Original Article

Dissecting the trade-off of grain number and size in wheat

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Main Conclusion: Principal component and meta-QTL analyses identified genetic loci affecting the trade-off of wheat grain number and size, which could provide opportunities to optimize local breeding strategies for further yield improvement.

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Abstract

Grain yield of wheat is complex, and its physiological and genetic bases remain largely unknown. Using the Forno/Oberkulmer recombinant inbred lines, this study validated the negative phenotypic relationships between thousand grain weight (TGW) and grain number components. This trade-off might be alleviated at the population level by early anthesis and at the shoot level by higher shoot biomass. Principal component (PC) analysis revealed three useful PCs, of which both PC1 and PC3 were positively associated with grain yield and grains m^{-2} through increased spikes m^{-2} (for PC1) or grains per spike (for PC3), while PC2 primarily reflected the trade-off of grain number and TGW. Quantitative trait locus (QTL) mapping detected eight and seven loci for PC1 and PC2, respectively, on chromosomes 1D, 2A, 3A, 3B, 4A, 4B, 5A and 7B, individually explaining 11.7–29.3% of phenotypic variations. Using the 1203 QTLs published previously, a meta-analysis was performed to reveal 12, 21, 37 and 54 genomic regions (MQTLs) affecting grains m^{-2}, spikes m^{-2}, grains per spike and TGW, respectively. Moreover, 67 MQTLs (96%) for grain number were coincided with the TGW MQTLs, with reverse phenotypic effects, suggesting intensive genetic trade-off between grain number and size. The *AGP2* gene, which encodes ADP-glucose pyrophosphorylase determining TGW, was found by haplotype analysis in the Forno/Oberkulmer population to affect grain number oppositely, indicating this trade-off at the gene level. Appropriate combinations of the QTLs/genes for local breeding targets such as higher grain number or larger grains, therefore, would be critical to achieve future yield gains.

Keywords: *AGP2*, Principal component analysis, QTL, Trade-off, Yield, Yield component

Abbreviations

BLUE	Best linear unbiased estimate
CS	Chinese Spring
IWGSC	International Wheat Genome Sequencing Consortium
LOD	Likelihood of odd
MQTL	Meta quantitative trait locus
PC	Principal component
QTL	Quantitative trait locus
RIL	Recombinant inbred line
TGW	Thousand grain weight

Introduction

Wheat (*Triticum aestivum* L.) is the most widely grown cereal in the world, with an annual acreage of more than 200 million hectares producing grains of over 700 million tons (www.fao.org/faostat/). As the world′s population is projected to 9.7 billion in 2050 (https://population.un.org/wpp/), the wheat production is required to be doubled to secure global food security (Ray et al. 2013). However, the current growth rates of wheat yield and production are around 1%, only half of what is needed to meet projected demand (Reynolds et al. 2012; Ray et al. 2013). A further physiological and genetic understanding of grain yield formation is essential to achieve future yield gains in wheat.

Grain yield of wheat is a product of grains per unit land area and individual grain weight. The former component can be divided into spikes per unit area and grains per spike. Factors such as seed rate, seedling establishment and tillering determine spike number. Seed rate is usually targeted according to local optimal plant population. Seedling establishment is affected by germination capacity, soil type, cultivation, sowing depth and occurrence of disease or pest attacks. A slightly low seedling establishment could have limited effect on grain yield due to compensatory tillering (Sylvester-Bradley et al. 2008). Tillering takes place approximately from the 3-leaf stage of seedlings to onset of stem extension, and then abortion of some young tillers ensues until anthesis (Tottman and Broad 1987). This process, which is strongly influenced by genotype, plant density, nutrient status and light environment, eventually defines spikes per plant. Spikelet initiation concurs with tillering, and a terminal spikelet is differentiated just before stem extension (Slafer 2012). A spike normally develops about 16−25 spikelets, each of which produces up to 10 florets. However, less than half of the florets in a spikelet survives and set grains, and the more distal florets are aborted around anthesis (Kirby 1988). There is evidence that wheat floret death and survival are linked to pre-anthesis spike growth (González et al. 2011). Both spikelet differentiation and floret fertility determine the final grains per spike.

Compared with grain number, individual grain weight is mainly formed after anthesis, although the pre-anthesis carpel development is believed to set an upper limit for grain growth (Calderini et al. 1999; Hasan et al. 2011; Xie et al. 2015). Dry matter for grain filling results generally from three pathways. The first source is the current photosynthesis contributed mainly by leaves. However, more studies revealed that the spikes, which are positioned at the top of canopy and stay green the longest, have photosynthetic capacity comparable to or even higher than that of flag leaves (Maydup et al. 2010; Sanchez-Bragado et al. 2014). The water-soluble carbohydrates (WSCs) accumulated in stems before anthesis are the second source for grain filling. These carbohydrates can provide up to 50% of dry

matter to grains under the stressed conditions (van Herwaarden et al. 1998; Rebetzke et al. 2008). At maturity, plant organs commence to senesce under programmed cell death, and parts of the degraded nutrients will be recycled and transported into grains (Distelfeld et al. 2014). This is an important source of nitrogen and other minerals deposited in grains. Apart from source size, sink capacity and characteristics affect individual grain weight as well. Grain dimensions including grain length, width and thickness, establishes an outer physical limit for grain filling, and is closely associated with grain weight (Xie et al. 2015). Meanwhile, endosperm cell number is considered as a key inner factor influencing the assimilation capacity of grains (Brocklehurst 1977). Dynamics of grain filling vary greatly between wheat genotypes, and an earlier, faster, longer grain filling favors grain growth, which is often accompanied with a faster grain water absorption (Lizana et al. 2010; Xie et al. 2015).

