Seed Biology

REVIEW

Open Access

https://doi.org/10.48130/seedbio-0024-0007

Seed Biology **2024**, 3: e007

Grain size control in wheat: toward a molecular understanding

Bo Wei[1*] and Yuling Jiao[2,3,4*]

[1] *National Key Laboratory of Wheat Improvement, Peking University Institute of Advanced Agricultural Sciences, Shandong Laboratory of Advanced Agricultural Sciences in Weifang, Weifang, Shandong 261325, China*

[2] *State Key Laboratory of Protein and Plant Gene Research, School of Life Sciences, Peking University, Beijing 100871, China*

[3] *Peking-Tsinghua Center for Life Sciences, Center for Quantitative Biology, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China*

[4] *Peking University Institute of Advanced Agricultural Sciences, Shandong Laboratory of Advanced Agricultural Sciences in Weifang, Weifang, Shandong 261325, China*

* Corresponding authors, E-mail: bo.wei@pku-iaas.edu.cn; yuling.jiao@pku.edu.cn

Abstract

Grain size is a major determinant of bread wheat (*Triticum aestivum*) yield, which has a broad impact on worldwide food security. Not surprisingly, grain size underwent extensive artificial selection during wheat domestication and breeding. Recent advances in wheat molecular genetics and genomics have facilitated the elucidation of the molecular basis underlying grain size. Grain size determination is the cumulative result of source strength, photoassimilate remobilization, and sink strength. Here, we systematically review the recent progress in the cloning and molecular mechanisms of genes that regulate grain size in wheat following the source-to-sink flow. In addition, we discuss possible strategies for overcoming the trade-off between grain size and grain number, as well as synergetic improvement of grain yield and grain quality.

Citation: Wei B, Jiao Y. 2024. Grain size control in wheat: toward a molecular understanding. *Seed Biology* 3: e007 https://doi.org/10.48130/seedbio-0024-0007

Introduction

During domestication and breeding, bread wheat (*Triticum aestivum*) underwent extensive artificial selection. The bread wheat genome is the result of two polyploidy events. Tetraploid wheat (AABB, 2n = 4x = 28) is the result of an ancient polyploidy event between *Triticum urartu* (AA, 2n = 2x = 14) and *Aegilops speltoides* (BB, 2n = 2x = 14). The second polyploidy event occurred approximately 10,000 years ago in the Fertile Crescent between presumably cultivated tetraploid wheat and wild *Aegilops tauschii* (DD, 2n = 2x = 14)[1], and the resulting hybrid (AABBDD, 2n = 6x = 42) is the bread wheat used today. Archaeobotanical evidence indicates that both the transitions from diploid wild einkorn (*Triticum monococcum* ssp *aegilopoides*; AmAm) to domesticated diploid forms (*T. monococcum* ssp *monococcum*) and from wild tetraploid emmer wheat (*Triticum turgidum* ssp *dicoccoides*; BBAA) to domesticated tetraploid cultivars (*T. turgidum* ssp *dicoccum*) are associated with a trend toward larger grains. For hexaploid wheat, grain size continues to be an important selection trait. Today, wheat grains provide one-fifth of the calories and a substantial proportion of protein consumed by humans[2]. The main attributes of grain size, including grain width, grain length, and length/width ratio, are greatly different among the hexaploid wheat varieties[3]. Compared to the early common wheat landraces, such as *T. aestivum* ssp. *spelta* and ssp. *macha*, grain width was dramatically increased and grain length was decreased in the early stages of the bread wheat breeding process[3]. Therefore, the wheat grain shape has changed from long and thin to shorter and wider in modern bread wheat varieties[3]. In addition, as wheat is a staple commercial crop, its market requirements also distinctly influence the selection of grain size and shape. Important attributes, such as grain density, uniformity, end-use quality, protein content, and trace elements are also associated with grain size, and directly influence grain yield and flour quality, which determines the market value of wheat grains[4].

Fertilization initiates grain development. Generally, the development process of wheat grains is divided into three stages: the cell division and expansion stage that occurs 1–14 d after anthesis (DAA), during which the basic structure of wheat grains are formed; the grain filling stage 14–28 DAA, during which the accumulation of starch and protein occurs and the grain dry weight increases by approximately twofold[5]; and grain maturation and desiccation that occurs from 28 DAA until complete maturation[6]. During the last stage, the grain filling slows and is complete by approximately 35 DAA. The fresh weight subsequently decreases rapidly until ~42 DAA due to desiccation[6].

During wheat grain development, gene expression, and translation profiles change dynamically[6–9]. A substantial change in the transcriptome occurs during grain development[6]. Moreover, different subgenomes of wheat are unbalanced at both the transcriptional and translational levels[7,8]. Dynamic chromatin landscape changes during embryogenesis is correlated with biased gene expression among homeolog gene triads and divergent expression among three subgenomes[9].

Although grain size is a key yield component in wheat[10], the large polyploid wheat genome substantially impedes linkage mapping of genes regulating grain size and identification of molecular functions. More information on wheat grain size control has been obtained by population genetic studies, in which putative regulatory genes or QTLs are found by genetic mapping or genome-wide association studies (GWASs). With the use of molecular markers, many QTLs related to grain size

or grain weight have been identified on all 21 wheat chromosomes in recent decades, and utilized in breeding[11–19]. Recently, with the rapid development of wheat genome sequencing, genetic mapping has been utilized to identify causal genes for grain traits[20,21]. A subset of these causal genes was independently confirmed by gene knockout and/or overexpression methods. There are excellent recent reviews centered on the molecular regulation pathways of grain size in rice and wheat[22–26]. In this review, we instead focused on the source-flow-sink system to summarize the genes involved in photosynthesis, and carbohydrate transportation, as well as their roles in grain size determination. We also investigated genes directly affect grain size and grain filling, with particular attention given to the regulation of starch and seed storage protein (SSP) biosynthesis (Fig. 1). We also discussed difficulties and feasible strategies for grain size improvement.

