Phenotypic variation in photosynthetic traits in wheat grown under field *versus* glasshouse conditions

Running title: Mismatch between field *versus* glasshouse-grown plants

Cristina R. G. Sales[1,a,*], Gemma Molero[2,b], John R. Evans[3], Samuel H. Taylor[1], Ryan Joynson[4,c], Robert T. Furbank[3], Anthony Hall[4], Elizabete Carmo-Silva[1,*]

[1]Lancaster Environment Centre, Lancaster University, Library Avenue, Lancaster LA1 4YQ, UK

[2]International Maize and Wheat Improvement Centre (CIMMYT) Int. Apdo. Postal 6-641, 06600 Mexico, DF, Mexico

[3]ARC Centre of Excellence for Translational Photosynthesis, Research School of Biology, The Australian National University, Canberra ACT 2601, Australia

[4]Organisms and Ecosystems, Earlham Institute, Norwich Research Park, Norwich NR4 7UG, UK

Present Addresses:

[a] Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK

[b] KWS Momont Recherche, 7 rue de Martinval, 59246 Mons-en-Pevele, France

[c] Limagrain Europe, CS 3911, 63720 Chappes, France

* Correspondence: e.carmo-silva@lancaster.ac.uk; cr673@cam.ac.uk

Emails and ORCID:

Cristina R. G. Sales:	cr673@cam.ac.uk	0000-0002-8748-7370
Gemma Molero:	gemma.molero@kws.com	0000-0002-6431-7563
John R. Evans:	john.evans@anu.edu.au	0000-0003-1379-3532
Samuel H. Taylor:	s.taylor19@lancaster.ac.uk	0000-0001-9714-0656
Ryan Joynson	ryan.joynson@limagrain.com	0000-0002-7979-4725
Robert T. Furbank:	robert.furbank@anu.edu.au	0000-0001-8700-6613
Anthony Hall:	anthony.hall@earlham.ac.uk	0000-0002-1806-020X
Elizabete Carmo-Silva:	e.carmosilva@lancaster.ac.uk	0000-0001-6059-9359

Date of submission: 02/03/2021

Number of tables: 2

Number of figures: 6

Supplementary data (number figures and tables): 4 tables and 9 figures

Word count (from introduction to the end of acknowledgments excluding M&M): 4214

Phenotypic variation in photosynthetic traits in wheat grown under field *versus* glasshouse conditions

Highlight: Wheat plants grown in the glasshouse show different physiological properties compared to plants grown under dynamic field conditions, highlighting the need to consider realistic environmental conditions when breeding for particular environments.

Abstract

Recognition of the untapped potential of photosynthesis to improve crop yields has spurred research to identify targets for breeding. The CO_2-fixing enzyme Rubisco is characterised by a number of inefficiencies and frequently limits carbon assimilation at the top of the canopy, representing a clear target for wheat improvement. Two bread wheat lines with similar genetic backgrounds and contrasting *in vivo* maximum carboxylation activity of Rubisco per unit leaf nitrogen ($V_{c,max,25}/N_{area}$) determined using high throughput phenotyping methods were selected for detailed study from a panel of 80 spring wheat lines. Detailed phenotyping of photosynthetic traits in the two lines using glasshouse-grown plants showed no difference in $V_{c,max,25}/N_{area}$ determined directly via *in vivo* and *in vitro* methods. Detailed phenotyping of glasshouse-grown plants of the 80 wheat lines also showed no correlation between photosynthetic traits measured via high throughput phenotyping of field-grown plants. Our findings suggest that the complex interplay between traits determining crop productivity and the dynamic environments experienced by field-grown plants needs to be considered when designing strategies for effective wheat crop yield improvement when breeding for particular environments.

Keywords: field, glasshouse, hyperspectral reflectance, photosynthesis, Rubisco, *Triticum aestivum.*

Abbreviations:

A_{CO2}, net CO_2 assimilation rate

A_{op}, operational A_{CO2}, i.e., at PAR of 500 µmol m^{-2} s^{-1} and 41 Pa CO_2

A_{sat}, A_{CO2} under saturating light, i.e., at PAR 1800 µmol m^{-2} s^{-1} and 41 Pa CO_2

Chl, chlorophyll

c_i, intercellular CO_2 concentration

c_{i_op}, c_i at PAR of 500 µmol m^{-2} s^{-1} and 41 Pa CO_2

c_{i_sat}, c_i at PAR of 1800 µmol m^{-2} s^{-1} and 41 Pa CO_2

c_{i_CJ}, c_i at which limitation of photosynthesis transitions from Rubisco to RuBP regeneration

Operating c_i, c_i at PAR of 500 µmol m^{-2} s^{-1} and 41 Pa CO_2

CO_2_r, air CO_2 concentration in the reference infra-red gas analyser

g_m, mesophyll conductance

GM2, grains per square meter

g_s, stomatal conductance

g_{s_op}, g_s at PAR of 500 µmol m^{-2} s^{-1} and 41 Pa CO_2

g_{s_sat}, g_s at PAR of 1800 µmol m^{-2} s^{-1} and 41 Pa CO_2

GY, grain yield

HI, harvest index

$J_{(A/ci)}$, electron transport rate estimated by A_{CO2}/c_i curve fitting

$J_{(HS)}$, electron transport rate estimated by hyperspectral reflectance

K_C, Michaelis-Menten constant for Rubisco in relation to CO_2

K_O, Michaelis-Menten constant for Rubisco in relation to O_2

LMA, leaf mass per area

L_s, stomatal limitation

N_{area}, leaf nitrogen content per unit leaf area

N_{mass}, leaf nitrogen content per unit dry mass

R_{day}, daytime rate of respiration

PAR, photosynthetic active radiation

TGW, thousand grain weight

T_p, triose phosphate utilization rate

TSP, total soluble protein

$V_{c,max,25(A/ci)}$, *in vivo* maximum carboxylation rate of Rubisco estimated by A_{CO2}/c_i curve fitting

$V_{c,max,25(HS)}$, *in vivo* maximum carboxylation rate of Rubisco estimated by hyperspectral reflectance

$V_{c,max,25}/N_{area}$, $V_{c,max}$ per unit leaf nitrogen

VPD_{leaf}, leaf to air vapour pressure difference

Z4.5, Z6.5, time (days after planting) at which Zadoks stages 4.5 or 6.5 were reached

Γ^*, photosynthetic CO_2 compensation point in the absence of mitochondrial respiration in the light

ϕ_{PSII}, quantum yield of photosystem II

Introduction

Global food demand is expected to double in the next fifty years or so due to the growing world population and dietary changes (Tilman and Clark, 2015). Wheat alone provides more than 20% of the calories and the protein for the world's population (Braun *et al*, 2010) and theoretical analyses estimate that genetic gains in wheat would have to increase at a rate of 2.4% per year to meet predicted global demand (Hawkesford *et al.*, 2013; Ray *et al.*, 2013). Past genetic gains in bread wheat have largely resulted from improvements in harvest index rather than increased biomass. Further large increases in harvest index are unlikely, but an opportunity exists for increasing biomass production and harvestable grain (Parry *et al*., 2011; Fischer *et al.*, 2014; Furbank *et al*., 2020).

Photosynthesis is the primary determinant of biomass production. The maximum theoretical efficiency with which the sun's energy can be captured as biomass by C_3 plants is around 4.6% (Zhu *et al*., 2008), although it rarely exceeds one-third of this value in wheat under field conditions (Parry *et al*., 2011). Improving conversion efficiency is a thriving area of research, with potential to significantly increase crop yields (Long *et al*., 2006; Zhu *et al*., 2010; Parry et al., 2011; Driever *et al.*, 2017; Yadav *et al.*, 2018; Simkin *et al.,* 2019). To investigate whether these attributes can be improved via breeding, the presence of existing genetic variation in a species germplasm is a prerequisite. Genetic variation in photosynthesis has been reported in wheat (Driever *et al.*, 2014; Gaju *et al.*, 2016; Carmo-Silva *et al.*, 2017; Pennacchi *et al.*, 2018; Molero *et al.*, 2019, Silva-Pérez *et al*., 2020). Despite plant primary production being dependent on photosynthesis, positive correlation between photosynthetic rates and yield is not always found (Murthy and Singh, 1979; Evans, 1983, Sadras *et al*., 2012; Driever *et al*., 2014). When considering yield increases achieved over the last century, one explanation for this lack of correlation is the dramatic impact of green revolution plant breeding strategies that increased allocation of primary production into yield components (reviewed by Gifford and Evans, 1981), a strategy that has been predicted to now be reaching its natural limit (Zhu *et al.*, 2010). Nonetheless, some studies have found positive correlations between flag leaf photosynthetic rates with grain yield in wheat (Gaju *et al.*, 2016; Carmo-Silva *et al*., 2017), but processes underlying the observed variation in photosynthesis and how it relates to yield warrant further study (Flood *et al.*, 2011; Lawson *et al.*, 2012).

It is well known that plant performance is highly affected by environmental conditions. Experiments under controlled or glasshouse conditions are often performed aiming to assess genetic yield potential; however, translation between results obtained under field and controlled conditions is challenging (reviewed by Poorter *et al.*, 2016), with some studies showing similar physiological responses across experiments (Lovell *et al.*, 2016) and others showing contrasting findings (Patterson *et al*., 1977; Silva-Pérez *et al*., 2020). The wheat photosynthetic tails (PStails) panel is a rich resource to understanding the underlying

processes that determine variation in CO_2 assimilation rates in wheat. The PStails panel is composed of 80 bread spring wheat lines (*Triticum aestivum* L.) assembled after screening a range of elite International Maize and Wheat Improvement Center (CIMMYT) spring wheat germplasm (Molero *et al*., 2017; 2019). The selection was based on lines contrasting for radiation use efficiency (RUE) at different growth stages, *in vivo* maximum carboxylation activity of Rubisco ($V_{c,max}$), and respiration. After phenotyping photosynthetic traits in this germplasm in the field, two lines that are genetically similar, but contrasting for $V_{c,max}$ per unit leaf nitrogen, yield and biomass at physiological maturity, were selected and further characterised in glasshouse conditions.

