Resistance to *Heterodera filipjevi* and *H. avenae* in Winter Wheat is Conferred by Different QTL

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ABSTRACT

The coexistence of cereal cyst nematode (CCN) species *Heterodera avenae* and *H. filipjevi*, often involving multiple pathotypes, is a limiting factor for wheat production in China. Some of the known genes for resistance to CCN are not effective against both nematode species, hence complicating breeding efforts to develop CCN-resistant wheat cultivars. Here, we demonstrate that the CCN resistance in wheat cultivar Madsen to both *Heterodera* spp. is controlled by different genetic loci, both of which originated from *Aegilops ventricosa*. A new quantitative trait locus (QTL), *QCre-ma7D*, was identified and localized in a 3.77-Mb genomic region on chromosome arm 7DL, which confers resistance to *H. filipjevi*. *QCre-ma2A* on chromosome arm 2AS corresponds to CCN resistance gene *Cre5* and confers resistance to *H. avenae*. This

QTL is a new locus on chromosome arm 7DL and is designated *Cre9*. Three competitive allele-specific PCR markers (BS00150072, BS00021745, and BS00154302) were developed for molecular marker-assisted selection of *Cre9* and locally adapted wheat lines with resistance to both nematode species were developed. *QCre-ma2A* on chromosome arm 2AS corresponds to CCN resistance gene *Cre5* and confers resistance to *H. avenae*. The identification of different loci underlying resistance to *H. filipjevi* and *H. avenae* and the development of adapted resistant entries will facilitate breeding of wheat cultivars that are resistant to these devastating nematodes in China.

Keywords: genetics and resistance

Wheat (*Triticum aestivum* L.) is one of the major staple food crops in the world. Global production and consumption of wheat were 752.8 and 738.2 million metric tons, respectively, in 2017 (FAO 2017). Approximately two-thirds of the global wheat production is consumed as food and the remaining one-third as feed and other uses. China is the leading wheat producer, with a current annual production of 129.2 million metric tons. Wheat production is constantly challenged by various pathogens. Historically, diseases caused by foliar fungal pathogens (e.g., rusts and powdery mildew), stem- or root-based diseases (e.g., sharp eyespot and common root rot), and spike diseases (e.g., Fusarium head blight) were the major biotic constraints to grain yield throughout China (Liu et al. 2016). Cereal cyst nematode (CCN)

(*Heterodera* spp.) is an emerging threat to wheat production in a wide range of winter- and spring-wheat-producing regions. Yield losses caused by CCN in China were estimated to be 11 to 35% and 10 to 28% in winter and spring wheat, respectively (Peng et al. 2015). CCN is also widespread in certain American, European, and Asian counties, as well as Australia, where it poses a considerable threat to grain crops (Smiley et al. 2017; Toumi et al. 2018). Yield loss of 22,000 tons was caused by CCN in the four northwestern states of the United States (Smiley et al. 2017). Nicol et al. (2009) reported that *Heterodera filipjevi* (Madzhidov) Stelter, the predominant CCN species in Turkey, could reduce wheat yields by 42 to 45%. Yield reductions caused by *H. filipjevi* in three winter wheat cultivars in Iran ranged from 20.4 to 24.8% (Karimipour Fard et al. 2018).

CCN is a complex group that consists of several *Heterodera* spp. (Smiley et al. 2017). *H. avenae* Wollenweber and *H. filipjevi* are economically important CCN species worldwide and are also the major species that occur in wheat fields in China. *H. avenae* is reported from 16 Chinese provinces and *H. filipjevi* is known to occur in at least 5 provinces (i.e., Henan, Anhui, Ningxia, Qinghai, Xinjiang, and Shandong) (Feng et al. 2018; Peng et al. 2009; Peng et al. 2016, 2018; Zhen et al. 2018). *H. filipjevi* and *H. avenae* occur in the same wheat fields in some areas. The situation is further complicated when multiple pathotypes of both CCN species occur in the same wheat fields (Peng et al. 2009; Peng et al. 2015).

Several resistance loci have been identified in wheat and its relatives (Ali et al. 2019; Smiley et al. 2017; Toumi et al. 2018). Among them, only *Cre1* and *Cre8* originated from common

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wheat; all other named resistance genes were introduced from wild related species. For example *Cre2*, *Cre5*, and *Cre6* came from *Aegilops ventricosa* Tausch; *Cre3* and *Cre4* from *A. tauschii* Coss.; *Cre7* from *A. triuncialis*; *CreX* and *CreY* from *A. variabilis* L.; *CreR* from *Secale cereale* L.; and *CreV* from *Dasypyrum villosum* L. Candargy. Genes *Ha1*, *Ha2*, *Ha3*, and *Ha4* have been described in barley (*Hordeum vulgare* L.). In addition, resistance quantitative trait loci (QTL) were mapped on wheat chromosomes 1A, 2A, 2B, 3A, 3B, 4A, 4B, 5B, and 7BL. However, no identified *Cre* gene confers complete resistance to both *H. avenae* and *H. filipjevi*.

The importance of CCN in wheat has attracted the attention of wheat breeders, growers, and agricultural administrators, resulting in substantial research in China (Peng et al. 2015; Riley and Qi 2015). Integrated control, including cultural practices, application of nematicides, and use of host resistance, has been proposed to reduce economic losses and limit spread of nematodes. Nematicides are associated with environmental concerns, and alternative methods for controlling CCN populations by rotation with non-host crops or summer fallow are not feasible in most Chinese cropping systems. Wheat monoculture is widely practiced in many areas; thus, resistant cultivars are the preferred means of control. In addition to reducing current-season damage, CCN-resistant cultivars are also reported to suppress nematode multiplication in infested fields (Cui et al. 2016; Safari et al. 2005), thus reducing the initial population density for subsequent cropping seasons. Unfortunately, the majority of current commercial Chinese winter wheat cultivars are highly susceptible (Li et al. 2012), and breeding efforts are needed to improve the resistance to local pathotypes.

Management of CCN by growing nematode-resistant cultivars was successful in Australia in the 1980s (Lewis et al. 2009). However, the lack of effective sources of resistance impedes the development of CCN-resistant wheat cultivars in most wheat breeding programs in China. Unlike Australia, where only *H. avenae* occurs, the coexistence of both *H. avenae* and *H. filipjevi*, as well as multiple pathotypes of each species, complicates efforts to genetically improve resistance in China (Feng et al. 2018; Li et al. 2010; Peng et al. 2015; Smiley et al. 2017). Thus, variation in the species and pathotypes present in different wheat fields must be considered when breeding for CCN resistance. The reported resistance genes or QTL are effective only against some *Heterodera* spp. or pathotypes (Imren et al. 2013; Smiley et al. 2017; Yuan et al. 2011). Moreover, most documented CCN resistance genes or QTL were characterized with *H. avenae*, and only a few cases with *H. filipjevi* (Pariyar et al. 2016; Zhang et al. 2016). Identification of effective sources of resistance to both *Heterodera* spp. is a high priority. Because previous genetic studies on resistance to CCN were conducted using only *H. avenae* or *H. filipjevi*, it is impossible to determine whether resistance to these species is based on common genes.

Cultivar Madsen (PI 511673), a soft white winter wheat cultivar adapted to the Pacific Northwest (PNW) region of the United States (Allan et al. 1989), is highly resistant to pathotypes of *H. filipjevi* and *H. avenae* that occur in China (Li et al. 2012; Wu et al. 2016). Based on phenotypic performance in field and greenhouse tests, CCN resistance of Madsen was believed to be inherited from the parent VPM-1 (Wu et al. 2016). The French wheat line VPM-1, which was developed from a complex cross that involves *A. ventricosa*, *T. persicum* Vav. Ex Zhuk., and the common wheat cultivar Marne carries *Cre5* for resistance to *H. avenae* (Jahier et al. 2001). However, *Cre5* is not effective against the Turkish pathotype of *H. filipjevi* (Dababat et al. 2014). Using Madsen as the resistance source, this study was initiated to (i) understand whether resistance to *H. filipjevi* and *H. avenae* is controlled by the same genetic factors, (ii) establish molecular marker-assisted selection (MAS) tools for efficient introgression of CCN resistance of Madsen, and (iii) develop CCN-resistant wheat germplasm adapted to local production conditions of China.