As seen from above, the yield and yield components of wheat are implicated in multiple biological processes spanning from sowing to maturity, indicating complexity. These traits are also related to each other. By summarizing the data of over 20 studies published previously, it was found that wheat grain yield is most closely associated with grains per unit area (average $r = 0.66$), followed by grains per spike (average $r = 0.43$), spikes per unit area (average $r = 0.32$) and individual grain weight (average $r = 0.32$) (Kuchel et al. 2007; McIntyre et al. 2010; Bennett et al. 2012; Maphosa et al. 2014; Lozada et al. 2018). A dilemma in wheat improvement is the reverse relationship between grain number and individual grain weight. Early studies showed that individual grain weight is negatively associated with grains per unit area (average $r = –0.44$ in 26 environments), spikes per unit area (average $r = –0.24$ in 33 environments) and grains per spike (average $r = –0.30$ in 103 environments) (Kuchel et al. 2007; McIntyre et al. 2010; Bennett et al. 2012; Schulthess et al. 2017; Guan et al. 2018; Lozada et al. 2018). The trade-off between grain number and size vary depending on growing environments and genotypes. For example, the same IDuWUE panel consisting of 189 durum elite accessions when grown in different Mediterranean countries showed distinct correlation coefficients between grains m^{-2} and individual grain weight, ranging from –0.39 to –0.99 (Maccaferri et al. 2011). Another example is the correlation coefficient between grains per spike and individual grain weight: it was –0.51 ($P < 0.01$) in the GABI-WHEAT population consisting of 373 European varieties (Schulthess et al. 2017) and –0.70 ($P < 0.01$) in the WAMI population consisting of 287 CIMMYT elite lines (Sukumaran et al. 2015), whereas the coefficient was positive ($r = 0.29$, $P < 0.01$) in the 723 Asian landraces (Liu et al. 2017a).

Being quantitative traits, the yield and yield components of wheat showed relatively low broad-sense heritability: on average, about 0.63 for each of grain yield, grains and spikes per unit area as well as grains per spike, and 0.78 for individual grain weight, estimated according to previous studies (Jia

et al. 2013; Deng et al. 2017; Liu et al. 2017a; Schulthess et al. 2017; Xu et al. 2017; Liu et al. 2018; Sukumaran et al. 2018; Zhai et al. 2018). These traits have been subjected to quantitative trait locus (QTL) mapping since 1990s, leading to identification of more than 1000 QTLs (Campbell et al. 1999; Börner et al. 2002; Gegas et al. 2010; Jia et al. 2013; Griffiths et al. 2015; Brinton et al. 2017; Deng et al. 2017; Schulthess et al. 2017; Shi et al. 2017; Xu et al. 2017; Liu et al. 2018; Sukumaran et al. 2018; Zhai et al. 2018; Yang et al. 2019). These QTLs distributed on all chromosomes of wheat genome, with diverse additive effects. Due to lack of a fine consensus genetic or physical map in the past, concurrence of these yield and yield component QTLs was not compiled in detail, which restricts a comprehensive understanding of the genetic structure of yield formation and utilization of the knowledge in high-yield breeding. With the development of next generation sequencing (NGS) technology, the genome references of Chinese Spring and other varieties become available (IWGSC 2018), which can be used to remove the restriction.

In this study, to determine pathways of yield formation in wheat, the relationships of grain yield with yield components, anthesis date and biomass per shoot were investigated in the Forno/Oberkulmer recombinant inbred line (RIL) population, with a focus on the trade-off of grain number and size. These traits were then subjected to principal component (PC) analysis, and the resulting PCs were used as quantitative variables for QTL mapping. An in-depth understanding of the genetic basis of yield components of wheat was accomplished by a meta-QTL and gene alignment analysis using the massive data published previously. The *AGP2* gene, encoding the large subunit of ADP-glucose pyrophosphorylase, was eventually taken as an example to explore its roles in the trade-off of grain number and size in the Forno/Oberkulmer population.

