View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Nottingham ePrints

Gaju, Oorbessy and deSilva, Jayalath G. and Carvalho, P. and Hawkesford, Malcolm J. and Griffiths, Simon and Greenland, Andrew and Foulkes, M.J. (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 . pp. 1-15. ISSN 1872-6852

Access from the University of Nottingham repository:
http://eprints.nottingham.ac.uk/46416/1/Gaju%20et%20al.%20FCR%202016%20193%201-15.pdf

Copyright and reuse:

The Nottingham ePrints service makes this work by researchers of the University of Nottingham available open access under the following conditions.

This article is made available under the Creative Commons Attribution Non-commercial No Derivatives licence and may be reused according to the conditions of the licence. For more details see: http://creativecommons.org/licenses/by-nc-nd/2.5/

A note on versions:

The version presented here may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the repository url above for details on accessing the published version and note that access may require a subscription.

For more information, please contact eprints@nottingham.ac.uk

Relationships between physiological traits, grain number and yield potential in a wheat DH population of large spike phenotype

Oorbessy Gaju [a], Matthew P. Reynolds [b], Debbie L. Sparkes[a], Sean Mayes[a], Gracia Ribas-Vargas[a] , Jose Crossa [b] and M. John Foulkes [a,*]

[a] *Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Leicestershire, LE12 5RD, UK*

[b] *CIMMYT International Maize and Wheat Improvement Center (CIMMYT), Km. 45, Carretera Mexico, El Batan, Texcoco, Mexico*

[*] Corresponding author at: *Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Leicestershire, LE12 5RD, UK.*
Tel.: +44 1159 516024; fax: + 44 1159 516060 .
E-mail address: John.Foulkes@nottingham.ac.uk (M. John Foulkes)

Abstract

Our objective was to investigate the relationships between spike traits, grain number and yield potential and their physiological basis in a doubled-haploid (DH) population derived from a cross between a CIMMYT spring wheat (*Triticum aestivum* L.) advanced line of large-spike phenotype (LSP2; +*Tin1 tiller inhibition gene*) and the UK winter wheat cultivar Rialto (R; -*Tin1*) of conventional spike phenotype. Field experiments were carried out in high radiation, irrigated conditions in NW Mexico in two seasons. Comparing the two groups of +*Tin1* and -*Tin1* DH lines, results showed the presence of the +*Tin1* allele for tiller inhibition increased spike partitioning index (spike DM / above-ground DM at GS61+5d ;SPI) from 0.32 to 0.34 (+6.3%) ($P< 0.01$) and grains spike^{-1} by 5.1 (+13.9%) ($P< 0.001$), but reduced spikes m^{-2} by 20.7 (-5.7%) ($P< 0.01$). Overall a significant increase in grains m^{-2} of 865 (+6.6%) was observed in +*Tin1* DH lines compared to -*Tin1* DH lines ($P< 0.05$), but the effect on grain yield was not statistically significant. Spike partitioning index was positively correlated with spike biomass per unit area amongst the 57 DH lines ($r = 0.58$, $P< 0.001$). There was a negative correlation between SPI and the spike partitioning index (grains per gram spike DM at GS61+5d; FE) ($r= -0.59$, $P< 0.01$); and for future application of large-spike phenotype it will be important to minimise this trade-off between SPI and FE. Our results indicated that introgressing the +*Tin1* allele into modern wheat germplasm may offer scope to increase grains spike^{-1} and grains m^{-2} in irrigated, high radiation environments.

]

Key words: Spike fertility, assimilate partitioning, tiller inhibition, awns, wheat breeding

1. Introduction

Wheat (*Triticum aestivum* L.) is globally one of the three most important cereal crops grown on more than 214 million hectares of land with an average grain yield of 2.83 t ha^{-1} (FAOSTAT, 2011). Since global demand for wheat is predicted to increase at a faster rate (Rosegrant & Agcaoili 2010) than the current annual genetic gains of ca. 1% (Shearman *et al.*, 2005; Fischer, 2007; Miralles and Slafer, 2007; Zhou *et al.*, 2007, Clarke et al., 2012), improvement in genetic yield potential will need to be accelerated. Genetic gains have historically been achieved by improvements in grain number per square metre (GN), with little change in individual grain weight (Foulkes *et al.*, 2009, 2011; Reynolds *et al.*, 2009, 2012). Semi-dwarf cultivars introduced in the 1960s and 1970s contributed large increases in GN associated with more fertile florets per spike as a consequence of increased assimilate partitioning to the spike during the pre-flowering period (Fischer, 1983). Since then, there have been continued improvements in both GN and HI in breeding programs worldwide (Reynolds *et al.*, 1999; Foulkes *et al.*, 2009; Peltonen-Sainio *et al.* 2009; Reynolds *et al*., 2011, 2012; Clarke et al., 2012).

Wheat is generally reported to be a sink-limited crop under favourable conditions with grain growth limited by the storage capacity of the grains for assimilate during the grain filling period (Borghi *et al*., 1986; Savin and Slafer, 1991; Fischer *et al*., 1998; Austin, 1999; Borras *et al*., 2004; Acreche and Slafer, 2009; Foulkes *et al.* 2011).. Therefore strategies to improve spike fertility are one of the most important avenues in the genetic improvement of yield potential (Slafer and Savin 1994; Reynolds *et al*., 2005, Fischer, 2007; Miralles and Slafer, 2007; Foulkes *et al*., 2011; Reynolds *et al.* 2012). In this respect, there is recent evidence from a study in spring wheat in the CIMMYT program in NW Mexico that novel large-spike phenotype (LSP) traits (e.g. high assimilate partitioning to spike, long rachis, high spikelet number per spike, high fertile florets per spikelet) may offer scope for increasing spike fertility and GN in future years (Gaju *et al*., 2009).

Large-spike "Gigas" phenotypes, having up to 30 spikelets, 9 grains per spikelet and individual grain weight of 63 mg, were characterised by Atsmon and Jacobs (1977), exhibiting tiller inhibition attributed to a single recessive gene *Tin1* on chromosome 1AS (Richards, 1988; Spielmeyer and Richards, 2004). In Australia under terminal drought, averaging effects in four pairs of near-isogenic lines, the *Tin1* gene increased grains per spike (+9%), but decreased spikes per square meter (-11%) and grain weight (-2%); with overall a

neutral effect on grain yield (Duggan *et al*., 2005). Elsewhere in Eastern Europe, crosses with wheats having novel tetrastichon spike morphology succeeded in boosting number of spikelets per spike (+10%), grains per spikelet (+9%) and GN (+18%) compared to the parent with normal spikes, although GN and individual grain weight were negatively correlated (Dencic, 1994). Motzo *et al*. (2004), examining the progeny of bread wheat genotypes Kite and Janz containing the *Tin1* gene crossed with the durum wheat (*Triticum turgidum* subsp. *durum*) cultivars Simeto and Valbelice, observed that the *Tin1* gene increased HI from 0.31 in freely tillering plants to 0.35 in uni- and bi-culm plants. Low tillering was associated with greater grain yields per spike, mainly resulting from greater spikelet fertility (up to 3.5 grains per fertile spikelet) and a lower incidence of sterile spikelets. In general, it can be concluded that restricted tillering to boost spike size in many cases worldwide did not increase grains per unit area due to a large degree of plasticity amongst yield components. So for future application of large-spike phenotype in breeding programs, it may be important to identify large-spike phenotypes associated with only moderate reductions in tillering capacity.

Restructured hexaploid wheat plant types exploiting heterosis were developed at CIMMYT during the 1990s, through a wide-crossing program involving *Agropyron elongatum* L., *Triticum polonicum* L. and *Triticum aestivum* L. var. Morocco wheat (Rajaram and Reynolds, 2001). These novel wheats, when grown as spaced plants, have intermediate tillering capacity (up to 10 tillers), long spikes (30 cm) and high spike fertility (up to 200 grains per spike). CIMMYT has developed new advanced lines derived from crosses with these novel wheats which have more grains per spike compared to modern CIMMYT releases when grown as spaced plants. Previously, we examined the CIMMYT wheat advanced line of large-spike phenotype (LSP2, +*Tin1*) compared with the check cultivar, Bacanora (-*Tin1*), in high radiation, irrigated field conditions in NW Mexico (Gaju *et al*., 2009). Results showed for LSP2 spikelets per spike (+4%), grains per spike (+5%) and individual grain weight (+10%) were increased compared to Bacanora, but grain yield was reduced (-8%) due to fewer spikes per square metre (-26%). In the present paper, we present the results of the field analysis of effects of the presence/absence of +*Tin1* in a doubled-haploid (DH) population derived from a cross between LSP2 and Rialto. Rialto is a UK winter wheat cultivar released in 1995 and was selected as a parental line due to its high expression of both sink-type (grains per spike) and source-type (radiation-use efficiency and stem carbohydrate reserves) traits amongst UK cultivars (Shearman *et al*., 2005). A degraining treatment (removal of 50% of the spikelets per spike) was carried out at GS61+14d) to assesses whether grain growth

amongst the +*Tin1* and -*Tin1* lines was limited by grain source or grain sink size. Grain growth responses to degraining (where assimilate supply per grain is increased by 100%) of ca. 0-10% are indicative of sink limitation of grain growth, whereas those of ca. 10-20% are indicative of co-limitation of grain growth by sink during the earlier phase of grain fill and source during the latter phase of grain fill (Acreche and Slafer, 2009).

The objectives of the present study were to examine the association of presence/absence of the *Tin1* gene with grains per m^2, grain yield and associated physiological traits and its physiological basis in a wheat LSP2 x Rialto DH population segregating for the *Tin1* gene.

2. Materials and Methods

2.1 Plant material

The CIMMYT spring wheat LSP2 for CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC was crossed with the UK winter wheat cultivar Rialto to generate a doubled-haploid (DH) population, using the maize pollination technique (Laurie and Bennett, 1986). A total of 138 lines were developed. However, only 57 DH lines were used in the present experiments carried out in Ciudad Obregon, NW Mexico. The LSP2 advanced line contains the dominant spring wheat *Vrn-A1* allele for vernalization response on chromosome 5A, whereas the winter wheat Rialto contains the recessive *vrn-A1* allele; similarly LSP2 contains the dominant *Ppd-D1a* allele for photoperiod insensitivity on chromosome 2D, whereas Rialto contains the recessive *Ppd-D1b* allele for photoperiod sensitivity. The progeny thus segregated for winter/spring vernalization and photoperiod sensitivity/insensitivity characteristics. The DH populations were initially grown as spike rows in a glasshouse under natural photoperiod at CIMMYT, El Batan, Mexico City in 2001-2 and 2002-3 and 57 of the LSP2 x Rialto (R) DH lines were selected for field analysis on the basis of acceptable flowering dates, i.e. those lines exhibiting photoperiod insensitivity and nil or low vernalization requirements.

