Functional gene assessment of wheat: breeding implication in Ningxia province

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Abstract

Background: The overall genetic distribution and divergences for cloned genes among wheat varieties that occurred during the breeding process of the past few decades in Ningxia province of China are poorly understood. Here we report the genetic diversities of 44 important genes underpinning grain yield, quality, adaptation and resistance in 121 Ningxia and 86 introduced wheat cultivars and advanced lines.

Results: Population structure indicated characteristics of genetic components of Ningxia wheats including landraces of particular genetic resources, introduced varieties with rich genetic diversities and modern cultivars in different times. Analysis of allele frequencies showed that dwarfing alleles Rht-B1b at Rht-B1 and Rht-D1b at Rht-D1, 1BL/1RS translocation, Hap-I at GW2-6B and Hap-H at Sus2-2B are present very frequently in Ningxia modern cultivars and introduced varieties from other
regions, but absent in landraces, indicating that the introduced wheat germplasm with numerous beneficial genes are vital for broadening genetic diversities of Ningxia wheat varieties. Large population differentiation occurred at adaptation genes between modern cultivars and well-adapted landraces. Founder parents have excellent allele combinations of important genes with a higher number of favorable alleles compared with modern cultivars. The gene flows manifested that six founder parents greatly contributed to breeding improvement in Ningxia province, in particular Zhou 8425B for yield related genes.

**Conclusions:** These results will greatly benefit for wheat breeding in Ningxia province and other areas with similar ecological environments.

**Keywords:** adaptation genes, founder parents, KASP, functional markers, quality genes, stress resistance genes, yield genes

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**Background**

Asia is the largest producer and consumer of wheat, with China and India as the two major countries in wheat production [1]. Wheat (*Triticum aestivum* L.) is widely cultivated in intricate geographical environments in China, reflecting its features of wide adaptability and high yield. Ningxia, as a north-western province of China with complex ecological types, has a long agricultural history of wheat cultivation. Since 1950s, wheat varieties have experienced five times of replacements and become the first cultivated cereal crops in Ningxia province. Only 14 wheat landraces, introduced varieties and founder parents play pivotal roles in the five-time update of wheat varieties. Therefore, it is essential to dissect the substance of wheat improvement in past several decades for directing future wheat breeding in Ningxia.

Bread wheat (AABBDD) has characteristics of large genome size, allopolyploid, highly complex repetitive genome contents that are shaped by two recent polyploidization events [2-5], domestication [6], gene flow from frequent intra- and inter-species introgression [7-8], and post-domestication selection aimed at
developing high-yielding locally adapted varieties [9]. Multiple factors drive the
evolution of wheat varieties, particularly many important genetic loci have been
selected during modern wheat breeding. Insight into these genetic loci is important to
understand phenotypic variations in adaptability, resistance to biotic and abiotic
stresses, processing and nutritional quality, and yield stability. The adaptation of
wheat to diverse environments is largely governed by genes for vernalization (Vrn-A1,
Vrn-B1 and Vrn-D1) [10], photoperiod (Ppd-D1 etc.) [11], and plant height (Rht-B1
and Rht-D1) [12]. Yield related genes include sucrose synthase genes TaSus1-7A, 7B,
TaSus2-2A, 2B for thousand-kernel weight and grain size [13-14], TaGW2-6A, 6B for
grain width [15-17], TaGS-D1 for grain size [18], TaCwi-A1 encoding cell wall
invertase [19], TaCKX6-D1 encoding cytokinin oxidase/dehydrogenase [20] and grain
length-associated gene TaGASR-A1 [21] in bread wheat. Assessing processing quality
is crucial in wheat quality improvement. Strong-gluten wheat varieties are
characterized by a combination of medium-high kernel hardness, acceptable protein
content, medium-strong dough and good extensibility, and representative varieties
included Yumai 34, Zhengmai 366 for both pan bread and noodles quality [22]. High-
and low-molecular-weight glutenin subunits (HMW-GS and LMW-GS) associated
with dough quality are influenced by Glu-1 and Glu-3 loci [23]. Flour color
responsible for noodle quality was influenced by several factors including polyphenol
oxidase (PPO) activity (Ppo-A1 and Ppo-D1) [24-25], phytoene synthase (PSY)
enzymes (Psy-A1, Psy-B1 and Psy-D1) [26-27], ζ (zeta)-carotene desaturase (ZDS)
enzymes (Zds-A1) [28] and peroxidase (Pod-A1) [29]. Kernel hardness, which has a
profound effect on milling and end-use quality, is largely determined by the Pina-D1
and Pinb-D1 genes encoding puroindoline a and puroindoline b proteins, respectively
[30]. Increasing biotic- and abiotic-stresses are major challenges with the impacts of
climate and environmental changes for wheat breeding. In the past decades, some
important stress resistance genes were cloned. Dehydration-responsive element
binding (DREB) proteins at Dreb-B1 locus are induced for improving drought
tolerance [31]. Fusarium head blight (FHB) devastates wheat production worldwide
and its resistance genes Fhb1 using recombinants [32-33] and Fhb7 in wheat distant
hybridization breeding [34] were cloned recently. The \textit{Lr34/Yr18/Pm38} locus conferring durable adult-plant resistance to multiple diseases is used in wheat breeding programs worldwide [30]. The \textit{1BL/1RS} translocation (\textit{1BL/1RS}) has been adopted widely in wheat breeding due to positive impacts on grain yield, adaptation, and particularly the presence of resistance genes to several diseases and pests although its translocation is associated with undesirable bread-making quality [35].

