Molecular Markers in Marker-Assisted Selection in Bread Wheat

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As one of the essential cereal crops, wheat provides 20% of the calories and proteins consumed by humans. Due to population expansion, dietary shift and climate change, it is challenging for wheat breeders to develop new varieties for meeting wheat production requirements. Marker-assisted selection (MAS) has distinct advantages over conventional selection in plant breeding, such as being time-saving, cost-effective and goal-oriented. Here gives a description of different molecular markers: sequence tagged site (STS), simple sequence repeat (SSR), genotyping by sequencing (GBS), single nucleotide polymorphism (SNP) arrays, exome capture, Kompetitive Allele Specific PCR (KASP), cleaved amplified polymorphic sequence (CAPS), semi-thermal asymmetric reverse PCR (STARP) and genotyping by target sequencing (GBTS).

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1. Molecular Markers

Molecular markers are pivotal components in wheat breeding. Markers, as a sign or flag, are associated with a QTL/gene. Since they are applied for gene mapping and MAS with varying degrees of efficiency, various marker technologies have been developed [1][2][3].

1.1. Sequence-Tagged-Site (STS) Marker

STSs are short and unique sequences that identify the specific loci. A large quantity of sequencing data and functional genes can be used for the development of STS markers. For example, peroxidase (POD) activity is correlated with flour color, processing and product quality. Through sequence blast against the wheat expressed sequence tag (EST) database, six STS markers were developed to characterize POD genes (*TaPod-A2*, *TaPod-A3*, *TaPod-B1* and *TaPod-D1*) [4]. Two complementary dominant functional markers were further designed to distinguish two haplotypes (*TaPod-D1a* and *TaPod-D1b*) with different POD activities [4].

In recent years, many STS markers from ESTs and sequencing datasets were developed in wild relatives, such as *Dasypyrum villosum* [5] and *Agropyron cristatum* [6]. A total of 507 STS markers of *Thinopyrum ponticum* were developed to discriminate chromosome $1J^s$ genetic stocks in wheat backgrounds by specific-locus amplified fragment sequencing (SLAF-seq) [7].

1.2. Simple Sequence Repeats (SSRs) Marker

SSRs are DNA stretches containing a variable number of short and simple sequence repeats. They are ubiquitous in wheat genomes, generally codominant and highly polymorphic, which allows for the direct detection of heterozygosity and makes them the marker of choice for a diversity of purposes, such as gene mapping and tagging [8]. Despite the frequent application of SSRs, there is limited potential in practical crop breeding. SSRs are also finite and distributed unevenly in a certain genome. It is challenging to properly identify precise information in terms of multiple alleles per locus and integrate or compare SSR data from different platforms or populations.

By developing new SSR markers using comparative genomics analysis, powdery mildew resistance gene *Pm52* was placed in a 0.21 cM genetic bin on chromosome arm 2BL in wheat [9]. The two *Pm52*-linked markers, Xicsl234 and Xicscl817, could expedite the mining of *Pm52* in the Liangxing99-derived materials. A total of 192 SSR markers were selected to evaluate 123 wheat stocks for lipoxygenase (LOX) activity, and association analysis found 22 marker loci with a significant correlation with LOX [10]. A novel QTL *QFhb.cau-7DL* for FHB resistance was identified from the wheat line AQ24788-83 (AQ) by genotyping recombinant inbred lines (RILs) using SSR, DArT and SNP markers [11]. This QTL was closely linked to the SSR marker gwm428, which could be used in the selection for *QFhb.cau-7DL* when it has polymorphisms in a given population.

1.3. Single Nucleotide Polymorphism (SNP) Markers

SNPs are the most common type of genetic variation, and are ideal markers for genetic discovery and molecular breeding. Their density varies from one per 370 bp to one per 540 bp in wheat [12]. SNPs are generally regarded as biallelic markers and classified based on nucleotide substitutions, genomic location or phenotype [3].

Next-generation sequencing (NGS) technology thoroughly promotes the development of SNP related platforms for genotyping in an ultra-high-throughput style, substantially improving QTL mapping and gene mining studies and genotyping large populations in a time-saving and cost-saving way, because these platforms can rapidly scan genomes at high-density with a range of multiplex levels and find robust allele calling with high call rates.

- **GBS-SNP**

Advances in NGS have greatly reduced the costs of DNA sequencing, so that genotyping by sequencing (GBS) is feasible for discovering and genotyping SNPs in wheat [13]. GBS is a high-throughput, cost-effective and rapid molecular tool for rating wheat complex genomes whose complexity is reduced through multiple enzyme digestions. GBS-SNPs provide better genome coverage and can be applied to construct high-density linkage maps, compared with conventional marker systems (e.g., SSRs) [14]. In addition, GBS does not depend on prior genome information for constructing genetic linkage maps. There are at least 15 restriction enzymes available and multiple genotyping strategies for GBS in crop plants [14][15]. However, there are some shortcomings. GBS is easily affected by the errors resulting from the low reads coverage and the poor capacity to identify true homozygotes [16]. Furthermore, GBS performance is extremely affected by the quality of the reference genome. The large genome size (16Gb) of hexaploid wheat and three homoeologous genomes also increases the occurrence of genotyping errors [1].

In wheat, GBS has been widely exploited for genomic selection for various traits. Using GBS technology, Hessian fly resistance genes *h4* [17] and *H7* [18] were mapped to chromosomes 1A and 6A, which explained 60.4–70.5% and 60.7–78.3% of the phenotypic variation, respectively. The GBS sequence data (102,147 SNP markers) of 439 elite spring wheat breeding lines were used for genome-wide association study (GWAS), which identified a QTL for FHB resistance on chromosome arm 1AL, explaining 5.3% of the total phenotypic variation [19]. The GWAS using 14,063 polymorphic GBS-SNP markers found eight QTL related with spot blotch disease resistance [20].

- **SNP arrays**

NGS technologies provide a surplus of sequencing data, which further enhance the development of chip-based marker platforms for high-throughput genotyping. Compared with NGS and PCR-based markers, SNP arrays are flexible in the light of sample and data point number customization, which helps its high-density scanning and high and powerful call rates. In wheat, various SNP solid chips are now available, including 15K [21], 35K [22], 55K [23], 90K [24], 660k [25] and 820K [26] in different platforms. Among them, each SNP array has its own advantages and disadvantages. For example, Wheat 820K SNP array were mainly customized by markers from wheat and its relatives [27] and designed for various stocks, such as landraces, synthetic hexaploids and wheat relatives, so that it is improper for breeders to specifically assess hexaploid germplasms. However, most markers of Wheat 660K SNP array were based on hexaploid and tetraploid wheats, emmer wheat and *Aegilops tauschii*, so they have a wide range of possible applications on wheat evaluation due to the genome-specificity, high-density and high-efficiency [28].

GBS-SNPs and chip-based SNPs are usually used together to discover loci of interest traits. Using 90K array and GBS SNPs, eight QTL for stripe rust resistance were identified on chromosomes 1A, 2A, 2B, 4A, 4B, 6B and 7D, and two of them were novel [29]. Flanking markers closely linked to green bug resistance gene *Gb7* and Hessian fly resistance gene *H32* were located on chromosome arms 7DL and 3DL, respectively [30].

