# Identification of a recessive gene *YrZ15-1370* conferring adult plant resistance to stripe rust in wheat-*Triticum boeoticum* introgression line

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**Research Article**

**Keywords:** Triticum boeoticum, Triticum aestivum, wheat stripe rust, adult-plant resistance, BSR-Seq

**Posted Date:** March 5th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-278043/v1

## Abstract

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most damaging diseases of wheat globally and resistance is the effectively control strategy. *Triticum boeoticum* Boiss (*T. monococcum* L. ssp. *aegilopoides*, 2n = 2x = 14, $A^bA^b$) accession G52 confers a high level of adult-plant resistance against a mixture of the Chinese prevalent *Pst* races. To transfer the resistance to common wheat, a cross was made between G52 and susceptible common wheat genotype Crocus. A highly resistant wheat-*T. boeoticum* introgression line Z15-1370 ($F_5$ generation) with 42 chromosomes was selected cytologically and by testing with *Pst* races. In order to map the resistance gene(s), $F_1$, $F_2$, and $F_{2:3}$ generations of the cross between Z15-1370 and stripe rust susceptible common wheat Mingxian169 were developed. Genetic analysis revealed that the resistance in Z15-1370 was controlled by a single recessive gene, temporarily designated *YrZ15-1370*. Using the bulked segregant RNA-Seq (BSR-Seq) analysis, *YrZ15-1370* was mapped to chromosome 6AL and flanked by markers *KASP1370-3* and *KASP-1370-5* within a 4.3 cM genetic interval corresponding to 1.8 Mb physical region in the Chinese Spring genome, in which a number of disease resistance-related genes were annotated. *YrZ15-1370* differed from previously *Yr* genes identified on chromosome 6A based on its position and/or origin. The *YrZ15-1370* would be a valuable resource for wheat resistance improvement and the flanking markers developed here should be useful tools for marker-assisted selection (MAS) in breeding and further cloning the gene.


## Introduction

Common wheat (*Triticum aestivum* L.) is one of the most important staple cereal crops for mankind, providing approximately 19% of the food calories and over 20% of the protein consumed by the world population (Braun et al. 2010). Stripe rust (*Puccinia striiformis* f. sp. *tritici*, *Pst*) is one of the most serious fungal diseases affecting wheat production worldwide (Wellings 2011). Breeding resistant cultivars is the most economical and sustainable method for stripe rust control.

Host resistance of wheat against *Pst* could be classified as either all-stage resistance (ASR) or adult-plant resistance (APR). Whereas ASR can be observed in seedling stage and is effective through all stages of plant growth, APR is susceptible in seedling stage and mainly effective at the late stages of plant growth (Chen and Kang 2017). The ASR confers high levels of resistance that is mostly race-specific and is vulnerably overcome by the emergence of new virulent races (Ellis et al. 2014). In contrast, APR generally provides a partial level of resistance that is non-race specific and, in some cases, has proven to be more durable than ASR (Ellis et al. 2014; Fu et al. 2009; Lagudah et al. 2009).

To date, more than 80 stripe rust resistance genes (*Yr1-Yr83*) have been permanently named (Gessese et al. 2019; Pakeerathan et al. 2019; Li et al. 2020). Most of them belonging to ASR genes have been overcome due to the rapidly evolving of new *Pst* races (Wellings et al. 2011). APR genes, such as *Yr18* (Lagudah et al. 2009), *Yr29* (William et al. 2003), and *Yr46* (Herrera-Foessel et al. 2011), provide durable partial resistance to several fungal pathogen species. Among the officially designated genes, only a few genes are recessive genes (e.g. *Yr6*, *Yr23*, and *Yr51*), whereas the remaining genes are dominant.

*Triticum boeoticum* (2n = 2x = 14, $A^bA^b$), the wild progenitor of *T. monococcum* L. ssp. *monococcum* (2n = 2x = 14, $A^mA^m$), exhibits high variability in a number of biotic and abiotic stress responses (Castagna et al. 1995; Harjit-Sngh et al. 1998; Vasu et al. 2000; Anker and Niks 2001; Lebedeva and Peusha 2006; Rey et al. 2015). Several resistance genes have been transferred from *T. boeoticum* to wheat, such as *PmTb7A.1*, *PmTb7A.2*, and *Pm25* for powdery mildew resistance (Abdelnaby et al. 2015; Chhuneja et al. 2015; Shi et al. 1997); *Sr22* for stem rust resistance (Gerechter-Amitai et al. 1971; The 1973, 1975; Paull et al. 1994). The *T. boeoticum* accessions are also valuable resistance source for wheat stripe rust (Chhuneja et al. 2008). For example, *QYrtm.pau-5A* confers APR to stripe rust has been mapped on the chromosome $5A^b$ of *T. boeoticum* accession pau5088 and was successfully transferred to hexaploid wheat using *T. durum* as a bridge (Chhuneja et al. 2008).

Although the potential of *T. boeoticum* for wheat improvement has been recognized for a long time, the available genetic diversity remains largely underexploited (Rey et al. 2015). In the current study, a resistant wheat-*T. boeoticum* introgression line Z15-1370 was obtained from common wheat cultivar Crocus as female crossed with *T. boeoticum* accession G52 as male. The objectives of this study were: (1) to evaluate the response of Z15-1370 to *Pst*; (2) to characterize the Z15-1370 by multicolor fluorescence in situ hybridization (mc-FISH), singlecolor genomic in situ hybridization (sc-GISH), multicolor genomic in situ hybridization (mc-GISH), and 55K SNP array; (3) to identify the stripe rust resistance gene in Z15-1370 using Bulked Segregant RNA-Seq (BSR-Seq) analysis.

## Materials And Methods

### Plant materials

Common wheat Crocus, *T. boeoticum* accession G52 and their derivative line Z15-1370 (selected from $F_5$ progenies of Crocus ×pG52) were used in this study. Crocus and G52 were kindly provided by George Fedak at the Ottawa Research and Development Centre in Canada. The wheat-*T. boeoticum* introgression line Z15-1370 was crossed with common wheat Mingxian169 to develop $F_1$, $F_2$, and $F_{2:3}$ populations, which were used in stripe rust resistance assessments and gene mapping. Mingxian169, widely used as a spreader for stripe rust evaluation, is highly susceptible to currently prevailing Chinese *Pst* races (Wang et al. 2017).

### Cytological observations

Multicolor FISH was conducted based on the methods provided by Tang et al. (2014) and Zhao et al. (2018). Oligo-pTa535 and Oligo-pSc119.2 were used as probes to differentiate individual chromosomes of Z15-1370. All probes were labeled with either 6-carboxyfluorescein (6-FAM) or 6-carboxytetramethylrhodamine (Tamra) by the TsingKe Biological Technology Company (Chengdu, China).