MATERIALS AND METHODS

Plant materials. Madsen (pedigree: VPM1/Moisson 951/2/2*Hill-81) was resistant to CCN, but susceptible to powdery mildew. The CCN-susceptible but powdery mildew-resistant winter wheat cultivar Liangxing 99 (pedigree: Ji 91102/Lumai 14/PH85-16) is widely grown throughout major production regions of China. A recombinant inbred line (RIL)₂₋₉ population containing 187 F₂₋₉ lines was developed from a cross between Madsen and Liangxing 99. The parents of Madsen—VPM-1 (PI 519303), VPM-1/Moisson 951, Hill 81 (Citr 17954), and *A. ventricosa* line #11—were included in the molecular characterization of the resistance QTL. Winter wheat cultivars Wenmai 19 and Zhongzuo 9504 were included as susceptible controls in CCN and powdery mildew tests, respectively.

Phenotyping CCN reactions in field assessments. Separate field assessments were carried out at Xuchang and Xingyang in Henan Province. Previous research using morphological and molecular approaches had determined that *H. filipjevi* pathotype Hfc-1 was the major soilborne pathogen at the Xuchang site, and *H. avenae* pathotype Ha43 occurred at the Xingyang site (Cui et al. 2016; Fu et al. 2011; Li et al. 2010; Yuan et al. 2010). A randomized complete block design with two replications was used in all the field assessments. Each plot consisted of two 1-m rows with a 25-cm row spacing. At the late grain-fill stage (growth stage 77) (Zadoks et al. 1974), 7 to 10 plants in soil cylinders were collected from each plot to determine the number of white females on the roots; numbers were averaged to obtain a phenotypic reactions for each line and plot. Field management, including irrigation, fertilization, and weed control, was based on local practices. The phenotypes of cultivars based on mean number of white females on the roots were classified as resistant (<5.0 females), moderately resistant (5 to 10 females), moderately susceptible (10 to 14 females), susceptible (15 to 25 females), and highly susceptible (>25 females) (Nicol et al. 2009).

Phenotyping CCN reactions in greenhouse trials. Second-stage juveniles (J2s) of *H. filipjevi* and *H. avenae* were hatched from cysts isolated from infested soil collected from Xuchang and Xingyang field sites. Plants were grown singly in polyvinyl chloride tubes (diameter = 4 cm and height = 13 cm). Each plant was inoculated separately with 600 J2s of each nematode species. Five plants were tested for each entry and entries were arranged in randomized complete blocks. The inoculation method, plant growth conditions, and determination of white females on the roots were carried out as described previously (Cui et al. 2016; Wu et al. 2016).

Assessment of powdery mildew reactions at the seedling and adult plant stages. Seedlings of the RIL population and parents to *Blumeria graminis* f. sp. *tritici* isolates E09, E20, E23, and B11 were tested following a method described previously (Wu et al. 2019; Zou et al. 2017). Fifteen two-leaf-stage plants of each entry were inoculated with each isolate. Infection types (IT) recorded 2 weeks postinoculation were based on a 0-to-4 scale, where plants with IT 0 to 2 were considered to be resistant and those with IT 3 to 4 were susceptible. This test was repeated three times.

To evaluate adult plant resistance, wheat entries were planted with a randomized complete block design with three replicates at Changping Experimental Station of Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China, during the 2015–16 growing season. Zhongzuo 9504 was grown as a control and a spreader of the pathogen. A mixture of isolates (virulence patterns V1, V3a, V3b, V3c, V3e, V3f, V4a, V4b, V5a, V6, V7, V8, V17, V19, V23, V25, V30, V34, V35, and VXBD) was inoculated on the spreader in early April 2016. When the powdery mildew symptoms were fully developed on the susceptible control plants, disease severities on flag leaves were recorded at weekly

intervals to determine area under the disease progress curve (AUDPC). The last scores were regarded as the maximum disease severities (MDS).

Observation of agronomic traits. Lines ML99-18 and ML99-46 and their parental cultivars Madsen and Liangxing 99 were grown at Changping Experimental Station in three cropping seasons (2014 to 2016) and Xuchang and Xingyang in the 2015 and 2016 cropping seasons using a randomized complete block design with two replicates. The agronomic traits, heading date, plant height, spike length, number of spikelets per spike, number of sterile spikelets per spike, and number of seed per spike for 10 plants were determined as previously described (Qiu et al. 2017).

Single-nucleotide polymorphism genotyping and molecular analysis. Genomic DNA was extracted from 5-day-old fresh leaf tissue (approximately 100 mg) of each wheat entry using the standard cetyltrimethylammonium bromide method (Sharp et al. 1988). All members of the Madsen × Liangxing 99 RIL population and the parents, as well as the parental wheat cultivars used to develop Madsen, were genotyped by the 90K Illumina iSelect Wheat SNP chip (Wang et al. 2014). The allele calling of single-nucleotide polymorphisms (SNP) was performed in the Illumina iScan reader equipped with the Genome Studio software (version 2011.1; Illumina Inc., San Diego, CA, U.S.A.). The manual option of Genome Studio (version 1.9.4) with polyploidy clustering (version 1.0.0) was used to determine the genotypic clusters for each SNP based on the data that were derived from all described genotypes. Eight simple sequence repeat (SSR) markers on chromosome 2A associated with *Cre5* were also used to construct a genetic linkage map. Primers VENTRIUP/LN2 and VRGA-F11/VRGA-R5 were specific for gene *Vrg1D* from *A. ventricosa* and were used to detect *Cre5* (Fang et al. 2011; Wu et al. 2016). In addition, *Pm52* was genotyped using SSR marker *Xgwm120* (Zhao et al. 2013). DNA amplification was carried out in a 20- μ l reaction mixture with the following profile: one cycle of denaturation at 94°C for 5 min, followed by 34 cycles of 94°C for 30 s, 50 to 60°C (depending on primers) for 30 s, and 72°C for 30 s. The reaction was terminated after an elongation step at 72°C for 5 min. PCR products were separated in 8% polyacrylamide gels and visualized by silver staining.

Linkage map construction and QTL mapping. Linkage maps were constructed with JoinMap 4 (Van Ooijen 2006). SNP with poor quality, over 10% missing data, or distortion from the expected segregation ratio were excluded from further analysis. Marker ordering was conducted with the maximum-likelihood algorithm, and map distance was calculated with the Haldane function. The distance between markers was converted from “Haldane centimorgans” to “Kosambi centimorgans” (Van Ooijen 2006). Interval mapping was used to detect single-locus QTL for different environments separately by MapQTL 6.0 (Van Ooijen 2009). The logarithm of odds (LOD) threshold was determined with 1,000 permutations in the Permutation Test process to declare the presence of a QTL at 95% significance level. MapChart 2.2 software was used to draw the genetic map and detect QTL (Voorrips 2002).

