear regression models. The first approach looked at the relationship between

the parameters of the two groups (image-based and traditional methods) for each cultivar

individually, but with all the development stages (Individual models for Bobwhite and Cadenza). In

the second approach, the linear regression was fitted using the whole dataset to analyse the

generalist capacity of the regression and analyse the effect of the cultivars. Two models were built

for this purpose: one model considered only the regression between the variables of the two groups

without the cultivar effect (Generalist model), and the other model considered the cultivar effect

(cv.) and its interaction (Int.) with the considered parameter (Interaction model).

All the results from the first approach are presented in supplementary data (Supplementary Tables

S1 to S4). A subsample is presented in the results section. A subset of five image-based variables

were selected and compared to six parameters derived from the CO_2 and light curve response (V_{cmax},

J, g_m, k_c^c, $R_{photoresp}$ and A_{max}), to six parameters resulting from the biochemical analysis (TSP, Chl a, Chl

b, Rubisco content, V_i and V_t) and five parameters collected by the handheld device (SPAD, Φ(II)FP,

Φ(II)FM, Φ(II)HP and F_v/F_mHP). The image-based variables selected were the fluorescence

parameters collected with the Field Scanalyzer (F_v/F_mSc), and with the Phenocenter (F_v/F_mPc, Φ(II)$_{532}$

and Φ(NPQ)$_{1077}$) as well as the NDVI calculated from the reflectance data.

ANOVAS were conducted to analyze the results of the regressions for each arrangement. The results

of the F-test of the ANOVA (F and p-values) and the adjusted coefficient of the determination (R^2_{adj})

were used to evaluate the regressions. Tables 1-5 display the F and p-values and R^2_{adj} for the

Individual Models (Bobwhite then Cadenza), the Generalist Models and the Interactions Models. In

Interactions Models, the F and p-values of the ANOVA for the parameter in the y-axis are referred to

as F (Par.) and p (Par.), respectively. The cultivar effect F (cv.) and p (cv.) and the interaction effect

between cultivars F (Int.) and p (Int.) are also noted. For p (Par.), a value lower than 0.05 suggests a

significant linear correlation between the two analysed parameters. A p-value lower than 0.05 for p

(cv.) and p (Int.) indicates that the intercepts and the slopes of the fitted regression for each cultivar

are significantly different. The p (Model) and R^2_{adj} are, respectively, the general p-value and the R^2_{adj}

of the linear regression with the line effect. The cultivar effect tests the hypothesis that the intercept

is not different between the two cultivars. A significant effect indicates a statistical difference

between the intercept of the two cultivars as well as the two regression lines are mostly parallels.

The interaction effect tests the null hypothesis that there is no difference in slopes between the two

cultivars, which indicates that both cultivars are responding similarly.

The residuals of the linear regressions were evaluated for normality, homoscedasticity (homogeneity

of variances) and independence by visually checking the Q-Q, residuals vs fitted values and

histogram of residuals' plots, and also by the Shapiro-Wilk test. The most relevant linear regressions

were plotted (Supplementary Fig. S3-S7) and the results were presented in tables 1 to 5. The R

software was used for the analyses with the following packages $ggpmisc$, $stats$ and $olsrr$ (R Core

Team, 2020).

RESULTS

Date and cultivar effect

Among the presented variables, two main patterns were observed (Fig. S3, Supplementary Table S5).

The first showed a significant decrease of the values for both cultivars from booting (Z4.5) to 21 days

post anthesis (Z6.5+21) for all the variables derived from the gas-exchange (V_{cmax}, J, g_m, k_c^c, $R_{photoresp}$

and A_{max}), for the biochemical parameters related to the Rubisco (TSP, Rubisco, V_i and V_t) as well as

for $\Phi(II)HP$ and F_v/F_mHP. The same was observed for the image variable $\Phi(NPQ)_{1077}$. The second

pattern showed a contrasted behaviour between the two cultivars for $\Phi(II)FM$ and the image

variables F_v/F_mSc, F_v/F_mPc, and NDVI with a significant decrease overtime for Cadenza, whilst no

significance differences were observed for Bobwhite.

The chlorophyll related parameters (Chl a, Chl b and SPAD) showed an increase of the values peaking

at Z6.5+7, followed by a decrease for Cadenza and no significant variation for Bobwhite. For $\Phi(II)_{532}$,

the values decreased significantly Z6.5+14 for Cadenza, whilst for Bobwhite the values dropped

significantly after booting with no more variation for the rest of the experiment. Finally, $\Phi(II)FP$ did

not show significant variation for either cultivar with, however, a decreasing trend observed for

Cadenza at Z6.5+14 and Z6.5+21.

Field Scanalyzer

Dark-adapted chlorophyll fluorescence (F_v/F_mSc)

The F_v/F_mSc calculated from images of the Field Scanalyzer for whole plants (Fig. 2) showed high

correlations with most of the parameters measured by traditional methods (Table 1; Supplementary

Fig. S3). Cadenza showed significant correlations with all the parameters, with the highest fitting for

V_i (R^2_{adj} = 0.75) and the lowest fitting for k_c^c and Chl b (R^2_{adj} = 0.33). For Bobwhite, the regressions

between F_v/F_mSc and (i) the gas-exchange parameters were all significant (R^2_{adj} = 0.32 − 0.61), (ii) the

biochemical parameters were mostly all significant (R^2_{adj} = 0.24 − 0.62) except for the Chl a and b

content and (iii) the handheld devices were significant only for $\Phi(II)FP$ (R^2_{adj} = 0.29) and F_v/F_mHP

(R^2_{adj} = 0.43).

The choice of the cultivar appeared to impact the relationship between the traditional parameters

and the imaging F_v/F_mSc (Fig. 3). The Interaction Models (Table 1) confirmed that most of the

parameters displayed a significant cultivar (cv.) effect except for the parameters k_c^c, $R_{photoresp}$, A_{max},

$\Phi(II)FP$, $\Phi(II)HP$, F_v/F_mHP. When the cv. effect was significant, the R^2_{adj} from the Interaction Model

increased compared to the Generalist Model. On the other hand, the R^2_{adj} between both models

were quite similar when no significant cv. effect was observed. No significant interaction between

the parameters and cultivar effect was observed, except for Chl a and b, indicating that in most

cases, there is no significant difference between the slopes of the regression of the parameters and

F_v/F_mSc of each cultivar.

Normalized Difference Vegetation Index (NDVI)

The regressions analysis between the NDVI measured by the Field Scanalyzer and other parameters

displayed a completely different pattern to the previous observation with F_v/F_mSc. All the linear

regressions were significant for Cadenza, whereas no significant regressions were observed for

Bobwhite (Table 2; Supplementary Fig. S4). Cadenza showed high correlations for most of the

parameters, regardless of the type of measurement with R^2_{adj} ranging from 0.55 to 0.84 for the gas-

exchange parameters, 0.31 to 0.79 for the biochemical parameters, and 0.42 to 0.75 for handheld

devices. The Generalist Models were clearly impacted by the lack of responsiveness of the ground

variables to changes in NDVI for Bobwhite, displaying lower but still significant R^2_{adj} for most

parameters (R^2_{adj} = 0.16 − 0.53), except for Chl b and SPAD. However, the Interaction Models did not

show a clear cultivar effect for all the parameters. Significant cv. effects were only observed for V_{cmax}

among the gas-exchange parameters, for Chl a and Chl b for the biochemical parameters and for

SPAD, Φ(II)FP and F_v/F_mHP. Incorporating the cv. effect into the Interaction Model improved the

regressions in most cases (R^2_{adj} = 0.29 − 0.60), except for g_m. Significant interactions were found for

Chl a, b SPAD and Φ(II)FM, confirming the different behaviour of Bobwhite in its responses to NDVI

(Fig. 3).

