Uncovering genomic regions controlling plant architectural traits in hexaploid wheat using GWAS

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Lingqiang Wang  
lqwang@mail.hzau.edu.cn  
Huazhong Agricultural University College of Plant Science and Technology  
Corresponding Author

Ali Muhammad  
Huazhong Agricultural University College of Plant Science and Technology

Weicheng Hu  
Huazhong Agricultural University College of Plant Science and Technology

Jinsheng Yu  
Zhejiang A and F University

Shahid Ullah Khan  
Huazhong Agricultural University College of Plant Science and Technology

Guosheng Xie  
Huazhong Agricultural University College of Plant Science and Technology

Jibin Wang  
Guangxi University Guangxi Agriculture College

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Abstract

**Background:** Wheat is a staple food crop worldwide. Plant height is a key factor in plant architecture as it plays a crucial role in lodging and thus affects yield and quality. Genome-wide studies are mostly applied in crop plants, due to its advanced genotyping technologies, identification of novel loci, and improved statistical approaches.

**Results:** In this study, the population was genotyped by using Illumina iSelect 90K single nucleotide polymorphism (SNP) assay and finally 22,905 high-quality SNPs were used to perform a genome-wide association study (GWAS) for plant architectural traits employing four multi-locus GWAS (ML-GWAS) and three single-locus GWAS (SL-GWAS) models. As a result, 174 and 97 significant SNPs controlling plant architectural traits were detected by four ML-GWAS and three SL-GWAS methods, respectively. Among these SNP makers, 43 SNPs were commonly detected, including seven across multiple environments and thirty-six across multiple methods. Interestingly, five most stable SNPs (Kukri_c34553_89, RAC875_c8121_1490, wsnp_Ex_rep_c66315_64480362, Ku_c5191_340, and tplb0049a09_1302) consistently detected across multiple environments and methods, possibly played a role in modulating plant height and flag leaf length. When comparing ML-GWAS methods, pLARmEB was the most powerful and accountable for the detection of 49 significant SNPs that mostly contributed to plant height (36 SNPs). However, in SL-GWAS the FarmCPU model detected most of the significant SNPs. Moreover, a total of 152 candidate genes were found that are likely to be involved in plant growth and development which may provide insightful information related to plant architectural traits.

**Conclusion:** Altogether, our results reveal 174 and 97 significant SNPs controlling plant architectural traits using four ML-GWAS and three SL-GWAS methods, respectively. The detection of the stable loci across multiple environments and methods, possibly play a role in modulating plant architectural traits in hexaploid wheat, and finally will contribute
to the discovery of valuable SNP loci for marker-assisted selection (MAS) in wheat molecular breeding.

**Background**

Wheat (*Triticum aestivum* L) is the most important food crop cultivated worldwide on about 200 million hectares and provides 20% of the total needs of the world population [1-3]. Wheat is among the three major cereal crops ranked its position next to maize and rice annually produced [4]. For the first time in human history, wheat cultivation has produced a sufficient amount of food, which in turn, supported the development of cities and contributed to the rise of human civilization in Middle Eastern empires of Babylon and Egypt [5]. High grain yield is one of the critical breeding goals in developing cultivars. Wheat has been grown in China for more than 4,000 years and is currently cultivated in 10 agricultural zones based on its different kinds, cultivation season, effects of temperature and photoperiod [6-8]. Crop yield is a complex trait significantly associated with thousand grain weight, grains number per spike and fertile spikes/m². Moreover, kernel shape, plant height, effective tillers per plant, flag leaf length and flag leaf width traits can also affect yield through effects on photosynthetic ability, grain filling duration and dry matter translocation [9-12]. Larger grains are the potential indicator of grain yield, apart from this increased grain size is positively associated with seeding growth and early maturity [13]. Using semi-dwarf cultivars contained dwarfing genes (*Rht1* and *Rht2*) were the main reason for the success of the Green Revolution [11].