The present study focused on establishing the extent of photosynthetic diversity across the PStails panel and characterizing the two selected lines in detail. The initial aims of this study were to (i) identify lines in the PStails panel with contrasting photosynthetic traits but similar genetic background under field conditions; and (ii) establish the photosynthetic properties of the two contrasting lines through detailed phenotyping under glasshouse conditions. The lack of correspondence between most of the physiological properties displayed by the two genotypes under field *versus* semi-controlled environment led to a third objective: (iii) to evaluate the correlation for photosynthetic and yield related traits determined under glasshouse *versus* field conditions across the PStails panel. The findings support the need to carefully define aims and design experiments given the lack of correlation between traits determined in plants of the wheat PStails panel grown under field *versus* glasshouse conditions.

Materials and methods

PS tails panel: field conditions - plant material and growth

The photosynthetic tails (PStails) panel is composed of 80 bread wheat lines (*Triticum aestivum* L.) selected from 150 lines of the High Biomass Association Panel (HiBAP; Molero *et al*., 2019) and from 370 lines of the Bread Wheat Diversity Panel (Molero *et al*., 2017; Table S1), based on genetic diversity identified with genetic analysis and lines contrasting for radiation use efficiency (RUE) at different growth stages, *in vivo* maximum carboxylation activity of Rubisco ($V_{c,max}$), and respiration (data not published). The panel was evaluated in the field for two years (2016-2017 and 2017-2018) under fully irrigated conditions at the International Wheat Yield Partnership Phenotyping Platform (IWYP-Hub) situated at the International Maize and Wheat Improvement Centre (CIMMYT) Experimental Station Norman E. Borlaug (CENEB) in the Yaqui Valley, near Ciudad Obregon, Sonora, Mexico (27°24' N, 109°56' W, 38 masl). Maximum and minimum temperature, and maximum solar radiation (W m^{-2}) during the two years field experiments (Fig. 1B and C) are from the weather station located about 2 km from the experimental station (http://www.siafeson.com/remas/index.php). Experimental design was an alpha-lattice with two replications in raised beds (2 beds per plot, 0.8 m wide) with two rows per bed (0.24 m between rows) and 4 m long. Seeding rates were 102 Kg ha^{-1}. Appropriate weed disease and pest control were implemented to avoid yield limitations. Plots were fertilized with 50 kg N ha^{-1} (urea) and 50 kg P ha^{-1} at soil preparation, 50 kg N ha^{-1} with the first irrigation and another 150 kg N ha^{-1} with the second irrigation.

PS tails panel: field conditions - hyperspectral reflectance measurements and SPAD

The full PS tails panel was screened under field conditions using hyperspectral reflectance. Flag leaves were measured between 11 h and 14 h at booting stage (Zadoks stage between 4.3 to 4.5), anthesis (Zadoks 6.5; Zadoks *et al.*, 1974) and grain filling (seven days after anthesis) using the protocol described by Silva-Perez *et al.* (2018). A FieldSpec®3 (Analytical Spectral Devices, Boulder, CO, USA) full range spectroradiometer (350–2500 nm) was coupled via a fibre optic cable to a leaf. A mask was used to reduce the leaf-clip aperture and a black circular gasket was pasted to the mask to avoid leaf damage and to eliminate potential entry of external light through the edges. One reflectance measurement was made per leaf lamina, and two measurements per plot measuring total of two plots per entry. Leaf nitrogen content per unit leaf area (N_{area}), leaf nitrogen content per unit dry mass (N_{mass}), $V_{c,max,25}$ per unit leaf nitrogen ($V_{c,max,25(HS)}/N_{area}$), electron transport rate ($J_{(HS)}$) and SPAD (indication for chlorophyll content) were calculated, as described in Silva-Perez *et al.* (2018).

PS tails panel: field conditions - photosynthetic measurements

Flag leaf photosynthetic rate was measured as carbon uptake using a LI-6400XT portable infrared gas analyser system (LI-COR, Lincoln, Nebraska, USA) approximately at booting stage (Zadoks stage between 4.3 to 4.5; Zadoks *et al.*, 1974). The flag leaf net CO_2 assimilation rate (A_{CO2}) was estimated at PAR of 1800 µmol m^{-2} s^{-1}, air CO_2 concentration in the reference analyser (CO_2_r) of 40 Pa, 300 µmol s^{-1} flow rate and block temperature of 25 °C (here called A_{sat} as it was under saturating light). The average value of leaf vapour pressure deficit (VPD_{leaf}) inside the chamber was 1.2 kPa across years.

PS tails - field conditions: phenology and yield components

Phenology of the plots was recorded at initiation of booting (Zadoks stage 4.5), heading (Zadoks stage 5.5) anthesis (Zadoks stage 6.5) and at physiological maturity (Zadoks stage 8.7; Zadoks *et al*., 1974) when 50% of the plants reached the phenological stage, as described by Pask *et al*., 2012. Plant height was measured as the length of five individual shoots per plot from the soil surface to the tip of the spike, excluding the awns.

At physiological maturity, determination of grain yield (GY) and yield components was conducted using standard protocols (Pask *et al*., 2012). A sample of 50 fertile shoots was taken from the area of the plot harvested to estimate yield components. The sample was oven-dried, weighed and threshed to allow calculation of harvest index (HI), biomass at physiological maturity, thousand grain weight (TGW) and grains per square meter (GM2). Grain yield was determined on a minimum of 4 m^2. To avoid edge effects arising from extra solar radiation reaching border plants, under yield potential conditions, 50 cm of the plot edges were discarded before harvesting. From the harvest of each plot, a subsample of grains was weighed before and after drying (oven-dried to constant weight at 70 °C for 48 h) and the ratio of dry to fresh weight was used to determine dry GY and TGW. GM2 was calculated as [(GY/TGW) × 1000]. Total biomass at physiological maturity was calculated from GY/HI.

PS tails panel: field conditions - DNA extraction and genotyping

Plant material was obtained from 5 plants per panel accession from field trials conducted in the CIMMYT field station in Ciuidad Obregon, Mexico. DNA was subsequently extracted from flag leaf material using a standard Qiagen DNeasy miniprep kit following the manufacturers protocols. Extracted DNA integrity and purity were determined using a Nanodrop2000 and quantified using the Qubit HS assay kit. All members of the PStails panel were subjected to enrichment capture sequencing using a custom MyBaits 12Mbp, 120bp RNA probe set based on the capture used by Gardiner *et al*. (2018) and Joynson *et al*. (2021). Enrichment capture was performed with no protocol modifications on libraries created using a standard Truseq preparation and fragment size of ~300-400bp. Each library pool contained 8 dual indexed samples that were pooled prior to capture enrichment. Enriched pools were then sequenced

using a Novaseq 6000 with 150bp paired-end reads. Variants were called from the subsequent data following the protocols outlined in Joynson *et al*. (2021). The resulting single nucleotide polymorphisms (SNPs) for each panel member were combined and utilised for population genetics analysis, after filtering for <10% missing data and >5% minor allele frequency (MAF) 269,390 SNPs were retained. To determine genetic similarity between lines SNPs were subjected to PCA analysis carried out in Python using Scikit learn, the first 2 eigenvectors were plotted. Further genetic comparison was made for two lines selected from the field experiment with contrasting phenotypes, but appeared genetically similar (51 and 64, see further detail below). 964,107 genome wide SNP loci were compared between the two lines to determine genomic regions of similarity and difference. SNPs were placed into 5Mbp bins of genomic sequence and the number of sites with identity by state (IBS) between the two lines within each bin was deduced with a custom script written in Python.

Two contrasting lines: glasshouse conditions - plant material and growth

Based on field data experiments (Table 1, Table S1), two contrasting wheat lines for $V_{c,max,25(HS)}/N_{area}$ (at tillering, anthesis and grain filling stages), grain yield, and total biomass, but genetically similar (Fig. S1), were evaluated in more detail under controlled conditions. Their cross names are TITMOUSE and BCN/WBLL1//PUB94.15.1.12/WBLL1, and here they are referred to as 51 and 64, respectively. Line 64 is a high yielding line generated by strategic crosses, with a Mexican landrace background (PUB94.15.1.12), Bacanora (BCN, high grain number) and Weebill (Weebill, high grain weight) in its pedigree. Line 51 is a comparatively lower yielding line selected from the systematic screening of 70,000 genetic resources under drought and heat based on its performance under these conditions. It is a Mexican elite line with the pedigree PI/3/INIA66/CIANO//CAL/4/Bluejay 'S' from the 70's (selection history CM30136-2Y-2M-2Y-0M).

Seeds of lines 51 and 64 were sown in 3 L pots containing commercial compost mix (Petersfield Growing Medium, Leicester, UK). Twelve replicate plants of each line were grown in a glasshouse at 26/18°C day/night with a photoperiod of 16h. Natural light was supplemented with high pressure sodium lamps (SON-T 400 W, Philips Lighting, Eindhoven, The Netherlands) when external light was lower than 200 W m^{-2}. When in use, the supplementary lights provide a minimum of ~500 µmol m^{-2} s^{-1}, measured at canopy level using a LI-190R sensor (LI-COR, Lincoln, Nebraska, USA). Pots, each containing 1 plant, were distributed randomly in the glasshouse, and watered daily to field capacity. Line 51 shows faster development, therefore seeds from line 64 were sown 12 days before line 51, so that plants of the two lines reached booting (Zadoks stage 4.5; Zadoks *et al*., 1974) and were analysed at the same time.

