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GENOME-WIDE ASSOCIATION STUDY OF YUNNAN-SPECIFIC WHEAT VARIETIES UNDER CONDITIONS OF DROUGHT STRESS

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Abstract

Drought is associated with serious declines in crop yield. In this study, we investigated and analyzed the effect of drought on wheat yields. We considered 263,742 single nucleotide polymorphisms (SNPs) obtained from genotyping-by-sequencing (GBS) and a genome-wide association study (GWAS) using PH (plant height), DTM (days to maturity), TGW (thousand grain weight), NGS (number of grains per spike), EL (ear length), FLL (flag leaf length), and FLW (flag leaf width) as indices. We identified 60 significant SNPs associated with EL, 41 with PH, 24 with DTM, 17 with FLL, 5 each with NGS and FLW, and 3 with TGW. We further identified 77 putative and potential genes within 100 kb from the major SNPs. We found that these genes encode transcription factors and hormones and are involved in enzyme activity, transmembrane transport, signal transduction, epidermal wax synthesis, metabolism, growth and development, pigment, stomata, root hairs, and stress tolerance. Collectively, our results provide valuable insights into the genetic basis of drought resistance in bread wheat. Further studies that predict candidate genes will help interpret the drought resistance mechanism of wheat, and aid the selection and breeding of drought-resistant wheat varieties suitable for different environmental conditions.

Key words: Wheat, Drought stress, GWAS, Gene prediction.

Introduction

Wheat is a crucial food crop that has considerable value in meeting the global food security needs (Curtis & Halford, 2014). It is a major source of carbohydrates and protein worldwide; the demand for wheat is increasing with the global population. Consequently, wheat production must increase to meet the long-term strategic agricultural production needs ((Maulana *et al*., 2018). China is both the largest producer and consumer of wheat worldwide. Drought poses a challenge to economic development and food security as it directly limits the growth and yield of staple crops like wheat (Mupangwa *et al*., 2008). Plant growth or survival depends largely on water availability; drought affects crop growth and development, thus limiting yield and gains in yield (Kumar *et al*., 2018). Global warming is resulting in decreased rainfall and increased water consumption, thereby increasing the severity of droughts worldwide. Drought decreases plant yields by up to 50%, causing substantial losses in crop yield and economic damage with in terms of agricultural production (Akpinar *et al*., 2013). Wheat is vulnerable to changes in rainfall patterns, increased temperatures, and the co-occurrence of drought and heat stress. Drought-resistant breeding is the key to improving the sustainable production of wheat; therefore, breeding drought-tolerant wheat varieties has become a priority to ensure food security (Budak *et al*., 2013). The phenomenon of drought tolerance involves many genes, transcription factors, hormones, and proteins, and by extension, so do the desired trait(s). This complexity limits the use of traditional breeding methods for cultivating drought-tolerant wheat varieties (Budak *et al*., 2015; Wang & Qin, 2017). Although these established strategies improve wheat resistance, yield, and quality, the total annual yield increase is < 1% (Sukumaran *et al*., 2018a).

Numerous attempts have been made to improve drought resistance through the conventional breeding of crop varieties, including wheat. In recent years, numerous molecular marker–assisted selection techniques (related to drought genes) have been reported. Results showed that the synthetic hexaploid wheat developed by crossing *Aegilops tauschii* with bread wheat (*Triticum aestivum* L.) carried alleles that conferred tolerance to various biotic and abiotic stresses (Ogbonnaya *et al*., 2013). Synthetic hexaploid wheat and its specific derivatives exhibit the potential to adapt to drought and heat stress (Afzal *et al*., 2017) and achieve higher yields (Jafar *et al*., 2016) under conditions of drought stress. Hence, traditional breeding methods that use original gene pools with superior genetic variation to promote genetic improvements in wheat have achieved great success. Several studies have focused on mapping genomic loci and quantitative trait loci (QTLs) that influence various major agronomic traits to facilitate molecular marker–assisted selection to affect yield increase in wheat experiencing increasing drought and other severe yield–reduction stresses. (Pinto *et al*., 2010; Alexander *et al*., 2012) Many markers, genes, and QTLs associated with traits controlled by multiple genes have been identified on the 21 chromosome pairs in bread wheat. These genomic resources are important for understanding the genetic mechanisms underlying drought tolerance and other limiting factors in polyploid economic crops (Edae *et al*., 2014; Sukumaran *et al*., 2015).

Although marker-assisted breeding is widely used in breeding, it does not play an important role in improving wheat varieties and adaptations to complex and variable environments; instead, breeding relies heavily on direct phenotypic identification to improve the performance of wheat plants in harsh environments (Fleury *et al*., 2010). Therefore, marker-assisted selection has a relatively small effect with respect to improving drought resistance in crops. This "small" effect is likely attributed to a complex genetic model, a strong genotype-environment interaction, and low heritability under conditions of drought stress (Abou-Elwafa, 2016; Noorka & Tabasum, 2015). Moreover, the general QTL method can only identify low-resolution genomics and is limited to biparental populations. In recent years, the rapid development of genotypic and phenotypic techniques has contributed greatly to our understanding of the complex physiological and genetic mechanisms underlying polygenic-controlled traits such as drought tolerance (Sukumaran *et al*., 2018b). Wheat genome-wide association studies (GWAS) have become increasingly valuable and focus on the yield and related traits (Liu *et al*., 2017; Sanu *et al*., 2017). The goal of GWAS is to identify markers, QTLs, and genes associated with key agronomic traits and to provide guidance for molecular marker–assisted breeding, gene mining, and gene penetration (Edae *et al*., 2014). In recent years, several high-throughput single-nucleotide polymorphism (SNP) arrays—9 K (Cavanagh *et al*., 2013), 35 K (Allen *et al*., 2016), 90 K (Wang *et al*., 2014), 660 K (Cui *et al*., 2017), 820 K (Winfield *et al*., 2016), and TaBW280K (Rimbert *et al*., 2018)—have been extensively used for identifying marker SNPs for use in wheat breeding. The identified SNPs have been used in the following GWAS: European spring wheat and winter wheat, International Maize and Wheat Improvement Center spring wheat, American elite wheat, wheat genotypes in Kazakhstan and Russia, and Chinese bread wheat varieties (Zanke *et al*., 2014; Sukumaran *et al*., 2015; Lin *et al*., 2016; Sun *et al*., 2017; Turuspekov *et al*., 2017). Rahimi et al. (Rahimi *et al*., 2019) used GWAS on 298 Iranian bread wheat varieties and landraces to investigate and analyze the genetic basis of agronomic traits for two seasons using 10,938 SNPs under normal water and rain irrigation conditions. Similarly, Jamil et al. (Jamil *et al*., 2019) conducted precise GWAS to classify chromosomal regions associated with various agronomic traits and yield traits. Qaseem et al. (Qaseem *et al*., 2019) identified marker-trait associations (MTAs) on all wheat chromosomes under conditions of irrigation and drought. MTAs for drought tolerance have been found on different varieties of wheat chromosomes, such as 1A, 2A, 4A, 5A, 2B, 7B, 2D, 3D, and 4D (Sukumaran *et al*., 2018b; Gahlaut *et al*., 2019; Mathew *et al*., 2019). Yield and yield-related traits assessed under drought conditions were used to identify QTLs associated with drought resistance in wheat (Bhatta *et al*., 2018; Zhang *et al*., 2018). Among the key agronomic traits of dry land cereal crops—especially in water-deficient environments—decrease in plant height (PH) is closely related to harvest index (Asif & Kamran, 2011). Yield components related to drought screening included the number of effective tillers, number of spikelets per spike, number of grains per spike (NGS), and thousand-grain weight (TGW). The strategy of reducing the number of days to heading and maturity during breeding significantly helps avoid the negative impacts of drought (Lopes *et al*., 2012). Appropriate PH, ear length (EL), and other agronomic traits also increase grain yield (Zhang *et al*., 2016). Chromosome regions 1B, 2B, 5A, 5B, and 6B have been reported to be associated with PH, yield, and drought resistance (Mathews *et al*., 2008; Pinto *et al*., 2010; Alexander *et al*., 2012). These reports have focused on identifying and dissecting the genetic basis of quantitative traits —including drought tolerance—through association analysis of genomic region mapping and agronomic traits (Tuberosa & Salvi, 2006; Tester & Langridge, 2010; Bevan *et al*., 2017).

Consequently, the comprehensive identification of a large number of QTLs by GWAS can help identify alleles or haplotypes that may be uncommon in crop varieties yet may be incorporated into the existing pool of drought resistance–related genes (Bevan *et al*., 2017). In the present study, the main wheat varieties and excellent germplasm resources of Yunnan Province were subjected to paired-end (PE)-150 sequencing using the Illumina HiSeq sequencing platform (Illumina, San Diego, CA, USA) for GWAS. We identified SNPs related to drought resistance traits, such as days to maturity (DTM), PH, TGW, NGS, EL, flag leaf width (FLW), and flag leaf length (FLL). Our results provide a basis for selective genomic breeding.

