



Phenotypic and genetic analysis to identify secondary physiological traits for improving grain yield in wheat considering anthesis time variability

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Abstract Identification of secondary traits in mapping populations is usually hindered by the strong effect of anthesis time. Thus, considering the variability in time to anthesis in combination with an accurate phenotyping of mapping populations and available molecular tools is a possible way for recognising secondary traits to improve yield potential. The aim of this work was to identify secondary traits to perform indirect selection for grain yield (GY) in a bread wheat mapping population consisting of 124 doubled haploid lines, derived from the cross between two soft red winter wheat genotypes with contrasting photoperiod

response. Genomic regions linked to different traits were analysed under two environments. The population showed a strong effect of time to anthesis over GY, as expected. A large variability in GY was observed but only two QTLs were detected on chromosome 5A for this trait, which co-localized with QTLs of time to anthesis. GY variation was partially associated with above-ground dry matter at maturity (AGDM) and to a lesser extent with harvest index (HI). Detected QTLs for grains per m² (GN), grain weight, AGDM and HI explained between 25 and 61% of the GY additive genetic variance. GN, defined as the product between spike dry weight at anthesis (SDW) and fruiting efficiency (FE), was the main numerical component that explained most of the variation in GY. Five QTLs were detected for SDW, which did not co-localize with QTLs of time to anthesis and captured ca. 50% of the additive genetic variance, while there was a weak relationship between

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NG and FE. When genotypes were filtered by similar anthesis time and plant height, SDW was identified as a promising secondary trait to be targeted for indirect selection of GY.

Keywords Extreme phenotypes · Grain number · Phenology · Quantitative trait loci · Spike dry weight

Abbreviations

AGDM	Above-ground dry matter at maturity (g m ⁻²)
AGDM-AN	Above-ground dry matter at anthesis (g m ⁻²)
CGR-AN	Crop growth rate during pre-anthesis (g m ⁻² °Cd ⁻¹)
EM-AN	Duration of the phase from emergence to anthesis (days or °Cd)
EM-FL	Duration of the phase from emergence to flag leaf appearance (days or °Cd)
FE	Fruiting efficiency (grains g ⁻¹)
FL-AN	Duration of the phase from flag leaf appearance to anthesis (days or °Cd)
GN	Number of grains per m ² (grains m ⁻²)
GW	Grain weight (mg grain ⁻¹)
GY	Grain yield (g m ⁻²)
HI	Harvest index
HI-AN	Harvest index at anthesis
PH	Plant height at maturity (cm)
SDW	Spike dry weight at anthesis (g m ⁻²)

Introduction

Wheat production increases are critically needed to meet food demand by the growing population in the near future (Godfray et al. 2010; Reynolds et al. 2011; McKersie 2015). Success in increasing wheat production by increasing yield per unit area is crucial since it is not possible to massively expand the sowing area. Yield potential has been successfully increased

through traditional breeding in the past, but in the last few years a lack of improvement in yield gain under field conditions has become evident in ca. 39% of the wheat world area (Ray et al. 2012). Considering that wheat global demand is growing at a faster rate than current genetic gains (Ray et al. 2012) and taken into account that wheat is one of the species that has received intense selection pressure, it is necessary to complement conventional breeding with physiological traits as indirect selection criteria. The identification of physiological traits, combined with available molecular tools, can provide a comprehensive model for understanding the genotype–phenotype relationship and the genotype × environment interaction to complement conventional breeding aimed at improving genetic gains in yield (Hammer et al. 2006; van Eeuwijk et al. 2010; Paux et al. 2012). This has led to a large investment in wheat genomic projects, which have been accompanied by a remarkable increase of the map-based identification of target genes for the improvement of grain yield (GY). However, an important weakness that emerges from this approach is that phenotyping of mapping populations under field conditions is expensive and time-consuming and consequently limits the number of samples to be taken and its size, especially when involving complex physiological traits.

GY can be analysed through two conceptual frameworks. The first one considers GY as the product between above-ground dry matter at maturity (AGDM) and the proportion of biomass partitioned to the grains (i.e. harvest index, HI). Genetic improvement of GY in wheat in the last century has been achieved through increases in harvest index, with null (Austin et al. 1980; Siddique et al. 1989; Calderini et al. 1999; Slafer and Andrade 1991; Lo Valvo et al. 2018) or sporadic (Shearman et al. 2005) differences in AGDM production when old and modern genotypes are compared. In current cultivars, HI in wheat is near 0.50 (Lo Valvo et al. 2018) and close to the maximum theoretical HI of 0.62 estimated by Austin et al. (1980), suggesting that only minor increases in this trait would be expected into the future. Approaches for improving AGDM, determined by the duration of the crop cycle and crop growth rate, are becoming more important in current research (Reynolds et al. 2011). In this sense, modifications in crop phenology (Miralles and Richards 2000; Slafer et al. 2001) and increases in crop photosynthetic capacity (Reynolds et al.

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2011, 2017) have been proposed as promising traits to improve wheat yield potential.

The second framework analyses GY in terms of its main numerical components: grain number per unit area (GN) and grain weight (GW). Changes in GY through genetic progress have been traditionally associated with changes in GN rather than GW (Calderini et al. 1999; Peltonen-Sainio et al. 2007; Fischer 2008; Lo Valvo et al. 2018). Grain number is mostly determined during the period around anthesis (Fischer 1985), which is critical for potential yield determination. Dry weight of the spike at the end of this critical period (or at anthesis) explains the number of grains established per m² and has been identified as a good predictor of final GY (Slafer and Andrade 1993; Fischer 2011). Fruiting efficiency (i.e. the number of grains set per unit of spike dry weight at anthesis or per unit of chaff dry weight at maturity) is the other trait that defines GN (Abbate et al. 1998; Slafer et al. 2015; Fischer 2011). One aspect to be taken into account is that GN and GW are frequently negatively associated (Slafer and Andrade 1989). But there was no association observed between GN and GW in some elite wheat populations (Rathey et al. 2009), and GW has increased in several cultivars released by CIMMYT in the last decade without negatively affecting GN (Aisawi et al. 2015), which is an incentive to continue increasing GY through increases in GN.

Identification of secondary physiological traits associated with yield potential in a breeding program has two common issues: (1) using the traditional approach of phenotyping at field level is practically impossible to carry out due to the number of plots involved in a typical program, and (2) the strong trade-off between time to anthesis and GY complicates the identification of independent secondary traits (Woodruff and Tonks 1983). Therefore, physiological traits can be identified indirectly by using molecular markers linked to target traits that do not co-localize with those related to phenology. An alternative approach, well suited for complex traits, consists in screening segregating populations for a potential indirect selection trait and identifying tails from the trait distribution that represent phenotypic extremes (Rebetzke et al. 2017). The smaller set of selected lines can then be evaluated more extensively for yield, expensive traits, or traits difficult to be measured.

The objective of the present study was to identify secondary traits to perform indirect selection for GY taking into account the variability in time to anthesis and its impact over other traits. Therefore, we measured the phenotypic variability of traits related to GY potential in a soft red winter wheat mapping doubled haploid population (Zhou et al. 2017). Genomic regions linked to these traits were analysed under two environments. As variability in time to anthesis is one of the main attributes explaining wheat adaptability and its wide expansion around the world (Kamran et al. 2014), we focus our analysis in a wheat population with variation in anthesis time, derived from a genotype with the Ppd-D1b photoperiod sensitive allele (MD01W233-06-1) and a photoperiod insensitive genotype (Ppd-D1a; SS8641) (Zhou et al. 2017). Additionally, lines of similar plant height and phenology were selected in each of the high and low tails for spike dry weight and crop growth rate at anthesis to evaluate the impact to those traits in yield and its physiological and numerical components.