Prior studies have identified chromosomal regions associated with yield-related traits, either by QTL mapping using linkage maps [14-17] or by genome-wide association study[18-21]. However, the standard QTL mapping using bi-parental crosses only detect
information on two alleles at a given locus with low resolution, and unable to detect QTLs
in complex and genetically diverse traits [22]. In addition, the evaluation of the mapping
population is a time consuming and costly process. As a complement to conventional QTL
mapping, GWAS analysis provides an opportunity to analyze the diverse genetic
background of complex traits with more accuracy [23]. GWAS has three obvious
advantages compared to QTL mapping: (1) it shortens the time of constructing
populations; (2) it can be used to evaluate a wider range of germplasms for phenotyping
many traits with one cycle of genotyping; (3) it offers much higher resolution for fine
mapping [20, 24]. Currently research have been accomplished through GWAS in wheat
[25], maize [26], rice [27], soybean [28] and cotton [29]. Comparatively hexploid wheat
has larger genome (≈ 15.8 GB) than rice (≈ 400 Mb) and maize (≈ 2.32 Gb) [25]. Over the
past ten years, the absence of fully sequenced reference genome has limited the gene
discovery of wheat and recent advances in functional genomics have provided breeders
with a new impetus to achieve their goals [30].
However, substantially more work is required because of the experimental bottleneck
emerging from the absence of inconsistency among studies, and the utilization of low-
density marker platforms in gene mapping studies. To achieve better understanding with
high resolution, larger number of molecular markers are preferred for mapping population.
Currently, the best alternate choice for wheat breeder is the utilization of single
nucleotide polymorphism (SNP) to completely detect candidate genes controlling complex
traits [20]. Comparatively, the high-density wheat Illumina iSelect array comprising about
90,000 SNPs has been extensively adapted than SSR marker due to their efficiency, high
density, stable inheritance, low cost, representativeness and capability of automatic
detection [25, 31].
To address these issues the present study was designed to use genome-wide association
study (GWAS) approach employing high-density wheat iSelect 90K SNP array. Our main objectives were to 1) investigate marker-trait associations (MTAs) for plant architecture-related traits. 2) detect candidate genes responsible for corresponding morphological traits. Indeed, this study will provide useful insights by integrating three single-locus and four newly developed multi-locus GWAS methods, and further establish a regulatory network in wheat breeding programs.

Results

Statistical analysis of phenotypic traits

In the present study, we evaluated wheat germplasm collections for plant architectural traits, including plant height, flag leaf length, flag leaf width and the number of tillers per plant. The phenotypic characteristics for plant height across four environments and flag leaf length, flag leaf width and number of tillers per plant across two environments as shown in Additional file 1 and Fig. 1. All the traits exhibited the normal distribution pattern each year, indicating the quantitative nature of these traits (Fig. 1). Descriptive statistics revealed large phenotypic variations for all the traits as given in Additional file S1. PH ranged from 48.40 to 124.82 cm with coefficient of variations (CVs) ranged from 11.09–16.11%. The FLL varied from 16.06 to 31.57 cm, FLW varied from 1.22 to 2.75 cm and for tillers, the number of tillers per plant ranged from 8.10 to 14.67. Analysis of variance indicated highly significant differences (P < 0.001) for all the studied traits (Additional file 2).

Broad sense heritability was also estimated for PH, FLL, FLW and number of tillers with values ranged from 0.569 (FLW) to 0.794 (PH), suggesting the stability of these traits. The correlation analysis revealed significant correlation between different environments for each of the four traits, indicating the consistency of these traits across various
environments (Fig. 2). Furthermore, PH was significantly and positively correlated with FLL in almost all the environments. However, they correlated significantly but negatively with FLW in most of the environments indicating competition of these traits to assimilate at the plant growth stage. Finally, the relatively weak correlation of tillers with other traits suggested the independency of this trait.

Population Structure Analysis

Population structure is important due to the large number of diverse genotypes used in the study may produce false associations between the phenotypic values and unlinked markers. Therefore, a comprehensive analysis of population structure is prerequisite for evaluating successful association mapping. The number of subpopulations were calculated by the rate of change in the log probability of data between successive K-values. \( \Delta K \) was calculated for increasing the number of K value determined by STRUCTURE analysis according to the procedure of Evanno [32]. At \( k = 2 \), a break in the slope was observed followed by flattening of the curve (Fig. 3a). Hence, the most likely number of subpopulations was two (\( k = 2 \)) (Fig. 3b). Moreover, this result was confirmed by PCA based on standardized covariance of genetic distances of SNP markers (Fig. 3c).

Gwas For Plant Architectural Traits

Four multi-locus GWAS (ML-GWAS) models: FASTmrMLM, FASTmrEMMA, mrMLM, and pLARmEB, and three single-locus GWAS (SL-GWAS) models: FarmCPU, MLM, and MLMM implemented by Genomic Association and Prediction Integrated Tool (GAPIT) in R were used to detect the association signals. In ML-GWAS the SNPs with the critical logarithm of odds (LOD) score of 3 or greater than 3 were regarded as significant SNPs for associated traits. In SL-GWAS the screening criteria for detecting significant SNPs were \( P = 1/m \) (m is the number of total SNPs). To obtain more reliable results, the SNPs that were
simultaneously detected in at least two years or by at least two methods were considered as most stable SNPs. A total of 113 and 62 significant SNPs were identified by ML-GWAS and SL-GWAS methods, respectively (Fig. 4a).

Gwas Using Four Multi-locus Models

ML-GWAS models i.e. FASTmrMLM, FASTmrEMMA, mrMLM, and pLARmEB screened 47, 32, 46, and 49, significant SNPs, respectively for PH (117 SNPs) across four environments and for FLL (24), FLW (15) and Tillers (18) across two environments (Fig. 4b, Table 1-3).