Materials and methods

Plant materials and field trials: We used a total of 335 materials provided by the Institute of Biotechnology and Germplasm Resources, Yunnan Academy of Agricultural Sciences (Kunming, China). The main germplasm resources were local varieties/lines (265), *Triticum aestivum* ssp. *yunnanense* King (46), and varieties bred (24) represented the wheat varieties and lines cultivated in Yunnan. The material was planted at the Institute of Agricultural Sciences of Baoshan, Yunnan Province (98°43′ E, 25°38′ N). Each material was seeded with 50 grains per row with a row length of 2 m, row spacing of 0.3 m, and plant spacing of 4 cm. Moisture management was provided with two treatments, three replicates each, i.e., drought stress (DS) and well-watered (WW). A protective row was established around the planted rows. Irrigation was carried out early on to ensure that both treatments impacted growth early, and the relative water content was controlled at 80% ± 5% of the field water capacity. Irrigation was based on the method described by Ali (Ali, 2011). The WW test group was irrigated when the soil water content reached 40–50%, and DS irrigation occurred when the soil capacity reached 25–30% (water consumption in this latter group was approximately 60% of the WW test group). The water content in the DS group was limited to 35% of the field water capacity starting at the heading period (Zadoks *et al*., 2010). In the WW group, irrigation continued uninterrupted until maturity. The soil in the experimental area was covered with a special rainproof system to reduce the influence of rainwater on the experiment. All ecological conditions, including light, day, and night temperature, relative humidity, sowing methods, field management, and data acquisition, were constant throughout the experiment.

Field data collection and phenotypic data analysis: Three plants with robust and consistent growth were selected from the middle of each row. The following traits were observed based on their average values: PH from base to top of the spike at maturity (excluding awn); EL (excluding awn); NGS, seed number per ear; FLL tip to ear; FLW at the middle; and thousand kernel weight (TKW) as the weight of 1000 seeds measured with an electronic balance at maturity. Days to maturity (DTM) were calculated by the number of days taken from sowing until all spikes turned to their specific color. Phenotypic data were analyzed using Excel 2013 to calculate the average value (AV), standard deviation (SD), and coefficient of variation (CV) of each trait in the two water treatments. Significance and correlation analyses were conducted using SPSS Version 25.0 (IBM Corp., Armonk, NY, USA).

DNA extraction and genotypic data analysis: Genomic DNA was extracted from mixed leaves of three-leaf seedlings using the CTAB (cetyltrimethylammonium bromide) method, performed using plant DNA kits (All-Style Gold Biotechnology Co., Beijing, China). First, agarose gel electrophoresis was used to analyze the integrity of the DNA, which was further assessed for purity by NanoDrop™ (Thermo Fisher Scientific, Waltham, MA, USA). Then, Qubit was used to quantify the DNA. The quality of the sequencing data was controlled; high-quality sequencing data were compared with the reference genome (161010_Chinese_Spring_v1.0 _pseudomolecules.fa) using BWA (mem-t4-k32-m) (Li & Durbin, 2009).

Genotypic data from 335 wheat germplasms were obtained using the genotyping-by-sequencing (GBS) technique (Elshire *et al*., 2011). In total, 6 988 290 SNPs were detected using Samtools (Li *et al*., 2009). After filtering with dp (3), Miss (0.4), and MAF (0.05), 263 742 high-quality SNPs were obtained for a more in-depth analysis. These SNPs were used to annotate gene variations detected in multiple genomes using the ANNOVAR software (Wang *et al*., 2010).

Population analysis: After SNP detection, the individual SNPs were used to calculate the distance between populations. The formula for the p-distance between two individuals, i and j, is

$$D_{ij} = \frac{1}{L}\sum_{l=1}^{L} d_{ij}^{(l)}$$

where L is the length of a high-quality SNP region.

TreeBest (http://treesoft.sourceforge.net.shtml) was used to calculate the distance matrix. Based on this matrix, a phylogenetic tree was constructed using the neighbor-joining method. Bootstrap values were obtained after up to 1000 calculations. GCTA (http://cnsgenomics.com/software/gcta/pca.html) was used to calculate eigenvectors and eigenvalues and R software PCA (principal component analysis) was used for mapping. First, the Plink-PED (Chang *et al*., 2015) input file was created, then the genetic structure and pedigree information of the population were constructed using the Admixture software (Alexander *et al*., 2009).

GWAS: Before GWAS, phenotypic data were preprocessed by marking and eliminating data larger than three standard deviations as outliers. A mixed linear model (MLM) (Yu *et al*., 2006) was used to analyze the association of traits. The population genetic structure was a fixed effect and individual relatedness was a random effect. To adjust for the effects of population structure and individual relatedness, we used the following formula:

$$y = X\alpha + Z\beta + W\mu + e$$

where y is the phenotypic trait, X is the indicative matrix of the fixed effect, α is the estimation parameter of the fixed effect, Z is the indicator matrix of the SNP, β is the effect of the SNP, W is the indicator matrix of random effect, μ is the predicted random individual, and e is the random residual; compliance to $e \sim (0, \delta_e^2)$.

At the same time, GEMMA (http://www.xzlab.org/software.html) correlation analysis was conducted for the related traits of different populations based on the correlation of significance (p-value) and potential candidate SNPs were screened. For GWAS results, when $-\log_{10}(P) = 5$, the SNP was considered to be significantly associated with the trait and was represented in Manhattan plots (MP) and quantile-quantile (Q-Q) plots.

Candidate gene identification: A BLASTx search was conducted against the International Wheat Genome Sequencing Consortium (IWGSC) RefSeq v1.0 to identify candidate genes and their functional annotation (Bellec *et al*., 2018). Using National Center for Biotechnology Information (NCBI), genes or proteins with SNP marker DNA sequences similar to those that impart drought-related traits were screened out to investigate potential functions, if any, and to prepare a list of potential candidate genes for future verification.

Results

Phenotypic variation and correlation analysis: The frequency distributions of the seven traits measured in the population are shown in Figure S1. The results revealed that EL, TGW, PH, NGS, FLL, FLW, and DTM were either normally or nearly normally distributed, indicating that these characteristics are complex quantitative traits regulated by multiple genes and are suitable for identifying SNPs. Analysis of variance (ANOVA) (Table 1) showed that DTM and NGS were not significantly different between WW and DS, but PH, EL, FLL, FLW, and TGW decreased significantly. Comparison of the CVs in the WW and DS environments indicated that the CVs for all indices in both environments were more than 10%, except for DTM. In the WW environment, CVs ranged from 13.52% to 25.92%. In contrast, CVs ranged from 15.05% to 30.78% in the DS environment, indicating that the growth and development of all characteristics were affected by drought stress. Based on the correlation analysis of the shapes (Table 2), it was found that, under WW and DS conditions, the relationships among all characteristics, except for FLL and PH, were significantly correlated. Under WW conditions, there were 18 pairs of significantly correlated characteristics, while under DS conditions, there were 19 pairs of significantly correlated traits.