1.4. Exome Capture

Given the advances in NGS techniques and reduced sequencing costs, it is now feasible to perform whole genome sequencing (WGS) to identify the genetic variants in a mapping population [31]. Nevertheless, WGS remains expensive in wheat due to its large and complex genome, making it necessary to have adequate coverage. To overcome this limitation, exome capture technology is an alternative solution to cover most gene coding regions [32]. On the other hand, the exome variants are the coding sequence information, which can facilitate the finding of the causative genes. On the contrary, using traditional techniques, forward mapping is a lengthy, multi-step process based on linked markers identification on the target trait in a mapping population and the candidate genes sequencing in a broad genetic region. Using this technology, some useful genes were identified, such as the leaf rust resistance genes (*Lr1*, *Lr10*, *Lr21* and *Lr34*) and novel genes (on chromosomes 1A and 3D) [33], the stripe rust resistance locus *QYr.ucw-1BL* [34] and reduced plant height gene *Rht-B1* [35]. Using bulked segregant exome capture sequencing (BSE-Seq) by combining the exome capture and sequencing of bulked segregant pools, a wheat yellow leaf mutant gene *ygl1* was quickly mapped [36].

1.5. SNP-Converted Markers

SNPs have shown many benefits, including high quantity, high density, high genetic stability and easy automatic detection. Thus, NGS technologies (GBS and exome capture) and SNP arrays have been rapidly replacing traditional markers and are now widely applied in genetic studies and molecular breeding. The linked SNP markers are usually converted to fluorescence-based or gel-based markers, including KASP, CAPS and STARP described below, before they are further verified in wheat populations and finely mapped using haplotype analysis and MAS.

- **KASP**

Based on both PCR and fluorescence detection, Kompetitive Allele Specific PCR (KASP) detection technology meets both low- and high-throughput genotyping requirements. For KASP assays, the two forward primers have the 5′ tags carrying the standard FAM (5’-GAAGGTGACCAAGTTCATGCT-3’) or HEX (5’-GAAGGTCGGAGTCAACGGATT-3’) fluorescence when amplified in KASP master mix. A common genome-specific reverse primer is also needed, and the total amplicon length usually ranges from 50 to 125 bp. The PCR results are read with a microplate reader and visualized using an SNP allele calling software, such as KlusterCaller (LGC Genomics, Middlesex, UK). The genotypes in each cluster are analyzed to reveal their association with phenotypic data. Currently, the vast majority of studies choose KASP technologies to convert linked SNP markers [37].

- **CAPS**

Cleaved amplified polymorphic sequence (CAPS) technology combines PCR amplification and restriction enzyme treatment. A CAPS marker usually amplifies the same sized bands for the two alleles. If polymorphism exists in the restriction site near the SNP loci of one allele, the restriction enzyme digestion cleaves one amplified sequence and make different fragments in length, which can be easily analyzed by gel electrophoresis. However, in some cases, the SNP between alleles does not generate any polymorphic restriction site. This kind of SNP could be utilized for the development of ‘derived CAPS’ (dCAPS, [38]).

- **STARP**

Semi-thermal asymmetric reverse PCR (STARP) is another innovative way to perform flexible SNP genotyping depending on a similar competitive PCR reaction without requiring the third-party KASP master mix [39]. In this method, genotyping assay is conducted under unique PCR conditions using two universal priming element-adjustable primers (PEA-primers) [also called asymmetrically modified allele-specific primers (AMAS-primers)] and their common reverse primer. The two PEA-primers each have one base substituted at the third or fourth base of their 3′ regions to greatly increase the amplification specificity of the two alleles. In addition, a 4-10 bp insertion tailed at the 5′ end of one AMAS primer comes into the final PCR products between two genotypes, allowing the discrimination of allelic variants in traditional gel electrophoresis [40]. Wu et al. [40] developed 56 gene-specific STARP markers for 46 genes for wheat quality, tolerance to biotic and abiotic stresses, grain yield and adaptation-related traits, which provided a powerful and reliable marker toolkit for wheat breeding programs.

1.6. Genotyping by Target Sequencing (GBTS)

Molecular detection technologies have been profoundly innovated, from gel-based and fluorescence-based to solid chip-based and now liquid chip-based, as well as possibly automated genotyping platforms in the future. Solid-based SNP arrays from different platforms have been playing a vital role in gene discovery for genotyping large populations in wheat, but some shortcomings of their applications in crop improvement exists, such as low customization efficiency, little flexibility, expensive equipment and high cost [41]. On the contrary, GBTS can compensate for their limits [42][43]. GBTS is mainly divided into multiplex PCR-based target sequencing (GenoPlexs) and probe-in-solution-based target sequencing (GenoBaits). GBTS can accurately capture sequences at random positions and lengths, except for the areas with highly repeated sequences. In addition, it is upgradable so that newly mapped important loci can be joined into an existing marker panel without resynthesis. Using these two techniques, a set of marker panels can be developed to meet almost all the requirements of marker applications in the fields. In wheat, a few studies have begun to apply GBTS in genetic studies [44][45]. GBTS will potentially be used in wheat improvement on a large scale in the future.

2. Loci and Markers of Potential Applications in MAS

Many QTL/genes of interest and their tightly linked markers or functional markers (FMs) from linkage analysis, association analysis, gene cloning and sequencing have been reported in wheat [46][47][48]. More than 200 QTL underpinning yield-related traits have been documented in different wheat genotypes in last decade [49]. Around 150 FMs for important genes have been reported to select desirable characteristics in wheat [1]; 97 FMs that identify 93 alleles at 30 loci in wheat have been documented, including 56, 27 and 14 FMs for processing quality, agronomic and disease resistance traits, respectively [50]. These markers are of potential value in MAS for improving wheat agronomic traits, such as biotic stresses, yield-related traits and quality characters. Numerous loci for agronomic traits have been reported in recent years.

2.1. Loci for Resistance to Biotic Stresses

Wheat is exposed to many biotic threats, such as fungi, viruses and insects, which heavily affect its yield and the quality. On average, yield loss in wheat production is about 21.5% due to pathogens and pests [51]. Plant pathogens are ever-evolving at a high pace through mutations or recombinations. FHB, powdery mildew and rusts are the three main menaces; therefore, it is of vital significance to find novel genes against them.

FHB is a serious disease in vast wheat-growing areas of the globe where rainfall frequently occurs during flowering time and leads to heavy reductions in wheat yield worldwide. A total of 50 QTL, controlling different types of FHB resistance, have been reported with unique chromosome locations [52][53]. Among them, only seven QTL have been officially named, from *Fhb1* on 3B [54] to *Fhb7* on 7D [55], which have been described in detail [53]. *Fhb1* from cultivar Sumai3 is the most frequent selection of FHB resistance in many breeding programs [56]. Two functional markers (TaHRC-GSM and TaHRC-KASP) for *Fhb1* were developed based on the critical sequence deletion of the putative histidine-rich calcium-binding protein (*TaHRC*) and validated to be diagnostic in different types of

populations [57]. Recently, a novel major QTL for FHB resistance (*QFhb-2DL)* was identified on chromosome 2D (Type II) in a Chinese wheat cultivar Ji5265, which can explain ~30% of the phenotypic variation for FHB resistance [58]. Two linked KASP markers (KASP10238 and KASP12056) were proved to be diagnostic in 2065 wheat accessions.