Materials and methods

Experimental conditions and plant material

Two field experiments were carried out at the experimental field (34° 35' S, 58° 29' W; 26 m.a.s.l.) of the Department of Plant Production of the School of Agriculture, University of Buenos Aires, on a silty clay loam soil, classified as Vertic Argiudoll, during 2013 and 2014 growing seasons. In both growing seasons, 124 doubled haploid lines of soft red winter wheat (*Triticum aestivum* L.), its parents [(MD01W233-06-1 (Costa et al. 2010) and SS8641 (Johnson et al. 2007), named throughout the present work as P1 and P2, respectively)] and a commercial bread wheat check cultivar (Baguette 31) were evaluated for different quantitative physiological traits. Genotypes were tested in an augmented block design (Federer and Raghavarao 1975) arranged into 15 blocks with unreplicated treatments while the check cultivar was assigned within the blocks with 22% of frequency. Each genotype was sown in three rows with 150 cm long and 17.5 cm intervals. Experiments were sown on June 13, 2013 and June 6, 2014 at a seed rate of 230 plants m². Sowing in each row was done by hand placing the seeds equidistantly at a distance of

2.5 cm on 150 cm linear strips of biodegradable paper tape covered with tissue paper, which was then seeded on ploughed soil. Before sowing, soil samples (0–60 cm) were taken to determine N-nitrates and phosphorous concentration (Bray and Kurtz 1945). Nitrogen fertilizer (urea) was applied one week after emergence until reaching a total equivalent of 120 kg N ha^{-1} in both seasons. Soil P content was high ($> 25 \text{ ppm}$) and, therefore, P fertilizer was not applied. For the control of pests and diseases, insecticides (chlorpyrifos-ethyl 48 g a.i. per litre at Z58) and fungicides (azoxystrobin 200 a.i. per litre plus cyproconazole 80 g a.i. per litre at Z37 and at Z62) were applied in both seasons. Weeds were removed by hand. The experiments were irrigated to complement natural rainfall throughout both growing seasons.

Phenotypic evaluation

Physiological traits related to yield were evaluated in all doubled haploid lines, its parents and the check cultivar. Growth stages of emergence (Z11, EM), flag leaf appearance (Z41, FL), anthesis (Z69, AN), and maturity (Z94, MT) were determined according to the scale proposed by Zadoks et al. (1974). Each phenological stage was identified when at least 50% of the individuals of each experimental unit reached the mentioned stage. Time between two phenological stages was measured in days and in thermal time units. Thermal time was calculated as follows:

$$TT_{xy} = (T_{mxy} - T_b)D_{xy} \quad (1)$$

where TT_{xy} is the thermal time between the x and y stages ($^{\circ}\text{Cd}$), T_{mxy} is air mean daily temperature between the x and y stages ($^{\circ}\text{C}$), T_b is base temperature (0°C ; Cao and Moss 1989), and D_{xy} is duration in days between the x and y stages (days).

Biomass production was quantified at anthesis and at maturity by sampling 0.40 m of linear row at each stage. Plants were cut as close to the ground as possible. The number of stems and spikes were counted. Plants were then separated into individual organs (i.e. leaves, stem plus sheaths, and spike). Dry mass was weighed after oven-drying at 60°C for 72 h. Plant height (PH) was recorded at maturity in 4 main stems for each experimental unit from the ground to spike insertion. Crop growth rate during pre-anthesis ($\text{g m}^{-2}^{\circ}\text{Cd}^{-1}$, CGR-AN) was estimated as the ratio

between total above-ground biomass at anthesis and thermal time from emergence to anthesis. Harvest index (HI) at anthesis was estimated as the ratio between spike dry matter and above-ground dry matter at anthesis. At maturity, spikes were threshed and the numerical yield components were determined (number of spikes, grains per spike, grains per m^2 , grain weight). Grain weight was estimated from two subsamples of 50 grains. Harvest index at maturity was estimated as the ratio between GY and AGDM. Fruiting efficiency coefficient (FE) was calculated at maturity as:

$$FE_i = GN_i CHAFF_i^{-1} \quad (2)$$

where FE_i is the fruiting efficiency coefficient of the i genotype (grains g^{-1}), GN is the number of grains at maturity per unit area (grains m^{-2}), and $CHAFF$ is the dry weight of the vegetative components of the spike at maturity (g m^{-2}).

Statistical analysis of phenotypic trait data

Best linear unbiased predictors (BLUPs) (Henderson 1975) were computed for each genotype for each experimental year, in a model in which genotype and block were treated as random effects (Piepho et al. 2003). We used a SAS code in PROC MIXED v 9.1 (SAS Institute 1999) developed for augmented designs (Wolfinger et al. 1997). Estimated BLUPs values were then used for all subsequent analysis. Pearson phenotypic correlation between traits and its significance was performed with InfoStat (Di Rienzo et al. 2010). BLUPs across environments were used in a principal component analysis to analyze trait correlations. Phenotypic correlation between traits was estimated using the cosine of the angles formed by trait vectors.

Genetic map construction and QTL analysis

The 124 doubled haploids and the parental lines were genetically characterized with 4981 molecular markers (4956 SNPs, 24 SSRs and 1 morphological marker) that were assigned to 39 linkage groups representing all 21 wheat chromosomes. After excluding co-segregating markers, the final linkage map was constructed with 859 markers that spanned 1977.62 cM in length. A QTL analysis was carried out using the inclusive composite interval mapping

(ICIM) proposed by Li et al. (2007) with the BIP option from QTL IciMapping software (available from <http://www.isbreeding.net>). As composite interval mapping, ICIM method uses a linear regression model:

$$y_i = b_0 + \sum_{j=1}^{m+1} b_j x_{ij} + e_i \quad (3)$$

where y_i is the trait value of the i th individual in the mapping population, b_0 is the overall mean of the model, x_{ij} is a dummy variable for the genotype of the i th individual at the j th marker, b_j is the regression coefficient of the phenotype on the j th marker conditional on all other markers, and e_i is the residual error that is assumed normally distributed.

When building Eq. 3, ICIM uses all marker information by selecting the most important markers and setting to zero the unselected ones through a stepwise regression. To avoid over-fitting a probability of 0.0001 was used (Li et al. 2008). Thus, for a testing position in a specific interval ($k, k + 1$) the phenotypic values from Eq. 4 were adjusted by:

$$\Delta y_i = y_i - \sum_{j \neq k, k+1} \hat{b}_j x_{ij} + e_i \quad (4)$$

where $\hat{b}_j$ is the estimate of b_j in Eq. 3. Phenotypic values were adjusted by all markers retained in the regression equation except the two markers flanking the current interval. The adjusted phenotype Δy_i contains the position and additive effect information of the QTL in the current testing position and excludes the influence of the QTL located on other interval or other chromosomes (Wang et al. 2012). A LOD score of 3 and a step size of 2 cM were used for QTL analysis.

After individual QTL detection, a multi-locus multi-environment mixed model was fitted with random effects for genotype (G) and experiment specific residual error variance (E). Genotype by environment interaction was excluded from the model due to a better AIC in the following model.

$$Y = Env + \underline{G} + \underline{E} \quad (5)$$

where Y is the vector of all phenotypic observations, Env represents fixed situation-specific means, while $\underline{G}$ and $\underline{E}$ are the random genotypic and residual effects, respectively.