Modern breeding has imposed selection for improved productivity that largely influences the frequency of superior alleles for genetic loci underpinning traits of breeding interest. Therefore, molecular diagnosis for the allelic variations of genes is important to manipulate beneficial alleles in wheat molecular breeding. Enhanced capacity in sequencing, along with the availability of high-quality genome sequences of bread wheat, has allowed researchers to deploy specific favorable alleles using molecular markers. Currently, 157 functional markers documented for more than 100 loci underpinning adaptability, resistance to biotic and abiotic stresses, quality and grain yield were converted into high-throughput KASP assays [36]. Such approaches will promote assessing the distribution of functional genes of wheat germplasms and applications in wheat breeding.

Our objectives for this study were to evaluate genetic structure, diversity, divergence and allelic variations of wheat germplasm resources in Ningxia province of China using KASP assays of 44 cloned genes for adaptation, stress resistance, quality, and grain yield. Genetic characteristics were exhibited in 207 bread wheat varieties, landraces and advanced lines including founder parents and varieties from Ningxia and other regions. Gene flow and allelic frequency implicate the distribution of important functional genes, which may contribute to improvement selection of future wheat breeding in Ningxia province and provide a robust guiding breeding foundation for other regions and countries with similar ecological environments.

\textbf{Methods}

\textbf{Plant materials and DNA extraction}

A representative sampling of wheat germplasm consisted of 207 wheat varieties,
including 121 Ningxia varieties and 86 introduced varieties (Table S1). The latter were introduced in Ningxia province over the past decades and played a huge role in local wheat breeding. The Ningxia varieties included 13 landraces and 108 modern cultivars and advanced lines. In addition, six founder parents among 207 wheat varieties used in this study include Moba 66, Abbondanza, Beijing 8, Orofen, Xiaoyan 6 and Zhou 8425B. Genomic DNA was extracted from fresh leaves in each accession using the CTAB method [37].

**KASP genotyping of functional genes**

Conventional functional markers were summarized based on 44 cloned wheat genes for grain yield, quality, adaptation and stress resistance [30]; these markers were converted into KASP assays [36] that were widely exploited to characterize wheat germplasm resources [38-41]. A total of 44 KASP arrays developed from cloned genes were used for genotyping in this study (Table S2).

KASP assays were detected with 5.0 μL mixtures containing 2.2 μL of 40 ng/μL DNA, 2.5 μL of 1 x KASP V4.0 2X Master mix (KBS-1016-017), 0.04 μL Mg²⁺, 0.056 μL of primer mixture, and 0.204 μL ddH₂O and the following amplification programs: hot start at 95°C for 15 min, followed by ten touchdown cycles (95°C for 20 s; touchdown at 65°C initially and decreasing by 1°C per cycle for 25 s), followed by 30 additional cycles of annealing (95°C for 10 s; 57°C for 60 s) [41]. KASP genotyping were performed using QuantStudio™ 7 Flex (Applied Biosystems by Life Technologies, U.S.), and visualized by QuantStudio™ Real-time PCR software v.1.3 (Applied Biosystems by Life Technologies, U.S.).

**Population structure and phylogenetic analysis**

A neighbor-joining tree was constructed in PowerMarker v3.25 [42] and visualized in MEGA 7 [43] using genotypic data of 44 genes. The first three eigenvectors of principal coordinate analysis (PCA) were performed using the R package Adegenet v2.0.1 [44]. Population structure of 207 accessions based on 44 functional genes was evaluated using Structure 2.3.4 with a burn-in period at 50,000 iterations and a run of
500,000 replications of Markov Chain Monte Carlo (MCMC) [45]. Then the number of populations was estimated based on the ΔK model [46].

Allele numbers and frequencies were calculated for all loci. Genetic diversities were evaluated by PowerMarker v3.25, and the student’s t-test was further used to compare the effects of two genotypes at a threshold probability of $P < 0.05$. Genetic flow and $F$-statistics ($Fst$) were measured of population differentiation with POPGENE software [47].

**Results**

**Genotyping and population structure**

Genotyping of 207 wheat varieties using 44 KASP assays identified allelic variations at 44 loci (Table S1). All selected KASP assays showed clear clustering results of varieties (Figure S1). In total, these loci underpin grain yield (10), quality (14), adaptation (6), and stress resistance (14).