After stripping off the FISH oligo probes, the same slides were analyzed by sc-GISH and mc-GISH. Sc-GISH was conducted according to Wang et al. (2019a). Total genomic DNA from G52 was labeled with fluorescein-12-dUTP (Roche Diagnostics Australia, Castle Hill, NSW) using nick translation. Total genomic DNA of wheat Chinese Spring was used for blocking. The probe to blocker ratio was ~1 : 2.2. Chromosomes were counter-stained with DAPI and pseudo-colored red. Mc-GISH was conducted based on the methods provided by Han et al. (2004). Total genomic DNA of *T. urartu* was labeled with digoxigenin-11-dUTP and that of *Ae. tauschii* with biotin-16-dUTP using the nick translation method. Total genomic DNA of *Ae. speltoides* was used for blocking. Hybridization signals were visualized and captured using an Olympus BX-63 epifluorescence microscope equipped with a Photometric SenSys DP70 CCD camera (Olympus, Tokyo, Japan). Raw images were processed using Photoshop v.7.1 (Adobe Systems Inc., San Jose, CA, USA).

Chromosome pairing observation in pollen mother cells (PMCs) was performed as described by Zhang et al. (2007). For meiotic analysis, at least 50 PMCs were observed for Z15-1370. Ring bivalents (ring II) and rod bivalents (rod II) were counted, and their average numbers were calculated.

### SNP genotyping

Genomic DNA was extracted from fresh leaves using a plant genomic DNA kit (Tiangen Biotech, Beijing, China). Chip-based genotyping was carried out using the Wheat 55K SNP array containing 53,063 markers by CapitalBio Technology (Beijing, China) (www.capitalbio.com). Markers showed homozygous genotype among Z15-1370, G52, and Crocus were used to analyze the G52 donor segments in Z15-1370. The ratios of same SNP to the total SNPs scored between Z15-1370 and its two parents were calculated using a sliding window of 10 Mb and step length of 1 Mb as described by Hao et al. (2019). Only results from windows with >30 markers were treated as effective data. The genome regions of Z15-1370 covered by windows with higher ratio of same SNP to G52 compared to that of Crocus and values larger than 0.6 were defined as the G52 introgression fragments. Graphical representations were constructed using the R package ggplot2 (v.2.2.1) (Wickham 2016).

### Stripe rust resistance response

Crocus, G52, Z15-1370, and $F_1$ individuals derived from Mingxian169×Z15-1370 were tested for seedling stripe rust resistance at growth chamber. The highly virulent *Pst* race CYR34 (virulent on *Yr1, Yr6, Yr7, Yr8, Yr9, Yr10, Yr17, Yr18, Yr24, Yr26, Yr27, Yr28, Yr29, Yr31, Yr43, Yr44, YrExp2*, and *YrSP*) (Wang et al. 2019b) was used to inoculate the plants at the second leaf stage. Urediniospores used for inoculation of leaf tissue were first suspended in isododecane and then sprayed as described previously (He et al. 2020). Disease severity was evaluated and characterized 14-21 days after inoculation using a 0-9 scale of infection type (IT) (Line and Qayoum 1992). Plants with ITs 1-3, 4-6, and 7-9 were considered resistant, intermediate resistant, and susceptible, respectively.

A field evaluation for adult-plant stripe rust resistance of parents, $F_1$ and $F_2$ individuals as well as their corresponding $F_{2:3}$ families was performed at the experimental field of the Triticeae Research Institute, Sichuan Agricultural University, Wenjiang. The highly susceptible spreader variety SY95-71 and Mingxian169 were planted around the experimental field as spreader rows. A mixture of the Chinese prevalent races (CYR32, CYR33, CYR34, Zhong4, and HY46), kindly provided by the Research Institute of Plant Protection, Gansu Academy of Agricultural Sciences, was used to inoculate the adult plants. Stripe rust response was scored on a 1-9 scale (Wellings and Bariana 2004), with 1 being highly resistant and 9 highly susceptible. ITs were recorded for three times at 7-day intervals when susceptibility of flag leaves of SY95-71 was fully expressed. The final rating of each wheat line was used for analysis.

### Bulked segregant RNA-Seq (BSR-Seq)

The phenotypically contrasting $F_{2:3}$ families against stripe rust races in the field were used to construct the resistant and suscptible RNA pools for RNA-Seq. Equal amounts of RNA from 30 homozygous resistant and 30 homozygous susceptible families each were pooled for conducting bulked segregant analysis Li et al. 2020). The RNA samples were sequenced on the Illumina HiSeq platform at the Beijing Novogene Technology (Beijing, China) (https://www.novogene.com/). Sequence quality was controlled using software Trimmomatic v0.36 (Bolger et al. 2014). RNA reads of the resistant and susceptible bulks were aligned to the Chinese Spring reference genome sequence v1.0 (IWGSC et al. 2018) using software STARv2.5.1b (Dobin et al. 2013). The unique and confident alignments were applied to call SNP variants using software GATK v3.6 (McKenna et al. 2010). The SNP variants with *P*-values of Fisher's exact test (FET) $<1e^{-8}$ and allele frequency difference (AFD) >0.6 were considered to be associated with the disease resistance and further used as templates to develop SNP markers (Li et al. 2020).

### Kompetitive allele-specific PCR (KASP) Assays

The resistance-associated SNPs and the 500 bp flanking sequences served to design the KASP primers and tested polymorphisms on the parental lines, the resistant and susceptible DNA bulks. Polymorphic markers that could be reliably scored were genotyped on the $F_2$ segregation population of Mingxian169 ×Z15-1370. For each KASP assay, 10 µl reaction volum containing 5 µl of 2 KASP mastermix (Biosearch Technologies), 1.4 µl primer mix (mixture of 0.168 µM each forward A1 and A2 primers and 0.42 µM of reverse primer), 100 ng of genomic DNA and 2.6 µl of $ddH_2O$ was prepared. The CFX96Touch$^{TM}$ real-time PCR detection system (BioRad, USA) was used for amplification under the following conditions: 15 min at 94 ºC, 10 touchdown cycles of 20 s at 94 ºC, 60 s at 65-57 ºC (decreasing by 0.8 ºC per cycle), and 32 cycles of 20 s at 94 ºC, 60 s at 57 ºC.

### Data analysis

The Chi-square ($\chi^2$) tests were used to determine goodness of fit for the observed segregation and expected ratios of the $F_2$ and $F_3$ populations. Linkage analysis was performed using MAPMAKER/EXP v3.0b (Lander et al. 1987). The Kosambi function was used to convert recombination values to genetic distances (Kosambi 1943). A logarithmic odds (LOD) ratio of 3.0 and maximum distance of 50.0 cM was set as a threshod for the declaration of linkage. The genetic linkage map was drawn using Mapdraw V2.1 software (Liu and Meng 2003).

