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Analysis of wheat (*Triticum aestivum* L.*)* resistance to cereal cyst nematode *Heterodera filipjevi* by genome-wide association mapping

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“This work is dedicated to my parents”

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Table of contents

Contents	Page number
Abstract	i
Zusammenfassung	iv
List of tables	vii
List of figures	viii
Acronyms and abbreviations	xii
1. Introduction	1
1.1. Wheat	1
1.2. Cereal cyst nematode	2
1.3. Biology of *Heterodera.filipjevi*	4
1.4. Host resistance to cereal cyst nematode	7
1.5. Molecular markers	8
1.6. Single nucleotide polymorphic marker	10
1.7. Quantitative trait loci	11
1.8. Association mapping	12
1.9. Linkage disequilibrium	14
1.10. Population structure	15
1.11**.** *In-silico* identification of genes linked to QTLs	17
1.2. Objectives	19
2. Identification and characterization of resistance to the cereal cyst nematode *Heterodera filipjevi* in winter wheat	20
3. Genome-wide association study in wheat identifies resistance to the cereal cyst nematode *Heterodera filipjevi*	30
4. GWAS in wheat identifies an amino acid transporter gene orthologue in Arabidopsis promoting nematode susceptibility	42
5. Discussion and conclusions	67
5.1. References	75
6. Annex	88
6.1. Supplementary materials for "Identification and characterization of resistance to the cereal cyst nematode *Heterodera filipjevi* in winter wheat"	88
6.2. Supplementary materials for "Genome-wide association study in wheat identifies resistance to the cereal cyst nematode *Heterodera filipjevi*"	100
6.3. Supplementary materials for "GWAS in wheat identifies an amino acid transporter gene orthologue in Arabidopsis promoting nematode susceptibility"	117
6.4. Acknowledgements	123
6.5. Curriculum vitae	124

Abstract

The cereal cyst nematode *Heterodera filipjevi* is an important plant parasite causing substantial yield loss in wheat. The use of resistant cultivars is the preferable method to manage this nematode, however, only few resistance sources are identified in cereals and no durable resistant cultivar is available for *H. filipjevi* in wheat. In this study, a collection of 290 winter wheat accessions was screened for resistance against *H. filipjevi.* The plants were infected with nematode juveniles and the number of developing females and cysts was counted. One percent of the wheat accessions was ranked as resistant, 16% as moderately resistant, 41% as moderately susceptible, 26% as susceptible, and 15% as highly susceptible. To understand the underlying resistance mechanism, nematode invasion, development, and reproduction were analyzed in one resistant accession Nudakota and three moderately resistant accessions Ekonomka, Katea and Lantian 12 and compared with susceptible accession Bezostaya 1. Invasion rate, and number of females and cysts per plant were significantly lower in Nudakota, Ekonomka, Katea and Lantian 12 compared to Bezostaya 1. No significant differences in plant height, plant weight, root length, root weight, and root volume were recorded for inoculated plants compared to non-inoculated plants. The different responses of wheat accessions observed in this study suggested genetic variation within the screened population. Therefore, it was decided to identify relevant quantitative trait loci (QTLs) and genes using genome-wide association studies. Association mapping is a powerful approach to detect associations between phenotypic variation and genetic polymorphisms; in this way favorable traits such as nematode resistance can be located in the genome. A mapping panel of 161 wheat accessions was genotyped by 90K iSelect SNP bead chip and was analyzed for QTL identification. These accessions were phenotyped in two nematode infection assays under controlled conditions. High variation was revealed in single nucleotide polymorphisms (SNPs) on wheat genomes A, B, and D. Linkage disequilibrium decayed across wheat genome was < 3cM. Population structure and principle component analysis