The variance components of random terms were extracted following the procedure described by Millet et al. (2016), and the standard deviations were expressed as a percentage of the general phenotypic mean. Any effect in the model can contribute to differences in phenotype for individual genotypes within a range of $\pm$ twice the standard deviation (SD). This range was also expressed as a percentage of the general mean. Then for testing all the QTL within the same model, we fitted a multi-environment model with multiple QTLs:

$$Y = Env + PC + \sum_{q \in Q} QTL_q^{Env} + \underline{G} + \underline{E} \quad (6)$$

with $QTL_q^{Env} = QTL_q + (QTL \times Env)_q$. We fitted environment-specific QTL effects that are the sum of a QTL main effect and a QTL by environment interaction term. The set of QTL used in the model was determined by a backward elimination process over the complete set of QTLs. To assess the amount of genetic (co) variance explained by the QTLs, we compared the estimated variance components in Eq. 5 with those obtained in Eq. 6.

Allelic effects of all candidate QTLs were extracted from Eq. 6. When no indication is provided, a positive effect indicates that the allele of the donor parent increased the trait value, while a negative effect indicates that the alternative allele increased the trait value. This procedure was used to test the amount of genetic variance explained by the detected QTL in GY and their components. Also, due to the high variability in anthesis time and its already known effect over GY (see results section), we tested the multi-locus multi-environment model by including anthesis time QTL using the same logic described above. Additionally, to identify potential secondary traits, we tested the amount of genetic variance of GY (and GN) explained by QTLs from traits other than GY.

Extreme phenotype selection

From 124 doubled haploids varying in spike dry weight at anthesis (SDW) and crop growth rate at anthesis (CGR-AN), 5 lines of similar PH and phenology were selected in each of the high and low tails for each trait, totalizing 10 lines per trait. The commercial wheat check genotype used in the experiments was also included as a reference. To compare

the difference between tails in each environment for different traits, data was treated as follow:

$$Z_{ij} = 100 * \left(\frac{\text{Target tail}_{ij} - \text{control tail}_{ij}}{\text{control tail}_{ij}} \right) \quad (7)$$

where Z_{ij} is the tail difference (%) for the i trait in the j environment, Target tail_{ij} is the mean value of the high tail for the i trait in the j environment, and control tail_{ij} is the mean value of the low tail for the i trait in the j environment. A combined analysis of variance was undertaken across environments for all traits. Differences in tail means (high vs low tails of SDW and CGR-AN) were tested statistically.

Results

Environmental conditions

Emergence date took place 15 days after sowing in 2013 and 17 days after sowing in 2014, without differences among genotypes. Mean temperature from emergence to maturity averaged 15.5 °C in 2013 and 16.2 °C in 2014, with a mean temperature during pre-anthesis of 9.6 °C in 2013 and 10.4 °C in 2014 (Fig. 6 suppl. material). Mean solar radiation from emergence to maturity was 15.9 and 14.5 MJ m⁻² d⁻¹ in 2013 and 2014, respectively; but distribution of the solar radiation throughout the crop cycle differed between years: in the second experimental year higher radiation values were observed during the anthesis phase (18.3 MJ m⁻² d⁻¹ in 2013 and 19.4 MJ m⁻² d⁻¹ in 2014) and along the grain filling period (19.0 MJ m⁻² d⁻¹ in 2013 and 21.0 MJ m⁻² d⁻¹ in 2014) than in the first one (Fig. 6 suppl. material). During the first growing season abundant rain occurred during the late vegetative period (Fig. 6 suppl. material) determining brief periods of waterlogging that affected negatively biomass production.

A large variability in anthesis time drove grain yield variation

The doubled haploid lines largely varied in phenology, principally due to variation in the pre-anthesis period (EM-AN), which ranged from 1434 to 2000 °Cd (representing from 113 to 135 days), without significant differences between years (Fig. 7 suppl.

material; Table 5 suppl. material). This variability was mostly observed in the period from emergence to flag leaf appearance (EM-FL), which was 1496 °Cd (109 days) in 2013 and 1370 °Cd (110 days) in 2014 (Fig. 7 suppl. material; Table 5 suppl. material), while the period between flag leaf emergence and anthesis (FL-AN) showed a narrow variation, lasting 280 °Cd (15 days) in 2013 and 312 °Cd (16 days) in 2014 (Fig. 7 suppl. material; Table 5 suppl. material). Parental lines were close to the mean value of all doubled haploid lines and transgressive segregation was evident for the three phenological phases (EM-AN, EM-FL, FL-AN) (Fig. 7 suppl. material; Table 5 suppl. material). Seven quantitative trait loci (QTLs) for the duration of the EM-AN period and seven QTLs for EM-FL period (Table 1) were identified, with three QTLs in common between them. No QTL was detected for the duration of the FL-AN period confirming the lack of genotypic variability for this period. Detected QTLs were stable across environments (Table 1) and co-localized with *Ppd-A1* and *Ppd-D1* genes (Table 1), suggesting that anthesis time variability was related to photoperiodic sensitivity. Delays on the duration of the phase EM-FL had a negative effect on GY (Table 6 suppl. material) and its numerical components (GN, GW) (Table 6 suppl. material).

GY largely varied across environments ($p < 0.05$) and across genotypes, showing a genotypic variability of $\pm 37\%$ around the mean value (Table 2), ranging from 285 to 406 g m⁻² in 2013 and from 341 to 750 g m⁻² in 2014 (Table 5 suppl. material). It is important to highlight that, as was stated above, abundant rain occurred during the late vegetative period that produced brief periods of waterlogging during 2013 growing season. These events determined yellowish in the expanded leaves (visual observation) affecting negatively aerial biomass and thereby yield. Despite the large variability of GY, only two QTLs were detected on chromosome 5A, which co-localized with QTLs of anthesis time (Table 1). The presence of the P1 allele in the QTL advanced anthesis time by 63 and 110 °Cd⁻¹ determining a positive effect on GY by 55 g m⁻² in 2013 and 76 g m⁻² in 2014 (Table 1). Both QTLs captured 49% of the additive genetic variance for GY (Table 2). Due to the impact of anthesis time over GY, QTLs from both traits were considered in the same multi-locus multi-environment model capturing 63% of the additive genetic variance

Table 1 QTLs for duration of the emergence-anthesis phase and the emergence-flag leaf appearance phase, grain yield, above-ground dry matter at physiological maturity, harvest index, grain number, grain weight, spike dry weight at anthesis, crop growth rate at anthesis, and fruiting efficiency