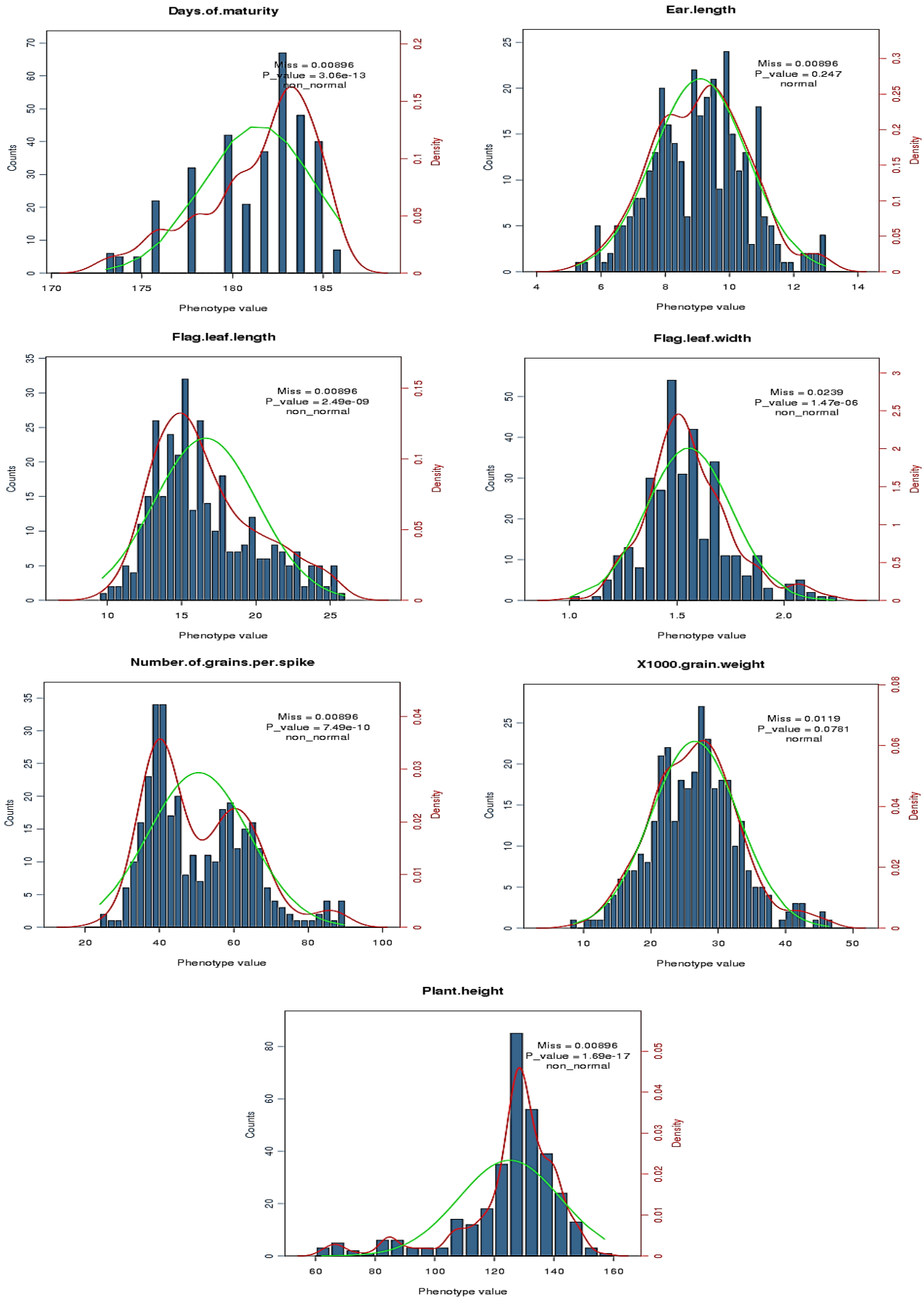

Fig. S1. Frequency distribution of observed traits.(A, Days of maturity; B, Ear length; C, Flag leaf length; D, Flag leaf width; E, Number of grains per spike; F, Plant height; G, X1000 grain weight).

Table 1. Performance of drought tolerance-related traits of used in this study.

Trait	Well-watered				Drought stress				DS/WW
	Range	Mean	SD	CV%	Range	Mean	SD	CV%	
DTM	173-188	182.40	3.13	1.74	172-186	181.00	3.13	1.73	0.99
EL	6.17-13.33	9.47	1.41	14.91	3.73-12.17	7.61	1.49	19.53	0.8235**
FLL	9.90-27.33	17.09	3.48	20.35	6.67-24.33	13.65	3.52	25.78	0.8218**
FLW	1.17-2.53	1.62	0.22	13.52	0.5-1.93	1.07	0.22	20.99	0.6618**
NGS	24-99	51.00	13.81	27.25	23-98	49.68	13.83	27.80	0.97
PH	62.33-159.67	122.95	17.97	14.62	50-145	113.19	17.03	15.05	0.9219**
TGW	9.29-51.28	27.59	7.15	25.92	3.49-41.92	21.63	6.66	30.78	0.8315**

Note: PH, Plant height; EL, Ear length; NGS, Number of grains per spike; FLL, Flag leaf length; FLW, Flag leaf width; TGW, X1000 grain weight; DTM, Days of maturity; SD, Standard deviation; CV, Coefficient of variation

Table 2. Correlation matrix for all traits under drought stress (DS, above diagonal) and well-watered conditions (WW, under diagonal).

Trait	DTM	EL	FLL	FLW	NGS	PH	TGW
DTM	1.00	0.45**	0.22**	0.32**	-0.53**	-0.51**	0.42**
EL	0.33**	1.00	0.12*	0.27**	-0.34**	-0.36**	0.34**
FLL	0.24**	0.04	1.00	0.21**	0.07	0.08	0.17**
FLW	0.35**	0.30**	0.18**	1.00	-0.26**	-0.43**	0.44**
NGS	-0.21**	-0.36**	-0.05	-0.29**	1.00	0.61**	-0.45**
PH	-0.46**	-0.36**	0.00	-0.45**	0.50**	1.00	-0.36**
TGW	0.32**	0.39**	0.16**	0.48**	-0.39**	-0.34**	1.00

Note: PH, Plant height; EL, Ear length; NGS, Number of grains per spike; FLL, Flag leaf length; FLW, Flag leaf width; TGW, X1000 grain weight; DTM, Days of maturity *, ** Significant at $p \leq 0.05$, 0.01, respectively

Genotypic identification and population stratification analysis: The sequencing data were filtered to obtain high-quality clean data. The final sequencing data volume was 623.073 Gb, with an average of 1.860 Gb per sample. The sequence quality was high, and the GC distribution did not reveal any abnormalities. The average comparison rate of the population samples was 99.62%. The similarity of the comparison results of the reference genomes for each sample met the analysis requirements and had good depth and degree of coverage. A total of 263,742 high-quality SNPs were screened for subsequent analyses. The SNP annotation results are shown in Figure S2. The majority of the variations were found in the intergenic regions (97.7787%). The percentage of variation at splice sites (2 bp in introns near exon/intron boundaries) was the lowest (4×10^{-4}%), and a genome-wide transition/ transversion (Ts/Tv) ratio of 2.38 was observed. The chromosomal distribution of these SNP loci was not uniform; most were concentrated in the A (47%) and B genomes (47%). Some were present in the D genome (5%), and 1% were not located in the genome (Fig. S3). Using the filtered SNPs to analyze phylogenetic relationships among 335 wheat lines to construct a phylogenetic tree and PCA maps, we attempted to determine the genetic diversity and background of the materials. We constructed a phylogenetic tree based on the SNPs detected (Fig. S4A). Most of the wheat from the same region in Yunnan Province converged, yet some of the materials originally from the northwest and southwest also converged, as did the materials from the northeast and the northwest. Nearly half of the materials were obtained from southwestern Yunnan, while the remainder were obtained from the northeast, southeast, south, northwest, southwest, and five central regions, essentially the same sources as for our materials. PCA (Fig. S4B) also confirmed this result.

Association analysis: Based on GWAS, we screened 155 SNPs with significant phenotypic associations ($-\log_{10}(p) > 5$). These results are shown in Fig. 1, Fig. S5, and Table 3. Sixty SNPs were associated with EL, 41 with PH, 24 with DTM, and 17 with FLL. The minimum numbers of NGS, FLW, and TGW SNPs were five, five, and three, respectively. Significant SNPs of EL were located on chromosomes 1A, 3A, 5A, 2B, 5B, and 2D; up to 30 were located on 5A. PH SNPs were located on chromosomes 2A, 4A, 6A, 7A, 1B, 3B, and 6B, with a maximum of 18 on chromosome 7A. DTM SNPs were located on chromosomes 2A, 3A, 7A, 2B, and 3B, with a maximum of nine on chromosome 3B. FLL SNPs were located on chromosomes 3B, 4A, and 7D, with a maximum of 12 on chromosome 4A. The five NGS SNPs were located on chromosome 3B, five FLW SNPs were located on chromosome 7B, and one TGW SNP was located on chromosomes 1B, 5B, and 7A. It is worth noting that $-\log_{10}(p) > 6$ for the DTM SNPs on chromosome 3 (PeakSNP 102343044), EL SNPs on chromosome 5A (PeakSNP 649811563), FLL SNPs on chromosome 4A (PeakSNP 181875054), PH SNPs on chromosome 3B (PeakSNP 556322650 and PeakSNP 574788489), and PH SNPs on chromosomes 6B (PeakSNP 121202054) and 7A (PeakSNP 15214073). Of these, $-\log_{10}(p) > 7$ for the PH SNPs on chromosome 7A (PeakSNP 15214073). These loci should be investigated further for the presence of possible drought-resistance genes. We also found that the distribution of these significantly associated SNPs in the A, B, and D genomes was 98 (63%), 47 (30%), and 10 (7%), respectively (Fig. 2). At the chromosomal level, 5A had the most SNPs (30) and 1A, 6A, 6B, and 7D had the least (one each). It should be noted that there was no significant association of SNP sites on chromosomes 1D, 3D, 4B, 4D, 5D, and 6D.