Materials and methods

Source and statistical analyses of phenotypic data

A mapping population including 226 RILs was produced from a cross between the bread wheat variety ‘Forno’ and the spelt variety ‘Oberkulmer’ (Messmer et al. 1999). Two field experiments were conducted at the University of Nottingham Farm, Leicestershire, United Kingdom, in 2011–2012 and 2012–2013 cropping seasons. The traits, including grain yield (in t ha^{-1}), grains m^{-2}, spike m^{-2}, grains per spike, thousand grain weight (TGW, in g), anthesis date (in degree days) and biomass per shoot (in g) of the RILs, were investigated, as described in Xie et al. (2017). Best linear unbiased estimates (BLUEs) were generated for each trait over replicates and years using TASSEL v5 (Bradbury et al. 2007), and used to conduct Pearson correlations. The BLUEs of all traits were also subjected to

principal component analysis using the Pearson correlation method. General statistical analyses were undertaken using Minitab v17 (Minitab Inc., USA) and XLSTAT v2018.1 (Addinsoft Inc., USA).

QTL mapping

QTL mapping for the traits investigated in the Forno/Oberkulmer RIL population was previously carried out (Xie et al. 2017). In this study, instead, the principal components derived from these yield-associated traits were performed for QTL detection by interval mapping and multiple-QTL model mapping using MapQTL v6 (Van Ooijen 2009). A QTL was declared significant when its logarithm of odds (LOD) was higher than the genome-wide significance threshold computed by a permutation test with 1,000 iterations. The genetic distributions of the QTLs detected were displayed using MapChart v2.2 (Voorrips 2002).

Meta-QTL analysis

The previously published studies were screened to identify the QTLs associated with grains m^{-2}, spikes m^{-2}, grains per spike and TGW in wheat. Information of each QTL was collected, including genetic position, flanking/closest markers, LOD value (LOD $\geq$ 3 required), additive effect, the parent conferring the increasing allele, phenotypic variation explained (R^2) and the number of environments where it was detected. The sequences of flanking/closest markers or their primers were used for blast alignment to project these QTLs onto the Chinese Spring (CS) genome reference IWGSC RefSeq v1.0 (IWGSC 2018). This resulted into QTL clusters along chromosomes. A meta-QTL (MQTL) was defined as the physical distance that was covered by more than 80% individual QTLs within a cluster. The chromosomes of CS genome and MQTLs, together with the physical positions of the iSelect 90K SNPs (Wang et al. 2014), were drawn using MapChart v2.2 (Voorrips 2002). Moreover, the genes known to regulate grain number and TGW were also collected from literatures, and anchored onto the CS genome by blast alignment of the gene sequences.

Haplotype analysis of *AGP2* in the Forno/Oberkulmer RIL population

Genomic sequence of the CS *AGP2* gene (GenBank: AJ563452.1) was employed for blast alignment in the 10^+ Wheat Genomes database (http://www.10wheatgenomes.com/). A total of 17 hexaploid and tetraploid varieties were used to determine the *AGP2* haplotypes, including bread wheat Robigus, Paragon, Claire, Cadenza, Jagger, Norin61, ArinaLrFor, Julius, Lancer, Landmark, Mace, Stanley, SY_Mattis and Weebill_1, durum wheat Kronos, wild emmer Zavitan, and spelt accession PI190962.

Based on the two simple sequence repeat (SSRs) variations in the promoter region, i.e. $(GGC)_n$ at position –1721 bp and $(GCCAG)_n$ at position –1777 bp, a molecular marker was developed to diagnose different haplotypes of *AGP2*. The primers were designed by Premier 5.0 (http://www.premierbiosoft.com/), with a forward primer: AGGTGAGACGAACCGAACGTG, and a reverse primer: TGCCGTGGAATGCTCTCCGT. Genomic DNA of the Forno/Oberkulmer RILs was extracted from the young leaves using SDS method (Ma and Sorrells 1995). Polymerase chain reaction (PCR) was accomplished in a volume of 12.5 μL, containing 20 ng template DNA, 4 pmol forward and 4 pmol reverse primers, 0.25 μL TaKaRa deoxynucleotide triphosphate (dNTP) Mixture, 0.4 U TaKaRa *Taq*™ DNA Polymerase and TaKaRa 1× PCR Buffer (Mg^{2+} plus). The amplification reactions were performed on a PE9600 thermal cycler (Perkin Elmer, Norwalk, CT, USA), following the cycling conditions: 94°C for 5 min, 35 cycles of 94°C for 40 s, 60°C for 40 s, 72°C for 40 s, and a final extension step at 72°C for 5 min. The PCR products were mixed with 1× DNA Loading Dye (Thermo Fisher Scientific), and run on an 8% (w/v) acrylamide/bisacrylamide (19:1) gel for about 80 min. The resulting band patterns were visualized by staining in 0.1% (w/v) silver nitrate solution and subsequently in 1% (v/v) formaldehyde prepared in 2% NaOH solution.

Results

Phenotypic correlations among yield and yield components in the Forno/Oberkulmer RIL population

There were significant variations between the bread wheat parent Forno and spelt parent Oberkulmer and between their resulting RILs in each of grain yield, grains m^{-2}, spikes m^{-2}, grains per spike and TGW (Xie et al. 2017). Based on the variations, phenotypic correlation and regression analyses among grain yield and yield components were performed. Grain yield showed a strong, positive relationship with grains m^{-2} (R^2 = 0.81, P < 0.01) (Fig. 1a), followed by spikes m^{-2} (Fig. 1b), but no significant relationship with grains per spike. There was only a weak, positive association between grain yield and TGW (R^2 = 0.04, P < 0.05) (Fig. 1c). As expected, all grain number subcomponents including grains m^{-2}, spikes m^{-2} and grains per spike, were negatively associated with TGW (R^2 = 0.19–0.24, P < 0.01) (Fig. 1d-f).

