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Genome-wide association study of wheat quality traits

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Abstract

Background

Wheat (*Triticum aestivum* L.) is one of the three major food crops worldwide. Improving quality-related traits is key to optimizing grain and dough processing and enhancing end-use properties.

Results

In this study, 314 wheat accessions were subjected to a genome-wide association study using the 55K single nucleotide polymorphism (SNP) array. Ten quality traits were assessed in six different environments. A total of 102 significant marker-trait associations (MTAs) were identified, and 11 stable MTAs were detected. Among them, MTA45, associated with dough development time, and MTA81, associated with maximum resistance, were stable across four environments. However, the favorable alleles associated with dough rheological properties at the MTA45 and MTA81 loci had significant negative effects on yield-related traits. Kompetitive allele-specific PCR markers for the two MTAs were designed. Meta-QTL analysis of 237 QTL yielded 78 MQTL, with an average confidence interval reduced from 12.94 cM to 8.04 cM, and 19 of these MQTL co-localized with GWAS signals, further supporting the reliability of these loci. Genomic prediction analysis showed that the identified MTAs significantly improved prediction accuracy compared to using all SNPs, with GBLUP performing best for MTA-based selection and random forest for genome-wide selection. Polygenic score analysis delineated the genetic architecture of 10 wheat quality traits, with five being influenced by a few major-effect SNPs and 5 by a background of small-effect SNPs. Additionally, 66 MTAs were co-identified by both polygenic score analysis and genome-wide association study methods.

Conclusion

This study provides valuable genetic resources and reliable molecular markers for high-quality wheat breeding.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops worldwide, providing approximately 20% of the global population's energy and protein intake, as well as serving as a significant source of dietary fiber [1]. Although wheat breeding's main focus is on increasing yields, it is also used to increase quality-related traits. Among them, grain traits and dough rheological properties collectively determine its processing quality [2]. Basic components, such as protein and starch, directly influence the functional performance of flour in various food applications. Therefore, the nutritional value, processing characteristics, and economic benefits of these components have garnered widespread attention [3]. Furthermore, wheat physicochemical properties and dough rheological

parameters have become important bases for the systematic evaluation of wheat quality and applicability [4].

The evaluation of wheat quality encompasses two major categories: grain characteristics and dough processing properties. Among the quality traits, grain protein content (GPC), test weight (TW), wet gluten content (WGC), and moisture content (MC) are crucial indicators. GPC, serving as a core parameter for assessing nutritional value, and WGC are both controlled by polygenes, and relevant QTLs have been extensively reported [5–7]. TW is key for market grading and pricing, and it can effectively predict flour yield [8, 9]. MC is primarily related to grain storage stability, and its ratios with other components influence processing quality [6]. The effects of quality traits need to be ultimately reflected in dough rheological properties (such as development time, stability, and extensibility) to have decisive roles in wheat processing [10]. Water absorption (WA) is a critical parameter for evaluating wheat flour quality, as it directly affects gluten network formation and thus ultimately shapes dough properties [11]. Dough stability time (DST), which reflects the dough's resistance to overmixing, is a critical trait that influences the quality of wheat-based foods and determines the end-use of wheat [12, 13]. Similarly, as a fundamental rheological parameter, dough development time (DDT) plays a critical role in determining the end-use processing quality of wheat [14, 15]. Extensibility (EXT), a key dough rheological property, determines the quality and end-use applications of flour products [16]. Using extension area (EA) as a selection criterion can effectively improve EXT and assist in optimizing dough strength [17]. The maximum extensional resistance (Rmax) of wheat dough strongly influences the uniaxial extensional rheology of dough and is a key parameter for determining whether dough meets processing requirements [18].

Genome-wide association studies (GWAS), which provide higher resolutions and greater allelic diversity than quantitative trait loci (QTLs) mapping, significantly improve the efficiency of dissecting complex traits [19, 20]. Building on the advantages of a GWAS, genomic selection (GS) further leverages genome-wide markers and statistical models to predict complex traits, enabling accelerated breeding without the need for extensive phenotyping [21, 22]. Wheat quality traits are typically controlled by multiple genes [23], and Polygenic scores (PGS), based on the polygenic inheritance theory of complex traits, effectively predicts an individual's genetic predisposition for a target trait by aggregating the effects of genome-wide markers [24, 25].

Numerous QTLs and marker-trait associations (MTAs) of quality traits have been mapped across all 21 chromosomes in wheat. The QTLs or MTAs for GPC have mainly been detected on Chromosomes 2A, 2B, and 6A [7, 12, 26–30]. Notably, the *GPC-B1* gene, cloned on Chromosomes 6A, 6B, and 6D, is a key regulator of senescence and enhances the remobilization of protein and micronutrients to developing grains [31]. For EXT, QTLs have been primarily mapped on Chromosomes 1D, 2B, 5A, and 7A, and the glutenin gene *Gli-2* has been cloned on Chromosomes 6A, 6B, and 6D [32, 33].

QTLs or MTAs for TW have mainly been detected on Chromosomes 1A, 4B, and 6D [6, 7, 30]. QTLs or MTAs for WGC have mainly been detected on Chromosomes 3A, 6A, and 7D [4, 6, 12, 28, 34–36]. QTLs

for MC have mainly been detected on Chromosomes 2A, 4D, 5D, and 6D [6]. QTLs for WA have mainly been detected on Chromosomes 5D and 7A [2, 4, 12]. For DST, QTLs have been primarily mapped on Chromosomes 1B, 2B, and 3B [2, 4, 12]. For DDT, QTLs have been primarily mapped on Chromosomes 1A, 2A, and 2D [2, 4]. No corresponding genes have been cloned for the above QTLs. Unlike the above quality traits, only a limited number of QTLs have been reported for EXT and Rmax in wheat. To date, no well-characterized or stably verified loci have been documented for the two traits.

Meta-QTL (MQTL) analysis enables the integration of QTL information from independent studies to identify consensus genomic hotspots governing target traits, thereby reducing the confounding effects of genetic background and environmental variation [37, 38]. By projecting QTL onto a consensus genetic map, this approach refines their confidence intervals and yields more robust and stable MQTL regions [39, 40]. In wheat, MQTL analysis has been successfully applied to a range of traits, including yield-related traits [41], disease resistance [42–44], abiotic stress tolerance [45], and grain quality traits [46]. With the availability of the high-quality Chinese Spring reference genome (IWGSC RefSeq v2.1), MQTL intervals can now be anchored to physical maps at a finer resolution, which facilitates the identification of candidate genes underlying consensus QTL regions [41, 47]. However, the integration of MQTL analysis with GWAS-defined marker-trait associations for wheat quality traits has been relatively limited. Further integration of these approaches could provide independent validation of GWAS signals and help prioritize genomic regions that are consistently associated with quality traits across diverse populations and environments.