The LSP2 (+*Tin1*) x Rialto (R) (-*Tin1*) population was segregating for the *Tin1* gene; and the 57 lines of this population were genotyped for three SSR markers in the vicinity of the gene on the short arm of chromosome 1 (Gdm33, W49 and Wms136). The 57 lines examined in the present study were also genotyped for the *Rht-B1a (tall) and Rht-B1b (semi-*

dwarf) gene for gibberellic acid-insensitivity and plant height using a perfect marker (Ellis *et al*., 2002); LSP2 (*Rht-B1b* for semi-dwarf allele) and Rialto (*Rht-B1a* for tall allele). Both parental lines possessed the *Rht-D1b* semi-dwarf allele of the *Rht-D1* gene. The 57 DH lines were also segregating for the presence and absence of awns (LSP2 awned and Rialto unawned).

2.2 Site and experimental treatments

The experiments were located at CIMMYT experimental station near Ciudad Obregon, North West Mexico located at 27° 20' N, 109° 54' W, 38 m above sea level in the state of Sonora. The soil type at the experimental station is a coarse sandy clay, mixed montmorillonitic type caliciorthid, low in organic matter and slightly alkaline (pH 7.7) in nature (Sayre *et al*., 1997). One experiment was carried out examining 57 DH lines from the LSP2 x R DH population in each of 2004-5 in 2005-6. The experimental design was an alpha-lattice with two replicates. Plot size was 5 x 1.6 m on raised beds (2 beds per plot; 2 rows per bed). The width of the bed was 80 cm, with 30 cm between rows. In the experiment in 2004-5 the LSP2 parental line was not included but in 2005-6 it was. The UK winter wheat Rialto parent was not included in the field experiments since it is photoperiod sensitive (*Ppd-D1b*) and has a vernalization requirement (*Vrn-A1*) and is unadapted to the growing conditions in NW Mexico. In addition, in 2005-6, a subset of eight DH lines from the LSP2 x R population was sown in a third experiment in four replicates using a randomised block design. Plot size was 5 x 1.6 m on raised beds (2 beds per plot; 2 rows per bed) with duplicate plots, one designated for growth analysis and the other for machine-harvested yield. The subset of eight DH lines was selected to represent two groups of *+Tin1* and *-Tin1* lines which were overall balanced for anthesis date and plant height, and with the eight individual DH lines representing restricted ranges for anthesis date and plant height.

2.3 Plot management

The experiments were sown on 24 November 2004 and 22 November 2005 with 80 g seed per plot (approximately 300 seeds m^{-2}). In each experiment, plots were irrigated using a gravity-based system with flood irrigations four to six times during the crop cycle at 3- to 4-week intervals to supply adequate moisture to avoid water stress during the growing season.

In each season, 150 kg ha^{-1} nitrogen fertilizer as urea was applied in a two-split program; the first half was applied to the seed bed during land preparation shortly before planting and the other at the time of the first irrigation close to the onset of stem elongation. Fifty kg ha^{-1} P_2O_5 was applied during land preparation to the seed bed. One hundred and forty g ha^{-1} Pirimicarb (2-dimethylamino-5,6-dimethylpyrimidin-4-yl-dimethyl-carbamate) was applied to control aphids during vegetative development. Herbicides and fungicides were applied as necessary to minimise weeds and diseases. No plant growth regulator was applied.

2.4 Crop measurements

Dates of flowering (GS61) (Tottman and Broad, 1987) and physiological maturity (when 50% of the shoots had no flag leaf or spike green area and less than 10% of the stem remained green) were recorded for each plot in all years.

2.4.1 Measurements at GS61+5d in LSP2 x R (57 lines) experiments

Plant material was sampled on the actual calendar date that the lines reached the stage (i.e. genotypes were sampled on different dates). In 2005, growth of the above-ground plant material was analyzed in two 50 cm length rows of the bed (= 0.4 m^2), situated at least 50 cm from the end of the plot. Plants were cut off at ground level in all cases. The fresh weight of the harvested plant material was recorded. Fifty fertile shoots (those with a spike) were randomly selected from the sample and the fresh weight and dry weight (after drying in oven for 48 hours at 75 ^oC) were recorded. Twenty fertile shoots were then taken randomly from the remaining sampled material and spikes were removed from the shoots at the spike collar. Both the spikes and straw were weighed after drying for 48 hours at 75 ^oC. In 2006, growth of the above-ground plant material was analyzed in 12 randomly sampled fertile shoots cut at ground level from each plot at GS61+5d. The spikes were separated from the straw, and the dry weight of each component recorded after drying for 48 hours at 75 ^oC.

In each year, rachis length and spikelets per spike were recorded on 12 randomly sampled fertile shoots per plot. In 2005, the 12 fertile shoots were randomly selected from the 20 used for DM partitioning. In 2006, the 12 fertile shoots were those used for DM partitioning. The assessments were carried out before the spikes were placed into the oven.

2.4.2 Measurements at GS31, GS41 and GS61+5d in LSP2 x R (8DH lines) experiment

For the sub-set of eight DH lines in 2006, plant material was sampled in one 0.4 m^2 area per plot as described above at GS31 (onset of stem extension), GS41 (early booting) and at GS61+5d. The number of fertile and infertile shoots in a 25% subsample (by fresh weight) was counted. At GS31 and GS41, infertile shoots were classified as those that either had no green area or for which the newest fully expanded leaf was completely senesced; the remaining shoots were classified as fertile. At GS61+5d, fertile shoots were classified as those with a spike; the remaining shoots were classified as infertile. The weight of the infertile shoots was recorded after drying for 48 h at 75°C. The fertile shoots were separated into (i) spikes, (ii) dead leaf lamina, (iii) green leaf lamina, and (iv) stem with attached leaf sheath. Green leaf lamina area was measured using a leaf area meter (LI3050A/4; LICOR, Lincoln, NE). The green area of the stem plus attached leaf sheath and of the spikes was calculated by assuming the shape of the organs to be a cylinder and applying the formulas: (i) π(diameter) x (length), for the stem plus attached leaf sheath and (ii) [π(diameter) x (length)] + [π(diameter/2)^2] for the spike. A calliper was used to measure the diameter of the stem or spike at its midpoint and a ruler to measure the length. Aboveground dry weight was measured on an additional 50% subsample (by fresh weight) from the original sample after drying for 48 h at 75°C. Dry matter of crop components (leaf lamina, stem and leaf sheath, etc.) was obtained by weighing components of the 25% subsample after drying for 48 h at 75°C. Rachis length and spikelet number per spike were recorded on 12 randomly sampled spikes per plot at GS61. The percentage of water-soluble carbohydrate (WSC) content in stems and attached leaf sheaths was estimated at GS61+5d in 10 randomly sampled fertile shoots per plot, using the anthrone method of Yemm and Willis (1954) as described by Gay *et al.* (1998).

Interception of photosynthetically active radiation (400-700 nm; PAR) was measured using a Sunfleck Ceptometer (Delta-T Devices, Burwell, Cambridge, UK) in all plots at 2- to 3-wk intervals from GS31 to GS61+5d. Readings were taken on cloudless, sunny days between 11.00 and 14.00 h above the crop and at ground level diagonally across the rows. Readings of the reflected PAR were taken by inverting the ceptometer approximately 5 cm above the crop. Radiation-use efficiency (RUE) was calculated over the period from GS31 to GS61+5d as the ratio of the aboveground dry matter increment between samplings to PAR interception over the same period. Values of daily fractional PAR interception were obtained by interpolation between readings of fractional interception; and these were applied to the daily incident solar radiation to calculate daily radiation interception, assuming PAR was

equal to 0.5 solar radiation (Monteith, 1972). Values for RUE were calculated individually for each plot, and the plot values subjected to analysis of variance (ANOVA).

In addition to the above measurements, degraining of spikes was performed 14 days after anthesis (GS61) on the subset of eight DH lines. Twelve spikes per plot were tagged to carry out the degraining treatment in which all spikelets were removed from one side of the spike and 12 control shoots were also tagged. At harvest, the 12 degrained shoots and the 12 control shoots were sampled in each plot. The spikes were threshed separately and their grains counted and weighed after drying for 48 h at 75°C.

2.4.3 Combine yield and growth analysis at harvest

In each experiment, after physiological maturity (PM) was reached, yield was measured by machine harvesting a plot area of 4.8 m^2 in each plot. Averaging across 57 LSP2 x R DH lines, PM occurred on 15 and 19 April in 2005 and 2006, respectively. Prior to machine harvesting, a random sub-sample of 100 spike-bearing shoots was removed from each plot by cutting at ground level. The plant material was dried for 48 h at 75^{o}C and weighed, and the spikes were then threshed. Dry weight of grains from 100 spikes was recorded. From this lot, 200 grains were randomly counted and weighed. Using these data, estimates of individual grain weight, all yield components, harvest index and final above-ground biomass were calculated.

2.4.4. Statistical analyses

Data collected in field experiments were subjected to ANOVA, where replications and incomplete blocks within replications were regarded as random effects and genotype was a fixed effect. For spike traits (spikelet number, rachis length etc), the mean value for the 12 spikes per plot was calculated and these plot means were then subjected to ANOVA. For ANOVAs across years, Bartlett's test (P = 0.05) was used to test for the homogeneity of variances, and years were regarded as random effects. The *Tin1* effect was tested as a contrast in the ANOVA model with one degree of freedom. Treatment means were compared using the least significant difference of the means of Fisher.

Since there were large differences in anthesis date (AD) amongst the DH lines, for all traits ANOVA was performed with AD as co-variable and probabilities presented for the statistical significance of the presence/absence of *Tin1* are those from ANOVA including AD as a covariable..The adjusted means from the ANOVA with AD as a covariable are used for

correlation and regression analysis. Regression analysis with a standard linear model was applied to two-year genotype means to calculate linear relationships between traits. Regression coefficients are presented for all variables for the linear regressions together with degrees of freedom for the error residual term in the regression model. Phenotypic correlations (Pearson's correlation coefficient) between traits were calculated using two-year genotype means. All analysis was carried out using Genstat version 15.1 (VSN International, Hemel Hempstead UK).

3. Results

3. 1 Growing conditions in experiments

Temperatures were similar in the two seasons, except during January (i.e. early-to-mid stem extension; GS31/33) when daily mean temperatures were on average 1.9 ^{o}C cooler in 2006 than 2005. Daily mean temperature increased during both seasons, from about 16^{o}C at onset of stem extension to 18^{o}C at anthesis to 21^{o}C at harvest (Table 1). The 2004-5 season was brighter than 2005-6 during grain filling in March and April with a cumulative total solar radiation for these two months of 1619 and 1472 MJ m^{-2}, respectively.