Traits associated with the trade-off of grain number and TGW

Anthesis dates and biomass per shoot of the Forno/Oberkulmer RILs were measured, and both showed significant variations between genotypes. Phenotypic correlation analysis revealed that anthesis dates

were negatively associated with grains m^{-2} (R^2 = 0.16, P < 0.01) (Fig. 2a), TGW (R^2 = 0.09, P < 0.01) (Fig. 2c) and grain yield (R^2 = 0.24, P < 0.01) (Fig. 2d), but positively associated with grains per spike (R^2 = 0.15, P < 0.01) (Fig. 2b). This suggested that in the RIL population early anthesis contributed to both increased grains m^{-2} and TGW, leading to a higher grain yield, although it decreased spike fertility somewhat. Therefore, it appears that an amendment of anthesis dates could alleviate the reverse relationship between grain number and TGW at the population level that eventually facilitated yield improvement.

Biomass per shoot showed negative associations with grains m^{-2} (R^2 = 0.15, P < 0.01) (Fig. 3a), spikes m^{-2} (R^2 = 0.41, P < 0.01) (Fig. 3b) and grain yield (R^2 = 0.06, P < 0.01) (Fig. 3e). However, it was positively associated with both grains per spike (R^2 = 0.30, P < 0.01) (Fig. 3c) and TGW (R^2 = 0.13, P < 0.01) (Fig. 3d). Compared to early anthesis, increased individual shoot biomass improved the balance of grain number and TGW at the shoot level but not at the population level, which reduced the population size and grain number per unit area, leading to a decrease in final grain yield.

Identification of principal components underlying the yield-associated traits

Considering the significant relationships between these yield-associated traits, principal component analysis was performed to reveal common factors underlying them. As a result, four principal components, i.e. PC1, PC2, PC3 and PC4, were extracted, individually explaining 41.3%, 25.4%, 19.2% and 7.3% of the total trait variations (Table 1).

PC1 was positively loaded with grain yield (0.78), grains m^{-2} (0.81) and spikes m^{-2} (0.65), and negatively loaded with grains per spike (–0.46), anthesis date (–0.66) and biomass per shoot (–0.75) (Table 1, Fig. 4a, b). This suggested that a higher PC1 score was associated with accelerated anthesis, more spikes and grains per unit area and higher grain yield, though with a penalty of reduced shoot biomass and grains per spike. Therefore, PC1 represented a pathway increasing grain yield by larger population size.

Grains m^{-2} (–0.33), spikes m^{-2} (–0.45) and grains per spike (–0.65) exhibited negative loadings on PC2, whereas TGW (0.92) had a high, positive loading on this PC (Table 1, Fig. 4a), indicating that PC2 represented the trade-off of grain number and size. PC2 was also negatively loaded with anthesis date, but showed little effect on grain yield (Table 1, Fig. 4a).

For PC3, grain yield (0.57), grains m^{-2} (0.45), grains per spike (0.52) and biomass per shoot (0.57) showed positive loadings, and spikes m^{-2} (–0.40) showed a negative loading (Table 1, Fig. 4b). This

suggested the other pathway boosting grain yield by higher shoot biomass and more grains per spike, rather than more spikes m^{-2}, to increase grains m^{-2}. PC4 was mainly loaded with anthesis date (0.59) (Table 1).

QTLs responsible for the principal component scores

The principal component scores underlying the yield-associated traits were subsequently used as quantitative indices to identify their genetic basis. A total of eight and seven QTLs for PC1 and PC2, respectively, were identified in the Forno/Oberkulmer RIL population (Table 2, Fig. 5), but no QTL was found for PC3 and PC4. For PC1, Forno and Oberkulmer both provided half of the QTLs detected. *QPc1-5A.2*, with a LOD of 8.5, was the strongest QTL coming from Forno, solely explaining 29.3% of phenotypic variation. It was located on the long arm of chromosome 5A and flanked by molecular markers *Xpsr1194–Xpsr918b*. The second QTL with large effect on PC1 from Forno was *QPc1-4B*, followed by *QPc1-4A.1* and *QPc1-1D*. The four QTLs from the spelt Oberkulmer increasing PC1 scores included *QPc1-3A.1*, *QPc1-3A.2*, *QPc1-4A.2* and *QPc1-5A.1*. All were detected in the A sub-genome: two on chromosome 3A, and one on each of chromosomes 4A and 5A. *QPc1-3A.1*, *QPc1-3A.2* and *QPc1-5A.1* were major QTLs, with R^2 ranging from 17.5–19.2%.

