



# OPEN Genetic analysis identifies key loci for traits and resistance in Qinghai plateau wheat F<sub>2</sub> populations

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The Qinghai Plateau's high-altitude conditions present significant challenges for wheat cultivation, demanding varieties with enhanced adaptability and stress resistance. However, the genetic basis for key traits like grain yield and stress tolerance in wheat adapted to these conditions remains poorly understood. This knowledge is crucial for developing wheat varieties that can thrive in the harsh environment of the Qinghai Plateau. This study aimed to uncover the genetic foundation of key traits by analyzing five F<sub>2</sub> populations from crosses between Qinghai Plateau spring wheat varieties and CIMMYT germplasm. We used a target sequencing breeder chip to assess phenotypic variation in six parental lines. We identified potential genes for all-stage resistance (APR) and adult plant resistance (ASR) to stripe rust. Genotyping lines across the F<sub>2</sub> populations revealed 99 loci/genes associated with ten key traits, with 35 showing genetic separation. Notably, the number of stripe rust resistance genes in the parental lines ranged from 4 to 9, with the F<sub>2</sub> lines aggregating between 0 and 9 genes. Grain-related genes were aggregated in varying numbers, with the majority of lines carrying two genes. Gene frequency analysis revealed significant variation across populations, contributing valuable data for marker-assisted breeding and advancing wheat molecular breeding research.

**Keywords** QINGHAI plateau, Spring wheat, CIMMYT, Genetic analysis, Marker-assisted selection (MAS)

Wheat (*Triticum aestivum* L.), a cornerstone of global food security, supplies essential dietary energy to about 40% of the global population<sup>1</sup>. Amid rapid global population growth and climate change challenges, wheat demand has reached unprecedented levels. By 2050, wheat production is projected to need a significant increase to meet escalating demand<sup>2,3</sup>. However, significantly improving wheat yield genetically faces challenges. Traditional breeding methods, limited by technology, struggle to make breakthroughs, and increasing environmental and biotic stresses severely challenge wheat productivity<sup>4</sup>. Fortunately, rapid advances in molecular biology and genomics have opened new avenues for wheat breeding, with molecular breeding emerging as a key approach for genetic enhancement. Recently, genomics and molecular biology have significantly aided in identifying genes and loci associated with phenotypic traits in wheat and its relatives. In wheat stripe rust resistance, 86 named resistance genes (*Yr1*-*Yr86*) have been identified across 21 chromosomes. Among them, 58 genes show adult plant resistance (APR), and the other 28 are all-stage resistance (ASR) genes<sup>5</sup>. Nine genes have been cloned to date, including *Yr5*, *Yrsp*, *Yr7*, *Yr10*, *Yr15*, *Yr36*, *Yr18*, *YrU1*, and *Yr46*. Four genes show pleiotropic resistance: *Yr18/Lr34/Pm38/Sr57*, *Yr29/Lr46/Pm39/Sr58*, *Yr30/Lr27/Pmx/Sr2*, and *Yr46/Lr67/Pm46/Sr55*<sup>6</sup>. Research indicates that *Yr5*, *Yr15*, *Yr32*, and *Yr76* alone are effective against the V26 (CYR34) pathogen population<sup>7</sup>. CIMMYT has significantly advanced the use of adult plant resistance, with about 60% of its materials possessing APR genes, especially *Yr18/Lr34/Sr57/Pm38*, now widely cultivated worldwide. Several resistance genes exhibit additive effects, and pyramiding of multiple adult plant resistance genes, like *Yr18/Lr34/Sr57/Pm38*, *Yr29/Lr46/Sr58/Pm39*, and *Yr46/Lr67/Sr55/Pm46*, has bred varieties with durable resistance to multiple diseases<sup>8,9</sup>. The durable multi-disease resistance genes *Yr18*, *Yr29*, *Yr30*, and *Yr46* show high susceptibility to stripe rust at the seedling stage, suggesting limited efficacy of individual genes. In China's wheat resistance breeding, pyramiding multiple adult plant resistance genes significantly boosts durable resistance to stripe rust<sup>10</sup>. Scientists have identified and cloned several key genes controlling grain size and weight via comparative genomics. These include *TaGW2*, *TaGS1a*, *TaTGW6*, *TaGS5-3 A*, *TaFlo2-A1*, and *TaTPP-6AL1*. *TaGW2* family members (*TaGW2-6A*, *TaGW2-6B*,

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and *TaGW2-6D*) differentially and additively affect grain weight and size. A particular *TaGW2-6A* haplotype is significantly linked to higher thousand-grain weight and width, effectively increasing grain weight in marker-assisted selection<sup>11</sup>. *TaSus2-2B*, a sucrose synthase gene in wheat, is primarily expressed during endosperm development and is closely associated with yield traits. This gene family is mapped to wheat chromosomes 2A, 2B, and 2D<sup>12</sup>. *CWI*, a cell wall invertase and one of three invertases, is closely related to crop yield. This  $\beta$ -fructofuranosidase hydrolase enzyme catalyzes the irreversible conversion of sucrose to glucose and fructose, and the wheat gene *TaCwi-A1* is associated with grain weight<sup>13</sup>. In wheat, transgenic expression of chloroplast glutamine synthetase (GS) increases nitrogen uptake and yield, with the GS family member *TaGS5-AL* also contributing<sup>14</sup>. Moreover, *TaCwi-A1*, *TaGW2-6A*, and *TaTGW6-4 A* are found in 49 major Qinghai wheat varieties, with materials carrying the top three haplotype combinations (*TaCwi-A1a/Hap6A-A/TaTGW64Aa*) showing the highest thousand-grain weight, marking them as the optimal gene combination for thousand-grain weight in Qinghai wheat<sup>15</sup>. The complexity of the wheat genome presents challenges for developing molecular markers and cloning genes. However, advances in whole-genome sequencing and the release of wheat and relatives' genome sequences<sup>16–19</sup> have aided fine mapping and cloning of adult plant resistance genes in wheat. In wheat genetic mapping, molecular marker use has expanded, with marker types increasing from initial RFLP markers<sup>20</sup> to PCR-based RAPD<sup>21</sup> and SSR markers<sup>22,23</sup>. A series of SNP chips for wheat, including 660 K, 90 K, 55 K, 35 K, and 16 K, have been developed. These chips offer high throughput, low cost, time efficiency, and stable results, providing a scientific basis for rapidly and precisely selecting elite disease-resistant varieties in breeding. This accelerates the development of high-yield, high-quality wheat varieties, crucial for global wheat breeding<sup>24</sup>. In 2023, Northwest A&F University compiled wheat whole-genome data from 2000 to the present, encompassing chip data, QTL, MetaQTL, GWAS sites, and cloned functional genes, to create a wheat whole-genome gene/QTL database. Using data processing, transformation, and SNP screening with GenoBait technology, a wheat whole-genome disease resistance molecular breeding chip was developed, including the GBW16K molecular breeding background chip and the GBW0.1 K functional marker prospect selection chip. The GBW16K chip includes 14,868 SNP regions evenly spread across the wheat genome, while the functional marker chip covers 101 SNP regions for 60 major breeding targets with high heritability and gene typing reliability<sup>25</sup>. The Qinghai-Tibet Plateau's unique ecology, marked by high altitude, low temperatures, diseases, and water scarcity, impedes agricultural technology development and adversely affects spring wheat cultivation, a key regional crop. There is an urgent need to develop spring wheat varieties that are high-yielding, high-quality, and resistant to multiple diseases, with durable resistance<sup>26</sup>. Over 50 years, the Northwest Institute of Plateau Biology, Chinese Academy of Sciences, has developed 26 new "Gaoyuan" and "Qingmai" varieties, characterized by large spikes, high yield, lodging resistance, and drought tolerance. For example, Gaoyuan 448, cultivated widely in Qinghai, is known for high yield, water efficiency, and lodging resistance and has long been the control variety in Qinghai's regional wheat trials. However, continuous mutation of stripe rust pathogens in Qinghai has reduced Gaoyuan 448's resistance, resulting in severe disease and yield loss. Thus, enhancing resistance genes to improve Gaoyuan 448's resistance is crucial for regaining its high-yield traits and prolonging its lifespan. CIMMYT has led efforts to enhance wheat varieties' yield potential and resistance to biotic and abiotic stresses<sup>27</sup>. Studies indicate that CIMMYT-bred wheat lines can be directly cultivated on the Qinghai-Tibet Plateau<sup>28</sup> as parental material to leverage their superior genetics for local variety improvement. In this study, we selected key Qinghai-Tibet Plateau spring wheat varieties—'Abodanza (ABO)', 'Heshangtuo (HST)', 'Gaoyuan 448 (GY448)', 'Qingmai NO 1 (QM1)', and 'Qingchun 38 (QC38)'—as maternal donors, and MUCUY, a CIMMYT-introduced, high-resistant, and high-quality material from 2018, as the paternal donor. We created hybrid combinations and established F<sub>2</sub> populations. this study eliminated paternal contribution noise and directly quantified maternal genetic effects on offspring traits by maintaining a constant paternal genome across five genetically diverse maternal populations. Using the GBW0.1 K functional marker chip, we analyzed the frequency of key genes in five F<sub>2</sub> populations, characterized the genetics of resistance, grain, and dwarf genes in F<sub>2</sub>, uncovered genetic patterns of key traits, provided a theoretical basis for marker-assisted breeding, and supported wheat molecular breeding research with data.