Env	Chr.	Position	Left_Marker	Right_Marker	LOD	PVE (%)	Add	LeftCI	RightCI
Duration of emergence-anthesis phase (°Cd)									
2013	2A	19	XPpd-A1	2AS_5289049_8858	6.1	12.3	44.8	16.5	24.5
	2D	54	2DS_5375380_1169	XPpd-D1	5.0	10.6	– 41.6	47.5	61.5
	5A	81	5AL_2794409_6138	5AL_2748687_5315	3.6	6.7	– 33.4	79.5	81.5
	5A	107	5AL_2784434_4621	5AL_2809951_162	11.1	24.5	– 63.5	105.5	108.5
	5B	68	5BL_10917817_3505	5BL_10884756_2450	3.9	7.4	34.4	66.5	69.5
2014	5A	107	5AL_2784434_4621	5AL_2809951_162	12.8	33.0	– 109.1	103.5	108.5
	2D	56	2DS_5375380_1169	XPpd-D1	3.6	8.6	– 55.6	47.5	61.5
Duration of emergence-flag leaf phase (°Cd)									
2013	2A	16	2AS_5309087_9086	2AS_5306357_2235	6.2	9.5	45.2	12.5	16.5
	2D	64	XPpd-D1	2DS_5382880_5243	9.5	16.7	– 60.2	62.5	68.5
	5A	107	5AL_2784434_4621	5AL_2809951_162	17.4	33.3	– 85.5	105.5	108.5
2014	2A	17	XPpd-A1	2AS_5289049_8858	4.6	9.7	50.1	16.5	19.5
	2D	64	XPpd-D1	2DS_5382880_5243	5.1	11.8	– 55.3	62.5	71.5
	5A	81	5AL_2794409_6138	5AL_2748687_5315	4.5	9.5	– 50.2	79.5	81.5
	5A	107	5AL_2784434_4621	5AL_2809951_162	10.4	24.7	– 80.6	104.5	108.5
Grain yield (g m ⁻²)									
2013	5A	101	IWA4207	5AL_2812590_39455	10.7	33.3	55.0	99.5	102.5
2014	5A	108	5AL_2809951_162	5AL_2803630_4943	7.1	21.8	76.5	105.5	108.5
Above ground dry matter at maturity (g m ⁻²)									
2013	5A	100	IWA4207	5AL_2812590_39455	8.4	27.2	113.1	99.5	102.5
2014	5A	73	5AL_2678682_64	5AL_2798538_722	3.1	9.8	108.1	68.5	77.5
	5B	28	5BL_10785394_286	5BL_10886215_8167	3.3	10.4	– 109.9	27.5	29.5
Harvest index									
2013	1B	19	1BS_3458552_2656	1BS_3484153_4290	4.9	10.5	0.0	18.5	21.5
	2D	44	2DS_5351730_1417	2DS_5375380_1169	5.9	14.5	0.0	36.5	46.5
	4A	1	4AS_6011855_802	4AS_6010640_35237	3.3	7.0	0.0	0.0	1.5
	5A	107	5AL_2784434_4621	5AL_2809951_162	5.7	12.6	0.0	104.5	108.5
2014	2D	39	2DS_5351730_1417	2DS_5375380_1169	3.8	11.8	0.0	29.5	52.5
	5A	107	5AL_2784434_4621	5AL_2809951_162	7.7	22.7	0.0	104.5	108.5
Grain number (# m ⁻²)									
2013	5A	101	IWA4207	5AL_2812590_39455	7.2	23.7	1392.4	99.5	102.5
2014	5A	108	5AL_2809951_162	5AL_2803630_4943	5.2	16.7	2145.7	105.5	108.5
	5B	65	5BL_10915544_8072	5BL_10917817_3505	3.2	9.9	– 1626.9	59.5	66.5
Grain weight (mg grain ⁻¹)									
2013	1B	4	1BS_3484067_14330	1BS_3477882_7381	4.3	6.1	– 1.0	0.0	7.5
	2B	60	IWA3868	2BS_3514149_4373	4.6	6.5	1.0	59.5	60.5
	3A	145	3AL_4354018_2106	3AL_4402578_4115	10.7	17.4	– 1.6	143.5	147.5
	4D	64	4DL_14366503_1367	4DL_6952434_82	3.8	5.4	– 0.9	62.5	73.5
	5A	62	Xbarc100	5AL_2808417_2826	10.6	17.1	1.6	58.5	66.5
2014	5A	60	Xbarc100	5AL_2808417_2826	5.6	18.9	1.4	58.5	65.5
Spike dry weight at anthesis (g m ⁻²)									
2013	5A	100	IWA4207	5AL_2812590_39455	3.3	11.6	15.4	98.5	101.5

Table 1 continued

Env	Chr.	Position	Left_Marker	Right_Marker	LOD	PVE (%)	Add	LeftCI	RightCI
2014	4B	40	4BS_4955467_2552	4BS_4959487_4643	5.9	14.8	49.8	31.5	47.5
	6B	58	6BS_2980939_1278	Xbarc101	3.2	7.0	– 33.1	51.5	60.5
	7D	87	7DS_3940684_560	3DL_6915964_5472	3.8	8.3	– 36.0	77.5	91.5
Crop growth rate at anthesis ($\text{g m}^{-2} \text{ }^{\circ}\text{Cd}^{-1}$)									
2013	5A	111	5AL_2803630_4943	5AL_2811200_3157	3.5	9.9	0.0	107.5	120.5
2014	7A	77	7AS_4073295_163	7AS_4256393_3907	4.2	13.5	0.1	73.5	78.5
Fruiting efficiency (grains g^{-1})									
2013	2B	25	2BS_5198867_18223	IWA2407	3.3	9.2	4.7	24.5	31.5

Each line contains information of the QTL, the situation where that QTL was significant, its position, flanking markers, LOD score, proportion of the phenotypic variance explained (PVE), its allelic effect (for allele P1) (Add) and its confidence interval (LeftCI and RightCI)

Table 2 Variance components of the different mixed models for grain yield

Statistical model	Type	G ^a	Res ^b
Model 1: Multi-envt	Variance components (g m^{-2}) ²	6742.7	11,108.9
Model 2: Multi-envt, multi locus	Variance components (g m^{-2}) ²	3427.2	10,876.6
Model 3: Multi-envt, multi locus, QTL EM-AN	Variance components (g m^{-2}) ²	2470.8	10,152.5
Model 1: Multi-envt	SD as % of mean	18.7	24.0
Model 2: Multi-envt, multi locus	SD as % of mean	13.3	23.8
Model 3: Multi-envt, multi locus, QTL EM-AN	SD as % of mean	11.3	22.9
QTLs (diff. Var. comp. Model 1–2)		49	2
QTLs (diff. Var. comp. Model 1–3)		63	9

Model 1 contains genotypes and environments. Model 2 is similar to Model 1 but includes the QTLs of grain yield. Model 3 is similar to Model 2 but also includes anthesis time QTLs. The general mean of grain yield was 438 g m^{-2}

^aGenotype

^bExperiment-specific residual error variance

(Table 2) and confirming the effect of anthesis time QTLs over GY.

Crop growth and partitioning

GY variation across environments was significantly associated with AGDM and, to a lesser extent, with HI (Table 6 suppl. material). Within each year, AGDM particularly explained a high proportion of GY variability in 2013 ($r = 0.90$ $p < 0.001$ vs $r = 0.46$ $p < 0.001$ for HI), while GY variation in 2014 was explained by changes in AGDM ($r = 0.59$ $p < 0.001$) as well as in HI ($r = 0.67$ $p < 0.001$) (Fig. 1).

A large genotypic variability was observed in AGDM (Fig. 6 suppl. material) with variations of $\pm 19\%$ around the mean value (Table 7 suppl. material). Three environmentally unstable QTLs were detected for this trait explaining 59% of the additive genetic variance (Tables 1; Table 7 suppl. material). On the other hand, six QTLs were detected for HI, which were also unstable across environments except for a QTL on chromosome 5A that also co-localized with a QTL for anthesis time (Table 1). QTL co-localization and its opposite additive effect between traits helped to explain the negative genotypic correlation observed between HI and the duration of the pre-anthesis period (Table 6 suppl. material). Also, combining QTLs of