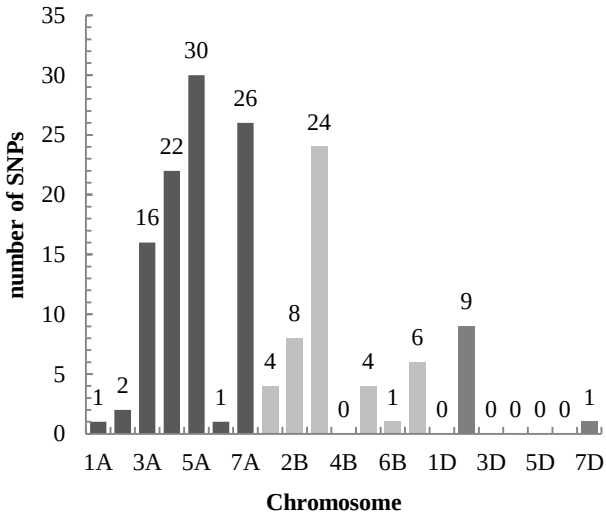

Fig. 2. The distribution of significant SNPs across different wheat genomes and chromosomes.

Prediction of significant association sites and candidate genes: We retained a total of 77 putative and potential genes within 100 kb of the most significant SNP sites (Table 4). We found that these genes mainly encoded transcription factors or hormones and were involved in enzyme activity, transmembrane transport, signal transduction, cuticular wax synthesis, metabolism, growth and development, pigment, stomata, root hairs, and stress-tolerance. Among these, 37 were EL-related (2B4, 2D9, 3A11, 5A10, 5B3), followed by 17 for PH (1B3, 3B5, 4A3, 7A5, 7B1), eight for DTM (2B4, 3A3, 7A1), five for FLW (7B5), five for NGS (3B5), four for FLL (3B4), and one for TGW (5B1). These genes were distributed both upstream and downstream of significant SNP sites. Generally, we believe that higher feasibility genes are located closer to significant SNP sites. For example, TRAESCS2B01G551700 (–2.819) on chromosome 2B, TRAESCS2D01G039800 (5.046) on chromosome 2D, TRAESCS3B01G507100 (3.676) on chromosome 3B, TRAESCS3B01G236000 (3.53) and TRAESCS3B01G 236100 (–2.632) on chromosome 3B, TRAESCS5A01 G473600 (4.585) and TRAESCS5A01G473800 (–8.496) on chromosome 5A, TRAESCS7B01G431700 (9.535) and TRAESCS7B01G431800 (1.443) on chromosome 7B. All these genes are located very close to significant SNPs. It is worth noting that $-\log_{10}(p) > 6$ for EL genes TRAESCS5A01G473400, TRAESCS5A01G473500, and TRAESCS5A01G473600 on chromosome 5A and PH genes TRAESCS3B01G346600, TRAESCS3B01 G346700, TRAESCS3B01G346800, TRAESCS3B01G 363300, and TRAESCS3B01G363400 on chromosome 3B. For PH genes TRAESCS7A01G034500, TRAESCS7 A01G034600, TRAESCS7A01G034700, and TRAESCS7 A01G034800 on chromosome 7A, $-\log_{10}(p) > 7$. Most notably, TRAESCS7A01G271700 was associated with both DTM and PH. Of all the genes associated with drought-related traits, most had only one significant SNP targeted to one gene, but EL (TRAESCS3A01G333200, TRAESCS5A01G473400, TRAESCS5A01G473500, TRAESCS5A01G473600, TRAESCS5A01G473700, TRAESCS5A01G473800, TRAESCS5A01G473900, TRAESCS5A01G474000, TRAESCS5A01G474100, TRAESCS5A01G474200) and PH (TRAESCS7A01 G034500, TRAESCS7A01G034600) had two or more significant SNPs. Six SNPs of EL TRAESCS5A 01G473700 may be located in this gene and four SNPs of TRAESCS5A01G473400, TRAESCS5A01G473500, and TRAESCS5A01G473600 were also localized in this gene. The genes mentioned above should be the focus of further study.

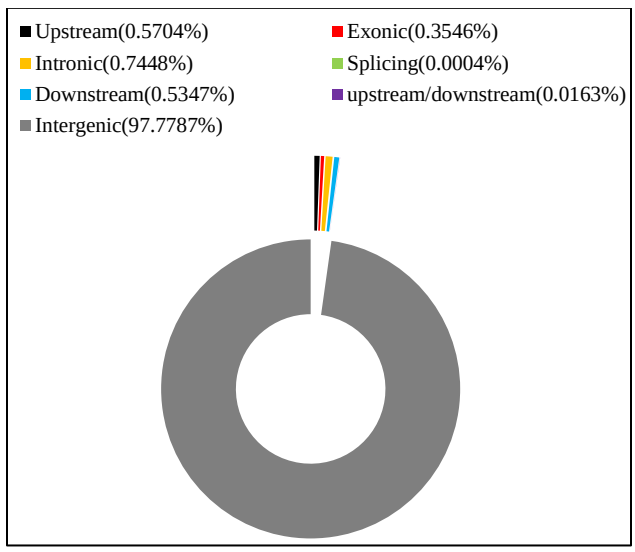

Fig. S2. Annotation results of polymorphic SNPs.
Notes: Upstream: gene upstream 1 Kb region. Exonic: mutation located in the exonic region. Intronic: variation located in the intronic region. Splicing: mutation occurs at the splicing site (2bp near the exon/intron boundary in the intron). Downstream: gene downstream 1 Kb region. Upstream/ Downstream: Upstream 1 kb of a gene and downstream 1 kb of another gene.

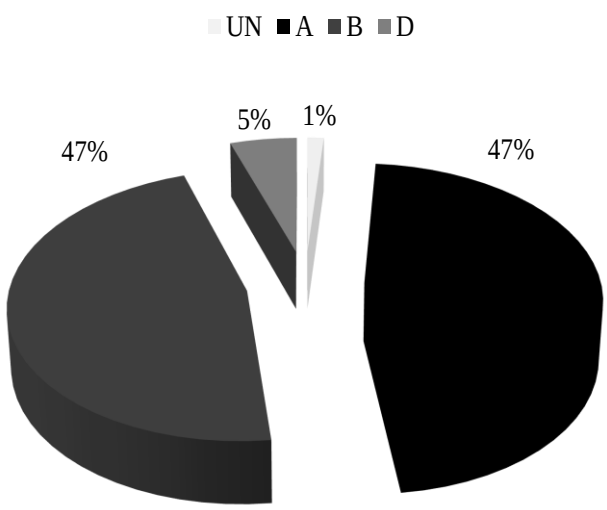

Fig. S3. Distribution of polymorphic SNPs on different wheat genomes. UN, Unknown; A, A genomes; B, B genomes; D, D genomes.

Table 3. Summary of significant SNPs with −log10(P) > 5.

Trait	Chr	Start	End	Length_Signal(bp)	Num_snp	Peak SNP	–Log10 (p)	Ref Base	Alt Base	MAF
DTM	chr2A	224994027	225194027	200001	1	225094027	5.23422642	T	C	0.075099
	chr2B	747718350	747918350	200001	4	747818350	5.2779877	C	G	0.144788
	chr3A	248095218	248295218	200001	1	248195218	5.1817914	A	C	0.127358
	chr3A	375656532	375856532	200001	2	375756532	5.17519584	A	C	0.145105
	chr3B	102243044	102443190	200147	3	102343044	6.45122953	T	C	0.105351
	chr3B	105565290	105840779	275490	5	105740779	5.4066396	C	T	0.056054
	chr3B	216590530	216790530	200001	1	216690530	5.60289603	C	T	0.231518
	chr7A	273542466	273742466	200001	1	273642466	5.04128077	A	G	0.052941
	chr7A	279393451	279593649	200199	3	279493649	5.57430588	T	A	0.059353
	chr7A	283521333	283721426	200094	2	283621333	5.11841417	C	T	0.072816
	chr7A	284863485	285063485	200001	1	284963485	5.37699419	G	A	0.063934
EL	chr1A	259635073	259835073	200001	1	259735073	5.18067313	A	G	0.069132
	chr2B	217377990	217577990	200001	4	217477990	5.81902616	T	C	0.146341
	chr2D	14348864	14548864	200001	9	14448864	5.31277711	T	C	0.406746
	chr3A	9800047	10000047	200001	10	9900047	5.57876373	C	T	0.137993
	chr3A	226499227	226699227	200001	1	226599227	5.75625673	T	C	0.198962
	chr3A	577632852	577832985	200134	2	577732852	5.11713931	G	A	0.255172
	chr5A	647854932	648054932	200001	1	647954932	5.02255041	T	A	0.121495
	chr5A	649711563	649911669	200107	12	649811563	6.33047672	T	C	0.113924
	chr5A	649843747	650043927	200181	4	649943919	5.79843793	G	A	0.116719
	chr5A	650019000	650219084	200085	10	650119084	5.56166243	C	T	0.108359
	chr5A	650858083	651058083	200001	1	650958083	5.0420547	G	A	0.129032
	chr5A	652435620	652635751	200132	2	652535620	5.71883727	C	T	0.109034
	chr5B	520947323	521147323	200001	3	521047323	5.42026052	T	C	0.23399
FLL	chr3B	750767415	750967415	200001	4	750867415	5.37574452	A	G	0.07767
	chr4A	181775054	181975054	200001	1	181875054	6.77210584	C	T	0.108766
	chr4A	307692861	307892861	200001	1	307792861	5.25284325	C	T	0.086441
	chr4A	308376369	308576369	200001	1	308476369	5.27415017	T	C	0.058608
	chr4A	351513686	351713686	200001	1	351613686	5.7314604	C	A	0.059028
	chr4A	353580244	353780346	200103	3	353680258	5.38946631	A	G	0.066946

Table 3 (Cont'd.).