In this study, high-quality single nucleotide polymorphism (SNP) data and multi-environment phenotypic records of 10 wheat quality traits were used to identify stable MTAs and select candidate genes. By analyzing the genetic effects of major SNPs, favorable allelic variations were elucidated, and Kompetitive allele-specific PCR (KASP) functional markers were developed for marker-assisted breeding. Five GS models were employed to systematically evaluate the predictive abilities of both MTA sets and genome-wide SNP sets for target traits. PGS was applied to decipher the genetic architectures of traits, and results from the optimal model were correspondingly verified using stable MTAs. This research provides valuable genetic resources and practical molecular markers for wheat quality improvement, establishing a theoretical basis and practical approach for genomics-assisted breeding.

Results

Phenotypic variation and correlation analysis

Based on the BLUP values, a comprehensive statistical analysis was performed for the 10 quality traits. The coefficients of variation were observed to vary considerably among traits, with the lowest value found in MC (1.69%) and the highest in Rmax (19.09%). Moderate to high broad-sense heritability was observed for traits such as TW (0.42), WA (0.54), DST (0.56), DDT (0.56), and Rmax (0.55), while other traits exhibited lower heritability, including GPC (0.30), WGC (0.30), MC (0.03), EXT (0.19), and EA (0.39). The absolute values of skewness and kurtosis for all traits were below 1, indicating generally

symmetrical distributions (Table S2). Together with the frequency distribution histograms (Fig. 1), these results suggest that all traits approximately follow a normal distribution.

Correlation analysis between quality- and yield-related traits

The correlation analysis revealed complex relationships among quality- and yield-related traits (Fig. S1). Among the grain quality traits, GPC was positively correlated with WGC at an extremely significant level ($P < 0.001$). In contrast, GPC exhibited extremely significant negative correlations with TW and MC. A significantly negative correlation was also observed between WGC and MC. No significant correlation was found between TW and MC. For the dough rheological parameters, the majority of traits (DST, DDT, EA, EXT, and Rmax) demonstrated mutually extremely significant positive correlations with each other. However, the correlation between WA and EXT was not extremely significant ($P < 0.01$).

Among the associations between grain quality and dough rheological traits, GPC showed extremely significant positive correlations with DST, DDT, EA, EXT, and Rmax, but an extremely significant negative correlation with WA. WGC was extremely significantly and positively correlated with DST, DDT, EA, EXT, and Rmax, whereas its correlation with WA was not significant. MC was negatively correlated with all dough rheological traits (DST, DDT, WA, EA, EXT, and Rmax) at an extremely significant level. TW showed extremely significant positive correlations with WA, EA, and Rmax, significant positive correlations ($P < 0.05$) with DDT and EXT, and no significant correlation with DST (Fig. S1).

The correlation analysis revealed multiple significant associations between yield- and quality-related traits. YPP was significantly negatively correlated with GPC, WGC, DDT, and EXT. TKW showed significant negative correlations with GPC, WGC, and DST. PH was significantly negatively correlated with TKW and YPP, and significantly positively correlated with GPC, WGC, DDT, EXT, and EA. PL was significantly negatively correlated with YPP and TKW, and significantly positively correlated with GPC, DDT, and EXT. No significant correlations were observed between PN or SN with any of the quality parameters examined (Fig. S1).

GWAS on quality traits

The genotyping of 314 wheat germplasm resources using a 55K SNP array identified 24,889 high-quality SNP loci. This panel exhibited a weak population structure, with a mean LD decay distance of 4.1 Mb [19].

In total, 102 significant MTAs (Table S3) were detected in at least two environments (including BLUE datasets). The Manhattan plots illustrating the MTAs for each trait are presented in Fig. 2. The largest number of MTAs was 34 for WA, followed by 12 for DST, 12 for DDT, 12 for WGC, 11 for GPC, 7 for EXT, 6 for EA, 6 for Rmax, 5 for TW, and 4 for MC. The total numbers of significant MTAs from the A (37) and B (35) subgenomes were approximately 2.4 times the number from the D (30) subgenome. Chromosome

3A carried the highest count (13), whereas Chromosome 2A contained the fewest (1) (Table S3). The quantile–quantile plots indicating the robustness of the GWAS model are shown in Fig. S2.

A total of 11 significant MTAs for GPC were identified, distributed across 6 chromosomes: 2D, 3A, 5D, 6A, 6D, and 7A. Among them, eight MTAs were detected specifically for GPC, whereas two MTAs (MTA30 and MTA78) co-localized with WGC, and one MTA (MTA89) co-localized with EXT. A total of five significant MTAs for TW were identified, distributed across 3 chromosomes: 1A, 2D, and 5A. MTA27 was stably detected specifically for TW, with no co-localization observed with other traits (Table S3). A total of 12 significant MTAs for WGC were identified, distributed across 7 chromosomes: 1A, 3A, 5A, 5D, 6D, 7A, and 7B. MTA31 was stably detected specifically for WGC, whereas two MTAs (MTA30 and MTA78) co-localized with GPC (Table S3). A total of four significant MTAs for MC were identified, distributed across three chromosomes: 3A, 4A, and 4D. All four MTAs (MTA29, MTA38, MTA53, and MTA63) were detected specifically for MC, with no co-localization observed with other traits (Table S3).

A total of 34 significant MTAs for WA were identified, distributed across 11 chromosomes: 1D, 2B, 2D, 3B, 3D, 4D, 5B, 5D, 6D, 7B, and 7D. MTA25, MTA72, MTA86, and MTA95 were stably detected for WA, whereas one locus, MTA86, co-localized with DDT and Rmax. A total of 12 significant MTAs for DST were identified, distributed across 10 chromosomes: 1A, 2B, 3A, 3B, 3D, 4B, 4D, 5B, 7A, and 7D. All the MTAs were detected specifically for DST, with no co-localization observed with other traits. Notably, one locus, MTA45, was consistently detected across multiple environments. A total of 12 significant MTAs for DDT were identified, distributed across 10 chromosomes: 1A, 1B, 2A, 3A, 4A, 5B, 5D, 6A, 6D, and 7A. In total, 9 MTAs were detected specifically for DDT, and 3 loci (MTA13, MTA81, and MTA86) co-localized with Rmax. Additionally, MTA86 co-localized with WA (Table S3). A total of 7 significant MTAs for EXT were identified, distributed across 5 chromosomes: 3B, 4B, 5A, 7A, and 7D. In total, 6 MTAs were detected specifically for EXT, with one locus, MTA89, co-localizing with GPC (Table S3). A total of 6 significant MTAs for EA were identified, distributed across four chromosomes: 1B, 2B, 3B, and 6A. MTA7 and MTA46 were stably detected specifically for EA with no co-localization observed with other traits (Table S3). A total of 6 significant MTAs for Rmax were identified, distributed across 6 chromosomes: 1B, 2A, 4A, 5B, 6A, and 6D. Three loci (MTA13, MTA81, and MTA86) co-localized with DDT, and two Rmax-associated loci, MTA70 and MTA81, were stably detected across multiple environments (Table S3).