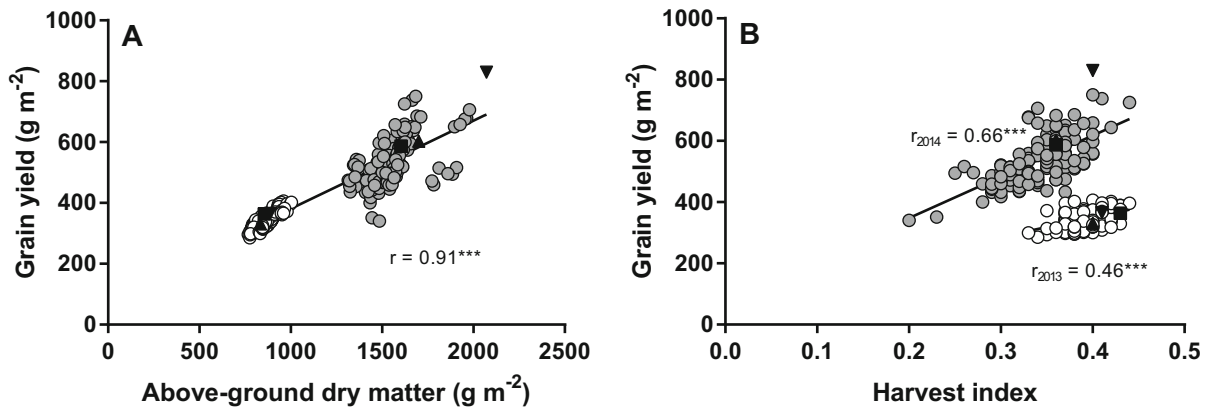


Fig. 1 Relationship between grain yield and above-ground dry matter (a), and harvest index (b) for a wheat doubled haploid population (circles), its parents (P1, black triangles; P2, black inverted triangles) and a check cultivar (black squares) grown

under field conditions in 2013 (white circles) and 2014 (grey circles). Correlation coefficient r and significance of the linear regression between variables ($***p < 0.001$) is indicated

anthesis time together with those of HI in a multi-locus multi-environment model increased the additive genetic variance captured by almost 10% (from 43 to 52%; Table 7 suppl. material).

Dissecting grain yield in its physiological components

When dissecting GY into its numerical components, we observed that variation in GY was strongly associated with changes in GN (Fig. 2; Table 6 suppl. material), which varied from 7055 to 14,014 grains m⁻² in 2013 and from 12,022 to 28,675 grains m⁻² in 2014 (Table 5 suppl. material). Three QTLs for GN,

which co-localized with QTLs of anthesis time, were detected on chromosomes 5A and 5B (Table 1). These QTLs captured 58% of the additive genetic variance, which increased to 62% when considering anthesis time QTLs (Table 7 suppl. material). On the other hand, no relation was observed between GY and GW (Fig. 2, Table 6 suppl. material). Grain weight ranged from 31 to 38 mg grain⁻¹ in 2013 and from 18 to 32 mg grain⁻¹ in 2014 (Table 5 suppl. material). Furthermore, it was affected by the duration of the FL-AN period rather than by anthesis time (Table 6 suppl. material). A total of six QTLs were detected for GW (Table 1), which captured 46% of the additive genetic variance (Table 7 suppl. material). Parental lines

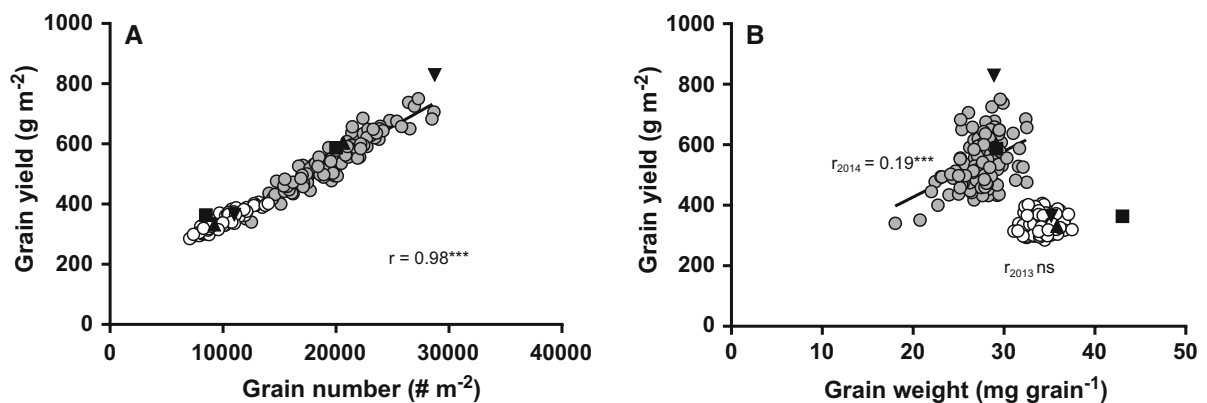


Fig. 2 Relationship between grain yield and grain number per m² (a), and grain weight (b) for a wheat doubled haploid population (circles), its parents (P1, black triangles; P2, black inverted triangles) and a check cultivar (black squares) grown

under field conditions in 2013 (white circles) and 2014 (grey circles). Correlation coefficient r and significance of the linear regression between variables ($***p < 0.001$; ns, non-significant $p \geq 0.05$) is indicated

showed GY and GN values near to the population mean in 2013, while in 2014 they performed better than the mean (Table 5 suppl. material). Overall, doubled haploid population showed transgressive segregation for GY and its numerical components, with enhanced trait variability beyond parental line limits (Fig. 7 suppl. material).

Grain number m^{-2} was dissected into SDW (Fig. 3a) and fruiting efficiency (FE, measured as number of grains established per unit of chaff; Fig. 3b). Both traits showed large variation between years and among genotypes. SDW showed a genotypic variation of $\pm 31\%$ (Table 8 suppl. material) ranging from 114 to 165 g m^{-2} with a mean value of 138 g m^{-2} in 2013, and from 158 to 288 g m^{-2} with a mean value of 202 g m^{-2} in 2014 (Fig. 7 suppl. material; Table 5 suppl. material). SDW was significantly related to changes in GN across environments (Fig. 3a). Four QTLs were detected for SDW, which did not co-localize with QTLs of anthesis time (Table 1) and captured 55% of the additive genetic variance (Table 8 suppl. material). Genotypic variation in SDW was associated with CGR-AN (Fig. 3c), which varied from 0.33 to 0.68 $\text{g m}^{-2} \text{ }^{\circ}\text{Cd}^{-1}$ in 2013 and from 0.6 to 1.02 $\text{g m}^{-2} \text{ }^{\circ}\text{Cd}^{-1}$ in 2014 (Fig. 6 suppl. material; Table 5 suppl. material). Two QTLs were detected for CGR-AN on chromosomes 5A and 7A (Table 1), explaining 52% of the additive genetic variance despite the low genotypic variance of the model (Table 8 suppl. material). Fruiting efficiency showed a wide range of variation from 44 to 127 grains g^{-1} in 2013 and from 72 to 145 grains g^{-1} in 2014 (Fig. 7 suppl. material; Table 5 suppl. material). Despite its wide genotypic variability, there was only a weak relationship between NG and FE (Fig. 3b) in each environment.

Average GN per unit area was significantly associated with CGR-AN ($r = 0.85$, $p < 0.001$) and SDW ($r = 0.83$, $p < 0.001$), while FE showed a weaker ($r = 0.52$, $p < 0.001$) and GW a negative ($r = -0.75$, $p < 0.001$) association with GN observed across both environments (Fig. 3). Medium to low phenotypic correlations were also observed at the genetic level with QTLs from SDW and CGR-AN explaining 38 and 39% of the additive genetic variance of GN (Table 9 suppl. material).