## Materials and methods

### Plant materials

'ABO', introduced to China in 1956, has become a key variety for promotion in the northwestern wheat regions. In addition to its direct use in cultivation, 'ABO' is a superior parent in wheat breeding in Qinghai, owing to its exceptional germplasm traits. Over the decades, numerous wheat varieties cultivated in Qinghai have inherited the 'Abodanza' germplasm lineage<sup>29</sup>. It is popular among Qinghai farmers and boasts excellent quality, robust drought resistance, consistent yields, and adaptability to extensive management practices. 'HTS', a high-quality local variety from Gansu's arid regions, is renowned for its drought tolerance, adaptability to poor soils and salinity, deep sowing capabilities, and high-quality flour. 'Qing Chun 38', developed by the Crops Institute of Qinghai Academy of Agricultural and Forestry Sciences, is a wheat variety resulting from a cross between Canadian red wheat and winter wheat 03702/W97208. In 2007, 'Qing Chun 38' yielded 466.5 kg/mu in Huangzhong County, Qinghai Province, marking an 11.1% increase over the control variety, 'Gaoyuan 448' spring wheat<sup>30</sup>. 'GY448', a predominant variety in Qinghai's irrigated regions, was developed by the Northwest Plateau Institute of Biology, Chinese Academy of Sciences, and has the pedigree 'Qing Chun 533'/'Plateau602'. The 'Gaoyuan 448' yield in Huangzhong, Qinghai, reached 6912 kg/ha in 2000, as reported by<sup>31</sup>. In 2007, 'Gaoyuan 448' yields in the Qaidam Basin of Qinghai varied from 9.75 to 10.50 t/ha under high water and fertilizer conditions and from 6.75 to 9.00 t/ha under average conditions. 'Qing Mai NO1', developed by the Northwest Plateau Institute of Biology, Chinese Academy of Sciences. In 2023, 'Qing Mai NO 1' was the control variety in Qinghai's spring wheat regional trials, with yields ranging from 7080–12210 kg/ha across seven sites, averaging 8550 kg/ha. Mucuy (CIMMYT ID: 5663955), a result of "Mutus" selfed twice with "Akuri", demonstrates high field resistance to yellow rust (YR)

and leaf rust (LR) in multiple countries, including China, but shows moderate resistance under greenhouse conditions at the seedling stage. During the resistance gene mining of Mucuy, seven YR and four LR resistance loci were identified, including the known genes *Yr29/Lr46* and a novel locus *QYr.cim-3DS*, all contributing to its durable rust resistance<sup>32</sup>. This study chose five predominant wheat varieties from Qinghai as maternal parents: ABO, HST, GY448, QM 1, and QC 38, and established corresponding genetic populations comprising 78 families from ABO×Mucuy (POP1), 74 families from HST×Mucuy (POP2), 47 families from QC 38×Mucuy (POP3), 214 families from GY448×Mucuy (POP4), and 278 families from QM1×Mucuy (POP5), totaling five F<sub>2</sub> families. These parents and genetic populations were cultivated at the Haidong Ecological Agricultural Experimental Station, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, located in Haidong City (102°19'32"E, 36°28'60"N, Qinghai Province; the station sits in a typical transitional zone between the Loess Plateau and the Qinghai-Tibet Plateau, at 2016 meters above sea level, with an average annual temperature of 3.2 to 8.6°C, annual precipitation ranging from 319.2 to 531.9 mm, evaporation rates between 1275.6 to 1861 mm, average annual sunshine duration of 2708 to 3636 hours, and a frost-free period of approximately 90 days.

DNA extraction and a target sequencing breeder chip

Test material sampling was performed at the three-leaf stage, and leaf DNA was extracted using the CTAB method<sup>33,34</sup>. DNA quality control was conducted using a NanoDrop One instrument. We evaluated 101 functional genes associated with various traits, including 41 disease-resistance genes (e.g., *QYr.nwafu-3BS*, *QYr.nwafu-4BL*). KASP marker detection was performed according to the protocol by Rasheed et al. (GBW0.1 K), with the analysis conducted at Northwest A&F University.