Kompetitive allele-specific PCR assay. Kompetitive allele-specific PCR (KASP) assays were developed to validate putative QTL-linked SNP. Primers designed using the online software PolyMarker (Ramirez-Gonzalez et al. 2015) included the standard FAM (5'-GAAGGTGACCAAGTTCATGCT-3') or HEX (5'-GAAGGTCGGAGTCAACGGATT-3') tails and the targeted SNP at the 3' end. The common primer was designed to ensure that the amplicon was less than 120 bp. KASP markers were also used to genotype individual RIL. KASP assays were performed in 5- μ l reaction mixtures prepared by mixing DNA at 10 to 20 ng μ l⁻¹, 2.5 μ l of 1× KASP master mixture, and 0.056 μ l of primer mixture. The primer mixture was prepared by mixing 46 μ l of double-distilled H₂O, 30 μ l of common primer (100 μ M), and 12 μ l of each

tailed primer (100 μ M). The following profile was used in PCR amplification: denaturing at 95°C for 15 min, 10 touchdown cycles (95°C for 20 s; touchdown at 65°C initially and then decreasing by -1°C per cycle for 25 s), and 30 cycles of annealing at 95°C for 10 s and 57°C for 60 s (Rasheed et al. 2016). Data analysis was performed manually using Kluster Caller software (LGC, Hoddesdon, U.K.).

Bulk segregant analysis and RNA-sequencing. In total, 20 resistant and 20 susceptible RIL, based on the reaction to *H. filipjevi* over 3 years of field testing, were used to construct contrasting RNA bulks. Total RNA was isolated from leaf tissues of the representative plants of each selected RIL with the TruSeq RNA Sample Prep Kit (Illumina, Inc.). RNA-seq analysis was conducted in the IlluminaHiSeq 4000 (Beijing Southern Genome Research Technology Co., Ltd., Beijing, China). The raw sequencing reads were trimmed for quality control using software Trimmomatic v0.36 with default parameters. The clean reads were aligned to a wheat whole-genome draft assembly using software STAR v2.5.1b (Clavijo et al. 2017), with a mismatch rate of <5%. Uniquely mapped read pairs were used for SNP calling using “HaplotypeCaller” in GATK v3.6 software (McKenna et al. 2010). The SNP were subjected to bulk segregant analysis (BSA) after removing SNP with sequencing depth less than six. Variants that had allele frequency differences >0.6 and *P* value of Fisher’s exact test on read count data < 10⁻⁸ were categorized as resistance-associated variants and used as templates for marker development (Wang et al. 2017). In total, 165 candidate SNP potentially linked with resistance to *H. filipjevi* were identified on chromosome arm 7DL.

Physical localization of CCN resistance QTL. Sequences of SNP linked to the target QTL were used as queries for BLASTN searches by alignment with the Chinese Spring whole-genome assembly sequences (IWGSC RefSeq v1.0) (International Wheat Genome Sequencing Consortium 2018) and *Aegilops tauschii* genome sequences (Aet v4.0) (Luo et al. 2017) using the basic search with default parameter settings. Gene annotations were provided by the online programs EnsemblPlants (<http://www.plants.ensembl.org>) and NCBI (<https://www.ncbi.nlm.nih.gov/>).

Statistical analysis. Genotypic and nongenotypic variance components of the traits investigated were determined using a residual maximum-likelihood (REML) procedure. All factors in the REML model were regarded as random; genotype was regarded as a fixed factor and environment and replicates were random. The same model was used to obtain adjusted means, which were then used for all statistical and QTL analyses. Fisher’s Protected least significant difference was used in multiple comparisons to differentiate the significance of differences between white female means, MDS, AUDPC, and different agronomic traits at *P* < 0.05. GenStat (18th edition; VSN International Ltd., Hemel Hempstead, U.K.) was used in all statistical analyses.

RESULTS

Assessment of the Madsen × Liangxing 99 RIL population with *H. filipjevi* and *H. avenae*. Multiple assessments in the field (*H. filipjevi* at Xuchang and *H. avenae* at Xingyang) and greenhouse tests with the same nematode species conducted from 2009 to 2017 demonstrated that Madsen was resistant to pathotype Hfc-1 of *H. filipjevi* and Ha43 of *H. avenae*, with ranges of white females of 0.1 to 5.0 and 1.6 to 9.4 per plant, respectively, in all tests. These results were based on the presence of fewer white females per plant for Madsen than for Liangxing 99 and the susceptible control Wenmai 19 (Table 1). Liangxing 99 differed from Madsen, with susceptible phenotypes to both CCN species having a range of white females of 16.3 to 140.9 and 16.2 to 39.3 per plant for *H. filipjevi* and *H. avenae*, respectively. Wenmai 19 always had more white females than Madsen in the phenotyping experiments (*P* < 0.05).

Using the same methods, three field tests and two greenhouse tests with *H. filipjevi* and one field test and one greenhouse test with *H. avenae* were conducted for the 187 RIL derived from Madsen × Liangxing 99. The distribution of mean number of white females per line for the RIL population was continuous (Supplementary Fig. S1), indicating quantitative inheritance of resistance to *H. filipjevi* and *H. avenae* in Madsen.

Construction of genetic linkage map. Genotyping of the RIL population with the Illumina iSelect 90K SNP array identified 11,715 polymorphic SNP between Madsen and Liangxing 99. After quality control on the mapping population, 3,219 SNP at 2,466 loci and eight *Cre5*-linked markers were used to produce a genetic linkage map that consisted of 37 linkage groups and spanned 4,576.11 centimorgans (cM) in length, with an average density of 1.42 cM/marker (Supplementary Table S1).

QTL for resistance to *H. filipjevi*. A QTL for resistance to *H. filipjevi*, designated *QCre-ma7D*, was consistently detected on chromosome arm 7DL and explained 13.6 to 16.5% of the phenotypic variation across field tests during 2013 to 2015 (E1, E2, and E3) (Fig. 1A; Table 2). The closest flanking SNP markers were BS00077148 and BS00151654. Madsen contributed the positive additive effect for fewer white females on host roots.

To confirm the localization of the QTL, two RNA bulks consisting of 20 resistant or 20 susceptible RIL identified in the field tests during 2013 to 2015 (E1, E2, and E3) were subjected to BSA and RNA sequencing (BSR-seq) analysis. In total, 165 candidate SNP were putatively associated with the resistant phenotype; 154 were anchored onto Chinese Spring chromosome arm 7DL (616,901,638 to 636,769,409 bp), with most located in the genomic region of *QCre-ma7D* (Fig. 2). Twenty KASP markers were designed based on candidate SNP (Supplementary Table S2); five (*Xics7D-27*, *Xics7D-61*, *Xics7D-99*, *Xics7D-115*, and *Xics7D-165*) were incorporated into the linkage map of 7DL. *QCre-ma7D* was also consistently identified on chromosome arm 7DL in greenhouse tests in 2015 and 2016 (E4 and E5), explaining 19.3 and 42.0% of the phenotypic variation, respectively (Fig. 1B; Table 2). *QCre-ma7D* was mapped in the interval between markers *Xics7D-27* and BS00129645 on chromosome arm 7DL in the integrated genetic linkage map.

QTL for resistance to *H. avenae*. When challenged by *H. avenae* in a field test (E6) and a greenhouse test (E7), another QTL, designated *QCre-ma2A*, was detected on chromosome arm

2AS, explaining 15.4 and 19.6% of the phenotypic variation, respectively (Fig. 1C; Table 2). The flanking markers of *QCre-ma2A* were BARC1138 and BS00163573. The marker VRGA-F11/VRGA-R5, which is linked to gene *Vrga1* on the 2NS chromosome segment from *A. ventricosa* and was used to detect *Cre5* (Seah et al. 2000), was located in the same interval as *QCre-ma2A*.