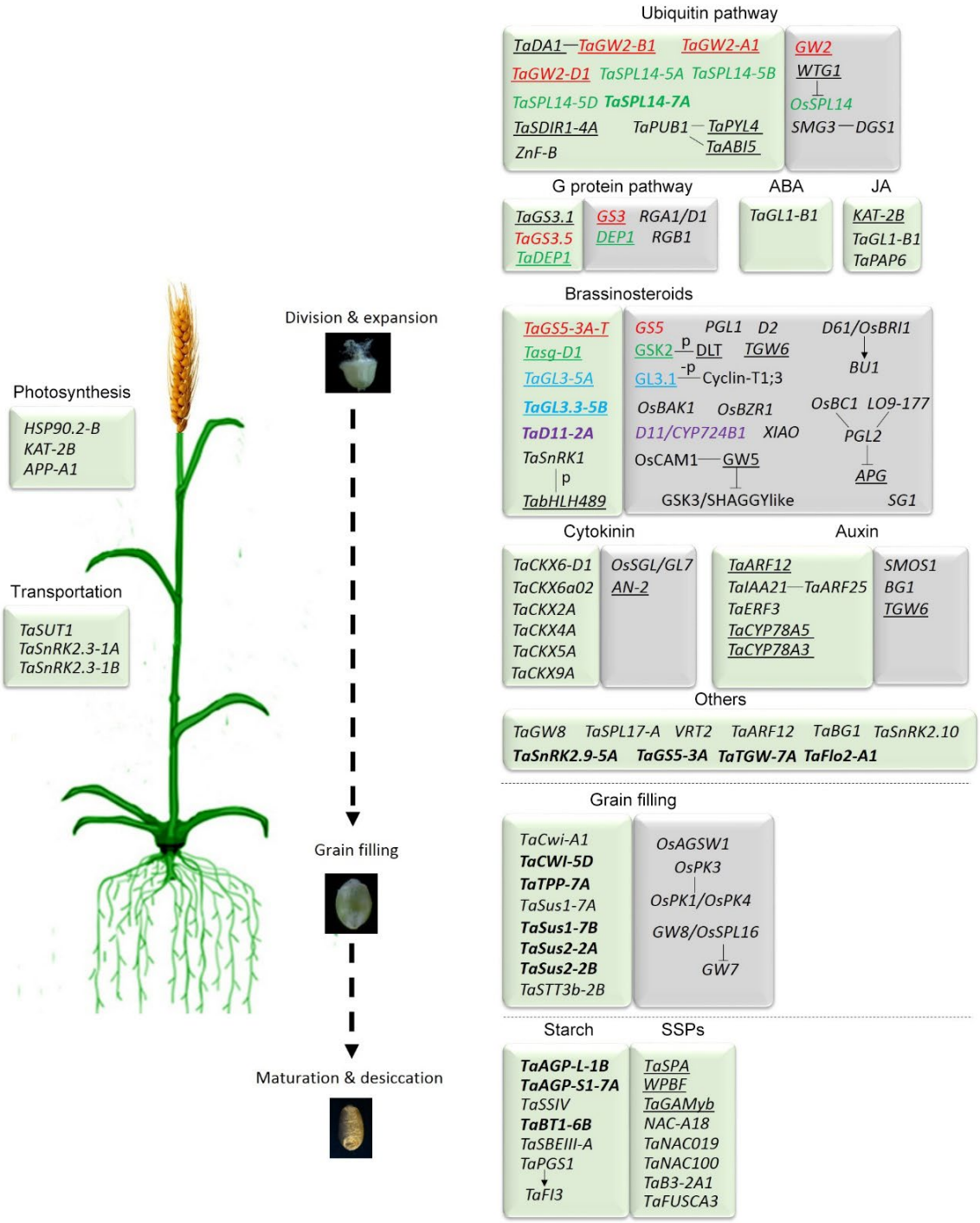

Fig. 1 Genes and genetic pathways regulating grain size in bread wheat and rice. The components without underlines are positive regulators of grain size, and those with underlines are negative regulators. The short connecting lines represent the proteins that physically interact. The bond genes were selected during wheat breeding. The genes with the same colors are homologous gene between wheat and rice. References for the individual genes are listed in Supplemental Tables S1 & S2 for wheat and rice genes, respectively.

Photosynthesis

During photosynthesis, solar energy is utilized and drives the accumulation of plant biomass and sink storage, such as in crop grains[27]. Genes related to chloroplast and chlorophyll synthesis play important roles in photosynthetic efficiency and capacity and influence wheat grain size. Recently, three genes related to chlorophyll content and the photosynthetic rate have been identified as grain size regulators[20,21,28]. Map-based cloning identified *keto-acyl thiolase 2B* (*KAT-2B*) as the causal gene for a large-grain mutant. Analysis of *KAT-2B* overexpressing lines revealed that *KAT-2B* can positively regulate grain size, weight, and yield. In addition, the leaf area and chlorophyll content increase in *KAT-2B*-overexpressing lines, indicating that the photosynthetic capacity is enhanced[21]. *HSP90.2-B* is the causal gene for the *CO_2 ASSIMILATION RATE AND KERNEL-ENHANCED1* (*CAKE1*) gene cloned from durum wheat[20]. It encodes a cytosolic molecular chaperone folding nascent preprotein that is crucial for the localization of nuclear-encoded photosynthesis units in chloroplasts. Mutation of *HSP90.2-B* led to a decreased photosynthetic rate, decreased grain size, and an 80% decrease in grain yield[20]. Similarly, the *CAKE2* gene has been mapped to *ASPARTIC PROTEASE1* (*APP-A1*), and a premature stop mutation increases the photosynthetic rate, grain size, and grain weight. The APP-A1 protein can degrade PsbO which is an important member of photosystem II[28]. By producing carbohydrates, photosynthesis is the first step in the source-flow-sink. Identification of genes that enhance photosynthetic capacity and efficiency is a constructive and effective way to increase sink storage at the source.

Transportation

The long-distance transport of photosynthetic products is an essential bridge connecting the source and the sink and is key to the growth and development of sink tissues, such as grains and fruits. Sucrose is one of the main photosynthetic products and is remobilized by sucrose transporters (SUTs) in the vasculature leading to the grain. Among the 14 wheat sucrose transporter genes *TaSUTs*, *TaSUT1* homeologous genes on chromosomes 4A, 4B, and 4D are expressed predominantly in the stem, leaf sheath, rachis, lemma, and developing grain, and their expression levels are significantly correlated with grain size and weight[29]. The peduncle is the key connection between the spike and the stem and plays important roles in the transportation of water-soluble carbohydrates[30]. Association analysis revealed that *TaSnRK2.3-1A* and *TaSnRK2.3-1B* are significantly associated with the length of the peduncle and penultimate node, as well as with grain size and weight. Additionally, one haplotype of *TaSnRK2.3-1B* was shown to be associated with increased grain weight and decreased plant height, whereas another was associated with increased grain weight, increased stem water-soluble carbohydrate contents, and decreased plant height; thus these two haplotypes were considered elite[31].

Size determination

Genes involved in grain development may directly determine grain size. Cloning and functional analysis of such genes have been a key research direction for grain size[24,26]. Since the large polyploid wheat genome substantially hinders the use of forward genetic methods and molecular functional identification, many grain size regulatory genes in wheat were studied by reverse genetic analysis of genes homologous to rice grain size regulators or by association analysis, which revealed homologs of rice grain size regulators. Here, we organized the genes related to grain size in wheat according to known regulatory pathways.

Ubiquitin-proteasome pathway

Ubiquitin–proteasome degradation is extensively utilized by plants to regulate development. Several grain-size regulatory genes were found to encode ubiquitin–proteasome degradation pathway proteins, including E2, E3 and their regulators. *GRAIN WIDTH2* (*GW2*) encodes a RING-type E3 ubiquitin ligase in rice[32]. Downregulation of three homeologous *TaGW2* genes by RNA interference (RNAi) leads to significant increases in grain width and grain weight[33]. *TaGW2-B1* has stronger effects on grain size regulation than *TaGW2-D1* does, and *TaGW2-B1* and *TaGW2-D1* double gene knockdown plants have significantly larger grain sizes than single gene knockdown plants[34]. *TaDA1* negatively regulates grain size in wheat. Downregulation of *TaDA1* increases grain size and grain weight. In addition, TaDA1-A physically interacts with TaGW2-B1 and subsequently affects grain size in an additive way[35].

Salt and drought-induced RING finger1 (SDIR1), is a RING-type E3 ubiquitin ligase that has been identified as a key player in the response to both salinity and drought stresses[36]. The grain size of the *TaSDIR1-4A*-silenced lines increased, and one haplotype showed a significant association with increased grain size and greater grain weight[37]. Another E3 ligase TaPUB1 can interact with TaPYL4 and TaABI5, both of which are components of the ABA signaling pathway, and subsequently induces the degradation of TaPYL4 and TaABI5. *TaPUB1* overexpressing plants exhibit larger grains, whereas the corresponding RNAi lines exhibit smaller grains[38]. Similarly, ZnF-B, which is also a RING-type E3 ligase, positively regulates grain size[39].

Rice OsOTUB1 is a deubiquitinating enzyme, whose mutant exhibits decreased grain width and thickness[40]. Rice OsSPL14 is degraded by OsOTUB1, which controls grain development[41]. In wheat, upon cleavage by miR156, the triple mutant of *TaSPL14-5A*, *-5B* and *-5D* exhibited reduced grain size and weight[42]. For another *TaSPL14* member *TaSPL14-7A*, the preferred haplotypes are correlated with increased grain size and weight, which underwent positive selection during wheat breeding worldwide[43].