Table 1 here

3.2 Grain yield, yield components, spike traits, plant height and anthesis date of 57 DH lines

Averaging across years, grain yield, above-ground DM at harvest ($AGDM_H$) and HI amongst the 57 lines ranged from 221-706 g m^{-2}, 565-1904 g m^{-2} and 0.22-0.52, respectively ($P< 0.001$; Table 2). The lines differed for spike traits in the following ranges: spikelets spike^{-1} (18.1-28.7), spikes m^{-2} (211-542), grains spike^{-1} (23.1-58.3), grains m^{-2} (7,655-18,697) and grain weight (19.5-51.7 mg) ($P< 0.05$; Table 2). Within these ranges, the distribution of lines was skewed towards the upper end of the range for GY, but approximated to a normal distribution for most other traits (data not shown). Variation above the LSP2 parent was observed in 2006 for GY (+19%), grains m^{-2} (+46.6%), spikelets spike^{-1}

(+28.9%), grains spike^{-1} (+44.8%) and grain weight (+14.5%) (Table 2). Averaged across years, anthesis date (GS61) ranged amongst lines from 21 February to 6 April and plant height from 51.1 to 103.6 cm (P< 0.001; Table 2 and Fig. 1), reflecting segregation for developmental and plant height (*Rht-B1*) genes. In the present study, effects of presence/absence of the *Tin1* gene on spike fertility and yield potential traits are mainly analyzed averaged across the groups of DH lines (+*Tin1* and -*Tin1*) which overall were balanced for anthesis date and plant height, minimising any potential confounding effects of variation in anthesis date and plant height. In addition, the effect of *Tin1* was tested using anthesis date as cavariable in the ANOVA as described above. .

Table 2 here

3.3 Effects of the presence/absence of Tin1 gene in set of 57 LSP2 x R DH lines

In total 13 out of the 57 lines possessed the tiller inhibition +*Tin1* gene. The ANOVA with the *Tin1* gene showed that averaging across years +*Tin1* decreased spikes m^{-2} (-5.7%, P< 0.01) and increased grains m^{-2} (+6.6%, P< 0.05), grains spike^{-1} (+13.9%, P< 0.001) and grains spikelet^{-1} (+11.3%, P< 0.01) (Table 3). In 2005, +*Tin1* conferred a non-significant increase in grains m^{-2} (+6.6%,), associated with an increase in grains spike^{-1} (+6.0%, P< 0.01). In 2006, +*Tin1* decreased spikes m^{-2} (-11.7%, P< 0.05) and increased grains m^{-2} (+6.7%, *P< 0.001*), grains spike^{-1} (+22.0%, P< 0.001) and grains spikelet^{-1} (+20.4%, P< 0.001). Overall *Tin1* increased grains m^{-2} from 13,071 (-*Tin1*) to 13,936 (+*Tin1*) (+6.6%; P< 0.05), the effect of +*Tin1* on grain yield was not statistically significant. The +*Tin1* allele also increase spikelets spike^{-1} overall from 23.3 to 23.9 (P< 0.05).There was a year x *Tin1* interaction for grains spike^{-1} (P< 0.01); the increase with +*Tin1* was relatively greater in 2006 than in 2005. There was also a year x +*Tin1* interaction for spike partitioning index (spike DM/above-ground DM at GS61+5d; SPI); the presence of +Tin1 increased SPI overall from 0.32 to 0.34 (P< 0.001), but the effect was only significant in individual yuears in 2005. In addition, the year x ±*Tin1* interaction was significant for the spike fertility index (grains per g spike DM at GS61+5d); in this case +*Tin1* allele increased SFI from 38.8 (-*Tin1*) to 45,5 grains g^{-1} DM (+*Tin1*) in 2006 (P< 0.001), but there was not significant effect in 2005.

, Overall there was no effect of +*Tin1* on anthesis date. There was a small overall effect for plant height to decrease with *Tin1* from 74.4 (-*Tin1*) to 73.3 (+*Tin1*) cm ($P< 0.05$) .

Table 3 here

3.4 *Relationship between grain m^{-2} and it determinants and plant height for Tin1 groups*

For each of the +*Tin1* and -*Tin1* groups there was positive linear regression between grains spike-1 and grains pikelet-1 ($R^2 = 0.76$ and 0.81, respectively, $P< 0.001$; Fig. 1a), whereas the relationships between grains spike^{-1} and spikelets spike^{-1} was not statistically significant (Fig. 1b). Therefore spikelet fertility was the main determinant influencing variation in grains per spike amongst the DH lines rather than spikelets spike^{-1}, albeit there was overall increase in spikelets spike^{-1} in the +*Tin1* (23,9) compared to -*Tin1* (23.3) groups of lines ($P< 0.05$), Table 3) . The linear positive relationship of grains m^{-2} on grains spike^{-1} was stronger in the +*Tin1* lines ($R^2 = 0.27$, $P< 0.05$) than the -*Tin1* lines ($R^2 = 0.19$, $P< 0.07$) (Fig. 1c). The range in plant height within the +*Tin1* (51 - 93 cm) and -*Tin1* (51 - 104 cm) groups of lines was broadly similar (Fig. 1d); there was no relationship between plant height and grains m-2 in either of the +*Tin1* and -*Tin1* groups or across all 57 DH lines.

Figure 1 here

3.5 *Interactions between Tin1 gene and Rht-B1b gene and presence/absence of awns*

The dwarf (*Rht-D1b*/*Rht-B1b*) genotypes (64 cm) showed decreased plant height compared to the semi-dwarf (*Rht-B1a*/*Rht-D1b*) genotypes (84 cm; -23%, $P< 0.01$), and decreased grain yield (-20.4%, $P< 0.01$), associated with increases in both HI (from 0.37 to 0.39, $P = 0.053$; Table 4) and $AGDM_H$ (from 1090 to 1178 g m^{-2} $P< 0.05$). There was a decrease in grains spike^{-1} (from 39.5 to 36.4, $P< 0.01$)and a decrease in grain weight (,36.3 to 30.7 mg $P< 0.001$) in the dwarf compared to the semi-dwarf group of lines. However, the effects of *Tin1* were generally observed consistently in both the semi-dwarf and dwarf backgrounds, e.g. for grains m^{-2}, grains spike^{-1} and spike partitioning index. For two of the 15 traits the *Tin1* x *RhtB1b* interaction was significant: above-ground DM and rachis length ($P< 0.05$). The +*Tin1* allele increased biomass in the semi-dwarf background; and had a neutral effect in the dwarf

background. The increase in rachis length with *Tin1* was relatively greater in the semi-dwarf background than the dwarf background (Table 4).

Of the 57 DH lines, 30 were awned and 27 were unawned. Averaging across years, the presence of awns did not have a statistically significant effect on grain yield, but decreased grains m^{-2} (-9.1%; $P< 0.001$) and increased grain weight (+16.2%; $P< 0.001$) (Table 5). The effect of awns was also not statistically significant on either anthesis date or plant height. There was *Tin1* x awned/unawned interaction for grain yield ($P< 0.001$), In the unawned DH lines, +*Tin1* increased grain yield (+14.0%); but in the awned lines it decreased grain yield (-7.4%). A similar interaction was observed for grains m^{-2} ($P< 0.05$)

Tables 4 and 5 here

3.7 *Effects of +Tin1 gene in sub-set of 8 LSP2 x R DH lines in 2006*

The subset of eight DH lines was selected for detailed study of physiological traits in the additional field experiment in 2006. The DH lines were chosen to represent a narrow range for anthesis date and plant height and overall to be balanced across +*Tin1* and -*Tin1*groups of lines, (Table 6). There were only small differences in either anthesis date (19 March -*Tin1* versus 18 March +*Tin1*)) or plant height (70 cm -*Tin1* versus 69 cm +*Tin1*) between the groups of lines. The plant density at GS31 did not differ amongst the eight DH lines in the range 141 - 169 plants m^{-2}, or between the +*Tin1* (151 plant m^{-2}) and –*Tin1* (160 plants m^{-2}) group of DH lines (Table 7). At GS61+5d, +*Tin1* decreased spikes m^{-2} (-13%; $P= 0.06$) and increased grains $spike^{-1}$ (+19.0%; $P< 001$). . There was a trend for +*Tin1* to increase grains m^{-2} (+11.0%: $P= 0.09$), although the effect of +*Tin1* on grain yield was not statistically significant.

Overall green canopy area, light extinction coefficient (k), aboveground DM at GS61+5d and radiation interception from GS31 to GS61+5d and were not affected by the presence/absence of the *Tin1* allel (Table 7). Spike DM partitioning at GS61+5d was increased with +*Tin1* from 0.28 to 0.33 (+14%; $P< 0.001$), so that spike DM per m^2 at

GS61+5d was boosted from 308 g m^{-2} in the -*Tin1* group of DH lines to 363 g m^{-2} (+17.9 %; $P< 0.05$) in the +*Tin1* lines. The effects of +*Tin1* on the spike partitioning index and stem soluble carbohydrate accumulation at GS61+5d were not statistically significant in the subset of eight DH lines.

Tables 6 and 7 here

The responses of grain weight to the degraining treatment imposed at GS61+ 14d are shown in Figure 2. A response of grain weight of ca. +0-10% to degraining would indicate sink limitation of grain growth and of ca. +10-20% co-limitation by source and sink (Acreche *et al.,* 2008). The response of grain weight to degraining did not differ significantly between the +*Tin1* (+10.6%) and the -*Tin1* (+14.7%) groups of lines. In the degrained shoots, where source per grain is theoretically increased by 100% in the degrained spikes (assuming no negative feedback on photosynthesis), the final grain dry weight is an indicator of potential grain weight; the grain weight in degrained shoots did not differ significantly between the +*Tin-1* lines and -*Tin1* lines.

Fig. 2 here

4. Discussion

Firstly, we discuss the physiological basis of effects of the presence/absence of the *Tin1* gene on grains m^{-2} and grain yield, then we consider further effects of large-spike phenotype on grains m^{-2} and grain yield determined independently of the *Tin1* gene and lastly we consider the prospects for exploiting +*Tin1* and large-spike phenotype in breeding for enhanced grains m^{-2} and yield potential.

4.1 Physiological basis of effects of presence/absence of Tin1 on grains m^{-2} and grain yield

The reduction in spikes m^{-2} with +*Tin1* (-6% in the 57 DH lines and -13% in the subset of 8 DH lines) was smaller than reported in previous field investigations, e.g. -30% in Australia in wheat grown at 170 seeds m^{-2} under high nitrogen fertilizer input (Duggan *et al*., 2005). Plant density was likely similar in our study to that of Duggan *et al*. (2005).

Although plant density was not recorded in the experiments examining 57 DH lines in 2005 and 2006, in the experiment examining the subset of 8 DH lines in 2006 plant density was on average 156 plants m^{-2}. Moreover, this experiment was located in the same field as the experiment examining 57 DH lines in 2006 with the same sowing date, seed rate, irrigation and other management inputs, so plant densities were likely similar in these two experiments. The experimental field and seed rate used for the 2005 and 2006 experiments examining the 57 DH lines was the same and sowing dates and other plot management were similar, so plant density was also probably close to 156 plants m^{-2} in the 2005 experiment. *Tin1*decreased spikes m^{-2} by 6% in the present study (57 DH line experiments), but there was a wide range of quantitative variation within the *Tin1* group of lines (240 - 520 spikes m^{-2}). This suggested that in the +*Tin1* lines, axillary buds were at least partially released from tiller inhibition during tiller development by modifying tiller promoting genes.