For PC2 corresponding to the trade-off of grain number and size, all three QTLs from Oberkulmer were also detected in the A sub-genome (Table 2, Fig. 5). *QPc2-2A.1* and *QPc2-2A.2* both were mapped on chromosome 2A, with a similar additive effect on PC2. *QPc2-4A* for PC2 was overlapped with *QPc1-4A.1*, the only genomic region associated with both PC1 and PC2. *QPc2-4A* increasing the PC2 scores came from Oberkulmer, resulting in accelerated anthesis and increased TGW but decreased grain number. On the other hand, *QPc1-4A.1* from Forno increased the PC1 scores, leading to earlier anthesis, more spikes and grains per unit area and higher grain yield accompanied with decreases in biomass and grains per shoot. Four QTLs for PC2 came from Forno: two (*QPc2-3B.1* and *QPc2-3B.2*) located on chromosome 3B and two (*QPc2-7B.1* and *QPc2-7B.2*) on chromosome 7B. These QTLs explained 12.5–19.8% of the PC2 variation, with an additive effect ranging from 0.56–0.90.

Meta-QTLs for yield components in wheat

To establish a genetic framework of yield components across different genotypes of wheat, a meta-QTL analysis was carried out using the data of 149 studies published previously. A total of 58, 127, 484 and 534 QTLs associated with grains m^{-2}, spikes m^{-2}, grains per spike and TGW, respectively, were collected and compiled (Börner et al. 2002; Gegas et al. 2010; Jia et al. 2013; Griffiths et al. 2015;

Brinton et al. 2017; Deng et al. 2017; Schulthess et al. 2017; Shi et al. 2017; Xu et al. 2017; Liu et al. 2018; Sukumaran et al. 2018; Zhai et al. 2018; Yang et al. 2019). These QTLs were projected onto the CS genome sequence (IWGSC 2018), leading to identification of 12, 21, 37 and 54 MQTLs for grains m^{-2}, spikes m^{-2}, grains per spike and TGW (Fig. 6 and Tables S1–S4). The resulting MQTLs clustered in about 52 genomic regions across all 21 chromosomes, and about 80% of them were mapped in the distal regions of chromosomes. Most MQTLs had minor effects on the yield components, and 60% of them explained less than 10% of phenotypic variations. Robust and stable MQTLs were found, for example, on chromosomes 1B (27–73 Mb for grains m^{-2}; 555–684 Mb for TGW), 3A (464–552 Mb for grains m^{-2}), 4B (4–119 Mb for spikes m^{-2}, grains per spike and TGW), 4D (8–122 Mb for TGW), 5A (6–84 Mb for TGW), 6A (428–612 Mb for TGW), and 7A (581–733 Mb for TGW).

MQTL coincidences between grain number and individual grain weight were frequently found (Fig. 6). Specifically, 67 MQTLs (96%) for grain number subcomponents were colocalized with 38 TGW MQTLs (70%); that is, only 3 and 16 MQTLs for grain number and grain weight were relatively independent of each other. More importantly, the coincident loci were generally associated with an increase in TGW but a reduction in one or more grain number subcomponents, and *vice versa*, based on observations in individual studies where both grain number and grain weight were analyzed. However, a few cases showing positive QTL pleiotropic effects on both grains per spike and TGW were seen, for example, on chromosomes 2D, 4B, 4D, 6B and 7A (Quarrie et al. 2006; McIntyre et al. 2010; Guan et al. 2018; Li et al. 2018; Liu et al. 2018). In addition, Sukumaran et al. (2018) reported a locus on 6A having positive effects on grains m^{-2}, TGW and grain yield.

Genes regulating yield components in wheat

To date about 36 genes (inclusive of homoeologs if functionally analyzed) associated with grain number were identified in wheat, including the HD-Zip I transcription factor *GNI1*, anthesis-related genes *Ppd-1*, *TaFT-2*, *VRN-A1*, *VRN3* and *Eps*, spike architecture genes *Q*, *BH-1*/*WFZP* and *B1*, plant dwarfing genes *Rht-B1*, *Rht-D1*, *Rht4*, *Rht5* and *Rht13*, vacuolar and cell-wall invertase genes *Ivr1*, *Ivr3*, *Ivr5* and *TaCWI-4A*, sucrose-encoding genes *1-SST* and *6-SFT*, RING E3 ubiquitin ligase gene *TaGW2-6A*, and axillary meristem regulation factor *TaMOC1* (Table S5).

Among the grain number genes, *GNI1*, *Ppd-B1*, *Ppd-D1*, *Q*, *WFZP-A/D*, *B1*, *Rht-B1*, *Rht-D1*, *Rht4*, *TaGW2*, *6-SFT* and *TaCWI-4A* also affect individual grain weight, generally with opposite effects (Table S6). Additional 28 grain weight genes were identified and most of them were isolated based on homology with other crops like rice and maize, including the carbohydrate synthesis genes *AGP2*,

TaSus1, *TaSus2*, *TaFlo2* and *TaTPP*, the phytohormone accumulation and signaling genes *TaGASR7*, *TaCKX4*, *TaCKX6*, *TaTGW6*, *TaGS5*, *Tasg-D1*, *TaGL3* and *TaCYP78A3*, the transcriptional regulatory factors *TaSPL20* and *TaSPL21*, the G-protein pathway subunit *TaGS-D1*, cell wall invertase genes *TaCWI-A1* and *TaCWI-5D*, glutamine synthetase gene *TaGS1a*, and the stress-responsive genes *TaSAP1* and *Tabas1* (Table S6).