G protein signaling

The G protein signaling pathway plays important roles in various developmental processes in plants and animals. Generally, the G protein complex has three subunits, Gα, Gβ and Gγ. In rice, both the Gα-encoding gene *RGA1* and the Gβ-encoding gene *RGB1* positively regulate grain size[44–46]. Rice *GRAIN SIZE3* (*GS3*), which encodes a noncanonical Gγ subunit, was identified as the causal gene that determines grain length[47,48]. Alternative splicing of the heterotrimeric G-protein encoding gene *TaGS3* controls wheat grain size. Different alternative splicing isoforms of *TaGS3* have different functions related to grain size control[49]. The splicing isoforms *TaGS3.2–3.4* have no effect on grain size, whereas the splicing isoform *TaGS3.1* overexpression line has deduced grain weight and grain length. In contrast, upregulation of *TaGS3.5* can significantly increase grain weight and grain length[49].

Rice *DENSE AND ERECT PANICLE1* (*DEP1*) shares homology with rice *GS3*, and a gain-of-function mutant of *DEP1* with a truncated ORS domain shows decreased grain size, grain weight, and dense and erect panicles and increased grain number per panicle and grain yield per plant[50]. The *TaDEP1* locus was also identified within a key QTL correlated with grain thickness by GWAS. It has been proposed that the function of *TaDEP1* is conserved, and rice *DEP1* is a regulator of grain weight and size[51].

Phytohormones

The phytohormone brassinosteroids (BRs) regulate multiple plant growth and development processes[23,52]. The BR signaling component rice OsGSK2, which encodes an ortholog of *Arabidopsis* BIN2, negatively regulates grain size[53], and phosphorylates DWARF AND LOW-TILLERING (DLT, also known as OsGRAS32, DWARF62, and GRAIN SIZE6)[54]. Wheat *Tasg-D1* is an ortholog of *OsGSK2* and plays a conserved role in negatively regulating grain size and weight[55].

The causal gene of a rice grain length QTL, *qGL3*/*GL3.1*, is *OsPPKL1*, which encodes a Ser/Thr phosphatase with a Kelch-like repeat domain[56–58]. OsPPKL1 dephosphorylates cyclin-T1;3 and subsequently regulates cell division in spikelets. A reduction in OsPPKL1 activity results in increased grain length and grain yield without affecting grain quality. In fact, the *qgl3* allele has been utilized in breeding[56]. The wheat homologous gene *TaGL3-5A* harbors a SNP in the 11th exon that leads to an amino acid change, and the resulting *TaGL3-5A-G* allele is correlated with increased grain size and grain weight[59]. *OsPPKL2* and *OsPPKL3* are two *OsPPKL1* homologs in the rice genome[56]. *OsPPKL1* and *OsPPKL3* decrease grain length, whereas *OsPPKL2* increases grain length. *TaGL3.3-5B* is the homolog of *OsPPKL3*, with one allele associated with large grain size and under selection in breeding[60]. Taken together, the results of these studies in rice have shown that BR signaling plays important roles in regulating grain development, not only in grain size and grain filling but also grain number per panicle. Wheat homologous genes are frequently found in seed trait QTLs, suggesting possible conservative roles in seed development. Wheat *TaD11-2A* positively regulates the endogenous BR content, and *TaD11-2A* mutants exhibit dwarfism and small grains. In addition, wheat *TaD11-2A* is significantly associated with plant height, grain size, and grain yield per plant, with an elite haplotype that has been positively selected during wheat breeding[61].

In addition to BR, auxin also plays important roles in determinating grain size. Rice *THOUSAND-GRAIN WEIGHT 6* (*TGW6*) encodes indole-3-acetic acid (IAA)-glucose hydrolase, which catalyzes the transformation of IAA-glucose back to IAA and regulates early endosperm development. *TGW6* negatively regulates grain size and weight without affecting grain quality[62]. Wheat *TaIAA21* negatively regulates grain length, width, and weight. TaIAA21 can physically interact with TaARF25 and subsequently positively regulates grain size and weight in tetraploid wheat. TaARF25 further positively regulates the expression of *TaERF3*, whose mutants exhibit reduced grain size and weight. Therefore, *TaIAA21* is a negative regulator of grain size and weight *via* interaction with the TaARF25–TaERF3 module in wheat[63].

Overexpression of *TaCYP78A5* specifically in the maternal integument significantly enhanced grain size, grain weight, and grain yield per plant in field trials. *TaCYP78A5* is involved in the auxin synthesis pathway and increases auxin accumulation in the ovary. *TaCYP78A5* expression prolongs the proliferation of maternal epidermal cells and increases the number of seed coat cells through auxin mediated flowering delay, thereby promoting grain enlargement[64]. Similarly, upregulation of *TaCYP78A3* expression can increase grain size by regulating the number of cells in the seed coat[65].

Rice *OsCKX2* controls grain number[66], and several wheat homologs of *OsCKX2* are associated with grain size traits. An 18-bp deletion in the 2nd intron of *TaCKX6-D1*[67] and a novel allele of *TaCKX6a02*[68] are associated with grain size and weight. In addition, *TaCKX2A_2*, *TaCKX4A_2*, *TaCKX5A_3*, and *TaCKX9A_2* are also significantly associated with increased grain weight in the Chinese wheat micro-core collection (MCC) and a landrace wheat population[69].

PYLs, which are abscisic acid (ABA) receptors, respond to plant drought stress. Overexpression of *TaPYL1-1B* improved drought tolerance and increased grain size and weight. The elite allele *TaPYL1-1B* confers not only drought tolerance but also increases grain size and yield[70].

TaGL1-B1 has been identified as a regulator of grain length by GWAS. A mutant of *TaGL1-B1* exhibits dramatically shorter grain lengths, and *TaGL1-B1*-overexpressing lines exhibit increased grain lengths. Moreover, TaGL1-B1 physically interacts with TaPAP6 which positively regulates grain length. The content of jasmonic acid (JA) is significantly increased in both the *TaGL1-B1*- and *TaPAP6*-overexpressing lines, suggesting that the JA pathway may influence grain length[71].

Other regulators of grain size

Among the genes affecting grain size, some are not associated with the abovementioned pathway. Wheat *TaGW8* is an ortholog of rice *GW8/OsSPL16* that increases grain size and grain yield by promoting cell division and grain filling[72]. The *TaGW8-B1a* haplotype is associated with greater grain size and greater grain weight, which is in contrast with the *TaGW8-B1b* haplotype, which lacks a 276-bp indel in the first intron[73]. Using GWAS, wheat *TaSPL17* was identified as a positive regulator of grain size and grain number by regulating spikelet and floret meristem development, which in turn leads to increased grain yield per plant[74]. An elite *TaSPL17* haplotype results in wider and longer grains and greater grain weight, accompanied by more spikelets per spike[74].

Polish wheat (*Triticum turgidum* ssp. *polonicum*, previously known as *Triticum polonicum*) is a subspecies of tetraploid wheat with long glumes, lemmas and grains. Map-based cloning identified a MADS-box TF encoding gene *VEGETATIVE TO REPRODUCTIVE TRANSITION2* (*VRT2*) as the causal gene underlying the long-glume *P1* locus[75,76]. Ectopic expression of *VRT-A2* in hexaploid bread wheat causes longer glumes and grains, leading to larger grain sizes, confirming that the *VRT-A2* expression level affects glume and seed length[75].

Two members of the wheat *TaSnRK2* family are reportedly involved in the regulation of grain size[77,78]. Two favored *TaSnRK2.9-5A* haplotypes are associated with higher grain weight and have been positively selected in wheat breeding in China[77]. The *TaSnRK2.10* haplotype is significantly associated with larger grain size[78].

Using GWAS, wheat *TaARF12* was identified as a plant height and spike length regulator[51]. Knockout of *TaARF12* can significantly increase grain number per spike and result in higher grain weight[79].