Phenocenter

Dark-adapted chlorophyll fluorescence (F_v/F_mPc)

The F_v/F_mPc calculated through the IMAGING-PAM at the Phenocenter for the flag leaves (Fig. 1) also

showed that the linear regressions were strongly affected by the cultivar (Table 3; Supplementary

Fig. S5). Cadenza demonstrated high and significant correlations with all the parameters measured

by gas exchange (R^2_{adj} = 0.57 − 0.85), biochemical analysis (R^2_{adj} = 0.35 − 0.76) or handheld device

(R^2_{adj} = 0.50 − 0.82), with the highest fits observed for J, Φ(II)FM and F_v/F_mHP (R^2_{adj} = 0.85, 0.81 and

0.82, respectively) and the lowest fits for Chl a and b (R^2_{adj} = 0.44 and 0.35, respectively). For

Bobwhite, significant regressions were only observed between F_v/F_mPc and the parameters from the

biochemical analysis, Chl a and Chl b and the parameters from the handheld devices, Φ(II)HP (R^2_{adj} =

0.32, 0.25 and 0.45, respectively). All the regression showed a significant R^2_{adj} for the Generalist

Models. Since most of the regressions for Bobwhite were non-significant, it was expected that a

cultivar effect would be present for most parameters.However, the Interaction Models only showed

a significant cv. effect for A_{max}, SPAD, Φ(II)FP, Φ(II)HP and F_v/F_mHP. This indicates that for these five

parameters, the offset of the regression differs significantly between cultivars. The only significant

interaction was found for $\Phi(II)HP$, indicating that in this case, the slopes were significantly different

between cultivars only for $\Phi(II)HP$. In general, the R^2_{adj} between the Generalist and the Interaction

Models were similar, with the exception of the five parameters that had a significant cv. effect. For

those, improvements to the regressions were achieved by considering the cv. effect into the

Interaction Models.

Photosystem II quantum efficiency ($\Phi(II)_{532}$)

The $\Phi(II)_{532}$ was calculated through the Phenocenter IMAGING-PAM, at 532 µmols m^{-2} s^{-1} photons

during a light curve at flag leaf level (Fig. 1). The regressions between $\Phi(II)_{532}$ and most of the

parameters displayed a significant cultivar effect except for Chl a, b and SPAD (Table 4,

Supplementary Fig. S6). Cadenza presented significant correlations with all the parameters

measured either by gas-exchange (R^2_{adj} = 0.59 − 0.84), biochemical analysis (R^2_{adj} = 0.32 − 0.83) or

handheld device (R^2_{adj} = 0.31 − 0.90), with the highest fittings for J, A_{max}, TSP, V_t and F_v/F_mHP (R^2_{adj} =

0.82, 0.84, 0.82, 0.83 and 0.90, respectively) and the lowest fittings for Chl b and $\Phi(II)FM$ (R^2_{adj} =

0.32 and 0.31, respectively). For Bobwhite, the regressions between $\Phi(II)_{532}$ and the gas-exchange

parameters were all significant (R^2_{adj} = 0.36 − 0.63), whereas the regression with the biochemical

parameters and the handheld devices parameters were only significant for TSP, V_i, V_t, $\Phi(II)FP$ and

F_v/F_mHP (R^2_{adj} = 0.37, 0.27, 0.31, 0.28 and 0.34, respectively). As already indicated, most of the

parameters (except for Chl a, b, and SPAD) showed a significant cv. effect, indicating that the

intercept of linear regressions for each of the cultivars was significantly different. Among those

parameters V_{cmax}, g_m, k_c^c, $R_{photoresp}$, TSP, Rubisco, V_i, $\Phi(II)FP$ and $\Phi(II)HP$ did not show significant

interactions, indicating that the slopes of the linear regression for each cultivar were not significantly

different. The relationship between $\Phi(II)_{532}$ and those nine parameters followed a similar pattern,

with only intercepts differing between cultivar. As expected, the Generalist Models were affected by

the cultivar. The R^2_{adj} was not significant for A_{max}, $\Phi(II)FP$, $\Phi(II)HP$ and F_v/F_mHP, and was low to

moderate, but significant, for the other parameters (R^2_{adj} = 0.14 − 0.44). Considering the cultivar

effect in the Interaction Model substantially improved the models for all the parameters, with R^2_{adj}

ranging from 0.32 to 0.77.

Non-photochemical quenching ($\Phi(NPQ)_{1077}$)

The $\Phi(NPQ)_{1077}$, calculated using the Phenocenter IMAGING-PAM, at 1077 µmols m^{-2} s^{-1} photons

during a light curve at flag leaf level was showing significant linear regressions for most of the

parameters and for both cultivars (Table 5; Supplementary Fig. S7). However, in this case, Cadenza

presented significant correlations for only a few parameters measured (i) by gas exchange: V_{cmax}, J,

$R_{photoresp}$ and A_{max} (R^2_{adj} = 0.24 – 0.49), (ii) by biochemical analysis: TSP, Rubisco, V_i and V_t (R^2_{adj} = 0.35

– 0.46), and by handheld device: SPAD, $\Phi(II)FM$, $\Phi(II)HP$ and F_v/F_mHP (R^2_{adj} = 0.28 – 0.54) with the

best fits for J, A_{max}, TSP, SPAD and F_v/F_mHP (R^2_{adj} = 0.49, 0.47, 0.46, 0.47 and 0.54, respectively). For

Bobwhite, the regressions with (i) the gas-exchange parameters were all significant (R^2_{adj} = 0.78 -

0.89), (ii) the biochemical parameters were mostly significant (R^2_{adj} = 0.36 – 0.76), except for the Chl

a and b content, and (iii) the handheld devices were significant only for $\Phi(II)FP$, $\Phi(II)FM$ and F_v/F_mHP

(R^2_{adj} = 0.25, 0.21 and 0.65, respectively). It should be noted that for $\Phi(NPQ)_{1077}$, the R^2_{adj} were

higher for Bobwhite for the gas-exchange and biochemical parameters, as well as for F_v/F_mHP of the

handheld devices. The Generalist Models showed signification R^2_{adj} for most of the parameters (R^2_{adj}

= 0.23 – 0.63), except for Chl a, b and SPAD. The Interaction Models showed significant cv. effects for

V_{cmax}, J, TSP, Chl a and b, V_t, SPAD and $\Phi(II)FM$, indicating a significantly difference between the two

cultivars. However, for these parameters, except for SPAD, the interactions were not significant,

indicating a similar slope for the regressions for both cultivars. Nonetheless, including the cv. effect

in the Interaction Models improved the R^2_{adj} for the aforementioned parameters compared to the

Generalist Models, whilst having little effect on the R^2_{adj} for the other parameters compared to the

Generalist Models.