Overall grains m^{-2} was increased by 7% in the +*Tin1* group; this was associated with more grains per spike (+14%). The increase in grains per spike was associated mainly with more grains per spikelet (+11%), with only a small increase in spikelets $spike^{-1}$ (+3%). Our previous work indicated in a growth-room experiment that the LSP2 parental line produced 5 more spikelets $spike^{-1}$ than a check CIMMYT cultivar (Bacanora) and that the thermal duration of spikelet primordia production was primarily responsible for the increased spikelet number (Gaju *et al*., 2009). However, in the field the increase in spikelets per spike for LSP2 compared to the Bacanora check was only 4% compared to 31% in the spaced plants in the growth-room experiment. In the present study, the increase in spikelets $spike^{-1}$ with +*Tin1* of 3%, was broadly consistent with the findings of Gaju *et al*. (2009).

Increased grains per m^2 with +*Tin1* was overall associated with higher SPI (both in 57 DH line expeirments and 8 DH line subset experiment). Spike DM per m^2 at anthesis was similaraly increased with *Tin1* in the 57 line and 8 DH line experiments. Enhanced spike DM per m^2 therefore appeared to be one mechanism driving the increase in grains m^{-2} with +*Tin1*. Hoever, there was also a contrinution fro the spikelet fetrtility index accorid n the results of the 57 DH lines experiments. The SFI averaging across years was increased with +*Tin1,* although the effect was only significant individual years in 2006 . Overall our results showed that the physilogica basis sof increased grain m^{-2} with *Tin1* depended partly on the season, with SPI the predominant mechanism in 2005 and SFI in 2006.

Encouragingly present results suggested increased SPI with +*Tin1* was not associated with a trade-off with SFI; nor was there a trade-off between SPI and potential grain weight. In previous work on large spike phenotype, increased SPI was associated with decreases in both FE and potential grain weight (as indicated by the grain DW in the degrained treatment) (Gaju *et al*., 2009).

The mechanism underlying the differences in SFI in the present study cannot be certain. Slafer and Andrade (1993) observed higher grains m^{-2} amongst bread wheat genotypes was associated with allocating a higher proportion of spike DM to reproductive (developing florets) rather than structural (rachis, glumes and paleas) organs within the spikes. A higher concentration of soluble carbohydrate in the spikes in slower-growing spikes was shown to increase fertile florets per ear (Ghiglione *et al*., 2008).

The positive effect of +*Tin1* on grains m^{-2} did not overall translate in the present study to a positive effect on grain yield. There was an apparent interaction with +*Tin1* increasing grain yield (+14.0%) in the unawned lines; but decreasing grain yield (-7.4%) in the awned lines. However, this interaction must be interpreted cautiously since the anthesis dates were not completely balanced in the four ±*Tin1*/±awns groups; and the relatively earlier anthesis date for +*Tin1* lines compared to -*Tin1* lines in the unawned background *(+Tin1* 3 days earlier than -*Tin1*) than in the awned background *(+Tin1* 2 days later than -*Tin1*) could partly account for this apparent interaction. The overall neutral effect of +*Tin1* on grain yield reflected a trade-off between grains m-2 and grian weight. This trade-off could be due to a trade-off between grains m^{-2} and potential grain weight or alternatively a dilution of post-anthesis assimilate supply amongst the increased grain number affecting final grain weight. Present results were not conclusive with regard to these two alternatives. There was no significant effect of presence/absence of +*Tin1* on the final grain weight in degrained spikes, an indicator of potential grain weight. Furthermore, there was no significant effect of presence/absence of +*Tin1* on source-type traits: e.g. green canopy area at anthesis, RUE during the stem-elongation period or stem WSC at GS61+5d. However, the similar grain growth responses to degraning between the +*Tin1* and -*Tin1* groups indicated source: sink balance did not differ between the lines. Further investigations with the degraining treatment applied across all 57 lines as well as additional source-sink manipulation treratments, e.g. defoliation at GS61+14 d, across all 57 DH lines are required to ascertain the physiological basis of the trade-off between grains m^{-2} and grain weight in the DH lines. Encouragingly, similar grain weight responses to degraining for +*Tin1* and -*Tin1* suggested

that +*Tin1* is not associated with impeded vascular connections to grain sites as has been postulated in relation to shrivelled grain of many large-spike lines.

The present analysis provides a quantitative assessment of the physiological basis of effects of *Tin1* on grains per m^2 and grain yield in a relatively uniform genetic background. For example, similar comparisons across groups of DH lines were used to examine effects of an awn suppressor gene and *Rht* genes on grain yield and yield components in a winter wheat Beaver x Soissons DH population (Aravinda Kumar et al., 2011). It is recognised that the present results on the effects of the presence/absence of *Tin1* should be confirmed by a more precise analysis of near isogenic lines for *Tin1* gene derived from individual LSSP2 x R DH lines. .

4.2 Implications for plant breeding

Large-spike phenotype associated with restricted tillering has been of interest to breeders in recent decades with a view to boosting grains m^{-2}, but there are several factors which have so far limited its utility. Firstly, there is the consideration of the extent to which large-spike phenotype is expressed at higher plant densities in a crop environment. Our results indicated that an increase in grains $spike^{-1}$ and grains m^{-2} was obtained with +*Tin1* at standard agronomic plant densities in spite of the decrease in spikes m^{-2}. These results therefore indicated potential scope for commercial exploitation of *Tin1* in optimal high radiation, irrigated environments, if *Tin1* could be combined with traits conferring ability to maintain. or ideally, increase individual grain weight. Such a conclusion would also be supported by results in a recent field experiment in the UK where +*Tin1* decreased spikes m^{-2} (-6.4%) and increased grains m^{-2} (+14.5%) and grain yield (+7.8%) (Aiswai, 2011). To combine high grain number and grain weight in the same genotype has however been to date a difficult task for wheat breeding. One strategy to avoid the trade-off between grain number and grain weight was proposed by Gaju *et al*. (2009) by selecting genotypes with hiuigh spikelets $spike^{-1}$ and high rachis length per spikelt number of spikelets which were shown to have higher grain number $spike^{-1}$ and grain weight. An alternative strategy may be to cross parental lines contrasting in grain number and grain weight as a way to combine both desired traits (Bustos *et al*., 2013). Those authors reported the two highest yielding lines in a spring wheat DH population derived from a cross between Bacanora (high grain number) and Weebil (high

grain weight) attained between 22 and 31% increment in grain yield compared to the parents in a field experiment in southern Chile.

In furure work the *+Tin1* germplasm should be grown at still higher plant densities of approximately 200 to 400 m^{-2} to examine if the gain in grains per spike can be maintained at these higher levels of interplant competition. Exploitation of large-spike-phenotype traits by breeders in the longer term will also depend on maintaining lodging resistance. Useful traits in this respect to select for may be crown roots that spread widely and wide stems with increased material strength of the stem wall (Berry *et al*., 2004). In this regard, large-spike-phenotype may have an advantage since stem material strength is readily expressed at low plant population density while spike size can compensate for low density.

Acknowledgements

The authors would like to express their gratitude to Univ. Nottingham, CIMMYT and the Australian Council for International Agricultural Research (ACIAR) for supporting this research. They also thank Eugenio Perez and Araceli Torres for technical support with field experiments.

References

Abbate, P.E., Andrade, F.H., Lazaro, L., Bariffi, J.H., Berardocco, V.H., Marturano, F. 1998. Grain yield increase in recent Argentine wheat cultivars. Crop Sci 38, 1203-1209.

Abbate, P.E, Andrade, F.H., Culot, J.P., 1995.The effects of radiation and nitrogen on number of grains in wheat. J. Agric. Sci. (Camb.),124, 351-360.

Abbate, P.E, Andrade, F.H., Culot, J.P., Bindraban, P.S., 1997. Grain yield in wheat:Effects of radiation during spike growth period. Field Crops Res. 54, 245-257.

Acreche, M.M, Briceno-Felix, G., Sanchez, J.A.M, Slafer, G.A., 2008. Physiological bases of genetics gains in Mediterranean bread wheat yield in Spain. Eur. J. Agron. 28, 162-170.

Acreche, M.M., Slafer, G.A. (2009). Grain weight, radiation interception and use efficiency as affected by sink-strength in Mediterranean wheats released from 1940 to 2005. Field Crops Res.110, 98-105.

Aisawi, K. A. B. (2012) Physiological processes associated with genetic progress in yield potential of wheat. PhD Thesis. University of Nottingham pp. 295

583 Aravinda Kumar, B.N., Azam-Ali,S.N., Snape, J.W., Weightman, R.M., Foulkes, M.J.
584 2011.Relationships between carbon isotope discrimination and grain yield in winter wheat
585 under well-watered and drought conditions. Journal of Agricultural Science,149, 257-272.
586 Atsmon, D., Jacobs E.,1977. A newly bred 'gigas' form of bread wheat (*Triticum aestivum*
587 L.). Morphological features and thermo-photoperiodic responses. Crop Sci.17, 31-35.
588 Austin, R.B., 1999. Yield of Wheat in the United Kingdom: Recent Advances and Prospects.
589 Crop Sci. 39, 1604–1610.
590 Bancal, P., Soltani, F., 2002. Source-sink partitioning. Do we need Münch? J. Exp. Bot. 53,
591 1919-1928.
592 Berry, P.M., Sterling, M,.. Spink, J.H Baker, C.J. Sylvester-Bradley, R., Mooney, S.J., Tams,
593 A.R.Ennos, A.R. 2004. Understanding and reducing lodging in cereals. Adv. Agron. 84:217–
594 271.
595 Borghi, B., Corbellini, M., Cattaneo, M., Fornasari, M.A., Zuccchelli, L., 1986. Modification
596 of the source/sink relationship in bread wheat and its influence on grain yield and protein
597 content. Journal of Agronomy and Crop Science 157, 245-254.
598 Borras, L., Slafer, G.A, Otegui, M.E. 2004. Seed dry weight response to source sink
599 manipulation in wheat, maize and soybean: a quantitative reappraisal. Field Crops Res. 86,
600 131-146.
601 Bustos, D.V., Hasan, A.K., Reynolds, M.P., Calderini, D.F. 2013. Combining high grain
602 number and weight through a DH population to improve grain yield potential of wheat in
603 high-yielding environments. Field Crops Research, 145, 106-115
604 Clarke S, Sylvester-Bradley R, Foulkes J, Ginsburg D, Gaju O, Werner P, Jack P, Flatman E, Smith-
605 Reeve L. **2012.** Adapting wheat to global warming or 'ERYCC' Earliness and Resilience for Yield in
606 a Changing Climate. HGCA Research and Development Report 496. Home-Grown Cereals
607 Authority, London.
608 Dencic, S. (1994). Designing a wheat ideotype with increased sink capacity. Plant Breeding
609 112, 311-317.
610 Dofing, S.M. (1996). Near isogenic analysis of uniculm and conventional-tillering barley
611 lines. Crop Sci. 36, 523-526.
612 Donald, C. M., 1979. A barley breeding program based on an ideotype. Journal of
613 Agricultural Science **93**, 261-269.
614 Donald, C.M., 1968. The breeding of crop ideotypes. Euphytica 17, 385-403.