Wheat *TaGS5-3A* has two alleles that are discriminated by a G/T SNP in the coding region, and the *TaGS5-3A-T* allele was associated with larger grain size and higher grain weight in a population analysis[80,81]. *Schizosaccharomyces pombe* expressing *TaGS5-3A-T* was shown to exhibit higher total serine carboxypeptidase activity than *TaGS5-3A-G*[80]. In addition, transgenic experiments in rice showed that *TaGS5-3A-T* overexpression lines are superior to *TaGS5-3A-G* overexpression lines in grain width, length and weight[80]. Furthermore, population analysis indicated that *TaGS5-3A-T* is a favored haplotype under positive selection[80].

TaTGW-7A transcription levels were found to be positively associated with grain size and grain weight using the Chinese mini-core collection with 501 varieties. The elite allele *TaTGW-7Aa* exhibits higher expression levels and has been under strong and positive selection during wheat breeding[82].

TaFlo2-A1, an ortholog of rice *FLOURY ENDOSPERM2*, is associated with grain size and weight in bread wheat[83]. Population genetics analysis was used to identify the *TaFlo2-A1b* allele as a positively selected haplotype[83].

Ectopic expression of *TaBG1*, the homologous gene of rice *BIG GRAIN1*, leads to a larger grain size, but transgenic lines exhibit a trade-off in grain number per plant, resulting in no significant overall increase in gain yield[84].

Grain filling

The grain filling stage is a key step that determines grain weight and grain size. During this stage, starch, protein, and other organic matter produced through photosynthesis are assimilated in wheat grains. The duration and velocity of grain filling are two factors that affect grain size and weight. Cell wall invertases catalyze the irreversible hydrolysis of sucrose to glucose and fructose and play a key role in sink strength. Based on the nucleotide polymorphisms at the *TaCwi-A1* locus, one haplotype was found to be associated with lower grain weight, whereas another was found to be associated with higher grain weight[85]. Similarly, a *TaCWI-5D* haplotype was shown to be distinctly associated with greater grain weight and is strongly selected during wheat polyploidization, domestication, and breeding[86]. TaSus converts sucrose to fructose and UDP-glucose during seed development. Both *TaSus1* and *TaSus2* are associated with grain size regulation[87,88]. The trehalose-6-phosphate phosphatase-encoding gene, *TaTPP-7A*, was identified as a putative grain size regulator that is expressed specifically in developing grains and significantly influences grain filling. Identification of *TaTPP-7A*-overexpressing wheat plants confirmed that *TaTPP-7A* is a key regulator of source-flow-sink interactions and sucrose allocation. Population genetic analysis revealed that *TaTPP-7A* is a domestication- and breeding-selected target gene[89].

As an important posttranslational protein modification, asparagine N-glycosylation, which is catalyzed by the oligo saccharyl transferase (OST) complex, plays essential roles in plant development[90]. STAUROSPORINE AND TEMPERATURE SENSITIVE3 (STT3) is one of the subunits in the OST complex that catalyzes the subunit encoding oligosaccharyl transferase[91]. Overexpression of *TaSTT3b-2B* distinctly increases grain size and weight by increasing the expression of genes encoding starch synthase, and sucrose synthase[92].

Starch and SSPs

Starch and seed storage proteins (SSPs) are the main components of wheat grains; they account for approximately 70% and 13%, respectively, of the weight of ripen grains and play essential roles in determining grain weight, size and quality[93]. Several key genes involved in starch synthesis, including ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS), starch-branching enzyme (SBE), and debranching enzyme (DBE), have been studied in wheat. AGPase catalyzes the conversion of glucose-1-Pi to ADP-glucose, which is a rate-limiting enzyme in the starch synthesis pathway[94]. The AGPase tetrameric complex comprises two large subunits (LSUs) and two small subunits (SSUs)[94]. A haplotype of *TaAGP-L-1B* and a haplotype of *TaAGP-S1-7A* are associated with larger grain size and greater weight, respectively, in modern wheat cultivars, and their additive effects on the determination of grain size have been detected in wheat populations[95]. Starch synthesis IV (SSIV) is an important starch synthesis isoform, and its mutation reduces the number of starch granules per chloroplast in wheat. An elite SSIV allele is significantly associated with grain size and weight[96]. *BRITTLE1* (*BT1*) is responsible for the transport of ADP-glucose which is essential for starch synthesis in *Arabidopsis*[97]. Knocking down the expression of *TaBT1-6B* in wheat decreases grain size and starch content[98]. *TaBT1-6B-Hap1* and *TaBT1-6B-Hap2* are two useful alleles under selection during modern wheat breeding[98]. In addition, a polymorphism in a starch-debranching enzyme-encoding gene, *TaSBEIII-A*, is associated with grain size and grain weight[99]. *T. aestivum Positive Regulator of Grain Size 1* (*TaPGS1*) is a bHLH transcription factor (TF) encoding gene that is strongly expressed in the endosperm at 10-20 d postanthesis in wheat. Ectopic expression of *TaPGS1* increases grain weight (up to 13.81% in wheat and 18.55% in rice) and grain size with decreasing starch granule size (smaller and tighter) in the proteinaceous matrix[100]. Furthermore, *TaPGS1* can regulate the expression of *TaFI3* by binding to the E-box motif in its promoter region.

SSPs are also essential determinants not only of wheat grain weight but also of the end-use quality of wheat flour[101,102]. SSPs include glutenin and gliadins. Based on their mobilities in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), glutenin can be further divided into high-molecular-weight glutenin subunits (HMW-GSs) and low-molecular-weight glutenin subunits (LMW-GSs)[102]. Gliadins consist of α/β-, γ-, ω- and δ-gliadin based on their different primary structures[103]. The expression and abundance of SSPs are regulated by the complicated network of TFs[104]. Wheat storage protein activator (TaSPA), a bZIP TF, is expressed specifically in grains and binds to the GCN4-like binding motif in the promoter of *LMWG-1D1*[105]. Wheat prolamin-box binding factor (WPBF) is a DNA-binding TF with one finger (Dof) that can bind to the promoter regions of gliadin-encoding genes, and LMW-GS and HMW-GS-encoding *TaGlu-1By8* and *-1Dx2* loci[106,107]. TaGAMyb, which is a companion of the GCN5-like histone acetyltransferase, regulates the expression of the HMW-GS 1Dy encoding gene by binding to its promoter directly[108].

The starch content in grains directly determines grain size and weight. However, the increase in SSPs usually occurs at the cost of grain weight. Therefore, the coordination of the starch content and SSPs content is crucial for balancing of grain size, weight and quality. Three NAC (NAM-ATAF-CUC) TFs, NAC-A18,

TaNAC019 and TaNAC100, are coordinators of starch and SSP accumulation[109–111]. Ectopic expression of *NAC-A18* in rice dramatically decreased the starch content and increased the SSP content, grain size and weight. Moreover, NAC-A18 can upregulate the expression of *TaLMW-D6* and *TaLMW-D1* by binding directly to their promoters and suppressing the expression of *TaGBSSI-A1* and *TaGBSSI-A2*. TaNAC019 binds to specific motifs in the promoters of *TaGlu-1Bx*, *TaGlu-1By*, *TaGlu-1Dx*, *TaSuSy1*, and *TaSSIIa* and positively regulates their expression. Compared to those of the wild type, the triple mutant of three homeologous *TaNAC019* exhibits lower gluten contents and smaller grain sizes with shorter grain widths and lengths[109]. In contrast, TaNAC100 negatively regulates the expression of *GLU-1* through binding to its promoter. Overexpression of *TaNAC100* suppresses *Bx14*, *By15* and *Dx2* expression and further decreases their protein levels[110]. Similarly, the TF TaB3-2A1 binds to the *cis*-element CCRM1-1 in the promoter of *GLU-1*. *TaB3-2A1* overexpression significantly reduces the contents of HMW-GSs and other seed storage proteins but increases the starch content, leading to increased grain size, including grain length and width[112]. Another B3-superfamily TF, TaFUSCA3, interacts with TaSPA and subsequently activates *Bx7* by binding to its promoter[113].