To date, more than 69 powdery mildew resistance loci (*Pm1-Pm69*) have been catalogued in wheat [59]. Among these named genes, some new alleles were found and more information is available for better utilization. For example, a previously uncategorized *Pm60* allele was discovered from *T*. *urartu* and two molecular markers (M-Pm60-S1 and M-Pm60-S2) were developed to distinguish the functional *Pm60a* allele from the non-functional *Pm60a'* allele, which were helpful for precisely identifying the *Pm60* allele [60]. Furthermore, many new loci were identified, including MIIW39 [61], *PmKN0816* [62] and *Qpm-3BL* [63]. A new adult plant resistance (APR) gene, *QPm.caas-3BS*, to powdery mildew pathogen was delimited from the RILs derived from the cross Zhou8425B/Chinese Spring. One linked SNP STARP marker was developed and validated on 103 wheat cultivars, showing that *QPm.caas-3BS* could reduce maximum disease severity by 5.3% [24].

Up to now, 82 leaf rust (*Lr*) resistance genes, 84 stripe rust (*Yr*) resistance genes and 63 stem rust (*Sr*) resistance genes have been permanently cataloged in wheat [59]. In addition to them, most genes can be chosen for improving wheat rust resistance, such as *Lr22a* [64], *Lr80* [65], *YrAS2388* [66] and *Sr13* [67]. Some genes have not been extensively used in wheat cultivars, such as the broadly effective resistance gene *Lr22a* [64]. Three linked KASP markers (Kwh636, Kwh637 and Kwh638) were developed to reliably detect the presence or absence of *Lr22a*, which can facilitate *Lr22a* selection in MAS [64]. Some genes confer APR, such as *QLr.cau-2BL* [68] from wheat landrace Hongmazha, while *QLr-2BS* is a valuable all-stage resistance gene [69]. Their linked SSR or KASP markers were developed and verified in genetic populations and were potentially useful for introducing them into commercial wheat cultivars. A new APR to stripe rust loci, *QYr.AYH-5BL* from Chinese wheat landrace Anyuehong (AYH), was stably detected in all environments and could explain 13.6–21.4% of the phenotypic variation [69]. Its linked KASP markers have potential value for MAS to improve stripe rust resistance in breeding programs. One KASP and several STARP markers were developed to identify *Sr13* haplotypes [67]. Both KASPSr13 and rwgsnp37 were robust markers for *Sr13* and could be used by geneticists and breeders. STARP markers rwgsnp38, rwgsnp39 and rwgsnp40 could be ideally used to discriminate four haplotypes.

2.2. Loci for Resistance to Abiotic Stresses

In addition to biotic threatens, wheat growth and development are also badly impacted by various abiotic stresses. About 50% crop losses are, on average, caused by abiotic factors, including drought (9%), heat (20%), cold (7%) and other forms of stresses [37].

Improving water absorption capacity is a good means to improve drought tolerance in crops. Roots are in charge of the uptake of water from soil; strong root architecture is beneficial for crops to absorb water stored in soil and avoid drought stress [70]. There are numerous QTL/genes related to root traits as well as their reported linked markers [25][70][71][72]. TaWRKY51 is a positive regulator contributing to the root system and grain yield (GY). Hap-2A-I is a

favorable haplotype for large spike, and Hap-2B-II and allele-G are elite haplotypes/alleles for long root. Their functional markers have been developed for the utilization in the MAS [73]. In addition to root-related genes, other markers for drought tolerance genes have been reported. Sixteen functional KASP markers for 16 alleles related to drought tolerance were used to assess 153 Pakistani wheat cultivars released from 1953 to 2016 [74]. Favored haplotypes of five genes were unconsciously pyramided and selected during selection breeding, while six genes had lower frequencies in favorable haplotypes among those stocks.

Winter hardiness is also a crucial breeding goal, since it is vital for wheat to adapt to harsh winter conditions. *Fr-A2* is a major QTL for frost resistance on chromosome 5A, and its polymorphisms contribute to the variation in winter hardiness [75]. Two KASP markers (S1862541 and S1298957) could differentiate two haplotypes of *Fr-A2*. Among 11 cold tolerance QTL found by genotyping a panel of 768 wheat germplasms using GBS, two significantly associated SNPs of *qCT5A.3* were converted into KASP markers and were validated successfully in a F_2 population [76].

Lodging is also an important concern for reducing wheat yield and grain quality. Caffeic acid 3-O-methyltransferase (COMT) is a key enzyme involved in lignin biosynthesis contributing to lodging resistance and has two allelic variants [77]. A codominant Indel marker was developed to validate the association between allelic patterns and stem lignin content, showing that *TaCOMT-3B*a was the excellent haplotype.

Additionally, wheat yield is seriously influenced by extremely hot weather, terminal heat stress in particular. It affects approximately 40% of the wheat-cultivating regions of the world. Sihag et al. [78] identified two microRNA (miRNA)-based SSR markers (miR159c and miR165b), which showed specific alleles and discriminated terminal heat-tolerant genotypes from the susceptible genotypes.

2.3. Loci for Yield-Related Traits

Agronomic traits such as plant height (PH), heading date (HD), spike length (SL) and thousand kernel weight (TKW) are critical factors affecting wheat yield. Many genes were cloned and their haplotypes were analyzed, such as vernalization genes *Vrn1* [79], *Vrn2/ZCCT1* [80] and *Vrn3* [81]; and PH-related genes *Rht-B1b*, *Rht-D1b* [82], *Rht8* and *Rht24* [83]. These genes have been widely utilized in wheat breeding.

As a polygenic trait, TKW is the most stably inherited determinant of yield potential, exhibiting higher heritability values than overall yield and yield components. Many genes controlling grain weight as well as their haplotypes were analyzed, such as *TaAGP* [84], *TaSus1* [85], *TaSDIR1* [86], *TaDA1* [87] and *KAT-2B* [88]. Fourteen distinct haplotypes of six *TaCKX* genes were explored by single-molecule real-time (SMRT) sequencing and seven functional markers were then developed (four Indel and three CAPS markers). Among them, six specific haplotypes were significantly correlated with high TKW and short plant height (PH) [89]. TaTAP46-5A (Type 2A-phosphatase-associated protein) has been proven to influence kernel size and TKW in transgenic wheat, which was rarely selected (less than 1%) in conventional breeding according to phenotype and experience [90]. A KASP functional marker could be used to select it to improve breeding efficiency. *TaSDIR1-4A*, a Salt- and Drought-Induced RING

Finger 1 (SDIR1) member, was found to affect TKW [91]. A dCAPS marker can be used to differentiate good haplotypes from two genotypes.

In addition, a series of QTL for grain weight were finely mapped, including *QTKW.caas-4BS* [92], *QTKW.caas-5DL* [93], *QTgw.caas-5B* [94] and *QYld.aww-1B.2* [95]. Duan et al. [86] dissected a major and stable QTL, *QGW4B.4-17*, for TKW with an increase of 2.19–3.06g and high phenotypic variation explained (PVE) of 22.5–36.3%. The corresponding CAPS marker was developed and verified in 205 wheat cultivars and showed a highly significant correlation with TKW.