### Candidate gene analysis

The corresponding sequences of markers *KASP-1370-3* and *KASP-1370-5* linked to *YrZ15-1370* were used to BLAST against the genomes of common wheat cv. Chinese Spring (IWGSC et al. 2018) and *T. urartu* accession G1812 (Ling et al. 2018). Genomic sequences in the target intervals of the reference genomes were explored to predict candidates using BLAST search against the Triticeae Multi-omics center database (http://202.194.139.32/).

## Results

# Cytological characterization of the introgression line Z15-1370

Mc-FISH and mc-GISH analysis indicated that the Z15-1370 has 42 chromosomes with 14 A-genome, 14 B-genome, and 14 D-genome chromosomes, respectively (Fig. 1a, b). Sc-GISH using *T. boeoticum* genomic DNA as a probe confirmed the existence of 14 chromosomes from A-genome in Z15-1370 (Fig. 1c). All 42 chromosomes of Z15-1370 paired as bivalents in PMCs (observed PMCs > 50) at metaphase I (Fig. 1d), with the pairing configuration 17.45 ring II (±1.00) + 3.55 rod II (±1.00), suggesting a normal meiosis.

### Genetic analysis of the stripe rust resistance in Z15-1370

The introgression line Z15-1370 and its male parent G52 were susceptible (IT=7-9; in a 0-9 scale, Line and Qayoum 1992) to *Pst* race CYR34 at the seedling stage (Fig. 2a), whereas both of them showed high resistance (IT=1-2; in a 1-9 scale, Wellings and Bariana 2004) to the mixture *Pst* races (CYR32, CYR33, CYR34, Zhong4, and HY46) (Fig. 2b) at the adult plant stage. The common wheat Mingxian169 and Crocus were completely susceptible (IT=9). The resistant Z15-1370 was then crossed with Mingxian169 to develop $F_1$, $F_2$, and $F_{2:3}$ populations for genetic analysis of the adult plant stripe rust resistance in Z15-1370. The infection type of all $F_1$ plants was similar to that of the susceptible parent Mingxian169 (Fig. 2b). The $F_2$ population segregated in 136 resistant(IT=1-3) and 406 susceptible(IT=7-9), fitting a 1R : 3S ratio ($\chi^2 = 0.001$, p = 0.98) (Fig. 2b, Table 1), indicating that the stripe rust resistance was conferred by a single recessive gene temporarily designated as *YrZ15-1370*. The $F_{2:3}$ population consisting of 273 families showed a segregation of 134 (homozygous resistant): 276 (heterozygous): 123 (homozygous susceptible) ($\chi_{1:2:1}^2 = 1.128$, p=0.569), in agreement with the results from $F_2$ population (Table 1).

### BSR-Seq analysis of the RNA bulks with contrasting responses to stripe rust

The RNA samples of the resistant bulk and the susceptible bulk were subjected to RNA-seq analysis, which generated 43,174,297 and 43,082,237 raw reads, respectively. After quality control, 42,279,816 and 42,104,773 high-quality reads from the resistant bulk and susceptible bulk were uniquely mapped to the Chinese Spring reference genome, respectively. A total of 914 SNPs ($P < 1e^{-8}$ and AFD > 0.6) were identified from these reads using GATK software (Fig. 3a). One hundred and sixteen of them were enriched in a 10 Mb genomic interval (597Mb-606 Mb) on the long arm of chromosome 6A (Fig. 3b, c) in the Chinese Spring reference genome, which were regarded as the candidate SNPs linked to *YrZ15-1370*.

### Molecular mapping of *YrZ15-1370*

Sixty-three out of the 116 clustered SNPs on 6AL were chosen to develop KASP markers. Five of them were successfully converted into KASP markers (*KASP-1370-2, KASP-1370-3, KASP-1370-5, KASP-1370-6*, and *KASP-1370-7*) and scored reliably on the parents as well as the resistant and susceptible bulks (Table 2). Subsequently, these KASP markers were used to genotype 273 $F_2$ plants derived from the cross between resistant Z15-1370 and susceptible Mingxian169. Linkage analysis indicated that *KASP-1370-5* was potentially mapped 2.5 cM proximal and *KASP1370-3* was placed 1.8 cM distal to *YrZ15-1370* (Fig. 4a).

### Evaluation of the G52 introgression segments on 6A chromosome of Z15-1370

The five KASP markers used for developing the genetic map described above were also used to genotype common wheat Crocus, G52 and their derivative line Z15-1370, as well as Mingxian 169. All tested markers exhibited identical haplotypes between Z15-1370 and G52 while distinct from those of Crocus and Mingxian 169 (Table 3). SNP genotyping analysis revealed five potential G52 donor segments on chromosome 6A of Z15-1370 (0-3.0 Mb, 6.0-24.0 Mb, 43.1-82.1 Mb, 405.2-434.0 Mb, 519.0-607.8 Mb, based on the physical positions of Chinese Spring). One of them (519.0-607.8 Mb) overlapped the genome region of *YrZ15-1370* defined by its linked KASP markers (600.4-604.4 Mb) (Fig. 4).

### Gene analysis of the *YrZ15-1370*genomic region

The sequences of closely linked markers *KASP-1370-3* and *KASP-1370-5* were blasted against the Chinese Spring genome and the *T. urartu* genome to obtain their physical positions, respectively. The *YrZ15-1370* was physically mapped to the region between the 601.5 Mb to 603.3 Mb positions of 1.8 Mb in the Chinese Spring 6AL chromosome and between 557.4 Mb to 560.2 Mb (2.8Mb) in the *T. urartu* 6AL chromosome. A total of 71 and 59 predicted genes exist in the target physical regions in Chinese Spring reference genome and *T. urartu* reference genome, respectively (IWGSC RefSeq v1.0; *T. urartu* G1812 Tu2.0, Supplementary Table S1, S2).

## Discussion

*Triticum boeoticum* represents a valuable source of disease resistance for wheat improvement (Gill et al. 1988; Ma et al. 1997; Ahmed et al. 2014), whereas the transferring of the disease resistance genes from this species has been relatively lagging behind. In the present study, a new stripe rust resistance gene, temporarily named *YrZ15-1370*, was mapped on 6AL in a wheat-*Triticum boeoticum* introgression line Z15-1370.

A previous study had reported the transferring of the stripe rust resistance gene *QYrtb.pau-5A* from *T. boeoticum* to hexaploid wheat, using *T. durum* as a bridging species (Chhuneja et al. 2008). In the current study, the resistant line Z15-1370 was obtained by direct hybridization between common wheat and *T. boeoticum.* SNP genotyping analysis revealed that part chromatin of *T. boeoticum* G52 was successfully introgressed into Z15-1370. The genomic interval of

one G52 donor fragment on 6AL of Z1370 was 88.8Mb (519.0-607.8 Mb), which overlapped the physical interval of *YrZ15-1370* locus (601.5 to 603.3 Mb) in the Chinese Spring reference genome (Fig. 4b). The SNP genotyping together with the KASP assay (Table 3) demonstrated that the *YrZ15-1370* segment on 6AL was derived from the $6A^b$ chromosome of *T. boeoticum*.