Among these SNPs, 30, 8, 5, and 4 were detected by FASTmrMLM for PH, FLL, FLW, and Tillers, respectively (Fig. 4b, Table 1). By using FASTmrEMMA the number of significant SNPs identified for the above-mentioned traits were 24, 4, 1, and 3, respectively. Also 27, 6, 9, and 4 significant SNPs were detected by mrMLM approach for the said traits, respectively. Finally, the pLARmEB model identified 36, 6, 0, and 7 significant association signals with PH, FLL, FLW, and Tillers, respectively (Fig. 4b, Table 1).

Table 1
Common SNPs detected through different ML-GWAS methods associated with plant architectural traits

| Trait | Years | Methods (1-4) | SNP               | Chr | Position (bp) | LOD > 3 | r² (%) |
|-------|-------|---------------|-------------------|-----|---------------|---------|--------|
| PH    | 2015  | 1,2,4         | BobWhite 24364_73 | 3B  | 1551850       | 3.84–5.19 | 2.41–5.93 |
|       | 2015  | 2,4           | BS000890 76 51    | 5A  | 2414392       | 3.47–4.94 | 1.49–3.53 |
|       | 2015  | 2,4           | D_contig10 675 778 | 2D | 9580166      | 3.1–3.58  | 1.31–2.35 |
|       | 2015  | 1,3           | RAC875_c3 4971 137 | 7A  | 3550660       | 5.21–9.59 | 4.03–9.99 |
|       | 2015  | 1,2           | wsnp_Ex_c 39592 468 49607 | 5A | 2274396       | 3.20–3.94 | 1.35–3.70 |
|       | 2015  | 1,2           | wsnp_Ex_rep_c 67296 65839761 | 4D | 2143869       | 3.38–7.15 | 5.21–6.17 |
|       | 2016  | 2,3,4         | Excalibur_03094 523 | 7D  | 4302086       | 4.27–7.19 | 2.22–5.60 |
|       | 2016  | 1,2,4         | Ku_c5191_340      | 6B  | 3397322       | 4.54–7.23 | 4.23–8.44 |
|       | 2016  | 2,4           | Kukri 0459_8_614  | 2A  | 87562         | 4.26–6.13 | 2.25–2.38 |
|       | 2016  | 1,4           | Kukri_c345 53 89  | 2B  | 887912        | 3.22–6.43 | 2.32–3.42 |
|       | 2016  | 1,2,3,4       | RAC875_c8 121 1490 | 3A | 1272572       | 4.98–9.25 | 5.67–9.34 |
|       | 2016  | 1,4           | wsnp_Ex_rep_c 66315 64480362 | 6B | 3350805       | 3.12–6.78 | 3.10–5.73 |
Methods (1-4): Corresponding four multi-locus GWAS methods i.e. mrMLM, FASTmrMLM, FASTmrEMMA, and pLARmEB, respectively. \( r^2 \) (%) represents proportion of total phenotypic variation explained by each SNP. PH (Plant height); FLL (Flag leaf length); FLW (Flag leaf width); TILL (Number of tillers per plant).

To validate the findings, we further compared the results across multiple environments and 6 and 1 SNPs were co-identified in at least two of the environments for PH and FLL, respectively (Fig. 4c, Table 2). These environment-stable SNPs were located on different chromosomes. For PH there was one SNP on chromosome 2B, one on 3A, one located on 3B, one on 6A and two SNPs located on chromosome 6B. The LOD scores ranged from 3.05 to 7.92. One stable SNP across two environments located on chromosome 5A associated with FLL with LOD value ranging from 3.88 to 6.55 (Table 2). Comparing the results across different methods, we found 36 common SNPs were co-detected simultaneously by at least two approaches (Fig. 4c, Table 1). Among these, four significant SNPs (RAC875_c8121_1490, Ku_27771_508, Tdurum_contig42962_2138, BS00022127_51) were detected by all four methods (Table 1).
Table 2
Stable SNPs co-detected in multiple environments associated with plant height and flag leaf length

| Trait | Years | SNP | Chr | Position (bp) | LOD > 3 | r² (%) |
|-------|-------|-----|-----|---------------|---------|--------|
| PH    | 2016, 2017 | Kukri_c34553_89 | 2B | 887912 | 4.60–6.43 | 1.95–2.32 |
|       | 2016, 2017 | RAC875_c8121_1490 | 3A | 1272572 | 5.05–7.07 | 5.76–13.88 |
|       | 2015, 2017, 2018 | IACX3190 | 3B | 1429597 | 5.05–6.35 | 4.29–8.51 |
|       | 2017, 2018 | GENE_3659_1 | 6A | 2991495 | 3.44–3.86 | 1.50–2.54 |
|       | 2016, 2017 | wsnp_Ex_rep_c66315_64480362 | 6B | 3350804 | 3.67–6.78 | 3.10–4.63 |
|       | 2015, 2017, 2018 | Ku_c5191_340 | 5A | 3397322 | 3.05–7.92 | 0.72–4.39 |
| FLL   | 2017, 2018 | tplb0049a09_1302 | 5A | 2453837 | 3.88–7.17 | 5.04–13.14 |

PH (Plant height); FLL (Flag leaf length). r² (%) represents proportion of total phenotypic variation explained by each SNP.