The neighbor-joining analysis divided 207 varieties into two groups, designated as Ningxia and Others, respectively (Figure 1A), in agreement with PCA (Figure 1B). The number of subpopulation (K) was plotted against the ΔK calculated from the Structure, and the peak of the broken line graph was observed at $K = 2$ (Figures 1C, S2), demonstrating that the population was basically divided into two subgroups. The first subgroup mainly referred to as landraces and cultivars from Ningxia provinces (Ningxia), while the second mainly consisted of introduced varieties from foreign countries and other provinces in China (Others). Moreover, accessions from Ningxia grouped into two clades for landraces and modern cultivars, respectively (Figure S3). This indicated characteristics of genetic components of Ningxia wheats, in which landraces, introduced varieties and modern cultivars in different times together formed wheat breeding process.

**Genetic divergence between Ningxia and Others**

To further clarify the large genetic differences between germplasms from Ningxia and Others, genetic diversities and variations were assessed. There was apparent
difference in genetic diversity at 44 loci controlling yield, quality, adaptation and stress resistance between Ningxia wheat germplasms and Others (Figure 2A). Further exploration indicated a higher genetic diversity at ten grain yield loci in the group Others compared with Ningxia wheat varieties \((P < 0.01)\), whereas Ningxia had a higher genetic diversity than Others at 14 quality genes \((P < 0.05)\) (Figure S4A, B). Among them, genetic diversities estimated at Cwi-4A, GS-D1, Sus2-2B and Sus1-7B loci for yield were abundant in the Others subgroup, while genes of Glu-B1, Glu-D1, Pina-D1 and Zds-A1 for quality showed much higher genetic diversities in the Ningxia subgroup relative to Others (Table S3). In addition, we also found that genetic divergence was most obviously at quality genes, followed by yield genes (Figure 2B). An in-depth analysis showed an evident genetic divergence at some loci such as Cwi-4A \((0.035)\), Sus2-2B \((0.035)\) for grain yield, Pinb-D1 \((0.057)\) and Zds-A1 \((0.064)\) for quality for Ningxia and Others subgroups (Table S3). To investigate genetic divergences on gene level, we evaluated allele frequencies at genes for grain yield, quality, adaptation and stress resistance as indicated below.

**Grain yield genes**

Among ten yield-related genes, alleles of Hap-4A-C (Cwi-4A), GS-D1a (GS-D1), Hap-A (GW2-6A) and Hap-1 (GW2-6B) for larger grain size and TKW were predominant in the subgroup Ningxia compared with Others (Figure 2C), whereas at Sus2-2B, the allele Hap-H associated with higher TKW was more frequently in the subgroup Others. Besides, the desirable alleles at Sus1-7A and TGW6 loci tended to occur in higher frequencies in both two subgroups. However, the favorable alleles of H1c and Hap-H at GASR-Al and MOC-7A, respectively, were rarely present in two subgroups, indicating that these were not selected by breeders.

**Quality genes**

At 14 loci for quality traits, a higher frequency of Glu-D1d encoding high-molecular-weight glutenin subunits (HMW-GS) Dx5+Dy10 occurred more frequently in the subgroup Ningxia \((44\%)\) than Others \((27\%)\) (Figure 2D). The Ppo-Alb, Pod-Alb and Zds-A1a alleles, associated with lower PPO activity, higher POD activity and lower yellow pigment content, respectively, were more frequent in
the subgroup Ningxia than Others. In contrast, the frequencies of HMW-GSs Ax1 or Ax2* and Pinb-D1b for hard grain texture were significantly higher in the subgroup Others. Additionally, the majority of two subgroups had high proportion of Pinb-B2b for hard grain texture, IBL-IBL, and Psy-A1b, Psy-D1a and Psy-B1a or b for low yellow pigment content, whereas the minority had alleles of Pina-D1b, Pds-B1b and Wx-B1b at Pina-D1, Pds-B1 and Wx-B1 loci, respectively.

**Adaptation genes**

Concerning adaptation genes, genetic diversities in two subgroups were not significantly different (Figure S4C). Allelic variations had minor differences at Rht-B1, Rht-D1, Vrn-B1, Vrn-D1 and Ppd-D1 in both subgroups Ningxia and Others except at Vrn-A1 locus (Figure S5A), indicating that spring-type alleles of Vrn-B1b and Vrn-D1a, photoperiod-insensitive allele Ppd-D1a, Rht-B1b and Rht-D1b reducing plant height distributed consistently in two subgroups.

**Stress resistance genes**

Loci for stress resistance included PHS1, Sdr-B1, VP-1B and MFT-A1 associated with seed dormancy, 1-fehw3 and Dreb-B1 related to drought stress, Lr14a, Lr34, Lr68, Yr15 and Fhb1 for disease resistance, DRO-5A and DRO-5B for root architectures. The favorable alleles Sdr-B1a, Vp-1Bc and Dreb-B1a were higher in the subgroup Ningxia than Others (Figure S5B), whereas the favorable alleles of PHS+ at PHS1, PHS+ at MFT-A1 and Lr14a occurred in higher frequencies in the subgroup Others compared with Ningxia. The drought-resistance alleles of Westonia type were not significantly different between two subgroups Ningxia and Others, while alleles Lr68+, Yr15+ and Fhb1+ were rarely present in two subgroups.