To date, three stripe rust resistance genes (*Yr38, Yr42*, and *Yr81*) have been assigned on chromosome 6A. *Yr81* located in the short arm of chromosome 6A, was detected in an Australian common wheat landrace Aus27430 (Gessese et al. 2019). *Yr38* (Marais et al. 2006) and *Yr42* (Marais et al. 2009) are present in translocated segments from wild relatives *Aegilops sharonensis* (2n = 14, $S^{sh}S^{sh}$) and *Ae. neglecta* (2n = 28, UUMM), respectively. All these genes confer ASR to stripe rust, whereas *YrZ15-1370* is an APR gene. To date, only one APR gene *QYrtb.pau-5A* from *T. boeoticum* had been mapped, while this gene was located on chromosome 5A (Chhuneja et al. 2008). Taken together, *YrZ15-1370* reported here is a new stripe rust resistance gene found in *T. boeoticum*.

Seventy-one and 59 genes in the *YrZ15-1370* genomic region were annotated in Chinese Spring and *T. urartu*, respectively (Supplementary Table S1, S2). Among them, six genes in CS (*TraesCS6A01G584000*, *TraesCS6A01G384700*, *TraesCS6A01G384900*, *TraesCS6A01G385000*, *TraesCS6A01G385100*, and *TraesCS6A01G385500*) and four genes (*TuG1812G0600004115.01*, *TuG1812G0600004116.01*, *TuG1812G0600004117.01*, and *TuG1812G0600004133.01*) in *T. urartu* had predicted protein domains (receptor-like kinase, serine-threonine/tyrosine-protein kinase, and MYB-like transcription factor) that have been previously associated with plant defense responses to pathogens (Chen et al. 2020; Klymiuk et al. 2018; Noman et al. 2019). These disease resistance-related genes could be used further for fine mapping and cloning of *YrZ15-1370*.

Among stripe rust resistance genes listed in the wheat gene catalogue, the majority show dominant inheritance, whereas a small number of them, such as *Yr51* (Randhawa et al. 2014), *yrCH45* (Yang et al. 2016), *yrGn22* (Li et al. 2016), and *yrMY37* (Ren et al. 2015) were recessive and usually confer all-stage resistance. *YrZ15-1370* in wheat-*Triticum boeoticum* line Z15-1370 is another case of recessive gene, while confer adult plant resistance. Most of the reported APR genes do not confer adequate levels of resistance when present alone. For example, APR genes *Yr18* (Lagudah et al.2009), *Yr29* (William et al.2003), *Yr36*, and *Yr46* (Herrera-Foessel et al.2011) provide partial resistance to a wide specturm of *Pst* races. The *YrZ15-1370* gene identified during this study provides a high level of APR to a mixture of the Chinese prevalent races (CYR32, CYR33, CYR34, Zhong4, and HY46), indicating its suitability for wheat stripe rust resistance improvement. *YrZ15-1370* should be stacked with other known *Yr* genes to achieve durable resistance. Flanking markers *KASP-1370-3* and *KASP-1370-5* would be used for marker assisted selection (MAS) in wheat resistance breeding.

## Declarations

### Author contribution statement

D.C. L., L.Z., L.H., M.H., Z.Y., and S.N. designed the experiments. M.Z., X.L., T.P., D.W., D.Y.L., X.J.C., B.J., H.L., and X.C. performed the experiments. D.C.L., L.Z., L.H., M.H., and M.Z. discussed results and wrote the paper.

### Acknowledgments

We thank George Fedak at the Ottawa Research and Development Centre in Canada for providing Crocus and G52 seeds. This research was supported by the National Natural Science Foundation of China (31671682, 31671689).

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

## References

Ahmed S, Bux H, Rasheed A, Gul Kazi A, Rauf A, Mahmood T, Mujeeb-kazi A (2014) Stripe rust resistance in *Triticum durum-T. monococcum* and *T. durum-T. urartu* amphiploids. Australas Plant Path 43:109-113

Anker CC, Niks RE (2001) Prehaustorial resistance to the wheat leaf rust fugus, *Puccinia triticina*, in *Triticum monococcum* (s.s.). Euphytica 117:209-215

Bariana H, Forrest K, Qureshi N, Miah H, Hayden M (2016) Adult plant stripe rust resistance gene *Yr71* maps close to *Lr24* in chromosome 3D of common wheat. Mol Breeding 36(7):98

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120

Braun HJ, Atlin G, Payne T (2010) Multi-location testing as a tool to identify plant response to global climate change. In: Reynolds MP (ed) Climate change and crop production. CABI, London pp115-138

Castagna R, Borghi B, Huen M, Salamini F (1995) Integrated approach to einkorn wheat breeding. In Hulled wheats. Promoting the conservation and use of underutilized and neglected crops 4. In: Proc Wrst Intl. workshop on Hulled wheat, Castelvecchio Pascoli, Tuscany, Italy. IPGRI, Rome, Italy pp21–22

Chen SS, Rouse MN, Zhang WJ , Zhang XQ, Guo Y, Briggs J, Dubcovsky J (2020) Wheat gene *Sr60* encodes a protein with two putative kinase domains that confers resistance to stem rust. New Phytol 225(2):948-959

Chhuneja P, Kaur S, Garg T, Ghai M, Kaur S, Prashar M, Bains NS, Gole RK, Keller B, Dhaliwal HS, Singh K (2008) Mapping of adult plant stripe rust resistance genes in diploid A genome wheat species and their transfer to bread wheat. Theor Appl Genet 116(3): 313-324

Chhuneja P, Yadav B, Stirnweis D, Hurni S, Kaur S, Elkot AFA, Keller B, Wicker T, Sehgal S, Gill BS, Singh K (2015) Fine mapping of powdery mildew resistance genes *PmTb7A.1* and *PmTb7A.2* in *Triticum boeoticum* (Boiss.) using the shotgun sequence assembly of chromosome 7AL. Theor Appl Genet 128(10): 2099-2111

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR (2013) STAR: Ultrafast universal RNA-seq aligner. Bioinformatics 29(1):15-21

Elkot AFA, Parveen C, Satinder K, Saluja M, Keller B, Singh K (2015) Marker assisted transfer of two powdery mildew resistance genes *PmTb7A.1* and *PmTb7A.2* from *Triticum boeoticum* (Boiss.) to *Triticum aestivum* (L.). Plos One 10(6): e128297

Ellis JG, Lagudah ES, Spielmeyer W, Dodds PN (2014) The past, present and future of breeding rust resistant wheat. Front Plant Sci 5:641

Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen X, Sela H, Fahima T, Dubcovsky J (2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science 323: 1357-1360

Gerechter-Amitai ZK, Wahl I, Vardi A, Zohary D (1971) Transfer of stem rust seedling resistance from wild diploid einkorn to tetraploid durum wheat by means of a triploid hybrid bridge. Euphytica 20(2): 281-285