We further checked the co-detected common SNPs simultaneously in multiple environments and different methods and detect five most stable SNPs (Kukri_c34553_89, RAC875_c8121_1490, wsnp_Ex_rep_c66315_64480362, Ku_c5191_340, and tplb0049a09_1302). Among these, one SNP was associated with FLL and the rest of four were identified for PH across multiple environments and different years (Fig. 4c, Table 3). Comparatively, the four ML-GWAS models i.e. FASTmrMLM, FASTmrEMMA, mrMLM, and pLARmEB, pLARmEB was the most powerful and useful approach, that detected 49 significant association signals, mostly associated with PH (36 SNPs), however FASTmrEMMA was the least effective approach accountable for 32 significant SNPs (Fig. 3b). The Manhattan and QQ plots of the above four ML-GWAS methods for plant height, flag leaf length, flag leaf width and number of tillers are presented in Additional file 7 (a-e).

Table 3
Stable SNPs identified simultaneously in different environments and different methods associated with plant height and flag leaf length

| Trait | Years | Methods (1–4) | SNP | Chr | Position (bp) | LOD > 3 | r² (%) |
|-------|-------|---------------|-----|-----|---------------|---------|--------|
| PH    | 2016, 2017 | 2016 (1,4), 2017 (1,2) | Kukri c34553_89 | 2B | 887912 | 3.22–6.43 | 1.95–3.42 |
|       | 2016, 2017 | 2016 (1,2,3,4), 2017 (1,2,3) | RAC875_c8121_1490 | 3A | 1272572 | 4.98–9.25 | 5.67–13.88 |
|       | 2016, 2017 | 2016 (1,4) | wsnp_Ex_rep_c66315_64480362 | 6B | 3350804 | 3.12–6.78 | 3.10–5.73 |
|       | 2016, 2017 | 2016 (1,4) | Ku_c5191_340 | 6B | 3397322 | 3.05–7.92 | 0.72–4.39 |
| FLL   | 2017, 2018 | 2017 (1,2) | tplb0049a09_1302 | 5A | 2453837 | 3.88–7.17 | 5.04–13.14 |

Methods (1-4): Corresponding four multi-locus GWAS methods i.e. mrMLM, FASTmrMLM, FASTmrEMMA, and pLARmEB, respectively. r² (%) represents proportion of total phenotypic variation explained by each SNP. PH (Plant height); FLL (Flag leaf length).

Gwas Using Three Single-locus Models

Three single-locus GWAS (SL-GWAS) methods i.e. FarmCPU, MLM, and MLMM were used to further analyzed the results of the same plant architectural traits. A total of 62 significant SNPs were detected by the three SL-GWAS methods for the above mentioned traits across multiple environments (Fig. 4d, Additional file 3). In the three SL-GWAS methods, FarmCPU
detected 56 significant SNPs, MLM detected 19, and MLMM detected 22 significant SNPs associated with different traits in multiple environments (Fig. 4d, Additional file 3). We further checked the common SNPs detected by all three SL-GWAS methods across multiple environments and methods and found three most stable SNPs i.e. RAC875_c8121_1490, BS00049008_51, and tplb0049a09_1302 were repeated consistently by all methods in most of the environments (Additional file 3). By comparing the results of all three SL-GWAS methods, the FarmCPU is the most effective and reliable approach accountable for 56 SNPS for all the traits, while MLM was the least effective method responsible for 19 SNPs detection.

Traits Having Common Associations

SNPs associated with more than one trait are very useful for marker-assisted selection. A total of five SNPs were detected associated with more than one trait across multiple environments (Additional file 4). Among these, one SNP on chromosome 5A (Excalibur_01167_1207) associated with PH and FLW. The rest of four pleiotropic SNPs were associated with PH and FLL across multiple environments. The presence of pleiotropic effects of these SNPs controlling plant height and flag leaf length were confirmed by the correlation analysis (Fig. 2). These pleiotropic SNPs i.e. Jagger_c6772_80, RAC875_c8121_1490, BS00089954_51, and Ku_c5191_340 were located on chromosome 1A, 3A, 3B, and 6B. Moreover, these pleiotropic associations suggest that the aforementioned SNPs have multifaceted role in plant architectural traits and highlight the significance of flag leaf length and width to plant height.

Candidate Genes Identification

To further understand the genetic basis of plant architectural traits, we detected several candidate genes that were surrounding the peak SNPs. Interestingly, several major
candidate genes that were directly associated with the consensus SNPs had exact same annotations (Fig. 5 and Additional file 6). For instance, several putative candidate genes for PH and FLL, annotated as Laccase which is used for lignin polymerization to help in a variety of functions in plant development [33]. Similarly, the putative genes responsible for PH and FLL annotated as Cysteine proteinase inhibitor, has a function in plant growth and defense [34]. Seven genes annotated as Glutathione S-transferase responsible for resistance against biotic stress [35]. Additionally, six putative candidate genes surrounding significant SNPs associated with number of tillers have annotations as F-box family protein, involved in plant vegetative and reproductive growth [36]. Further examples are given in Fig. 5 and Additional file 6. These results will provide useful information for future work and suggest that the likely gene families are important for plant architectural traits.