Physical localization of *QCre-ma7D* and comparative genomic analysis. Markers flanking *QCre-ma7D*, *Xics7D-27*, and BS00129645 were blasted against the Chinese Spring reference genome sequence. *QCre-ma7D* was located in an approximately 3.77-Mb (618,226,160 to 622,003,384 bp) genomic region on chromosome 7D of Chinese Spring containing 61 predicted genes (Supplementary Table S3). Among them, eight were annotated as disease resistance protein (nucleotide-binding site leucine-rich repeat class) family genes (*TraesCS7D01G520800.1*, *TraesCS7D01G521900.1*, *TraesCS7D01G522200.1*, *TraesCS7D01G522300.1*, *TraesCS7D01G525700.1*, *TraesCS7D01G525800.1*, *TraesCS7D01G526300.1*, and *TraesCS7D01G526600.1*) and eight were protein kinase family genes (*TraesCS7D01G521200.1*, *TraesCS7D01G525000.1*, *TraesCS7D01G525100.1*, *TraesCS7D01G526200.1*, *TraesCS7D01G526400.1*, *TraesCS7D01G526500.1*, *TraesCS7D01G526700.1*, and *TraesCS7D01G526800.1*). In addition, there was a programmed cell death protein 2 gene (*TraesCS7D01G521500.1*), a coiled-coil domain containing protein 14 gene (*TraesCS7D01G521600.1*), and protein-enhanced disease resistance 2 gene (*TraesCS7D01G523200.1*) in this genomic region. The orthologous region in *A. tauschii* chromosome 7D spanned approximately 4.23 Mb (622,673,980 to 626,908,644) containing 49 predicted genes, of which 8 were predicted to encode the disease resistance-associated proteins (Fig. 3).

Origins of *QCre-ma7D* and *QCre-ma2A*. The wheat cultivars and lines in the pedigree of Madsen were analyzed with the iSelect 90K SNP assay. Genotypes of 17 SNP markers flanking *QCre-ma7D* in *A. ventricosa*, Madsen, VPM-1, and VPM-1/Moisson 951 were similar, with very few exceptions, whereas the genotypes of Hill 81 were quite different from the other parents (Supplementary Table S4). The genotypes of these markers in Liangxing 99 were similar to Hill 81. Together, these data provided evidence that *QCre-ma7D* in Madsen was derived from *A. ventricosa* via VPM-1 and VPM-1/Moisson 951.

Development of diagnostic KASP markers for detection of *QCre-ma7D*. Of the 17 *QCre-ma7D*-linked SNP, 10 were

TABLE 1. Mean number of white females per plant in Madsen, Liangxing 99, and the susceptible control Wenmai 19 in response to *Heterodera filipjevi* and *H. avenae* in field and greenhouse tests^z

Year	<i>H. filipjevi</i>			<i>H. avenae</i>		
	Madsen	Wenmai 19	Liangxing 99	Madsen	Wenmai 19	Liangxing 99
Field tests						
2009	0.5 a	140.9 c	38.6 b	—	—	—
2010	0.5 a	86.0 c	58.3 b	—	—	—
2011	0.1 a	104.8 b	—	4.4 a	34.7 b	—
2012	0.1 a	45.5 b	—	4.6 b	22.7 b	—
2013	5.0 a	16.3 b	12.0 b	1.6 a	39.0 b	—
2014	3.6 a	26.6 b	18.6 b	3.7 a	39.3 c	17.5 b
2015	2.6 a	21.0 b	14.5 b	6.6 a	36.0 c	18.2 b
2016	—	—	—	8.2 a	26.1 b	25.7 a
2017	4.5 a	52.1 c	23.2 b	—	—	—
Mean	2.1	61.7	17.1	4.2	34.3	17.9
SD	2.1	44.8	4.9	2.1	6.4	3.9
Inoculation tests						
2012	0.5 a	20.5 b	—	2.0 a	21.4 b	—
2013	4.2 a	22.2 b	20.8 b	5.1 a	65.0 b	—
2014	3.4 a	25.0 b	21.0 b	5.3 a	16.2 b	14.5 b
2015	1.3 a	29.3 b	—	9.4 a	55.2 c	36.8 b
2016	—	—	—	7.4 a	20.4 b	14.6 b
Mean	2.4	24.3	20.9	5.8	35.6	22.0
SD	1.7	3.8	0.1	2.8	22.7	12.8

^z Means followed by different letters within a line are significantly different ($P < 0.05$) based on least significant difference test; — indicates not tested and SD = standard deviation.

converted to KASP markers. Six specific and four semispecific KASP assays were carried out using the complete Madsen × Liangxing 99 RIL population, the parents, and a “heterozygous” control composed of equal amounts of DNA from Madsen and Liangxing 99. KASP markers BS00150072, BS00021745, and BS00154302 showed a clear clustering for precise genotypes that were consistent with the genotyping results from 90K iSelect SNP arrays (Fig. 4; Supplementary Table S5), thus demonstrating that these KASP markers were linked and able to detect *QCre-ma7D*.

Effects of *QCre-ma7D* and *QCre-ma2A* on reactions to *H. filipjevi* and *H. avenae*. Effects of QTL *QCre-ma7D* and *QCre-ma2A* and their allelic combinations on reaction to *H. filipjevi* and *H. avenae* were further investigated by grouping the RIL population according to genotypes of the *QCre-ma7D* flanking markers (*Xics7D-27* and BS00150072) and *QCre-ma2A*-linked markers (VRGA-F11/VRGA-R5) on chromosomes 7D and 2A, respectively. *QCre-ma7D*-carrying lines (17 lines), *QCre-ma2A*-carrying lines

(38 lines), two-QTL-carrying lines (36 lines), and no-QTL-carrying lines (36 lines) were identified from the Madsen × Liangxing 99 RIL population. Fifteen lines randomly selected from each of the four genotypes each were tested in the field against *H. filipjevi* and *H. avenae* in 2017, with 10 plants for each line. When challenged by *H. filipjevi*, the mean number of white females per plant for the lines carrying both QTL and *QCre-ma7D* only were not different from that of Madsen but were significantly less than for the other two genotypes ($P < 0.05$); *QCre-ma2A*-carrying lines (13.3 ± 6.7), and no-QTL-carrying lines (15.2 ± 7.0) were both moderately susceptible to *H. filipjevi* (Fig. 5). When tested in a field infested by *H. avenae*, the mean number of white females on *QCre-ma2A*-carrying lines (8.3 ± 2.1), two-QTL-carrying lines (9.1 ± 2.3), and Madsen (8.9 ± 1.7) were similar and significantly less than those for the no-QTL-carrying lines (12.0 ± 4.1) and *QCre-ma7D*-carrying lines (13.0 ± 2.9) ($P < 0.05$) (Fig. 5). Liangxing 99 was consistently susceptible to both nematode species.

Fig. 1. A, Genetic linkage map for *QCre-ma7D* on chromosome arm 7DL conferring resistance to *Heterodera filipjevi* based on genotyping data generated by the 90K iSelect SNP assay and phenotypic data from environments E1, E2, and E3; B, genetic linkage map integrating the 90K iSelect SNP and bulk segregant analysis and RNA sequencing single-nucleotide polymorphism (SNP) markers with the phenotypic data from all the five environments; and C, genetic linkage map for *QCre-ma2A* on chromosome arm 2AS conferring resistance to *H. avenae*. The mapping population consisted of 187 recombinant inbred lines from the cross of Madsen × Liangxing 99. Phenotypic assessments for *H. filipjevi* resistance were conducted in field tests in 2013 (E1), 2014 (E2), and 2015 (E3) and greenhouse tests in 2015 (E4) and 2016 (E5). Phenotypic assessments for *H. avenae* resistance were conducted in the greenhouse (E6) and field (E7) in 2016.