Genetic correlations between GY and traits related to biomass accumulation, partitioning and to the numerical components of GY in the doubled haploid

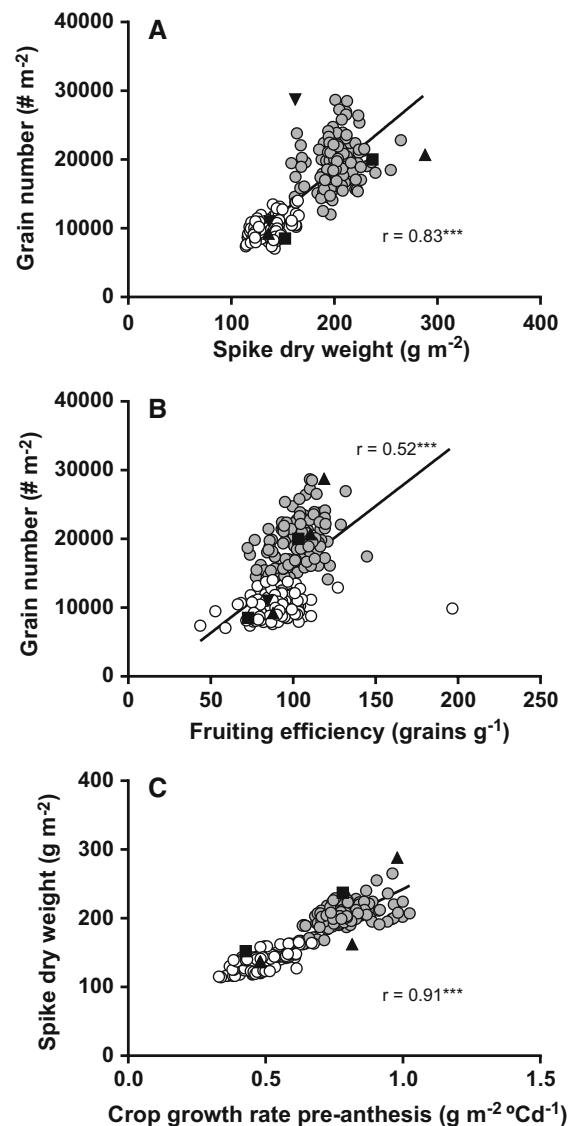


Fig. 3 Relationship between grain number per m^2 and spike dry weight at anthesis (a) and fruiting efficiency (b); and between spike dry weight at anthesis and crop growth rate during pre-anthesis (c) for a wheat doubled haploid population (circles), its parents (P1, black triangles; P2, black inverted triangles) and a check cultivar (black squares) grown under field conditions in 2013 (white circles) and 2014 (grey circles). Correlation coefficient r and significance of the linear regression between variables (***) $p < 0.001$ is indicated

population across environments are illustrated in Fig. 4. Principal components analysis explained almost 67% of the total variability among genotypes using the first two axes. GY was positively associated with GN ($r = 0.98$, $p < 0.001$), AGDM ($r = 0.91$, $p < 0.001$), and HI ($r = -0.29$, $p < 0.001$). A

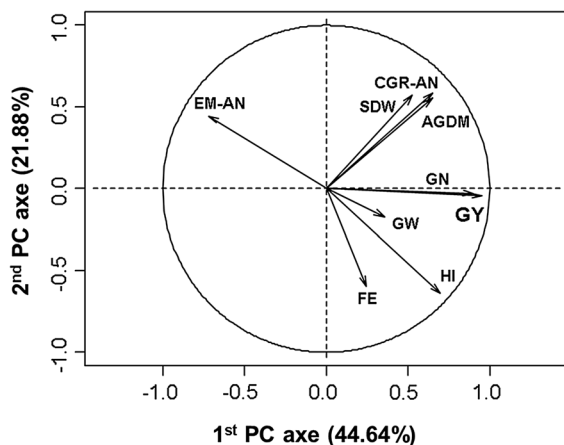


Fig. 4 Principal component analysis of grain yield (GY), above-ground dry matter (AGDM), harvest index (HI), grain number per m^2 (GN), grain weight (GW), spike dry weight at anthesis (SDW), fruiting efficiency (FE), crop growth rate during pre-anthesis (CGR-AN), and duration of the emergence-anthesis period (EM-AN) for the BLUPs of a wheat doubled haploid population across all environments (mean values in 2013–2014). Percentage of variability explained by each principal component (PC) is indicated between brackets on each axis

negative relationship was observed between GY and GW (Fig. 4), associated with the year effect (Fig. 2; Table 6 suppl. material). Results were in line with the proportion of genotypic variance explained for GY. QTLs for GN, GW, AGDM and HI explained 61, 25 and 46 and 46% of the GY additive genetic variance, respectively (Table 3).

Anthesis time showed a negative association with all GY traits except for GW (Fig. 4; Table 6 suppl. material). The most relevant negative associations with the delay in anthesis time were GY ($r = -0.58$, $p < 0.001$), GN ($r = -0.55$, $p < 0.001$), and AGDM ($r = -0.44$, $p < 0.001$). The impact of anthesis time over GY and GN was also observed at the genetic level when including QTLs of anthesis time for predicting both traits (Table 3; Table 9 suppl. material). The proportion of additive genetic variance ranged from 55 to 66% for GY and from 50 to 62% for GN (Table 3; Table 9 suppl. material).

Overall, we identified several secondary traits related to GY that could be targeted for indirect selection. The most promising traits were SDW and CGR-AN as they explained an important portion of GY variance and because they are measured early in the cycle, speeding up the selection process. From

these two traits, SDW seems to be independent from time to anthesis as QTLs for both traits did not co-localize (Table 1) and explained a different proportion of the additive genetic variance.

Performance of extreme genotypes with similar cycle to anthesis and plant height

An alternative approach for selection consists in identifying tails of secondary trait distribution. Those tails represent extreme phenotypes, which were constrained to be phenotypically similar for other adaptive attributes such as anthesis time and PH. Based on QTL results, we chose SDW and CGR-AN as secondary traits to select phenotypic tails (5 genotypes per tail and trait; Table 10 suppl. material) considering similar anthesis time and PH (Fig. 5; Table 11 suppl. material).

Environment effects were large and significant for all measured traits (Table 4), reflecting the contrasting amount of incident solar radiation around anthesis between years and the slight waterlogging damage that occurred at the beginning of the crop cycle in 2013 (Fig. 6 suppl. material). For example, mean GY for the high SDW selected tails varied from 285.1 g m^{-2} in 2013 to 737.6 g m^{-2} in 2014 (Table 11 suppl. material).

Mean anthesis time was similar and not statistically different for the selected tails (Tables 4; Table 11 suppl. material). Averaged across years, anthesis time was 1749 and 1792 °Cd for the high and low SDW tails, respectively. Similarly, for the CGR-AN tails, anthesis time was 1783 °Cd for the low CGR-AN tail and 1788 °Cd for the high CGR-AN tail. Differences in PH were also not statistically significant between tails (Table 4) being 53 and 54 cm for CGR-AN low and high tails, and 52 cm for both SDW tails. Anthesis time of the check genotype averaged 1742 °Cd while its average PH was 49 cm (Fig. 7 suppl. material; Table 5 suppl. material).

SDW varied significantly between tails across years (Table 4), and it was higher in 2014 (197.3 g m^{-2}) than in 2013 (133.1 g m^{-2}). The lowest SDW tail value of 148.4 g m^{-2} represented a reduction of 18.5%, compared to the high SDW tail (182.0 g m^{-2}). This reduction was consistent with the 23% reduction in GN and the 21% reduction in GY in both years (Fig. 5A). However, reductions in GN and GY were significantly higher in 2014 (38.4% for

Table 3 Variance components of multi-locus multi-environment mixed models for grain yield. QTLs from specific traits were used to predict grain yield (Trait QTLs)

Trait	Model 1: Trait QTLs					Model 2: Trait QTLs + an the sis time QTLs					Diff explained Var GY (%) ^f
	Nb QTL ^a	Var GY ^b	Var Res ^c	%Var GY ^d	%Var Res ^e	Nb QTL ^a	Var GY ^b	Var Res ^c	%Var GY ^d	%Var Res ^e	
Grain yield (without QTLs)	0	6742.7	11,108.9	–	–	0	6742.7	11,108.9	–	–	–
Grain yield	2	3427.2	10,876.6	49	2	7	2470.8	10,152.5	63	9	14
Above-ground dry matter	3	3664.5	10,469.7	46	6	8	2880.9	9805.0	57	12	12
Harvest index	4	3625.5	10,818.6	46	3	8	2796.7	9964.2	59	10	12
Grain number	3	2618.7	10,751.6	61	3	8	2379.4	10,169.5	65	8	4
Grain weight	5	5036.2	11,088.3	25	0.2	10	2320.9	10,334.0	66	7	40
Spike dry weight at anthesis	4	3441.4	11,114.3	49	0	9	2838.6	9893.7	58	11	9
Crop growth rate	2	3418.6	10,887.2	49	2	7	2484.4	9663.9	63	13	14
Fruiting efficiency	1	6747.4	11,176.2	0	– 1	6	3055.4	10,377.4	55	7	55