**Genetic divergence between landraces and modern cultivars in Ningxia accessions**

To evaluate the population differentiation during breeding improvement in Ningxia province, we investigated the genetic structure based on 44 genes (Figures 3A, S3). The results showed that wheat accessions from Ningxia clustered in two clades, i.e. landraces and modern cultivars, respectively. Therefore, we further analyzed genetic
relationship between landraces and modern cultivars. We found a higher genetic diversity in modern cultivars compared with landraces (Figure 3B). Moreover, the difference of genetic diversities was clearly manifested in adaptation-related genes, while no significant differences between two groups were observed in other genes controlling yield, quality and resistance (Figure S6). Population differentiation (Fst) and gene frequency against the four types of genes were also analyzed to reveal substantial divergences between two subgroups below.

**Adaptation genes**

The $Fst$ between modern cultivars and landraces was very high at $Vrn-A1$ (0.39), followed by $Rht-B1$ (0.16) (Figure 4A). Similarly, the spring-type allele $Vrn-A1a$ at $Vrn-A1$ influencing vernalization occurred frequently in modern cultivars (57%), but absent in landraces (Figure 4B). In contrast, $Vrn-B1b$ associated with spring type was predominant both in modern cultivars (58%) and landraces (82%); and $Vrn-D1a$ for spring type retained a towering scaling in modern cultivars (57%) and landraces (86%) (Figure S7). The dwarfing allele $Rht-B1b$ ($Rht-B1$) was present in 28% modern cultivars but absent in landraces (Figure 4B). A similar situation did happen in another dwarfing gene $Rht-D1b$. Interestingly, the photoperiod-insensitive allele ($Ppd-D1a$) predominated in modern cultivars and landraces (Figure S7).

**Stress resistance genes**

For genes controlling stress resistance, the $Fst$ among two subgroups at $Lr34$ and $1-fehw3$ loci were extremely high compared with other resistance genes, which were 0.55 ($Lr34$) and 0.43 ($1-fehw3$), respectively (Figure 4C). Allele frequency analyses showed that the favorable alleles of $Lr34+$ and Westonia type at $1-fehw3$ were predominant in landraces (Figure 4D). The genetic differentiation ($Fst$) of $DRO-5B$, $Lr68$, $PHS1$, $VP-1B$ and $Lr14a$ was 0.30, 0.22, 0.22, 0.14 and 0.12, respectively (Table S4), whereas corresponding favorable allele frequencies had distinct differences between modern cultivars and landraces (5% vs 54%, 5% vs 47%, 50% vs 92%, 67% vs 30%, 22% vs 0%) (Figure S8).

**Quality genes**

For quality genes, the most extreme genetic differentiation occurred in $Pod-A1$ (0.23),
followed in Pinb-D1 (0.24) between two subgroups (Figure 4E). The majority of modern cultivars (55%) had the Pod-A1b allele, whereas few landraces had this allele. The hard grain texture allele (Pinb-D1B) was frequently present in modern cultivars (38%), but it was absent in landraces (Figure 4F), which was verified at genetic differentiation between two subgroups (Figure 4E). Additionally, we found that loci including Glu-D1, PSY1-D1 and Pinb2-B2 had obvious genetic difference (Table S4), and corresponding allele frequencies were significantly different between cultivars and landraces (Figure S9).

Grain yield genes

For yield-related genes, the most significant difference occurred in TaGW2-6B (Figure 4G), at which the favorable allele Hap-1 was predominant in modern cultivars (76%), but absent in landraces (Figure 4H). At TGW6, Cwi-4A and GS-D1 loci, the favorable allele frequencies were higher in modern cultivars than in landraces (Figure S10), whereas a contrary situation was observed at GASR-A1, Sus1-7A and GW2-6A loci.