Gessese M, Bariana H, Wong D, Hayden M, Bansal U (2019) Molecular mapping of stripe rust resistance gene *Yr81* in a common wheat landrace Aus27430. Plant Dis 103(6): 1166-1171

Gill RS, Dhaliwal HS, Multani DS (1988) Synthesis and evaluation of *Triticum durum– T. monococcum* amphiploids. Theor Appl Genet 75: 912-916

Han FP, Liu B, Fedak G, Liu ZH (2004) Genomic constitution and variation in five partial amphiploids of wheat-*Thinopyrum intermedium* as revealed by GISH, multicolor GISH and seed storage protein analysis. Theor Appl Genet 109(5):1070-1076

Hao M, Zhang LQ, Zhao LB, Dai SF, Li AL, Yang WY, Xie D, Li QC, Ning SZ, Yan ZH, Wu BH, Lan XJ, Yuan ZW, Huang L, Wang JR, Zheng K, Chen WS, Yu M, Chen XJ, Chen MP, Wei YM, Zhang HG, Kishii M, Hawkesford M J., Mao L, Zheng YL, Liu DC (2019) A breeding strategy targeting the secondary gene pool of bread wheat: introgression from a synthetic hexaploid wheat. Theor Appl Genet 132:2285-2294

Harjit Singh, Grewal TS, Dhaliwal HS, Pannu PPS, Bagga PPS (1998) Sources of leaf rust and stripe rust resistance in wild relatives of wheat. Crop Improv 25:26-33

He Y, Feng LH, Jiang Y, Zhang LQ, Yan J, Zhao G, Wang JR, Chen GY, Wu BH, Liu DC, Huang L, Fahima T (2020) Distribution and nucleotide diversity of *Yr15* in wild emmer populations and Chinese wheat germplasm. Pathogens 9: 212

Herrera-Foessel SA, Lagudah ES, Huerta-Espino J, Hayden M, Bariana HS, Singh D, Singh RP (2011) New slow-rusting leaf rust and stripe rust resistance genes *Lr67* and *Yr46* in wheat are pleiotropic or closely linked. Theor Appl Genet 122(1):239-249

Klymiuk V, Yaniv E, Huang L, Raats D, Fatiukha A, Chen SS, Feng LH, Frenkel Z, Krugman T, Lidzbarsky G, Chang W, Jääskeläinen MJ, Schudoma C, Paulin L, Laine P, Bariana H, Sela H, Saleem K, Sørensen CK, Hovmøller MS, Distelfeld A, Chalhoub B, Dubcovsky J, Korol AB, Schulman AH, Fahima T (2018) Cloning of the wheat *Yr15* resistance gene sheds light on the plant tandem kinase-pseudokinase family. Nat Commun 9(1):3735

Kosambi DD (1943) The estimation of map distance from recombination values. Ann Eugenics 12:172–175

Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J , Spielmeyer W (2009) Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. Theor Appl Genet 119(5):889-898

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg LA, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181

Lebedeva TV, Peusha HO (2006) Genetic control of the wheat *Triticum monococcum* L. resistance to powdery mildew. Russ J Genet 42:60-66

Li JB, Dundas I, Dong CM, Li GR, Trethowan R, Yang ZJ, Hoxha S, Zhang P (2020) Identification and characterization of a new stripe rust resistance gene *Yr83* on rye chromosome 6R in wheat. Theor Appl Genet 133:1095-1107

Li Q, Ma D, Li Q, Fan Y, Shen X, Jing J, Kang Z (2016) Genetic analysis and molecular mapping of a stripe rust resistance gene in Chinese wheat differential Guinong22. J Phytopathol 164(7-8):476-484

Li YH, Shi XH, Hu JH, Wu PP, Qiu D, Qu YF, Xie JZ, Wu QH, Zhang HJ, Yang L, Liu HW, Zhou Y, Liu ZY, Li HJ (2020) Identification of a recessive gene *PmQ* conferring resistance to powdery mildew in wheat landrace Qingxinmai using BSR-Seq analysis. Plant Dis 104(3):743-751

Line R F, Qayoum A.Virulence, aggressiveness, evolution, and distribution of races of Puccinia striiformis (the cause of stripe rust of wheat) in North America, 1
87. Technical Bulletin-United States Department of Agriculture, 1992 (1788)

Ling HQ, Ma B, Shi XL, Liu H, Dong LL, Sun H, Cao YH, Gao Q, Zheng SS, Li Y, Yu Y, Du HL, Qi M, Lu HW, Yu H, Cui Y, Wang N, Chen CL, Wu HL, Zhao Y, Zhang JC, Li YW, Zhou WJ, Zhang BR, Hu WJ, Eijk MJ, Tang JF, Witsenboer HM, Zhao SC, Li ZS, Wang DW, Liang CZ (2018) Genome sequence of the progenitor of

wheat a subgenome triticum urartu. Nature 557(7705):424-428

Liu B, Liu TG, Zhang ZY, Jia Qiu-zhen, Wang BT, Gao L, Peng YL, Jin SL, Chen WQ (2017) Discovery and pathogenicity of CYR34, a new race of *Puccinia striiformis* f. sp. *tritici* in China. Acta Phytopathol Sin 47(5):681-687

Liu RH, Meng JL (2003) MapDraw: a Microsoft Excel macro for drawing genetic linkage maps based on given genetic linkage data. Heraditas 3:317-321

Ma H, Singh RP and Mujeeb-Kazi A (1997) Resistance to stripe rust in durum wheats, A-genome diploids, and their amphiploids. Euphytica 94: 279-286

Marais GF, Mccallum B, Marais AS (2006) Leaf rust and stripe rust resistance genes derived from aegilops sharonensis. Euphytica 149(3): 373-380

Marais F, Marais A, Mccallum B, Pretorius Z (2009) Transfer of leaf rust and stripe rust resistance genes *Lr62* and *Yr42* from aegilops neglecta req. ex Bertol. to common wheat. Crop Science 49(3): 871-879

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, KernytskyA, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA (2010) The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20:1297-1303

Michikawa A, Yoshida K, Okada M, Sato K, Takumi S (2019) Genome-wide polymorphisms from RNA sequencing assembly of leaf transcripts facilitate phylogenetic analysis and molecular marker development in wild einkorn wheat. Mol Genet Genomics 294: 1327-1341

Noman A, Hussain A, Adnan M, Ifnan MK, Furqan AM, Madiha Z, Ali KK, He SL (2019) A novel MYB transcription factor CaPHL8 provide clues about evolution of pepper immunity againstsoil borne pathogen. Microb Pathogenesis 137:103758

Pakeerathan K, Bariana H, Qureshi N, Wong D, Hayden M, Bansal U (2019) Identification of a new source of stripe rust resistance *Yr82* in wheat. Theor Appl Genet 132: 3169-3176