A second multi-locus multi-environment mixed models for grain yield was performed using QTLs of specific traits but also including anthesis time QTLs (Trait QTLs + anthesis time QTLs)

^aNumber of QTLs used for predicting grain yield

^bAdditive genetic variance of grain yield captured by the multi-locus multi-environment model

^cResidual variance of the multi-locus multi-environment model

^dProportion of the additive genetic variance of grain yield captured by the multi-locus multi-environment model

^eProportion of the residual variance of the multi-locus multi-environment model

^fDifference between the proportion of genotypic explained variance using model 2 vs model 1. Lower differences indicates a lower effect of anthesis time over the evaluated trait

GN and 38.2% for GY) than in 2013 (7.5 for GN and 4.2% for GY). Above-ground dry matter and harvest index at anthesis were, as expected, significantly higher for the high SDW tail (18.4% for AGDM and 9.5% for HI at anthesis) (Table 4, Fig. 5a).

Crop growth rate, as expected, was significantly higher in the high than in the low CGR-AN tail (Table 4). Average CGR-AN was 0.563 g m⁻² °Cd⁻¹ for the low tail and 0.707 g m⁻² °Cd⁻¹ for the high tail (Table 11 suppl. material). Differences in CGR-AN were evident in 2014 (30%) while in 2013 it was similar between tails (Table 11 suppl. material), probably due to the general low range of variation in 2013. Indeed, differences in CGR-AN were consistent with differences in GN and GY being 6 and 28% in 2014 and – 8 and – 2% in 2013, respectively (Fig. 5b). Taken together, our results suggest that SDW may be a more accurate option than CGR-AN

when seeking for a secondary trait to indirectly select for GY.

Discussion

Due to the need to increase world-wide food production, there has been an increased interest to identify novel traits that could be used in wheat breeding programs to increase GY. Doubled haploid populations are extremely valuable in genetic studies for identifying traits of interest to improve breeding for GY. However, identify secondary traits in this type of populations is complicated due to the high variability usually observed in time to anthesis and its strong impact over GY. Two different ways for dealing with this issue were suggested in the present study linking phenotypic and genetic information, and/or selecting

Table 4 Mean squares from analysis of variance for different traits measured on spike dry weight at anthesis (SDW) and crop growth rate in pre-anthesis (CGR-AN) tails assessed in two consecutive years (2013 and 2014)

Variance component	EM-AN duration (°Cd)	PH (cm)	SDW (g m ⁻²)	AGDM-AN (g m ⁻²)	CGR-AN (g m ⁻² °Cd)	HI-AN	Number of spikes m ⁻²	FE (# g ⁻¹)	GN (# m ⁻²)	GW (mg grain ⁻¹)	AGDM (g m ⁻²)	HI	GY (g m ⁻²)
Tail	3860 ns	5.6 ns	2615***	14***	0.0573***	0.00033*	3025 ns	169 ns	25,000,000**	5.6 ns	11.8 ns	0.00228 ns	16,077**
Experiment	54,243**	1786.2***	41,913***	572.4***	1.0980***	0.0026***	289,340***	970**	840,000,000***	496.6***	906.7***	0.05476***	325,694***
Tail * experiment	5755 ns	16 ns	1717**	10.2***	0.071***	0.0001 ns	2211 ns	19 ns	17,000,000*	3.9 ns	7.4 ns	0.00093 ns	12,333*
Pooled error	4189	18.6	186	1.2	0.0043	0.000079	2160	110	5,000,000	4.0	5.4	0.00097	3136

EM-AN, emergence-anthesis duration in thermal time units; PH, plant height at maturity; AGDM-AN, above-ground dry matter at anthesis; HI-AN, harvest index at anthesis; FE, fruiting efficiency; GN; grain number; GW, grain weight; AGDM, above-ground dry matter at maturity; HI, harvest index at maturity; GY, grain yield; ns, not significant ($p \geq 0.05$)

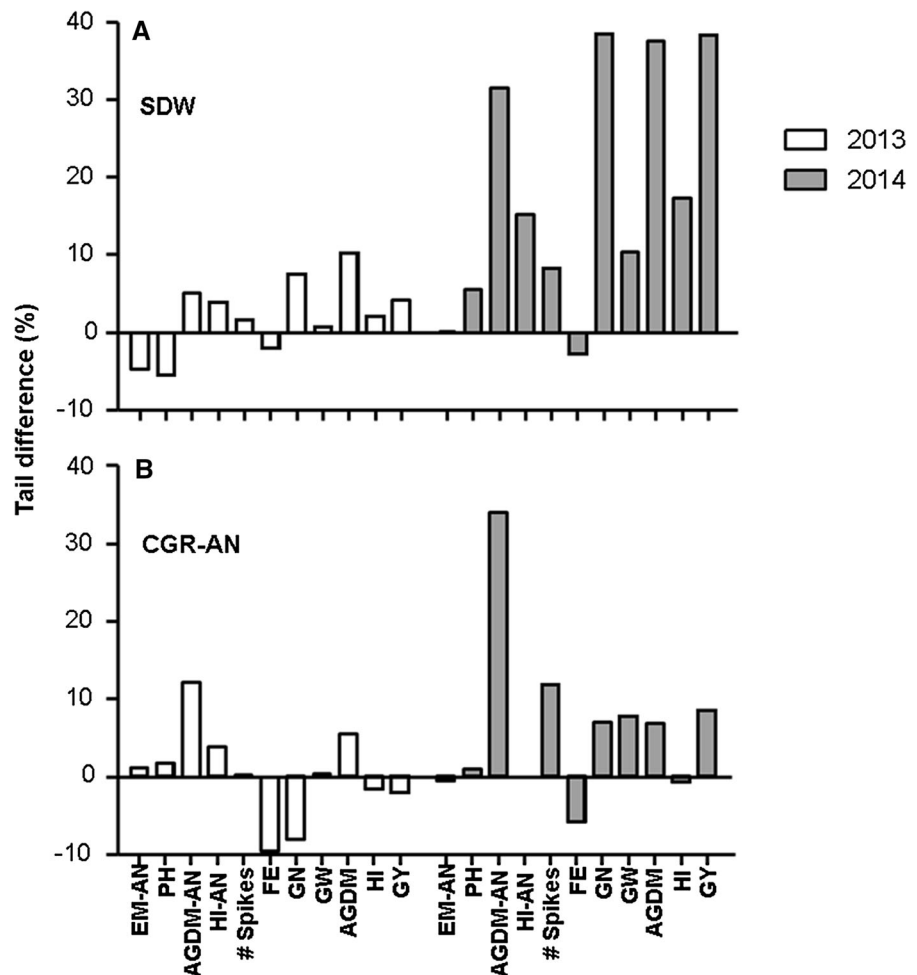
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

extreme genotypes for secondary traits with common phenology and PH. A proof of concept was performed for eliminating the noise associated with time to anthesis when identifying secondary traits using a doubled haploid wheat mapping population characterized by a wide variability in anthesis time. We evaluated traits related to phenology and GY in terms of numerical components as well as biomass traits, combining genotyping and phenotyping techniques as alternative to provide tools to improve selection in wheat breeding programs, attempting to reduce the gap between genotyping and phenotyping (which is a bottleneck nowadays; Mira et al. 2019).