Two contrasting lines: glasshouse conditions - photosynthetic CO_2 responses and leaf sampling

Two LI-6800F portable infrared gas analyser systems (software version 1.3.17, LI-COR, Lincoln, Nebraska, USA) were used to assess photosynthetic parameters in the two wheat genotypes. Response curves of A_{CO2} to the intercellular CO_2 concentration (c_i) combined with quantum yield of photosystem II, Φ_{PSII} (F_m'-F_t/F_m') from chlorophyll fluorescence (using a multiphase flash) were measured in the mid-section of the flag leaf when the plants reached Zadoks stage between 4.3 to 4.5 (Zadoks *et al*., 1974). In all measurements, leaf temperature was maintained at 25°C, VPD_{leaf} at *ca* 1.3 kPa, PAR of 1500 µmol m^{-2} s^{-1}, and flow rate of 500 µmol s^{-1}. Leaves were enclosed in the cuvette and induced to steady-state at 43 Pa CO_2_r; with this CO_2_r it was obtained a CO_2_s concentration in sample analyser (CO_2_s) of 40.6 ± 2.8 Pa, close to the current 41 Pa atmospheric concentration (NOAA, 2021). CO_2_r was then stepped down through 35, 27, 20, 15 and 5 Pa, and increased to 43, 48, 53, 58, 63, 68, 73, 79, 85 and 95 Pa. Before data for each step was logged, the reference and sample gas analyser signals were matched. The minimum and maximum wait time for stability were 60 and 120 s, respectively.

The response of A_{CO2} to c_i was modelled as described by Taylor *et al.* (2020), but using temperature dependent constants derived for wheat (Silva-Pérez *et al.*, 2017; Table S2). The relationship between A_{CO2} and [CO_2] was described using a version of the FvCB model (von Caemmerer and Farquhar 1981; Farquhar *et al*., 1980) with a simple function for limitation by triose-phosphate utilisation (Sharkey *et al.*, 2007). The approach of Gu *et al*. (2010) was used, where all possible carboxylation limitation-state combinations were tested, given the required order of limitation states along the c_i axis (Rubisco limited < electron transport limited < triose-phosphate utilisation limited) and the minimum number of data necessary for each limitation state (N ≥ 2 when Michaelis constants for Rubisco catalysis of carboxylation, K_C, and oxygenation reactions, K_O; and photosynthetic CO_2 compensation point in the absence of mitochondrial respiration in the light, Γ^*, are fixed). The R Language and Environment function *optim* (R Core Team, 2018) was used to minimise the distribution-wise cost function, and the model with the lowest cost function value was accepted after checking for admissibility, and, if necessary, testing for co-limited 'swinging points' (Gu *et al*., 2010).

Mean leaf temperatures measured in the LI-6800F were used to predict Γ^*, K_C and K_O, using values for wheat (Silva-Pérez *et al*., 2017; Table S2). We compared three alternative parameterisations for mesophyl conductance (g_m): $g_m \sim \infty$ (approximated by setting g_m to 1×10^6 µmol m^{-2} s^{-1} Pa^{-1}); g_m = 5.5 µmol m^{-2} s^{-1} Pa^{-1}, consistent with Silva-Pérez *et al.*, 2017; and estimation of g_m from the data. Of these, only $g_m \sim \infty$ both credibly predicted limitation states indicated by Φ_{PSII} (e.g., Busch and Sage, 2017) and usually led to fitted values of day

respiration (R_{day}) > 0. Values for $V_{c,max,25(A/ci)}$, $J_{(A/ci)}$ and triose phosphate utilization (T_p) are thus apparent rates that may underestimate true values obtained with a finite estimate of g_m. Similarly, while the CO_2 compensation point, Γ, is a close match for the data, and c_i-transitions marking boundaries between A_C, A_J and A_P were broadly consistent with trends in Φ_{PSII}, they depend on the value assigned to g_m.

Stomatal limitation (L_s) was calculated from the A_{CO2}/c_i curve (Farquhar and Sharkey, 1982). An example of a fitted A_{CO2}/c_i response curve and the different parameters derived from it can be seen in Fig. S2. Intrinsic water use efficiency (iWUE) was calculated as A_{CO2}/g_s.

After the A_{CO2}/c_i response curve, leaves were acclimated back to steady-state at 43 Pa CO_2_r. Once steady-state was reached, a sample incorporating the leaf lamina surface inside the cuvette was freeze-clamped within 10 seconds of opening the chamber (rapidly cooled to the boiling point of liquid N_2). Measurement of leaf width of the frozen sample and the width of any gap between the leaf edge and the tong perimeter enabled precise calculation of the sampled area (Carmo-Silva *et al*., 2017). Samples were stored at -80°C until extraction.

Two contrasting lines: glasshouse conditions - biochemistry

Leaf homogenates were extracted from the samples (3.1 cm^2 total area) previously harvested and stored at –80 °C by grinding the leaves at 4 °C with an ice-cold pestle and mortar containing 0.8 ml of extraction buffer (according to Carmo-Silva *et al*., 2017 with slight modifications, as described in Sales *et al*., 2020). The homogenate was clarified by centrifugation at 14,000 *g* and 4 °C for 1 min and the supernatant was immediately used for measuring Rubisco activity at 25 °C, by incorporation of $^{14}CO_2$ into acid-stable products, according to Parry *et al.* (1997) and as detailed in Sales *et al*. (2020). Initial and total Rubisco activities were determined, and activation state was calculated from the ratio of initial and total activities.

Rubisco and total soluble protein (TSP) contents were determined in the same supernatant, by the ^{14}C-CABP binding assay (Whitney *et al*., 1999) and Bradford method (Bradford, 1976) with bovine serum albumin as standard, respectively.

Chlorophyll (Chl) determination followed the method described by Wintermans and de Mots (1965). A 20 µL aliquot of homogenate was taken before centrifugation and added to 480 µL ethanol, mixed by inversion and kept in the dark for at least 4 h. After centrifugation, Chl content was determined by the absorbance at 649 and 665 nm, using a microplate reader (SPECTROstar Nano, BMG LabTeck, Aylesbury, UK).

A leaf sample adjacent to the region used for gas-exchanges was collected, oven-dried at 70 °C and ground to a fine powder using a ball mill (Retsch MM400, Retsch UK Limited, Castleford, UK). Subsamples containing 6-8 mg of leaf powder were wrapped into tin capsules

and analysed for carbon and nitrogen in % using an elemental analyser (VARIO Micro Cube, Hanau, Germany).

PStails panel: glasshouse conditions - plant material and growth

Addressing the unexpected lack of correspondence between phenotypic properties displayed by the two contrasting genotypes under field *versus* glasshouse environment conditions, data were analysed for the 80 lines that compose the PStails panel, plus the UK modern spring wheat cultivar Paragon, grown in glasshouse conditions for detailed phenotyping. The ambient conditions in the glasshouses were the same as described in the section "Two contrasting lines: glasshouse conditions - plant material and growth". Four replicates were used, with one plant of each genotype represented in each of four replicate blocks. Due to space constraints, two blocks were grown at the same time in one glasshouse while the other two blocks were planted 17 days later in a second glasshouse set to the same environmental conditions. Maximum and minimum temperature in the two glasshouses during the experimental period are shown in Fig. 1E. Solar radiation measured with a LP02 pyranometer (Campbell Scientific, Logan, Utah, USA) by the closest weather station to the experimental location (http://es-websupp.lancs.ac.uk/hazelrigg/) is shown in Fig. 1F.

Plants were grown in 3 L pots containing commercial compost mix (Petersfield Growing Medium, Leicester UK). Plants within each block were distributed according to a random design using Edgar II Experimental Design Generator and Randomiser (Brown, 2005), and were watered daily to field capacity.

PStails panel: glasshouse conditions - photosynthetic measurements

Three LI-6400XT portable infrared gas analyser system (LI-COR, Lincoln, Nebraska, USA) were used to assess photosynthetic parameters in the wheat genotypes. Response curves of A_{CO2} to c_i were performed in the mid-section of the flag leaf when the plants reached a Zadoks stage between 4.3 to 4.5 (Zadoks *et al.*, 1974). In all measurements, leaf temperature was maintained at 25 °C, VPD_{leaf} at *ca.* 1.3 kPa, PAR of 1500 µmol m^{-2} s^{-1}, and flow rate between 200 and 300 µmol s^{-1}. Leaves were enclosed in the cuvette and induced to steady-state at 40 Pa CO_2_r. CO_2_r was then stepped down through 30, 20, 10, and 7 Pa, and increased to 40, 45, 55, 70, 100 and 120 Pa. After the A_{CO2}/c_i response curve, leaves were acclimated back to steady-state at 40 Pa CO_2_r and PAR of 1800 µmol m^{-2} s^{-1}; then PAR was stepped down through 1500, 1000, 500, 250, 120, 50 and 25 µmol m^{-2} s^{-1}. Before data for each step was logged, the reference and sample gas analyser signals were matched; the minimum and maximum wait time for stability were 60 and 120 s, respectively.

A_{CO2} measured in the light response curves at PAR of 500 µmol m^{-2} s^{-1} is referred to as the operational photosynthetic rate (A_{op}), i.e., similar ambient light to the ambient growth

conditions; and at PAR 1800 µmol m^{-2} s^{-1} as A_{sat}, i.e., saturating light. A_{CO2}/c_i response curves were fitted according to the photosynthesis model of Farquhar, von Caemmerer and Berry (Farquhar *et al.,* 1980) using the Plantecophys R package (Duursma, 2015) and $V_{c,max,25(A/ci)}$ and $J_{(A/ci)}$ were estimated. T_p was fitted but data is not presented here as not all lines showed T_p limitation. Default settings were used for the other parameters.