These genes affecting grain number and grain weight were anchored on the CS reference genome, and compared for their physical positions with the MQTLs identified (Fig. 6). It was found that 30 (83%) genes controlling grain number were coincident with the MQTLs for grain number, while 27 (66%) genes controlling grain weight were coincident with the MQTLs for TGW. The genes controlling both grain number and grain weight, including *Ppd-B1*, *Ppd-D1*, *Q*, *WFZP-A/D*, *B1*, *Rht-B1*, *Rht-D1*, *Rht4*, *6-SFT* and *TaCWI-4A*, were also colocalized with the MQTLs controlling both yield components.

Effects of *AGP2* haplotypes on yield and yield components

The *AGP2* gene encodes the large subunit of ADP-glucose pyrophosphorylase that is a key enzyme catalyzing the synthesis of ADP-glucose as the precursor for starch accumulated in the endosperm of wheat grains (Thorneycroft et al. 2003). It is a well-known gene regulating TGW in wheat (Hou et al. 2017), coincident with the MQTL *QTgw-1B.3* with stable, large effect on TGW (Table S4, Fig. 6). At the same location, there was also a stable MQTL *QGps-1B.3* for grain number. To determine possible pleiotropic effects of *AGP2* on yield and yield components, a diagnostic marker of this gene was developed and used to genotype the RILs of Forno/Oberkulmer. A clear marker polymorphism between the two parents was seen (Fig. 7a). Genotyping of the RILs showed that, on average, the lines with the Oberkulmer haplotype of *AGP2* (i.e. $AGP2^O$) displayed higher TGW (+3.2%, $P < 0.01$) than those with the Forno haplotype ($AGP2^F$) (Fig. 7b). In contrast, $AGP2^F$ contributed to more grains m^{-2} (+5.9%, $P < 0.05$) (Fig. 7c) and more grains per spike (+11.3%, $P < 0.01$) (Fig. 7d). As a consequence of compensation, this gene had little effect on grain yield.

Discussion

The present study revealed that grain yield was more closely associated with grains m^{-2} than with TGW, and there were negative relationships between grain number subcomponents and TGW, in line with previous reports (Kuchel et al. 2007; McIntyre et al. 2010; Bennett et al. 2012; Slafer et al. 2014; Lozada et al. 2018). Given the complexity and relatedness of grain yield and yield components, principal component analysis was conducted and identified three important PCs. Both PC1 and PC3 suggested the pathways of increasing grain yield by more grains m^{-2}, which supports that progress in wheat yield gain has mainly attributed to an increase in grain number (Shearman et al. 2005; Sanchez-Garcia et al. 2013; Lo Valvo et al. 2018). However, the two PCs achieved more grains m^{-2} by contrasting ways: PC1 functioned via more spikes m^{-2} (Route I: larger population size), and PC3 functioned via more grains per spike (Route II: larger individual spikes). Either Routes I or II is employed depending on breeding efforts and diverse agro-ecological systems. In China, more grains per spike have been preferred in breeding and contributed substantially to yield progress, while there has been no significant change in spikes m^{-2} since 1980s (Qin et al. 2015). A similar trend was also observed in Argentine (Lo Valvo et al. 2018) and Spain (Sanchez-Garcia et al. 2013; Chairi et al. 2018). This occurred likely because an optimal spike number per unit area can be easily met by adjusting seed rate. Therefore, future breeding to achieve further yield gain could focus on PC3 associated with fewer tillers but with higher individual shoot biomass and spike fertility, part of the wheat ideotypes as proposed by Donald (1968).

The second principal component PC2 reflected the trade-off of grain number subcomponents and TGW, even though it showed little association with grain yield. A higher PC2 score was associated with larger but fewer grains, and *vice versa*. Negative relationship between grain number and size is commonly seen in wheat (Kuchel et al. 2007; McIntyre et al. 2010; Bennett et al. 2012; Lozada et al. 2018) and other crop species (Sadras 2007; Gambín and Borrás 2010), but physiological understanding of the trade-off is limited. This study found that anthesis date and biomass per shoot played significant but contrasting roles in this trade-off. Earlier anthesis favored both more grains m^{-2} and high TGW, implying an improved balance between grain number and size at the population level. This was accompanied with reduced grains per spike, at least partly as a result of shorter duration of stem extension ($r = 0.49$, $P < 0.01$; details not shown) that might be detrimental to floret survival (González et al. 2011). Earlier anthesis was eventually associated with higher grain yield and thus would be preferred in breeding. By comparison, greater biomass per shoot contributed to more grains per spike and high TGW, indicating an improved balance between grain number and grain weight at the shoot

level. However, it showed associations with fewer spikes m^{-2}, giving rise to reduced grains m^{-2} and grain yield. These results were based on the fixed seed rate (250 seeds m^{-2}) implemented for all RILs. It can be speculated that, as discussed above, the spike number per unit area for a given cultivar has already been optimized by modifying seed rate in practice and usually not been a constraint of yield formation. Under the fixed spike number per unit area, improvement of individual shoot biomass by capturing more resources, therefore, would be promising step forward in the search for higher grain number and grain yield.