Many QTL/genes have pleiotropic effects on multiple traits. Two QTL clusters (*QSc/Sl.cib-5A* and *QSc/Sl.cib-6A*) have pleiotropic effects on plant height, TKW and grain length; their related KASP markers might be potentially applicable in wheat breeding [96]. The *FT-D1* gene controls spikelet number and heading date, and a robust STARP marker was reported to simplify and streamline MAS for this gene in wheat breeding [97]. A pleiotropic gene *TaCol-B5* can not only increase the number of spikelet nodes per spike, but can also produce more tillers and spikes, thereby improving the yield of transgenic wheat under field conditions. It was found in only 33 of 1657 stocks from a global collection of wheat germplasms. A diagnostic marker identifying the SNP involving the Ser269/Gly269 substitution was developed to plausibly accelerate this rare allele in a variety of genetic backgrounds [98].

2.4. Loci for Grain Quality

Wheat grain protein content (GPC) is a major end-use quality. Wheat quality can be improved by the manipulation of the main storage protein genes, many of which have been efficiently utilized for wheat quality improvement, such as *GluD1* (5 + 10) [99] and *GluB1* (17 + 18) [100]. A robust and reliable KASP marker toolkit was reported for HMW-GS at *Glu-A1*, *Glu-B1* and *Glu-D1* loci; polyphenol oxidase (PPO) activity genes (*Ppo-A1* and *Ppo-D1*) [101], which can distantly facilitate the selection and stacking of excellent genes in wheat breeding programs. Jiang et al. [98] successfully developed three KASP markers (Kgpc-2B, Kgpc-2D and Kgpc-4A) associated with GPC, which were applied to screen 15 lines with high GPC from 164 F_6 breeding lines, indicating their high selective efficiency.

Breeding wheat with a weak and extensible gluten feature is another aspect aiming to improve biscuit making quality. Nap Hal is an Indian wheat landrace with weak gluten because of *Glu-D1* double null [102]. The codominant marker (gwm642) tightly linked with *Glu-D1* double null could be applied to transferring it into high yielding backgrounds.

Pre-harvest sprouting is another aspect to ultimately affect the end product quality. Three novel QTL for pre-harvest sprouting tolerance (PHST) were detected based on the phenotypes of 192 wheat varieties (lines) and the corresponding genotypes [103]. SNP markers from 90K SNP array were tightly linked with these major QTL (*Qphs.ahau-1A*, *Qphs.ahau-3B* and *Qphs.ahau-6B*) and further converted to two CAPS and one dCAPS markers. The CAPS marker EX06323 for *Qphs.ahau-6B* co-segregated with a novel major QTL underlying PHST in a RIL population raised from the cross Jing 411/Wanxianbaimaizi. In addition, the allele EX06323-G revealed a highly significant correlation with all PHST traits in 374 wheat varieties.

Grain micronutrient content is also an important trait for the nutritional quality improvement for achieving biofortification. QTL and related markers for improving Fe or Zn contents in wheat were identified [104]. Among the many QTL/genes for Fe, Zn and Se contents in another review, two key QTL deserve attention, *QGZn.cimmyt-7B_1P2* and *QGFe.cimmyt-4A_P2*, which explained the largest PVE of 32.7% for Zn and 21.14% for Fe, respectively [105].

References

1. Rasheed, A.; Xia, X. From markers to genome-based breeding in wheat. Theor. Appl. Genet. 2019, 132, 767–784.
2. Paux, E.; Sourdille, P.; Mackay, I.; Feuillet, C. Sequence-based marker development in wheat: Advances and applications to breeding. Biotechnol. Adv. 2012, 30, 1071–1088.
3. Pandurangan, S.W.C.; Nilsen, K.; Kumar, S. Introduction to Marker-Assisted Selection in Wheat Breeding. In Accelerated Breeding of Cereal Crops; Springer Protocols Handbooks; Bilichak, A., Laurie, J.D., Eds.; Humana: New York, NY, USA, 2022.
4. Geng, H.; Shi, J.; Fuerst, E.P.; Wei, J.; Morris, C.F. Physical mapping of Peroxidase genes and development of functional markers for TaPod-D1 on bread wheat chromosome 7D. Front. Plant Sci. 2019, 10, 523.
5. Zhang, R.; Yao, R.; Sun, D.; Sun, B.; Feng, Y.; Zhang, W.; Zhang, M. Development of V chromosome alterations and physical mapping of molecular markers specific to Dasypyrum villosum. Mol. Breed. 2017, 37, 67.
6. Zhang, J.; Liu, W.; Lu, Y.; Liu, Q.; Yang, X.; Li, X.; Li, L. A resource of large-scale molecular markers for monitoring Agropyron cristatum chromatin introgression in wheat background based on transcriptome sequences. Sci. Rep. 2017, 7, 11942.
7. Wang, Y.; Cao, Q.; Zhang, J.; Wang, S.; Chen, C.; Wang, C.; Zhang, H.; Wang, Y.; Ji, W. Cytogenetic analysis and molecular marker development for a new wheat–Thinopyrum ponticum 1Js (1D) disomic substitution line with resistance to stripe rust and powdery mildew. Front. Plant Sci. 2020, 11, 1282.
8. Gupta, P.K.; Varshney, R.K. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 2000, 113, 163–185.
9. Wu, P.; Hu, J.; Zou, J.; Qiu, D.; Qu, Y.; Li, Y.; Li, T.; Zhang, H.; Yang, L.; Liu, H.; et al. Fine mapping of the wheat powdery mildew resistance gene Pm52 using comparative genomics analysis and the Chinese Spring reference genomic sequence. Theor. Appl. Genet. 2019, 132, 1451–1461.