Results

Phenotype in parents

Phenotypic variation analysis was conducted on six parents, focusing on stripe rust resistance (seedling and adult plant stages), field agronomic traits, and grain characteristics (Table 1). Assessments of stripe rust resistance at the seedling and adult plant stages indicated that ABO, QC38, and GY448 showed moderate resistance at the seedling stage but were highly susceptible at the adult plant stage, suggesting the presence of resistance genes that may be active throughout the growth period. In contrast, HST, QM1, and Mucuy exhibited high susceptibility at the seedling stage but resistance at the adult plant stage, indicating they may harbor genes that confer resistance during the adult plant phase. Mucuy had the highest tiller number among field agronomic traits, averaging 4.3 ± 0.58 (*P* > 0.05) tillers. QM1 had the most extended spike neck length, measuring 45.2 ± 1.5 (*P* > 0.05), while QC38 had the most extended spike length, at 14.2 ± 1.26 cm, (*P* < 0.001). Gaoyuan 448 had the highest number of grains per spike, with 74.0 ± 5.19 (*P* < 0.001). Regarding plant height, Heshangtuo was the tallest, at 142.8 ± 2.75 cm (*P* < 0.001), and Mucuy was the shortest, at 84 ± 6.6 cm (*P* < 0.001). Mucuy was the only parent with awns, while the others were awnless. QM1 had the largest average grain area, grain perimeter, average grain length and length-to-width ratio, measuring 20.34 ± 0.72 mm<sup>2</sup> (*P* < 0.001), 18.26 ± 0.34 mm (*P* < 0.001), 6.77 ± 0.14 mm (*P* < 0.001) and 1.70 ± 0.04 (*P* < 0.001), respectively. GY448 had the largest width with 4.20 ± 0.43 mm<sup>2</sup>.

Genotype in parents

The markers, chromosomal information, and associated traits for all tested genes are presented in Table 2. Specifically, *QYrsn.nwafu-2AS*, *QYrqin.nwafu-2BL* and *QYrsn.nwafu-3DL* were detected exclusively in HST. In contrast, *QYrqin.nwafu-2AL*, *QYr.nwafu-3BS*, and *QYrqin.nwafu-6BS* were found in most wheat parents. *Yr29*

Phenotype	Key indicators	ABO	HST	QC38	GY448	QM1	MUCUY
Stripe rust resistance	Adult Stage	100	20	100	100	40	0
	Seedling Stage	3	8	3	3	6	8
Agronomic traits	Tillering Number	4 ± 1 <sup>A</sup>	4.3 ± 2.3 <sup>A</sup>	3.7 ± 0.6 <sup>A</sup>	3.3 ± 1.5 <sup>A</sup>	3.7 ± 0.6 <sup>A</sup>	4.3 ± 0.6 <sup>A</sup>
	Spike Neck Node	43.5 ± 10.8 <sup>A</sup>	43 ± 12.2 <sup>A</sup>	43.2 ± 3.2 <sup>A</sup>	42.5 ± 1.6 <sup>A</sup>	45.2 ± 1.5 <sup>A</sup>	31.5 ± 2.3 <sup>A</sup>
	Spike length (cm)	10.67 ± 0.6 <sup>C</sup>	13.5 ± 0.5 <sup>AB</sup>	14.2 ± 1.2 <sup>A</sup>	11.4 ± 0.5 <sup>BC</sup>	12.2 ± 1.0 <sup>ABC</sup>	11 ± 0.5 <sup>C</sup>
	Spikelet Number (cm)	16 ± 0 <sup>C</sup>	18.67 ± 1.2 <sup>BC</sup>	20 ± 0 <sup>AB</sup>	22 ± 1.7 <sup>A</sup>	19.0 ± 1.0 <sup>B</sup>	16 ± 0 <sup>C</sup>
	Sterile Spikelet Number	0.67 ± 1.2 <sup>A</sup>	0 ± 0 <sup>A</sup>	0.3 ± 0.6 <sup>A</sup>	0 ± 0 <sup>A</sup>	0.3 ± 0.6 <sup>A</sup>	0 ± 0 <sup>A</sup>
	Spikelet Grain Number	51.33 ± 6.5 <sup>C</sup>	52.7 ± 5.0 <sup>C</sup>	67 ± 1.0 <sup>AB</sup>	74.0 ± 5.2 <sup>A</sup>	73.0 ± 2.0 <sup>A</sup>	54 ± 6.1 <sup>BC</sup>
	Plant Height (cm)	114.0 ± 7.0 <sup>B</sup>	142.8 ± 2.8 <sup>A</sup>	107.7 ± 2.1 <sup>BC</sup>	109.7 ± 0.6 <sup>B</sup>	95.3 ± 0.6 <sup>CD</sup>	84 ± 6.6 <sup>D</sup>
	Awn Character	Awnless	Awnless	Long Awn	Awnless	Long Awn	Long Awn
Grain traits	Area Mean (mm <sup>2</sup> )	17.58 ± 0.20 <sup>B</sup>	17.69 ± 0.24 <sup>B</sup>	17.12 ± 0.27 <sup>B</sup>	19.65 ± 0.27 <sup>A</sup>	14.2 ± 0.72 <sup>A</sup>	17.27 ± 0.67 <sup>B</sup>
	Perimeter Mean (mm)	16.54 ± 0.12 <sup>C</sup>	16.66 ± 1.10 <sup>C</sup>	16.72 ± 0.08 <sup>C</sup>	17.43 ± 0.09 <sup>B</sup>	18.26 ± 0.34 <sup>A</sup>	16.78 ± 0.32 <sup>C</sup>
	Length Mean (mm <sup>2</sup> )	6.05 ± 0.04 <sup>C</sup>	6.20 ± 0.03 <sup>BC</sup>	6.17 ± 0.02 <sup>C</sup>	6.15 ± 0.11 <sup>BC</sup>	6.77 ± 0.14 <sup>A</sup>	6.31 ± 0.08 <sup>B</sup>
	Width Mean (mm <sup>2</sup> )	3.79 ± 0.05 <sup>BC</sup>	3.75 ± 0.04 <sup>C</sup>	3.64 ± 0.05 <sup>C</sup>	4.20 ± 0.02 <sup>A</sup>	4.03 ± 0.16 <sup>AB</sup>	3.56 ± 0.09 <sup>C</sup>
	Length/Width Ratio	1.61 ± 0.02 <sup>C</sup>	1.66 ± 0.01 <sup>BC</sup>	1.71 ± 0.02 <sup>B</sup>	1.47 ± 0.03 <sup>D</sup>	1.70 ± 0.04 <sup>A</sup>	1.81 ± 0.03 <sup>A</sup>

**Table 1.** The phenotype of six parental accessions includes resistance, agronomic and grain traits. Groups with the same letter in the table are not significantly different (*P* > 0.05).

Gene name	ABO	HST	QC38	GY448	QM1	MUCUY
<i>QFhb.caas-3BL</i>	+	+				
<i>QFhb.hbaas-5AS</i>			+	+	+	+
<i>QFhb.caas-5AL</i>						+
<i>QYrsn.nwafu-1BL</i>	+			+	+	
<i>QYrxn.nwafu-1BL</i>	+	+				
<i>QYrsn.nwafu-2AS</i>		+				
<i>QYrqin.nwafu-2AL</i>		+	+	+	+	+
<i>QYrqin.nwafu-2BL</i>		+				
<i>QYr.nwafu-3BS</i>	+	+	+	+	+	
<i>QYrsn.nwafu-3DL</i>		+				
<i>QYrsn.nwafu-6BS</i>	+		+	+		
<i>QYrqin.nwafu-6BS</i>	+	+		+		+
<i>Yr5</i>		+				
<i>Yr17</i>						+
<i>Yr18</i>		+				
<i>Yr29</i>				+	+	+
<i>Yr75</i>	+					+
<i>Yr78</i>						
<i>Yr80</i>			+		+	+
<i>QGl-4 A</i>	+		+		+	
<i>TaSus2-2B</i>					+	
<i>TaCwi-A1</i>			+			
<i>Tabas1</i>	+		+		+	+
<i>TaGS5-A1</i>	+		+		+	
<i>TaGW2-6 A</i>	+			+	+	
<i>TaT6P</i>			+	+	+	+
<i>RHT-8</i>	+			+	+	
<i>Rht-D1</i>	+	+		+	+	+
<i>Rht24_AP2</i>		+	+			+