Allele analysis and candidate gene predictions for major MTAs

This study selected two multi-environmentally stable loci, MTA45 (AX-109989063) and MTA81 (AX-109478902) from 102 MTAs for further investigation. We subsequently conducted comprehensive analyses of their spatiotemporal allele frequency distributions, genetic effects on yield-related traits, and candidate genes within ± 5 Mb genomic regions.

The MTA45 locus on Chromosome 3B was significantly associated with DST. Among the 314 wheat accessions, the frequency of allele C (146) was higher than that of allele A (115). Compared to allele C, allele A significantly decreased several key dough rheological parameters, including DST by 7.9%, DDT by

8.3%, EXT by 7.8%, EA by 3%, and Rmax by 9.3% (Fig. S3A). In contrast, allele A increased TKW by 3.5% and YPP by 5.1%, and it showed no significant associations with PH, PL, PN, or SN (Fig. S3B). Allele A underwent significant positive selection during the breeding transition from landraces to modern varieties (Fig. S3C). The geographical distribution analysis indicated that the overall frequency of allele C exceeded that of allele A across the six continents studied (Fig. S4A). These results suggest that allele A has been preferentially selected in breeding programs due to its positive effects on yield-related traits, despite a trade-off with processing quality. Within the LD block containing MTA45, 109 candidate genes were identified. Four genes (*TraesCS3B03G0153400*, *TraesCS3B03G0153700*, *TraesCS3B03G0154700*, and *TraesCS3B03G0154800*) were proposed as core candidates. Their predicted functions are associated with lipid metabolism, hormone signaling, and iron homeostasis, which are biological processes that may contribute to quality-related traits (Table S4).

The MTA81 locus on Chromosome 6A was significantly associated with DDT and Rmax (Fig. 3A). Among the 314 wheat accessions, 278 accessions were classified into two groups based on the presence of either allele A (185 accessions) or allele C (93 accessions). Compared to allele C, allele A significantly decreased DST by 3.0%, DDT by 6.9%, EXT by 3.4%, and Rmax by 6.7% (Fig. 3D). However, no significant changes were observed in WA and EA. Allele A was also associated with reduced PH (6.5%) but increased YPP (5.4%) and TKW (4.5%). No significant associations were observed with PL, PN, and SN (Fig. 3B). Allele A underwent significant positive selection during the transition from landraces to modern varieties (Fig. 3C). The geographical distribution analysis indicated that the overall frequency of allele A exceeded that of allele C across the six continents studied (Fig. S4B). These results demonstrate that allele A has been preferentially selected in breeding due to its positive effects on yield-related traits, despite its negative association with dough quality. Within the MTA81 LD block, 252 candidate genes were identified. Three genes (*TraesCS6A03G0993000*, *TraesCS6A03G1016900*, and *TraesCS6A03G1022100*) were selected as core candidates based on their predicted involvement in fundamental metabolic regulation (Table S4).

Development of molecular markers for major stable MTAs used in MAS

In this study, we successfully converted the SNP marker AX-109989063 of MTA45 associated with SHL, along with the SNP marker AX-109478902 of MTA81 associated with GP, into robust KASP markers (Tables S1 and S5; Fig. S7A, B), enabling the efficient genotyping of germplasm resources. These markers will provide important resources for the Mol Breed of wheat.

The significant MTAs improved prediction accuracy and efficiency

The genomic prediction analysis across 10 wheat quality traits revealed that MTAs consistently outperformed genome-wide SNPs in nine traits (except for TW) (mean accuracy gain: +26.8%, $P < 0.001$).

For MTA-based predictions, GBLUP achieved the highest accuracy (0.614), particularly excelling in dough rheological properties, such as WA (0.706) and Rmax (0.686). In the full-genome analysis, RF performed best (0.495), showing robustness for minor-effect traits such as MC (0.339). Notably, RKHS maintained superior performance for protein-related traits (protein content/GPC: 0.693 under MTA). When jointly evaluating both marker sets, GBLUP and RKHS emerged as co-optimal models (composite accuracy: 0.544), with GBLUP recommended for dough characteristics and RKHS recommended for protein enhancement (Fig. 4; Table S6).

PGS of quality traits

Based on the GWAS and BLUE data, PGS were calculated for 10 quality traits to determine the optimal *P*-value threshold (P_T) for maximal variance explained (R^2). The genetic architecture was delineated by the optimal P_T and number of SNPs incorporated. Traits influenced by a polygenic background of numerous small-effect loci included TW (P_T = 0.0336, R^2 = 41.37%, 379 SNPs), GPC (P_T = 0.044, R^2 = 36.48%, 557 SNPs), MC (P_T = 0.1157, R^2 = 41.36%, 1,108 SNPs), EXT (P_T = 0.1621, R^2 = 37.71%, 1,432 SNPs), and EA (P_T = 0.0274, R^2 = 40.52%, 231 SNPs). Conversely, traits primarily governed by a few large-effect loci included WGC (P_T = 0.0001, R^2 = 27.46%, 4 SNPs), WA (P_T = 0.0033, R^2 = 41.52%, 88 SNPs), DDT (P_T = 0.0014, R^2 = 43.05%, 24 SNPs), DST (P_T = 0.0012, R^2 = 48.01%, 22 SNPs), and Rmax (P_T = 0.0017, R^2 = 39.74%, 29 SNPs). The optimal PGS models captured a subset of the MTAs identified by GWAS for each trait (Figs. S6 and S7; Table S7)

Meta-QTL analysis and co-localization with MTAs

A comprehensive literature survey yielded a total of 289 QTL associated with wheat quality traits, collected from studies published between 2008 and 2025 (Table S8). Of these, 237 QTL were successfully projected onto the high-density consensus genetic map, while the remaining 52 QTL were discarded due to the absence of common markers with the consensus map or excessively large confidence intervals.

Following meta-analysis, the 237 initial QTL were consolidated into 78 meta-QTL (MQTL), which were distributed across all 21 wheat chromosomes (Table S9). Among them, chromosome 3D harbored the fewest MQTL, with only one identified. The average confidence interval of the MQTL was reduced from 12.94 cM to 8.04 cM, representing a 1.6-fold improvement in mapping precision (Figs. S8 and S9; Table S9).

To validate the biological relevance of the MQTL and provide independent support for our GWAS findings, we compared their physical intervals with the positions of the significant MTAs detected in this study. This co-localization analysis revealed that 19 of the 78 MQTL overlapped with the significant MTAs detected in this study within a 5 Mb interval (Table S9).

Discussion

Wheat, as a major global food crop, plays a critical role in improving food processing adaptability and nutritional value through genetic enhancement of its quality traits [6]. However, research on the genetic mechanisms underlying quality traits still lags behind studies on yield. Moreover, these traits are highly influenced by genotype-by-environment interactions and cultivation practices, leading to extensive phenotypic variation [55]. Grain quality formation is a complex physiochemical process involving the synthesis and accumulation of multiple components such as proteins and starches, which directly determine flour processing quality and end-product applications [4]. This study employs GWAS, GS, and PGS to systematically dissect the genetic architecture of key quality traits in wheat, aiming to provide theoretical support and genetic resources for Mol Breed of high-quality wheat varieties and the analysis of phenotypic variation.