Although the abovementioned genes independently and coordinately regulate the contents of starch and SSPs, the intricate mechanisms underlying their regulatory pathway still need to be determined to accumulate additional genetic resources for genome-wide target editing.

Conclusions and perspectives

Grain size is a key agronomic trait in crop breeding. In recent decades, there has been exciting progress in research on grain size development in rice. Studies in wheat suggest that some grain size regulators have conserved functions in rice and other plants[32,34,35,114–116]. However, many of the observed associations in wheat remain unconfirmed by experiments. Often, a homolog of the rice grain size regulatory gene is found in a grain trait QTL by GWAS in wheat. It is often difficult to experimentally validate whether a favorable haplotype indeed promotes a grain trait in wheat, as often only a single SNP exists between two haplotypes, and we still cannot edit the genome precisely to reconstruct the haplotype in the desired genetic background. On the other hand, overexpression of an allele could lead to misleading results. Because of these difficulties, we sometimes know much about a wheat homolog of a rice grain regulator, including its interacting proteins and binding promoters, in the case of TFs; however, no solid data confirming its role in regulating a seed trait, such as for its rice homolog, have yet been obtained. Therefore, our understanding of wheat grain traits heavily relies on knowledge from rice, and the proposed roles of genes often need validation. For grain trait regulators that are not conserved in rice or have not yet been found in rice, we know little about these regulators in wheat. In addition, as an allopolyploid crop, bread wheat exhibits complex genetic regulation. For example, the effect of *TaGW2-B1* on grain size is significantly greater than that of *TaGW2-D1*, and the grain sizes of double mutants are substantially greater than those of single mutants[34]. The interactions among subgenomes need to be elucidated, especially by utilizing improved genetic transformation and editing technology.

Like for other crops, maintaining a balance in yield components are essential for improving wheat yield. Many seed trait QTLs or genes exhibit intensive genetic trade-offs between grain size/weight and seed number, which substantially impedes the utilization of these genes[117]. Only a few genes, such as *TaGSNE* in wheat and *OsOTUB1*, *OsSGL*, and *OsAGSW1* in rice, have been found to simultaneously increase both grain size and number and hold promise to improve wheat yield[40,118,119]. Recently, overexpression of an α-*expansin* gene specifically in early developing grain led to a significant increase in grain size without a negative effect on grain number, resulting in an increase in yield under field conditions[120]. Similarly, targeted expression of *TaCYP78A5* in integument also enhances grain weight without negative effects on grain number per spike. Therefore, modifying the expression of genes in specific organs, developmental periods, or biogenesis steps may be a feasible way to overcome the trade-off between the grain size/weight and grain number.

Additionally, grain size and weight usually have a negative correlation with grain quality, as indicated by the protein content and end-use quality[4]. Therefore, synergetic improvement of grain yield and end-use quality is also a goal for wheat breeding. In rice, both *OsPPKL1* (*qGL3*/*GL3.1*) and *TGW6* influence grain size but have no significant influence on grain quality[56,62], which are potentially useful for improving grain weight and quality. Additionally, some TFs can regulate the contents of starch and SSPs by binding directly to the promoters of related genes, such as TaNAC019 andNAC-A18[109,111]. Therefore, identifying additional genes that regulate grain size development and SSPs is still an effective way to coordinately improve grain size, weight, and quality.

Author contributions

The authors confirm contribution to the paper as follows: draft manuscript preparation: Wei B, Jiao Y. Both authors approved the final version of the manuscript.

Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Acknowledgments

This work was supported by the Key R&D Program of Shandong Province, China (ZR202211070163); the Shandong Provincial Natural Science Foundation (ZR2021ZD30 and ZR2022ZD22), the National Natural Science Foundation of China (32072061, 31921005 and 32230010), and National Key R&D Program of China (2019YFA0903900 and 2023YFE0101100).

Conflict of interest

The authors declare that they have no conflict of interest. Yuling Jiao is the Editorial Board member of *Seed Biology* who was blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer-review handled independently of this Editorial Board member and the research groups.

Supplementary Information accompanies this paper at (https://www.maxapress.com/article/doi/10.48130/seedbio-0024-0007)