DISCUSSION

Model performances and cultivar effect

The present study aimed to model photosynthetic parameters collected at leaf scale through gas-

exchange and by biochemical analysis using imaging technology. Five image variables collected at

pot scale in the field (NDVI and F_v/F_mSc) and at leaf scale in laboratory conditions (F_v/F_mPc, $\Phi(II)_{532}$

and $\Phi(NPQ)_{1077}$) were investigated as potential targets for further screening of photosynthetic

parameters in phenotyping trials. Overall, the results showed the possibilities to build linear models

to predict photosynthesis parameters based on the image variables, despite the varying

performance of the two cultivars. The Interaction Models proved that considering the cultivar effect

improved the models R^2_{adj} when the cultivar effect was significant compared to the Generalist

Models. The linear regression model performances were largely driven by the phenotypic variability

resulting from the stage of development of the two cultivars. As senescence progressed, a decrease

of the phenotypic values for each cultivar was observed for all the gas exchange parameters (V_{cmax}, J,

g_m, k_c^c, $R_{photoresp}$ and A_{max}) as well as the Rubisco related parameters (TSP, Rubisco, V_i, V_t), the

fluorescence parameters F_v/F_mHP collected with the HandiPEA and $\Phi(NPQ)_{1077}$ measured at the

Phenocenter. A contrasting behaviour was observed between Bobwhite and Cadenza for chlorophyll

variables (Chl a and b, SPAD) and the $\Phi(II)$ parameters measured by the handheld devices. The same

was observed for the other four image variables. Cadenza followed the same senescence pattern

with a decrease in phenotypic values with the senescence, explaining the good agreement between

the image and the ground variables. The lower model performances with $\Phi(NPQ)_{1077}$ were due to

the fact that a significant decrease of NPQ could only be seen at 21 days post anthesis (DPA). The

low model performances between the five image variables and the chlorophyll index resulted from

the fact that the chlorophyll amount increased up to 7 DPA before decreasing. In contrast, Bobwhite

displayed a different pattern with no significant variation of the phenotypic values across the five

dates of measurement for the chlorophyll variables (Chl a and b, SPAD), the $\Phi(II)$ variables as well as

for F_v/F_mSc, NDVI, F_v/F_mPc. While no significant decreases were observed for F_v/F_mSc, a decreasing

trend was still observed, which explains why the model performances were similar to Cadenza. A

decreasing trend was also observed for NDVI without significant differences between dates.

However, the small day-to-day fluctuations explained the absence of correlation with the

photosynthetic variables. The absence of trend and significant differences between dates of F_v/F_mPc

explained the absence of correlations with the ground variables for Bobwhite. The relatively good

performance of Bobwhite for $\Phi(II)_{532}$ seems to be due to the first date having a significant higher

value than the four other dates.

The observed patterns in Cadenza correspond to a typical wheat senescence pattern with a gradual

decrease in photosynthetic capacity along with the degradation of the chlorophyll (Thomas &

Howarth, 2000). On the other hand, Bobwhite is known as a slow maturaturing cultivar (Mackintosh

et al., 2006), and it was expected to see delayed decrease in phenotypic values for both

photosynthesis and chlorophyll variables, or a slower maturation compared to Cadenza, which

would correspond to a Type A or B stay green phenotype according to Thomas & Howarth (2000).

However, the current results suggest Bobwhite may belong to Type C (also called cosmetic stay-

green) which is described as undergoing a degradation of the photosynthetic activity as normal with,

however, no sign of degradation of the chlorophyll, which corresponds to the results observed in this

study, at least up to the end of the experiment at 21 days after anthesis. According to Thomas and

Ougham (2014), breeders tend to avoid using cosmetic stay-greens because they do not offer any

yield benefits. The opposite type, functional stay-green, is more desirable for breeding purposes as

photosynthesis is maintained longer during the maturation phase (Thomas & Ougham, 2014; Li *et*

al., 2022). However, screening populations for functional stray-green genotypes is complicated as it

would require gas-exchange measurements at multiple time points on a large number of individuals,

which is not feasible without a large number of instruments and trained users. Currently,

phenotyping studies on stay-green genotypes are based on screening materials with visual scoring,

SPAD, RGB images or spectral indices, without being able to distinguish cosmetic from functional

genotypes (Pinto *et al.*, 2016; Christopher *et al.*, 2018, Hassan *et al.*, 2021, Chapman *et al.*, 2021).

The last five years have seen successful studies predicting V_{cmax} and J using spectral data (Silva-Perez

et al., 2018; Meacham-Hensold *et al.*, 2019, Montes *et al.*, 2022). It would be interesting to see if

those approaches can overcome the challenges of identifying functional stay green phenotype in

field conditions.

Image variables vs traditional approaches

The results showed the image variables, tested at the plant scale (NDVI and F_v/F_mSc), and at the leaf

scale (F_v/F_mPc, $\Phi(II)_{532}$ and $\Phi(NPQ)_{1077}$) constitute good to strong estimators of the gas-exchange

(V_{cmax}, J, g_m, k_c^c, $R_{photoresp}$ and A_{max}) and the biochemical variables (TSP, Rubisco, V_i and V_t), when

senescence is driving the variability. As mentioned above, the model performances have been

strongly influenced by the cultivar disparity in term of the senescence patterns, resulting in strong

specificity between the ground variables and their relationship with the image-based variables. The

highest predictions for the gas-exchange variables were obtained with $\Phi(NPQ)_{1077}$ (R^2_{adj} = 0.78-0.89)

for the cultivar Bobwhite. NDVI, F_v/F_mPc and $\Phi(II)_{532}$ were also strong estimators for V_{cmax}, J and A_{max}

with the cultivar Cadenza. Lower performances were obtained using F_v/F_mSc but with acceptable

results ($R^2_{adj} \sim 0.50$). However, it was not possible to conclude which of the image variables was the

most relevant to investigate a particular parameter in the photosynthetic measurements collected

by gas-exchange or biochemical analysis.

NDVI has been used extensively over decades to monitor vegetation dynamics and quantify green

biomass at various scales (landscape to plot). It is well accepted that NDVI is not the most suitable

index to monitor instantaneous change in net CO_2 assimilation (Gamon *et al.*, 1995, Dobrowski *et al.*,

2005; Urban *et al.*, 2013). However, a few studies showed significant relationship between NDVI and

net CO_2 assimilation, mostly driven by the genetic variability as well as by the stages of development,

similarly to our study (Kumari *et al.*, 2012; Gonzalez-Dugo *et al.*, 2015; Camino *et al.*, 2018). Studies

in the literature looking at the link between NDVI and gas-exchange and biochemical parameters are

scarce to our knowledge. Letts *et al.* (2008) found an absence of correlation between A_{max} and NDVI

while Zhou *et al.* (2014) showed a high correlation with V_{cmax} on deciduous forest trees, mainly due

to the different phenological stages. Despite the absence of correlation observed for Bobwhite, due

to the reasons discussed, the Interaction Models showed that it was possible to use NDVI to predict

the different parameters related to carbon assimilation, either derived from A-C_i and A-Par curve

(V_{cmax}, J, g_m, k_c^c, $R_{photoresp}$, A_{max}) or from biochemical analysis (V_i and V_t) but not the Rubisco activation

state (Act, Table S3). The same was observed for N related parameters (TSP and Rubisco) and

chlorophyll pigment (Chl a and b, SPAD). Despite the strong discrepancy existing between the two

cultivars caused by their senescence behaviour, the Interaction Models showed that NDVI could be a

predictor for those variables with acceptable results. Gitelson *et al.* (1998, 2003) showed that NDVI

was far from the best index to predict chlorophyll content at leaf scale due to its lack of sensitivity

from medium chlorophyll concentration. However, other studies proved that, at the canopy scale,

NDVI might be a useful indicator if no other indices are available (Zarco-Tejada *et al.*, 2005; Camino

et al., 2018; Alordzinu *et al.*, 2021).