(PCA) revealed a very low genetic differentiation (K = 2, and PCA1 = 6.16%) within the 161 wheat accessions and needed no further corrections. Eleven novel quantitative trait loci (QTLs) on chromosomes 1AL, 2AS, 2BL, 3AL, 3BL, 4AS, 4AL, 5BL and 7BL were detected using a mixed linear model at false discovery rate ($P \leq 0.01$) that explained 43% of total genetic variation. The identified 11 novel QTLs can now be used in resistance breeding via marker-assisted selection. The four wheat accessions Bezenchukskaya 380, Lantian 12, T04/17, and Olifants have the highest markers allele frequency and can therefore be designated as sources of resistance. *In-silico* annotation of flanking sequences of the significant markers identified genes supposed to be involved in biotic and abiotic stresses. Eight of the 11 QTLs on chromosome 1AL, 2AS, 2BL, 3AL and 4AL were linked to putative genes known to be involved in plant-pathogen interactions. Two other QTLs on 3BL and one QTL on 7BL linked to putative genes known to be involved in abiotic stress and plant growth. Five of the QTLs identified on chromosomes 1AL, 2AS, 2BL, 3BL, and 5BL in this study were previously reported to be linked to resistance against *H. avenae,* while QTL IWB66494 on chromosome 2BL was reported to be linked to resistance against Fusarium head blight. Although the wheat genome is sequenced, annotation of a large number of genes and proteins is still limited. In order to characterize the candidate gene *TaAAT* we applied a comparative genomics approach and identified the orthologue gene *AtAAP6* in *Arabidopsis thaliana.* Nematode infection assays of an *AtAAP6* mutant Arabidopsis line revealed a significant reduction in average number of females, female size, and female-associated syncytia. Different methods showed that *AtAAP6* is upregulated in syncytia. We also found a strong expression of *TaAAT* in infected roots of two susceptible wheat accessions, whereas the expression remained unchanged in infected roots of two resistant accessions. We further analyzed the amino acid sequence of *AtAAP6* in 3 lowly susceptible and 3 highly susceptible *Arabidopsis* accessions and found glycine is substituted by alanine on a specific site in the

Abstract

AtAAP ORF in the lowly susceptible accessions. The molecular mechanism by which the exchange of a single amino acid in *AtAAP6* leads to reduced nematode development needs further investigation.

Keywords: amino acid transporter, association mapping, gene, cyst nematode, quantitative trait loci, single nucleotide polymorphism, *Triticum aestivum*

Der Getreidezystennematode *Heterodera filipjevi* ist ein bedeutender Schadereger im Weizenanbau. Der Einsatz von resistenten Sorten ist die Methode der Wahl, um Nematodenproblemen im Weizen zu begegnen. Allerdings wurden bis heute nur wenige Resistenzquellen in Getreiden identifiziert und es existieren keine resistenten Weizensorten für *H. filipjevi*. In der vorliegenden Arbeit wurden daher 290 Winterweizenlinien auf das Vorkommen von Resistenz gegenüber *H. filipjevi* untersucht. Die Pflanzen wurden mit Larven infiziert und anschließend auf die Bildung von Weibchen und Zysten untersucht. Ein Prozent der Linien wurden als resistent bewertet, während 16% als moderat resistent, 41% als moderat anfällig, 26% als anfällig und 15% als hoch anfällig eingestuft wurden. Um die zugrunde liegenden Resistenzmechanismen zu klären, wurden die Eindringung der Nematoden, sowie ihre Entwicklung und Reproduktion in der resistenten Linie Nudakota und den moderat resistenten Linien Ekonomka, Katea und Lantian 12 untersucht und mit der anfälligen Linie Bezostaya 1 verglichen. Die Eindringungsrate, die Anzahl der Weibchen und der Zysten waren in den resistenten Linien signifikant geringer als in der anfälligen Bezostaya 1. Die untersuchten Weizenlinien unterschieden sich im Vergleich zwischen inokulierten und nicht-inokulierten Pflanzen nicht in Größe, Gewicht, Wurzellänge, und Wurzelvolumen. Die unterschiedliche Wirtsreaktion der Weizenlinien auf *H. filipjevi* ließ auf eine entsprechende genetische Variabilität schließen. Daher war ein weiteres Ziel der Arbeit, entsprechende Quantitative Trait Loci (QTLs) bzw. Gene mit Hilfe genomweiter Assoziationsstudien zu identifizieren. Assoziationskartierung ist eine vielversprechende Methode um Assoziationen zwischen phänotypischer Variation und genetischen Polymorphismen zu detektieren; auf diese Weise können vorteilhafte Merkmale wie z.B. Nematodenresistenz im Genom lokalisiert werden. Hierzu wurde eine Auswahl von 161 Weizenlinien mit einem neuartigen 90K iSelect SNP Bead Chip genotypisiert und auf QTLs analysiert. Die Linien wurden auf der Basis von zwei nacheinander folgenden Infektionsversuchen unter kontrollierten