Genetic dissection of wheat quality and yield interplay

In the present study, we analyzed the genetic correlations among quality traits, and the results revealed a more comprehensive picture than previously reported. While the significantly positive correlation between GPC and WGC aligns with findings from previous research [15, 20, 23], and underscores their close association, it is crucial to consider this within a broader network of relationships. For instance, the observed negative correlations of GPC with TW and MC, and of WGC with MC, suggest potential trade-offs between protein content, grain density, and moisture. Furthermore, the strong positive correlations among most dough rheological parameters (DST, DDT, EA, EXT, and Rmax) indicate that these traits are often co-inherited. Notably, GPC and WGC showed consistent positive correlations, whereas MC was negatively correlated, with these dough strength-related parameters, underscoring their pivotal roles in determining processing quality.

Our study revealed a complex genetic interplay between yield- and quality-related traits in wheat. Correlation analyses confirmed significant negative correlations between yield components (YPP and TKW) and both protein-related traits (GPC and WGC) and dough rheological properties (DST, DDT, EXT, and Rmax). Notably, our allele analyses of MTA45 and MTA81 provided direct evidence for a relationship between yield and quality. For the MTA45 locus, allele A conferred substantial yield gains (YPP, + 5.1%, and TKW, + 3.5%) while negatively affecting quality traits (Rmax, -9.3%, and DST, -7.9%). The same results were observed for MTA81, in which the allele variation positively affecting yield had a negative effect on quality. These findings confirm the negative correlation between yield and protein-related traits [14].

Breeding selection indicates that the allele that enhances yield-related traits were positively selected during modern breeding (allele A for both MTA45 and MTA81); conversely, alleles enhancing quality were seldom selected in wheat breeding. Thus, there is still huge potential for quality improvement.

Co-localization with previously reported loci

To elucidate the biological relevance of the identified loci, we systematically compared their genetic positions with previously reported loci associated with quality-related traits. Among the 102 MTAs identified, 31.4% (32 loci) were co-localized with known genomic regions. For instance, MTA50 (3D) is associated with WA, co-localizing not only with a previously reported QTL for WA but also with multiple markers linked to grain quality traits, including GPC and WGC [12, 29, 56, 57]. Similarly, MTA67 (5A), associated with WGC, co-localizes with GPC [30, 36, 58].

To further explore the functional implications of the identified loci, we also investigated their potential roles in yield-related traits. Notably, MTA23 (2D: 632.7 Mb) was found to co-localize with a QTL for PH [19] and the *TaARF12-2D* gene, which has been implicated in height regulation [59]. Similarly, on Chromosome 7A, MTA93 (675.5 Mb) and MTA94 (676.3 Mb), associated with GPC and EXT, co-localized with multiple yield-related QTLs. These include JD_c1201_631 for GPC [30], MTA278 for spike length [19], as well as the following QTLs for spikes: *QSns.sau-7A* [60], *QTsn.cau-7A.4* [61], *QSPS-7A.3* and *QSPS-7A.4* [62], *qSn-7A.2* [63], and *QTS.perg-7A* [64]. MTA81 (6A) co-localizes with two documented QTLs, *QTkw.dms-6A.3* and *QGpc.dms-6A* [7]. This locus not only showed significant associations with DDT and Rmax, but also has a potential role in regulating TKW and GPC (Table S3). These co-localized regions suggest that there may be pleiotropic effects on both quality and yield components, highlighting their potential importance in breeding for multi-trait improvement.

Regarding multi-environmentally stable MTAs, multiple novel loci were detected. Among them, MTA25 (2D, 692.1 Mb) and MTA95 (7B, 16.4 Mb) were associated with WA; MTA31 (3A, 346 Mb) was linked to WGC; the co-localized loci MTA45 and MTA46 on Chromosome 3B (52.4–55.1 Mb) were associated with DST and EA, respectively; and MTA86 (6D, 489.4 Mb) was identified as a novel pleiotropic locus concurrently affecting WA, DDT, and Rmax (Table S3).

Validation of GWAS signals through meta-QTL analysis

As an independent line of evidence, we performed a meta-QTL analysis to evaluate whether the MTAs identified in this study overlap with consensus genomic regions derived from previously reported QTL. A total of 289 QTL associated with wheat quality traits were collected from the literature, of which 237 were successfully projected onto the consensus genetic map and consolidated into 78 MQTL distributed across all 21 chromosomes. The average confidence interval of these MQTL was substantially narrower than that of the initial QTL, reflecting the improved mapping resolution afforded by meta-analysis [37, 38]. Among the 78 MQTL, 19 co-localized with significant MTAs from the present GWAS, falling within the MQTL physical intervals or their 5 Mb flanking regions. This overlap provides external validation for nearly one-fifth of our GWAS signals and reinforces the robustness of these associations. Notably, MTA45, one of the two stable MTAs identified in this study, was among the co-localized loci, further supporting its reliability. The co-localized MQTL represent genomic regions that have been independently linked to quality traits across diverse genetic backgrounds and environments, thereby increasing confidence in their utility for marker-assisted breeding. Conversely, MTAs that did not co-localize with any MQTL may represent population-specific or novel associations requiring further investigation. Taken

together, the integration of meta-QTL and GWAS results strengthens the rationale for prioritizing these loci in wheat quality improvement programs.

Candidate gene analyses of the two core MTAs

As an initial step towards dissecting the genetic bases of quality traits, we performed candidate gene analyses of two stable MTAs, MTA45 and MTA81. There were 109 candidate genes of MTA45, including *TraesCS3B03G0153400*, which encodes sulfoquinovosyl transferase (*SQD2*), a key enzyme catalyzing the final step in sulfolipid biosynthesis. Beyond its conserved role in sulfolipid production under phosphate-limited conditions, SQD2 also participates in flavonoid glycosylation and influences carbon partitioning between primary and secondary metabolism [65–67]. SQD2-mediated flavonoid glycosylation enhances the reactive oxygen species scavenging capacity under osmotic stress, whereas its activity affects starch synthase functions and soluble sugar levels, potentially contributing to stable grain development and final quality traits [66, 68]. *TraesCS3B03G0153700* encodes the F-box protein GID2, a central component of the SCF E3 ubiquitin ligase complex that mediates the degradation of DELLA repressor proteins in the gibberellin signaling pathway [69, 70]. Through this molecular mechanism, GID2 regulates PH and spike development while potentially influencing grain quality traits by modulating photoassimilate partitioning and nutrient allocation during grain filling processes [71, 72]. *TraesCS3B03G0154700* and *TraesCS3B03G0154800* encode nicotianamine synthase 3 (NAS3), which catalyzes nicotianamine synthesis, a key metallochaperone critical for iron homeostasis. NAS3-mediated nicotianamine biosynthesis enhances iron and zinc accumulation in grain and facilitates long-distance metal transport within plants [73–75]. These functions directly contribute to improved mineral nutrition and grain quality traits.