The maximum efficiency of the photosystem II (F_v/F_m) is commonly used as an indicator of plant

health status and to monitor stress responses in large population screening, as it is extremely

difficult to screen population for gas-exchange, especially in field conditions (Pask *et al.*, 2012).

However, Pask *et al.* (2012) noted that F_v/F_m may not always be a reliable indicator of crop

performance. While F_v/F_m is often used as a proxy for photosynthetic performance in phenotyping,

its link with gas exchange measurements is not yet fully established. Some studies have found

positive correlations with mixed success for V_{cmax} (R^2 = 0.02 - 0.13 and R^2 = 0.11 to 0.47 in Zhuang *et*

al., 2020 and Mission *et al.*, 2006, respectively), J (R^2 = 0.09 – 0.28 in Zhuang *et al.*, 2020) and A_{max} (R^2

= 0.52, Ishida *et al.*, 2014). In the present study, the cultivar models performance for gas-exchange

(V_{cmax}, J, g_m, k_c^c, $R_{photoresp}$, A_{max}) and biochemical parameters (V_i, V_t) with F_vF_mPc ranged from 0.57 to

0.85 for Cadenza and are nearly zero for Bobwhite due to its senescence behaviour. The same would

have been expected at the whole plant scale (F_v/F_mSc). However, the R^2_{adj} range of the two cultivars

was between 0.32 to 0.75. Whilst results showed no significant differences between dates, a

decreasing trend was observed, leading to an increase in model performances for Bobwhite at the

whole plant scale. Despite the contrasting results between the two sets of measurements, the

Interaction models gave similar performances, ranging from 0.28 to 0.66. This suggests that F_v/F_m is

a reliable indicator of photosynthetic performance at a seasonal scale, regardless of the

measurement environment. Using F_v/F_m as a predictor of the chlorophyll content (Chl a, b and SPAD)

or the protein content variables (TSP, Rubisco) gave low to high performances depending on the

cultivar (*i.e.*, senescence behaviour) and the scale of the measurement. These findings are consistent

with the previous literature, which has reported high correlations between Fv/Fm and these

variables accross different stages of development (Peng *et al.*, 2017, Hailemichael *et al.*, 2016, Jia *et*

al., 2019) and/or under various nitrogen or irrigation treatments (Jia *et al.*, 2019, Zarco-Tejada *et al.*,

2013).

The PSII operating efficiency ($\Phi(II)$), also known as quantum efficiency, provides an overall

measurement of the efficiency of the PSII reaction centre in light conditions (Genty *et al.*, 1989). This

parameter indicates the proportion of light absorbed by chlorophyll associated with PSII that is used

in photochemistry, and is strongly correlated with net CO2 assimilation (McAusland *et al.*, 2013). It is
also affected by the degradation of the photosystem during senescence as demonstrared in Mu *et al.* (2010) and Dai *et al.* (2004) where $\Phi(II)$ began to decrease significantly after 15 DPA. Similarly,
Kong *et al.* (2015) observed a significant decrease in $\Phi(II)$ around the same time period. In the
present study significant differences in $\Phi(II)_{532}$, $\Phi(II)FM$ and $\Phi(II)HP$ for Cadenza were observed after
14 DPA, consistent with previous research (Fig. 3). However, variations between dates were not
uniform across instruments. No significant difference was observed between dates for Cadenza and
Bobwhite with $\Phi(II)FP$, as well as $\Phi(II)FM$ for Bobwhite. For $\Phi(II)HP$, values reached their peak at 7
DPA and were significantly different at 28 DPA, while for $\Phi(II)_{532}$, the highest values were at booting
and no significant differences were observed from anthesis to 28 DPA. The present study showed
that $\Phi(II)$ under moderate light is a good estimator for carbon assimilation related parameters
measured either by gas-exchange (V_{cmax}, J, g_m, k_c^c, $R_{photoresp}$, A_{max}) or derived from biochemical
analysis (V_i and V_t). Except for k_c^c, the model performances were better for Cadenza than for
Bobwhite, possibly due to the absence of significant differences between dates mentioned above.
High and positive correlations between $\Phi(II)$ and V_{cmax}, J, A_{sat} and g_m were also found in Sun *et al.* (2014), with variation caused by contrasted levels of ozone. Almaida *et al.* (2021) also observed
positive correlations with A_{max} and V_{cmax} resulting from genetic variability of the sugar cane cultivars
and wild types. Using $\Phi(II)$ as a predictor of the chlorophyll content will give reasonable results as
long as both variables behave similarly during senescence.

Non-photochemical quenching (NPQ) is one of the three main mechanisms for dissipating the energy
of the light absorbed by the chlorophyll pigments. NPQ competed with the photochemistry and the
re-emission of the energy as a photon of fluorescence (Porcar-Castell *et al.*, 2014). NPQ is a
photoprotective mechanism removing the excess excitation energy within the chlorophyll-containing
complexes (Murchie and Lawson, 2013). However, contradictory results have been reported in the
literature regarding changes of NPQ after anthesis and during the senescence in wheat. Some
authors observed an increase of NPQ during the period post anthesis period, with measurements
performed either in the morning or at midday (Dai *et al.*, 2004), and in different shading conditions
(Mu *et al.*, 2010). Xu *et al.* (2017) and Shao *et al.* (2013) observed an increase in NPQ peaking at 21
and 30 DPA, respectively, before decreasing. However, Kong *et al.* (2015) reported a significant
decrease of NPQ measured on the flag leaves from anthesis to 32 DPA, and Peng *et al.* (2021)
observed a decrease in NPQ between anthesis and maturity stages in wheat grown with various
nitrogen inputs. The results of the present study showed a decreasing trend in $\Phi(NPQ)_{1077}$ from
anthesis, becoming significant from 21 DPA for both cultivars compared to anthesis time. The
model's performances with the photosynthesis parameters derived from gas-exchange and from

biochemical analysis were higher for Bobwhite (R^2_{adj} = 0.78 - 0.89) than Cadenza, indicating a better
match between the senescence pattern of $\Phi(NPQ)_{1077}$ and those variables in Bobwhite. Negative
correlations with net CO_2 assimilation have been reported (Ishida *et al.*, 2014, Zarco-Tejada *et al.*,
2013), but no data concerning A_{max} or the other parameters derived from the A-C_i and A-PAR curves
could be found in the literature to our knowledge. Negative correlations with Chl a content have
been also reported in Ishida *et al.* (2014) and Zarco-Tejada *et al.* (2013), mainly driven by drought.

The handheld fluorescence devices were used as comparison with the fluorescence imagers, as well
as with NDVI. While the use of NDVI to estimate F_v/F_m and $\Phi(II)$ could be questionable, the results
did not differ much from those reported in the literature, and low to moderate relationships were
observed (Peng *et al.*, 2017; Tan *et al.*, 2012, Jia *et al.*, 2019). The difference in performance
between Bobwhite and Cadenza is attributed to their senescence behaviour, as discussed earlier.
Usually, indices such as PRI, red-edge indices and fluorescence band indices are preferred to report
variation in fluorescence. However, the hyperspectral image collection was done under non-optimal
outdoor conditions, with variable ambient illumination between the end of September and the
beginning of November in the UK. Consequently, the above-mentioned indices would not be reliable
to report the variations in fluorescence, as the conditions between acquisition dates were too
inconsistent.