Paull JG, Pallotta MA, Langridge P, The TT (1994) RFLP markers associated with *Sr22* and recombination between chromosome 7A of bread wheat and the diploid species Triticum boeoticum. Theor Appl Genet 89:1039-1045

Randhawa M , Bansal U , Valárik M, Klocová B, Doležel J, Bariana H (2014) Molecular mapping of stripe rust resistance gene *Yr51* in chromosome 4AL of wheat. Theor Appl Genet 127(2):317-324

Ren Y, Li SR, Xia XC, Zhou Q, He YJ, Wei YM, Zheng YL, He ZH (2015) Molecular mapping of a recessive stripe rust resistance gene *yrMY37* in Chinese wheat cultivar Mianmai 37. Mol Breeding 35(3):1-9

Rey E, Molnár I, Doležel J. Genomics of wild relatives and alien introgressions. In: Molnár-Láng M, Ceoloni C, Doležel J. (eds) Alien introgression in wheat. Springer, Cham, 2015

Shi AN, Leath S, Murphy JP (1998) A major gene for powdery mildew resistance transferred to common wheat from wild einkorn wheat. Phytopathology 88:144-147

Tang Z, Yang Z, Fu S (2014) Oligonucleotides replacing the roles of repetitive sequences pAs1, pSc119.2, pTa-535, pTa71, CCS1, and pAWRC.1 for FISH analysis. J Appl Genet 55:313-318

The TT (1973) Chromosome location of genes conditioning stem rust resistance transferred from diploid to hexaploid wheat. Nat New Biol 241(112): 256

The TT, McIntosh RA (1975) Cytogenetical studies in wheat VIII. Telocentric mapping and linkage studies involving *Sr22* and other genes in chromosome 7AL. Aust J Biol Sci 28(6):531-538

Vasu K, Harjit-Singh, Chhuneja P, Singh S, Dhaliwal HS (2000) Molecular tagging of Karnal bunt resistance genes of *Triticum monococcum* transferred to *Triticum aestivum* L. Crop Improv 27:33-42

Wang H, Zou SH, Li YW, Lin FY, Tang DZ (2020) An ankyrin-repeat and WRKY-domain-containing immune receptor confers stripe rust resistance in wheat. Nat Commun 11:1353

Wang J, Liu C, Guo XR, Wang K, Du LP, Lin ZS, Ye XG (2019) Development and genetic analysis of wheat double substitution lines carrying *Hordeum vulgare* 2H and *Thinopyrum intermedium* 2Ai-2 chromosomes. Crop J 7(2):163-175

Wang LC, Tang XR, Wu JH, Shen C, Dai MF, Wang QL, Zeng QD, Kang ZS, Wu YF, Han DJ (2019) Stripe rust resistance to a burgeoning *Puccinia striiformis* f. sp. *tritici* race CYR34 in current Chinese wheat cultivars for breeding and research. Euphytica 215: 68

Wang Y, Xie JZ, Zhang HZ, Guo BM, Ning SZ, Chen YX, Lu P, Wu QH, Li MM, Zhang DY, Guo GH, Zhang Y, Liu DC, Zou SK, Tang JW, Zhao H, Wang XC, Cao TJ, Yin GH, Liu ZY (2017) Mapping stripe rust resistance gene *YrZH22* in Chinese wheat cultivar Zhoumai 22 by bulked segregant RNA-Seq (BSR-Seq) and comparative genomics analyses. Theor Appl Genet 130:2191-2201

Wellings C, Bariana H (2004) Assessment scale for recording stripe rust responses in field trials. Cereal Rust Report Season 2004, Plant Breeding Institute-Cereal Rust Laboratory, University of Sydney 2: 1-2

Wellings CR (2011) Global status of stripe rust: a review of historic and current threats. Euphytica 179(1): 129-141

Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.

William M, Singh RP, Huerta-Espino J, Islas SO, Hoisington D (2003) Molecular marker mapping of leaf rust resistance gene *Lr46* and its association with stripe rust resistance gene *Yr29* in wheat. Phytopathology 93(2):153-159

Yang EN, Li GR, Li LR, Zhang ZY, Yang WY, Peng YL, Zhu YQ, Yang ZJ, Rosewarne GM (2016) Characterization of stripe rust resistance genes in the wheat cultivar chuanmai45. Int J Mol Sci 17(4):601

Zhao LB, Ning SZ, Yi YJ, Zhang LQ, Yuan ZW, Wang JR, Zheng YL, Hao M, Liu DC (2018) Fluorescence in situ hybridization karyotyping reveals the presence of two distinct genomes in the taxon *Aegilops tauschii*. BMC Genomics 19:3

Zhang LQ, Yen Y, Zheng YL, Liu DC (2007) Meiotic restriction in emmer wheat is controlled by one or more nuclear genes that continue to function in derived lines. Sex Plant Reprod 20: 159-166

## Tables

**Table 1** Genetic analysis of resistance to mixture *Pst* races in $F_1$, $F_2$ and $F_{2:3}$ families derived from Z15-1370 × Mingxian169

| Parents and cross | Generation[a] | No. of plants/families | Observed ratio[b] | | | Expected ratio | $\chi^2$ | *P-value* |
|---|---|---|---|---|---|---|---|---|
| | | | R | Seg | S | | | |
| Z15-1370 | $P_R$ | 20 | 20 | | | | | |
| Mingxian169 | $P_S$ | 20 | | | 20 | | | |
| $P_R \times P_S$ | $F_1$ | 20 | | | 20 | | | |
| | $F_2$ | 542 | 136 | | 406 | 1:3 | 0.001 | 0.98 |
| | $F_{2:3}$ | 533 | 134 | 276 | 123 | 1:2:1 | 1.128 | 0.569 |

[a] $P_R$: resistant parent Z15-1370; and $P_S$: susceptible parent Mingxian169

[b] R: homozygous resistant; Seg: segregating within an $F_{2:3}$ families; S: homozygous susceptible

**Table 2** Primer sequences of KASP markers used for genetic mapping of *YrZ15-1370*

| Maker | Physical Position (Mb) | Allele 1 primer[a] | Allele 2 primer[b] | Common/reverse primer |
|---|---|---|---|---|
| *KASP-1370-2* | 600.41 | GCATATAAGAGACGGGGTGAC | GCATATAAGAGACGGGGTGAG | AGCAGAACACATATACACAC |
| *KASP-1370-3* | 601.47 | AGGAGAAAGATGAGCCCAAAA | AGGAGAAAGATGAGCCCAAAG | CCAAGATCGTCCTCCTACTC |
| *KASP-1370-5* | 603.3 | TTCTAGTTGTAGCTGAGGAC | TTCTAGTTGTAGCTGAGGAT | CAGATGGCCATGAAGGCAGT |
| *KASP-1370-6* | 604.38 | CGACTAGCTAGCTAGCTACAA | CGACTAGCTAGCTAGCTACAG | GGCTGAGCGACGGATCATGG |
| *KASP-1370-7* | 604.44 | ATCAATGTAACCAAAATTGGG | ATCAATGTAACCAAAATTGGT | GAGCTAGTCCATGTGAATCG |