In addition to anthesis date and shoot biomass, a few other traits were reported to affect the trade-off of grain number and size (Dreccer et al. 2009; Rebetzke et al. 2016). Stem WSCs accumulated mainly during the pre-anthesis phase contribute to grain filling and final grain weight (van Herwaarden et al. 1998; Rebetzke et al. 2008). However, it was found that the wheat lines with more WSCs showed fewer grains m^{-2} linked to fewer spikes m^{-2}, which were compensated with more fertile florets/grains per spike and larger individual grains, resulting in comparable or higher yields (Dreccer et al. 2009). These are consistent with the effects of individual shoot biomass on the yield components presented in this study, as large individual shoots tend to accumulate more WSCs. Comparison of the awned-awnless near-isogenic lines showed that the awnless lines produced more grains m^{-2} and more grains per spike but significant smaller grains, leading to equivalent yields (Rebetzke et al. 2016; Sanchez-Bragado et al. 2020). The awned plants might allocate an amount of assimilates to developing awns prior to spike emergence, restricting spikelet and floret fertility (Rebetzke et al. 2016). From these instances, it is evident that the trade-off of grain number and size is established before anthesis according to resource allocation under maternal control (Sadras and Denison 2009). Environmental factors affecting resource availability before anthesis also affect this trade-off. For example, shading and water deficiency implemented during the pre-anthesis phase could reduce grain number, which was compensated by an increase in grain size, leading to a comparable grain yield (Ji et al. 2010; Labra et al. 2017). This trade-off, therefore, is sometimes beneficial for yield stability under stressed conditions.

Genetic basis of the yield and yield components in the Forno/Oberkulmer RIL population was revealed by PC QTL mapping. A total of eight and seven QTLs for PC1 and PC2 were detected, indicating that the PCs can be used as quantitative traits for genetic dissection. Among the QTLs for PC1 and PC2, *QPc1-5A.2* mapped on chromosome 5A was identified with the strongest effect on PC1. This QTL may correspond to the wheat domestication gene *Q*. In the same RIL population, an early study revealed that the *Q* allele relative *q* facilitated increases in grains m^{-2}, TGW and grain yield, but

a reduction in grains per spike (Xie et al. 2018), concurring with the function of PC1. The QTLs for PC1 and PC2 were not coincided across the genome with an exception of *QPc1-4A.1* for PC1 and *QPc2-4A* for PC2, both of which were located in the *Xglk315–Xpsr59a* interval of chromosome 4A. The allele of *QPc1-4A.1* increasing the PC1 scores came from Forno, and the allele of *QPc2-4A* increasing the PC2 scores came from Oberkulmer, suggesting that the alleles of the locus from the two parents had different roles in yield formation. *QPc1-4A.1* from the modern bread wheat Forno was beneficial for high yielding.

The PC QTLs were compared with the QTLs for individual traits in this RIL population (Xie et al. 2017). The PC1 QTLs on chromosomes 1D, 3A, 4A and 4B were co-located with those for spikes m^{-2}, grains m^{-2} and/or grain yield, with the increasing alleles from the same parents. *QPc1-5A.1* coming from Oberkulmer was coincident with the QTLs for early anthesis and decreased grains per spike. The other PC1 QTL on chromosome 5A, i.e. *QPc1-5A.2*, also contributed to more grains m^{-2}, higher grain yield and earlier anthesis, together with fewer grains per spike. For PC2, *QPc2-3B.1*, *QPc2-4A* and *QPc2-7B.2* were coincident with the QTLs for both grain number and TGW, and the increasing alleles for more/larger grains at those loci were conferred by opposite parents (Xie et al. 2017). The two QTLs for PC2 on chromosome 2A and *QPc2-3B.2* contributed to TGW. Of them, *QPc2-2A.1* was also mapped to the locus controlling the partitioning of dry mass to spikes at anthesis, a trait affecting the trade-off of grain number and size: it was positively associated with grains per spike ($r = 0.47$, $P < 0.01$) but negatively associated with TGW ($r = –0.49$, $P < 0.01$). *QPc2-7B.1* was not co-located with any QTL for grain number or TGW, but co-located with the QTL for the growth duration from flag leaf emergence to anthesis (GS39-61; Tottman and Broad 1987). GS39-61 showed a positive relationship with grains per spike ($r = 0.30$, $P < 0.01$), but a negative relationship with TGW ($r = –0.23$, $P < 0.01$). The results of QTL coincidences concur with the roles of PC1 and PC2 in determination of the yield and grain number/size trade-off based on analyses of factor loadings. PC analysis is useful to reveal the pathways underlying complex traits of crops in a simpler manner. The resultant PCs calculated based on a limited number of investigated traits, may be loaded by other traits potentially participating in the same pathways, e.g. partitioning of dry mass to spikes at anthesis and GS39-61 on PC2 for grain number/size trade-off in this study, suggesting predictability.