**Table 2.** Distribution of known gene markers in the six parental accessions.

were detected in all parents. *TaCwi-A1* was detected solely in QC 38, and *Tasus2-2B* was detected only in QM1. *QYr.nwafu-3BS* was detected in all Tibetan Plateau wheats except Mucuy. *Yr17* and *QFhb.caas-5AL* were detected exclusively in Mucuy, with no segregation observed across all populations. HST harbored the most stripe rust resistance genes or loci, totaling 9. Gaoyuan 448 and Mucuy each had seven stripe rust resistance genes or loci. ABO and QM1 each possessed six stripe rust resistance genes or loci. In comparison, QC38 had the fewest stripe rust resistance genes or loci, with only four detected. Regarding genes associated with grain yield, QM1 showed the highest aggregation degree, including five genes. QC38 contained 4, ABO had 3, and GY448 and Mucuy had 2. However, no grain-related genes were detected in HST. Aside from QC38 detecting one gene for genes related to plant height, all other parents aggregated two dwarfing genes.

## Genotype in five population

### Stripe rust-resistant genes

All 16 stripe rust loci/genes detected in the population were identified. The distribution of the number of resistance genes across the Qinghai plateau wheat  $F_2$  families followed a normal distribution (Fig. 1). The maximum number of accumulated resistance genes was nine, with only eight families accumulating nine. The most common accumulation was five resistance genes in 237 families, accounting for 34.30%. A significant number of families accumulated six resistance genes, totaling 166 and representing 24.02% of the total. Families with seven and four resistance genes were similarly frequent, constituting 15.62% and 16.06% of the total, respectively. Families with three and eight resistance genes were comparably rare, comprising 5.78% and 4.34% of the total, respectively. No families had a single gene; five lacked any detected resistance gene loci.

Most detected gene loci are located on chromosome 1BL, including *QYrsn.nwafu-1BL*, *Yr29*, and *QYrxn.nwafu-1BL* (Fig. 2). *QYrsn.nwafu-1BL* showed segregation in POP2 with a gene frequency (refers to the proportion of a specific allele of a gene in a population) of 50.68%. *QYrxn.nwafu-1BL* showed segregation in POP1 and POP2 with gene frequencies of 55.13% and 45.27%, respectively. *Yr29* with Gene frequencies in POP1, POP2, and POP3 were 54%, 47%, and 46.81%, respectively. Two genes are located on chromosome 2 A: *QYrsn.nwafu-2AS* and *QYrqin.nwafu-2AL*. *QYrsn.nwafu-2AS* showed segregation in POP1, POP2, and POP3 with gene frequencies of 50%, 47.97%, and 68.93%, respectively. *QYrqin.nwafu-2AL* showed segregation in POP2, POP4, and POP5 with gene frequencies of 50.67%, 49.77%, and 49.47%, respectively. The *Yr17* gene was not detected in POP1,

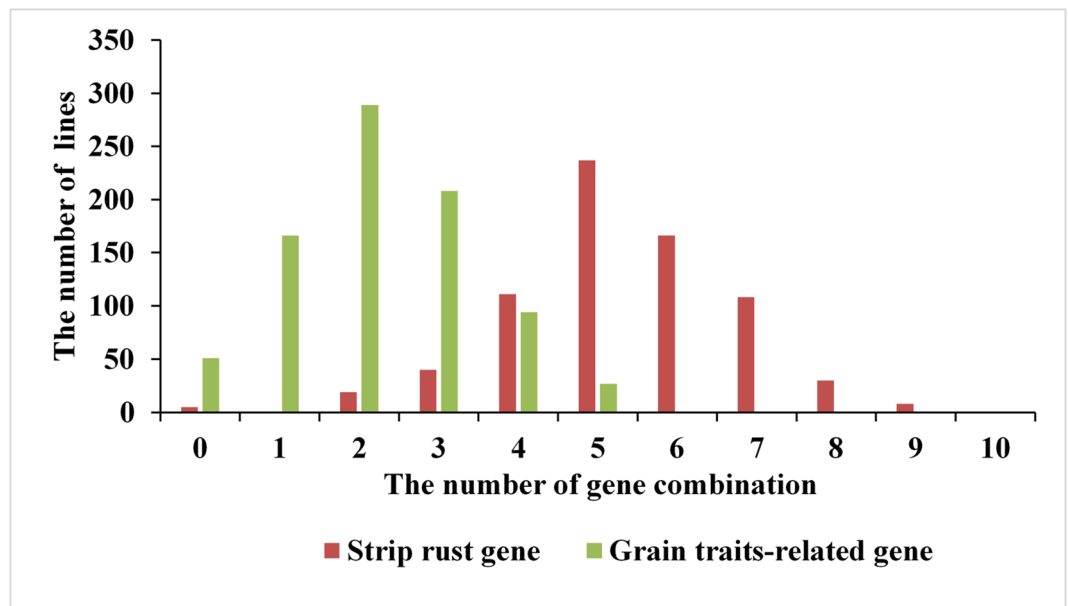


Fig. 1. The number of stripe rust gene combinations in the five populations.

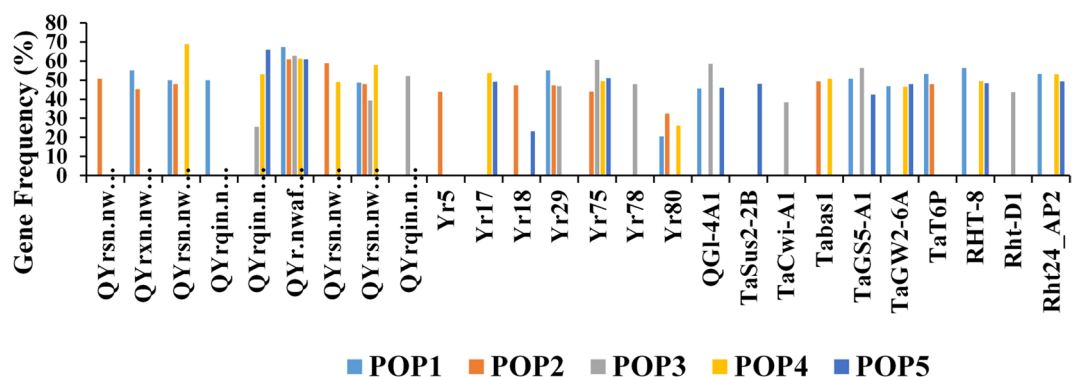
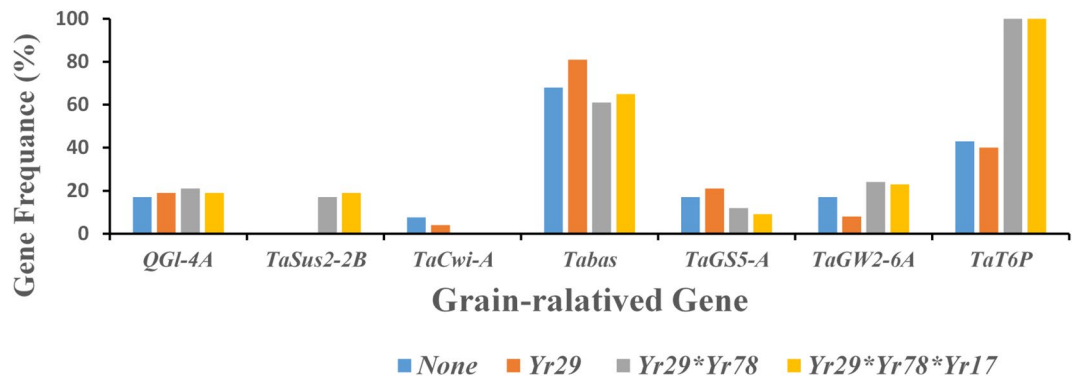