PStails panel: glasshouse conditions - phenology and yield components

The time to reach booting (Zadoks stage 4.5) and 50% of anthesis (Zadoks stage 6.5; Zadoks *et al*., 1974) was recorded for each plant. At the end of the experiment when plants reached physiological maturity (Zadoks stage 8.7), plant height was measured as the length of the main tiller from the soil surface to the tip of the spike excluding the awns. Determination of yield components was conducted using adapted protocols from Pask *et al*. (2012). Each plant was sampled, threshed, oven-dried and weighed to allow calculation of GY, HI and biomass at physiological maturity was calculated on individual plants. From the harvest of each plant, a subsample of grains was weighed before and after drying (oven-dried to constant weight at 70 °C for 48 h). GY was calculated as grain weight at 85% dry matter, and the ratio of dry grain weight to total dry aboveground biomass was used to determine HI.

Statistical analyses

For the field work data, adjusted means were calculated for each trait by combining data from the 2 years. Days to heading and days after irrigation were used as covariate separately (fixed effect) only when its effect was significant (P<0.05). For phenology, only days after irrigation was used as a covariate. The analysis of variance was conducted with the general linear model (GLM) procedure from META R version 6.01 (Alvarado *et al*., 2017), with all the effects of years (Y), blocks within replications, replications within years, replications, genotypes (G) and GxY being considered as random effects.

For the glasshouse experiment with the full PStails panel, the statistical analyses followed the same procedure described above, but the random effects were the different glasshouse (GH) blocks/replications, G, and GxGH. Adjusted means were calculated for each trait using position in the GH as covariate (fixed effect) when its effect was significant. For the gas-exchange data, the LI-6400XT (three systems) and time of the day when measurements were performed were used as covariates when their effects were significant.

All figures were prepared in RStudio (version 1.4.1103; RStudio Team, 2021) using ggplot2 package (Wickham, 2006). For the boxplots comparing lines 51 and 64, outliers were detected and excluded, using the Tukey's fences method, where outliers are defined as extreme values that are 1.5 times the inter-quartile range (1.5 IQR) below the first quartile or 1.5 IQR above the third quartile. The Shapiro-Wilk test was performed to evaluate if the data

was normally distributed, and F-test applied to test for homogeneity in the variances of each set of data (for lines 51 and 64). As no significant difference between the variances were found, parametric *t*-test was applied to test the significance of differences between mean values obtained for each trait for the two lines.

For the linear regressions, Pearson correlation coefficients and probabilities were computed and visualized in RStudio using the packages Hmisc (Harrell, 2019) and corrplot (Wei and Simko, 2017).

Results

Two lines with contrasting $V_{c,max,25(HS)}/N_{area}$ traits and similar genetic background under field conditions

Based on two years of field experiments with the PStails panel of 80 bread wheat lines (Table S1), lines 51 and 64 were selected for detailed characterisation in glasshouse conditions as these lines showed contrasting results for high throughput phenotyping-estimated maximum rate of Rubisco carboxylation normalised per unit leaf nitrogen ($V_{c,max,25(hs)}/N_{area}$). Line 51 showed lower $V_{c,max,25}/N_{area}$ at tillering, anthesis and grain filling stages, and comparatively lower GY and biomass at physiological maturity than line 64 (Table 1).

To determine the overall level of diversity within the PStails panel, genetic characterisation was carried out using PCA analysis (Fig. S1A). This analysis split the panel into two main subpopulations across the first eigenvector. To study this similarity in further detail all genome wide SNPs for lines 51 and 64 were compared. Overall, ~4.7Gbp of sequence between the two genotypes were at least 90% similar, represented by ~940 5Mbp bins of genomic sequence across the genome. Chromosomes with the largest regions of similarity (Fig. S1B) were 2D where 76% of the chromosome had >90% similarity followed by 2A (75%), 4A (74%), 1B (72%), 1A (67%) and 3B (50%). The least similar chromosome between the two lines was 7B in which 62% of sequences had SNP similarity of less than 20%.

Detailed analysis of phenotypic traits showed no difference in $V_{c,max,25(A/ci)}/N_{area}$ in glasshouse-grown wheat contrasting lines 51 and 64

The response of A_{CO2} to c_i for the wheat lines 51 and 64 showed divergence between the two genotypes only at the highest CO_2 concentrations (Fig. 2A). The genotypes did not differ in $V_{c,max,\ 25(A/ci)}$ and $J_{(A/ci)}$, both corrected for 25 °C (Table 2), however $J/V_{c,max}$ was greater and hence the c_i at which the limitation of photosynthesis transitions from Rubisco to RuBP regeneration (c_{i_CJ}) occurred at higher c_i values for 51 (38.8 ± 0.6 Pa) than 64 (34.4 ± 0.9 Pa). For both lines, this transition was above the operating c_i, i.e. that obtained at the current atmospheric level of 41 Pa and PAR of 500 µmol m^{-2} s^{-1}. Furthermore, line 64 showed consistent limitation by T_p, which was not detected in any biological replicates for line 51 (Table 2). The stomatal response to c_i showed that line 51 had lower stomatal conductance at all c_i points compared to 64 (Fig. 2B). This result was consistent with the stomatal limitation (L_s) estimated from the A_{CO2}/c_i response curve, higher for line 51 than 64 (Table 2). Due to the lower g_s, the intrinsic water use efficiency (iWUE) in line 54 was higher than in 64 when c_i became higher than 35 Pa (Fig. S3).

Line 51 had a 13% greater N content per unit leaf area compared to line 64 (Fig. S4). These results were consistent with the total soluble protein amounts in the leaves (Fig. 3A),

with line 51 investing more resources into greater amounts of protein than 64. Rubisco amounts and activities did not differ significantly between lines (Fig. 3B-D), while chlorophyll *a*, *b*, total Chl and carotenoids contents were ~24% greater in line 51 (P<0.001) than in 64 (Table S3).

Considering that the main parameter used to select lines 51 and 64 from the field experiment was the difference in grain yield and $V_{c,max,25}/N_{area}$ (estimated through hyperspectral reflectance), *in vivo* and *in vitro* parameters were normalised to N content in the leaves, in order to understand variation in N use efficiency between the lines with contrasting yield. No significant differences were found in $V_{c,max,25(A/ci)}$ (Table 2), Rubisco initial and total activities, and Rubisco amounts between the lines when normalised by N content (Fig. S5). On the other hand, total Chl/N_{area} and carotenoids/N_{area} were significantly higher in the line 51 than in 64, consistent with results expressed per leaf area (Table S3).

Natural variation in photosynthetic traits amongst the PS tails wheat panel grown under glasshouse conditions

The lack of significant differences in Rubisco activity between the two wheat lines (Fig. 3; Table 2) was further supported by phenotyping of photosynthetic traits across the full PStails panel in glasshouse conditions. The rate of A_{CO2} measured at ambient CO_2 and the irradiance experienced by plants in the greenhouse (A_{Q500}) represents a close approximation to the operational photosynthetic rates (A_{op}). No significant phenotypic variation in A_{Q500} (P=0.429) or A_{sat} (P=0.669) was observed within the PStails lines (Fig. 4).

$V_{c,max,25(A/ci)}$ and $J_{(A/ci)}$, both determined from the A_{CO2}/c_i response curves (Fig. 5), did not differ significantly among glasshouse-grown plants of the different lines (P=0.884 and P=0.380, respectively). The parameters A_{sat}, $V_{c,max,25(HS)}$ and $J_{(HS)}$ described above were plotted for the field experiment (Fig. S6) to show how the results compared between field *versus* glasshouse experiment. These results were obtained at booting stage, and while A_{sat} was measured using an IRGA, $V_{c,max,25(HS)}$ and $J_{(HS)}$ were estimated using hyperspectral reflectance. Again, no significant phenotypic variation was found in $V_{c,max,25(HS)}$ (P=0.719) or $J_{(HS)}$ (P=0.480). On the other hand, A_{sat} was significantly different between the lines (P<0.001) and generally lower for the field-grown than the glasshouse-grown plants.

HI correlated with $V_{c,max,25}$ under field conditions but the correlation shifted to J under glasshouse-conditions

Fig. 6 shows the correlation matrices between parameters measured under field (Fig. 6A) or glasshouse (Fig. 6B) conditions. In the field dataset (Fig. 6A), A_{sat}, i.e., A_{CO2} measured at PAR of 1800 µmol m^{-2} s^{-1}, and $V_{c,max,25(HS)}$ were positively correlated with HI, whilst under

glasshouse conditions (Fig. 6B) only the photosynthetic parameter $J_{(A/ci)}$ correlated with HI, consistent with electron transport limiting photosynthesis at lower irradiance.

Total above ground biomass correlated strongly and positively with GY in both environments (r = 0.91 in the field and r = 0.87 in the glasshouse), and GY also correlated with straw biomass (r = 0.68). Interestingly, time to reach booting (Zadoks 4.5) and anthesis (Zadoks 6.5) did not correlate with yield parameters in field grown plants, but showed positive correlation with GY, total above-ground biomass and straw biomass in the glasshouse grown plants. While leaf mass per area (LMA) correlated with $V_{c,max,25}$, J and $V_{c,max,25}/N_{area}$ under field conditions, this leaf trait did not correlate with any photosynthetic parameter under glasshouse conditions. While different methods were used in the different environments, these results suggest a different set of limitations to plant productivity in glasshouse and field conditions.

The environment experienced by plants during growth strongly impacts photosynthetic traits

We investigated whether results from glasshouse conditions represented a robust assessment of potential performance under field conditions. The correlation between the values measured across the full PS tails panel grown under field *versus* glasshouse conditions for the different agronomic, photosynthetic, and yield traits are shown in Fig. 6C. The results obtained from glasshouse grown plants translated well to the field for the agronomic traits (Zadoks stage and height), and GY. However, photosynthetic traits did not show significant correlation between the two experimental conditions.