Dates

Received 6 October 2023; Accepted 19 April 2024; Published online 13 May 2024

References

1. Choulet F, Alberti A, Theil S, Glover N, Barbe V, et al. 2014. Structural and functional partitioning of bread wheat chromosome 3B. *Science* 345:1249721
2. Dubcovsky J, Dvorak J. 2007. Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science* 316: 1862–66
3. Gegas VC, Nazari A, Griffiths S, Simmonds J, Fish L, et al. 2010. A genetic framework for grain size and shape variation in wheat. *The Plant Cell* 22:1046–56
4. Evers AD, Cox RI, Shaheedullah MZ, Withey RP. 1990. Predicting milling extraction rate by image analysis of wheat grains. *Aspects of Applied Biology* 25:417–26
5. Xie Q, Mayes S, Sparkes DL. 2015. Carpel size, grain filling, and morphology determine individual grain weight in wheat. *Journal of Experimental Botany* 66:6715–30
6. Shewry PR, Mitchell RAC, Tosi P, Wan Y, Underwood C, et al. 2012. An integrated study of grain development of wheat (cv. Hereward). *Journal of Cereal Science* 56:21–30
7. Xiang D, Quilichini TD, Liu Z, Gao P, Pan Y, et al. 2019. The transcriptional landscape of polyploid wheats and their diploid ancestors during embryogenesis and grain development. *The Plant Cell* 31:2888–911
8. Guo Y, Chen Y, Wang Y, Wu X, Zhang X, et al. 2023. The translational landscape of bread wheat during grain development. *The Plant Cell* 35:1848–67
9. Zhao L, Yang Y, Chen J, Lin X, Zhang H, et al. 2023. Dynamic chromatin regulatory programs during embryogenesis of hexaploid wheat. *Genome Biology* 24:7
10. Hao C, Jiao C, Hou J, Li T, Liu H, et al. 2020. Resequencing of 145 landmark cultivars reveals asymmetric sub-genome selection and strong founder genotype effects on wheat breeding in China. *Molecular Plant* 13:1733–51
11. Wang X, Dong L, Hu J, Pang Y, Hu L, et al. 2019. Dissecting genetic loci affecting grain morphological traits to improve grain weight via nested association mapping. *Theoretical and Applied Genetics* 132:3115–28
12. Jahani M, Mohammadi-Nejad G, Nakhoda B, Rieseberg LH. 2019. Genetic dissection of epistatic and QTL by environment interaction effects in three bread wheat genetic backgrounds for yield-related traits under saline conditions. *Euphytica* 215:103
13. Su Q, Zhang X, Zhang W, Zhang N, Song L, et al. 2018. QTL detection for kernel size and weight in bread wheat (*Triticum aestivum* L.) using a high-density SNP and SSR-based linkage map. *Frontiers in Plant Science* 9:1484
14. Cabral AL, Jordan MC, Larson G, Somers DJ, Humphreys DG, et al. 2018. Relationship between QTL for grain shape, grain weight, test weight, milling yield, and plant height in the spring wheat cross RL4452/'AC Domain'. *PLoS ONE* 13:e0190681
15. Cheng R, Kong Z, Zhang L, Xie Q, Jia H, et al. 2017. Mapping QTLs controlling kernel dimensions in a wheat inter-varietal RIL mapping population. *Theoretical and Applied Genetics* 130: 1405–14
16. Gu L, Wei B, Fan R, Jia X, Wang X, et al. 2015. Development, identification and utilization of introgression lines using Chinese endemic and synthetic wheat as donors. *Journal of Integrative Plant Biology* 57:688–97
17. Gao F, Wen W, Liu J, Rasheed A, Yin G, et al. 2015. Genome-wide linkage mapping of QTL for yield components, plant height and yield-related physiological traits in the Chinese wheat cross zhou 8425B/Chinese spring. *Frontiers in Plant Science* 6:1099
18. Cui F, Zhao C, Ding A, Li J, Wang L, et al. 2014. Construction of an integrative linkage map and QTL mapping of grain yield-related traits using three related wheat RIL populations. *Theoretical and Applied Genetics* 127:659–75
19. Huang XQ, Cöster H, Ganal MW, Röder MS. 2003. Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 106:1379–89
20. Yan Y, Wang ML, Guo YT, Ding CH, Niu KX, et al. 2023. *HSP90.2* promotes CO_2 assimilation rate, grain weight, and yield in wheat. *Plant Biotechnology Journal* 21:1229–39
21. Chen Y, Yan Y, Wu TT, Zhang GL, Yin H, et al. 2020. Cloning of wheat *keto-acyl thiolase 2B* reveals a role of jasmonic acid in grain weight determination. *Nature Communications* 11:6266
22. Gao Y, Li Y, Xia W, Dai M, Dai Y, et al. 2023. The regulation of grain weight in wheat. *Seed Biology* 2:17
23. Xiong M, Feng GN, Gao Q, Zhang CQ, Li QF, et al. 2022. Brassinosteroid regulation in rice seed biology. *Seed Biology* 1:2
24. Li N, Xu R, Li Y. 2019. Molecular networks of seed size control in plants. *Annual Review of Plant Biology* 70:435–63
25. Li W, Yang B. 2017. Translational genomics of grain size regulation in wheat. *Theoretical and Applied Genetics* 130:1765–71
26. Li N, Li Y. 2016. Signaling pathways of seed size control in plants. *Current Opinion in Plant Biology* 33:23–32
27. Batista-Silva W, da Fonseca-Pereira P, Martins AO, Zsögön A, Nunes-Nesi A, et al. 2020. Engineering improved photosynthesis in the era of synthetic biology. *Plant Communications* 1:100032
28. Niu KX, Chang CY, Zhang MQ, Guo YT, Yan Y, et al. 2023. Suppressing *ASPARTIC PROTEASE 1* prolongs photosynthesis and increases wheat grain weight. *Nature Plants* 9:965–77
29. Al-Sheikh Ahmed S, Zhang J, Ma W, Dell B. 2018. Contributions of *TaSUTs* to grain weight in wheat under drought. *Plant Molecular Biology* 98:333–347
30. Gaur A, Jindal Y, Singh V, Tiwari R, Kumar D, et al. 2022. GWAS to identify novel QTNs for WSCs accumulation in wheat peduncle under different water regimes. *Frontiers in Plant Science* 13:825687
31. Miao L, Mao X, Wang J, Liu Z, Zhang B, et al. 2017. Elite haplotypes of a protein kinase gene *TaSnRK2.3* associated with important agronomic traits in common wheat. *Frontiers in Plant Science* 8:368
32. Song XJ, Huang W, Shi M, Zhu MZ, Lin HX. 2007. A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nature Genetics* 39:623–30
33. Hong Y, Chen L, Du LP, Su Z, Wang J, et al. 2014. Transcript suppression of *TaGW2* increased grain width and weight in bread wheat. *Functional & Integrative Genomics* 14:341–49
34. Zhang Y, Li D, Zhang D, Zhao X, Cao X, et al. 2018. Analysis of the functions of *TaGW2* homoeologs in wheat grain weight and protein content traits. *The Plant Journal* 94:857–66
35. Liu H, Li HF, Hao CY, Wang K, Wang YE, et al. 2020. *TaDA1*, a conserved negative regulator of kernel size, has an additive effect with *TaGW2* in common wheat (*Triticum aestivum* L.). *Plant Biotechnology Journal* 18:1330–42
36. Zhang Y, Yang C, Li Y, Zheng N, Chen H, et al. 2007. SDIR1 is a RING finger E3 ligase that positively regulates stress-responsive abscisic acid signaling in *Arabidopsis*. *The Plant Cell* 19:1912–29
37. Wang J, Wang R, Mao X, Zhang J, Liu Y, et al. 2020. RING finger ubiquitin E3 ligase gene *TaSDIR1-4A* contributes to determination of grain size in common wheat. *Journal of Experimental Botany* 71:5377–88