Identification of the QTLs for PCs initiates a first step to understand wheat yield formation through the principal component analysis. In breeding, however, care must be taken for utilization of those QTLs in marker-assisted selection. First, the confidence intervals of these QTLs are still large and thus fine mapping is necessary to find out more closely linked molecular markers, thereby avoiding linkage

drags. Second, the QTLs for PC2 are associated with the trade-off between grain number and size, and their utilization must follow the local breeding targets, for either more grains per unit area or larger grains.

To establish a genetic framework of the yield determination in wheat, more than 1200 QTLs/genes published previously for yield components were compiled and aligned onto the CS reference genome. This work provides an overview regarding the numbers, distributions and effects of yield component MQTLs. Almost all MQTLs for grain number were coincided with those for individual grain weight, indicating intensive trade-off of the two yield components at the genome level. Additionally, 36 and 41 genes/homoeologs regulating grain number and grain weight, respectively, were previously identified, and most of them were coincided with the MQTLs for respective traits. Only 13 genes, including *GNI1*, *Ppd-B1*, *Ppd-D1*, *Q*, *WFZP-A*, *WFZP-D*, *B1*, *Rht-B1*, *Rht-D1*, *Rht4*, *TaGW2-6A*, *6-SFT* and *TaCWI-4A*, were functionally analyzed to have reverse, pleiotropic effects on both grain number and grain weight (Maphosa et al. 2014; Jiang et al. 2015; Liu et al. 2017b; Xie et al. 2018; Zhai et al. 2018; Golan et al. 2019). Whether or not the remaining genes have the pleiotropic effects on the two yield components is unknown. To clarify this, the grain weight gene *AGP2* was taken as an example for analysis of pleiotropy. It eventually displayed reverse effects on grain number, consistent with the MQTL *QGps-1B.3* for grain number identified by meta-QTL analysis. This gene functioned possibly through its role in starch biosynthesis pathway in anthers (Lalonde et al. 1997), as revealed in rice (Lee et al. 2016). For the other genes controlling the yield components, a comprehensive analysis of pleiotropy is therefore essential before utilization in breeding. Considering the intensive trade-off of grain number and size at the genome level, a simple addition of the yield component QTLs/genes may not work well to maximize grain yield potential. Instead, which and how many loci to be combined in a wheat cultivar should be carefully taken into account according to local breeding goals.

Author contribution statement

QX conceived the experiments, performed field trials and data analysis, and wrote the paper. DLS contributed to the experimental design and paper writing. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

Supplementary information

The online version contains supplementary material available at

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Figure captions

Fig. 1 Relationships among grain yield and yield components investigated in the Forno/Oberkulmer RIL population. Data of the RILs (n = 117) are the best linear unbiased estimates across the two years. * significant at $P < 0.05$; ** significant at $P < 0.01$

Fig. 2 Relationships of anthesis date with grain yield and yield components investigated in the Forno/Oberkulmer RIL population. Data of the RILs (n = 117) are the best linear unbiased estimates across the two years. ** significant at $P < 0.01$

Fig. 3 Relationships of biomass per shoot with grain yield and yield components investigated in the Forno/Oberkulmer RIL population. Data of the RILs (n = 117) are the best linear unbiased estimates across the two years. ** significant at $P < 0.01$

Fig. 4 Loading plots of PC1 vs PC2 (a) and PC1 vs PC3 (b). Circles indicate 100% of explained variance. Proportion of variances for the principal components are shown in parentheses

Fig. 5 Identification of the QTLs for PC1 and PC2 in the Forno/Oberkulmer RIL population. The 1-LOD support intervals of significant QTLs are shown as vertical bars. The parents (F: Forno, O: Oberkulmer) conferring increasing alleles are indicated in parentheses following the QTL names

Fig. 6 Identification of the meta-QTLs for yield components of wheat. The left scales indicate the physical distances of chromosomes, in Mb. On the chromosome bars, the positions of centromeres (red) and iSelect 90K SNPs (grey; Wang et al. 2014) are shown. The meta-QTLs are presented as vertical bars on the right of chromosomes (orange: TGW, blue: grain number), and the genes known to regulate grain weight (orange), grain number (blue) or both (black) are aligned on the left of chromosomes

Fig. 7 Haplotype effects of the *AGP2* gene on yield components in the Forno/Oberkulmer RIL population. (a) Polymorphism of the *AGP2* diagnostic marker between the parents Forno (F) and Oberkulmer (O). The numbers on the left indicate the band size of pUC19 DNA/*Msp*I Marker. (b–d) Average effects of the Forno (AGP^F, n = 50) and Oberkulmer (AGP^O, n = 65) haplotypes on the yield components in the RIL population. Bars represent means ± SD. * significant at $P < 0.05$, ** significant at $P < 0.01$