10. Zheng, W.Y.; Zhao, L.; Li, Y.M.; Li, J.; Zhu, Z.H.; Yao, D.N. Association analysis between Lipoxygenase activity and SSR markers in wheat grains. Cereal Res. Commun. 2021, 50, 297–303.
11. Ren, J.; Wang, Z.; Du, Z.; Che, M.; Zhang, Y.; Quan, W.; Wang, Y.; Jiang, X.; Zhang, Z. Detection and validation of a novel major QTL for resistance to Fusarium head blight from Triticum aestivum in the terminal region of chromosome 7DL. Theor. Appl. Genet. 2019, 132, 241–255.
12. Khan, M.K.; Pandey, A.; Choudhary, S.; Hakki, E.E.; Akkaya, M.S.; Thomas, G. From RFLP to DArT: Molecular tools for wheat (Triticum spp.) diversity analysis. Genet. Resour. Crop Ev. 2014, 61, 1001–1032.
13. Lehnert, H.; Berner, T.; Lang, D.; Beier, S.; Stein, N.; Himmelbach, A.; Kilian, B.; Keilwagen, J. Insights into breeding history, hotspot regions of selection, and untapped allelic diversity for bread wheat breeding. Plant J. 2022, 112, 897–918.
14. Rajendran, N.R.; Qureshi, N.; Pourkheirandish, N. Genotyping by Sequencing Advancements in Barley. Front. Plant Sci. 2022, 13, 931423.
15. Wang, N.; Yuan, Y.; Wang, H.; Yu, D.; Liu, Y.; Zhang, A.; Gowda, M.; Nair, S.K.; Hao, Z.; Lu, Y.; et al. Applications of genotyping-by-sequencing (GBS) in maize genetics and breeding. Sci. Rep. 2020, 10, 16308.
16. Reyes, V.P.; Kitonv, J.K.; Nishiuchi, S.; Makihara, D.; Doi, K. Utilization of Genotyping-by-Sequencing (GBS) for Rice Pre-Breeding and Improvement: A Review. Life 2022, 12, 1752.
17. Niu, F.; Xu, Y.; Liu, X.; Zhao, L.; Bernardo, A.; Li, Y.; Liu, G.; Chen, M.S.; Cao, L.; Hu, Z.; et al. The Hessian fly recessive resistance gene h4 mapped to chromosome 1A of the wheat cultivar ‘Java’ using genotyping-by-sequencing. Theor. Appl. Genet. 2020, 133, 2927–2935.
18. Liu, G.; Liu, X.; Xu, Y.; Bernardo, A.; Chen, M.; Li, Y.; Niu, F.; Zhao, L.; Bai, G. Reassigning Hessian fly resistance genes H7 and H8 to chromosomes 6A and 2B of the wheat cultivar ‘Seneca’ using genotyping-by-sequencing. Crop Sci. 2020, 60, 1488–1498.
19. Liu, Y.; Salsman, E.; Fiedler, J.D.; Hegstad, J.B.; Green, A.; Mergoum, M.; Zhong, S.; Li, X. Genetic mapping and prediction analysis of FHB resistance in a hard red spring wheat breeding population. Front. Plant Sci. 2019, 10, 1007.
20. Tomar, V.; Singh, D.; Dhillon, G.S.; Singh, R.P.; Poland, J.; Joshi, A.K.; Singh, P.K.; Bhati, P.K.; Kumar, S.; Rahman, M.; et al. New QTLs for spot blotch disease resistance in wheat (Triticum aestivum L.) using genome-wide association mapping. Front. Genet. 2021, 11, 613217.
21. Chen, S.; Zhang, Z.; Sun, Y.; Li, D.; Gao, D.; Zhan, K.; Cheng, S. Identification of quantitative trait loci for Fusarium head blight (FHB) resistance in the cross between wheat landrace N553 and elite cultivar Yangmai 13. Mol. Breed. 2021, 41, 24.

22. Mu, J.; Huang, S.; Liu, S.; Zeng, Q.; Dai, M.; Wang, Q.; Wu, J.; Yu, S.; Kang, Z.; Han, D. Genetic architecture of wheat stripe rust resistance revealed by combining QTL mapping using SNP-based genetic maps and bulked segregant analysis. Theor. Appl. Genet. 2019, 132, 443–455.
23. Xiong, H.; Li, Y.; Guo, H.; Xie, Y.; Zhao, L.; Gu, J.; Zhao, S.; Ding, Y.; Liu, L. Genetic mapping by integration of 55K SNP array and KASP markers reveals candidate genes for important agronomic traits in hexaploid wheat. Front. Plant Sci. 2021, 12, 628478.
24. Jia, A.; Ren, Y.; Gao, F.; Yin, G.; Liu, J.; Guo, L.; Zheng, J.; He, Z.; Xia, X. Mapping and validation of a new QTL for adult-plant resistance to powdery mildew in Chinese elite bread wheat line Zhou8425B. Theor. Appl. Genet. 2018, 131, 1063–1071.
25. Fan, X.; Zhang, W.; Zhang, N.; Chen, M.; Zheng, S.; Zhao, C.; Han, J.; Liu, J.; Zhang, X.; Song, L.; et al. Identification of QTL regions for seedling root traits and their effect on nitrogen use efficiency in wheat (Triticum aestivum L.). Theor. Appl. Genet. 2018, 131, 2677–2698.
26. Winfield, M.O.; Allen, A.M.; Burridge, A.J.; Barker, G.L.A.; Benbow, H.R.; Wilkinson, P.A.; Coghill, J.; Waterfall, C.; Davassi, A.; Scopes, G.; et al. High-density SNP genotyping array for hexaploid wheat and its secondary and tertiary gene pool. Plant Biotechnol. J. 2016, 14, 1195–1206.
27. Allen, A.M.; Winfield, M.O.; Burridge, A.J.; Downie, R.C.; Benbow, H.R.; Barker, G.L.A.; Wilkinson, P.A.; Coghill, J.; Waterfall, C.; Davassi, A.; et al. Characterization of a wheat breeders' array suitable for high-throughput SNP genotyping of global accessions of hexaploid bread wheat (Triticum aestivum). Plant Biotechnol. J. 2017, 15, 390–401.
28. Sun, C.; Dong, Z.; Zhao, L.; Ren, Y.; Zhang, N.; Chen, F. The Wheat 660K SNP array demonstrates great potential for marker-assisted selection in polyploid wheat. Plant Biotechnol. J. 2020, 18, 1354–1360.
29. Yang, Y.; Basnet, B.R.; Ibrahim, A.M.H.; Rudd, J.C.; Chen, X.; Bowden, R.L.; Xue, Q.; Wang, S.; Johnson, C.D.; Metz, R.; et al. Developing KASP markers on a major stripe rust resistance QTL in a popular wheat TAM 111 using 90K array and genotyping-by-sequencing SNPs. Crop Sci. 2019, 59, 165–175.
30. Tan, C.; Yu, H.; Yang, Y.; Xu, X.; Chen, M.; Rudd, J.; Xue, Q.; Ibrahim, A.M.; Garza, L.; Wang, S.; et al. Development and validation of KASP markers for the greenbug resistance gene Gb7 and the Hessian fly resistance gene H32 in wheat. Theor. Appl. Genet. 2017, 130, 1867–1884.
31. Schneeberger, K. Using next-generation sequencing to isolate mutant genes from forward genetic screens. Nat. Rev. Genet. 2014, 15, 662–676.
32. He, F.; Pasam, R.; Shi, F.; Kant, S.; Keeble-Gagnere, G.; Kay, P.; Forrest, K.; Fritz, A.; Hucl, P.; Wiebe, K.; et al. Exome sequencing highlights the role of wild-relative introgression in shaping the adaptive landscape of the wheat genome. Nat. Genet. 2019, 51, 896–904.