38. Zhang G, Yang J, Zhao X, Li Q, Wu Y, et al. 2021. Wheat TaPUB1 protein mediates ABA response and seed development through ubiquitination. *Plant Science* 309:110913
39. Song L, Liu J, Cao B, Liu B, Zhang X, et al. 2023. Reducing brassinosteroid signalling enhances grain yield in semi-dwarf wheat. *Nature* 617:118–124
40. Huang K, Wang D, Duan P, Zhang B, Xu R, et al. 2017. *WIDE AND THICK GRAIN 1*, which encodes an otubain-like protease with deubiquitination activity, influences grain size and shape in rice. *The Plant Journal* 91:849–60
41. Miura K, Ikeda M, Matsubara A, Song XJ, Ito M, et al. 2010. *OsSPL14* promotes panicle branching and higher grain productivity in rice. *Nature Genetics* 42:545–49
42. Cao J, Liu K, Song W, Zhang J, Yao Y, et al. 2021. Pleiotropic function of the *SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE* gene *TaSPL14* in wheat plant architecture. *Planta* 253:44
43. Cao L, Li T, Geng S, Zhang Y, Pan Y, et al. 2023. *TaSPL14-7A* is a conserved regulator controlling plant architecture and yield traits in common wheat (*Triticum aestivum* L.). *Frontiers in Plant Science* 14:1178624
44. Ashikari M, Wu J, Yano M, Sasaki T, Yoshimura A. 1999. Rice gibberellin-insensitive dwarf mutant gene *Dwarf 1* encodes the α-subunit of GTP-binding protein. *Proceedings of the National Academy of Sciences* 96:10284–89
45. Fujisawa Y, Kato T, Ohki S, Ishikawa A, Kitano H, et al. 1999. Suppression of the heterotrimeric G protein causes abnormal morphology, including dwarfism, in rice. *Proceedings of the National Academy of Sciences* 96:7575–80
46. Utsunomiya Y, Samejima C, Takayanagi Y, Izawa Y, Yoshida T, et al. 2011. Suppression of the rice heterotrimeric G protein β-subunit gene, *RGB1*, causes dwarfism and browning of internodes and lamina joint regions. *The Plant Journal* 67:907–16
47. Fan C, Xing Y, Mao H, Lu T, Han B, et al. 2006. *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theoretical and Applied Genetics* 112:1164–71
48. Li S, Liu Y, Zheng L, Chen L, Li N, et al. 2012. The plant-specific G protein γ subunit AGG3 influences organ size and shape in *Arabidopsis thaliana*. *New Phytologist* 194:690–703
49. Ren X, Zhi L, Liu L, Meng D, Su Q, et al. 2021. Alternative splicing of *TaGS3* differentially regulates grain weight and size in bread wheat. *International Journal of Molecular Sciences* 22:11692
50. Huang X, Qian Q, Liu Z, Sun H, He S, et al. 2009. Natural variation at the *DEP1* locus enhances grain yield in rice. *Nature Genetics* 41:494–97
51. Li A, Hao C, Wang Z, Geng S, Jia M, et al. 2022. Wheat breeding history reveals synergistic selection of pleiotropic genomic sites for plant architecture and grain yield. *Molecular Plant* 15:504–19
52. Kim EJ, Russinova E. 2020. Brassinosteroid signalling. *Current Biology* 30:R294–R298
53. Yoo MJ, Albert VA, Soltis PS, Soltis DE. 2006. Phylogenetic diversification of *glycogen synthase kinase 3/SHAGGY-like kinase* genes in plants. *BMC Plant Biology* 6:3
54. Tong H, Liu L, Jin Y, Du L, Yin Y, et al. 2012. *DWARF AND LOW-TILLERING* acts as a direct downstream target of a GSK3/SHAGGY-Like Kinase to mediate Brassinosteroid responses in rice. *The Plant Cell* 24:2562–77
55. Cheng X, Xin M, Xu R, Chen Z, Cai W, et al. 2020. A single amino acid substitution in STKc_GSK3 kinase conferring semispherical grains and its implications for the origin of *Triticum sphaerococcum*. *The Plant Cell* 32:923–34
56. Zhang X, Wang J, Huang J, Lan H, Wang C, et al. 2012. Rare allele of *OsPPKL1* associated with grain length causes extra-large grain and a significant yield increase in rice. *Proceedings of the National Academy of Sciences* 109:21534–39
57. Qi P, Lin YS, Song XJ, Shen JB, Huang W, et al. 2012. The novel quantitative trait locus *GL3.1* controls rice grain size and yield by regulating Cyclin-T1;3. *Cell Research* 22:1666–80
58. Hu Z, He H, Zhang S, Sun F, Xin X, et al. 2012. A Kelch motif-containing serine/threonine protein phosphatase determines the large grain QTL trait in rice. *Journal of Integrative Plant Biology* 54: 979–90
59. Yang J, Zhou Y, Wu Q, Chen Y, Zhang P, et al. 2019. Molecular characterization of a novel *TaGL3-5A* allele and its association with grain length in wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 132:1799–1814
60. Wang C, Zhang L, Xie Y, Guo X, Zhang Y, et al. 2022. A superior allele of the wheat gene *TaGL3.3-5B*, selected in the breeding process, contributes to seed size and weight. *Theoretical and Applied Genetics* 135:1879–91
61. Xu H, Sun H, Dong J, Ma C, Li J, et al. 2022. The brassinosteroid biosynthesis gene *TaD11-2A* controls grain size and its elite haplotype improves wheat grain yields. *Theoretical and Applied Genetics* 135:2907–23
62. Ishimaru K, Hirotsu N, Madoka Y, Murakami N, Hara N, et al. 2013. Loss of function of the IAA-glucose hydrolase gene *TGW6* enhances rice grain weight and increases yield. *Nature Genetics* 45:707–11
63. Jia M, Li Y, Wang Z, Tao S, Sun G, et al. 2021. TaIAA21 represses TaARF25-mediated expression of *TaERFs* required for grain size and weight development in wheat. *The Plant Journal* 108: 1754–67
64. Guo L, Ma M, Wu L, Zhou M, Li M, et al. 2022. Modified expression of *TaCYP78A5* enhances grain weight with yield potential by accumulating auxin in wheat (*Triticum aestivum* L.). *Plant Biotechnology Journal* 20:168–82
65. Ma M, Wang Q, Li Z, Cheng H, Li Z, et al. 2015. Expression of *TaCYP78A3*, a gene encoding cytochrome P450 CYP78A3 protein in wheat (*Triticum aestivum* L.), affects seed size. *The Plant Journal* 83:312–25
66. Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, et al. 2005. Cytokinin oxidase regulates rice grain production. *Science* 309:741–45
67. Zhang L, Zhao YL, Gao LF, Zhao GY, Zhou RH, et al. 2012. *TaCKX6-D1*, the ortholog of rice *OsCKX2*, is associated with grain weight in hexaploid wheat. *New Phytologist* 195:574–84
68. Lu J, Chang C, Zhang HP, Wang SX, Sun G, et al. 2015. Identification of a novel allele of *TaCKX6a02* associated with grain size, filling rate and weight of common wheat. *PLoS ONE* 10:e0144765
69. Shoaib M, Yang W, Shan Q, Sun L, Wang D, et al. 2020. *TaCKX* gene family, at large, is associated with thousand-grain weight and plant height in common wheat. *Theoretical and Applied Genetics* 133:3151–63
70. Mao H, Jian C, Cheng X, Chen B, Mei F, et al. 2022. The wheat ABA receptor gene *TaPYL1-1B* contributes to drought tolerance and grain yield by increasing water-use efficiency. *Plant Biotechnology Journal* 20:846–61
71. Niaz M, Zhang L, Lv G, Hu H, Yang X, et al. 2023. Identification of *TaGL1-B1* gene controlling grain length through regulation of jasmonic acid in common wheat. *Plant Biotechnology Journal* 21:979–89
72. Wang S, Wu K, Yuan Q, Liu X, Liu Z, et al. 2012. Control of grain size, shape and quality by *OsSPL16* in rice. *Nature Genetics* 44:950–54
73. Yan X, Zhao L, Ren Y, Dong Z, Cui D, et al. 2019. Genome-wide association study revealed that the *TaGW8* gene was associated with kernel size in Chinese bread wheat. *Scientific Reports* 9:2702
74. Liu Y, Chen J, Yin C, Wang Z, Wu H, et al. 2023. A high-resolution genotype–phenotype map identifies the *TaSPL17* controlling grain number and size in wheat. *Genome Biology* 24:196
75. Liu J, Chen Z, Wang Z, Zhang Z, Xie X, et al. 2021. Ectopic expression of *VRT-A2* underlies the origin of *Triticum polonicum* and *Triticum petropavlovskyi* with long outer glumes and grains. *Molecular Plant* 14:1472–88