Discussion

The initial objective of this study was to identify lines within the PStails panel with contrasting photosynthetic traits but similar genetic background, with the aim of using these lines to generate a double haploid population to further identify markers associated with these photosynthetic traits. Such a population would serve as a resource to identify segregation for multiple traits including $V_{c,max}$, biomass production, and Rubisco activity. Using results obtained from two years of field experiment, two lines, here called 51 (low tail) and 64 (high tail), were selected (Table S1). Although the two genotypes showed similar genetic background (Fig. S1), line 51 had lower $V_{c,max(HS)}/N_{area}$ (measured at anthesis and grain filling stage but not at initiation of booting), total biomass and GY compared to line 64 (Table 1; Table S1). When the two genotypes were characterised as part of the PS tail panel at booting stage in the glasshouse, results were not consistent with some of the findings under field conditions. In the glasshouse environment, both lines showed low GY and low total biomass compared to the whole panel; the yield advantage of line 64 under field conditions (Table S1) was lost in the glasshouse environment (Table S4). There was some indication for a difference in $V_{c,max(A/ci)}/N_{area}$ between genotypes measured under glasshouse conditions, although this was not significant (P=0.123), and the absolute values were similar to those obtained in the field experiments at anthesis and grain filling stages (Tables 1 and 2). Overall, our findings highlight the influence of growth environment on the physiological characteristics of wheat and suggest caution when assessing genetic yield potential and variation in photosynthetic traits to inform strategies for crop improvement.

While the detailed characterisation of the two lines 51 and 64 under glasshouse conditions did not find significant differences between them in $V_{c,max,25(A/ci)}/N_{area}$ (Table 2), some other differences were detected. For both lines, this transition was above the operating c_i, i.e. that obtained at the current atmospheric level of 41 Pa and PAR of 500 μmol m^{-2} s^{-1}, suggesting that Rubisco activity was limiting photosynthesis in the glasshouse-grown plants. Limitation by T_p was identified in line 64 at c_i as low as 49 Pa, but no such effects were found for c_i values as high as 70 Pa in line 51. The leaves of line 51 had greater N (Fig. S4), chlorophyll content (Table S3), and iWUE (Fig. S3), especially at high c_i, than line 64. Another clear difference was that the operating c_i was lower for line 51, and consistent with this, L_S was greater in 51 than in 64 (Table 2). It is interesting to notice that $V_{c,max,25(A/ci)}/N_{area}$, which showed similar absolute values between field (Table 1) and glasshouse (Table 2) experiments, is associated with a shift in L_S and operating c_i. In addition, the J:$V_{c,max}$ ratio was significantly greater for genotype 51 than 64 which results in a higher c_i for the transition from Rubisco- to electron transport-limited A_{CO2}.

It is well known that Rubisco capacity and photosynthetic rate are highly correlated and therefore, estimation of modelled parameters reflecting Rubisco capacity ($V_{c,max}$) is

essential to evaluate photosynthetic performance across different elite crops germplasm (von Caemmerer, 2000; Furbank *et al*., 2020). $V_{c,max}$ combined with photosynthetic electron transport capacity (*J*), another modelled parameter, are more robust than single-point A_{CO2} measurements to assess photosynthetic performance in C_3 plants as they are independent of diurnal variation in g_s (von Caemmerer, 2000; Condon *et al*., 2004; Feng *et al*., 2018; Silva-Pérez *et al*., 2020). When screening for photosynthetic capacity it is not desirable that the measured parameters vary much due to diurnal changes in the surrounding environment (e.g., soil water availability, light) as it can lead to an underestimation of potential photosynthesis (Condon *et al*., 2004; Silva-Pérez *et al*., 2020). Furthermore, these parameters have been recently incorporated into a modelling tool that connects leaf-level photosynthesis to crop yield, and highlighted that increases in $V_{c,max}$, and *J* increase the simulated wheat yields (Wu *et al*., 2019). Existing genotypic variation in $V_{c,max}$ and *J*, therefore, should be exploited in breeding programs aiming to improve wheat yield.

The number of studies exploring natural variation in $V_{c,max}$ and *J* in wheat has been increasing (Driever *et al*., 2014; Jahan *et al*., 2014; Carmo-Silva *et al*., 2017). However, these parameters are frequently derived from measuring the response of A_{CO2} to c_i, which is time-consuming and not easily achievable under field conditions. An alternative method using leaf reflectance technique to estimate $V_{c,max}$ and *J* has been well established in many species (Doughty *et al*., 2011; Serbin *et al*., 2012; Ainsworth *et al*., 2014; Yendrek *et al*., 2017), including wheat (Silva-Pérez *et al*., 2018; 2020, Khan *et al*., 2021). This method can dramatically increase phenotyping throughput and shows a correlation around 0.6-0.7 with photosynthetic parameters predicted via gas-exchange (Silva-Pérez *et al*., 2018). In the current work, however, $V_{c,max,25}$ and *J* estimated via leaf reflectance under field conditions did not correlate with these parameters estimated via gas-exchange in the glasshouse experiment (Fig. 6C). This lack of correlation might be due to the different techniques used or the environmental growth conditions, even though parameters such as $V_{cmax,25}$ derived from leaf reflectance seems to be unaffected by the leaf temperature at which reflectance is measured, as shown by Khan *et al*. (2021).

The lack of correlation between results obtained with field grown and glasshouse grown plants highlights the complexity of comparing results obtained in different environments (Poorter *et al*., 2016). Many factors may contribute to the observed differences, but some of the most important are light quantity and quality, as well as the growth temperatures. Plants in the field were exposed to a broader temperature range (lower minimum and higher maximum), and higher maximum daily solar radiation compared to glasshouse conditions (Fig. 1). Even though light under controlled conditions fluctuated much less than under field conditions, plants did not experience saturating light, which would strongly affect processes dependent on light, such as photosynthesis (Poorter *et al*., 2013; 2016). Plants grown under

glasshouse conditions in the UK get exposed to relatively low light levels, which means that photosynthesis operation under J limitation is expected, and limitations by $V_{c,max}$ are less frequent. This is highlighted by the evident difference between the lines 51 and 64 in nitrogen allocation. Differences in $V_{c,max,25}$ were not detected between the lines at any growth stages under field conditions (Table 1) or at booting stage under glasshouse conditions (Table 2). The differences in $V_{c,max,25}$ were detected only when normalised by N content. Although both lines had the same amount of N and SPAD under field conditions, line 51 showed significantly higher nitrogen (Fig. S4) and Chl contents (Table S3) than line 64 under glasshouse conditions. These results indicate that plants optimise nitrogen allocation to pigments under glasshouse conditions, probably as a strategy to acclimate to low irradiance (Evans, 1989), leading to a higher $J_{(A/ci)}/N_{area}$ in glasshouse conditions (Table 2) than $J_{(HS)}/N_{area}$ in field grown-plants (Table 1).

Another important factor to be considered under field conditions is the higher temperatures and consequently higher VPD_{leaf} than in the glasshouse, and the more dynamic environment, *e.g.*, air movement. These factors are likely to drive more frequent stomatal limitation and consequently it can lead to $V_{c,max}$ limitation more frequently than under glasshouse conditions. This is consistent with the relationship between g_s (Fig S7A) and c_i (Fig S7B) measured in the plants grown under field *versus* glasshouse conditions, since plants under field conditions showed, in general, lower g_s and c_i than glasshouse grown plants.

The timing of phenological phases influences crop yield and is sensitive to photoperiod and cumulative temperature (Richards, 1991; Gómez-Macpherson and Richards, 1997). The number of days to reach anthesis (Zadoks stage 6.5) was significantly correlated between field and glasshouse experiments (r = 0.57; Fig S8), but the crop cycle was shorter in the glasshouse than in the field. While, for reasons of repeatability, environmental settings are manipulated to obtain a reasonable degree of constancy throughout the growth cycle in glasshouse experiments, the same is not observed in the field, where seasonal progression is a natural complement to progress through phenological stages. In Mexico, temperature and solar radiation were lower at the beginning of the field trial and increased during the crop cycle (Fig. 1). Such increases in photoperiod and temperature should be considered in experiments under glasshouse conditions that aim to assess crop yield for specific environments.

It is noteworthy that under glasshouse conditions plants were growing individually in pots, which contrasts with the higher plant density experienced under field conditions. In wheat, the number of tillers per plant is strongly affected by sowing density (Lloveras *et al.*, 2004) and genetic variation for tillering capacity has been reported (Fischer *et al.*, 2019). The relationship between tiller number in plants grown under field and glasshouse environments (Fig. S9) shows that lines 51 and 64 did not differ in the number of tillers per m^2 measured in the field but under glasshouse conditions line 64 produced significantly more tillers per plant

(11±3) than line 51 (6±2). Plasticity in ear number affects grain yield (Sadras and Rebetzke, 2013) and could contribute to explain the differences observed between the two growing environments. Plant density can have a range of effects in above and belowground responses (Wang *et al.*, 2021). Plant growth in large containers under glasshouse conditions may be an accessible alternative to translate yield results between field *versus* glasshouse experiments. Hohmann *et al*. (2016) have shown high accuracy predicting yield in oilseed rape using this technique. Use of similar sowing densities to those recommended in the field, and reduced constraints on root development in these large containers, led to above-ground architecture similar to that of field-grown plants. Studies with other crops comparing the impact of pot size in plant physiology and yield (Poorter *et al.*, 2012) would be useful to inform future studies aiming to assess natural variation in photosynthetic traits.

Improving photosynthesis offers untapped potential to increase crop yields (Long *et al*., 2006; Zhu *et al*., 2010; Parry et al., 2011; Simkin *et al.,* 2019). With the increasing number of experiments under controlled conditions, as part of efforts to identify genetic variation in photosynthesis for crop yield improvement, the findings presented here suggest caution in designing experiments so that the environmental conditions are closely aligned with the conditions experienced by plants in their target environment and throughout the growth cycle. Field trials complemented with enhanced phenotyping methods under controlled conditions is one of the best approaches to produce reliable data for breeders (Byrne *et al.*, 2022). However, not all researchers have access to the field and/or high-throughput phenotyping platforms. Alternative solutions to bridge the gap between field and glasshouse/controlled conditions experiments include higher grade growth cabinets and glasshouses that can be programmed simulating environmental fluctuations experienced by plants under field conditions. However, these types of technologies are not broadly accessible due to their high costs. Furthermore, light intensities in plant growth facilities rarely reach the same level experienced by plants grown under field conditions in the tropics, which can be an obstacle (reviewed by Poorter *et al.*, 2016), specially for crops like wheat, where the light response saturates at fairly high light intensities above those achieved by most growth cabinets.