Trait	Chr	Start	End	Length_Signal(bp)	Num_snp	Peak SNP	–Log10 (p)	Ref Base	Alt Base	MAF
	chr4A	368243032	368443032	200001	1	368343032	5.88045281	T	C	0.078859
	chr4A	370308940	370509004	200065	2	370409004	5.91136148	A	G	0.058621
	chr4A	395454084	395654176	200093	2	395554176	5.77886965	G	A	0.068106
	chr7D	327712180	327912180	200001	1	327812180	5.64311769	A	G	0.058252
FLW	chr7B	699833020	700033020	200001	5	699933020	5.45018657	C	T	0.370175
NGS	chr3B	368750328	368950328	200001	5	368850328	5.29148622	G	A	0.200382
PH	chr1B	384261597	384461597	200001	3	384361597	5.33751775	T	A	0.053763
	chr2A	484750557	484950557	200001	1	484850557	5.11846956	T	C	0.123746
	chr3B	128153480	128353480	200001	1	128253480	5.74532893	C	T	0.408714
	chr3B	556222650	556422650	200001	3	556322650	6.03340369	C	A	0.06872
	chr3B	574688489	574888489	200001	2	574788489	6.20411303	A	T	0.055336
	chr4A	108672921	108872921	200001	2	108772921	5.60802454	G	C	0.052716
	chr4A	137995244	138195244	200001	1	138095244	5.29737554	T	G	0.072607
	chr4A	138910533	139110533	200001	1	139010533	5.57343279	A	G	0.06891
	chr4A	139060944	139271170	210227	3	139161056	5.28267391	G	T	0.065705
	chr4A	139202735	139402921	200187	2	139302921	5.66198021	G	C	0.070313
	chr4A	382767264	382967264	200001	1	382867264	5.11767883	C	T	0.053292
	chr6A	207340045	207540045	200001	1	207440045	5.11705338	G	T	0.051887
	chr6B	121102054	121302054	200001	1	121202054	6.08342305	A	G	0.058642
	chr7A	15041544	15314073	272530	12	15214073	7.08916495	A	G	0.050654
	chr7A	266239842	266439842	200001	1	266339842	5.06567813	G	A	0.077982
	chr7A	278760161	278960161	200001	1	278860161	5.37778888	A	G	0.093272
	chr7A	284906728	285106728	200001	1	285006728	5.02239911	C	T	0.065141
	chr7A	331530281	331730450	200170	2	331630450	5.25184276	C	T	0.498494
	chr7A	449378331	449578331	200001	1	449478331	5.12040649	C	T	0.071646
	chr7B	167433694	167633694	200001	1	167533694	5.38081773	A	G	0.067797
TGW	chr1B	323785485	323985485	200001	1	323885485	5.13102453	C	T	0.054656
	chr5B	316881270	317081270	200001	1	316981270	5.27285491	T	C	0.110938
	chr7A	281403729	281603729	200001	1	281503729	5.57634592	A	T	0.086751

Note: DTM, Days of maturity; EL, Ear length; FLL, Flag leaf length; FLW, Flag leaf width; NGS, Number of grains per spike; PH, Plant height; TGW, X1000.grain.weight; Length_Signal, Length of the associated genomic region; Num_snp,Snp number; Peak SNP, Most significant SNP sites; Ref Base, Reference base; Alt Base, Altered base; MAF, Minor Allele Frequency

Table 4. Trait-associated genes summary.

Trait	(IWGSC) gene ID	Chr	Pos	Gene length (bp)	Dist (kb)	–Log10 (p)	Sig snp_ num	Description
DTM	TraesCS2B01G551600	2B	747818350	339	31.936	5.277987695	1	ATP-binding cassette sub-family A member 7
	TraesCS2B01G551700	2B	747818350	339	-2.819	5.277987695	1	6,7-dimethyl-8-ribityllumazine synthase
	TraesCS2B01G551800	2B	747818350	339	-36.547	5.277987695	1	Brefeldin A-inhibited guanine nucleotide-exchange protein 1
	TraesCS2B01G551900	2B	747818350	9631	-47.173	5.277987695	1	F-box family protein
	TraesCS3A01G192100	3A	248195218	2741	-23.139	5.181791399	1	Cyclin
	TraesCS3A01G210200	3A	375756532	4518	-12.57	5.175195842	1	bZIP transcription factor
	TraesCS3A01G210300	3A	375756532	6821	-60.8	5.175195842	1	Serine/threonine-protein phosphatase 6 regulatory subunit 3
	TraesCS7A01G271700	7A	284963485	1959	-93.261	5.37699419	1	Armadillo repeat only
EL	TraesCS2B01G226600	2B	217477990	1567	79.716	5.819026157	1	Remorin
	TraesCS2B01G226700	2B	217477990	5226	68.258	5.819026157	1	40S ribosomal protein S27
	TraesCS2B01G226800	2B	217477990	5221	62.878	5.819026157	1	DNAse I-like superfamily protein
	TraesCS2B01G226900	2B	217477990	1244	43.44	5.819026157	1	Leucine-rich repeat receptor-like protein kinase family protein
	TraesCS2D01G039000	2D	14448864	2038	98.494	5.312777115	1	Transmembrane protein, putative (DUF594)
	TraesCS2D01G039100	2D	14448864	1137	89.752	5.312777115	1	Sulfotransferase
	TraesCS2D01G039200	2D	14448864	588	73.155	5.312777115	1	Ribonuclease H-like superfamily protein
	TraesCS2D01G039300	2D	14448864	1007	67.739	5.312777115	1	Acidic chitinase
	TraesCS2D01G039400	2D	14448864	1989	52.15	5.312777115	1	Receptor lectin kinase
	TraesCS2D01G039500	2D	14448864	4397	43.296	5.312777115	1	Acetyltransferase component of pyruvate dehydrogenase complex
	TraesCS2D01G039600	2D	14448864	8428	28.067	5.312777115	1	Glutamyl-tRNA(Gln) amidotransferase subunit A
	TraesCS2D01G039700	2D	14448864	11170	14.984	5.312777115	1	Glutamyl-tRNA(Gln) amidotransferase subunit A
	TraesCS2D01G039800	2D	14448864	712	5.046	5.312777115	1	Ethylene-responsive transcription factor
	TraesCS3A01G013600	3A	9900047	4421	85.349	5.578763734	1	O-acyltransferase WSD1
	TraesCS3A01G013700	3A	9900047	2798	53.564	5.578763734	1	O-acyltransferase WSD1
	TraesCS3A01G013800	3A	9900047	3137	44.244	5.578763734	1	Fatty acyl-CoA reductase
	TraesCS3A01G013900	3A	9900047	1452	10.251	5.578763734	1	NBS-LRR-like resistance protein
	TraesCS3A01G014000	3A	9900047	931	-31.643	5.578763734	1	Fatty acyl-CoA reductase
	TraesCS3A01G014100	3A	9900047	1424	-32.896	5.578763734	1	Fatty acyl-CoA reductase
	TraesCS3A01G014200	3A	9900047	1002	-50.85	5.578763734	1	NBS-LRR-like resistance protein
	TraesCS3A01G014300	3A	9900047	549	-53.039	5.578763734	1	DNA topoisomerase
	TraesCS3A01G014400	3A	9900047	831	-64.389	5.578763734	1	DNA topoisomerase
	TraesCS3A01G014500	3A	9900047	9435	-88.414	5.578763734	1	Disease resistance protein RPM1
	TraesCS3A01G333200	3A	577732852	2407	96.635	5.117139313	2	DNA-directed RNA polymerase II
	TraesCS5A01G471300	5A	647954932	1254	-11.043	5.022550407	1	Serpin
	TraesCS5A01G473400	5A	649811563	4792	28.933	6.330476716	4	Multiple inositol polyphosphate phosphatase 1
	TraesCS5A01G473500	5A	649811563	804	26.394	6.330476716	4	Ubiquitin family protein
	TraesCS5A01G473600	5A	649811563	1791	4.585	6.330476716	4	Cinnamoyl-CoA reductase 4
	TraesCS5A01G473700	5A	649943747	3381	-79.942	5.79843793	6	30S ribosomal protein S12
	TraesCS5A01G473800	5A	650119000	3229	-8.496	5.561662427	2	Ethylene-responsive transcription factor
	TraesCS5A01G473900	5A	650119000	1314	-22.858	5.561662427	2	F-box domain containing protein, expressed

Table 4 (Cont'd.).