The variation of F_v/F_m measured by the two imagers reflected the variation of F_v/F_m measured using
the HandyPea for Cadenza. For Bobwhite, although no significance difference was observed between
date for F_v/F_mSc at the plant scale, the observed decreasing trend explained the relatively good
correlation with the value collected with HandyPea. The measurements resulting from the
Phenocenter did not show variations between time points, while the data collected with the
HandyPea on the same leaves showed a significant decrease over time. The results are unexpected
as the leaves were cut from the plants just before being imaged by the fluorescence of the
Phenocenter, and no sensible explanation could be found.

The variation of $\Phi(NPQ)_{1077}$ was better reflected by the variation of F_v/F_m than by $\Phi(II)$, in agreement
with Kong et al (2015), where the decrease in NPQ appeared to be more concomitant with the
decrease of F_v/F_m than with the decrease of $\Phi(II)$. The authors also mentioned a regional accordance
of imaging F_v/F_m and NPQ across the leaves, concluding that this would indicate that NPQ is closely
associated with the quantum efficiency of PSII photochemistry.

Throughput consideration of the different approaches

Reynolds *et al.* (2012) noted that breeders are interested in reducing the size of their test
populations to achieve the desired genotype at a reasonable probability and cost, even if the
methods may be imperfect from a physiological and theoretical standpoint. In the case of
photosynthetic traits, gas-exchange and biochemical analysis represent the gold standards but are
also considered low throughput. The time required to perform an A-C_i followed by an A-PAR curve
takes approximately 50-60 minutes per flag leaf for an experienced user in controlled conditions.
Biochemical analysis involves destructive sampling that also requires processing (freeze drying,
grinding, etc) before quantification of TSP, Rubisco and enzymatic reactions. Handheld fluorescence
instruments are used for pre-screening of populations for photosynthesis performance using either
$\Phi(II)$ or F_v/F_m as they are quite fast. Measuring $\Phi(II)$ with the Fluorpen is straightforward and takes
only 10 seconds per leaf, potentially allowing for measurement of 360 leaves per hour, which is the
fastest instrument to our knowledge. However, as shown in this study, the correlations with image
fluorescence are weaker than those obtained with the HandyPea and the FMS2.

The advantages of HTP methods are the higher phenotyping capacity and, in some cases, the
reduced necessity of human intervention. However, the throughput is highly dependent of the type
of the technologies used. For example, in the case of the Phenocenter, an operator is required to
program the execution, but the measurements are automatic and there is the possibility to perform
an automatic loading of sample, pre inserted in the machine. The time frame for measuring F_v/F_m,
followed by a light curve for $\Phi(II)$ and $\Phi(NPQ)$, on three flag leaves is around 40 minutes, including
the 30 minutes of dark adaptation. The system should be able to host several boxes of three leaves
for dark adaptation, allowing to measure 18 leaves per hour in total. The Field Scanalyzer requires an
operator to program the platform, but the platform runs automatically over the full experiment once
it is launched. The throughput of the fluorescence camera is 40 plots (or pots) per hour, but this
includes the time it takes to move the camera between targets. The visible diameter of the flash
used to induce fluorescence is 1.2 to 1.4 meters, which can also trigger a response to light in nearby
plots. Therefore, the device must be carefully routed to measure each plot in complete dark
adaptation, which decreases the camera's throughput. The hyperspectral camera, set up in medium
spatial resolution (Sadeghi-Tehran *et al.*, 2021), allows collection of 26 scans per hours. As the area
covered is about 1.8 x 1.5 m, the number of plots or pots scanned will vary depending on the size of
targets. The two phenotyping platforms discussed in the previous paragraph offer significant
advantages over gas-exchange measurements in terms of speed and reduced human intervention.
However, their throughput is still quite low compared to handheld fluorescence instruments, which
are commonly used as proxy of photosynthesis. Nonetheless, spectral data are still promising for
fluorescence and photosynthetic traits. The Fraunhofer Line Depth (FLD) can be used to retrieve sun-

induced fluorescence (Porcar-Castell *et al.*, 2014) and the last decade have seen an increase of
prediction of V_{cmax} and J using the full range of spectral data (Silva-Perez *et al.*, 2018; Meacham-
Hensold *et al.*, 2019, Montes *et al.*, 2022). Most of those developments have relied on single
spectroradiometer, as they are much faster than the imager mounted on ground-based system.
However, hyperspectral imaging with UAV provides data on large scale, enabling access to those
traits at lower spatial resolution (Zarco-Tejada *et al.*, 2013; Camino *et al.*, 2018, 2019).

Conclusion

The present study showed that photosynthetic traits obtained either by gas-echange or biochemical
analysis could be monitored and quantified susccesfully using imaging technologies as long as the
senescence is driving the variability. Despite the contrasted behaviour between the two cultivars,
Bobwhite and Cadenza, linear regression models which account for both the cultivar and the
interaction effects, further improved the modelling of photosynthesis indicators. Future work will be
necessary to evaluate the link between the image variables and gas-exchange during earlier stages
of development. The results also showed the importance of prior knowledge of the stay green type
of the cultivar used, as it can strongly impact the outcomes. It also emphasises the need to develop
sensitive enough approaches to differentiate cosmetic from functional stay green genotypes that
breeders can use for photosynthetic traits.

Supplementary data

Th following supplementary data are available at the JXB online:

Fig. S1. Flag leaf holder for Phenocenter analysis

Fig. S2. Pots settings under the Field Scanalyzer

Fig. S3. Linear regressions between Fv/FmSc and selected variables

Fig. S4. Linear regressions between NDVI and selected variables

Fig. S5. Linear regressions between F_v/F_mPc and selected variables

Fig. S6. Linear regressions between $\Phi(II)_{532}$ and selected variables

Fig. S7. Linear regressions between $\Phi(NPQ)_{1077}$ and selected variables

Table S1. Linear regressions parameters between images and A-C_i curves

Table S2. Linear regressions parameters between images and A-PAR curves

Table S3. Linear regressions parameters between images and biochemical variables

Table S4. Linear regressions parameters between images and handhled devices variables

Table S5. Mean and standard deviation for each variables per date and per cultivar

Dataset S1: Phenotypic data

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Author contributions

JPP and NV designed and performed the experiments and analysed data; TA performed the image
acquisition at the PhenoCenter (CHAP), PST performed the image extraction and analysis from the
Field Scanalyzer; DO and ECS peformed the biochemical analyses; NV and JPP wrote the manuscript;
NV, JPP, TA, PST, DO, ECS and MJH revised and edited the manuscript; JPP, ECS and MJH enabled to
secure funding for the project leading to this publication. All authors reviewed and approved the
final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Data availability

All data supporting the findings of this study are available within the paper and within its
supplementary data published online.