Another approach with increasing application in plant sciences is the integration of machine learning with high-throughput phenotyping. Machine learning enables the search for patterns in large datasets containing multiple traits, instead of analysing each factor individually (Ma *et* al., 2014; Singh *et al*., 2016). Recent examples of studies combining plant phenotyping with machine learning to predict photosynthetic traits in tobacco (Fu *et al*., 2019) and wheat (Furbank *et al*., 2021) showed that this approach improved prediction of photosynthetic traits from leaf hyperspectral reflectance. However, it is important to keep in mind that these studies are dependent on large datasets, and high-throughput techniques.

675 Furthermore, the complexity of the machine learning concepts requires expert knowledge for
676 accurate interpretation of results (Ma *et al*., 2014).
677 The complex interplay of traits determining crop productivity in dynamic environments
678 experienced by field-grown plants (reviewed by Murchie *et al*., 2018) should be considered
679 when designing strategies for effective improvement of wheat crop yields. Our findings
680 suggest that when breeding for particular environments, an improved match between
681 phenotypes in field and glasshouse environments will be achieved when experiments are
682 designed so that key conditions are aligned with the cropping cycle in the target breeding
683 environment.
684
685

Supplementary data

Table S1. Summary of two years field experiment results.

Table S2. Kinetic constants used for $V_{c,max,25(A/ci)}$ estimation.

Table S3. Chlorophyll and carotenoid contents for lines 51 and 64.

Table S4. Summary of glasshouse experiment results.

Fig. S1. PCA for the PS tails and SNPs distribution.

Fig. S2. Example of a fitted A_{CO2}/c_i response curve.

Fig. S3. iWUE for lines 51 and 64.

Fig. S4. Carbon and nitrogen content for lines 51 and 64.

Fig. S5. Rubisco parameters normalised to N content for lines 51 and 64.

Fig. S6. A_{sat}, $V_{c,max,25(HS)}$, and $J_{(HS)}$ in the PStails panel grown under field conditions.

Fig. S7. g_s and c_i relationships between glasshouse and field grown plants.

Fig. S8. Relationships between time to reach Zadoks stage 6.5 in glasshouse and field grown plants.

Fig. S9. Relationships between the number of tillers in glasshouse and field grown plants.

Acknowledgements

This research was funded by the Biotechnology and Biological Sciences Research Council (BBSRC) through the International Wheat Yield Partnership project *Using next generation genetic approaches to exploit phenotypic variation in photosynthetic efficiency to increase wheat yield* (IWYP64; BB/ N020871/2). We thank Catarina Sobral (Lancaster University) for her valuable support during the glasshouse experiments; Carlos-Robles Zazueta (University of Nottingham) for helping with yield components in the field grown panel, Dr Francisco Pinto (CIMMYT) for providing the environmental data for the field seasons; James Heath for coordinating and curating Hazelrigg weather data collection at Lancaster University; Maureen Harrison and Geoff Holroyd (Lancaster University) for managing the plant growth facilities at Lancaster University.

Author contributions

AH, ECS, JRE and RTF obtained funding; GM, designed and performed field experiments; ECS and CRGS designed glasshouse experiments; CRGS performed glasshouse experiments; RJ performed genotyping analysis; SHT, GM and CRGS performed data analysis; CRGS and ECS wrote the manuscript with input from GM, SHT and JRE; all authors read, edited and approved the manuscript.

Data availability statement

The data presented in this publication are available at the data repository used by Lancaster University: (doi to be added)

Conflict of interest

The authors have no conflicts to declare.

References

Alvarado G, López M, Vargas M, Pacheco Á, Rodríguez F, Burgueño J, Crossa J. 2017 META-R (Multi Environment Trail Analysis with R for Windows) Version 6.01.

Ainsworth EA, Serbin SP, Skoneczka JA, Townsend PA. 2014. Using leaf optical properties to detect ozone effects on foliar biochemistry. Photosynthesis Research **119,** 65–76.

Bradford MM. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry **72**, 248–254.

Braun HJ, Atlin G, Payne T. 2010. Multi-location testing as a tool to identify plant response to global climate change. In: Reynolds MP, ed. Climate change and crop production. Surrey, UK: CABI Climate Change Series, 115–138.

Brown JKM. 2005. Experimental Design Generator and Randomiser. http://www.edgarweb.org.uk/. Accessed November 2017.

Busch FA, Sage RF. 2017. The sensitivity of photosynthesis to O_2 and CO_2 concentration identifies strong Rubisco control above thermal optimum. New Phytologist **213**, 1036–1051.

Byrner T, Grant J, Kock-Appelgren P, Förster L, Michel T, Miricescu A, Thomas WTB, Graciet E, Spink J, Ng CKY, Barth S. 2022. Improving phenotyping in winter barley cultivars towards waterlogging tolerance by combining field trials under natural conditions with controlled growth condition experiments. European Journal of Agronomy **133,** 126432.

Carmo-Silva E, Andralojc PJ, Scales JC, Driever SM, Mead A, Lawson T, Raines CA, Parry MAJ. 2017. Phenotyping of field-grown wheat in the UK highlights contribution of light response of photosynthesis and flag leaf longevity to grain yield. Journal of Experimental Botany **68,** 3473–3486.

Condon AG, Richards RA, Rebetzke GJ, Farquhar GD. 2004. Breeding for high water-use efficiency. Journal of Experimental Botany **55**, 2447–2460.

Doughty CE, Asner GP, Martin RE. 2011. Predicting tropical plant physiology from leaf and canopy spectroscopy. Oecologia **165**, 289–299.

Driever SM, Lawson T, Andralojc PJ, Raines CA, Parry MA. 2014. Natural variation in photosynthetic capacity, growth, and yield in 64 field- grown wheat genotypes. Journal of Experimental Botany **65,** 4959–4973.

Driever SM, Simkin AJ, Alotaibi S, Kisk SJ, Madgwick PJ, Sparks CA, Jones HD, Lawson T, Parry MA, Raines CA. 2017. Increased SBPase activity improves photosynthesis and grain yield in wheat grown in greenhouse conditions. Philosophical Transactions of the Royal Society B: Biological Sciences **372,** 1730.

Duursma RA. 2015. Plantecophys – an R package for analysing and modelling leaf gas exchange data. PLoS One **10,** e0143346.

Evans JR. 1983. Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). Plant Physiology **72,** 297–302.

Evans JR. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia **78**, 9–19.

Farquhar GD, Sharkey TD. 1982. Stomatal conductance and photosynthesis. Annual Review of Plant Physiology **33**, 317–345.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta **149,** 79–90.

Feng Z, Calatayud V, Zhu J, Kobayashi K. 2018. Ozone exposure- and flux-based response relationships with photosynthesis of winter wheat under fully open air condition. The Science of the Total Environment **619– 620**, 1538–1544.

Fischer RA, Byerlee D, Edmeades G. 2014. Crop yields and global food security. Canberra, ACT, Australia: ACIAR.

Fischer RA, Moreno Ramos OH, Ortiz Monasterio I, Sayre KD. 2019. Yield response to plant density, row spacing and raised beds in low latitude spring wheat with ample soil resources: and update. Field Crops Research **232,** 95–105.

Flood PJ, Harbinson J, Aarts MG. 2011. Natural genetic variation in plant photosynthesis. Trends in Plant Science **16,** 327–335.

Fu P, Meacham-Hensold K, Guan K, Bernacchi CJ. 2019. Hyperspectral leaf reflectance as proxy for photosynthetic capacities: an ensemble approach based on multiple machine learning algorithms. Frontiers in Plant Science **10,** 730.

Furbank RT, Sharwood R, Estavillo GM, Silva-Pérez V, Condon AG. 2020. Photons to food: genetic improvement of cereal crop photosynthesis. Journal of Experimental Botany **71,** 2226–2238.

Furbank RT, Silva-Pérez V, Evans JR, Condon AG, Estavillo GM, He W, Newman S, Poiré R, Hall A, He Z. 2021. Wheat physiology predictor: predicting physiological traits in wheat from hyperspectral reflectance measurements using deep learning. Plant Methods **17,** 108.

Gaju O, DeSilva J, Carvalho P, Hawkesford MJ, Griffiths S, Greenland A, Foulkes MJ. 2016. Leaf photosynthesis and associations with grain yield, biomass and nitrogen-use efficiency in landraces, synthetic-derived lines and cultivars in wheat. Field Crops Research **193,** 1–15.

Gardiner L-J, Joynson R, Omony J, Rusholme-Pilcher R, Olohan L, Lang D, Bai C, Hawkesford M, Salt D, Spannagl M, Mayer KFX, Kenny J, Bevan M, Hall N, Hall A.

2018. Hidden variation in polyploid wheat drives local adaptation. Genome Research **28,** 1319–1332.

Gifford RM, Evans LT. 1981. Photosynthesis, carbon partitioning, and yield. Annual Review of Plant Physiology **32,** 485–509.

Gómez-Macpherson H, Richards RA. 1997. Effect of early sowing on development in wheat isolines differing in vernalisation and photoperiod requirements. Field Crops Research **54,** 91–107.

Gu L, Pallardy SG, Tu K, Law BE, Wullschleger SD. 2010. Reliable estimation of biochemical parameters from C_3 leaf photosynthesis-intercellular carbon dioxide curves. Plant, Cell and Environment **33**, 1852–1874.