33. Liu, F.; Zhao, Y.; Beier, S.; Jiang, Y.; Thorwarth, P.H.; Longin, C.F.; Ganal, M.; Himmelbach, A.; Reif, J.C.; Schulthess, A.W. Exome association analysis sheds light onto leaf rust (Puccinia triticina) resistance genes currently used in wheat breeding (Triticum aestivum L.). Plant Biotechnol. J. 2020, 18, 1396–1408.
34. Cobo, N.; Wanjugi, H.; Lagudah, E.; Dubcovsky, J. A high-resolution map of wheat QYr.ucw-1BL, an adult plant stripe rust resistance locus in the same chromosomal region as Yr29. Plant Genome. 2019, 12, 180055.
35. Mo, Y.; Howell, T.; Vasquez-Gross, H.; de Haro, L.A.; Dubcovsky, J.; Pearce, S. Mapping causal mutations by exome sequencing in a wheat TILLING population: A tall mutant case study. Mol. Genet. Genom. 2018, 293, 463–477.
36. Dong, C.; Zhang, L.; Chen, Z.; Xia, C.; Gu, Y.; Wang, J.; Li, D.; Xie, Z.; Zhang, Q.; Zhang, X.; et al. Combining a new exome capture panel with an effective varBScore Algorithm accelerates BSA-based gene cloning in wheat. Front. Plant Sci. 2020, 11, 1249.
37. Kaur, B.; Mavi, G.S.; Gill, M.S.; Saini, D.K. Utilization of KASP technology for wheat improvement. Cereal Res. Commun. 2020, 48, 409–421.
38. Hrubá, M. dCAPS method: Advantages, troubles and solution. Plant Soil Environ. 2007, 53, 417–420.
39. Long, Y.M.; Chao, W.S.; Ma, G.J.; Xu, S.S.; Qi, L.L. An innovative SNP genotyping method adapting to multiple platforms and throughputs. Theor. Appl. Genet. 2017, 130, 597–607.
40. Wu, Y.; Li, M.; He, Z.; Dreisigacker, S.; Wen, W.; Jin, H.; Zhai, S.; Li, F.; Gao, F.; Liu, J.; et al. Development and validation of high-throughput and low-cost STARP assays for genes underpinning economically important traits in wheat. Theor. Appl. Genet. 2020, 133, 2431–2450.
41. Rasheed, A.; Hao, Y.; Xia, X.; Khan, A.; Xu, Y.; Varshney, R.K.; He, Z. Crop breeding chips and genotyping platforms: Progress, challenges, and perspectives. Mol. Plant 2017, 10, 1047–1064.
42. Guo, Z.; Wang, H.; Tao, J.; Ren, Y.; Xu, C.; Wu, K.; Zou, C.; Zhang, J.; Xu, Y. Development of multiple SNP marker panels affordable to breeders through genotyping by target sequencing (GBTS) in maize. Mol. Breed. 2019, 39, 37.
43. Xu, Y.; Yang, Q.; Zheng, H.; Xu, Y.; Sang, Z.; Guo, Z.; Peng, H.; Zhang, C.; Lan, H.; Wang, Y.; et al. Genotyping by target sequencing (GBTS) and its applications. Sci. Agric. Sin. 2020, 53, 15.
44. Hou, J.; Liu, Y.; Hao, C.; Li, T.; Liu, H.; Zhang, X. Starch metabolism in wheat: Gene variation and association analysis reveal additive effects on kernel weight. Front Plant. Sci. 2020, 11, 562008.
45. Qiao, L.; Li, H.; Wang, J.; Zhao, J.; Zheng, X.; Wu, B.; Du, W.; Wang, J.; Zheng, J. Analysis of genetic regions related to field grain number per spike from Chinese wheat founder parent Linfen 5064. Front Plant. Sci. 2022, 12, 808136.

46. Liu, S.; Banik, M.; Yu, K.; Park, S.J.; Poysa, V.; Guan, Y. Marker-assisted selection (MAS) in major cereal and legume crop breeding: Current progress and future directions. Int. J. Plant Breed. 2007, 1, 74–88.
47. Arabzai, M.G.; Gul, H. Application techniques of molecular marker and achievement of marker assisted selection (MAS) in three major crops rice, wheat and maize. Int. J. Appl. Sci. Biotechnol. 2021, 8, 82–93.
48. Gupta, P.K.; Langridge, P.; Mir, R.R. Marker-assisted wheat breeding: Present status and future possibilities. Mol. Breed. 2010, 26, 145–161.
49. Cao, S.; Xu, D.; Hanif, M.; Xia, X.; He, Z. Genetic architecture underpinning yield component traits in wheat. Theor. Appl. Genet. 2020, 133, 1811–1823.
50. Liu, Y.; He, Z.; Appels, R.; Xia, X. Functional markers in wheat: Current status and future prospects. Theor. Appl. Genet. 2012, 125, 1–10.
51. Savary, S.; Willocquet, L.; Pethybridge, S.J.; Esker, P.; McRoberts, N.; Nelson, A. The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 2019, 3, 430–439.
52. Liu, S.; Hall, M.D.; Griffey, C.A.; McKendry, A.L. Meta-Analysis of QTL Associated with Fusarium Head Blight Resistance in Wheat. Crop Sci. 2009, 49, 1955–1968.
53. Bai, G.; Su, Z.; Cai, J. Wheat resistance to Fusarium head blight. Can. J. Plant Pathol. 2018, 40, 336–346.
54. Cuthbert, P.A.; Somers, D.J.; Thomas, J.; Cloutier, S.; Brulé-Babel, A. Fine mapping Fhb1, a major gene controlling fusarium head blight resistance in bread wheat (Triticum aestivum L.). Theor. Appl. Genet. 2006, 112, 1465.
55. Guo, J.; Zhang, X.; Hou, Y.; Cai, J.; Shen, X.; Zhou, T.; Xu, H.; Ohm, H.W.; Wang, H.; Li, A.; et al. High-density mapping of the major FHB resistance gene Fhb7 derived from Thinopyrum ponticum and its pyramiding with Fhb1 by marker-assisted selection. Theor. Appl. Genet. 2015, 128, 2301–2316.
56. Hao, Y.; Rasheed, A.; Zhu, Z.; Wulff, B.B.H.; He, Z. Harnessing wheat Fhb1 for Fusarium resistance. Trends Plant Sci. 2020, 25, 1–3.
57. Su, Z.; Jin, S.; Zhang, D.; Bai, G. Development and validation of diagnostic markers for Fhb1 region, a major QTL for Fusarium head blight resistance in wheat. Theor. Appl. Genet. 2018, 131, 2371–2380.
58. Li, H.; Zhang, F.; Zhao, J.; Bai, G.; Amand, P.S.; Bernardo, A.; Ni, Z.; Sun, Q.; Su, Z. Identification of a novel major QTL from Chinese wheat cultivar Ji5265 for Fusarium head blight resistance in greenhouse. Theor. Appl. Genet. 2022, 135, 1867–1877.