Fig. 2. Gene frequency distribution of lines in five populations.

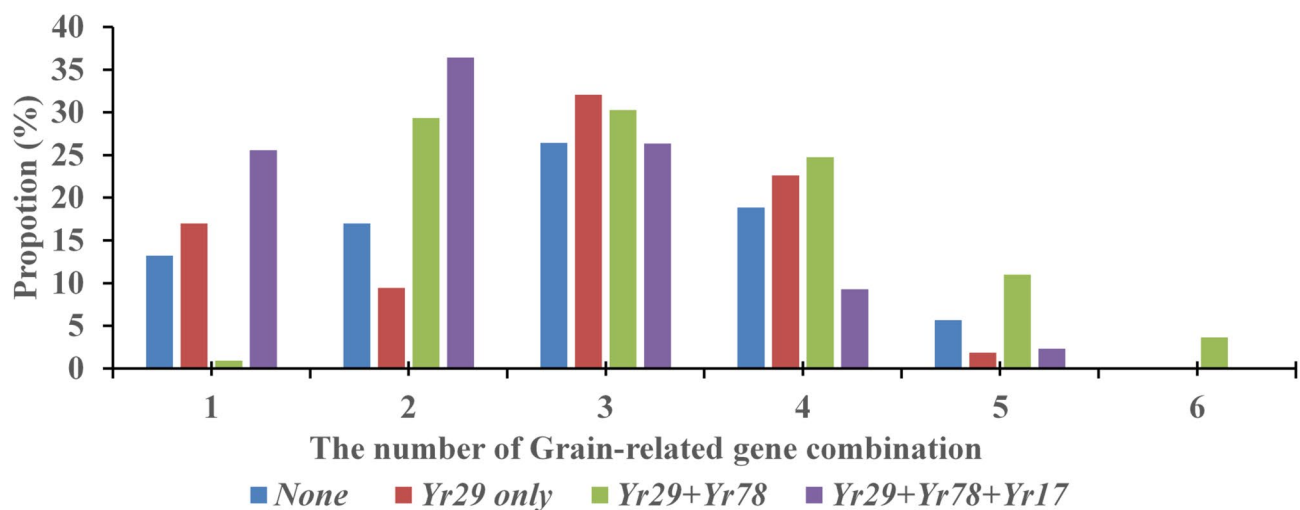
POP2, and POP3, but in POP4 and POP5, the gene frequencies were 53.74% and 49.10%, respectively. One gene detected on chromosome 2B is *QYrqin.nwafu-2BL*. *QYrqin.nwafu-2BL* showed segregation in POP1, POP2, and POP4, with gene frequencies ranging from 36.54 to 62.16%. Two genes are located on chromosome 3B: *QYr.nwafu-3BS* and *Yr80*. *QYr.nwafu-3BS* showed segregation in all populations, with gene frequencies ranging from 60.89 to 67.31%. *Yr80* showed segregation in POP1, POP2, and POP4, with gene frequencies ranging from 20.51 to 32.43%. *QYrsn.nwafu-3DL* showed segregation in POP2 and POP4 with gene frequencies of 58.78% and 49.07%, respectively. Three genes are located on chromosome 6B: *QYrsn.nwafu-6BS* showed segregation in POP1, POP3, and POP4, with gene frequencies ranging from 39.36 to 57.94%. *QYrqin.nwafu-6BS* showed a gene frequency of 52.13% in POP3. *Yr75*, located on chromosome 7 A, showed gene frequencies ranging from 43.91 to 60.63% in all populations except POP1.

#### Grain size and yield trait-related genes

We tested six genes linked to grain weight and yield, locating them on chromosomes 2A, 2B, 3A, 6A, 6B, and 7A. In the Qinghai plateau wheat  $F_2$  families, the aggregation of grain-related genes was normally distributed (Fig. 1), with 36.03% of families aggregating two genes, the most common occurrence. Families aggregating one or three genes were similarly frequent, comprising 24.02% and 21.85% of the total, respectively. The maximum number of grain-related genes in a single family was five, representing just 2.75% of the total. Families lacking these genes made up 7.23% of the total. *TaSus2-2B* segregated in POP5 with a frequency of 48.03%; *TaCwi-A1* segregated in POP3 with a frequency of 38.29%; *Tabas1* segregated in POP2 and POP4 with frequencies of 49.32% and 50.70%, respectively; *TaGS5-A1* segregated in POP1, POP3, and POP5 with frequencies ranging from 42.50 to 56.38%; *TaGW2-6A* segregated in POP1, POP3, and POP5 with frequencies from 46.49 to 51.38%;



**Fig. 3.** Grain-related gene frequency distribution of lines with the combinations *Yr29\*Yr17*, *Yr29\*Yr78*, and *Yr29\*Yr17\*Yr78*, and none of those.



**Fig. 4.** The number of Grain-related genes lines with the combinations *Yr29\*Yr17*, *Yr29\*Yr78*, and *Yr29\*Yr17\*Yr78*, and none of those.