TABLE 2. Quantitative trait loci (QTL) for resistance to *Heterodera filipjevi* and *H. avenae* in the Madsen × Liangxing 99 recombinant inbred line population

QTL	Nematode	Env ^x	Chr ^y	Closest marker	LOD ^z	LOD threshold	Exp (%)	Additive
<i>QCre-ma7DE1</i>	<i>H. filipjevi</i>	E1	7D	BS00120482, BS00122126	6.2	3.0	16.2	-2.82
<i>QCre-ma7DE2</i>	<i>H. filipjevi</i>	E2	7D	BS00096149, BS00154302	5.8	3.3	16.5	-1.44
<i>QCre-ma7DE3</i>	<i>H. filipjevi</i>	E3	7D	BS00166614	5.7	3.2	13.6	-0.99
<i>QCre-ma7DE4</i>	<i>H. filipjevi</i>	E4	7D	BS00127843, BS00149529	6.4	3.3	19.3	-3.37
<i>QCre-ma7DE5</i>	<i>H. filipjevi</i>	E5	7D	BS00096149, BS00154302	20.4	3.5	42.0	-5.45
<i>QCre-ma2AE6</i>	<i>H. avenae</i>	E6	2A	BS00163573	8.9	3.3	19.6	-2.01
<i>QCre-ma2AE7</i>	<i>H. avenae</i>	E7	2A	BS00146293	4.6	3.0	15.4	-1.28

^x Environments: E1 = field test of *H. filipjevi* in 2013, E2 = field test of *H. filipjevi* in 2014, E3 = field test of *H. filipjevi* in 2015, E4 = greenhouse test of *H. filipjevi* in 2015, E5 = greenhouse test of *H. filipjevi* in 2016, E6 = greenhouse test of *H. avenae* in 2016, and E7 = field test of *H. avenae* in 2016.

^y Chromosome.

^z Logarithm of odds.

These findings demonstrate that *QCre-ma7D* conferred resistance to *H. filipjevi* and *QCre-ma2A* conferred resistance to *H. avenae*.

Development of CCN-resistant wheat lines adapted to local conditions. Based on the genotypes of markers specific for *QCre-ma7D* (*Xics7D-27*, BS00150072, BS00021745, and BS00154302) and *QCre-ma2A* (*VRGA-F11/VRGA-R5*), lines ML99-18 and ML99-46 were selected to test for CCN and powdery mildew resistance, and agronomic traits. Both lines carried *QCre-ma7D* and *QCre-ma2A* from Madsen (Supplementary Table S6) and were resistant to both *H. filipjevi* and *H. avenae* during the 4 years of field testing and three greenhouse tests (Supplementary Table S7). These lines were also resistant to four *B. graminis* f. sp. *tritici* isolates at the seedling stage and moderately resistant to powdery mildew at the adult plant stage, as was the Liangxing 99 parent (Supplementary Table S8). The *Xgwm120* marker allele associated with *Pm52* (Zhao et al. 2013) was present in both lines, indicating that they had inherited *Pm52* from Liangxing 99. Agronomic traits such as plant height, spike length, number of seed per spike, and heading dates of these lines resembled their locally adapted parent Liangxing 99 (Supplementary Table S9).

DISCUSSION

Madsen is highly resistant to different pathotypes of the cyst nematodes *H. filipjevi* and *H. avenae*, both of which are indigenous

to China (Wu et al. 2016). This study further confirmed the stability of CCN resistance of Madsen in multiple-year field tests in wheat fields where both CCN species occur. A high-density genetic linkage map was generated by genotyping a Madsen × Liangxing 99 RIL population with the 90K iSelect SNP chip. QTL *QCre-ma7D* and *QCre-ma2A* were detected on chromosome arms 7DL and 2AS, respectively. Results of BSR-seq analysis using contrasting RNA bulks from *H. filipjevi*-resistant and -susceptible plants provided further evidence for the presence of *QCre-ma7D* on chromosome arm 7DL. *QCre-ma7D* was effective against *H. filipjevi* but not against *H. avenae* whereas *QCre-ma2A* conferred resistance to the latter species but not the former. Because a gene for resistance to *H. filipjevi* has not been reported on chromosome arm 7DL, *QCre-ma7D* represents a new locus conferring resistance and we named it *Cre9*. *QCre-ma2A* is presumed to be *Cre5* because Madsen carries the 2NS chromosomal segment harboring *Cre5* and other disease resistance genes from VPM-1 (Wu et al. 2016).

Several genes or QTL for nematode resistance have been reported. Most, except for *CreV* from *D. villosum* (Zhang et al. 2016), were characterized using *H. avenae* races. Previous tests have indicated that only *CreR* from rye (*S. cereale*) and *CreV* from *D. villosum* conferred resistance to the *H. filipjevi* pathotype used in this study (Cui et al. 2012; Zhang et al. 2016). *Cre9* is a new locus for resistance to CCN because it resides on chromosome arm 7DL, in which no previously characterized CCN resistance gene was located. The occurrence of

Fig. 2. Distribution of 165 candidate single-nucleotide polymorphisms (SNP) generated by bulk segregant analysis and RNA sequencing (BSR-seq) analysis and 90K iSelect SNP assay that were linked to *QCre-ma7D* on chromosome 7D.

H. filipjevi in China was reported quite recently (Feng et al. 2018; Li et al. 2010; Peng et al. 2018; Zhen et al. 2018) and is increasingly being reported in other countries (Karimipour Fard et al. 2017; Renčo et al. 2018; Smaha et al. 2018; Smiley et al. 2017; Toumi et al. 2018). Resistance to *H. filipjevi* has been identified in some wheat genotypes (Li et al. 2012; Moatamedi et al. 2018; Pariyar et al. 2016). In contrast to *H. avenae*, only a few genetic studies have involved *H. filipjevi* (Pariyar et al. 2016; Zhang et al. 2016). Consequently, identification of *Cre9* provides a new, potentially valuable source of resistance.

QCre-ma2A was associated with the marker VRGA-F11/VRGA-R5, which is specific for the *A. ventricosa* 2NS chromosome segment harboring *Cre5* (Seah et al. 2000). Previous research demonstrated that Madsen inherited the 2NS segment from VPM-1 (Wu et al. 2016). *Cre5* conferred resistance to *H. avenae* pathotypes in France (Jahier et al. 2001), Australia (Ogbonnaya et al. 2001), Spain (Montes et al. 2003), and uncharacterized pathotypes from the PNW of the United States (Smiley et al. 2013). However, it was not effective against *H. filipjevi* isolates in Turkey

Fig. 3. A, *QCre-ma7D* physical interval on chromosome arm 7DL and synteny between B, *Triticum aestivum* 'Chinese Spring' and C, *Aegilops tauschii* in the corresponding genomic interval.

(Dababat et al. 2014). We observed that some of the Madsen-derived lines that carry the 2NS chromosomal segment were not resistant to *H. filipjevi*. We also observed that wheat cultivar Tianmin 668 was resistant to *H. avenae* pathotype Ha43 but susceptible to *H. filipjevi* pathotype Hf-1 in field tests (Cui et al. 2016). The present dissection of genetic loci conferring

resistance to the two *Heterodera* spp. in Madsen is consistent with that observation.

Three *H. avenae*-resistance genes (*Cre2*, *Cre5*, and *Cre6* on 6M^v, 2N^v, and 5N^v chromosome segments, respectively) were transferred onto the corresponding homoeologous wheat chromosomes originated from *A. ventricosa*. Although the

Fig. 4. Scatter plots for three Kompetitive allele-specific PCR (KASP) assays showing clustering of different genotypes. **A**, KASP marker BS00150072; **B**, KASP marker BS00021745; and **C**, KASP marker BS00154302. NTC = nontemplate control.

Fig. 5. Mean number of white females of cereal cyst nematode (CCN) species *Heterodera avenae* and *H. filipjevi* per plant for the four genotypic classes with different combinations of alleles at *QCre-ma7D* and *QCre-ma2A* in the field test in 2017. Genotypes: Madsen, LX99 = Liangxing 99, 7D+2A = genotypes with *QCre-ma7D* and *QCre-ma2A*, 7D = genotypes with *QCre-ma7D*, 2A = genotypes with *QCre-ma2A*, and No QTL = genotypes with neither *QCre-ma7D* nor *QCre-ma2A*.

wheat-*A. ventricosa* line H-93-8 carrying *Cre2* possesses two substituted chromosomes—namely, M^v(5A) and 7M^v(7D)—*Cre2* was located in a separate translocated 6M^v segment (Romero et al. 1998). The 2NS chromosome segment was transferred from *A. ventricosa* to wheat chromosome arm 2AS in wheat line VPM-1 (Jahier et al. 2001). Rust resistance genes *Yr17*, *Lr37*, and *Sr38* are located in the 2NS chromosome segment (Dyck and Lukow 1988; Robert et al. 1999). Madsen also carries other QTL or gene on chromosomes 1AS, 3BS, and 6BS conferring all-stage resistance and high-temperature adult-plant resistance (Liu et al. 2018). Gene *Rkn3* for resistance to the root-knot nematode *Meloidogyne incognita* (Kofoed & White) Chitwood and *M. javanica* (Treub) Chitwood is also present in Madsen (Williamson et al. 2013).