76. Adamski NM, Simmonds J, Brinton JF, Backhaus AE, Chen Y, et al. 2021. Ectopic expression of *Triticum polonicum VRT-A2* underlies elongated glumes and grains in hexaploid wheat in a dosage-dependent manner. *The Plant Cell* 33:2296–319
77. Ur Rehman S, Wang J, Chang X, Zhang X, Mao X, et al. 2019. A wheat protein kinase gene *TaSnRK2.9-5A* associated with yield contributing traits. *Theoretical and Applied Genetics* 132:907–19
78. Zhang ZG, Lv GD, Li B, Wang JJ, Zhao Y, et al. 2017. Isolation and characterization of the *TaSnRK2.10* gene and its association with agronomic traits in wheat (*Triticum aestivum* L.). *PLOS One* 12:e0174425
79. Kong X, Wang F, Wang Z, Gao X, Geng S, et al. 2023. Grain yield improvement by genome editing of *TaARF12* that decoupled peduncle and rachis development trajectories via differential regulation of gibberellin signalling in wheat. *Plant Biotechnology Journal* 21:1990–2001
80. Ma L, Li T, Hao C, Wang Y, Chen X, et al. 2016. *TaGS5-3A*, a grain size gene selected during wheat improvement for larger kernel and yield. *Plant Biotechnology Journal* 14:1269–80
81. Wang S, Yan X, Wang Y, Liu H, Cui D, et al. 2016. Haplotypes of the *TaGS5-A1* gene are associated with thousand-kernel weight in Chinese bread wheat. *Frontiers in Plant Science* 7:783
82. Hu MJ, Zhang HP, Liu K, Cao JJ, Wang SX, et al. 2016. Cloning and characterization of *TaTGW-7A* gene associated with grain weight in wheat via SLAF-seq-BSA. *Frontiers in Plant Science* 7:1902
83. Sajjad M, Ma X, Habibullah Khan S, Shoaib M, Song Y, et al. 2017. *TaFlo2-A1*, an ortholog of rice *Flo2*, is associated with thousand grain weight in bread wheat (*Triticum aestivum* L.). *BMC Plant Biology* 17:164
84. Milner MJ, Bowden S, Craze M, Wallington EJ. 2021. Ectopic expression of *TaBG1* increases seed size and alters nutritional characteristics of the grain in wheat but does not lead to increased yields. *BMC Plant Biology* 21:524
85. Ma D, Yan J, He Z, Wu L, Xia X. 2012. Characterization of a cell wall invertase gene *TaCwi-A1* on common wheat chromosome 2A and development of functional markers. *Molecular Breeding* 29:43–52
86. Jiang Y, Jiang Q, Hao C, Hou J, Wang L, et al. 2015. A yield-associated gene *TaCWI*, in wheat: its function, selection and evolution in global breeding revealed by haplotype analysis. *Theoretical and Applied Genetics* 128:131–43
87. Hou J, Jiang Q, Hao C, Wang Y, Zhang H, et al. 2014. Global selection on sucrose synthase haplotypes during a century of wheat breeding. *Plant Physiology* 164:1918–29
88. Jiang Q, Hou J, Hao C, Wang L, Ge H, et al. 2011. The wheat (*T. aestivum*) *sucrose synthase 2* gene (*TaSus2*) active in endosperm development is associated with yield traits. *Functional & Integrative Genomics* 11:49–61
89. Liu H, Si X, Wang Z, Cao L, Gao L, et al. 2023. *TaTPP-7A* positively feedback regulates grain filling and wheat grain yield through *T6P-SnRK1* signalling pathway and sugar-ABA interaction. *Plant Biotechnology Journal* 21:1159–75
90. Rose JKC, Lee SJ. 2010. Straying off the highway: trafficking of secreted plant proteins and complexity in the plant cell wall proteome. *Plant Physiology* 153:433–36
91. Aebi M. 2013. N-linked protein glycosylation in the ER. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1833:2430–37
92. Zhu X, Rong W, Wang K, Guo W, Zhou M, et al. 2022. Overexpression of *TaSTT3b-2B* improves resistance to sharp eyespot and increases grain weight in wheat. *Plant Biotechnology Journal* 20:777–93
93. Dale EM, Housley TL. 1986. Sucrose synthase activity in developing wheat endosperms differing in maximum weight. *Plant Physiology* 82:7–10
94. Dickinson DB, Preiss J. 1969. Presence of ADP-glucose pyrophosphorylase in shrunken-2 and brittle-2 mutants of maize endosperm. *Plant Physiology* 44:1058–62
95. Hou J, Li T, Wang Y, Hao C, Liu H, et al. 2017. *ADP-glucose pyrophosphorylase* genes, associated with kernel weight, underwent selection during wheat domestication and breeding. *Plant Biotechnology Journal* 15:1533–43
96. Irshad A, Guo H, Zhang S, Gu J, Zhao L, et al. 2019. EcoTILLING reveals natural allelic variations in starch synthesis key gene *TaSSIV* and its haplotypes associated with higher thousand grain weight. *Genes* 10:307
97. Kirchberger S, Tjaden J, Neuhaus HE. 2008. Characterization of the *Arabidopsis* Brittle1 transport protein and impact of reduced activity on plant metabolism. *The Plant Journal* 56:51–63
98. Wang Y, Hou J, Liu H, Li T, Wang K, et al. 2019. *TaBT1*, affecting starch synthesis and thousand kernel weight, underwent strong selection during wheat improvement. *Journal of Experimental Botany* 70:1497–511
99. Irshad A, Guo H, Ur Rehman S, Wang X, Gu J, et al. 2021. Identification of single nucleotide polymorphism in *TaSBEIII* and development of KASP marker associated with grain weight in wheat. *Frontiers in Genetics* 12:1484
100. Guo X, Fu Y, Lee YRJ, Chern M, Li M, et al. 2022. The PGS1 basic helix-loop-helix protein regulates *Fl3* to impact seed growth and grain yield in cereals. *Plant Biotechnology Journal* 20:1311–26
101. Ma S, Han W, Li L, Zheng X, Wang X. 2019. The thermal stability, structural changeability, and aggregability of glutenin and gliadin proteins induced by wheat bran dietary fiber. *Food & Function* 10:172–79
102. Payne PI. 1987. Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Annual Review of Plant Physiology* 38:141–53
103. Cho K, Beom HR, Jang YR, Altenbach SB, Vensel WH, et al. 2018. Proteomic profiling and epitope analysis of the complex α-, γ-, and ω-gliadin families in a commercial bread wheat. *Frontiers in Plant Science* 9:818
104. Ravel C, Fiquet S, Boudet J, Dardevet M, Vincent J, et al. 2014. Conserved *cis*-regulatory modules in promoters of genes encoding wheat high-molecular-weight glutenin subunits. *Frontiers in Plant Science* 5:621
105. Albani D, Hammond-Kosack MC, Smith C, Conlan S, Colot V, et al. 1997. The wheat transcriptional activator SPA: a seed-specific bZIP protein that recognizes the GCN4-like motif in the bifactorial endosperm box of prolamin genes. *The Plant Cell* 9:171–84
106. Zhu J, Fang L, Yu J, Zhao Y, Chen F, et al. 2018. 5-Azacytidine treatment and *TaPBF-D* over-expression increases glutenin accumulation within the wheat grain by hypomethylating the *Glu-1* promoters. *Theoretical and Applied Genetics* 131:735–46
107. Dong G, Ni Z, Yao Y, Nie X, Sun Q. 2007. Wheat Dof transcription factor WPBF interacts with *TaQM* and activates transcription of an alpha-gliadin gene during wheat seed development. *Plant Molecular Biology* 63:73–84
108. Guo W, Yang H, Liu Y, Gao Y, Ni Z, et al. 2015. The wheat transcription factor TaGAMyb recruits histone acetyltransferase and activates the expression of a high-molecular-weight glutenin subunit gene. *The Plant Journal* 84:347–59
109. Gao Y, An K, Guo W, Chen Y, Zhang R, et al. 2021. The endosperm-specific transcription factor TaNAC019 regulates glutenin and starch accumulation and its elite allele improves wheat grain quality. *The Plant Cell* 33:603–22
110. Li J, Xie L, Tian X, Liu S, Xu D, et al. 2021. *TaNAC100* acts as an integrator of seed protein and starch synthesis exerting pleiotropic effects on agronomic traits in wheat. *The Plant Journal* 108:829–40
111. Wang X, Liu Y, Hao C, Li T, Majeed U, et al. 2023. Wheat *NAC-A18* regulates grain starch and storage proteins synthesis and affects grain weight. *Theoretical and Applied Genetics* 136:123
112. Xie L, Liu S, Zhang Y, Tian W, Xu D, et al. 2023. Efficient proteome-wide identification of transcription factors targeting *Glu-1*: A case

study for functional validation of TaB3-2A1 in wheat. *Plant Biotechnology Journal* 21:1952–65
113. Sun F, Liu X, Wei Q, Liu J, Yang T, et al. 2017. Functional characterization of TaFUSCA3, a B3-superfamily transcription factor gene in the wheat. *Frontiers in Plant Science* 8:1133
114. Li Q, Li L, Yang X, Warburton ML, Bai G, et al. 2010. Relationship, evolutionary fate and function of two maize co-orthologs of rice *GW2* associated with kernel size and weight. *BMC Plant Biology* 10:143
115. Bednarek J, Boulaflous A, Girousse C, Ravel C, Tassy C, et al. 2012. Down-regulation of the *TaGW2* gene by RNA interference results in decreased grain size and weight in wheat. *Journal of Experimental Botany* 63:5945–55
116. Xia T, Li N, Dumenil J, Li J, Kamenski A, et al. 2013. The ubiquitin receptor DA1 interacts with the E3 ubiquitin ligase DA2 to regulate seed and organ size in *Arabidopsis*. *The Plant Cell* 25:3347–59
117. Xie Q, Sparkes DL. 2021. Dissecting the trade-off of grain number and size in wheat. *Planta* 254:3
118. Li T, Jiang J, Zhang S, Shu H, Wang Y, et al. 2015. *OsAGSW1*, an *ABC1*-like kinase gene, is involved in the regulation of grain size and weight in rice. *Journal of Experimental Botany* 66:5691–701
119. Khan N, Zhang Y, Wang J, Li Y, Chen X, et al. 2022. TaGSNE, a WRKY transcription factor, overcomes the trade-off between grain size and grain number in common wheat and is associated with root development. *Journal Experimental Botany* 73:6678–96
120. Calderini DF, Castillo FM, Arenas-M A, Molero G, Reynolds MP, et al. 2021. Overcoming the trade-off between grain weight and number in wheat by the ectopic expression of expansin in developing seeds leads to increased yield potential. *New Phytologist* 230:629–40