Trait	(IWGSC) gene ID	Chr	Pos	Gene length (bp)	Dist (kb)	–Log10 (p)	Sig snp_ num	Description
	TraesCS5A01G474000	5A	650119000	1158	-28.218	5.561662427	2	F-box domain containing protein, expressed
	TraesCS5A01G474100	5A	650119000	498	-32.618	5.561662427	2	Cortactin-binding protein 2
	TraesCS5A01G474200	5A	650119000	1359	-40.78	5.561662427	2	F-box family protein
	TraesCS5B01G337400	5B	521047323	699	76.473	5.420260521	1	B-box zinc finger family protein
	TraesCS5B01G337500	5B	521047323	1549	14.655	5.420260521	1	Zinc finger protein CONSTANS-like protein
	TraesCS5B01G337600	5B	521047323	2882	-90.849	5.420260521	1	Ankyrin repeat family protein, putative, expressed
FLL	TraesCS3B01G507000	3B	750867415	2250	80.907	5.37574452	1	ENTH/ANTH/VHS superfamily protein
	TraesCS3B01G507100	3B	750867415	2888	3.676	5.37574452	1	Transmembrane protein 45B
	TraesCS3B01G507200	3B	750867415	555	-33.223	5.37574452	1	F-box domain containing protein
	TraesCS3B01G507300	3B	750867415	321	-34.066	5.37574452	1	PEBP (phosphatidylethanolamine-binding protein) family protein
FLW	TraesCS7B01G431500	7B	699933020	16899	94.423	5.450186566	1	Transcription initiation factor TFIID subunit 1
	TraesCS7B01G431600	7B	699933020	3031	89.875	5.450186566	1	XH/XS domain-containing family protein
	TraesCS7B01G431700	7B	699933020	13584	9.535	5.450186566	1	Transcription initiation factor TFIID subunit 1
	TraesCS7B01G431800	7B	699933020	3252	1.443	5.450186566	1	transmembrane protein
	TraesCS7B01G431900	7B	699933020	577	-20.676	5.450186566	1	Protein PLANT CADMIUM RESISTANCE 2
NGS	TraesCS3B01G236000	3B	368850328	234	3.53	5.291486217	1	RNA-binding (RRM/RBD/RNP motifs) family protein
	TraesCS3B01G236100	3B	368850328	369	-2.632	5.291486217	1	Orf101b
	TraesCS3B01G236200	3B	368850328	255	-15.88	5.291486217	1	Ankyrin repeat and SOCS box protein 15
	TraesCS3B01G236300	3B	368850328	699	-32.036	5.291486217	1	Cytochrome c biogenesis C
	TraesCS3B01G236400	3B	368850328	1689	-82.434	5.291486217	1	Gag polyprotein
PH	TraesCS1B01G211100	1B	384361597	2419	63.385	5.337517749	1	Antiholin-like protein lrgB
	TraesCS1B01G211200	1B	384361597	2128	60.594	5.337517749	1	Choline-phosphate cytidylyltransferase
	TraesCS1B01G211300	1B	384361597	4120	21.548	5.337517749	1	CoA ligase family protein
	TraesCS3B01G346600	3B	556322650	7054	48.697	6.033403693	1	Flap endonuclease 1
	TraesCS3B01G346700	3B	556322650	1743	-26.861	6.033403693	1	Cytochrome P450
	TraesCS3B01G346800	3B	556322650	1497	-45.278	6.033403693	1	F-box protein
	TraesCS3B01G363300	3B	574788489	8130	81.6	6.204113034	1	Stomatal closure-related actin-binding protein 1
	TraesCS3B01G363400	3B	574788489	1860	-39.62	6.204113034	1	DNA-directed RNA polymerase II
	TraesCS4A01G097700	4A	108772921	5346	19.017	5.608024539	1	U-box domain-containing protein
	TraesCS4A01G097800	4A	108772921	1377	-98.642	5.608024539	1	Dof zinc finger protein
	TraesCS4A01G113900	4A	138095244	1179	-75.467	5.297375539	1	DUF1262 family protein
	TraesCS7A01G034500	7A	15141544	751	58.865	7.089164955	2	Glutathione S-transferase
	TraesCS7A01G034600	7A	15141544	3266	52.437	7.089164955	2	NBS-LRR disease resistance protein
	TraesCS7A01G034700	7A	15214073	1773	-60.46	7.089164955	1	Leucine-rich repeat receptor-like protein kinase family protein
	TraesCS7A01G034800	7A	15214073	1098	-69.344	7.089164955	1	F-box protein
	TraesCS7A01G271700	7A	285006728	1959	-50.018	5.022399112	1	Armadillo repeat only
	TraesCS7B01G135900	7B	167533694	1011	-41.329	5.380817732	1	Caffeoyl-CoA O-methyltransferase
TGW	TraesCS5B01G172600	5B	316981270	414	-77.995	5.272854912	1	Zinc finger-like protein

Note: Negative and positive values for distance indicate the gene is upstream and downstream of the SNP, respectively; Sig_snp_num,the number of significant SNPs located in this gene