Based on the marker genotypes and reactions to nematode infestation for cultivars involved in the pedigree of Madsen, *Cre9* is also derived from *A. ventricosa* via VPM-1. This is another CCN resistance locus originating from this tetraploid *Aegilops* sp. Madsen was commercialized in the PNW region of the United States in 1987 (Allan et al. 1989). This winter wheat cultivar carries gene *Pch1* for resistance to eyespot (caused by *Oculimacula yallundae* (Wallwork & Spooner) Crous & W. Gams and *O. acufiformis* (Boerema, R. Pieters)), making it the primary source of eyespot resistance in that region. Gene *Pch1* was mapped to a 7NS chromosome segment that was translocated from *A. ventricosa* onto wheat chromosome arm 7DL (Strausbaugh and Murray 1989). Using the RIL population derived from Madsen × Liangxing 99 cross, the locus for resistance to *O. yallundae* was localized in the same region of chromosome arm 7DL as *Cre9* for resistance to *H. filipjevi* (unpublished data). Therefore, *Cre9* is likely to be located in the same introgressed segment as *Pch1*.

Wheat cultivars resistant to *H. filipjevi* are not necessarily resistant to *H. avenae* and vice versa (Mokabli et al. 2002). In order to develop durable and broad-spectrum resistance in cultivars, pyramiding different resistance genes into a single genotype to avoid rapid breakdown of resistance is a popular breeding strategy. Barloy et al. (2007) transferred genes *CreX* and *CreY* from *A. variabilis* Eig. into common wheat using MAS; these gene-stacked lines performed better in the presence of *H. avenae* (Ha12 pathotype) than the single-gene introgression lines. Because Madsen carries two resistance QTL, there is great potential for using it as a parent in breeding for resistance to both CCN species. However, the direct use of resistance to CCN from Madsen is limited by its late flowering time in China. Madsen is also susceptible to powdery mildew, a major foliar disease in most winter-wheat-producing regions where CCN occurs. A prebreeding step has been carried out to incorporate CCN resistance from Madsen into local wheat parents by crossing it with the locally adapted winter wheat cultivars. Advanced lines resistant to both nematode species and powdery mildew have been developed, and those lines—such as H192 (PI 678374) and H782 (PI 678375)—have flowering times similar to local Chinese winter wheat cultivars Liangxing 99, Jimai 22, Yannong 21, and Jimai 19 (Qiu et al. 2017; Sun et al. 2015). This study also produced some lines from the mapping population of Madsen × Liangxing 99 (i.e., ML99-18 and ML99-46) with resistance to CCN and powdery mildew, as well as improved agronomic performance. Those lines have been used as sources of resistance to CCN and were provided to breeders for developing CCN-resistant cultivars. In addition, KASP and SSR markers linked to *Cre9* and *Cre5* will facilitate introgression of these resistance genes from Madsen into different wheat backgrounds through molecular MAS strategies.

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LITERATURE CITED

- Ali, M. A., Shahzadi, M., Zahoar, A., Dababat, A. A., Toktay, H., Bakhsh, A., Nawaz, M. A., and Li, H. J. 2019. Resistance to cereal cyst nematodes in wheat and barley: An emphasis on classical and modern approaches. *Int. J. Mol. Sci.* 20:432.
- Allan, R. E., Peterson, C. J., Rubenthaler, G. L., Line, R. F., and Roberts, D. E. 1989. Registration of 'Madsen' wheat. *Crop Sci.* 29:1575-1576.
- Barloy, D., Lemoine, J., Abelard, P., Tanguy, A. M., Rivoal, R., and Jahier, J. 2007. Marker-assisted pyramiding of two cereal cyst nematode resistance genes from *Aegilops variabilis* in wheat. *Mol. Breed.* 20:31-40.
- Clavijo, B. J., Venturini, L., Schudoma, C., Accinelli, G. G., Kaithakottil, G., Wright, J., Borrill, P., Kettleborough, G., Heavens, D., Chapman, H., and Lipscombe, J. 2017. An improved assembly and annotation of the allohexaploid wheat genome identifies complete families of agronomic genes and provides genomic evidence for chromosomal translocations. *Genome Res.* 27:885-896.
- Cui, L., Gao, X., Wang, X. M., Jian, H., Tang, W. H., Li, H. L., and Li, H. J. 2012. Characterization of interaction between wheat roots with different resistance and *Heterodera filipjevi*. *Acta Agron. Sin.* 38:1009-1017.
- Cui, L., Sun, L., Gao, X., Song, W., Wang, X. M., Li, H. L., Liu, Z. Y., Tang, W. H., and Li, H. J. 2016. The impact of resistant and susceptible wheat cultivars on the multiplication of *Heterodera filipjevi* and *H. avenae* in parasite-infested soil. *Plant Pathol.* 65:1192-1199.
- Dababat, A. A., Erginbaş-Orakci, G., Toktay, H., Imren, M., Akin, B., Braun, H. J., and Dreisigacker, S. 2014. Resistance of winter wheat to *Heterodera filipjevi* in Turkey. *Turk. J. Agric. For.* 38:180-186.
- Dyck, P. L., and Lukow, O. M. 1988. The genetic analysis of two interspecific sources of leaf rust resistance and their effect on the quality of common wheat. *Can. J. Plant Sci.* 68:633-639.
- Fang, T. L., Campbell, K. G., Liu, Z. Y., Chen, X., Wan, A., Li, S., Liu, Z., Cao, S., Chen, Y., Bowden, R. L., and Carver, B. F. 2011. Stripe rust resistance in the wheat cultivar Jagger is due to *Yr17* and a novel resistance gene. *Crop Sci.* 51:2455-2465.
- FAO. 2017. Food Outlook. Biannual Report on Global Food Markets. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.
- Feng, Y. X., Wang, D., Xuan, Y. H., Chen, L. J., Wang, Y. Y., Liu, X. Y., Chen, Y., Lu, X. P., Zhu, X. F., and Duan, Y. X. 2018. Identification and phylogenetic analysis of nine cyst nematode populations from main field crops in China. *J. Anim. Plant Sci.* 28:791-802.
- Fu, B., Yuan, H. X., Zhang, Y., Hou, X. S., Nian, G. L., Zhang, P., and Xing, X. P. 2011. Molecular characterisation of cereal cyst nematodes in winter wheat on the Huang-Huai floodplain of China using RFLP and rDNA-ITS sequence analyses. *Australas. Plant Pathol.* 40:277-285.
- İmren, M., Toktay, H., Bozbuga, R., Dababat, A. A., and Elekçioğlu, H. İ. 2013. Pathotype determination of the cereal cyst nematode (*Heterodera avenae* Wollenweber, 1924) in the Eastern Mediterranean Region in Turkey. *Turk. J. Entomol.* 37:13-19.
- International Wheat Genome Sequencing Consortium. 2018. Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science* 361:eaar7191.
- Jahier, J., Abelard, P., Tanguy, M., Dedryver, F., Rivoal, R., Khatkar, S., Bariana, H. S., and Koebner, R. 2001. The *Aegilops ventricosa* segment on chromosome 2AS of the wheat cultivar 'VPM1' carries the cereal cyst nematode resistance gene *Cre5*. *Plant Breed.* 120:125-128.
- Karimipour Fard, H., Pourjam, E., Tanha Maafi, Z., and Safaie, N. 2017. Cereal cyst nematode, (*Heterodera filipjevi* (Madzhidov, 1981) in wheat fields of Chaharmahal and Bakhtiari Province, Iran and its distribution based on interpolation by geographic information system. *J. Agric. Sci. Technol.* 19:1185-1196.
- Karimipour Fard, H., Pourjam, E., Tanha Maafi, Z., and Safaie, N. 2018. Assessment of yield loss of wheat cultivars caused by *Heterodera filipjevi* under field conditions. *J. Phytopathol.* 166:299-304.
- Lewis, J. G., Matic, M., and McKay, A. C. 2009. Success of cereal cyst nematode resistance in Australia: History and status of resistance screening systems. Pages 137-142 in: *Cereal Cyst Nematodes: Status, Research and Outlook*. Proc. First Workshop Int. Cereal Cyst Nematode Initiative. Antalya, Turkey.
- Li, H. J., Cui, L., Li, H. L., Wang, X. M., Murray, T. D., Conner, R. L., Wang, L. J., Gao, X., Sun, Y., Sun, S. C., and Tang, W. H. 2012. Effective resources in wheat and wheat-*Thinopyrum* derivatives for resistance to *Heterodera filipjevi* in China. *Crop Sci.* 52:1209-1217.