615 Duggan, B.L., Richards, R.A., van Herwaarden, A.F, Fettell, N.A. 2005. Agronomic
616 evaluation of a tiller inhibition gene (tin) in wheat. I. Effect of yield, yield components and
617 grain protein. Aust. J. Agric. Res. 56, 169-178.
618 Ellis, M.H., Spielmeyer, W., Gale, K.R., Rebetzke, G.J. &. Richards, R.A. 2002. "Perfect"
619 markers for the Rht-B1b and Rht-D1b dwarfing genes in wheat. Theor. Appl. Genet. **105**,
620 1038-1042
621 Fischer, R. A., 2007. Understanding the physiological basis of yield potential in wheat. J.
622 Agric. Sci. (Camb.) 145, 99–113.
623 Fischer, R.A., 1983. Growth and yield of wheat. In *Potential productivity of field crops under*
624 *different environments*. Edited by Smith, W.H and Bante, S.J, International Rice Research
625 Institute, pp 129-154, Los Banõs, Phillipines.
626 Fischer, R.A., Rees, D., Sayre, K.D., Lu, Z.M., Condon, A.G.,Larque Saavedra, A.
627 1998.Wheat yield progress associated with high stomatal conductance and photosynthetic
628 rate, and cooler canopies. Crop Sci. 38, 1467-1475.
629 Foulkes, M.J., Reynolds, M.P., Sylvester-Bradley, R. 2009. Genetic improvement of grain
630 crops: yield potential. In: Sadras VO, Calderini D, editors. Crop physiology applications for
631 genetic improvement and agronomy. The Netherlands: Elsevier; p. 235-256.
632 Foulkes, M.J., Slafer, G.A., Davies, W.J., Berry, P.M., Sylvester-Bradley, R., Martre, P.,
633 Calderini, D.F, Griffiths, S., Reynolds, M.P., 2011. Raising yield potential of wheat. III.
634 Optimizing partitioning to grain while maintaining lodging resistance. J. Exp. Bot. 62, 469-
635 486.
636 Gaju, O., Reynolds, M.P., Sparkes, D.L,. Foulkes, M.J. 2009 Relationships between large-
637 spike phenotype, grain number, and yield potential in spring wheat. Crop Sci. 49, 961-973.
638 Gay, A.P., Stokes, D.J., Weightman, R.M., Sylvester-Bradley, R. 1998. How to run a
639 reference crop. HGCA Research and Development Project Rep. 151. Home-Grown Cereals
640 Authority, London.
641 Ghiglione, O.G., Gonzalez, F.G., Serrago, R., Maldonado, S.B., Chilcott, C., Cura, J.A.,
642 Miralles, D.J., Zhu, T. and Cassel, J.J. 2008. Autophagy regulated by day length determines
643 the number of fertile florets in wheat. Plant Journal 55, 1010-1024.
644 González F.G., Terrile I.I., Falcón M.O., 2011. Spike Fertility and Duration of Stem
645 Elongation as Promising Traits to Improve Potential Grain Number (and Yield): Variation in
646 Modern Argentinean Wheats. Crop Sci. 51, 1693-1702.

647 Hanif, M., Langer, H.M., 1972.The vascular system of the spikelet in wheat (*Triticum*
648 *aestivum*). Ann. Bot. 36, 721-727.
649 Laurie, D.A., Bennett, M.D., 1986 Wheat x maize hybridisation. Canadian Journal of
650 Genetics and Cytology 28, 313-316.
651 Lazaro, L., Abbate, P.E., 2011. Cultivar effects on relationship between grain number and
652 photothermal quotient or spike dry weight in wheat. J. Agric. Sci. (Camb.) **148**, 83-93.
653 Minchin, P.E.H., Thorpe, M.R.,Farrar, J.F. 1993. A simple mechanistic model of phloem
654 transport which explains sink priority. J. Exp. Bot. 44, 947-955.
655 Miralles, D.J., Slafer, G.A. 2007. Sink limitations to yield in wheat: how could it be reduced?
656 Paper presented at international workshop on increasing wheat yield potential, CIMMYT,
657 Obregon, Mexico, 20-24 March 2006. J. Aric. Sci. (Camb.)145, 99-113.
658 Monteith, J.R. 1972. Solar radiation and productivity in tropical ecosystems. J. App. Eco.
659 9,747-766.
660 Motzo, R., Giunta, F., Deidda, M., 2004. Expression of a tiller inhibitor gene in the progenies
661 of interspecific crosses *Triticum aestivum* L. x *T. turgidum* subsp. durum. Field Crops Res.
662 85, 15-20.
663 Peltonen-Sainio, P., Jauhiainen, L., and Laurila, I. P. 2009. Cereal yield trends in northern
664 European conditions: Changes in yield potential and its realisation. Field Crops Res. 110: 85-
665 90.
666 Rajaram, S., Reynolds, M. P., 2001. *International Wheat Breeding.* Abstracts of XVIth
667 EUCARPIA Congress, Edinburgh, Scotland, 'Plant Breeding: Sustaining the Future' 10-14
668 September 2001. EUCARPIA.
669 Reynolds, M.P., Foulkes, M.J., Furbank, R., Griffiths, S., King, J., Murchie, M., Parry, M.,
670 Slafer, G. 2012. Achieving yield gains in wheat. Plant, Cell Enviorn. 35, 1799-1823.
671 Reynolds, M., Bonnett, D., Chapman, S.C., Furbank, R.T., Manès, Y., Mather, D.E., Parry,
672 M.A.J., 2011. Raising yield potential of wheat. I. Overview of a consortium approach and
673 breeding strategies. J. Exp. Bot. 62, 532-549.
674 Reynolds, M., Foulkes, M.J., Slafer, G.A., Berry, P., Parry, M.A.J, Snape, J.W., Angus, W.J.
675 2009. Raising yield potential in wheat. . J. Exp. Bot. 60, 1899-1918.
676 Reynolds, M.P., Pellegrineschi, A., Skovmand, B. 2005. Sink limitation to yield and biomass:
677 a summary of investigations in spring wheat. Ann. App. Biol. 146, 39-49.
678 Reynolds, M.P., Rajaram, S., Sayre, K.D., 1999. Physiological and genetic changes of irrigated
679 wheat in the post-green revolution period and approaches for meeting projected global demand.
680 Crop Sci. 39, 1611-1621.

Richards, R.A., 1988. A tiller inhibitor gene in wheat and its effect on plant growth. Aust. J. Agric. Res. 39, 749–757.

Rosegrant M.W., Agcaoili M. 2010. Global food demand, supply, and price prospects to 2010. International Food Policy Research Institute, Washington, DC, USA.

Savin, R., Slafer, G.A., 1991. Shading effects in the yield of an Argentinean wheat cultivar. J. Agric. Sci. (Camb.) 116, 1-7.

Sayre, K.D., Rajaram, S., Fischer, R.A., 1997. Yield potential progress in short bread wheats in northwest Mexico. Crop Sci. 37, 36-42.

Shearman, V.J, Sylvester-Bradley, R, Scott, R.K., Foulkes, M.J., 2005. Physiological processes associated with wheat yield progress in UK. Crop Sci. 45, 175-185.

Slafer, G.A. Andrade, F.H. 1993. Physiological attributes related to the generation of grain yield in bread wheat cultivars released at different eras. Field Crops Res. 31**,** 351–367.

Slafer, G.A., Savin, R. 1994. Source-sink relationships and grain mass at different positions within the spike in wheat. Field Crops Res. 37, 39-49.

Spielmeyer, W., Richards, R. 2004. Comparative mapping of wheat chromosome 1AS which contains the tiller inhibition gene (*tin*) with rice chromosome 5S. Theor. Appl. Genet. 109, 1303-1310.

Tottman, D.R, Broad, H., 1987. The decimal code for the growth stages of cereals with illustrations. Ann. Appl. Biol. 110, 441-454.

Yemm, E.W., Willis, A.J., 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochemistry Journal 57, 508-514.

Zhou, Y., He, Z.H., Suic, X.X., Xiaa, X.C., Zhang, X.K., Zhang, G.S. (2007). Genetic improvement of grain yield and associated traits in the northern china winter wheat region from 1960 to 2000. *Crop Science* 47, 245-253.

Table 1. Mean daily temperature, mean daily relative humidity (RH), monthly rainfall and daily solar radiation at CIMMYT experimental station at Ciudad Obregon in NW Mexico in 2004-5 and 2005-6

	2004-5					2005-6				
	Dec	Jan	Feb	Mar	Apr	Dec	Jan	Feb	Mar	Apr
Mean daily temp (^{o}C)	15.5	16.4	16.0	17.7	20.8	16.1	14.5	16.5	17.2	21.1
Mean daily RH (%)	62.2	72.3	75.0	61.1	47.3	58.2	57.4	66.1	62.3	47.2
Monthly rainfall (mm)	0.1	1.7	1.7	0.0	0.0	0.0	0.7	0.0	0.0	0.0
Daily solar rad. (MJ m^{-2})	13.9	14.8	17.4	25.5	27.6	15.8	16.1	19.3	22.7	25.6

Table 2. Maximum, minimum and mean values for anthesis date (GS61), spike traits at GS61+5d, grain yield (at 1000 g DM kg^{-1}), yield components and above-ground dry matter at harvest ($AGDM_H$) for 57 DH lines of LSP2 x R population in 2004-5 and 2005-6 and SEDs from cross-year ANOVA for year, DH line and interaction. Probability † < 0.10, * < 0.05, ** < 0.001, and *** < 0.001.

	Anthesis date	**Plant height (cm)**	**Grains m^{-2}**	**Grains $spike^{-1}$**	**Rachis length (cm)**	**Spikelets $spike^{-1}$**	**Spikes m^{-2}**	**Grain weight (mg)**	**Grain yield (g m^{-2})**	**$AGDM_H$ (g m^{-2})**
2005										
Min	18 Feb	46.8	6,813	26.2	10.2	18.9	224.6	20.8	204.4	672
Max	11 Apr	106.0	19,391	51.7	16.1	29.7	543.0	52.9	727.3	2176
Mean	10 Mar	72.2	13,322	38.2	12.9	24.1	354.6	35.2	460.8	1247
S.E.D. DH line (D.F.=56)	-	1.95	2,059	5.62	0.51	1.37	66.25	2.33	66.50	269.1
DH line Prob.	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001
2006										
Min	24 Feb	52.0	8,496	20.1	9.6	17.3	197.9	18.2	202.5	457
Max	1 Apr	101.9	17,966	64.8	15.1	27.6	541.4	50.5	646.5	1632
Mean	13 Mar	74.5	13,199	38.2	12.6	22.7	363.2	32.8	427.5	1072
LSP2	24 Feb	93.4	12,258	44.8	13.2	21.4	275.1	44.1	543.4	1064
S.E.D. DH line (D.F.=56)	-	3.39	1,442.7	6.42	0.58	1.41	56.47	2.15	37.85	116.4
DH line Prob.	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SED DH line vs LSP2 (D.F.=57)	-	2.344	1,440.1	6.41	0.59	1.40	56.61	2.14	38.71	152.71
Cross-Year ANOVA										
S.E.D. (D.F.=112) DH line	2.30***	1.97***	1259.2***	4.27***	0.39***	0.99***	43.68***	1.59***	38.28***	146.8***
S.E.D. (D.F.=2) Year	0.50*	0.30*	612.8	0.41	0.21	0.51	16.9	0.39*	15.8	94.2
S.E.D. (D.F. = 112) Yr*line	3.26***	2.78***	1869.1	6.01***	0.59***	1.47***	63.52*	2.27**	55.93*	226.3†

Table 3. Rachis length (R. Len.), spikletes per spike, spike DM partitioning index at anthesis (GS61) (Spike Part. Index), spike fertility index (SPI), soike DM per unit area at GS61 (Spike DM_{anth}), plant height, harvest index, above-ground dry matter (AGDM) at harvest, grain yield (at 1000 g DM kg^{-1}), yield components, and anthesis date (GS61) for groups of -*Tin1* (44 lines) and +*Tin1* (13 lines) lines of the LSP2 x Rialto DH population in 2005 and 2006. ns = not significant.