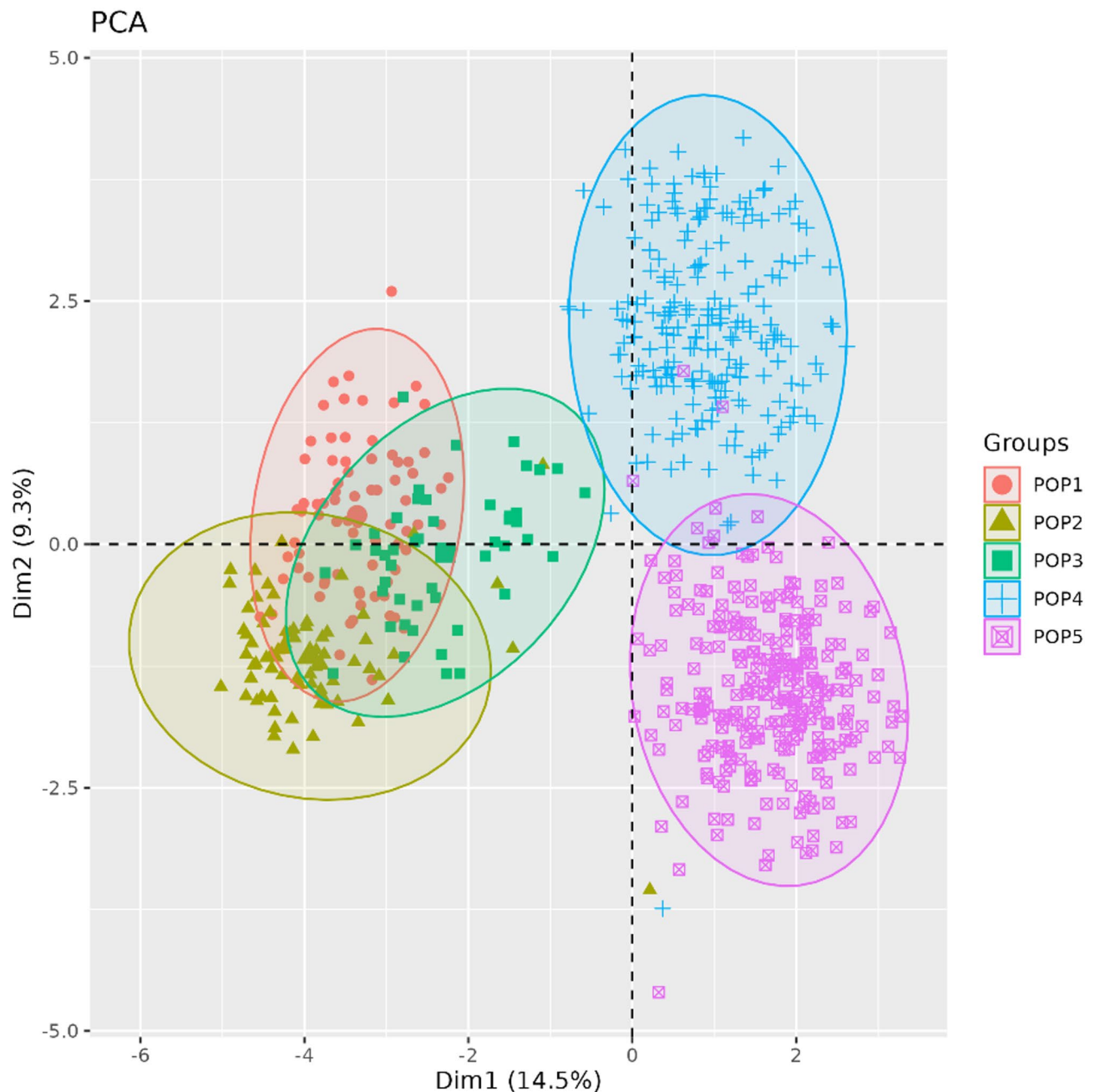
*TaT6P* segregated in POP1 and POP2 with frequencies of 53.21% and 47.97%, respectively; *QGl-4A2* segregated in POP1, POP3, and POP5 with frequencies of 45.51%, 58.51%, and 46.25%, respectively.

#### Plant height-related genes

We identified three genes associated with plant height: *RHT-8*, *Rht-24\_AP*, and *Rht-D1*. *RHT-8* showed segregation in POP1, POP4, and POP5, with gene frequencies ranging from 48.39 to 56.42%. *Rht-24\_AP* segregated in POP1, POP4, and POP5 with gene frequencies ranging from 49.28 to 53.21%. *Rht-D1* showed segregation in POP3 with a gene frequency of 43.62% (Fig. 2).

#### The gene infrequency and gene combination of grain-related gene

We analyzed the aggregation of grain-related genes based on the presence or absence of the stripe rust resistance genes *Yr29*, *Yr17*, and *Yr78* across five populations (Fig. 3). The analysis considered four scenarios: the absence of all three genes, the presence of *Yr29* alone, or their presence in various combinations. Results indicated that grain-related genes predominantly appeared in combinations of 2, 3 and 4 genes when all three were absent, peaking most frequently in 3 genes (26.42%), exhibiting a normal distribution centered around 3. When *Yr29* was present alone, grain-related gene aggregations (refer to co-localization or clustering of genes with similar functions, often on a chromosome) were most common in three-gene combinations (32.08%). With *Yr29* and *Yr78* co-aggregating, grain-related traits are predominantly associated with 2, 3, and 4 genes (29.30%, 30.28%, and 24.77%, respectively), exhibiting a normal distribution centered around five. Similarly, when *Yr29*, *Yr78*, and *Yr17* co-aggregated, the aggregation pattern peaked with 2 and 3 genes (36.43% and 26.35%). We analyzed the frequency changes of grain-related genes based on the presence or absence of the three stripe rust resistance genes *Yr29*, *Yr17*, and *Yr78* across five different populations, considering three possible combinations (Fig. 4). With the progressive pyramiding of the three genes, the number of pyramided grain-related genes stabilized at three. However, when *Yr29*, *Yr17*, and *Yr78* were pyramided together, the number of grain-related genes pyramided



**Fig. 5.** Principal component analysis of five population.

was predominantly two. This suggests that the pyramiding of these three genes (*Yr29*, *Yr17*, *Yr78*) does not interfere with (or: did not significantly alter) the pyramiding of grain-related genes. Initially, the trend in grain gene frequency changes was consistent in the absence of all genes and when *Yr29* was present alone. However, the frequency changes of grain-related genes were similar in combinations of *Yr29* + *Yr78* and *Yr29* + *Yr17* + *Yr78*. Genes including *TaSus2-2B*, *TaGW2-6 A*, and *TaT6P* exhibited increasing gene frequency trends in the *Yr29* + *Yr78* and *Yr29* + *Yr17* + *Yr78* combinations. In contrast, genes including *TaCwi-A*, *Tabas*, and *TaGS5-A* displayed decreasing gene frequency trends in the same combinations, *Yr29* + *Yr78* and *Yr29* + *Yr17* + *Yr78*. *QGL-4 A* remains unchanged under four situations.

#### Principal component analysis of six population

Principal component analysis (PCA) of five populations (POP1 to POP5) (Fig. 5) reveals that genetic samples are tightly clustered on the PCA plot. The PCA results indicated that the first two principal components (Dm1 and Dm2) accounted for 14.5% and 9.3% of the genetic variation, respectively. Along these two principal component dimensions, populations POP1, POP2, and POP3 clustered closely together with significant overlap, indicating a close genetic relatedness and high genetic similarity among these groups. In contrast, POP4 and POP5 were

positioned farther away from POP1 and POP2. While POP4 and POP5 showed partial overlap with each other, they were predominantly distributed in distinct regions of the PCA space. This spatial separation suggests lower genetic similarity between POP4 and POP5, and between POP1 and POP2. High genetic similarity among populations due to sharing the same paternal wheat variety. For example, Plateau GY448 and QM1 derive from the same parental source, 'Gaoyuan 602', accounts for the substantial overlap observed between POP4 and POP5 on the PCA plot. POP1 exhibits the smallest circle, indicating the most concentrated data, and intersects with POP2 and POP3 the most frequently. Conversely, POP4 displays the largest circle, indicating relatively dispersed data. Furthermore, despite considerable overlap, distinct separations are evident among populations, with greater distances indicating larger differences. POP2 is relatively distant from the other populations, possibly due to its more distant genetic relationship with them.