- Li, H. L., Yuan, H. X., Sun, J. W., Fu, B., Nian, G. L., Hou, X. S., Xing, X. P., and Sun, B. J. 2010. First record of the cereal cyst nematode *Heterodera filipjevi* in China. *Plant Dis.* 94:1505.
- Liu, L., Wang, M. N., Feng, J. Y., See, D. R., Chao, S. M., and Chen, X. M. 2018. Combination of all-stage and high-temperature adult-plant resistance QTL confers high-level, durable resistance to stripe rust in winter wheat cultivar Madsen. *Theor. Appl. Genet.* 131:1835-1849.
- Liu, W. C., Liu, Z. D., Huang, C., Lu, M. H., Liu, J., and Yang, Q. P. 2016. Statistics and analysis of crop yield losses caused by main disease and insect pests in recent 10 years. *Plant Prot.* 42:1-9.
- Luo, M. C., Gu, Y. Q., Puiui, D., Wang, H., Twardziok, S. O., Deal, K. R., Huo, N., Zhu, T., Wang, L., Wang, Y., McGuire, P. E., Liu, S. Y., Long, H., Ramasamy, R. K., Rodriguez, J. C., Van, S. L., Yuan, L. X., Wang, Z. Z., Xia, Z. Q., Xiao, L. C., Anderson, O. D., Ouyang, S. H., Liang, Y., Zimin, A. V., Perte, G., Qi, P., Bennetzen, J. L., Dai, X. T., Dawson, M. W., Müller, H. G., Kugler, K., Rivarola-Duarte, L., Spannagl, M., Mayer, K. F. X., Lu, F. H., Bevan, M. W., Leroy, P., Li, P. C., You, F. M., Sun, Q. X., Liu, Z. Y., Lyons, E., Wicker, T., Salzberg, S. L., Devos, K. M., and Dvořák, J. 2017. Genome sequence of the progenitor of the wheat D genome *Aegilops tauschii*. *Nature* 551:498-502.
- McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., and DePristo, M. A. 2010. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 20:1297-1303.
- Moatamedi, M., Bazgir, E., Esfahani, M. N., and Darvishnia, M. 2018. Genetic variation of bread wheat accessions in response to the cereal cyst nematode, *Heterodera filipjevi*. *Nematology* 20:859-875.
- Mokabli, A., Valette, S., Gauthier, J. P., and Rivoal, R. 2002. Variation in virulence of cereal cyst nematode populations from North Africa and Asia. *Nematology* 4:521-525.
- Montes, M. J., López-Braña, I., Romero, M. D., Sin, E., Andrés, M. F., Martín-Sánchez, J. A., and Delibes, A. 2003. Biochemical and genetic studies of two *Heterodera avenae* resistance genes transferred from *Aegilops ventricosa* to wheat. *Theor. Appl. Genet.* 107:611-618.
- Nicol, J. M., Ogonnaya, F., Singh, A. K., Bishnoi, S. P., Kanwar, R. S., Li, H., Chen, S., Peng, D., Bolat, N., Şahin, E., and Elekçioğlu, I. H. 2009. Current global knowledge of the usability of cereal cyst nematode resistant bread wheat germplasm through international germplasm exchange and evaluation. Pages 149-153 in: *Cereal Cyst Nematodes: Status, Research and Outlook*. I. T. Riley, J. M. Nicol, and A. A. Dababat, eds. CIMMYT, Ankara, Turkey.
- Ogonnaya, F. C., Seah, S., Delibes, A., Jahier, J., López-Braña, I., Eastwood, R. F., and Lagudah, E. S. 2001. Molecular-genetic characterisation of a new nematode resistance gene in wheat. *Theor. Appl. Genet.* 102:623-629.
- Pariyar, S. R., Dababat, A. A., Siddique, S., Erginbas-Orakci, G., Elashry, A., Morgounov, A., and Grundler, F. M. 2016. Identification and characterisation of resistance to the cereal cyst nematode *Heterodera filipjevi* in winter wheat. *Nematology* 18:377-402.
- Peng, D. L., Nicol, J. M., Li, H. M., Hou, S. Y., Li, H. X., Chen, S. L., Ma, P., Li, H. L., and Riley, I. T. 2009. Current knowledge of cereal cyst nematode (*Heterodera avenae*) on wheat in China. Pages 29-34 in: *Cereal Cyst Nematodes: Status, Research and Outlook*. I. T. Riley, J. M. Nicol, and A. A. Dababat, eds. CIMMYT, Ankara, Turkey.
- Peng, D. L., Peng, H., and Huang, W. K. 2015. Occurrence, distribution and integrated management of the cereal cyst nematodes (*Heterodera avenae* & *H. filipjevi*) in China. Pages 17-24 in: *Nematodes of Small Grain Cereals: Current Status and Research*. A. A. Dababat, H. Mumjanov, and R. W. Smiley, eds. Food and Agriculture Organization of the United Nations, Ankara, Turkey.
- Peng, H., Li, X., Cui, J. K., Peng, D. L., Huang, W. K., He, W. T., and Kong, L. A. 2016. First report of cereal cyst nematode, *Heterodera filipjevi*, on winter wheat from Anhui province, China. *Plant Dis.* 100:536.
- Peng, H., Zhang, Y. D., Cui, J. K., Li, X., Huang, W. K., Kong, L. A., He, W. T., and Peng, D. L. 2018. First report of cereal cyst nematode (*Heterodera filipjevi*) on winter wheat in Xinjiang Uygur Autonomous region, China. *Plant Dis.* 102:454.
- Qiu, D., Cui, L., Sun, Y., Zou, J. W., Zheng, C. X., Wang, X. M., Zhou, Y., and Li, H. J. 2017. Registration of H192 and H782, white winter wheat lines resistant to cereal cyst nematode and powdery mildew. *J. Plant Regist.* 11:71-74.
- Ramirez-Gonzalez, R. H., Uauy, C., and Caccamo, M. 2015. PolyMarker: A fast polyploid primer design pipeline. *Bioinformatics* 31:2038-2039.
- Rasheed, A., Wen, W. E., Gao, F. M., Zhai, S. N., Jin, H., Liu, J. D., Guo, Q., Zhang, Y. J., Dreisigacker, S., Xia, X. C., and He, Z. H. 2016. Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. *Theor. Appl. Genet.* 129:1843-1860.
- Renčo, M., Čermák, V., Tománková, K., and Čudejková, M. M. 2018. Morphological and molecular characterisation of *Heterodera filipjevi* (Mazhzhidov, 1981) from the Slovak Republic. *Nematology* 20:253-264.
- Riley, I. T., and Qi, R. D. 2015. Annotated bibliography of cereal cyst nematodes (*Heterodera avenae* and *H. filipjevi*) in China, 1991 to 2014. *Australas. Nematol. Newsl.* 26:1-46.
- Robert, O., Abelard, C., and Dedryver, F. 1999. Identification of molecular markers for the detection of the yellow rust resistance gene *Yr17* in wheat. *Mol. Breed.* 5:167-175.
- Romero, M. D., Montes, M. J., Sin, E., López-Braña, I., Duce, A., Martín-Sánchez, J. A., Andrés, M. F., and Delibes, A. 1998. A cereal cyst nematode (*Heterodera avenae* Woll.) resistance gene transferred from *Aegilops triuncialis* to hexaploid wheat. *Theor. Appl. Genet.* 96:1135-1140.
- Safari, E., Gororo, N. N., Eastwood, R. F., Lewis, J., Eagles, H. A., and Ogonnaya, F. C. 2005. Impact of *Cre1*, *Cre8* and *Cre3* genes on cereal cyst nematode resistance in wheat. *Theor. Appl. Genet.* 110:567-572.
- Seah, S., Miller, C., Sivasithamparan, K., and Lagudah, E. S. 2000. Root responses to cereal cyst nematode (*Heterodera avenae*) in hosts with different resistance genes. *New Phytol.* 146:527-533.
- Sharp, P. J., Kreis, M., Shewry, P. R., and Gale, M. D. 1988. Location of β -amylase sequences in wheat and its relatives. *Theor. Appl. Genet.* 75:286-290.
- Smaha, D., Mokri, F., Mustafa, I., Mokabli, A., and Dababat, A. A. 2018. First report of the cereal cyst nematode (*Heterodera filipjevi*) on wheat in Algeria. *Plant Dis.* 102:1860.
- Smiley, R. W., Dababat, A. A., Iqbal, S., Jones, M. G., Maafi, Z. T., Peng, D. L., and Subbotin, S. A. 2017. Cereal cyst nematodes: A complex and destructive group of *Heterodera* species. *Plant Dis.* 101:1692-1720.
- Smiley, R. W., Marshall, J. M., Gourlie, J. A., Paulitz, T. C., Kandel, S. L., Pumphrey, M. O., Garland-Campbell, K., Yan, G., Anderson, M. D., Flowers, M. D., and Jackson, C. A. 2013. Spring wheat tolerance and resistance to *Heterodera avenae* in the Pacific Northwest. *Plant Dis.* 97:590-600.
- Strausbaugh, C. A., and Murray, T. D. 1989. Inheritance of resistance to *Pseudocercospora herpotrichoides* in three cultivars of winter wheat. *Phytopathology* 79:1048-1053.
- Sun, X. Y., Cui, L., Sun, L., Sun, Y. L., Qiu, D., Zou, J. W., Wu, X. F., Wang, X. M., and Li, H. J. 2015. Development and identification of wheat lines H3714 and H4058 resistant to cereal cyst nematode. *Acta Agron. Sin.* 41:872-880.
- Toumi, F., Waeyenbergh, L., Viaene, N., Dababat, A. A., Nicol, J. M., Ogonnaya, F., and Moens, M. 2018. Cereal cyst nematodes: Importance, distribution, identification, quantification, and control. *Eur. J. Plant Pathol.* 150:1-20.
- Van Ooijen, J. W. 2006. JoinMap 4.0. Software for the Calculation of Genetic Linkage Maps in Experimental Populations. Kyazma B.V., Wageningen, The Netherlands.
- Van Ooijen, J. W. 2009. MapQTL 6. Software for the Mapping of Quantitative Trait Loci in Experimental Populations of Diploid Species. Kyazma B.V., Wageningen, The Netherlands.
- Voorrips, R. E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. *J. Hered.* 93:77-78.
- Wang, S. C., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., and Maccaferri, M. 2014. Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant Biotechnol. J.* 12:787-796.
- Wang, Y., Xie, J. Z., Zhang, H. Z., Guo, B. M., Ning, S. Z., Chen, Y. X., Lu, P., Wu, Q. H., Li, M. M., Zhang, D. Y., Guo, G. H., Zhang, Y., Liu, D. C., Zou, S. K., Tang, J. W., Zhao, H., Wang, X. C., Li, J., Yang, W. Y., Cao, T. J., Yin, G. H., and Liu, Z. Y. 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.
- Williamson, V. M., Thomas, V., Ferris, H., and Dubcovsky, J. 2013. An *Aegilops ventricosa* translocation confers resistance against root-knot nematodes to common wheat. *Crop Sci.* 53:1412-1418.
- Wu, L. F., Cui, L., Li, H. L., Sun, L., Gao, X., Qiu, D., Sun, Y. L., Wang, X., Murray, T. D., and Li, H. J. 2016. Characterization of resistance to the cereal cyst nematode in the soft white winter wheat 'Madsen'. *Plant Dis.* 100:679-685.
- Wu, P. P., Hu, J. H., Zou, J. W., Qiu, D., Qu, Y. F., Li, Y. H., Li, T., Zhang, H. J., Jiang, L., Liu, H. W., Zhou, Y., Zhang, H. J., Li, J. T., Liu, Z. Y., and Li, H. J. 2019. Fine mapping of the wheat powdery mildew resistance gene *Pm52* using comparative genomics analysis and the Chinese Spring reference genomic sequence. *Theor. Appl. Genet.* 132:1451-1461.
- Yuan, H. X., Sun, J. W., Yang, W. X., Xing, X. P., Wang, Z. Y., Riley, I. T., and Li, H. L. 2010. New pathotypes of *Heterodera avenae* (cereal cyst nematode) from winter wheat in Zhengzhou, Henan, China. *Australas. Plant Pathol.* 39:107-111.