	2005			2006			2005-6			
Trait	-*Tin1*	+*Tin1*	Prob.	-*Tin1*	+*Tin1*	Prob.	-*Tin1*	+*Tin1*	Prob. ±*Tin1*	Prob . ±*Tin1* x Year
Anthesis										
Anthesis date	71.1	70.3	n/a	74.20	74.35	n/a	72.7	72.3	n/a	n/a
R. Len. (cm)	12.78	13.11	0.08	12.48	12.77	0.04	12.6	12.9	0.005	ns
Spikelets $spike^{-1}$	23.9	24.8	0.02	22.62	22.90	Ns	23.3	23.9	0.020	ns
Spike Part. Index	0.32	0.34	0.00	0.33	0.33	Ns	0.32	0.34	0.001	0.019
SFI. (grns g^{-1} DM)	55.6	56.2	ns	38.89	45.49	0.002	47.3	50.8	ns	0.010
Spike DM_{anth} (g m^{-2})	299.4	352.2	0.01	365.1	343.5	ns	332.3	347.8	ns	0.013
Harvest										
Plant height (cm)	72.4	71.6	ns	74.45	73.0	0.010	73.4	72.3	0.003	ns
Grain yield (g m^{-2})	456.6	474.9	ns	425.4	426.1	ns	441.0	450.5	Ns	ns
AGDM (g m^{-2})	1222.6	1329.3	ns	1084.9	1030.1	0.03	1153.8	1179.7	Ns	ns
Harvest Index	0.38	0.37	ns	0.39	0.43	<0.001	0.388	0.399	0.001	<0.001
Grains m^{-2}	13125	13990	ns	13017	13883	0.013	13071.2	13936.4	0.013	ns
Spikes m^{-2}	354.0	356.5	ns	374.70	330.91	0.001	364.4	343.7	0.003	ns
Grains $spike^{-1}$	37.7	40.0	0.02	36.2	44.2	<0.001	37.0	42.1	<.0001	0.003
Grains $spikelet^{-1}$	1.59	1.62	ns	1.61	1.95	<0.001	1.60	1.79	<.0001	0.007
Grain weight mg	35.4	34.4	0.06	33.02	31.05	0.001	34.2	32.7	<.0001	ns

Table 4. Antheiss date, rachis length, spikelets per spike, , spike partitioning index, spike fertility index, plant height, harvest index, above-ground dry matter (AGDM) and grain yield (t ha^{-1}; 1000 g DM/kg) and yield components for semi-dwarf (*Rht-B1a* + *Rht-D1b* ; 31 lines) and dwarf (*Rht-B1b* + *Rht-D1b* ; 26 lines) groups of lines of the LSP2 x Rialto DH population. Probability † < 0.10, * < 0.05, ** < 0.001, and *** < 0.001.

Traits	**RhtB1b**		**RhtB1a**		**SED RhtB1 (DF = 195)**	**SED RhtB1 x *Tin1* (DF=195)**
	+Tin1	***-Tin1***	***+Tin1***	***-Tin1***		
Anthesis						
Anthesis date	16 Mar	17 Mar	14 Mar	10 Mar	1.558 ***	5.90 ns
Rachis length (cm)	13.06	12.81	13.72	12.58	0.162 ns	0.367 *
Spikelets $spike^{-1}$	24.3	23.7	24.5	23.1	0.33 ns	0.754 ns
Spike partitioning index	0.367	0.358	0.314	0.289	0.0062 ***	0.0142 ns
SFI (grains g^{-1} DM)	43.8	41.4	54.3	51.8	2.483 ***	5.62 ns
Harvest						
Plant height (cm)	62.5	64.2	84.4	82.9	1.63 ***	3.67 ns
Grain yield (t ha^{-1} 100% DM)	415.1	401.6	467.9	458.1	15.05 **	34.07 ns
AGDM (g m^{-2})	1073	1094	1325	1141	39.55 *	89.52 *
Harvest index	0.401	0.373	0.379	0.406	0.011 0.053†	0.0263 †
Grains m^{-2}	14040	13144	14179	12557	377.8 ns	855.2 ns
Spikes m^{-2}	353.1	386.5	331.5	346.3	12.00 ***	27.16 ns
Grains $spike^{-1}$	41.4	35.2	43.2	38.6	1.25 *	2.83 ns
Grains $spikelet^{-1}$	1.71	1.50	1.79	1.68	0.0589 **	0.129 ns
Grain weight (mg)	29.6	30.9	33.2	37.1	1.075 ***	2.43 ns

Table 5 . Antheiss date, rachis length, spikelets per spike, , spike partitioning index, spike fertility index, plant height, harvest index, above-ground dry matter (AGDM) and grain yield (t ha^{-1}; 1000 g DM/kg) and yield components for +*Tin1A*/awned (7 lines), -*Tin1A*/awned (23 lines), +*Tin1A*/unawned (6 lines) and -*Tin1A*/unawned (21 lines) groups of lines of the LSP2 x Rialto DH population. Values represent means across 2004-5 and 2005-6. Probability < 0.10 denoted by †

Traits	**Awned**		**Unawned**		**SED Awned/Unawned (DF = 109)**	**SED Tin1A x Awns (DF=109)**
	+Tin1A	***-Tin1 A***	***+Tin1A***	***-Tin1A***		
Anthesis						
Anthesis date	12 Mar	10 Mar	11 Mar	14 Mar	0.351 ns	4.39 ns
Rachis length (cm)	12.7	12.4	13.3	12.9	0.3456 ***	0.32 ns
Rachis length spikelet^{-1} (cm)	0.55	0.55	0.54	0.54	0.01436 ns	0.012 ns
Spikelets spike^{-1}	23.1	22.7	24.7	23.9	0.714 ***	0.65 ns
Spike partitioning index	0.341	0.325	0.333	0.322	0.01747 ns	0.0159 ns
SFI (grains g^{-1} DM)	45.7	45.9	56.8	48.7	5.705 ns	5.31 ns
Harvest						
Plant height (cm)	69.7	74.5	75.3	72.2	4.67 ns	4.14 ns
AGDM (g m^{-2})	1180	1213	1180	1088	87.05 ***	86.1 ns
Harvest index	0.383	0.381	0.417	0.394	0.0247 *	0.024 ns
Grain yield (t ha^{-1} 100% DM)	4.26	4.60	4.89	4.14	0.337 ns	0.315***
Grains m^{-2}	12574	12699	15525	13479	813.2 **	711.6*
Spikes m^{-2}	346.2	364.0	340.8	364.8	27.60 ns	24.01 ns
Grains spikelet^{-1}	1.69	1.58	1.90	1.63	0.12618 *	0.113*
Grains spike^{-1}	38.1	35.6	46.8	38.5	2.661 ***	2.33*
Grain weight (mg)	34.1	36.9	31.1	31.2	0.24 ***	2.15 ns

Table 6 *Grain* yield (t ha^{-1}; 1000 g DM kg^{-1}), harvest index (HI), above-ground dry matter (AGDM), grain yield (t ha^{-1}; 1000 g DM kg^{-1}) and yield components for *+Tin1A* and *-Tin1A* groups of lines of the LSP2 x Rialto DH population in 2005-6. Probability < 0.10 denoted by †

Lines	*Tin1A*	Grain yield (t ha^{-1})	Grains $spike^{-1}$	Spikes m^{-2}	Grains m^{-2}	AGDM (g m^{-2})	HI	Grain weight (mg)	Rachis length (cm)	Spikelets $Spike^{-1}$	Plant height (cm)	Anthesis date
1	*+Tin1A*	666.1	52.3	497.2	25981	1811	0.37	25.8	13.4	26.3	72.1	20 Mar
21	*+Tin1A*	710.4	45.5	453.8	20596	1712	0.42	34.6	13.2	23.7	70.6	15 Mar
31	*+Tin1A*	667.3	42.4	457.7	19327	1718	0.39	34.6	13.2	24.8	66.9	21 Mar
124	*+Tin1A*	558.2	47.0	444.3	20911	1443	0.39	26.7	14.5	26.3	65.9	17 Mar
Mean		650.5	46.8	463.3	21704	1671	0.40	30.4	13.575	25.3	68.9	68.9
7	*- Tin1A*	473.1	30.5	542.2	16390	1403	0.34	29.0	11.7	22.7	61.0	27 Mar
17	*- Tin1A*	704.3	37.3	565.0	20864	1946	0.36	33.8	11.6	22.1	93.9	16 Mar
116	*-Tin1A*	658.6	47.9	410.0	19604	1468	0.45	33.6	14.5	25.8	61.2	20 Mar
106	*- Tin1A*	682.4	41.6	529.2	22082	1713	0.40	31.1	13.2	26.2	61.9	16 Mar
Mean		629.6	39.3	511.6	19735	1632.5	0.38	31.9	12.75	24.2	69.5	69.5
SED (df=20)		32.61	2.282	23.56	1134.1	87.0	0.015	1.457	0.433	0.584	4.56	4.56
Prob.		ns	**	*	0.09	ns	ns	ns	0.065	ns	Ns	ns

Table 7 Fertile shoots per m^2, spike DM per m^2, above-ground DM per m^2 (AGDM) and spike partitioning index (SPI) at GS61+5d; fruiting efficiency (grains per gram spike DM at GS61+5d; FE), accumulated photosynthetically active radiation (PAR) from GS 31-61+5d, radiation use efficiency (RUE_{PAR}) from GS 31-61+5d, green area index (GAI) at GS61+5d, plant height at harvest and anthesis date for *+Tin1* and *- Tin1* groups of lines of the LSP2 x Rialto DH population in 2005-6. Probability < 0.10 denoted by †