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Tables

Table 1. Parameters of linear regressions between chlorophyll fluorescence (F_v/F_m) measured by the Field Scanalyzer and other measured parameters

Parameters			Bobwhite			Cadenza			Generalist Model			Interaction Model							
y		x	F	p	R^2_{adj}	F	p	R^2_{adj}	F	p	R^2_{adj}	F (Par.)	p (Par.)	F (cv..)	p (cv..)	F (Int.)	p (Int.)	p (Mod.)	R^2_{adj}
V_{cmax}	vs	F_v/F_mSc	12.7	0.003	0.46	17.9	0.001	0.55	12.8	0.001	0.29	18.3	0.000	14.2	0.001	0.0	0.954	0.000	0.50
J	vs	F_v/F_mSc	14.3	0.002	0.49	20.7	0.001	0.58	17.6	0.000	0.36	24.5	0.000	12.9	0.001	0.0	0.858	0.000	0.54
g_m	vs	F_v/F_mSc	22.5	0.000	0.61	17.1	0.001	0.54	25.3	0.000	0.46	31.3	0.000	6.9	0.014	1.7	0.202	0.000	0.56
k_c^c	vs	F_v/F_mSc	7.6	0.016	0.32	7.9	0.015	0.33	11.5	0.002	0.27	12.2	0.002	3.6	0.067	0.0	0.892	0.006	0.31
$R_{photoresp}$	vs	F_v/F_mSc	16.8	0.001	0.53	16.3	0.001	0.52	29.4	0.000	0.49	31.6	0.000	4.0	0.055	0.0	0.916	0.000	0.53
A_{max}	vs	F_v/F_mSc	13.2	0.003	0.47	19.1	0.001	0.56	44.8	0.000	0.60	43.0	0.000	0.9	0.365	0.0	0.943	0.000	0.58
TSP	vs	F_v/F_mSc	19.5	0.001	0.57	23.3	0.000	0.61	18.4	0.000	0.38	27.8	0.000	16.3	0.000	0.0	0.827	0.000	0.59
Chl a	vs	F_v/F_mSc	0.1	0.767	-0.07	18.3	0.001	0.55	5.3	0.029	0.13	8.7	0.007	13.6	0.001	6.0	0.021	0.000	0.47
Chl b	vs	F_v/F_mSc	0.8	0.392	-0.02	7.8	0.015	0.33	0.8	0.386	-0.01	1.1	0.297	9.5	0.005	5.3	0.030	0.006	0.31
Rubisco	vs	F_v/F_mSc	5.4	0.037	0.24	11.6	0.005	0.43	12.9	0.001	0.29	14.1	0.001	4.5	0.043	0.0	0.878	0.003	0.35
V_i	vs	F_v/F_mSc	13.5	0.003	0.47	42.6	0.000	0.75	32.0	0.000	0.52	45.0	0.000	13.3	0.001	0.1	0.736	0.000	0.66
V_t	vs	F_v/F_mSc	24.3	0.000	0.62	30.7	0.000	0.68	19.6	0.000	0.39	34.2	0.000	22.7	0.000	0.1	0.725	0.000	0.65
SPAD	vs	F_v/F_mSc	0.7	0.407	-0.02	13.1	0.003	0.46	1.0	0.331	0.00	1.9	0.181	25.4	0.000	2.8	0.104	0.000	0.48
Φ(II)FP	vs	F_v/F_mSc	6.8	0.022	0.29	9.8	0.008	0.39	31.7	0.000	0.51	30.2	0.000	0.1	0.723	0.5	0.493	0.000	0.49
Φ(II)FM	vs	F_v/F_mSc	2.4	0.145	0.09	26.0	0.000	0.64	11.0	0.003	0.26	16.1	0.000	12.7	0.001	2.1	0.155	0.000	0.49
Φ(II)HP	vs	F_v/F_mSc	2.5	0.136	0.10	26.6	0.000	0.65	28.2	0.000	0.48	26.3	0.000	0.0	0.980	0.0	0.834	0.000	0.45
F_v/F_mHP	vs	F_v/F_mSc	11.7	0.005	0.43	25.0	0.000	0.63	60.3	0.000	0.67	56.1	0.000	0.0	0.863	0.0	0.946	0.000	0.65

y and x, elements of the regression; p (Eq), p-value of the regression; R^2_{adj}, adjusted coefficient of determination of the regression; p (Eq) < 0.05, the regression is significant (rows in black);

Table 2. Parameters of linear regressions between the normalized difference vegetation index (NDVI) measured by the Field Scanalyzer and other measured parameters

Parameters			Bobwhite			Cadenza			Gen. Model			Line effect							
y		x	F	p	R^2_{adj}	F	p	R^2_{adj}	F	p	R^2_{adj}	F (Par.)	p (Par.)	F (cv.)	p (cv.)	F (Int.)	p (Int.)	p (Eq)	R^2_{adj}
V_{cmax}	vs	NDVI	0.7	0.405	-0.02	43.0	0.000	0.75	21.2	0.000	0.41	25.0	0.000	4.5	0.043	2.6	0.121	0.000	0.50
J	vs	NDVI	0.8	0.379	-0.01	74.0	0.000	0.84	31.6	0.000	0.51	38.2	0.000	3.8	0.063	4.1	0.053	0.000	0.60
g_m	vs	NDVI	1.2	0.302	0.01	18.3	0.001	0.55	16.3	0.000	0.35	15.3	0.001	0.1	0.794	0.3	0.587	0.006	0.30
k_c^c	vs	NDVI	1.2	0.298	0.01	36.4	0.000	0.72	27.3	0.000	0.48	28.1	0.000	1.1	0.307	1.8	0.188	0.000	0.49
$R_{photoresp}$	vs	NDVI	0.9	0.368	-0.01	23.2	0.000	0.61	24.9	0.000	0.45	24.7	0.000	0.0	0.954	1.7	0.202	0.000	0.45
A_{max}	vs	NDVI	0.9	0.352	-0.01	34.2	0.000	0.70	33.1	0.000	0.53	35.2	0.000	1.6	0.212	2.2	0.152	0.000	0.55
TSP	vs	NDVI	1.5	0.247	0.03	52.2	0.000	0.79	27.7	0.000	0.48	31.2	0.000	3.7	0.066	1.9	0.180	0.000	0.54
Chl a	vs	NDVI	3.1	0.101	0.13	13.3	0.003	0.47	6.6	0.016	0.16	9.5	0.005	5.0	0.035	9.4	0.005	0.001	0.42
Chl b	vs	NDVI	4.4	0.057	0.19	7.2	0.019	0.31	2.3	0.139	0.04	3.3	0.081	6.5	0.017	7.1	0.013	0.004	0.32
Rubisco	vs	NDVI	0.1	0.740	-0.07	37.0	0.000	0.72	20.0	0.000	0.40	21.8	0.000	0.8	0.369	3.7	0.065	0.000	0.45
V_i	vs	NDVI	0.4	0.564	-0.05	46.3	0.000	0.76	26.3	0.000	0.47	28.4	0.000	0.7	0.426	3.6	0.069	0.000	0.51
V_t	vs	NDVI	0.6	0.443	-0.03	49.2	0.000	0.78	22.0	0.000	0.42	25.5	0.000	3.8	0.062	2.7	0.110	0.000	0.50
SPAD	vs	NDVI	0.6	0.467	-0.03	14.6	0.002	0.49	3.9	0.059	0.09	7.1	0.013	18.7	0.000	6.4	0.017	0.000	0.50
Φ(II)FP	vs	NDVI	0.4	0.545	-0.05	11.2	0.005	0.42	18.9	0.000	0.38	22.0	0.000	4.6	0.042	2.0	0.170	0.000	0.47
Φ(II)FM	vs	NDVI	0.1	0.808	-0.07	29.9	0.000	0.67	14.3	0.001	0.32	18.7	0.000	3.6	0.069	6.8	0.015	0.000	0.47
Φ(II)HP	vs	NDVI	0.0	0.927	-0.08	13.0	0.003	0.46	9.6	0.004	0.23	10.5	0.003	3.2	0.084	1.3	0.261	0.007	0.29
F_v/F_mHP	vs	NDVI	0.6	0.466	-0.03	41.9	0.000	0.75	29.9	0.000	0.50	35.5	0.000	4.9	0.036	2.3	0.138	0.000	0.58

y and x, elements of the regression; p (Eq), p-value of the regression; R^2_{adj}, adjusted coefficient of determination of the regression; p (Eq) < 0.05, the regression is significant (rows in black);