There were 252 genes of MTA81, including *TraesCS6A03G0993000*, which encodes a P-loop NTPase domain-containing protein (LPA1). Loss-of-function mutations in this gene significantly reduce phytic acid accumulation in seeds while increasing inorganic phosphorus levels, thereby regulating grain phosphorus composition and nutritional quality [76, 77]. *TraesCS6A03G1016900* encodes phosphatidylinositol 4-phosphate 5-kinase 9 (PIP5K9). This enzyme catalyzes the production of phosphatidylinositol 4,5-bisphosphate and directly influences sugar metabolism by negatively regulating the activity of cytosolic invertase CINV1, thereby modulating sucrose hydrolysis and carbon allocation. PIP5K9 is also involved in regulating potassium ion homeostasis. These functions collectively affect the accumulation of nutrients during grain development, potentially influencing quality traits such as grain plumpness [78, 79]. *TraesCS6A03G1022100* encodes xyloglucan endotransglucosylase/hydrolase 8 (XTH8), an enzyme that modifies xyloglucan structure to regulate cell wall properties. This gene is highly expressed in vascular tissues, and its suppression leads to impaired plant growth. XTH8 likely influences grain quality traits by modulating cell wall characteristics in vascular tissues to enhance nutrient transport to developing grains and by directly regulating endosperm cell expansion, thereby potentially affecting grain size and plumpness [80, 81]. The above genes were predicted as candidate genes for MTA81.

Interplay of MTAs, PGS, and GS validates wheat quality architecture

To further validate the genetic contributions of these loci, PGS were determined based on the optimal *P*-value thresholds derived from multi-trait models. The resulting genetic predictions were highly consistent with the observed effects of the identified MTAs, providing independent validation of their functional importance. Based on the PGS models, the 10 traits were clearly divided into two distinct genetic architectures: 5 traits (WGC, WA, DST, DDT, and Rmax) were influenced by a limited number of large-effect SNPs, whereas the other 5 (GPC, TW, MC, EXT, and EA) were controlled by a polygenic background consisting of numerous small-effect SNPs. Notably, basic grain quality traits (GPC, TW, and MC) were largely governed by a polygenic background, whereas dough rheological parameters (DST, DDT, Rmax, etc.) tended to be influenced by major-effect loci. This pattern may reflect differences in the underlying biochemical pathways and selective pressures during wheat breeding.

In total, five GS models were implemented in parallel to compare the predictive accuracy of the MTA sets to genome-wide SNPs. Consistent with a previous report [19], the MTA-based models generally outperformed whole-genome approaches for most quality-related traits, with the GBLUP model achieving the highest accuracy when trained on MTAs. Notably, the RF algorithm exhibited superior performance in the whole-genome SNP setting, suggesting its robustness in capturing complex genetic interactions underlying quality traits.

These findings demonstrate that the focused utilization of stable MTAs can enhance genomic prediction efficiencies while providing functional insights into the genetic architecture of wheat quality. The integration of PGS and multi-model GS offers a scalable framework for prioritizing candidate loci and accelerating marker-assisted wheat breeding for improved end-use quality.

Conclusion

In total, 102 significant MTAs were identified and 11 stable MTAs were detected. Among them, MTA45 and MTA81 underwent allele analyses and candidate genes were predicted. In addition, KASP markers were developed for both. PGS further validated 66 MTAs having substantial effects on their associated traits. GS demonstrated the superior predictive ability of the MTA-based set over whole-genome SNPs. The GBLUP model achieved the highest accuracy using MTAs, while the RF model performed best using the whole-genome framework. Furthermore, meta-QTL analysis consolidated 237 initial QTL into 78 MQTL, of which 19 co-localized with GWAS signals, providing independent validation for these loci. This study provides functionally validated molecular markers and robust genomic strategies to facilitate quality improvement in wheat Mol Breed.

Methods

Plant materials and quality trait measurement

This investigation employed a natural population of 314 wheat accessions [48] for quality trait assessment across six geographic–seasonal combinations (Table S1): Qixia (37.34°N, 120.85°E) 2017–2018 (E1), Pulagu (121.41°E, 37.31°N) 2019–2020 (E2), Shijiazhuang (114.69°E, 37.89°N) 2019–2020 (E3), Shijiazhuang (114.69°E, 37.89°N) 2020–2021 (E4), Pulagu (121.41°E, 37.31°N) 2020–2021 (E5), and Ludong University (121.35°E, 37.51°N) 2020–2021 (E6). A randomized complete block design with two replications per location was implemented. Accessions were established in three-row plots with a 1.5-m row length and 20-cm row spacing, sown manually at 15 seeds per row, with crop management following locally recommended agronomic protocols.

Ten key quality traits were quantified using a near-infrared analyzer DA7200, employing established near-infrared calibration curves. The analyzed grain quality parameters were the following: GPC (%), TW (g/L), WGC (%), and MC (%). The analyzed dough rheological properties were the following: WA (%), DST (min), DDT (min), EXT (mm), EA (cm^2), and Rmax (kPa). Additionally, the following six yield-related traits were evaluated: plant height (PH, cm), panicle length (PL, cm), panicle number per plant (PN), spikelet number per spike (SN), thousand-kernel weight (TKW, g), and yield per plant (YPP, g).

Statistical analyses and genotyping

Phenotypic statistics and ANOVAs for 10 quality traits across six environments were conducted using SPSS v25.0 (SPSS, Chicago, IL, USA). With genotype as a fixed effect and environment as a random effect, best linear unbiased estimates (BLUE) were obtained via the lme4 package. Broad-sense heritability (H^2) was calculated using OGAStation 1.0 with the formula: H^2 = genetic variance / (genetic variance + environmental variance) × 100%. Trait correlation heatmaps were generated with corrplot, whereas frequency distribution histograms and normal curves were constructed in Origin 2021.

Genomic DNA was extracted from young leaves using an improved CTAB protocol. The genotyping of 314 wheat accessions was performed with the Wheat 55K SNP Array by Beijing Compass Biotechnology Co., Ltd (Beijing, China). After filtering with PLINK1.9 to remove low-quality SNPs exhibiting minor allele frequency ≤ 0.05, missing rate ≥ 10%, or heterozygosity ≥ 20%, 24,889 high-quality SNPs were retained for the GWAS. All the SNP markers were anchored to the wheat reference genome (IWGSC RefSeq v2.1) through a BLASTN alignment of flanking sequences.

GWAS

Building upon previously published population structure, kinship, and linkage disequilibrium (LD) data [19], this study conducted a GWAS for phenotypic values across six environments and their BLUEs using the BLINK model in R/GAPIT 3.4. Multi-environment Manhattan and quantile–quantile plots were generated with R/CMplot [49]. Significant MTAs were declared at a threshold of $P < 0.001$ ($-\log_{10}P \geq 3$), with MTAs detected in at least two independent environments (including BLUE) being considered as significant and those consistently detected in at least three environments being considered as stable.