[a]A1 primer labelled with FAM: GAAGGTGACCAAGTTCATGCT

[b]A2 primer labelled with HEX: GAAGGTCGGAGTCAACGGATT

**Table 3** Genotyping of G52, Z15-1370, Crocus, and Mingxian169 using KASP markers linked to *YrZ15-1370*.

| Parents | Marker genotype[a] | | | | |
|---|---|---|---|---|---|
| | *KASP-1370-2* | *KASP-1370-3* | *KASP-1370-5* | *KASP-1370-6* | *KASP-1370-7* |
| G52 | GG | GG | TT | GG | TT |
| Z15-1370 | GG | GG | TT | GG | TT |
| Crocus | CC | AA | CC | AA | GG |
| Mingxian169 | CC | AA | CC | AA | GG |

[a] AA, CC, GG, and TT represent the haplotype results of SNP genotyping

## Figures

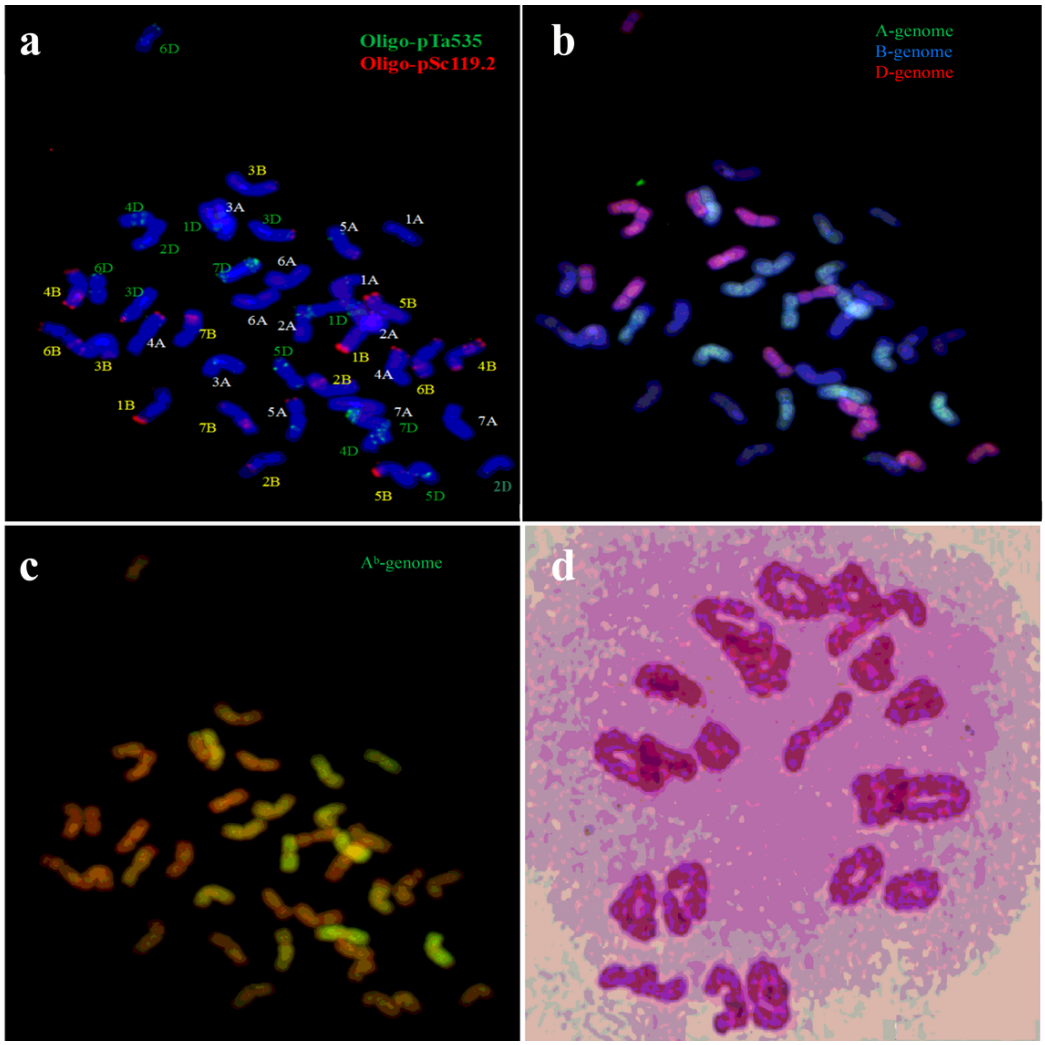


**Figure 1**

Cytogenetic identification of Crocus, G52, and Z15-1370 a, FISH pattern of metaphase chromosomes of Z15-1370. The probes Oligo-pSc119.2 (red) and Oligo-pTa535 (green) were used. b, mc-GISH patterns of metaphase chromosomes of Z15-1370. T. urartu genomic DNA (green) and Ae. tauschii genomic DNA (red) were used as probes for GISH analysis; c, sc-GISH patterns of metaphase chromosomes of Z15-1370. T. boeoticum genomic DNA was used as a probe (yellow-green) for GISH analysis; d, chromosome pairing of Z15-1370 in pollen mother cells at the metaphase I during the meiosis.

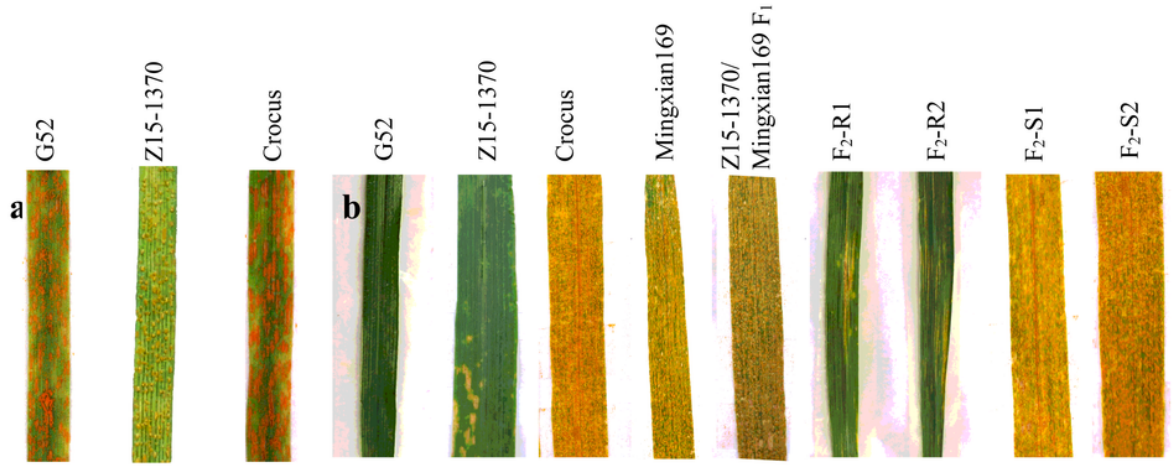


Figure 2

Stripe rust resistance phenotype in wheat lines carrying YrZ15-1370. a, stripe rust responses of Crocus, Z15-1370, and G52 to Pst race CYR34 at seedling stage. b, Stripe rust responses of Crocus, Z15-1370, G52 and the Z15-1370×Mingxian169 F1, F2 to the mixture Chinese Pst races at the adult plant stage. F2-R, resistant F2 plants; F2-S, susceptible F2 plants.