- Yuan, H. X., Zhang, F. X., Zhang, J. J., Hou, X. S., Li, H. J., and Li, H. L. 2011. Resistance of CIMMYT wheat germplasm to *Heterodera filipjevi* Xuchang population from Henan province, China. Acta Agron. Sin. 37:1956-1966.
- Zadoks, J. C., Chang, T. T., and Konzak, C. F. 1974. A decimal code for the growth stages of cereals. Weed Res. 14:415-421.
- Zhang, R., Feng, Y., Li, H., Yuan, H. X., Dai, J. L., Cao, A. Z., Xing, L. P., and Li, H. L. 2016. Cereal cyst nematode resistance gene *CreV* effective against *Heterodera filipjevi* transferred from chromosome 6VL of *Dasy-pyrum villosum* to bread wheat. Mol. Breed. 36:122.
- Zhao, Z. H., Sun, H. G., Song, W., Lu, M., Huang, J., Wu, L. F., Wang, X. M., and Li, H. J. 2013. Genetic analysis and detection of the gene *MILX99* on chromosome 2BL conferring resistance to powdery mildew in the wheat cultivar Liangxing 99. Theor. Appl. Genet. 126: 3081-3089.
- Zhen, H. Y., Peng, H., Zhao, H. H., Qi, Y. H., Huang, W. K., Kong, L. A., Liang, C., Wen, Y. H., and Peng, D. L. 2018. First report of cereal cyst nematode (*Heterodera filipjevi*) on winter wheat in Shandong Province, China. J. Integr. Agric. 17:1912-1913.
- Zou, J. W., Qiu, D., Sun, Y., Zheng, C. X., Li, J. T., Wu, P. P., Wu, X. F., Wang, X. M., Zhou, Y., and Li, H. J. 2017. *Pm52*: Effectiveness of the gene conferring resistance to powdery mildew in wheat cultivar Liangxing 99. Acta Agron. Sin. 43:332-342.