Genetic contribution from founder parents for Ningxia wheat cultivars

Founder parents, as one of important genetic resources, have greatly promoted improvement of wheat varieties in China since the 1950s. In this study, we analyzed genetic contributions of six founder parents, including Moba 66, Abbondanza, Beijing 8, Orofen, Xiaoyan 6 and Zhou 8425B, to modern cultivars in Ningxia province. To clearly understand the importance of founder parents, we counted the number of favorable alleles of genes for yield, quality, adaptation and stress resistance in these cultivars (Figure 5). Results showed that the number of favorable alleles for higher TKW in six founder parents ranged from three to seven at ten yield genes. The founder parent Xiaoyan 6 carried seven favorable alleles that were Hap-4A-C at Cwi-4A, GS-D1a at GS-D1, Hap-H at Sus1-7A and 2B, Hap-A at GW2-6A, Hap-1 at GW2-6B and TGW6-A1a at TGW6 (Figure S11). At 14 quality genes, the average favorable alleles were about six, ranging from five to nine. The founder parent Xiaoyan 6 carried the most of favorable alleles, which was Ax1 or Ax2* at Glu-A1, Glu-D1d at Glu-D1, Zds-A1a at Zds-A1, Pds-B1b at Pds-B1, Pod-A1b at Pod-A1,
Psy-A1b at Psy-A1, Psy-D1a at Psy1-D1, Psy-B1a or b at Psy-B1 and Wx-B1b at Wx-B1. All six founder parents carried Psy-A1b, Psy-D1a and Psy-B1a or b associated with low YP content except Psy-D1 for Zhou 8425B (Figure S12). For stress resistance, the founder parent Abbondanza had eight favorable alleles including PHS+ at PHS1, Vp-1Bc at VP-1B, PHS+ at MFT-A1, Westonia type at 1-fehw3, Dreb-B1a at Dreb-B1, Hap-5A-A at DRO-5A, Hap-5B-II at DRO-5B and Lr14+ at Lr14a (Figure S13). At six adaptation genes, the photoperiod-insensitive allele (Ppd-D1a) was present in all founder parents except Orofen. Besides, Moba 66, Xiaoyan 6 and Zhou 8425B had the dwarfing allele Rht-B1b. And Zhou 8425B also had another dwarfing allele Rht-D1b (Figure S14). Such evaluation of these founder parents from different types of functional genes allowed us to aim at contributions to breeding improvement in Ningxia province.

To compare differences at four types of genes between founder parents and modern cultivars, we investigated relationship number of alleles with the proportion of accessions. The 17% of founder parents had seven and nine favorable alleles at yield and quality genes, respectively, while the proportion was 7% on average in modern cultivars (Figures 5B, C). Most founder parents and modern cultivars had three to six allelic variations at resistance genes and carried dwarfing, spring-type and photoperiod-insensitive alleles at adaptation genes (Figures 5D, E).

The gene flow value at yield genes was 2.47 between modern cultivars and Zhou 8425B, which is most frequent among all founder parents, indicating that Zhou 8425B had the largest genetic exchange with modern cultivars and played an important role in yield potential in Ningxia wheats (Figure 6). At quality and resistance genes, all founder parents had nearly equal gene flow to modern cultivars, with an average gene flow of 0.60 at quality loci ranging from 0.48 to 0.76, and 0.53 at resistance loci ranging from 0.40 to 0.69. For adaptation genes, the gene flow values between founder parents Abbondanza, Orofen and modern cultivars were 0.82 and 0.63, respectively. In a word, founder parents with different favorable alleles pushed together improvement of Ningxia wheat cultivars.
Discussion

Population structure indicated wheat genetic components in Ningxia province

Ningxia province, located in north-western of China, is one of the major spring wheat grown regions and has been experienced five times of varietal replacements, i.e., direct utilization of ‘Quality Florence’, a wheat germplasm introduced from Australia in 1950s; efficient use of ‘Abbondanza’ wheat resource from Italy in 1960s; breeding of milestone variety ‘Doudi 1’ in 1970s; application and improvement of ‘Ningchun 4’ in 1980s, and release of ‘Ningchun 50’ in 2000s [48]. Looking back to wheat breeding history, it is not difficult to find foreign wheat germplasm that has played an important role in China, especially from establishment of the People’s Republic of China in 1949 to present. Of course, landraces with particular genetic resources have been applied to improve cultivars in breeding program. In this study, all varieties could be clustered into three subgroups based on the population structure analyses, namely modern cultivars, landraces and others, which showed their genetic differences based on 44 important functional genes (Figures 3A, S3). The three genetic divergent groups were in accordance with wheat breeding history of utilizing wheat genetic resources from other regions and local landraces in Ningxia province.

Ningxia, being diversified ecological types, is among the provinces that could benefit from the use of introduced germplasm and landraces from genebanks in China. To further clarify the contribution of introduced germplasm and landraces during the successive decades of breeding process in Ningxia province, genetic diversities and the frequency spectra divergence were evaluated based on 44 genes for yield, quality, adaptation and resistance. In this study, genetic diversities are most enriched for the subgroup Others comprising introductions outside of Ningxia province, followed by modern cultivars, and landraces have minimum genetic diversities at 44 important loci (Figures 2A, 3B). This indicates that conventional artificial hybridizing breeding using external resources to improve varieties has increased diversities by promoting gene exchanges and recombination in gene coding regions in particular important cloned functional genes for self-pollinated wheat [49]. Besides, introduced varieties
from other regions with rich genetic diversities facilitate local breeding improvement, which means introduced varieties made a significant genetic contribution to Chinese modern cultivars [41]. Allele frequency spectra divergence also supports this point on gene levels. Dwarfing alleles \( \text{Rht-B}1b \) at \( \text{Rht-B}1 \) and \( \text{Rht-D}1b \) at \( \text{Rht-D}1 \), the well-known green revolution genes that swept through China and occupied significantly positive influence on wheat breeding, are very widespread in Ningxia modern cultivars and introductions from other regions but absent in landraces (Figures 4B, S5A, S7), indicating that introduced wheat germplasm with numerous beneficial genes are vital for broadening the genetic diversities of Ningxia wheat cultivars. Similar types of genes also include \( 1B/1R \) translocation, \( \text{GW}2-\text{6B} \), and \( \text{Sus}2-\text{2B} \) (Figures 2D, S8, S10). As autochthonous traditional varieties, evaluation of wheat landraces stored in gene banks with highly beneficial untapped diversity and sources of stress adaptation should be used for wheat improvement [50]. Due to colonization of diverse ecological environments in the process of domestication and selections by ancient farmers in Ningxia province, landraces contain wider specific genetic loci than most breeding programs and form the basis of early wheat breeding, especially for China in pre-1950s. Zhou et al [5] highlights environmental stresses and independent selection efforts that have resulted in considerable genome-wide divergence at the population level in Chinese wheat landraces. Of course, this characteristic has been exploited in other countries, where the first improved varieties consisted of selections of local landraces [50], such as landrace population ‘Catalan de Monte’ in Spain [52-53] and ‘Turkey Red’ in the United States [54]. Overall, our results indicate the point that landraces with good adaptation and introduced varieties with wide diversities will co-promote wheat breeding in Ningxia province.