Harrell FE. 2019. Hmisc: harrell miscellaneous. R package (version 4.2-0). https://CRAN.R-project.org/package=Hmis

Hawkesford MJ, Araus J-L, Park R, Calderini D, Miralles D, Shen T, Zhang J, Parry MAJ. 2013. Prospects of doubling global wheat yields. Food and Energy Security **2,** 34–48.

Hohmann M, Stahl A, Rudloff J, Wittkop B, Snowdon RJ. 2016. Not a load of rubbish: simulated field trials in large-scale containers. Plant, Cell and Environment **39,** 2064–2073.

Jahan E, Amthor JS, Farquhar GD, Trethowan R, Barbour MM. 2014. Variation in mesophyll conductance among Australian wheat genotypes. Functional Plant Biology **41**, 568–580.

Joynson R, Molero G, Coombes B, Gardiner L-J, Rivera-Amado C, Pinera-Chaves FJ, Furbank RT, Reynolds MP, Hall A. 2021. Uncovering candidate genes involved in photosynthetic capacity using unexplored genetic variation in spring wheat. Plant Biotechnology Journal **19**, 1537–1552.

Khan HA, Nakamura Y, Furbank RT, Evans JR. 2021. Effect of leaf temperature on the estimation of photosynthetic and other traits of wheat leaves from hyperspectral reflectance. Journal of Experimental Botany **72,** 1271–1281.

Lawson T, Kramer DM, Raines CA. 2012. Improving yield by exploiting mechanisms underlying natural variation of photosynthesis. Current Opinion in Biotechnology **23,** 215–220.

Lloveras J, Manent J, Viudas J, Lópes A, Santiveri P. 2004. Seeding rate influence on yield and yield components of irrigated winter wheat in a Mediterranean climate. Agronomy Journal **96,** 1258–1265.

Long SP, Zhu XG, Naidu SL, Ort DR. 2006. Can improvement in photosynthesis increase crop yields? Plant, Cell and Environment **29,** 315–330.

Lovell JT, Shakirov EV, Schwartz S, Lowry DB, Aspinwall MJ, Taylor SH, Bonnette J, Palacio-Mejia JD, Hawkes CV, Fay PA, Juenger TE. 2016. Promises and challenges of eco-physiological genomics in the field: tests of drought responses in switchgrass. Plant Physiology **172**, 734–748.

Ma C, Zhang HH, Wang X. 2014. Machine learning for Big Data analytics in plants. Trends in Plant Science **12,** 798–808.

Molero G, Joynson R, Piñera-Chavez FJ, Gardiner LJ, Rivera-Amado C, Hall A, Reynolds MP. 2019. Elucidating the genetic basis of biomass accumulation and radiation use efficiency in spring wheat and its role in yield potential. Plant Biotechnology Journal **17,** 1276–1288.

Molero G, Piñera-Chavez FJ, Rivera-Amado C, Pinto F, Gimeno J, Sukumaran S, Reynolds MP. 2017. Phenotypic characterization of the International Wheat Yield Partnership-Hub (IWYP-HUB) panels. In: Reynolds MP, Molero G, McNab A, eds. Proceedings of the 3rd International TRIGO (wheat) yield potential workshop. Ciudad Obregón, Mexico, March 22–23, 2017 Proceedings. CENEB, CIMMYT, **64–73**.

Murchie EH, Kefauver S, Araus JL, Muller O, Rascher U, Flood PJ, Lawson T. 2018. Measuring the dynamic photosynthome. Annals of Botany **122,** 207-220.

Murthy KK, Singh M. 1979. Photosynthesis, chlorophyll content and ribulose diphosphate carboxylase activity in relation to yield in wheat genotypes. Journal of Agricultural Science **93,** 7–11.

NOAA. 2021. Global Monitoring Laboratory. Trends in atmospheric carbon dioxide. https://gml.noaa.gov/ccgg/trends/. Accessed October 2021. NOAA.

Parry MAJ, Andralojc PJ, Parmar S, Keys AJ, Habash D, Paul MJ, Alred R, Quick WP, Servaites JC. 1997. Regulation of Rubisco by inhibitors in the light. Plant, Cell and Environment **20**, 528–534.

Parry MAJ, Reynolds M, Salvucci ME, Raines C, Andralojc PJ, Zhu XG, Price GD, Condon AG, Furbank RT. 2011. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. Journal of Experimental Botany **62, 453–467.**

Pask A, Pietragalla J, Mullan D, Reynolds M, eds. 2012. Physiological breeding II: a field guide to wheat phenotyping. Mexico, DF: CIMMYT.

Patterson DT, Bunce JA, Alberte RS, Van Volkenburgh E. 1977. Photosynthesis in relation to leaf characteristics of cotton from controlled and field environments. Plant Physiology **59**, 384–387.

Pennacchi JP, Carmo-Silva E, Andralojc PJ Feuerhelm D, Powers SJ, Parry MAJ. 2018. Dissecting wheat grain yield drivers in a mapping population in the UK. Agronomy **8,** 94.

Poorter H, Anten NP, Marcelis LF. 2013. Physiological mechanisms in plant growth models: do we need a supra-cellular systems biology approach? Plant, Cell and Environment **36,** 1673–1690.

Poorter H, Bühler J, van Dusschoten D, Climent J, Postma JA. 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. Functional Plant Biology **39,** 839–850.

Poorter H, Fiorani F, Pieruschka R, Wojciechowski T, van der Putten WH, Kleyer M, Schurr U, Postma J. 2016. Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. New Phytologist **212,** 838–855.

R Core Team. 2018. R: a language and environment for statistical computing.

Ray DK, Mueller ND, West PC, Foley JA. 2013. Yield trends are insufficient to double global crop production by 2050. PLoS One **8,** e66428.

Richards RA. 1991. Crop improvement for temperate Australia: future opportunities. Field Crops Research **26,** 141–169.

RStudio Team. 2021. RStudio: Integrated development for R. Boston, MA: RStudio, PBC. http://www.rstudio.com/

Sadras VO, Lawson C, Montoro A. 2012. Photosynthetic traits in Australian wheat varieties released between 1958 and 2007. Field Crops Research **134,** 19–29.

Sadras VO, Rebetzke GJ. 2013. Plasticity of wheat yields is associated with plasticity of ear number. Crop and Pasture Science **64,** 234–243.

Sales CRG, Silva AB, Carmo-Silva E. 2020. Measuring Rubisco activity: challenges and opportunities of NADH-linked microtiter plate-based and ^{14}C-based assays. Journal of Experimental Botany **71**, 5302–5312.

Serbin SP, Dillaway DN, Kruger EL, Townsend PA. 2012. Leaf optical properties reflect variation in photosynthetic metabolism and its sensitivity to temperature. Journal of Experimental Botany **63,** 489–502.

Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL. 2007. Fitting photosynthetic carbon dioxide response curves for C_3 leaves. Plant, Cell and Environment **30**, 1035–1040.

Silva-Pérez V, De Faveri J, Molero G, Deery DM, Condon AG, Reynolds MP, Evans JR, Furbank RT. 2020. Genetic variation for photosynthetic capacity and efficiency in spring wheat. Journal of Experimental Botany **71,** 2299–2311.

Silva-Pérez V, Furbank RT, Condon AG, Evans JR. 2017. Biochemical model of C_3 photosynthesis applied to wheat at different temperatures. Plant, Cell and Environment **40**, 1552–1564.

Silva-Pérez V, Molero G, Serbin SP, Condon AG, Reynolds MP, Furbank RT, Evans JE. 2018. Hyperspectral reflectance as a tool to measure biochemical and physiological traits in wheat. Journal of Experimental Botany **69,** 483–496.

Simkin AJ, López-Calcagno PE, Raines CA. 2019. Feeding the world: improving photosynthesis efficiency for sustainable crop production. Journal of Experimental Botany **70,** 1119–1140.

Singh A, Ganapathysubramanian B, Singh AK, Sarkar S. 2016. Machine learning for high-throughput stress phenotyping in plants. Trends in Plant Science **21,** 110–124.

Taylor SH, Orr DJ, Carmo-Silva E, Long SP. 2020. During photosynthetic induction, biochemical and stomatal limitations differ between *Brassica* crops. Plant, Cell and Environment **43**, 2623–2636.

Tilman D, Clark M. 2015. Food, agriculture and the environment: can we feed the world and save the Earth? Daedalus **144,** 8–23.

von Caemmerer S. 2000. Biochemical models of leaf photosynthesis. Collingwood, Australia: CSIRO Publishing.

von Caemmerer S, Farquhar GD. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153**, 376–387.

Wang S, Li L, Zhou D-W. 2021. Root morphological responses to population density vary with soil conditions and growth stages: the complexity of density effects. Ecology and Evolution **11,** 10590–10599.

Wei T, Simko V. 2017. Visualization of a correlation matrix. R package corrplot: visualization of a correlation matrix (version 0.84). https://github. com/taiyun/corrplot

Whitney SM, von Caemmerer S, Hudson GS, Andrews TJ. 1999. Directed mutation of the Rubisco large subunit of tobacco influences photorespiration and growth. Plant Physiology **121**, 579–588.

Wickham H. 2006. ggplot2: elegant graphics for data analysis. New York: Springer-Verlag.

Wintermans JFGM, de Mots A. 1965. Spectrophotometric characteristics of chlorophylls a and b and their pheophytins in ethanol. Biochimica et Biophysica Acta **109**, 448–453.

Wu A, Hammer GL, Doherty A, von Caemmerer S, Farquhar GD. 2019. Quantifying impacts of enhancing photosynthesis on crop yield. Nature Plants **5**, 380–388.

Yadav SK, Khatri K, Rathore MS, Jha B. 2018. Introgression of UfCyt c6, a thylakoid lumen protein from a green seaweed *Ulva fasciata* Delile enhanced photosynthesis and growth in tobacco. Molecular Biology Reports **45**, 1745–1758.