Table 3. Parameters of linear regressions between chlorophyll fluorescence (F_v/F_m) measured by the Phenocenter and other measured parameters

Parameters			Bobwhite			Cadenza			Gen. Model			Line effect							
y		x	F	p	R^2_{adj}	F	p	R^2_{adj}	F	p	R^2_{adj}	F (Par.)	p (Par.)	F (cv.)	p (cv.)	F (Int.)	p (Int.)	p (Eq)	R^2_{adj}
V_{cmax}	vs	F_v/F_mPc	0.7	0.407	-0.02	44.3	0.000	0.76	32.1	0.000	0.52	31.3	0.000	0.2	0.662	1.1	0.308	0.000	0.50
J	vs	F_v/F_mPc	1.0	0.345	0.00	82.3	0.000	0.85	47.3	0.000	0.62	46.8	0.000	0.0	0.914	1.7	0.206	0.000	0.61
g_m	vs	F_v/F_mPc	0.1	0.756	-0.07	19.6	0.001	0.57	12.6	0.001	0.28	12.5	0.002	0.9	0.345	0.9	0.360	0.009	0.28
k_c^c	vs	F_v/F_mPc	0.0	0.923	-0.08	28.7	0.000	0.66	20.8	0.000	0.41	21.6	0.000	0.4	0.531	2.6	0.118	0.001	0.43
$R_{photoresp}$	vs	F_v/F_mPc	0.6	0.464	-0.03	28.2	0.000	0.66	23.7	0.000	0.44	25.3	0.000	2.8	0.108	1.1	0.304	0.000	0.47
A_{max}	vs	F_v/F_mPc	0.1	0.710	-0.07	42.7	0.000	0.75	21.1	0.000	0.41	28.9	0.000	9.8	0.004	2.5	0.129	0.000	0.57
TSP	vs	F_v/F_mPc	0.1	0.809	-0.07	45.4	0.000	0.76	27.1	0.000	0.47	27.8	0.000	0.0	0.949	2.8	0.109	0.000	0.49
Chl a	vs	F_v/F_mPc	7.5	0.017	0.32	11.8	0.004	0.44	23.1	0.000	0.43	22.5	0.000	1.3	0.272	0.0	0.921	0.001	0.42
Chl b	vs	F_v/F_mPc	5.6	0.035	0.25	8.4	0.012	0.35	15.2	0.001	0.33	16.1	0.000	3.5	0.071	0.0	0.957	0.002	0.36
Rubisco	vs	F_v/F_mPc	0.0	0.854	-0.07	31.0	0.000	0.68	18.6	0.000	0.38	19.9	0.000	0.3	0.562	3.6	0.070	0.001	0.42
V_i	vs	F_v/F_mPc	2.8	0.115	0.12	29.8	0.000	0.67	31.5	0.000	0.51	30.2	0.000	0.8	0.374	0.0	0.859	0.000	0.49
V_t	vs	F_v/F_mPc	1.5	0.246	0.03	46.1	0.000	0.76	34.9	0.000	0.54	33.0	0.000	0.1	0.797	0.4	0.523	0.000	0.51
SPAD	vs	F_v/F_mPc	0.2	0.681	-0.06	16.2	0.001	0.52	15.4	0.001	0.33	21.3	0.000	10.9	0.003	1.8	0.191	0.000	0.52
$\Phi(II)$FP	vs	F_v/F_mPc	0.1	0.737	-0.07	14.8	0.002	0.50	12.6	0.001	0.29	18.6	0.000	13.6	0.001	1.8	0.188	0.000	0.52
$\Phi(II)$FM	vs	F_v/F_mPc	3.0	0.105	0.13	61.6	0.000	0.81	54.8	0.000	0.65	52.2	0.000	0.2	0.623	0.4	0.529	0.000	0.63
$\Phi(II)$HP	vs	F_v/F_mPc	12.4	0.004	0.45	25.2	0.000	0.63	17.9	0.000	0.37	28.4	0.000	13.4	0.001	4.9	0.035	0.000	0.60
F_v/F_mHP	vs	F_v/F_mPc	1.7	0.211	0.05	65.0	0.000	0.82	22.3	0.000	0.42	35.8	0.000	18.7	0.000	0.3	0.582	0.000	0.64

y and x, elements of the regression; p (Eq), p-value of the regression; R^2_{adj}, adjusted coefficient of determination of the regression; p (Eq) < 0.05, the regression is significant (rows in black);

Table 4. Parameters of linear regressions between photosystem II quantum efficiency ($\Phi(II)_{532}$) measured by the Phenocenter and other measured parameters

Parameters			Bobwhite			Cadenza			Gen. Model			Line effect							
y		x	F	p	R^2_{adj}	F	p	R^2_{adj}	F	p	R^2_{adj}	F (Par.)	p (Par.)	F (cv.)	p (cv.)	F (Int.)	p (Int.)	p (Eq)	R^2_{adj}
V_{cmax}	vs	$\Phi(II)_{532}$	19.2	0.001	0.57	34.0	0.000	0.70	23.5	0.000	0.44	37.8	0.000	17.6	0.000	1.4	0.244	0.000	0.65
J	vs	$\Phi(II)_{532}$	17.9	0.001	0.55	65.9	0.000	0.82	21.6	0.000	0.42	48.5	0.000	32.3	0.000	4.6	0.041	0.000	0.74
g_m	vs	$\Phi(II)_{532}$	15.4	0.002	0.51	26.6	0.000	0.65	9.9	0.004	0.23	18.0	0.000	24.9	0.000	0.0	0.850	0.000	0.58
k_c^c	vs	$\Phi(II)_{532}$	24.9	0.000	0.63	21.0	0.001	0.59	12.7	0.001	0.29	22.0	0.000	22.0	0.000	0.5	0.478	0.000	0.59
$R_{photoresp}$	vs	$\Phi(II)_{532}$	16.4	0.001	0.52	41.2	0.000	0.74	8.6	0.007	0.21	21.7	0.000	41.5	0.000	2.8	0.108	0.000	0.68
A_{max}	vs	$\Phi(II)_{532}$	9.0	0.010	0.36	73.2	0.000	0.84	3.9	0.057	0.09	13.4	0.001	64.0	0.000	5.5	0.027	0.000	0.73
TSP	vs	$\Phi(II)_{532}$	9.3	0.009	0.37	65.0	0.000	0.82	19.6	0.000	0.39	36.3	0.000	21.9	0.000	4.1	0.052	0.000	0.67
Chl a	vs	$\Phi(II)_{532}$	0.9	0.370	-0.01	13.0	0.003	0.46	7.0	0.013	0.17	9.5	0.005	1.0	0.328	11.4	0.002	0.001	0.39
Chl b	vs	$\Phi(II)_{532}$	3.2	0.095	0.14	7.5	0.017	0.32	5.7	0.024	0.14	7.2	0.012	0.1	0.802	9.6	0.005	0.004	0.32
Rubisco	vs	$\Phi(II)_{532}$	4.2	0.061	0.19	30.9	0.000	0.68	9.4	0.005	0.22	14.7	0.001	14.8	0.001	3.0	0.094	0.000	0.50
V_i	vs	$\Phi(II)_{532}$	6.3	0.026	0.27	43.1	0.000	0.75	10.3	0.003	0.24	19.3	0.000	22.7	0.000	3.8	0.062	0.000	0.60
V_t	vs	$\Phi(II)_{532}$	7.3	0.018	0.31	69.7	0.000	0.83	20.1	0.000	0.40	34.8	0.000	18.3	0.000	4.3	0.048	0.000	0.65
SPAD	vs	$\Phi(II)_{532}$	0.3	0.609	-0.05	21.6	0.000	0.60	18.9	0.000	0.38	27.2	0.000	0.1	0.713	14.2	0.001	0.000	0.57
$\Phi(II)FP$	vs	$\Phi(II)_{532}$	6.5	0.024	0.28	7.2	0.019	0.31	0.2	0.623	-0.03	0.4	0.510	23.6	0.000	1.2	0.281	0.000	0.43
$\Phi(II)FM$	vs	$\Phi(II)_{532}$	2.6	0.132	0.10	47.0	0.000	0.77	15.6	0.000	0.34	25.4	0.000	11.3	0.002	8.2	0.008	0.000	0.59
$\Phi(II)HP$	vs	$\Phi(II)_{532}$	0.9	0.371	-0.01	25.5	0.000	0.64	0.4	0.545	-0.02	0.6	0.435	19.2	0.000	1.9	0.184	0.001	0.39