**Dissecting allele frequency identified selection direction of important genes**

The modern wheat breeding practices accompanying intensive selection pressure have always focused on economically important loci [55-56]. For each of those loci contributing to agronomic phenotypes, causal polymorphisms were identified with increased frequencies of favorable alleles consistent with selection during modern
breeding [57]. For improvement selection for Ningxia wheat from landraces to modern cultivars in this study, we found the evidence of convergent increases in allele frequencies at targeted genes. VP-1B is one of important seed dormancy genes for PHS tolerance during harvest [58] and the favorable allele Vp-1Bc was predominant with a frequency of 68% in Ningxia modern cultivars. Similarly, Hap-1 at GW2-6B locus influencing strongly kernel width and thousand kernel weight was observed in 76% modern cultivars, while the desirable allele was absent in landraces, indicating that breeders selected intensively the favorable allele at GW2-6B locus due to the demand for increasing grain yield in breeding. HMW-GS was influenced at Glu-D1 [23]. The allele Glu-D1d associated with strong gluten contents and superior bread-making quality attributes had relatively high frequency in modern cultivars compared with landraces, which is concordant with the reports of most cultivars in Pakistani and China [22, 55-56]. Besides, the favorable allele Hap-4A-C at Cwi-4A encoding CWI enzyme that converts sucrose to glucose associated with grain size was present in 85% of modern cultivars, showing the effective use of this gene in wheat molecular breeding.

For some important genes, their favorable alleles had maintained high values in varieties before modern wheat breeding [41]. The photoperiod insensitive allele, Ppd-D1a, was fixed with a frequency of 100% in both landraces and modern cultivars, showing that this gene is so important that it has been selected completely before modern breeding in Ningxia. As we know, flowering time is one of the most important developmental traits for wheat adaptability and yield stability in target environments, and the photoperiod insensitive allele at photoperiod response (Ppd1) is known to be major determinants of flowering time optimization [59]. Therefore, early flowering with varieties carrying photoperiod insensitive allele was fixed during long-time selection process. High yellow pigment content is favored for durum wheat pasta, but is considered undesirable for Chinese steamed bread and white noodles [60-62] and the alleles P3y-A1b and P3y-B1a/b are encouraged. The frequencies of two alleles were approached almost 100% and were fixed in both landraces and modern cultivars before breeding selection in Ningxia.
However, to breed perfect wheat varieties in Ningxia, these favorable alleles with minor frequencies in modern cultivars should be regarded. In this study, the root architecture related gene \textit{DRO-5B} is an IAA response gene which is responsible for reduced height and increased thousand kernel weight [63-64]. The favorable allele \textit{Hap-5B-II} was present in 54\% of landraces, but only 5\% of modern cultivars, indicating that this allele has not been focused by breeders in the past. Besides, \textit{H1c} at \textit{GASR-A1} locus which influences grain length were pre-dominant in landraces, but had a low frequency in modern cultivars. This comes to the point that breeding is a process of aggregating desirable genes and eliminating undesirable or even deleterious alleles. Low frequencies of favorable alleles for important genes in modern cultivars identified the improvement direction in future wheat breeding in Ningxia and are helpful to further performing breeding by design.