59. McIntosh, R.A.; Dubcovsky, J.; Rogers, W.J.; Xia, X.C.; Raupp, W.J.V. Catalogue of gene symbols for wheat. Annu. Wheat Newsl. 2022, 68, 68–81.
60. Zhao, F.; Li, Y.; Yang, B.; Yuan, H.; Jin, C.; Zhou, L.; Pei, H.; Zhao, L.; Li, Y.; Zhou, Y.; et al. Powdery mildew disease resistance and marker-assisted screening at the Pm60 locus in wild diploid wheat Triticum urartu. Crop J. 2020, 8, 252–259.
61. Qiu, L.; Liu, N.; Wang, H.; Shi, X.; Li, F.; Zhang, Q.; Wang, W.; Guo, W.; Hu, Z.; Li, H.; et al. Fine mapping of a powdery mildew resistance gene MlIW39 derived from wild emmer wheat (Triticum turgidum ssp. dicoccoides). Theor. Appl. Genet. 2021, 134, 2469–2479.
62. Wang, W.; He, H.; Gao, H.; Xu, H.; Song, W.; Zhang, X.; Zhang, L.; Song, J.; Liu, C.; Liu, K.; et al. Characterization of the powdery mildew resistance gene in wheat breeding line KN0816 and its evaluation in marker-assisted selection. Plant Dis. 2021, 105, 4042–4050.
63. Du, X.; Xu, W.; Peng, C.; Li, C.; Zhang, Y.; Hu, L. Identification and validation of a novel locus, Qpm-3BL, for adult plant resistance to powdery mildew in wheat using multilocus GWAS. BMC Plant Biol. 2021, 21, 357.
64. Sharma, J.S.; McCallum, B.D.; Hiebert, C.W. Development of single nucleotide polymorphism-based functional molecular markers from the Lr22a gene sequence in wheat (Triticum aestivum). Plant Breed. 2022, 141, 204–211.
65. Kumar, S.; Bhardwaj, S.C.; Gangwar, O.P.; Sharma, A.; Qureshi, N.; Kumaran, V.V.; Khan, H.; Prasad, P.; Miah, H.; Singh, G.P.; et al. Lr80: A new and widely effective source of leaf rust resistance of wheat for enhancing diversity of resistance among modern cultivars. Theor. Appl. Genet. 2021, 134, 849–858.
66. Hu, Y.; Huang, X.; Wang, F.; He, Y.; Feng, L.; Jiang, B.; Hao, M.; Ning, S.; Yuan, Z.; Wu, J.; et al. Development and validation of gene-specific KASP markers for YrAS2388R conferring stripe rust resistance in wheat. Euphytica 2021, 217, 206.
67. Gill, B.K.; Klindworth, D.L.; Rouse, M.N.; Zhang, J.; Zhang, Q.; Sharma, J.S.; Chu, C.; Long, Y.; Chao, S.; Olivera, P.D.; et al. Function and evolution of allelic variations of Sr13 conferring resistance to stem rust in tetraploid wheat (Triticum turgidum L.). Plant J. 2021, 106, 1674–1691.
68. Zhang, Y.; Wang, Z.; Quan, W.; Zhang, X.; Feng, J.; Ren, J.; Jiang, X.; Zhang, Z. Mapping of a QTL with major effect on reducing leaf rust severity at the adult plant growth stage on chromosome 2BL in wheat landrace Hongmazha. Theor. Appl. Genet. 2021, 134, 1363–1376.
69. Long, L.; Yao, F.; Guan, F.; Cheng, Y.K.; Duan, L.; Zhao, X.; Li, H.; Pu, Z.; Li, W.; Jiang, Q.; et al. A stable QTL on chromosome 5BL combined with Yr18 conferring high-level adult-plant resistance to stripe rust in Chinese wheat landrace Anyuehong. Phytopathology 2021, 111, 1594–1601.
70. Li, C.N.; Li, L.; Reynolds, M.P.; Wang, J.Y.; Chang, X.P.; Mao, X.G.; Jing, R.L. Recognizing the hidden half in wheat: Root system attributes associated with drought tolerance. J. Exp. Bot. 2021,

72, 5117–5133.

71. Ma, J.; Luo, W.; Zhang, H.; Zhou, X.H.; Qin, N.N.; Wei, Y.M.; Liu, Y.X.; Jiang, Q.T.; Chen, G.Y.; Zheng, Y.L.; et al. Identification of quantitative trait loci for seedling root traits from Tibetan semi-wild wheat (Triticum aestivum subsp tibetanum). Genome 2017, 60, 1068–1075.
72. Fleury, D.; Jefferies, S.; Kuchel, H.; Langridge, P. Genetic and genomic tools to improve drought tolerance in wheat. J. Exp. Bot. 2010, 61, 3211–3222.
73. Li, Y.Y.; Zhang, Y.F.; Li, C.A.; Chen, X.; Yang, L.L.; Zhang, J.; Wang, J.Y.; Li, L.; Reynolds, M.P.; Jing, R.L.; et al. Transcription factor TaWRKY51 is a positive regulator in root architecture and grain yield contributing traits. Front. Plant Sci. 2021, 12, 734614.
74. Rehman, S.U.; Sher, M.A.; Saddique, M.A.; Ali, Z.; Khan, M.A.; Mao, X.G.; Irshad, A.; Sajjad, M.; Ikram, R.M.; Naeem, M.; et al. Development and exploitation of KASP Assays for genes underpinning drought tolerance among wheat cultivars from Pakistan. Front. Genet. 2021, 12, 684702.
75. Pang, Y.; Wu, Y.; Liu, C.; Li, W.; Amand, P., St.; Bernardo, A.; Wang, D.; Dong, L.; Yuan, X.; Zhang, H.; et al. High-resolution genome-wide association study and genomic prediction for disease resistance and cold tolerance in wheat. Theor. Appl. Genet. 2021, 134, 2857–2873.
76. Würschum, T.; Longin, C.F.H.; Hahn, V.; Tucker, M.R.; Leiser, W.L. Copy number variations of CBF genes at the Fr-A2 locus are essential components of winter hardiness in wheat. Plant J. 2017, 89, 764–773.
77. Fu, L.P.; Xiao, Y.G.; Yan, J.; Liu, J.D.; Wen, W.E.; Zhang, Y.; Xia, X.C.; He, Z.H. Characterization of TaCOMT genes associated with stem lignin content in common wheat and development of a gene-specific marker. J. Integr. Agric. 2019, 18, 939–947.
78. Sihag, P.; Sagwal, V.; Kumar, A.; Balyan, P.; Mir, R.R.; Dhankher, O.P.; Kumar, U. Discovery of miRNAs and development of heat-responsive miRNA-SSR markers for characterization of wheat germplasm for terminal heat tolerance breeding. Front. Genet. 2021, 12, 699420.
79. Yan, L.; Loukoianov, A.; Tranquilli, G.; Helguera, M.; Fahima, T.; Dubcovsky, J. Positional cloning of the wheat vernalization gene VRN1. Proc. Natl. Acad. Sci. USA 2003, 100, 6263–6268.
80. Yan, L.; Loukoianov, A.; Blechl, A.; Tranquill, G.; Ramakrishna, W.; SanMiguel, P.; Bennetzen, J.L.; Echenique, V.; Dubcovsky, J. The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. Science 2004, 303, 1640–1644.
81. Yan, L.; Fu, D.; Li, C.; Blechl, A.; Tranquilli, G.; Bonafede, M.; Sanchez, A.; Valarik, M.; Yasuda, S.; Dubcovsky, J. The wheat and barley vernalization gene VRN3 is an orthologue of FT. Proc. Natl. Acad. Sci. USA 2006, 103, 19581–19586.

82. Peng, J.; Richards, D.; Hartley, N.; Murphy, G.; Devos, K.; Flintham, J.; Beales, J.; Fish, L.J.; Worland, A.J.; Pelica, F.; et al. ‘Green revolution’ genes encode mutant gibberellin response modulators. Nature 1999, 400, 256–261.

83. Tian, X.; Xie, L.; Fu, L.; Xu, D.; Fu, C.; Wang, D.; Chen, X.; Xia, X.; Chen, Q.; He, Z.; et al. Molecular mapping of reduced plant height gene Rht24 in bread wheat. Front. Plant Sci. 2017, 8, 1379.