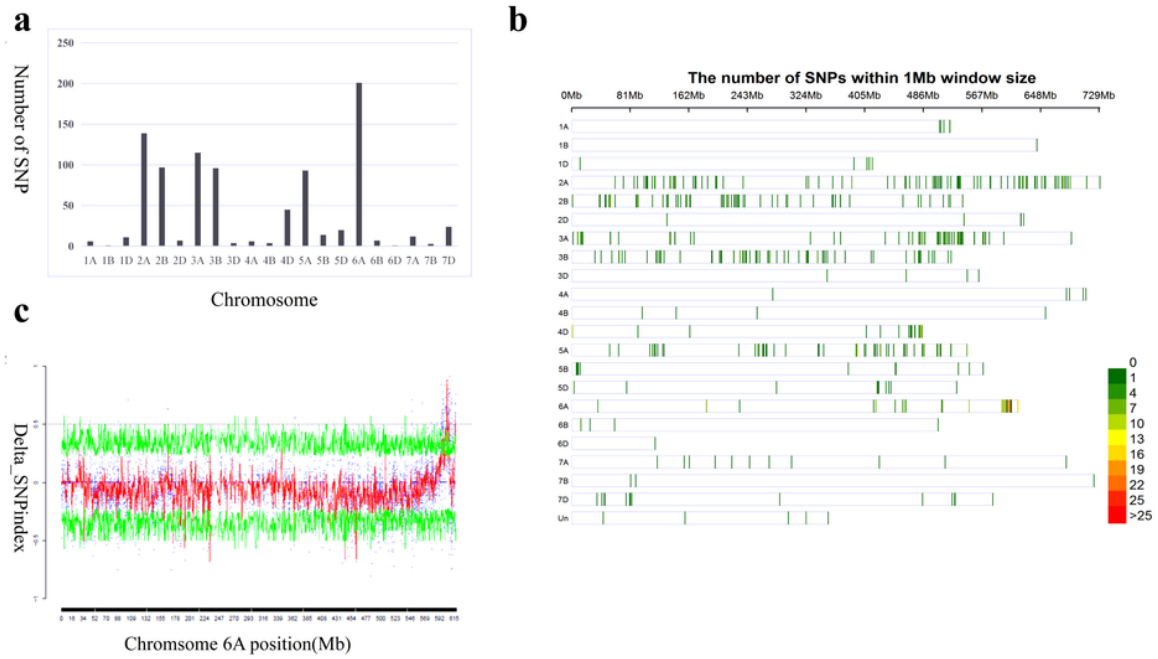


Figure 3

Distribution of SNPs from RNA bulks with contrasting responses to Pst. a, number of SNPs (AFD > 0.6, P-value < 1e−8) distributed on different wheat chromosomes; b, the enrichment of SNPs within 1Mb window size on wheat chromosomes; c, distribution of SNPs on chromosome 6A

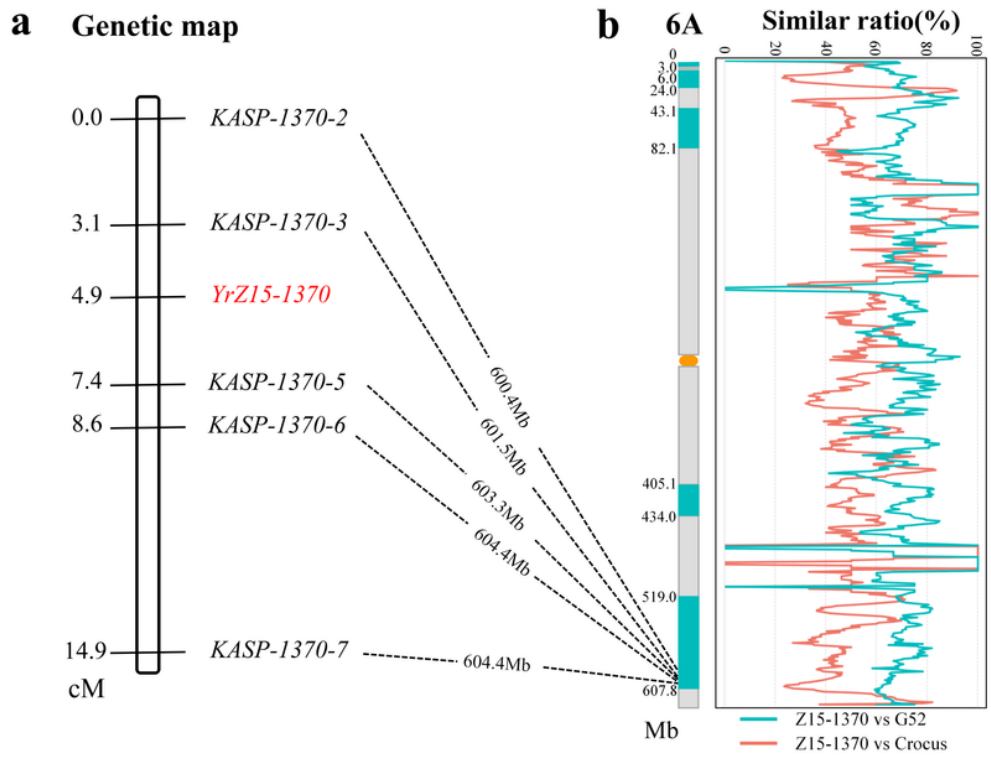


**Figure 4**

Chromosome 6AL linkage map of Z15-1370×Mingxian169 showing location of YrZ15-1370. a, linkage map of YrZ15-1370. b (left), graphic represents the G52 donor segments in chromosome 6A of Z15-1370. Blue rectangles indicate the G52 introgression segments. The physical positions of five linked KASP marker with YrZ15-1370 were indicated by dotted lines. b (right), graphic shows the similar ratio (%) calculated based on same SNP to the total SNPs scored between Z15-1370 and its two parents. A total of 2,277 SNP markers on chromosome 6A were obtained for the calculation. Blue curve, Z15-1370 vs. G52. Red curve, Z15-1370 vs. Crocus.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xlsx
- TableS2.xlsx