Prediction of candidate genes

To identify candidate genes, we combined genetic association signals with functional annotation data. Genomic regions flanking significant GWAS loci (± 5 Mb) were delineated using the *Triticum aestivum* Chinese Spring reference genome (IWGSC RefSeq v2.1). Gene annotations from the Triticeae-GeneTribe platform were analyzed to identify genes harboring putative biological functions. Functional annotations were retrieved from the UniProt knowledgebase. For gene prioritization, empirically validated functional evidence specific to *Triticum aestivum* L, *Oryza sativa*, or *Arabidopsis thaliana* in UniProt received primary weighting, complemented by conserved domain architecture analyses.

PGS

The PGS for wheat quality traits were generated using PRSice v2.3.3 to identify optimal *P*-value thresholds [50]. LD clumping was performed based on the 4.1-Mb LD decay profile of this array. The threshold maximizing R^2 was selected as optimal. Visualization was implemented using the ggplot2 package.

GS

Genomic predictions for 10 wheat quality traits were performed using five models, Genomic Best Linear Unbiased Prediction (GBLUP, implemented in rrBLUP), Bayesian LASSO (BL, BGLR), Bayes C (BC, BGLR), Reproducing Kernel Hilbert Space (RKHS, BGLR), and Random Forest (RF, ranger), through tenfold cross-validations. The following two complementary approaches were implemented: full-genome analysis incorporating all the SNPs, and MTA-focused analysis using significant markers only, both modeled as random effects. Prediction accuracies were determined by Pearson's correlation coefficients between observed and predicted phenotypic values.

Meta-QTL analysis and co-localization with GWAS-derived MTAs

A systematic literature search for QTL associated with wheat quality traits was conducted using the X-MOL academic platform (https://www.x-mol.com/) and PubMed (http://www.ncbi.nlm.nih.gov/pubmed), covering studies published between 2008 and 2025. Quality traits were categorized as follows: grain traits including GPC, TW, WGC, MC, dry gluten content, flour protein content, gluten index, grain hardness, and kernel hardness; and dough rheological properties including WA, DST, DDT, EXT, EA, Rmax, sedimentation volume, falling number, flour water absorption, dough tenacity, dough strength, mixing time, and mixing tolerance. For each QTL, the following information was collected: flanking markers, peak position, 95% confidence interval, LOD score, R^2, and mapping population type and size. Missing LOD scores were set to 3.0 and missing R^2 values to 10%. When confidence intervals were not reported, they were recalculated using the standard formulas: $530/(N \times R^2)$ for F_2 and backcross populations, $163/(N \times R^2)$ for recombinant inbred line populations, and $287/(N \times R^2)$ for doubled haploid populations, where N denotes population size.

A consensus genetic map was constructed using the R package LPmerge by integrating marker data from six high-density linkage maps: "Wheat_Composite_2004" and "Wheat_Consensus_SSR_2004" (GrainGenes, https://wheat.pw.usda.gov/GG3/), "Illumina iSelect 90K SNP Array" [51], "Wheat 55K SNP Array" [52], "Wheat 35K SNP Array" [53], and "Wheat consensus map version 4.0" (Diversity Arrays Technology, https://www.diversityarrays.com). All collected QTL were projected onto this consensus map using BioMercator v4.2.3; QTL that could not be reliably projected were discarded. Meta-analysis was performed using the Goffinet and Gerber method implemented in BioMercator.

To determine the physical coordinates of the resulting MQTL, flanking markers within each MQTL interval were curated, and their physical positions were obtained by alignment to the Triticum aestivum Chinese Spring reference genome (IWGSC RefSeq v1.0). An MTA identified in the present GWAS was considered co-localized with an MQTL if it fell within the MQTL physical interval or within a 5 Mb flanking region on either side.

Conversion of SNPs to KASP markers

The SNP genotyping of the MTA45 and MTA81 loci (Table S1) was carried out using KASP technology on the KASP™ platform (LGC Genomics, Hoddesdon, UK). Each 5-µL PCR reaction contained 2.5 µL of sample DNA (30 ng/µL), 2.5 µL of KASP Master Mix, and 0.07 µL of KASP Assay Mix. The thermal cycling protocol consisted of: an initial denaturation at 94°C for 15 min; 10 touchdown cycles (94°C for 20 s; annealing from 61°C to 55°C, decreasing by 0.6°C per cycle, for 60 s); and 26 additional cycles of 94°C for 20 s and 55°C for 60 s. Genotype calling was automated using the SNPline/Array Tape platform via dual-channel fluorescence detection (FAM: 465–510 nm; HEX: 528–560 nm), and results were validated using KlusterCaller software v2.4.0 [54].

Abbreviations

BLUE
Best linear unbiased estimator
DDT
dough development time
DST
dough stability time
EA
extension area
EXT
Extensibility
GPC
grain protein content
GS
genomic selection
GWAS

Genome-wide association study
KASP
Kompetitive Allele-Specific PCR
LD
Linkage disequilibrium
MC
moisture content
MQTL
Meta-QTL
MTA
Marker-trait association
PGS
polygenic scores
PH
plant height
PL
panicle length
PN
panicle number per plant
QTL
Quantitative trait loci
Rmax
maximum extensional resistance
SNP
Single nucleotide polymorphism
SN
spikelet number per spike
TKW
thousand-kernel weight
TW
test weight
WA
Water absorption
WGC
wet gluten content
YPP
yield per plant

Declarations

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Not applicable.

Author contribution

CZ, HG, YT and FC analyzed the data and drafted the manuscript. HG, YT, MT, YL, MX, BZ and NW performed phenotype evaluation. RQ, YW, and HS helped design the study and revised the manuscript. All of the authors have read and approved the final version of the manuscript.

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Data availability

Data is provided within the manuscript or supplementary information files, and the datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

These experiments complied with the ethical standards in China.

Clinical Trial

Not applicable.