84. Hou, J.; Li, T.; Wang, Y.; Hao, C.; Liu, H.; Zhang, X. ADP-glucose pyrophosphorylase genes, associated with kernel weight, underwent selection during wheat domestication and breeding. Plant Biotechnol. J. 2017, 15, 1533–1543.

85. Khalid, M.; Afzal, F.; Gul, A.; Amir, R.; Subhani, A.; Ahmed, Z.; Mahmood, Z.; Xia, X.; Rasheed, A.; He, Z. Molecular characterization of 87 functional genes in wheat diversity panel and their association with phenotypes under well-watered and water-limited conditions. Front. Plant Sci. 2019, 10, 717.

86. Duan, X.; Yu, H.; Ma, W.; Sun, J.; Zhao, Y.; Yang, R.; Ning, T.; Li, Q.; Liu, Q.; Guo, T.; et al. A major and stable QTL controlling wheat thousand grain weight: Identification, characterization, and CAPS marker development. Mol Breed. 2020, 40, 68.

87. Liu, H.; Li, H.; Hao, C.; Wang, K.; Wang, Y.; Qin, L.; An, D.; Li, T.; Zhang, X. TaDA1, a conserved negative regulator of kernel size, has an additive effect with TaGW2 in common wheat (Triticum aestivum L.). Plant Biotechnol. J. 2020, 18, 1330–1342.

88. Chen, Y.; Yan, Y.; Wu, T.T.; Zhang, G.L.; Yin, H.; Chen, W.; Wang, S.; Chang, F.; Gou, J.Y. Cloning of wheat keto-acyl thiolase 2B reveals a role of jasmonic acid in grain weight determination. Nat. Commun. 2020, 11, 6266.

89. Shoaib, M.; Yang, W.; Shan, Q.; Sun, L.; Wang, D.; Sajjad, M.; Li, X.; Sun, J.; Liu, D.; Zhan, K.; et al. TaCKX gene family, at large, is associated with thousand-grain weight and plant height in common wheat. Theor. Appl. Genet. 2020, 133, 3151–3163.

90. Zhang, Y.; Li, T.; Geng, Y.; Wang, Y.; Liu, Y.; Li, H.; Hao, C.; Wang, H.; Shang, X.; Zhang, X. Identification and development of a KASP functional marker of TaTAP46-5A associated with kernel weight in wheat (Triticum aestivum). Plant Breed. 2021, 140, 585–594.

91. Wang, J.; Wang, R.; Mao, X.; Zhang, J.; Liu, Y.; Xie, Q.; Yang, X.; Chang, X.; Li, C.; Zhang, X.; et al. RING finger ubiquitin E3 ligase gene TaSDIR1-4A contributes to determination of grain size in common wheat. J. Exp. Bot. 2020, 71, 5377–5388.

92. Li, F.J.; He, Z.H.; Liu, J.D.; Jin, H.; Cao, S.H.; Geng, H.W.; Yan, J.; Zhang, P.Z.; Wan, Y.X.; Xia, X.C. Genome-wide linkage mapping of yield-related traits in three Chinese bread wheat populations using high-density SNP markers. Theor. Appl. Genet. 2018, 131, 1903–1924.

93. Song, J.; Xu, D.; Dong, Y.; Li, F.; Bian, Y.; Li, L.; Luo, X.; Fei, S.; Li, L.; Zhao, C.; et al. Fine mapping and characterization of a major QTL for grain weight on wheat chromosome arm 5DL. Theor. Appl. Genet. 2022, 135, 3237–3246.
94. Zhao, D.; Yang, L.; Liu, D.; Zeng, J.; Cao, S.; Xia, X.; Yan, J.; Song, X.; He, Z.; Zhang, Y. Fine mapping and validation of a major QTL for grain weight on chromosome 5B in bread wheat. Theor. Appl. Genet. 2021, 134, 3731–3741.
95. Tura, H.; Edwards, J.; Gahlaut, V.; Garcia, M.; Sznajder, B.; Baumann, U.; Shahinnia, F.; Reynolds, M.; Langridge, P.; Balyan, H.S.; et al. QTL analysis and fine mapping of a QTL for yield-related traits in wheat grown in dry and hot environments. Theor. Appl. Genet. 2020, 133, 239–257.
96. Li, T.; Deng, G.; Su, Y.; Yang, Z.; Tang, Y.; Wang, J.; Qiu, X.; Pu, X.; Li, J.; Liu, Z.; et al. Identification and validation of two major QTLs for spike compactness and length in bread wheat (Triticum aestivum L.) showing pleiotropic effects on yield-related traits. Theor. Appl. Genet. 2021, 134, 3625–3641.
97. Chen, Z.; Ke, W.; He, F.; Chai, L.; Cheng, X.; Xu, H.; Wang, X.; Du, D.; Zhao, Y.; Chen, X.; et al. A single nucleotide deletion in the third exon of FT-D1 increases the spikelet number and delays heading date in wheat (Triticum aestivum L.). Plant Biotechnol. J. 2022, 20, 920–933.
98. Jiang, P.; Zhang, P.; Wu, L.; He, Y.; Li, C.; Ma, H.; Zhang, X. Linkage and association mapping and Kompetitive allele-specific PCR marker development for improving grain protein content in wheat. Theor. Appl. Genet. 2021, 134, 3563–3575.
99. Altpeter, F.; Popelka, J.C.; Wieser, H. Stable expression of 1Dx5 and 1Dy10 high-molecular-weight glutenin subunit genes in transgenic rye drastically increases the polymeric glutelin fraction in rye flour. Plant Mol. Biol. 2004, 54, 783–792.
100. Payne, P.I. Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Annu. Rev. Plant Physiol. Plant Mol. Biol. 1987, 38, 141–153.
101. Rasheed, A.; Wen, W.; Gao, F.; Zhai, S.; Jin, H.; Liu, J.; Guo, Q.; Zhang, Y.; Dreisigacker, S.; Xia, X.; et al. Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. Theor. Appl. Genet. 2016, 129, 1843–1860.
102. Ram, S.; Devi, R.; Singh, R.B.; Narwal, S.; Singh, B.; Singh, G.P. Identification of codominant marker linked with Glu-D1 double null and its utilization in improving wheat for biscuit making quality. J. Cereal Sci. 2019, 90, 102853.
103. Zhu, Y.; Wang, S.; Wei, W.; Xie, H.; Liu, K.; Zhang, C.; Wu, Z.; Jiang, H.; Cao, J.; Zhao, L.; et al. Genome-wide association study of pre-harvest sprouting tolerance using a 90K SNP array in common wheat (Triticum aestivum L.). Theor. Appl. Genet. 2019, 132, 2947–2963.

104. Gupta, P.K.; Balyan, H.S.; Sharma, S.; Kumar, R. Biofortification and bioavailability of Zn, Fe and Se in wheat: Present status and future prospects. Theor. Appl. Genet. 2021, 134, 1–35.

105. Gaikwad, K.B.; Rani, S.; Kumar, M.; Gupta, V.; Babu, P.H.; Bainsla, N.K.; Yadav, R. Enhancing the nutritional quality of major food crops through conventional and genomics-assisted breeding. Front. Nutr. 2020, 7, 533453.