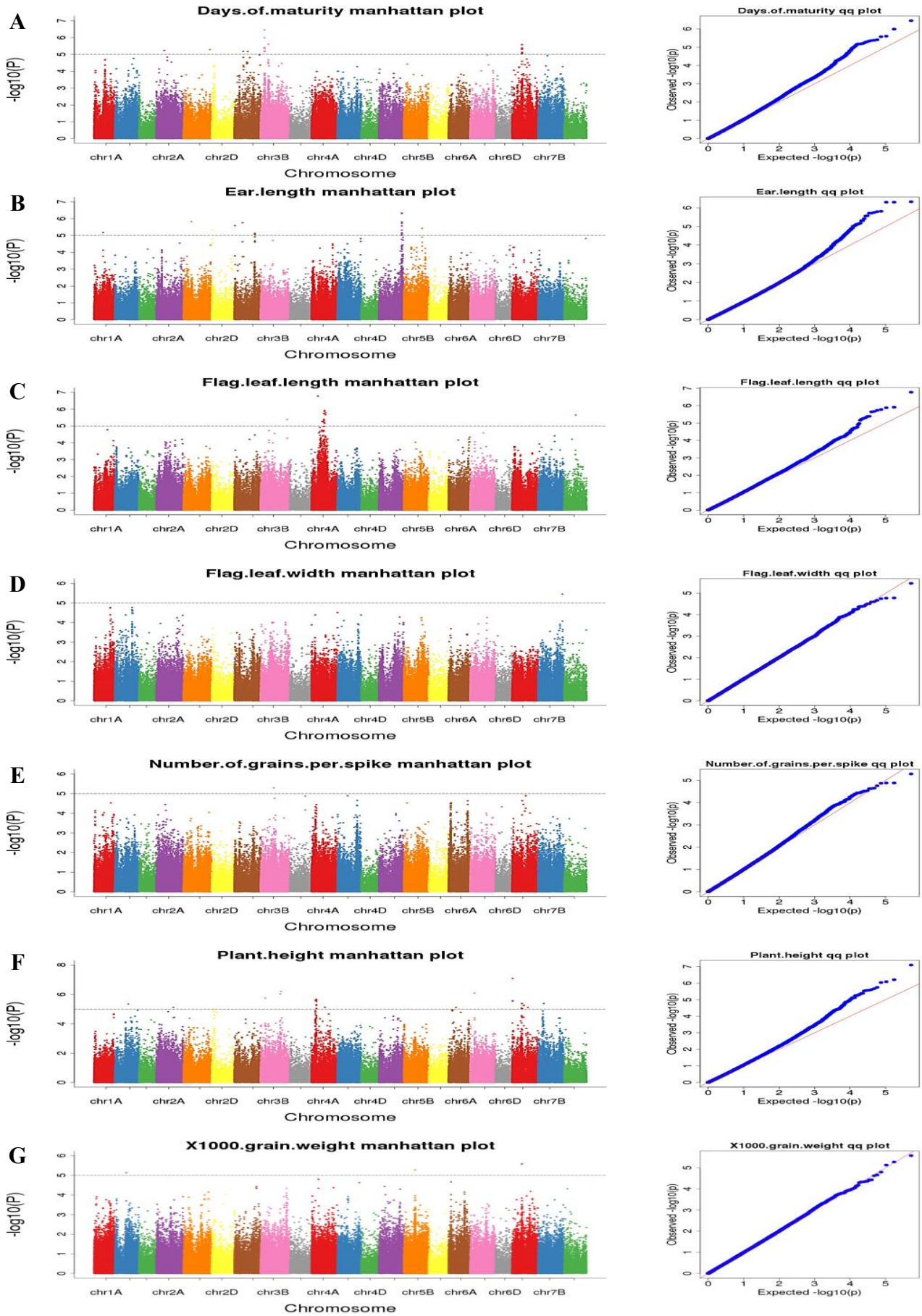

Fig. 1. Manhattan plots and QQ plots of genome-wide association studies (GWAS). QQ plot: Quantile-Quantile plots.(A, Days of maturity; B, Ear length; C, Flag leaf length; D, Flag leat width; E, Number of grains per spike; F, Plant height; G, X1000 grain weight).

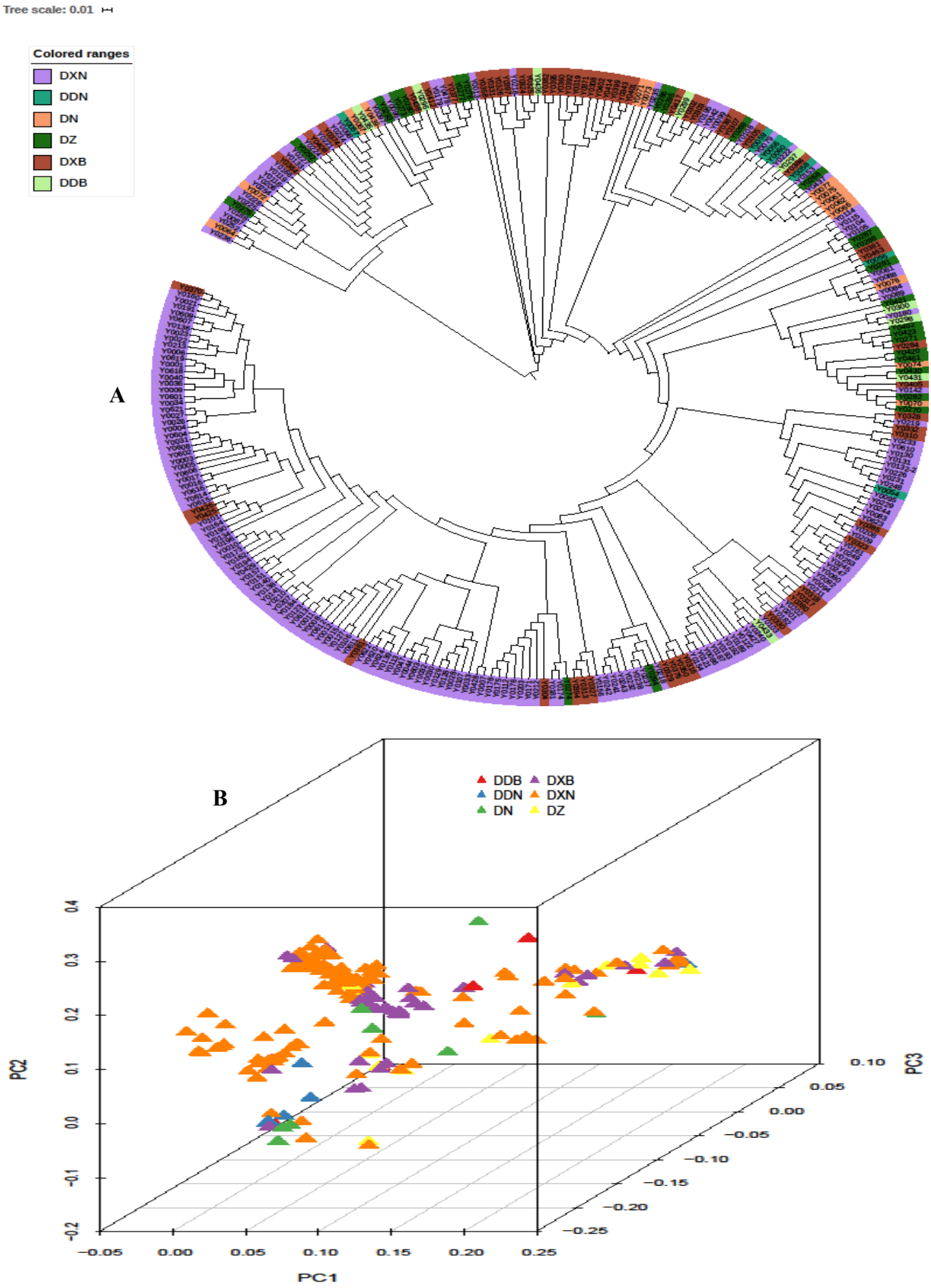

Fig. S4. Phylogenetic tree and principal component analysis(PCA).
Note: DDB, Northeast; DDN, Southeast; DN: South; DXB, Northwest; DXN, Southwest; DZ, Centre. (A) neighbor-joining tree of the 335 wheat lines; (B) PCA of the 192 wheat lines.

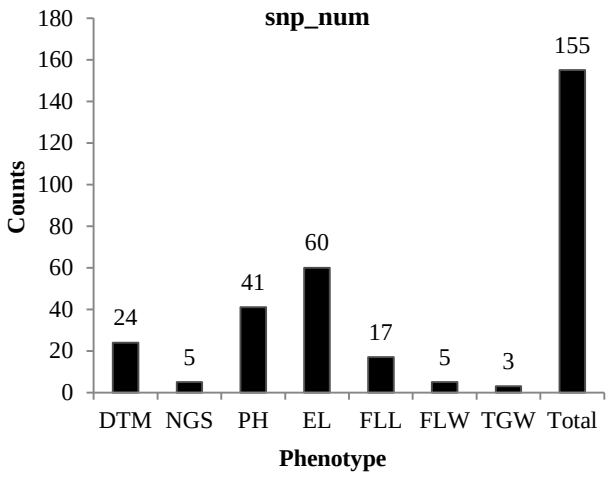

Fig. S5. Number of significant SNPs sites in the phenotype.

Discussion

Wheat is one of the important cereal crops in China and plays a valuable role in achieving food security. However, climate change threatens wheat productivity in many regions, making research and development on drought-resistant wheat crucial. In this study, GWAS of seven agronomic traits from 335 germplasm materials from Yunnan Province revealed SNPs significantly associated with shape and predicted possible candidate genes. We hope to study the introduction of new drought-resistant genes into high-quality wheat varieties in later experiments based on our findings.

In this study, field phenotypic data showed that most phenotypic indices in most varieties/lines decreased significantly under drought stress, indicating that these lines have substantial potential to exploit superior genes to improve drought resistance in high-quality wheat lines. The significant decrease in PH, TGW, FLL, FLW, and other directly related indices also confirmed the phenotypic plasticity of biomass accumulation. This plasticity can be used in wheat breeding for drought resistance to alleviate water shortages (Dalal *et al*., 2017). In this study, most selected indices showed significant correlation under two kinds of water conditions, suggesting that the selection of characteristics was reasonable and drought resistance resulted from multiple traits. This study collected phenotypic data from mature plants because early selection may not reflect later growth traits, especially those associated with drought tolerance (Mathew *et al*., 2019). In this study, the population was divided according to geographical regions, as reported previously (Liu *et al*., 2017). The phylogenetic tree and PCA showed that many materials from the northwest and southwest of Yunnan Province are clustered together, while materials from the northeast and the northwest also converge. We suspect that this could be directly related to the introduction, variation, and other region-specific characteristics. There was no obvious population structure in the selected material, indicating that the process of selection, domestication, and improved breeding may lead to a hidden population structure (Neumann *et al*., 2011). This can lead to a degree of genetic affinity between genotypes. Studying population structure helps trace potential parents and provides a basis for drought resistance breeding.