Consent for Publish

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

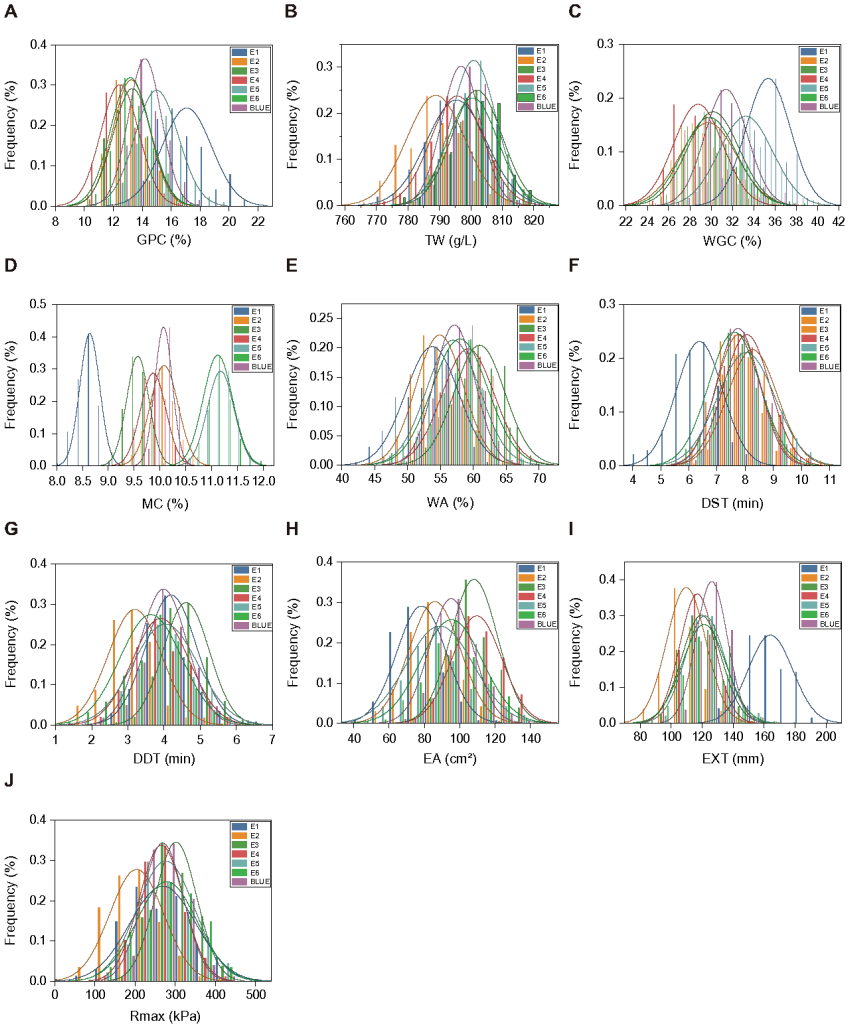


Fig 1. Frequency distribution of ten quality-related traits in six environments and their BLUE values.

A-J represent GPC (grain protein content), TW (test weight), WGC (wet gluten content), MC (moisture content), WA (water absorption), DST (dough stability time), DDT (dough development time), EXT (extensibility), EA (elastic area), and Rmax (maximum resistance).

Figure 1

See image above for figure legend.

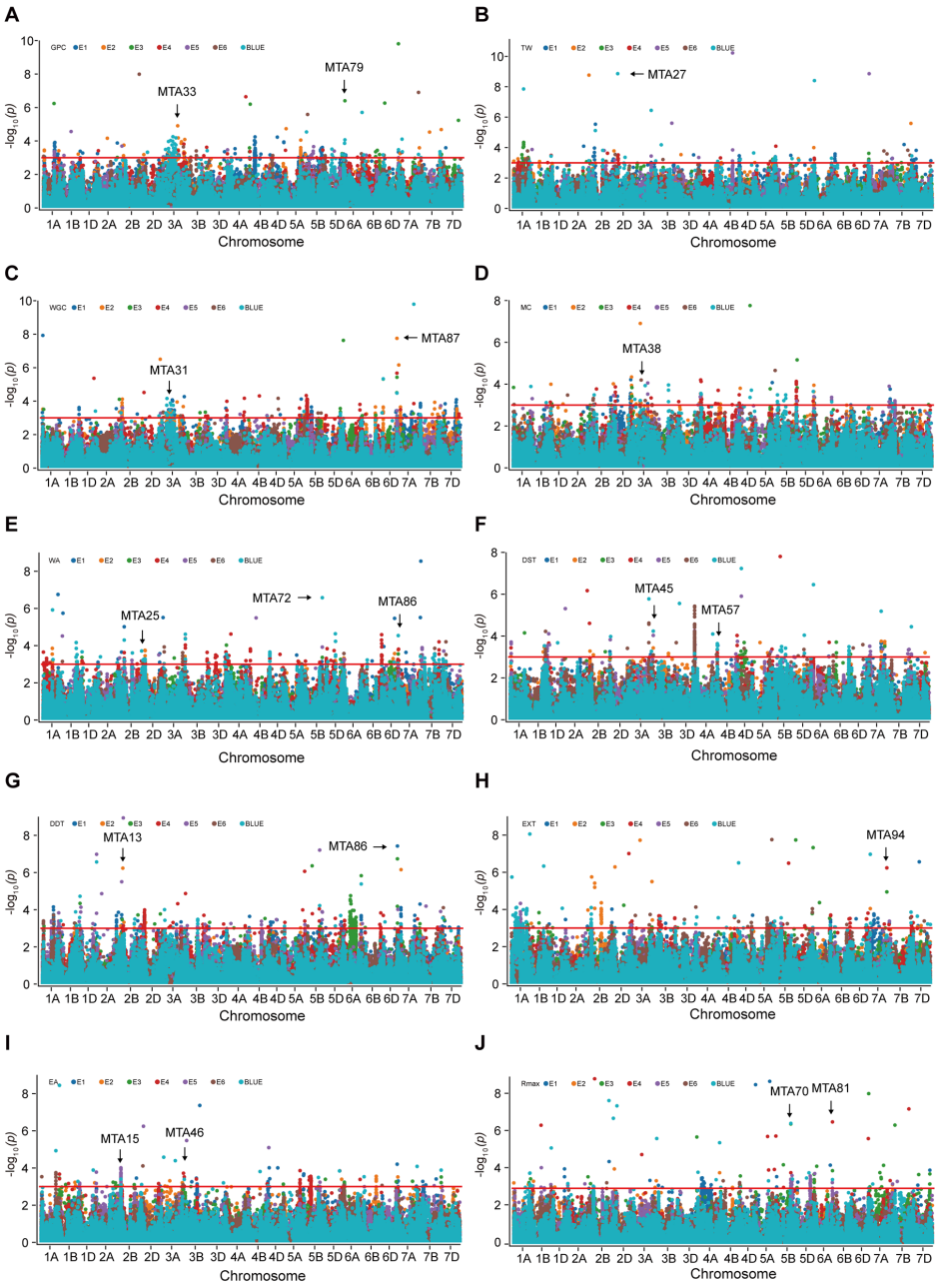


Fig 2. Manhattan plots from GWAS of ten grain quality traits.
A GPC, Grain protein content; **B** TW, Test weight; **C** WGC, Wet gluten content; **D** MC, Moisture content. **E** WA, Water absorption; **F** DST, Stability time; **G** DDT, Development time; **H** EXT, Extensibility; **I** EA, Extension area; **J** Rmax, Maximum resistance. Results are shown for six individual environments (E1-E6) and their combined Best Linear Unbiased Estimation (BLUE).

Figure 2

See image above for figure legend.