Discussion

Plant architecture related traits

In this study, we employed four multi-locus models: FASTmrMLM, FASTmrEMMA, mrMLM, and pLARmEB and three single-locus models i.e. FarmCPU, MLM, and MLMM to identify significant association with plant height, flag leaf length, flag leaf width, and number of tillers across multiple environments in hexaploid wheat. Plant height is a key factor in crop breeding as it plays a crucial role in reshaping plant architecture. In wheat, it greatly affects lodging and thus grain yield and quality [37]. Consequently, the identification of major dwarfing genes in green revolution to reduce plant height and improve yield was a major component in wheat breeding [38]. In this study, we identified significant SNPs across multiple years by different ML-GWAS approaches (Table 1-3, supplementary Fig. 1a-e). By integrated ML-GWAS results, a total of 24 stable SNPs consistently detected
by most of the ML-GWAS methods for PH (Table 1 and Fig. 4c). Most of these SNPs located on chromosome 5A and 7A. Earlier studies reported significant association signals with PH located on chromosome 5A and 7A [39-41]. It is revealed that chromosome 5A harboured the highest number of significant SNPs associated with plant architectural traits (Table 1). Several previous studies confirmed that chromosome 5A is the most useful and reproducible in wheat genome [42-45]. S Sukumaran, S Dreisigacker, M Lopes, P Chavez and MP Reynolds [22] evaluated spring wheat population for yield related traits and reported that most of the significant SNPs identified on chromosome 5A and 6A. Similarly, the SNPs on chromosomes 5A and 6A are most likely the MTAs reported previously [43, 46].

Among the four stable SNPs simultaneously detected across multiple environments and methods for PH (Table 3), two SNPs were located on chromosome 6B, revealing the significance of chromosome 6B which are consistent with the findings of [47-49] most of their significant SNPs located on chromosome 6B controlling FLW. Another SNP (Kukri_c34553_89) located on chromosome 2B with LOD ranging from 3.22-6.43, simultaneously detected in two environments by three ML-GWAS methods i.e. mrMLM, FASTmrMLM, and pLARmEB. J Chen, F Zhang, C Zhao, G Lv, C Sun, Y Pan, X Guo and F Chen [50] evaluated six quality related traits in Chinese wheat and detected Kukri_c34553_89 among the environment-stable SNPs and revealed the positive effects of the aforementioned SNP on harvest index across five environments. Furthermore, two consensus SNPs (RAC875_c8121_1490 and Ku_c5191_340), located on chromosome 3A and 6B respectively, significantly associated with plant height across multiple environments and methods. L Gao, G Zhao, D Huang and J Jia [51] constructed a selection map for domestication and improvement in wheat and reported both RAC875_c8121_1490 and Ku_c5191_340 in their study. Another candidate SNP (tplb0049a09_1302) located on
chromosome 5A, simultaneously detected by mrMLM and FASTmrMLM approaches and consistently repeated in two environments (Table 3). Similarly, tplb0049a09_1302 is reported earlier by Q-u Ain, A Rasheed, A Anwar, T Mahmood, M Imtiaz, Z He, X Xia and UM Quraishi [39], using 90K array to identify several genomic regions associated with yield related traits in historical wheat genotypes of Pakistan. Another SNP (BobWhite_c5694_1201) located on chromosome 4B is likely same to the QTL identified by J Zou, K Semagn, M Iqbal, H Chen, M Asif, A N’Diaye, A Navabi, E Perez-Lara, C Pozniak and R-C Yang [52], investigating the effect of 90K SNP array and QTL detection in a spring wheat population. For FLL three consensus SNPs were detected on chromosome 4B, 5A, and 6D (Table 1). F Li, W Wen, J Liu, Y Zhang, S Cao, Z He, A Rasheed, H Jin, C Zhang and J Yan [20] also reported significant SNPs associated with FLL on chromosome 5A and 6D. The SNP (BS00021881_51) associated with FLL simultaneously detected via two ML-GWAS approaches i.e. FASTmrMLM and pLARmEB was reported earlier in QTL mapping [53]. Two stable SNPs (BS00022127_51 and wsnp_BE499835B_Ta_2_5) associated with the number of tillers per plant corresponded to the previously reported SNPs in wheat [54, 55]. Among the stably detected SNPs for number of tillers, BS00022127_51 located on chromosome 7B, simultaneously detected by all four ML-GWAS methods (Table 1). F Li, W Wen, J Liu, Y Zhang, S Cao, Z He, A Rasheed, H Jin, C Zhang and J Yan [20] reported significant SNPs associated with number of tillers located on the same chromosomes 7B and 7D.