\textbf{Founder parents contain the combination of important functional genes}

Founder parents, which serve as important germplasm resources, play a pivotal role in update of new varieties [65]. They exhibited not only superior phenotypes, high combining ability, but also wide adaptation and prominent specific characteristic [66]. Previous studies found that genes controlling important traits were the combination rather than randomly distributed on chromosomes in founder parents [67-72]. For example, the pedigree analysis of rice \textit{Huanghuazhan} showed 61.79\% of 50 kb blocks are HTBs (\textit{Huanghuazhan} traceable blocks) together with the elite performance of \textit{Huanghuazhan}, and large scale of important genes locate on HTBs, supporting that they are the combination of elite allele of important genes [72]. In this study, counting number of favorable alleles at ten yield-related genes had successfully clarified 3~7 favorable alleles for higher TKW in six founder parents. Furthermore, \textit{Hap-4A-C} at \textit{Cwi-4A}, \textit{Hap-H} at \textit{Sus1-7A}, \textit{GS-D1a} at \textit{GS-D1}, \textit{Hap-A} at \textit{GW2-6A}, \textit{Hap-l} at \textit{GW2-6B} and \textit{TGW6-A1a} at \textit{TGW6} were conserved in founder parents \textit{Zhou 8425B}, \textit{Xiaoyan 6} and \textit{Abbondanza} except for \textit{GS-D1a} allele. For quality-related genes, the average number of favorable alleles was about six, and \textit{Psy-Alb}, \textit{Psy-D1a}, \textit{Psy-B1a} or \textit{b} associated with low YP content were conserved across all founder parents, showing
that favorable alleles of these important genes had been conserved in modern wheat breeding. Interestingly, six founder parents carried different favorable alleles at resistance and adaptation related genes, probably due to the reason that these genes are randomly selected to respond to varied environments, so that these founder parents can maintain high yield and good quality wherever cultivated.

Founder parents have excellent allele combinations of important genes for agronomical desirable traits, and many varieties have been derived from them. In this study, yield traits improvement of modern cultivars is the main achievement using founder parents in Ningxia wheat breeding. Gene flow was most frequent (2.47) comparing the modern cultivars with founder parents Zhou 8425B for yield related genes, meaning that founder parents especially from Zhou 8425B contributed greatly in yield improvement of Ningxia wheat. Zhou 8425B is a founder parent fitting current breeding needs with features of dwarfing, high yielding and disease resistance, and more than 300 wheat varieties (lines) such as AK58, Zhou 16 were bred from this parent [65, 72-73]. High yield is an ever-important objective of wheat breeding, and analysis of the breeding history of many crop species revealed the presence and roles of founder parents [67]. Li et al [75] found that Beijing 8 serving as a founder parent has contributed many loci in close proximity to the positions of known yield component genes conferring important traits in breeding. The pedigree analysis showed that inherited ancestor genome segments donated to rice variety Huanghuazhan extremely enrich in grain yield category [72].

**New era of functional markers is advanced through progress of cloning genes with published reference genome**

Functional markers that have strong associations with relevant phenotypes, are ideal for gene tagging and allelic variants can be diagnosed with functional genes in breeding [30, 76]. Liu et al. [30] documented 97 functional markers that detect 93 alleles at 30 loci in bread wheat. Rasheed et al [36] converted gel-based functional markers to high-throughput KASP markers. In this study, we evaluate molecular characterization and improvement history of Ningxia wheat breeding in terms of
important genes related with adaptation, stress resistance, quality and yield utilizing these KASP functional markers. However, an objective fact is that these in our research are only a few number of predicted genes relative to wheat whole genome. Currently, most gene mapping studies (both QTL and GWAS) could identify more genes controlling agronomic traits and have been converted to KASP markers [76]. More than 150 KASP markers for almost 100 functional genes were developed and 72 were validated in a bread wheat diversity panel [77]. With the innovations of whole-genome assemblies, revolutionized advances of reference genome sequences were recently gained for bread wheat ‘Chinese Spring’ [78] and its progenitors, T. turgidum spp. dicoccoides [79], Aegilops tauschii [80-81] and T. urartu [82]. These wheat genome data provide new opportunities to uncover genetic variation in traits of breeding interest and enable genome-based breeding to deliver wheat cultivars. High-throughput genotyping technology of KASP is now used in wheat not only for breeding by design but also for high-density genome-wide genotyping. These advances will promote wheat research to dig more important genes of targeting traits and ensure sustainable wheat production through developing widely adapted wheat cultivars with high yield and nutritional quality.

**Conclusions**

In this study, we report a comprehensive functional gene assessment of modern improved wheat based on 44 important genes underpinning grain yield, quality, adaptation and resistance in 207 cultivars and lines in Ningxia province. Introduced varieties from other regions with rich genetic diversities and landraces with well-adapted genetic resources have been applying to improve modern cultivars. Founder parents in particular Zhou 8425B for yield related genes contributed greatly to breeding improvement of wheat in Ningxia province. This work reports genetic characteristics on gene levels and advances improvement selection of future wheat breeding at Ningxia province.

**Additional files**
Additional file 1: Table S1. Detailed information of materials and their allelic variations of 44 genes used in this study.

Additional file 1: Table S2. Basic information including allelic variations and primer sequences for the 44 KASP assays.

Additional file 1: Table S3. Gene diversities and genetic differentiation ($Fst$) of 44 polymorphic genes between Ningxia and Others accessions.

Additional file 1: Table S4. Gene diversities and genetic differentiation ($Fst$) of 44 polymorphic genes between landraces and modern cultivars in Ningxia province.