## Discussion

Introducing new genetic resources can enrich the wheat genetic pool in China and broaden its genetic basis, an effective strategy for enhancing Chinese wheat varieties<sup>28</sup>. The primary source of improvement for the spring wheat variety in China is CIMMYT wheat. Mucuy exhibited high resistance to yellow rust (YR) across multi-year field trials in Mexico, Kenya, and India. Molecular mapping identified four leaf rust (LR) and six YR resistance loci (Refers to specific genomic regions associated with resistance traits) in Mucuy, with two co-located resistance loci—*QYr.cim-1BL/QLr.cim-1BL* and *QYr.cim-2AS/QLr.cim2AS*—corresponding to *Lr46/Yr29/Sr58/Pm39* and *Yr17*, respectively. This study detected seven promising alleles in MUCUY, including *QYrqin.nwafu-2AL*, *QYrqin.nwafu-6BS*, *Yr17*, *Yr29*, *Yr78* (6BS), *Yr75* (2BS), and *Yr80* (2AL).

*Yr29* is a prevalent and effective gene in Chinese common wheat. The previously identified QTLs—*Yr17*, *Yr78*, and *Yr29*—demonstrate strong additive effects in MUCUY resistance studies. The stripe rust severity in lines with *Yr29* + *Yr17*, *Yr29* + *Yr78*, and *Yr29* + *Yr17* + *Yr78* combinations was 8.8%, 27.2%, and 7.0%, respectively<sup>32</sup>. Stacking *Yr29* with other stripe rust resistance genes like *Yr24* and *Yr26* enhances resistance, with this synergistic effect validated across various environments<sup>35</sup>. The variable resistance effects of *Yr29* across different genetic backgrounds suggest that gene-gene and gene-environment interactions significantly influence disease resistance<sup>36</sup>. In our study, the *Yr17* gene was undetected in POP1, POP2, and POP3, suggesting that introgressing *Yr17* into the genotypes of HST, ABO, and QC38 is challenging. POP4 and POP5 pyramided the *Yr29* + *Yr17* combination, with 30% and 23% of lines already possessing this pyramid, respectively. Combining all-stage and high-temperature adult-plant (HTAP) resistance QTLs, as seen in the winter wheat cultivar Madsen, provides a robust approach to achieving high-level, durable resistance to stripe rust. Traditionally, *Yr17*'s effectiveness has been overcome by most Pst races. However, some studies suggest that *Yr17*'s resistance is due to multiple QTLs, including *QYrMa.wgp-2AS*, which seems to confer HTAP resistance rather than race-specific all-stage resistance. This implies that the 2NS chromosome from *Ae. ventricosa* may harbor additional resistance genes requiring further investigation<sup>37</sup>. *Yr78* confers partial resistance at the adult plant stage but is ineffective in seedlings. The effectiveness of *Yr78* against new Pst races in the western USA, Australia, and Asia, where the favorable IWA7257 allele is less common, is noteworthy<sup>38</sup>. A strong synergistic effect exists between *QYr.nwafu-6BL* and *QYrsnb.nwafu-2BL*, and multi-gene pyramiding can achieve higher resistance levels, a strategy applicable in breeding.

The *Yr75* gene is on chromosome 7 A in the Australian wheat variety Axe. *Yr75* differs from the typical slow rusting resistance (APR) genes *Yr29* and *Yr18*, potentially harboring a unique resistance mechanism to complement existing strategies<sup>39</sup>. In our study, the *Yr75* gene was identified in Abodanza and mucuy, with segregation observed in POP2, POP3, POP4, and POP5, exhibiting gene frequencies from 43.91 to 60.63%. However, this gene locus was not identified in previous QTL mapping studies on mucuy<sup>32</sup> and field resistance surveys for Abandon and mucuy did not demonstrate the gene's effectiveness. Additionally, Heshangtuo and Gaoyuan 776 showed moderate resistance at the adult plant stage, suggesting the accumulation of adult plant resistance genes in these varieties. The stripe rust gene combination in Heshangtuo includes *QYrxn.nwafu-1BL*, *QYrsn.nwafu-2AS*, *QYrqin.nwafu-2AL*, *QYrqin.nwafu-2BL*, *QYr.nwafu-3BS*, *QYrsn.nwafu-3DL*, *QYrqin.nwafu-6BS*, and *Yr18*, whereas Gaoyuan 776 combines *QYrxn.nwafu-1BL*, *QYrqin.nwafu-2AL*, *QYr.nwafu-3BS*, *Yr78*, and *Yr80*. However, determining which specific combinations are effective or if there are other undetected genes and loci requires further analysis.

Is there a positive correlation between the number of resistance genes and disease resistance? Using a breeding chip, we detected those six parents harbored 4–9 resistance genes each, with HST and Mucuy carrying 9 and 7 genes, respectively, showing the most robust field resistance, nearly immune. These results suggest a positive correlation between the number of resistance genes and disease resistance. However, previous multi-year, multi-environment studies on Mucuy revealed that not all genes are active, and various gene combinations yield different resistance effects, with the most effective being *Yr29* + *Yr17*, *Yr29* + *Yr78*, and *Yr29* + *Yr17* + *Yr78*<sup>32</sup>. Additional research has confirmed that combining 1–2 non-immune seedling resistance genes with 2–3 adult plant resistance genes can confer near-immune resistance under various environmental conditions at the adult plant stage, a key factor in the enduring resistance of CIMMYT wheat lines.

Furthermore, determining which combinations of the aggregated resistance genes in HST play a decisive role will require further analysis, including resistance evaluations across different family lines within the population. Analysis of 691 families indicated that the maximum number of aggregated resistance genes was nine, achieved by only nine families. Moreover, none of the offspring families exceeded the parental gene aggregation count, suggesting that elite wheat materials might be limited to accumulating no more than nine genes. In other studies, materials carrying various resistance genes, including *QYr.nwafu-4BL*, *Yr15*, *Yr17*, and others, those with 2–6 aggregated stripe rust resistance genes constituted 24, 22, 11, 14, and 3 individuals, respectively, totaling 94.87% of the population<sup>40</sup>. A study focusing on the Zhou 8425B line and its derivatives revealed that Zhou 8425B possesses the highest number of genes conferring resistance to stripe rust. In progeny varieties like Zhoumai

12 and Zhoumai 17, a pyramiding of four stripe rust resistance genes originating from Zhou 8425B has been achieved<sup>10</sup>.

*QGL-4 A*, *TaSus2-2B*, *TaCwi-A1*, *Tabas1*, *TaGS5-A1*, *TaGW2-6 A*, and *TaT6P* are high-impact genes associated with grain traits and yield in wheat. In a QTL analysis of the wheat population Zhongmai 578/Jimai 22, *TaSus2-2B* emerged as a potential candidate gene influencing several yield traits<sup>12</sup>. The *Hap-H* haplotype of the *TaSus2-2B* gene represented 44% of varieties in the 1990s, increasing to 56.9% in recent wheat varieties from China's second district. This indicates that selective breeding for high thousand-kernel weight (TKW) has increased the frequency of the high-TKW-associated *Hap-H* haplotype in the *TaSus2-2B* gene<sup>26</sup>. These genes can also exhibit genetic additive effects, where the combined effects are particularly potent. In this study, we detected seven grain-related genes, with the parent QM1 showing the highest aggregation of six grain-weight genes.