Significance Of Gwas Using High-density Genotyping

Sequencing larger data with new technology will provide the base to use high-density genotyping approach for quicker and cost-effective operations. Compared to tradition QTL mapping, GWAS has three major advantages: high resolution power for QTL identification, ability to detect more alleles, and less estimation time [56]. GWAS is mostly applicable in crop plants, due to its modern genotyping technologies, identification of novel alleles, and
improved statistical methods [56]. Crop genotyping has been a common approach since 1990s, but recently several improvements have been occurred in different types of polymorphisms and genotyping platforms [57]. Previously used methods depend on patterns of DNA digestion restriction and hybridization, randomly amplified PCR fragments, the advent of next generation sequencing (NGS) technologies enable genotyping in depth investigation with higher resolution [58, 59]. The most widely used NGS is single nucleotide polymorphisms (SNPs) which allow better detection power for markers associated with agronomic traits [60]. Previously genotyping efforts were not very effective in screening complex traits because the associated loci for desirable traits were not completely detected due to their week individual effects. The discovery of high-throughput genotyping and advanced bioinformatics tools now solve this issue by increasing the accuracy, while reducing the cost of genotyping [61, 62]. The application of Single Nucleotide Polymorphism (SNP) as a molecular marker provides better understanding of variation in an organism or individual part. Using SNPs provide the base for high throughput genotyping. Molecular markers are mostly used segregation in analysis, forensic examination, genetic mapping and diagnosis, and numerous biological applications [63–65]. Up to now, different genetic markers are being used in crop breeding but most of these are limited in their applications due to their unavailability and high cost of operation. SNPs are the most widely used markers having a wide range of applications in genome analysis [66, 67]

Combining strategy of SL and ML-GWASs can improve the power of GWAS

With the advent of molecular markers and genome studies, several association mapping approaches have been developed to reveal the genetic architecture of complex traits in crops [68, 69]. In earlier studies, mostly SL-GWAS methods were adopted to dissect complex traits, but only few SNPs for each trait have been identified due to its procedural
limitations. GLM has an obvious shortcoming of high false positive rate due to the absence of kinship among materials as covariate [70]. In MLM, due to the setting of very high threshold, many small-effect loci are missed [71]. To make up for the limitations of these methods, some multi-locus methodologies have recently been implemented, such as FASTmrMLM [72], FASTmr EMMA [73], mrMLM [71], and pLARmEB [74] are more effective approaches, which were used in this study. Using these models can improve the accuracy of SNPs with high detection power and less stringent criteria, which can effectively overcome the above issues. The most obvious advantage of these multi-locus models is that no Bonferroni multiple test correction is needed ([71, 73]. To study quantitative traits with the complex genetic background as the number of molecular markers is comparatively larger than sample sizes, it is recommended to simultaneously use multiple GWAS methods.

In the past ten years, several GWAS approaches have been used to identify significant SNPs, especially evaluating agronomic traits in common wheat (T. aestivum L.) traits using a single locus and two multi-locus approaches [75]. Conclusively, V Jaiswal, V Gahlaut, PK Meher, RR Mir, JP Jaiswal, AR Rao, HS Balyan and PK Gupta [75] verified that ML-GWAS has more detection power than SL-GWAS by revealing ten Marker Trait Associations (MTAs) through SL-GWAS while, 22 MTAs through multi locus mixed model (MLMM) and 58 MTAs through multi-trait mixed model (MTMM). A more recent study of Ward and his co-researchers utilized a conventional mixed linear model and recently developed FarmCPU approach to dissect the genetic architecture of yield related traits in winter wheat. Comparatively, FarmCPU detected 74 significant associations while, the single locus model only screened nine significant associations for different yield-related traits. Furthermore, FarmCPU approach is more complicated and less obvious to user than mix linear model, and hence more care should be taken during using FarmCPU algorithm [18]. Y Zhang, P
Liu, X Zhang, Q Zheng, M Chen, F Ge, Z Li, W Sun, Z Guan and T Liang [76] evaluated maize lines through a series of multi locus GWAS approaches to detect some novel loci responsible for lodging resistance. By comparing four multi-locus methods i.e. FASTmrEMMA, mrMLM, pLARmEB, and ISIS EM-BLASSO methods, it was confirmed that ISIS EM-BLASSO was the most effective approach for QTL identification [76]. The combination of two SL-GWAS and ML-GWAS methods contributes efficiently to detection of significant loci associated with pre-harvest sprouting tolerance in wheat [77]. SU Khan, J Yangmiao, S Liu, K Zhang, MHU Khan, Y Zhai, A Olalekan, C Fan and Y Zhou [68] combined SL and ML-GWAS approaches and revealed that ML-GWAS methods are more effective with high robustness and power of QTN detection than SL-GWAS in the genetic dissection of yield related traits of rapeseed genotypes. Li and his co-workers integrated the results of three single locus GWAS and three multi locus methods for fiber quality traits in upland cotton [78]. A total of 342 significant QTNs were detected of which 72 were consistently detected by at least two approaches or in at least two environments. According to C Li, Y Fu, R Sun, Y Wang and Q Wang [78], the power of QTN detection in association analysis can be improved by combining single locus and multi-locus GWASs.