Yendrek CR, Tomaz T, Montes CM, Cao Y, Morse AM, Brown PJ, McIntyre LM, Leakey AD, Ainsworth EA. 2017. High-throughput phenotyping of maize leaf physiological and biochemical traits using hyperspectral reflectance. Plant Physiology **173,** 614–626.

Zadoks JC, Chang TT, Konzak CF. 1974. A decimal code for the growth stages of cereals. Weed Research **14,** 415–421.

Zhu X-G, Long SP, Ort DR. 2008. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? Current Opinion in Biotechnology **19,** 153–159.

Zhu X-G, Long SP, Ort DR. 2010. Improving photosynthetic efficiency for greater yield. Annual Review of Plant Biology **61,** 235–261.

Table 1. Physiological traits measured on the flag leaves at booting (Zadoks 4.3-4.5), anthesis (Zadoks 6.5), and grain filling (seven days after anthesis; A+7) using hyperspectral reflectance; and yield traits determined at physiological maturity for the two wheat lines 51 and 64 grown for two years (Y16-17 and Y17-18) in northeast Mexico under fully irrigated conditions as part of the panel photosynthetic tails (PStails).

		Line		Student's *t*-test
	Parameter	**51**	**64**	**P value**
	GY (g m^{-2})	463 ± 14	612 ± 15	**<0.001**
	GM2 (grains m^{-2})	13392 ± 343	14256 ± 369	0.112
	TGW (g)	34.5 ± 0.5	42.9 ± 0.4	**<0.001**
	Total biomass (g m^{-2})	1106 ± 29	1371 ± 41	**0.004**
	HI	0.43 ± 0.01	0.45 ± 0.01	0.073
Grain Filling (A+7)	$V_{c,max,25(HS)}$ (µmol m^{-2} s^{-1})	140 ± 16	156 ± 19	0.657
	$V_{c,max,25(HS)}/N_{area}$ (µmol s^{-1} (g N)$^{-1}$)	56 ± 1	63 ± 1	**0.018**
	$J_{(HS)}$ (µmol m^{-2} s^{-1})	202 ± 27	219 ± 31	0.757
	$J_{(HS)}/N_{area}$ (µmol s^{-1} (g N)$^{-1}$)	75 ± 7	84 ± 7	0.504
	N_{area} (g m^{-2})	2.6 ± 0.1	2.6 ± 0.2	0.798
	N_{mass} (mg g^{-1})	55.5 ± 2.2	57.1 ± 3.4	0.785
	SPAD	49.6 ± 1.3	49.6 ± 2.2	0.989
	LMA (g m^{-2})	50.7 ± 1.1	47.6 ± 1.4	0.222
Anthesis	$V_{c,max,25(HS)}$ (µmol m^{-2} s^{-1})	102 ± 1	153 ± 22	0.127
	$V_{c,max(HS)}/N_{area}$ (µmol s^{-1} (g N)$^{-1}$)	53 ± 1	65 ± 3	**0.032**
	$J_{(HS)}$ (µmol m^{-2} s^{-1})	153 ± 6	221 ± 36	0.208
	$J_{(HS)}/N_{area}$ (µmol s^{-1} (g N)$^{-1}$)	70 ± 4	79 ± 6	0.358
	N_{area} (g m^{-2})	2.2 ± 0.1	2.7 ± 0.3	0.208
	N_{mass} (mg g^{-1})	45.1 ± 1.4	55.1 ± 3.3	0.079
	SPAD	46.0 ± 0.6	49.7 ± 1.4	0.115
	LMA (g m^{-2})	47.0 ± 1.8	50.0 ± 1.2	0.317
Initiation of Booting	$V_{c,max,25(HS)}$ (µmol m^{-2} s^{-1})	167 ± 5	169 ± 7	0.892
	$V_{c,max(HS)}/N_{area}$ (µmol s^{-1} (g N)$^{-1}$)	68 ± 1	68 ± 1	0.861
	$J_{(HS)}$ (µmol m^{-2} s^{-1})	228 ± 10	228 ± 16	0.998
	$J_{(HS)}/N_{area}$ (µmol s^{-1} (g N)$^{-1}$)	88 ± 3	88 ± 4	0.975
	N_{area} (g m^{-2})	2.6 ± 0.1	2.6 ± 0.1	0.978
	N_{mass} (mg g^{-1})	53.0 ± 1.9	52.3 ± 3.1	0.886
	SPAD	47.8 ± 0.9	49.0 ± 1.2	0.856
	LMA (g m^{-2})	52.7 ± 2.2	53.0 ± 3.1	0.958

Values are means ± SEM (n = 4, i.e., 2 biological replicates per year).

Table 2. Parameters estimated from the response curves of net CO_2 assimilation (A_{CO2}) to the intercellular CO_2 concentration (c_i) in the flag leaves of wheat lines 51 and 64 at booting stage grown under glasshouse conditions.

	Line		Student's *t*-test
Parameter	**51**	**64**	***P* value**
$V_{c,max,25(A/ci)}$ (μmol m^{-2} s^{-1})	136 ± 4	139 ± 5	0.671
$V_{c,max,25(A/ci)}/N_{area}$ (μmol s^{-1} $(g\ N)^{-1}$)	54 ± 2	62 ± 2	0.123
$J_{(A/ci)}$ (μmol m^{-2} s^{-1})	255 ± 7	247 ± 7	0.419
$J_{(A/ci)}/N_{area}$ (μmol s^{-1} $(g\ N)^{-1}$)	102 ± 5	109 ± 3	0.392
c_{i_J} (Pa)	38.7 ± 0.7	34.4 ± 0.9	**0.001**
$J/V_{c,max}$	1.87 ± 0.01	1.78 ± 0.02	**0.002**
c_{i_JP} (Pa)	NA	55.9 ± 1.9	NA
T_p (μmol m^{-2} s^{-1})	NA	17.0 ± 0.4	NA
Operating c_i (Pa)	28.1 ± 0.3	29.2 ± 0.3	**0.032**
R_d (μmol m^{-2} s^{-1})	0.42 ± 0.08	0.46 ± 0.13	0.790
L_s	0.22 ± 0.01	0.17 ± 0.01	**0.004**

Values are means ± SEM (n = 8-11 biological replicates). $V_{c,max,25(A/ci)}/N_{area}$ was calculated using N data from Fig. S4 (n = 5-6 biological replicates).

Figure legends

Fig. 1. Schematic description and meteorology from the (A-C) field and (D-F) glasshouse experiments conditions performed with the 80 wheat lines of the Photosynthetic tails (PStails) panel; (B, E) daily maximum and minimum air temperature, and (C, F) maximum solar radiation during the experiments. Weather data for the field experiments are from December 2016 to May 2017 (Years 16-17), and from December 2017 to May 2018 (Years 17-18), from the weather station (http://www.siafeson.com/remas/index.php) located about 2 km from CIMMYT Experimental Station Norman E. Borlaug (CENEB). Temperature data for the glasshouse experiments are from sensors located inside the glasshouse; solar radiation is from the weather station (http://es-websupp.lancs.ac.uk/hazelrigg/) located about 1 km from Lancaster University, from December 2017 to March 2018. Days after planting (DAP) in E and F are shown for the first experimental block; the second block was sown at 17 DAP (green arrow).

Fig. 2. (A) Response curves of net CO_2 assimilation (A_{CO2}), and (B) stomatal conductance (g_s) to the intercellular CO_2 concentration (c_i) in flag leaves of wheat lines 51 and 64 at booting stage grown under glasshouse conditions. Values are means ± SEM (n = 8-11 biological replicates).

Fig. 3. (A) Total soluble protein, (B) Rubisco amounts, and (C) Rubisco initial and (D) total activities in flag leaves of wheat lines 51 and 64 sampled at booting stage. Leaves were sampled after the A_{CO2}/c_i response curves, at steady state (PAR of 1500 µmol m^{-2} s^{-1} and 43 Pa CO_2_r). Boxplots show median (white line), mean (white x), inter-quartile range (IQR, box upper and lower edges), 1.5 times of IQR (whiskers) and individual data points (grey dots). Student's *t*-test *P* value is shown for each parameter. n = 8-10 biological replicates.

Fig. 4. Net CO_2 assimilation rates at booting stage of flag leaves at 40 Pa CO_2 and PAR of 500 µmol m^{-2} s^{-1} (A_{Q500} or A_{op}, A) or 1800 µmol m^{-2} s^{-1} (A_{sat}, B) in the 80 lines of the photosynthetic tails (PStails) panel plus the UK modern spring wheat cultivar cv. Paragon, grown under glasshouse conditions. A_{CO2} was measured during the light response curves. Cultivars are ranked according to increasing mean of each parameter. Boxplots show median, inter-quartile range (IQR, box upper and lower edges), and 1.5 times of IQR (whiskers). Grey dots are the adjusted means for n=3-4 experimental repetitions. The lines 51 and 64 are highlighted in green and orange, respectively.

Fig. 5. (A) Maximum carboxylation activity of Rubisco ($V_{c,max,25(A/ci)}$), and (B) electron transport rate ($J_{(A/ci)}$) estimated from the response curves of net CO_2 assimilation (A_{CO2}) to the intercellular CO_2 concentration (c_i) in the flag leaves of the 80 lines of the photosynthetic tails (PStails) panel plus the UK modern spring wheat cultivar cv. Paragon, grown under glasshouse conditions. Cultivars are ranked according to increasing mean of each parameter. Boxplots show median, inter-quartile range (IQR, box upper and lower edges), and 1.5 times of IQR (whiskers). Grey dots are the adjusted means for n=3-4 experimental repetitions. The lines 51 and 64 are highlighted in green and orange, respectively.

Fig. 6. Correlation matrices showing the significance of linear correlation between paired mean values among traits in (A) the field, and (B) the glasshouse experiments; and (C) between the two experiments for the 80 lines of the photosynthetic tails (PStails) panel. Numbers are Pearson product–moment correlation coefficients and increasingly significant correlations are indicated by increasingly darker shading.