Additional file 2: Figure S1. KASP genotyping at $Rht-D1$, $VP-1B$ and $Glu-A1$. Red and blue dots show homozygous varieties; Green dots show heterozygous varieties; Black dots show negative control; X shows missing types.

Additional file 3: Figure S2. Plot of Delta K against putative K ranging from 1 to 8.

Additional file 4: Figure S3. Population structure of all accessions based on Structure from K=2 to K=3.

Additional file 5: Figure S4. Genetic diversities on four types of genes between Ningxia and Others subgroups. (A) Genetic diversities on grain yield genes. (B) Genetic diversities on quality genes. (C) Genetic diversities on adaptation genes. (D) Genetic diversities on stress resistance genes.

Additional file 6: Figure S5. Allele frequencies between Ningxia and Others subgroups at adaptation (A) and stress resistance (B) genes.

Additional file 7: Figure S6. Genetic diversities on four types of genes between modern cultivars and landraces subgroups in Ningxia province. (A) Genetic diversities on adaptation genes. (B) Genetic diversities on stress resistance genes. (C) Genetic diversities on quality genes. (D) Genetic diversities on grain yield genes.

Additional file 8: Figure S7. Allele frequencies of adaptation genes between modern cultivars and landraces in Ningxia province.

Additional file 9: Figure S8. Allele frequencies of stress resistance genes between modern cultivars and landraces in Ningxia province.

Additional file 10: Figure S9. Allele frequencies of quality genes between modern cultivars and landraces in Ningxia province.
Additional file11: Figure S10. Allele frequencies of grain yield genes between cultivars and landraces in Ningxia province.

Additional file12: Figure S11. Distribution of allelic variations of grain yield genes in six founder parents.

Additional file13: Figure S12. Distribution of allelic variations of quality genes in six founder parents.

Additional file14: Figure S13. Distribution of allelic variations of stress resistance genes in six founder parents.

Additional file15: Figure S14. Distribution of allelic variations of adaptation genes in six founder parents.

Abbreviations

Fst: F-statistics; HWM-GS: High-Molecular-Weight Glutenin Subunits; KASP: Kompetitive Allele Specific PCR; PCA: Principal Coordinate Analysis; MCMC: Markov Chain Monte Carlo; TKW: Thousand Kernel Weight; SNP: Single Nucleotide Polymorphism; LGC: Laboratory of the Government Chemist; HTBs: Huanghuazhan Traceable Blocks

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Authors’ Contributions
Weijun Zhang carried out the experiments and wrote the manuscript; Junjie Zhao helped to carry out the experiments and analyzed the data; Jinshang He guided the use of instruments; Ling Kang prepared a part of figures for the manuscript; Xiaoliang Wang and Fuguo Zhang gave suggestions for the experiments; Chenyang Hao participated in the design of experiments and contributed to writing the manuscript; Xiongfeng Ma contributed to overall design of the experiments; Dongsheng Chen provided advice for data analysis and assisted in writing the manuscript. All authors have read and approved the final version.

**Availability of data and materials**

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable requests.

**Conflicts of interest/Competing interests**

The authors declare no conflicts of interest.
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Legends of Figures

Figure 1. The population structure of 207 wheat accessions based on 44 genes. (A) A neighbor-joining tree of all accessions. Different lines are presented in different colors. (B) Plots of first three principal components of all accessions. (C) Population structure of all accessions based on Structure.

Figure 2. Genetic divergence on all 44 genes between Ningxia and Others subgroups. (A) Genetic diversities between Ningxia and Others subgroups. (B) Genetic differentiation (Fst) between Genetic diversities on grain yield genes. (C) Allele frequency at grain yield genes between Ningxia and Others subgroups. (D) Allele frequencies of quality genes between Ningxia and Others subgroups.

Figure 3. A neighbor-joining tree and genetic diversity based on 44 genes between modern cultivars and landraces in Ningxia province.

Figure 4. Genetic differentiation (Fst) and allele frequencies of 44 genes between modern cultivars and landraces in Ningxia province. (A) (B) Fst and allele frequencies at adaptation genes between modern cultivars and landraces. (C) (D) Fst and allele frequencies of stress resistance genes between modern cultivars and landraces. (E) (F) Fst and allele frequencies of quality genes between modern cultivars and landraces. (G) (H) Fst and allele frequency of grain yield-related genes between modern cultivars and landraces.

Figure 5. Distributions of favorable alleles of yield, quality, stress resistance and adaptation genes in founder parents and modern cultivars. (A) Number of favorable alleles at four types of genes in six founder parents. (B) Proportion of accessions carrying different numbers of favorable alleles of yield genes in founder parents and modern cultivars. (C) Proportion of accessions carrying different numbers of favorable alleles of quality genes in founder parents and modern cultivars. (D) Proportion of accessions carrying different numbers of favorable alleles of stress resistance genes in founder parents and modern cultivars. (E) Proportion of accessions carrying different numbers of favorable alleles of adaptation genes in founder parents and modern cultivars.
Figure 6. Gene flow between six founder parents and modern cultivars at yield, quality, stress resistance and adaptation genes.