Lines	***Tin1A***	**Plants m^{-2}**	**Fertile shoots m^{-2}**	**Spike DM (g m^{-2})**	**AGDM (g m^{-2})**	**SPI**	**FE (grains g^{-1})**	**Stem WSC t ha^{-1}**	**Accumulated PAR (MJ m^{-2}) GS 31-61+5d**	**RUE_{PAR} (g MJ^{-1})**	**GAI**	**Plant height (cm)**	**Anthesis date**
1	*+Tin1*	158.3	494	350.8	1138	0.31	74.1	0.58	1969	1.17	9.43	72.1	20 Mar
21	*+Tin1*	140.8	417	310.9	1001	0.31	66.3	0.76	1756	1.15	7.73	70.6	15 Mar
31	*+Tin1*	147.5	505	438.9	1344	0.33	44.0	0.89	1968	1.36	8.73	66.9	21 Mar
124	*+Tin1*	157.5	451	352.7	999	0.35	59.3	0.70	1792	1.10	8.15	65.9	17 Mar
Mean *+Tin1A*		151.0	467	363.3	1121	0.33	60.9	0.73	1871	1.20	8.51	68.9	18 Mar
7	*- Tin1*	168.3	622	287.2	1159	0.25	57.1	0.80	2121	1.12	9.12	61.0	27 Mar
17	*- Tin1*	157.5	584	279.7	1154	0.24	74.6	1.09	1787	1.31	7.88	93.9	16 Mar
106	*- Tin1*	169.2	552	334.1	1154	0.29	66.1	0.90	1910	1.26	7.86	61.2	20 Mar
116	*-Tin1*	144.8	394	331.6	1054	0.32	59.1	0.79	1774	1.20	6.64	61.9	16 Mar
Mean *Tin1*	-	160.0	538	308.2	1130	0.28	64.2	0.90	1898	1.22	7.88	69.5	19 Mar
SED (df = 20)		5.90	35.8	19.85	62.8	0.0153	0.584	0.584	54.2	0.509	0.452	4.56	
Prob.		Ns	†	*	ns	**	ns	ns	ns	ns	ns	ns	

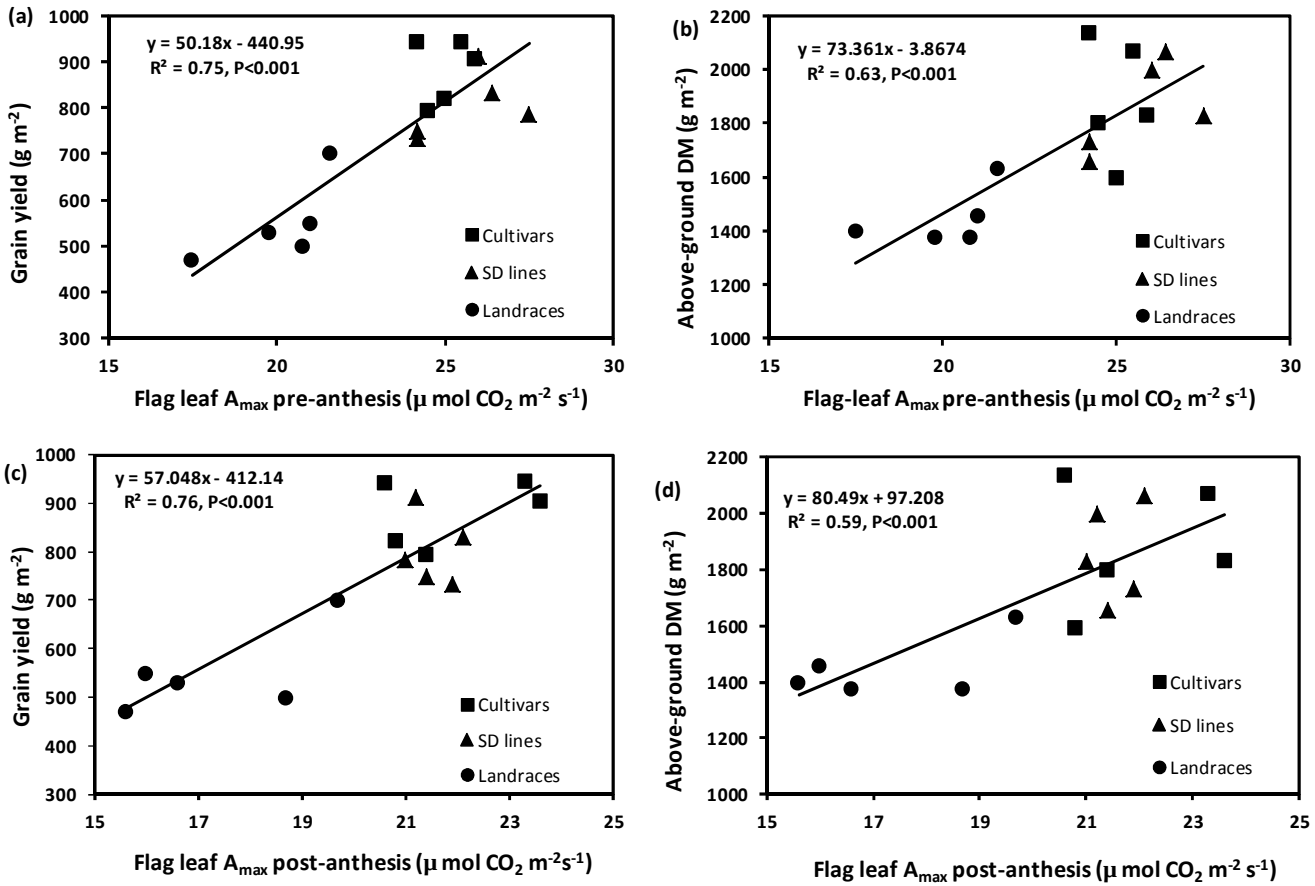

Fig. 1. Linear regression of a) grain yield (100% DM) on pre-anthesis flag-leaf photosynthetic rate (A_{max}), b) above-ground dry matter on pre-anthesis flag-leaf photosynthetic rate, c) grain yield on post-anthesis flag-leaf photosynthetic rate and d) above-ground dry matter on post-anthesis flag-leaf photosynthetic rate for 15 wheat genotypes (5 modern cultivars, 5 synthetic-derived (SD) lines and 5 landraces) in the high N treatment (values represent means of 2011 and 2012).

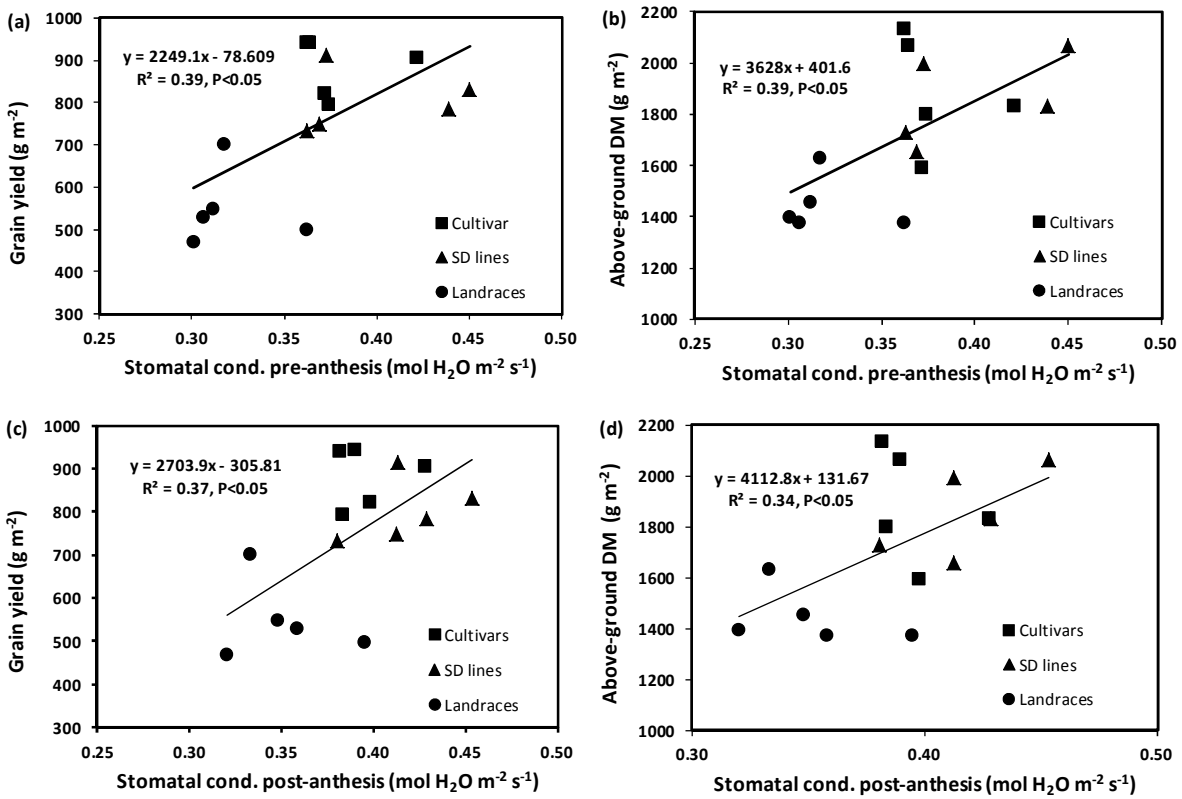

Fig. 2. Linear regression of a) grain yield (100% DM) on pre-anthesis flag-leaf stomatal conductance, b) above-ground dry matter on pre-anthesis flag-leaf stomatal conductance, c) grain yield on post-anthesis flag-leaf stomatal conductance and d) above-ground dry matter on post-anthesis flag-leaf stomatal conductance on 15 wheat genotypes (5 modern cultivars, 5 synthetic-derived (SD) lines and 5 landraces) in the high N treatment (values represent means of 2011 and 2012).