In this study, we detected a total of 113 and 62 significant SNPs by ML-GWAS and SL-GWAS approaches, respectively. Furthermore, 19 SNPs co-detected by using ML-GWAS and SL-GWAS methods together (Additional file 5). A comparison of the four ML-GWAS methods revealed that pLARmEB was more powerful and robust [68], than the other three models in the detection significant SNPs for plant architectural traits. Through integrating the results of four ML-GWAS and three SL-GAWS methods led to the verification of the significance of ML-GWAS models. However, some recent findings revealed the reliability of association studies can be improved by combining single-locus and multi-locus GWAS approaches [78-81].
Conclusion

We applied four multi-locus and three single-locus GWAS models to investigate the complex genetic background of plant architectural traits (PH, FLL, FLW, and TILL) in hexaploid wheat. A total of 271 significant SNPs associated with plant architectural traits across multiple environments and in different methods were detected by employing four multi-locus and three single-locus methods. Comparatively, 174 and 97 significant association signals were detected by ML-GWAS and SL-GWAS models, respectively which signifies the importance of ML-GWAS over SL-GWAS approaches. Taken together, the results of ML-GWAS revealed five most stable SNPs i.e. Kukri_c34553_89, RAC875_c8121_1490, wsnp_Ex_rep_c66315_64480362, Ku_c5191_340, and tplb0049a09_1302 which were consistently detected across multiple environments and methods. In total, 152 putative candidate genes for three traits were identified, which will provide useful information for future work and highlight the role of the likely gene families for plant architectural traits.

Materials And Methods

Plant materials and phenotyping

A total of 319 wheat germplasm accessions from the collection at the Hubei Agricultural Science Institute in Hubei Province, China, which represent a wheat gene pool adapted to central China and the Yangzi River region, were performed on the experimental farm of Huazhong Agricultural University, Wuhan, China for consecutive four years (2015–2018). Twenty individuals from each variety (line) were grown in two rows with a distance of 15 cm between plants in each row and 20 cm between rows. Field management essentially followed normal local wheat cropping practices. The lines were harvested individually at maturity to prevent seed contamination among lines. Four phenotypic traits were
evaluated, including plant height (PH) across four environments and the rest of three traits i.e. flag leaf length (FLL), flag leaf width (FLW), and the number of tiller per plant (TILL) across two environments. Plant height was measured after physiological maturity by measuring the distance between the stem base and the top of the spike excluding awns. Flag leaf length was measured as the distance from the base to the tip of the leaf. Flag leaf width as the width of the widest section of the leaf. Number of tiller was recorded by counting the total number of fertile tillers per plant.

**Genotyping**

A total of 319 wheat accessions were genotyped using the Illumina iSelect 90K SNP array [31]. Genotyping was performed by Beijing Compass Technology & investment Co. Ltd (http://www.bjcompass.com/), using the 90K wheat genotyping assay [31], following the manufacturer’s protocol. A quality preprocessing of genotyping data was done for sample call rate, SNP call rate, minor allele frequency (MAF) and Hardy–Weinberg equilibrium (HWE). This preprocessing was implemented in PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) [82].

**Statistical Analysis**

Descriptive analysis, ANOVA, correlation analysis and heritability estimates were conducted in the R statistical package [83]. The broad sense heritability for the traits was estimated by the formula $H^2 = VG/(VG + VE)$ where VG and VE represent estimates of genetic and environmental variance, respectively [84].

**Population Structure And Kinship Analysis**

Population structure using a Bayesian cluster analysis was estimated by STRUCTURE HARVESTER software [85]. A putative number of subpopulations ranging from $k = 1$ to 7 was assessed using 100,000 burn-in iterations followed by 500,000 recorded Markov-Chain
iterations. To estimate the sampling variance (robustness) of inferred population structure, 10 independent runs were carried out for each k. K was estimated using an ad-hoc statistic ΔK based on the rate of change in log probability of data between successive values [32]. Principle component analysis (PCA) was calculated by R software for evaluating the population structure and compared to the result of STRUCTURE [25]. Linkage disequilibrium (LD) among markers was calculated using observed vs. expected allele frequencies of the markers in TASSEL v.5.0 [39].

Genome-wide Association Studies

In this study, we used mrMLM software for four ML-GWAS (FASTmrMLM, FASTmrEMMA, mrMLM, and pLARmEB) and three SL-GWAS (FarmCPU, MLM, and MLMM) implemented by Genomic Association and Prediction Integrated Tool (GAPIT) in R [86]. Previously, SL-GWAS methods were mostly applied such as GLM and MLM. However, single-locus approaches have some limitations such as GLM leads to high false-positive rates (FPRs), while MLM utilizes Bonferroni corrections for loci detection to reduce the FPRs [87]. Though, this procedure is so stringent that results in missing significant SNPs [71]. Therefore, multi-locus GWAS approaches are the best alternatives. The stringent Bonferroni multiple test correction in the SL-GWAS analysis is substituted by a flexible selection criterion in multi-locus GWAS analysis, that reduces the possibility of missing out significant loci [71, 73]. The four ML-GWAS methods were performed with default parameters, and the screening criteria for significance were set with LOD scores 3 or > 3 [71, 73, 74, 88, 89]. However, for SL-GWAS models, the threshold for P-value was calculated based on the number of the markers (P = 1/n, n = total SNP used) according to the method of [90]. Significant markers were visualized with a Manhattan plot using Haploview 4.2 software [91]. Important p-value distributions (expected vs. observed p-values on a -log^{10} scale) were shown with a
quantile-quantile plot.