MTA analysis revealed associations between specific phenotypes and genetic variations within the genome, which may contribute to the discovery of genes that control traits. Although, only SNPs observed at $-\log_{10}(p) > 5$ were considered significant in this study, those observed at $-\log_{10}(p) < 5$ may also be useful for drought-tolerant breeding and may be located in areas of the genome that directly or indirectly affect their respective traits. Most of these significant SNPs were concentrated in the A genome, followed by the B genome, and finally the D genome; moreover, there was no significant association between SNP loci on chromosomes 4B, 1D, 3D, 4D, 5D, and 6D, which is at least partially consistent with previous studies (Qaseem *et al*., 2019). Studies by Learnmore (Learnmore *et al*., 2017) suggest that EL markers under drought stress originate from 3A, 6A, 7A, 1B, 2B, 4B, 5B, 6B, and 2D, respectively. In this study, SNPs related to EL were also detected on chromosomes 3A, 5A, 2B, and 2D. It has been reported that PH is related to chromosomes 2A, 6A, 7A, 1B, and 3B (Mathews *et al*., 2008; Pinto *et al*., 2010; Bellucci *et al*., 2015; Sheoran *et al*., 2019). In the present study, PH was located at 2A, 4A, 6A, 7A, 1B, 3B, and 6B, consistent with previous studies. Moreover, DTM-related regions were found on chromosomes 2A and 3B, as shown in previous studies (Sharma & Pandey, 2015; Sukumaran *et al*., 2018a). TGW SNPs were detected on chromosomes 7A, 1B, and 5B, a finding that has been previously reported (Zanke *et al*., 2015). Further, we found that, when different traits were located on the same chromosome, the phenotypes of these traits tended to be highly correlated. This finding is also consistent with previously reported results (Kashif & Khaliq, 2004; Zahid *et al*., 2008), suggesting that highly correlated traits are usually controlled by the same SNP (Dholakia *et al*., 2010). SNPs on chromosomes 3B, 4A, 7D, 3B, and 7B related to FLL, NGS, and FLW were also detected. This result may not be consistent with that of previous studies, but certain gene loci may have different effects on specific traits in different growing environments, leading to inconsistent associations between markers or loci and specific traits when environmental conditions change. However, the discovery of these chromosomal regions may also present a new concern in drought-resistant breeding.

In this study, a total of 77 genes that may be associated with drought resistance were predicted, almost all of which were derived from wheat and some from *Arabidopsis thaliana*. The sequence similarity of all predicted genes was 95%; the highest was 100%. Among them, 37 genes were related to EL, 17 to PH, eight to DTM, five each to FLW and NGS, four to FLL, and one to TGW. Based on the annotations, we identified certain genes. Among the EL-related genes, TRAESC S2B01G226600 occupying the 217.48 Mb region on 2B chromosome is related to drought resistance resistance and signal transduction and the regulation of the stress signal pathway and the activity of photosystem II (Checker & Khurana, 2013). TRAESCS2D01G039100

occupying the 14.45 Mb region of chromosome 2D plays a very important role in plant growth, development, and stress adaptation (Tang *et al*., 2014); recent studies have suggested thatTRAESCS2D01G039400 occupying the 14.45 Mb region, can improve drought resistance in millets (Zhao *et al*., 2016). TRAESCS3A01G013600 and TRAESCS3A01G013700 that occupy the 9.9 Mb region of chromosome 3A encode wax synthases (Li *et al*., 2018); TRAESCS3A01G013800 and TRAESCS3A01G014100 occupying the 9.9 Mb region are involved in plant drought resistance and the synthesis of epicuticular wax (Qiaoli *et al*., 2020). TRAESCS5A01G471300, occupying the 647.95 Mb region of chromosome 5A, is a serine endopeptidase inhibitor that is stably expressed (Simova-Stoilova *et al*., 2016). TRAESCS5B01G337600 that occupies the 521.05 Mb region of chromosome 5B is associated with anchorin repeat proteins and plays an essential role in plant growth and development. For PH-related genes, TRAESCS3B01G363300 occupying the 574.79 Mb region of chromosome 3B is associated with stomata (Zhao *et al*., 2011). TRAESCS4A01G097800 occupying the 108.77 Mb region of chromosome 4A, (Chen *et al*., 2020) regulates key plant-specific processes, including photosynthesis and carbohydrate metabolism. TRAESCS7A01G034500 occupying the 15.14 Mb region of chromosome 7A, (Mohammad *et al*., 2013), is related to glutathione, and significantly enhances drought tolerance in plants. NGS gene TRAESCS5B01G172600 (Dong *et al*., 2013) occupying the 316.98 Mb region of chromosome 5B has been found to increase the tolerance of transgenic *A. thaliana* to drought and salt stress.

We have also speculated on some possible drought-resistance genes. The DTM-associated gene TRAESCS2 B01G551700 occupying the 747.82 Mb region of chromosome 2B is associated with energy production via the respiratory chain, lipid oxidation, iron transport, various metabolic pathways, and riboflavin precursor synthetase (Deng *et al*., 2014); TRAESCS2B01G551800 plays a key role in intracellular vesicle transport (Jones *et al*., 2005); TRAESCS2B01G551900 is involved in the regulation of plant life processes and signal transduction by plant hormones. Modultion of the activity of transcription factors results in altered expression of downstream genes, thereby influencing plant stress resistance. TRAESCS3A01G210200 occupying the 375.76 Mb region of chromosome 3B is an important transcription factor (Huang *et al*., 2010). The EL-related gene TRAESCS2B01G226900 occupying the 217.478 Mb region of chromosome 2B may play a key role in plant growth, development, differentiation, and stress response (Liu *et al*., 2017). TRAESCS3A01G014300 occupying the 9.9 Mb region of chromosome 3A is related to the enzyme activity associated with drought resistance (Yanfei *et al*., 2019). TRAESCS5A01G473600 occupying the 647.95 Mb region of chromosome 5A encodes an important enzyme for lignin synthesis and a product of drought induction and enhances plant drought resistance (Li *et al*., 2016). The 650.1 Mb region of the 5A chromosome contains TRAESCS5A01G473900 and TRAESCS5A01G474000, which comprise genes encoding one of the largest regulatory protein families, i.e., the F-box protein family that can respond to abiotic stress (Jian *et al*., 2015). TRAESCS5B01G337400 occupying the 521.05 Mb region of chromosome 5B may also be associated with drought resistance in plants (Kobayashi *et al*., 2012). TRAESCS5B01G337500 occupying the 521.05 Mb region enhances drought and salt tolerance in *Arabidopsis* (Xin *et al*., 2019). The genes associated with FLL occupy the 750.87 Mb region of chromosome 3B. TRAESCS3B01G507000 is involved in membrane transport, protein, and lipid regulation and is necessary for metabolic absorption, cell growth, and development; moreover, its encoded proteins are involved in many aspects of cytokinesis (De Craene *et al*., 2011). For PH, TRAESCS1B01G211300 occupying the 384.36 Mb region of chromosome 1B was highly expressed under drought stress (Hiremath *et al*., 2011). TRAESCS3 B01G346700, occupying the 556.32 Mb region of chromosome 3B, contributes to plant drought resistance (Fangmeng *et al*., 2017). TRAESCS4A 01G097700 occupying the 108.77 Mb region of chromosome 4A can improve drought resistance (Hu *et al*., 2018). TRAESCS4A01G113900, occupying the 138.1 Mb region of chromosome 4A, is also associated with drought resistance in plants (Li *et al*., 2018). TRAESCS7A 01G271700 (Sakai *et al*., 2008) occupying the 285 Mb region of chromosome 7A is associated with root hair.

In addition to these genes, we suspect that other genes—directly and indirectly—affect the physiological response to drought. We plan to continue our study of the known genes to identify new genetic resources. Genes whose predicted function is unknown or undetected in wheat need be a focus of research to understand their role in wheat breeding under conditions of drought stress and to determine their relationship with drought resistance and yield traits. The validated genes can be further used for marker-assisted selection to improve drought resistance breeding in wheat.

Conclusions

In this study, seven characteristics related to drought resistance were evaluated by GWAS over an entire growth period using 335 local and self-bred wheat varieties, and SNPs related to basic agronomic traits of wheat were identified. The GWAS identified 155 significant SNPs, of which 60 were associated with EL, 41 with PH, 24 with DTM, 17 with FLL, 5 each with NGS and FLW, and 3 with TGW. Thus, a total of 77 candidate genes related to the drought response were identified or predicted. These genes encode transcription factors and hormone and are involved in enzyme activity, transmembrane transport, signal transduction, epidermal wax synthesis, metabolism, growth, pigment, stomata, root hair, and resistance. Interestingly, most of the potential candidate genes are described as being involved in abiotic stress responses. In conclusion, the biomaterials we obtained, the functional SNPs we discovered, and the potential drought response candidate genes we predicted will provide a theoretical basis for molecular marker–assisted breeding, thereby improving the drought resistance of wheat varieties.

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