In contrast, HST lacked high grain weight haplotypes and exhibited lower yields and grain traits. A positive correlation between the number of aggregated grain-related genes and grain size and yield may also exist. Among the 691 lines, those with two aggregated grain-related genes were the most common, with no lines aggregating six genes. The offspring's gene aggregation did not exceed the parental levels. However, determining if lines with superior yield and grain traits can be selected from these requires further verification via phenotypic and genotypic assessments of the families. Other studies have also shown additive effects among grain-related traits, like *TaGW2-6B* and *TaDA1*, which negatively regulate wheat grain weight and size<sup>42</sup>. In 49 primary Qinghai wheat varieties, *TaCwi-A1*, *TaGW2-6 A*, and *TaTGW6-4 A* were analyzed, with results indicating that materials carrying the *TaCwi-A1a* + *Hap6A-A* + *TaTGW64* combination had the highest thousand-grain weight<sup>15</sup>.

We analyzed the frequency changes of grain-related genes based on the presence or absence of the stripe rust resistance genes *Yr29*, *Yr17*, and *Yr78* across five distinct populations, considering three potential combinations. Initially, the trend in grain gene frequency changes was consistent in the absence of any genes and when *Yr29* was the sole gene present. However, similar frequency changes in grain-related genes were observed in the combinations of *Yr29* + *Yr78* and *Yr29* + *Yr17* + *Yr78*. Genes including *TaSus2-2B*, *TaGW2-6 A*, and *TaT6P* exhibited an increasing trend in frequency within both the *Yr29* + *Yr78* and *Yr29* + *Yr17* + *Yr78* combinations. In contrast, genes such as *TaCwi-A*, *Tabas*, and *TaGS5-A* showed a decreasing trend in frequency within the *Yr29* + *Yr78* and *Yr29* + *Yr17* + *Yr78* combinations. Resistance and yield are complex traits that influence plant physiology, morphology, and the environment. The relationship between molecular growth-defense trade-offs and disease-resistance-yield antagonism remains unclear. In crops, qualitative and quantitative resistance often correlates with genetic variations associated with reduced yield, though exceptions exist.

The wheat resistance gene *Sr26* for stem rust, and the barley gene *mlo* for powdery mildew are reported to result in average yield reductions of 9%, and 4.2%, respectively. However, a few studies have identified genes that can enhance resistance and yield. For example, the *OsGRF6* gene promotes high yields in rice with large panicles and significantly enhances resistance to bacterial blight<sup>43</sup>. This suggests that precise regulation of immune signals to allocate resources for growth without pathogens, and integrating multiple signaling pathways to reallocate resources for appropriate immunity during pathogen attacks, could synergistically enhance yield and disease resistance.

Analysis of the phenotype and genotype of six parental lines revealed that the genotypes of known genes in some parents correspond to phenotypic characteristics. For example, MUCUY contains seven adult-plant resistance genes, aligning closely with previous QTL results for MUCUY. GY448 contained the grain-related genes *TaGW2-6 A* and *TaT6p*, exhibiting a large grain area and width phenotype. Moreover, some genes did not improve grain weight or plant height, and in some cases, even performed worse than the absence of the gene. In the parent Heshangtong, despite the presence of *Rht-D1b* and *Rht24\_AP2* genes, the plant height reached 140 cm, with no significant reduction in stature. The combined effect of these two genes did not yield an additive decrease in plant height.

Molecular marker-assisted breeding (MAB) serves as a crucial tool in modern wheat breeding, with its application in disease-resistance breeding becoming increasingly widespread. Compared to conventional breeding, marker-assisted selection (MAS) can significantly enhance the efficiency of gene pyramiding. In the United States, utilizing marker-assisted backcrossing (MAS-BC) technology, 27 disease resistance genes and 20 quality-related alleles have been successfully transferred into 180 wheat lines through targeted breeding<sup>44</sup>. CIMMYT has pyramided genes such as *Yr29/Lr46/Pm39* or *Yr18/Lr34/Pm38*, along with other adult-plant resistance genes into single wheat cultivars. This effort has yielded a series of materials with multiple resistances, high resistance levels, and durable resistance, including varieties such as 'Pavon 76', 'Jupateco 73R', 'Attila', 'Kukuna', 'Vivitsi', 'Amadina', 'Chapio', and 'Tukuru'. The resistance of these varieties has been maintained for several decades<sup>8,45</sup>. The Wheat Breeders' Chip integrates effective markers for most developed functional genes, offering significant advantages to wheat breeders. It increases breeding speed, reduces costs, and enables the efficient pyramiding of genes associated with multiple traits. However, further exploration is needed better to apply the Breeder Chip technology in practical breeding programs. This may involve addressing several key questions, such as Which generation is optimal for detection? Which functional genes have substantial phenotypic effects, thus allowing for marker optimization? Furthermore, could chips be designed to be more region-specific for wheat varieties adapted to distinct growing areas, thereby improving their utility? This study utilized five populations sharing the same paternal parent but different maternal parents to analyze the distribution frequency of target genes in the  $F_2$  generation. We found that some genes cannot be successfully transmitted to offspring through hybridization. For example, *Yr17* failed to be transferred into ABO, HST, and QC38. However, GY448 and QM1 were able to combine *Yr29*, *Yr17*, and *Yr78* along with other grain-related genes. It is anticipated that in later stages, candidate breeding lines with both disease resistance and high yield will be developed. This result was to explore genotyping patterns in  $F_2$  populations, providing valuable data references for the application of breeder chip technology in wheat breeding programs within the Qinghai-Tibet Plateau wheat region. This study employed the GBW0.1K functional marker chip to analyze six main spring wheat varieties on the Qinghai

Plateau—ABO, HST, GY448, QM1, QC38—and 693 families across five F<sub>2</sub> populations for known genes. The analysis focused on the genetic characteristics of disease resistance, grain-related, and dwarf genes in the F<sub>2</sub> generation. GBW0.1K genotypic data correlated highly with phenotypic data, and normal gene segregation was observed within populations, demonstrating GBW0.1K's efficacy in molecular-assisted breeding and its applicability for future molecular breeding. Furthermore, validating the genetic effects of various genotypes within populations requires substantial phenotypic data, marking the next phase of our research. Enhancing the multi-resistance and durable resistance of the main cultivated varieties on the Qinghai Plateau through Mucuy is feasible; however, we must not neglect the possibility of undiscovered or newly discovered genes in these parents, warranting further research into gene discovery.

## Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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## Author contributions

DM-L, HQ-W, WJ-C, RJ-L and JC-S conceived of the project. DM-L and AHE-S wrote the paper. HQ-W and WJ-C performed the developmental analyses, FH-Y and JC-S evaluated phenotyping of the RILs. DM-L and RU-L performed Phenotype and KASP analyses.

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## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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