**Candidate Gene Analysis**

Candidate gene sites were aligned and downloaded from the ViroBLAST database (https://urgi.versailles.inra.fr/blast/docs/aboutviroblast.html). The R Package Pathway Association Study Tool (PAST) version 1.0.1 was used to identify genes around the peak SNPs with a window size of 200 kb. To find candidate genes or putative related proteins of SNP flanking-regions, BLASTx search was conducted for significant marker-trait associations (MTAs) against recently released genome sequence IWGSC RefSeq v1.0 [92].

**Declarations**

**Abbreviations**

GWAS: Genome-Wide Association Study; ML: Multi-locus; SL: Single-locus; SNP: Single Nucleotide Polymorphism; MAS: Marker-Assisted Selection; QTL: Quantitative Trait Loci; MTA: Marker-Trait Associations; PH: Plant Height; FLL: Flag Leaf Length; FLW: Flag Leaf Width; TILL: Tillers; CV: Coefficient of Variation; GAPIT: Genomic Association and Prediction Integrated Tool; NGS: Next Generation Sequencing; MAF: Minor Allele Frequency; MTAs: Marker-trait associations; HWE: Hardy–Weinberg Equilibrium; ANOVA: Analysis of Variance; LD: Linkage disequilibrium.

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**Authors’ contributions**

JW and LW conceived and designed the project, AM and WH performed the experiment, JY and SUK analyzed and interpreted the data, AM and GX drafted and revised the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing Interests**

The authors declare that they have no competing interests.

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**Supplementary Files Legend**

**Additional file 1.** Descriptive statistics of plant architectural traits across multiple
environments.

**Additional file 2.** Analysis of Variance (ANOVA) and heritability for plant height, flag leaf length, flag leaf width and no of tillers per plant.

**Additional file 3.** Significant SNPs detected through SL-GWAS method across different environments and methods.

**Additional file 4.** List of pleiotropic SNPs associated with more than one trait.

**Additional file 5.** Common SNPS detected through ML-GAWS and SL-GWAS methods.

**Additional file 6.** Summary of potential candidate genes identified for plant architectural traits.

**Additional file 7(a-e).** Manhattan and quantile-quantile (Q-Q) plots for plant architectural traits across different environments; **(a & b)** Manhattan and Q-Q plots for Plant height (2015-2018); **(c)** Manhattan and Q-Q plots for flag leaf length (2017-2018); **(d)** Manhattan and Q-Q plots for flag leaf width (2017-2018); **(e)** Manhattan and Q-Q plots for number of tillers (2017-2018).

**Figures**
Figure 1

Phenotype distributions for plant architectural traits (a) Plant height across four environments (2015, 2016, 2017, and 2018); (b) Flag leaf length (2017 and 2018); (c) Flag leaf width (2017 and 2018); (d) Number of tillers per plant (2017 and 2018).
Figure 2

Correlation among plant architectural traits across multiple environments. PH-Plant height (2015-2018), FLL-Flag leaf length (2017-2018), FLW-Flag leaf width (2017-2018) and TILL-Number of tillers per plant (2017-2018). * and ** indicate significant correlation at P < 0.05 and 0.01, respectively.
Figure 3

Population structure analysis of 319 wheat varieties based on unlinked SNP markers. (a) plot of ΔK value with putative k ranging from 2 to 6. (b) two major subpopulations k=2, indicated by two colors (red and green) represent the proportion of each subpopulation. (c) analysis of principal component analysis of the genotypic data.
Significant SNPs detected through different GWAS methods in multiple environments. The traits include PH (Plant height); FLL (Flag leaf length); FLW (Flag leaf width) and TILL (Number of tillers per plant). (a) Significant SNPs detected via ML-GWAS and SL-GWAS methods, (b) Significant SNPs detected through four ML-GWAS methods i.e. FASTmrMLM, FASTmrEMMA, mrMLM, and pLARmEB, (c) Significant SNPs detected across multiple years and different ML-GWAS methods, (d) Significant SNPs detected through three SL-GWAS methods i.e. FarmCPU, MLM, and MLMM.
Putative candidate genes responsible for important functions associated with plant architectural traits. PH (Plant height); FLL (Flag leaf length); TILL (Number of tillers per plant).

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

Additional file 7a-e. Manhattan and Q-Q plots.pptx
Additional file 1-5.docx
Additional file 6. Candidate genes.xlsx