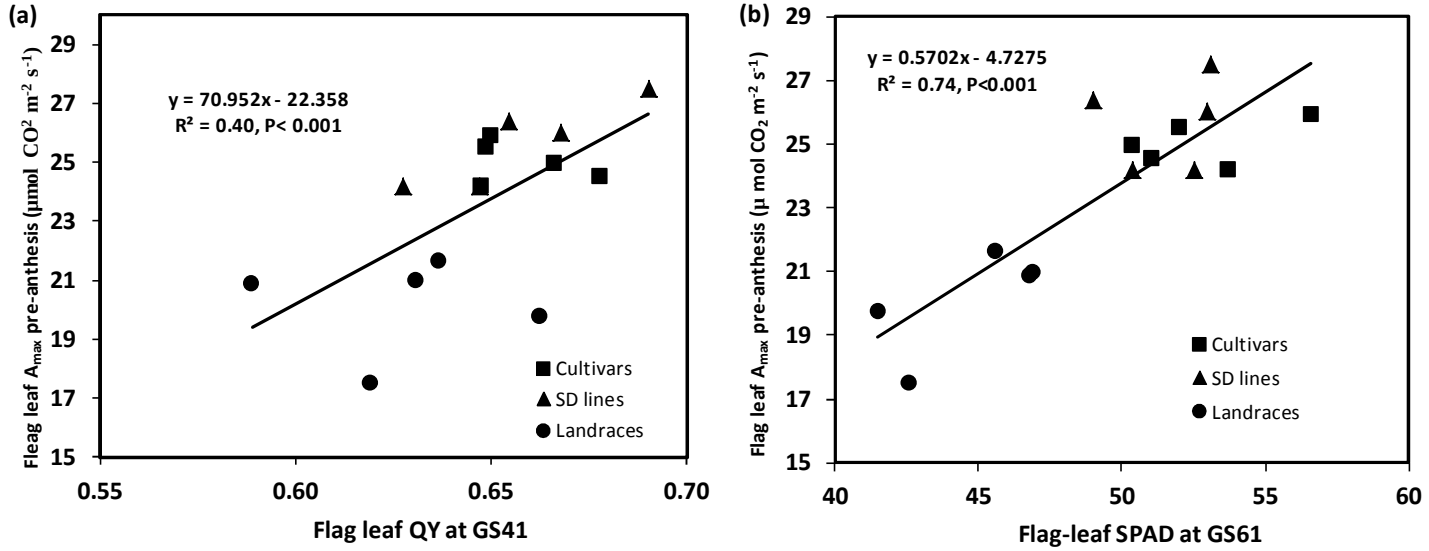

Fig. 3. Linear regression of: (a) pre-anthesis flag-leaf photosynthetic rate (A_{max}) on flag-leaf chlorophyll fluorescence quantum yield at onset of booting (GS41) and (b) flag-leaf A_{max} pre-anthesis on flag-leaf relative chlorophyll content (SPAD) at anthesis (GS61) for 15 wheat genotypes (modern cultivars landraces and synthetic-derived lines) in the high N treatment (values represent means of 2011 and 2012).

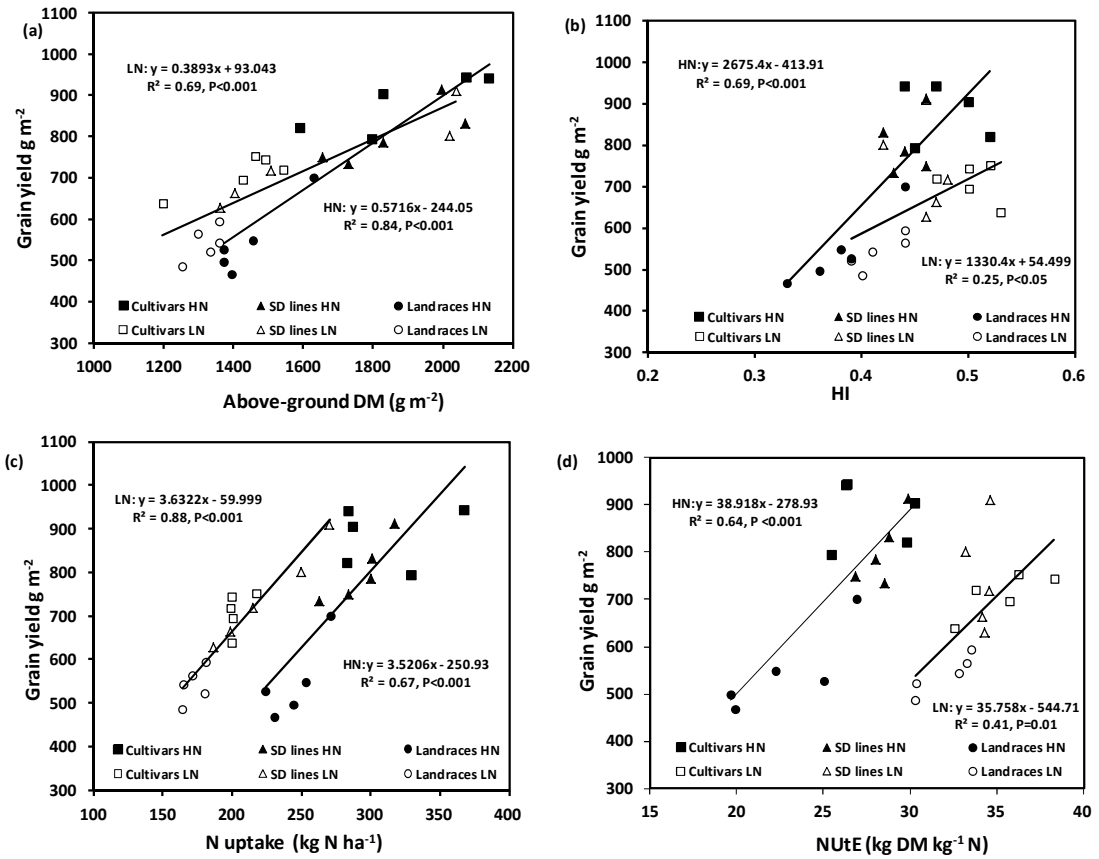

Fig. 4. Linear regression of (a) grain yield (100% DM) on above-ground dry matter, (b) grain yield on harvest index, (c) grain yield on above-ground N uptake at harvest and (d) grain yield on N-utilisation efficiency (NUtE) under HN (solid symbols) and LN (open symbols) conditions for 15 wheat genotypes (landraces, synthetic-derived (SD) lines and modern cultivars) (values represent means of 2011 and 2012).

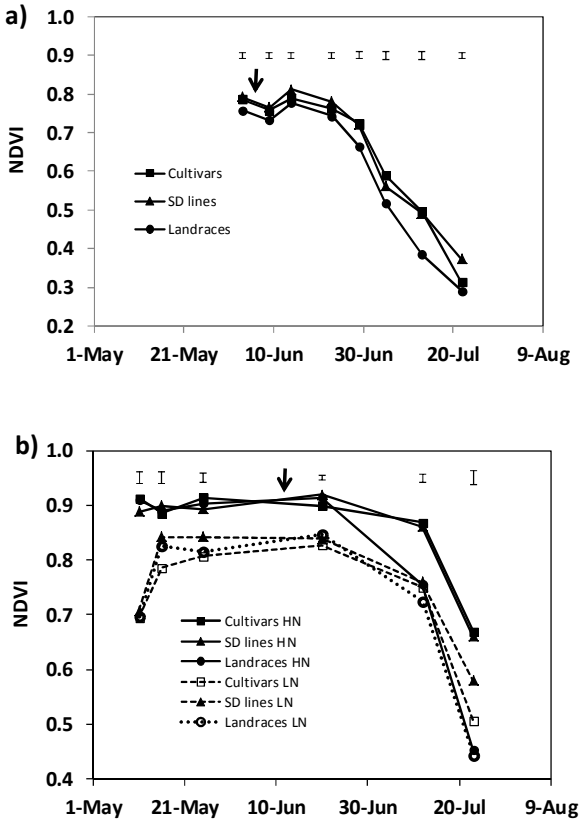

Fig. 5. NDVI for genotype groups (landraces, synthetic-derived (SD) lines and modern cultivars under a) high N (HN) in 2011 and b) high N (HN) and low N (LN) conditions in 2012. Error bars show LSD (5%) for genotype in 2011 and N x Genotype in 2012. Arrows indicate date of anthesis (GS61) averaged across genotypes under HN in 2011 and across genotypes under HN and LN conditions in 2012.

a) High N **b) Low N**

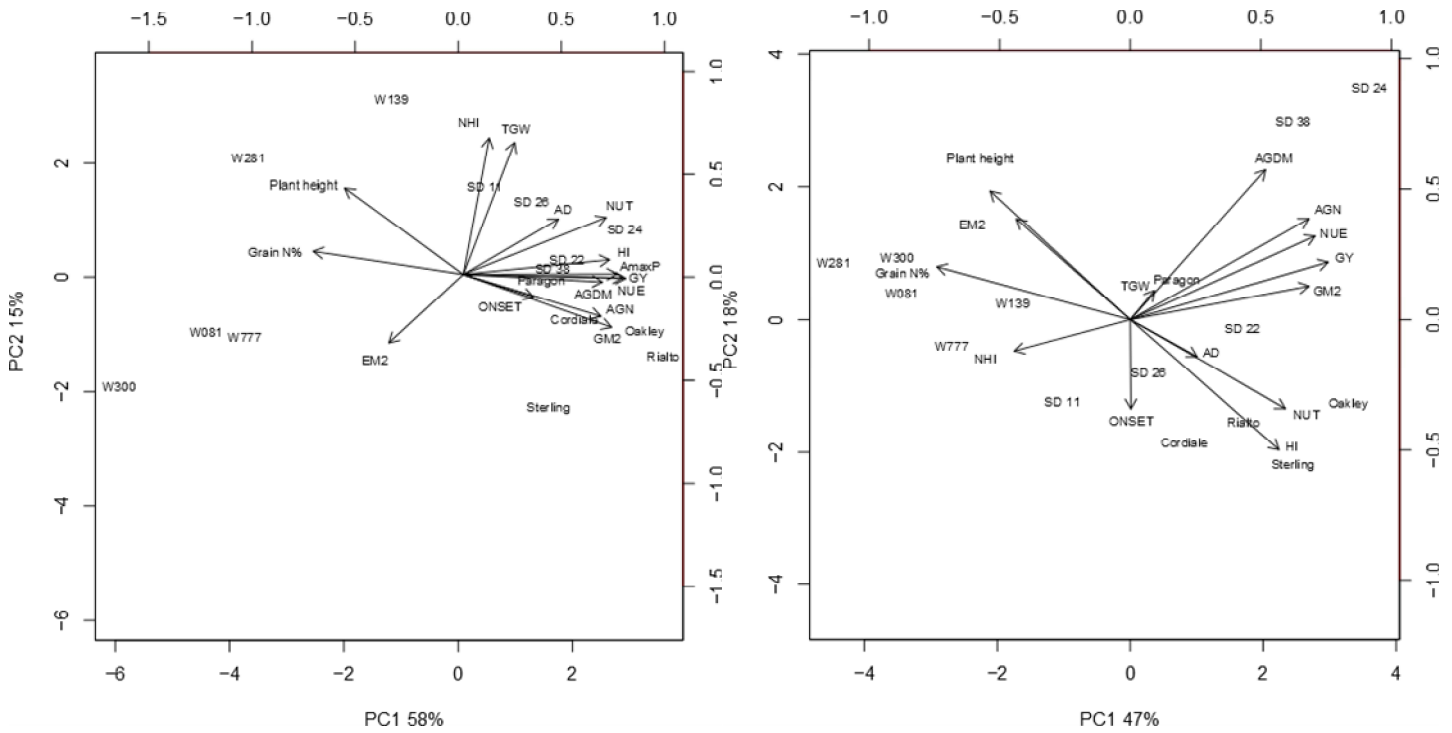

Fig. 6. Biplot for grain yield (GY), grains m^{-2} (GM2), N-use efficiency (NUE), N-utilization efficiency (NUT), N harvest index (NHI), above-ground N uptake at harvest (AGN), above-ground dry matter at harvest (AGDM), harvest index (HI), 1,000 grain weight (TGW), anthesis date (AD), onset of flag-leaf post-anthesis senescence (ONSET), grain N%, plant height and ears per m^2 (EM2) for 15 wheat genotypes under a) HN conditions and b) LN conditions (values represent means of 2011 and 2012).