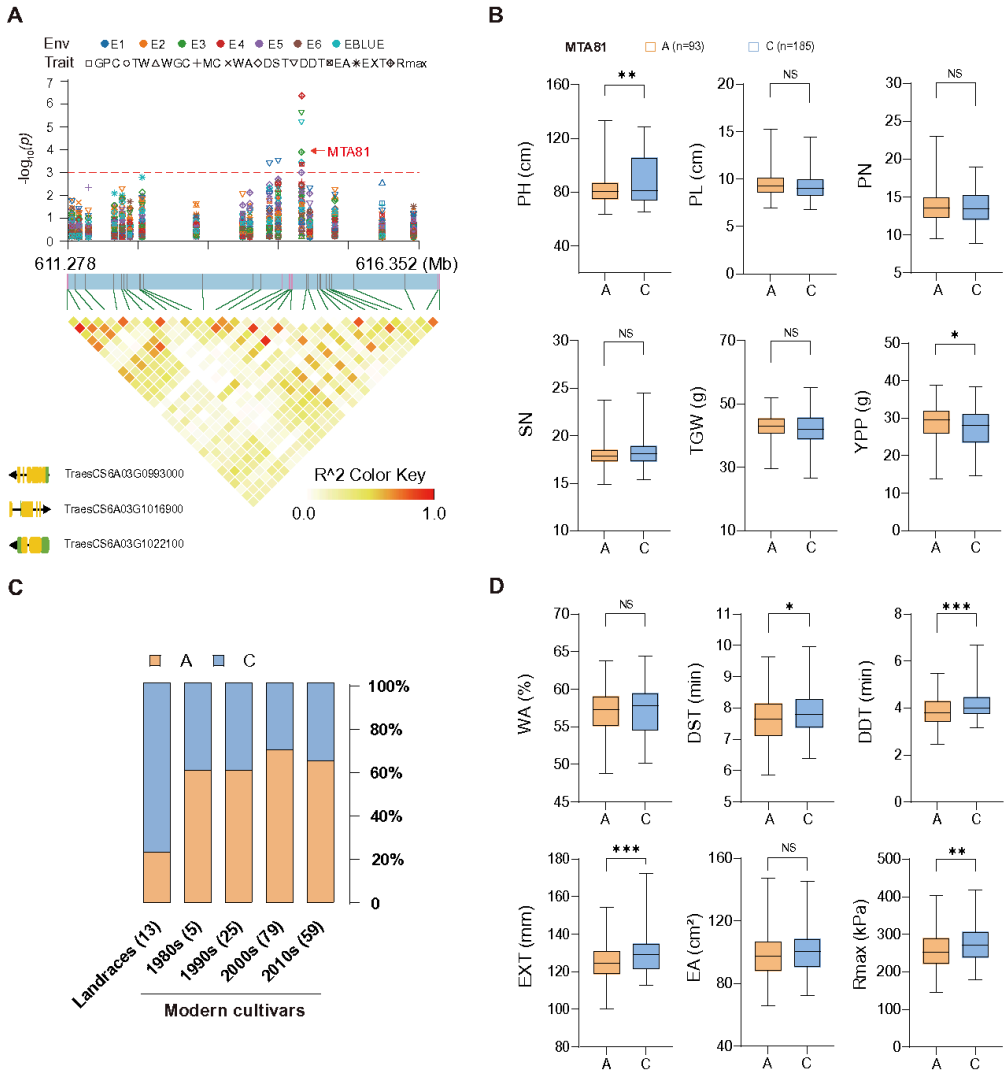


Fig 3. Genetic effects and candidate gene analyses of the MTA81 on Chromosome 6A associated with DDT and Rmax.
A Association analysis of nine quality-related traits under eight different environments in the LD block of MTA81. Red arrows represent the most significant MTAs associated with DDT and Rmax. **B** Phenotypic comparison between contrasting alleles at MTA81 for PH, PL, PN, SN, TGW, and YPP. Two-tailed Student's *t-tests* were performed between contrasting alleles at MTA81 (NS = not significant; *P < 0.05,***P < 0.001). **C** Haplotype block analysis of the MTA81 locus was performed for Rmax and DDT based on best linear unbiased estimates (BLUE) across multiple environments. **D** Changes in the frequencies of MTA81 alleles across the history of wheat breeding in China.

Figure 3

See image above for figure legend.

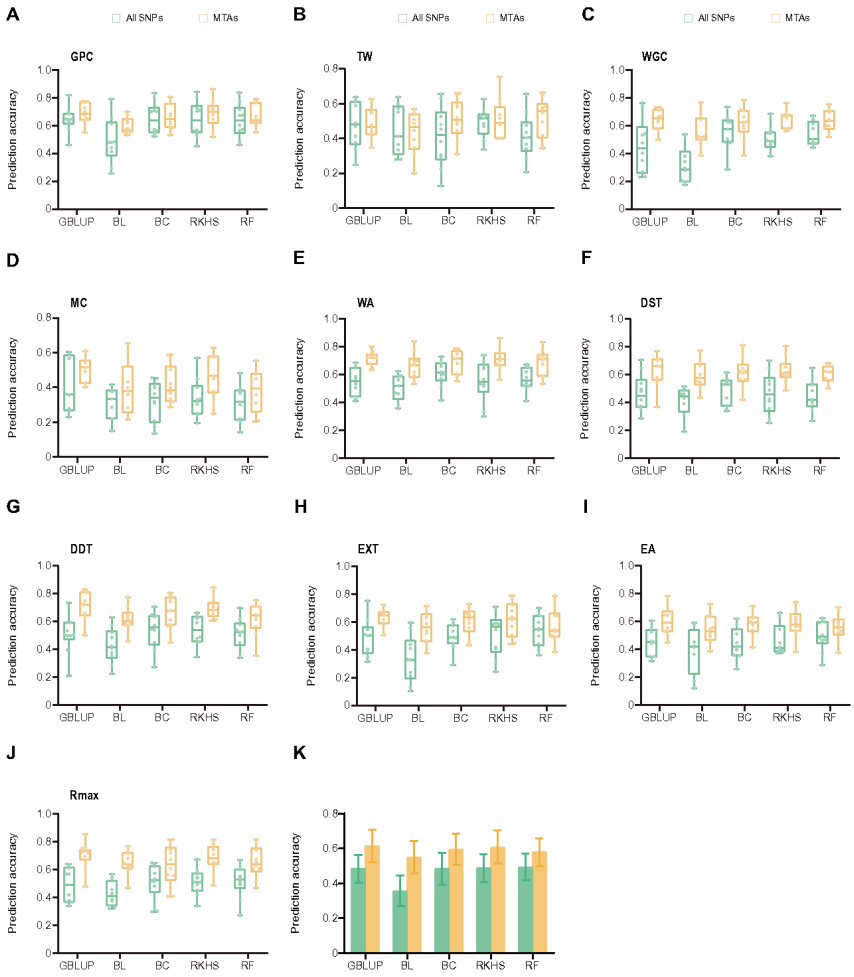


Fig 4. Comparative analysis of genome-wide prediction accuracy and efficiency between all the SNPs and MTAs.
A-J Genome-wide predictions for ten wheat quality traits (GPC, TW, WGC, MC, WA, DST, DDT, EXT, EA, Rmax) using five genomic selection models (GBLUP, BL, BC, RKHS, RF). **K** Comparative assessment of average computing times between all SNPs and stable MTAs for the ten quality traits using the five models. GBLUP, Genomic Best Linear Unbiased Prediction; BL, Bayesian Lasso; BC, BayesC; RKHS, Reproducing Kernel Hilbert Space; RF, Random Forest.

Figure 4

See image above for figure legend.

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