Changes in phenology modified grain yield

Variability in the duration of the vegetative phase was related to photoperiodic sensitivity as some of the seven QTLs for this trait co-localized with *Ppd-A1* and *Ppd-D1* genes. The genes *Ppd-A1*, *Ppd-B1* and *Ppd-D1*, located on chromosomes 2A, 2B and 2D, respectively, are major genes controlling response to photoperiod in wheat (Snape et al. 2001). The effect of photoperiod on time to anthesis is mainly mediated by its effects on the time to floral initiation and, consequently, the final number of leaves differentiated in the apex (Whitechurch and Slafer 2002). In the present study, the period from emergence to flag leaf appearance showed the largest variability among genotypes. The variation of the late reproductive period, measured in our study as FL-AN, has been proposed as an alternative to increase yield potential in wheat (Slafer et al. 2001); however, our data did not allow us to test that hypothesis because the population showed only slight variability in the duration of the FL-AN phase. García et al. (2011) demonstrated that selection of a longer FL-AN phase with similar cycle to anthesis is labile due to the high environmental influence of this trait. Additionally, García et al. (2011) showed that phenotypic variability evidenced on this trait was not clearly associated with major adaptation genes (i.e. *Ppd* and/or *Vrn*), suggesting that other minor genes could be also associated with that trait. Anthesis date and flag leaf appearance date shared three QTLs, which was consistent with the high and positive correlation between both stages. However, because other chromosomal regions were detected, this confirms that both traits are under multigenic control (Griffiths et al. 2009). Adjustment

Fig. 5 Percentage trait difference between high and low tails for spike dry weight at anthesis (SDW) (a), and crop growth rate during pre-anthesis (CGR-AN) (b), in 2013 (white columns) and 2014 (grey columns) growing conditions. EM-AN, emergence-anthesis duration; PH, plant height at maturity; AGDM-AN, above-ground dry matter at anthesis; HI-AN, harvest index at anthesis; # Spikes, number of spikes per m⁻²; FE, fruiting efficiency; GN, grain number; GW, grain weight; AGDM, above-ground dry matter at maturity; HI, harvest index at maturity; GY, grain yield



in phenology as determinant of GY is a key target which has been considered in traditional breeding programs (Slafer 2003) as well as in new ones (Tondelli et al. 2014).

The doubled haploid lines showed large differences in GY. Lines producing the lowest GY were those with the longest duration of the phase from emergence to anthesis. This was also observed at the genetic level as QTL for anthesis time improved the additive genetic variation explained by the multi-locus model by almost 15%. Transgressive segregation in time to anthesis is ubiquitous in plants and is a mechanism to cope with adaptation to different environmental conditions (Rieseberg et al. 1999). For the conditions explored in our study, the optimal time to anthesis for wheat is late enough to avoid eventual frosts but early enough to avoid decreasing photothermal coefficient (i.e. the ratio between the incident radiation and mean

air temperature above a base temperature) around anthesis and increasing temperatures during the grain filling period (Fig. 6 suppl. material). GY variability was associated with the duration of pre-anthesis phase, which impacted on partitioning, as well as with biomass accumulation during the crop cycle.

Grain yield depended on biomass production

Modern genetic improvement has increased wheat yield by changes in biomass partitioning (i.e. harvest index) rather than by increases in the total biomass produced by the crop at maturity (Austin et al. 1980; Siddique et al. 1989; Calderini et al. 1999; Slafer and Andrade 1991; Lo Valvo et al. 2018). However, HI of current genotypes is close to the maximum values suggesting that the alternative of achieving future increases in yield through improvements in biomass

production becomes more relevant (Miralles and Slafer 2007). Interestingly, in the present study differences in GY were strongly associated with differences in AGDM. Working with another population of 105 doubled haploid lines, García et al. (2013) also found a robust association between AGDM and GN (the main numerical component of GY). The lack of negative correlation between AGDM and HI, and the lack of QTL co-localization between AGDM and time to anthesis makes the lines with high biomass valuable genotypes to be studied further. Recent studies point out that source limitation is emerging in modern wheat cultivars under extreme stress conditions (Álvaro et al. 2008 for durum wheat; Bijanzadeh and Emam (2010), and Serrago et al. (2013) for bread wheat). Within this context, increasing AGDM becomes more and more relevant to improve yield.

Definition of the numerical components of grain yield

The number of grains established per unit area was, as expected, the main numerical component explaining variation in GY among doubled haploid genotypes. Loci related to grain yield and the number of grains were identified in chromosome 5A, which is a chromosome known for its contribution to yield traits (Table 1; Kato et al. 2000; Sukumaran et al. 2015; Brinton and Uauy 2019). In our study we integrated the definition of GN as the result of the product between SDW and FE. SDW at anthesis was a better predictor of GN than FE. Also, QTLs for SDW did not co-localize with those of anthesis time suggesting both traits were independent in this population. In addition, SDW was highly dependent on CGR-AN, suggesting that focusing on the partitioning to the spike at anthesis time would be helpful for identifying secondary traits for GY selection by avoiding, at least in part, the strong impact of anthesis time. The selection of phenotypes with extreme values for SDW and CGR-AN with similar phenology and PH allowed us to analyze GY in terms of different traits eliminating the effect of both variables on yield potential (Rebetzke et al. 2017). Results of our study showed that SDW is more precise, and simpler to measure as trait, than CGR-AN. Similarly, Martino et al. (2015) and Mirabella et al. (2016) also showed that SDW can be used for selection in breeding programs to increase

grains per unit area. Lines with high SDW also exhibited high AGDW at anthesis, indicating that SDW is a promising trait to select for GY. Increases in SDW at anthesis can be obtained through more production of assimilates and through increases in partitioning to the spike (Fischer 2011). Recently, Zhou et al. (2017) identified two major QTLs that explained ca. 31% of the phenotypic variation in spike length and fertile spikelet number per spike. The structure of the spike as trait to be considered for improving grain yield in wheat as received special attention during the last decade, with spike number per plant, spike length, and spikelet number per spike as traits to be considered (Wang et al. 2011; Patil et al. 2013). These traits can be easily measured through spike dry matter at anthesis, which is a trait that encompasses them. Richards et al. (2002) suggested that a useful trait to be used in breeding programs should be affordable and easy to measure and not be negatively related to other agronomic traits. It is interesting to note that there was no negative correlation between GN and GW, suggesting that gains in GY could be reached through increases in both components independently as has been postulated by Rattey et al. (2009) and Bustos et al. (2013), working with different progenies derived from parents with contrasting GY components. Promising alternatives for increasing GW in wheat (related to the control of early grain development) has been recently identified (Brinton et al. 2018). One aspect to be considered in the future is that a large part of the world wheat production is carried out under restrictive environmental conditions (such as high temperatures and water deficit). However, many of the works related to the identification of QTLs for grain yield in wheat were carried out under potential conditions and only a few of them screened mapping populations under abiotic stress conditions (e.g. Mason et al. 2010; Bennett et al. 2012; Sukumaran et al. 2018), which is a topic that deserves to be considered.

In conclusion, throughout the present work we used two approaches for analyzing GY taking into account the variability in time to anthesis; one based on biomass production and its partitioning to the grains and the other one based on establishment of the main numerical yield component (grain number, grain weight). Our results confirmed the importance of enhancing biomass production as a way to increase GY in wheat. Additionally, for genotypes with similar

time to anthesis and PH, improving SDW by partitioning of biomass to the spike at anthesis is a particular target to be considered as a further strategy to increase the number of grains established per unit area and, consequently, GY.

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