| F_v/F_mHP | vs | $\Phi(II)_{532}$ | 8.3 | 0.013 | 0.34 | 131.7 | 0.000 | 0.90 | 2.2 | 0.153 | 0.04 | 8.9 | 0.006 | 84.4 | 0.000 | 4.7 | 0.040 | 0.000 | 0.77 |

y and x, elements of the regression; p (Eq), p-value of the regression; R^2_{adj}, adjusted coefficient of determination of the regression; p (Eq) < 0.05, the regression is significant (rows in black);

Table 5. Parameters of linear regressions between non-photochemical quenching ($\Phi(NPQ)_{1077}$) measured by the Phenocenter and other measured parameters

Parameters			Bobwhite			Cadenza			Gen. Model			Line effect							
y		x	F	p	R^2_{adj}	F	p	R^2_{adj}	F	p	R^2_{adj}	F (Par.)	p (Par.)	F (cv.)	p (cv.)	F (Int.)	p (Int.)	p (Eq)	R^2_{adj}
V_{cmax}	vs	$\Phi(NPQ)_{1077}$	63.6	0.000	0.82	6.0	0.031	0.28	16.8	0.000	0.36	20.8	0.000	7.9	0.009	0.4	0.544	0.000	0.48
J	vs	$\Phi(NPQ)_{1077}$	75.7	0.000	0.84	13.5	0.003	0.49	30.0	0.000	0.51	36.5	0.000	7.7	0.010	0.1	0.743	0.000	0.60
g_m	vs	$\Phi(NPQ)_{1077}$	101.2	0.000	0.88	3.6	0.084	0.16	25.2	0.000	0.46	27.1	0.000	1.1	0.306	3.0	0.096	0.000	0.50
k_c^c	vs	$\Phi(NPQ)_{1077}$	50.2	0.000	0.78	4.0	0.069	0.19	17.3	0.000	0.37	17.3	0.000	1.6	0.217	0.3	0.583	0.002	0.37
$R_{photoresp}$	vs	$\Phi(NPQ)_{1077}$	112.5	0.000	0.89	5.1	0.043	0.24	26.3	0.000	0.47	24.8	0.000	0.2	0.634	0.2	0.651	0.000	0.44
A_{max}	vs	$\Phi(NPQ)_{1077}$	55.8	0.000	0.80	12.5	0.004	0.47	49.3	0.000	0.63	46.7	0.000	0.5	0.495	0.1	0.782	0.000	0.61
TSP	vs	$\Phi(NPQ)_{1077}$	20.9	0.001	0.59	12.0	0.005	0.46	21.3	0.000	0.42	24.2	0.000	5.4	0.029	0.3	0.612	0.000	0.49
Chl a	vs	$\Phi(NPQ)_{1077}$	0.0	0.852	-0.07	4.4	0.057	0.21	1.1	0.300	0.00	1.4	0.248	4.9	0.037	3.8	0.061	0.034	0.20
Chl b	vs	$\Phi(NPQ)_{1077}$	1.5	0.241	0.04	2.5	0.141	0.10	0.0	0.935	-0.04	0.0	0.926	6.3	0.019	3.9	0.059	0.034	0.20
Rubisco	vs	$\Phi(NPQ)_{1077}$	9.0	0.010	0.36	8.1	0.015	0.35	15.7	0.000	0.34	15.5	0.001	1.2	0.276	0.3	0.560	0.004	0.33
V_i	vs	$\Phi(NPQ)_{1077}$	24.1	0.000	0.62	9.1	0.011	0.38	24.3	0.000	0.45	24.1	0.000	1.8	0.193	0.0	0.855	0.000	0.45
V_t	vs	$\Phi(NPQ)_{1077}$	44.4	0.000	0.76	10.4	0.007	0.42	23.1	0.000	0.44	27.9	0.000	7.6	0.011	0.0	0.961	0.000	0.54
SPAD	vs	$\Phi(NPQ)_{1077}$	0.8	0.398	-0.02	12.6	0.004	0.47	2.1	0.161	0.04	4.2	0.050	24.0	0.000	6.0	0.022	0.000	0.53
$\Phi(II)$FP	vs	$\Phi(NPQ)_{1077}$	5.8	0.032	0.25	2.2	0.160	0.09	9.1	0.005	0.23	9.6	0.005	3.3	0.080	0.1	0.713	0.013	0.27
$\Phi(II)$FM	vs	$\Phi(NPQ)_{1077}$	4.8	0.047	0.21	11.7	0.005	0.45	9.8	0.004	0.24	11.9	0.002	6.3	0.019	1.4	0.247	0.002	0.37
$\Phi(II)$HP	vs	$\Phi(NPQ)_{1077}$	2.9	0.113	0.12	5.9	0.031	0.28	11.2	0.002	0.27	11.2	0.003	1.9	0.179	0.0	0.945	0.013	0.26
F_v/F_mHP	vs	$\Phi(NPQ)_{1077}$	26.5	0.000	0.65	16.2	0.002	0.54	49.4	0.000	0.63	51.7	0.000	3.1	0.089	0.1	0.733	0.000	0.65

y and x, elements of the regression; p (Eq), p-value of the regression; R^2_{adj}, adjusted coefficient of determination of the regression; p (Eq) < 0.05, the regression is significant (rows in black);

Figure legends

Fig. 1. Example Phenocenter images for chlorophyll fluorescence (F_v/F_m) and photosystem II quantum efficiency ($\Phi(II)$) analysis. $\Phi(II)_X$, photosystem II quantum efficiency at the light level of X µmols photons $m^{-2} s^{-1}$.

Fig. 2. Example Field Scanalyzer images for chlorophyll fluorescence (F_v/F_mSc). Z4.5, booting stage; Z6.5, anthesis; Z6.5 + 7d, 7 days post-anthesis; Z6.5 + 14d, 14 days post-anthesis; Z6.5 + 21d, 21 days post-anthesis.

Fig. 3. Mean and standard deviation for each variable for Bobwhite (orange) and Cadenza (blue) at booting (Z4.5), anthesis (Z6.5) and at 7-, 14- and 21-days post anthesis (Z6.5+7, +14, +21) for (A) gas-exchange, (B) biochemical, (C), handheld devices and, (D) image variables. Letters come from the post-hoc test *HSD.test* allowing multiple comparisons of treatments (here time point and cultivar) by means of Tukey. Different letters indicate a statistical difference between cultivar and/or date (a = 0.05).

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A – Gas-exchange

B – Biochemical analysis

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