Bedingungen phänotypisiert. Die Ergebnisse der Analyse brachten eine große Anzahl von Singulären Nukleotidpolymorphismen (SNP) zu Tage, die darauf hindeuteten, dass die Rekombinationsrate in den unterschiedlichen Teilgenomen von Weizen differiert. Das Kopplungsungleichgewicht betrug für das gesamte Weizengenom < 3cM. Die Analyse der Populationsstruktur mit Hilfe einer Hauptkomponentenanalyse (PCA) ergab eine sehr geringe genetische Differenzierung (K = 2; PCA1 = 6.16%) innerhalb der 161 Weizenlinien und erforderte daher keine weitere Korrektur. Insgesamt wurden 11 QTLs auf den Chromosomen 1AL, 2AS, 2BL, 3AL, 3BL, 4AS, 4AL, 5BL and 7BL mit Hilfe eines gemischten linearen Modells bei einer Fehlerwahrscheinlichkeit $P \leq 0.01$ detektiert, das 43% der genetischen Variabilität erklärte. Diese QTLs können nun in der Resistenzzüchtung mit Hilfe von markergestütztem Selektionsverfahren eingesetzt werden. Vier Weizenlinien, nämlich Bezenchukskaya 380, Lantian 12, T04/17 und Olifants wiesen die höchste Frequenz an Markerallelelen auf und konnten daher als potentielle neue Resistenzquellen beschrieben werden. Die *in-silico* Annotation der die Marker flankierenden Regionen führte zur Entdeckung von Genen, die im Zusammenhang mit biotischem und abiotischem Stress stehen. Acht der 11 QTLs auf den Chromosomen 1AL, 2AS, 2BL, 3AL and 4AL waren mit Genen gekoppelt, die im Zusammenhang mit Pflanzen-Pathogen Interaktionen stehen könnten. Zwei weitere QTLs auf Chromosom 3BL und ein QTL auf 7BL waren mit Genen gekoppelt, die eine Rolle bei abiotischem Stress und Pflanzenwachstum spielen könnten. Fünf QTLs auf den Chromosomen 1AL, 2AS, 2BL, 3BL, and 5BL, die in dieser Arbeit beschrieben werden, waren bereits in früheren Arbeiten in Zusammenhang mit Resistenz gegenüber dem Getreidezystennematoden *H. avenae* gebracht worden, QTL IWB66494 auf Chromosom 2BL mit Resistenz gegenüber Ährenfusariosen. Obwohl das Weizengenom mittlerweile sequenziert ist, ist die Annotation vieler Gene bzw. Proteine in den verschiedenen Linien noch sehr unvollständig. Zur Charakterisierung des Kanidatengens wurde daher ein vergleichender

genomischer Ansatz verfolgt, mit dessen Hilfe ein orthologes Gen *AtAAP6* in *Arabidopsis thaliana* identifiziert werden konnte. In Infektionsversuchen mit einer *ATAAP6* Mutantenlinie in Arabidopsis war die Anzahl und Größe der weiblichen Tiere reduziert, ebenso wie die Größe der induzierten Syncytien. Mit verschiedenen Methoden konnte gezeigt werden, dass dieses Gen in Arabidopsis in den nematodeninduzierten Syncytien verstärkt exprimiert wird. In Weizen war die Expression in infizierten Wurzeln zweier anfälligen Linien erhöht, wo hingegen in infizierten resistenten Pflanzen nach Infektion kein Anstieg gemessen werden konnte. Deshalb wurde die Aminosäuresequenz in anfälligen und weniger anfälligen Arabdidopsis-Linien analysiert. Dabei wurde festgestellt, dass in den weniger anfälligen auf einer Position der Sequenz von *AtAAP6* Glycin durch Alanin ersetzt ist. Allerdings ist der molekulare Mechanismus, der letztlich zu einer reduzierten Nematodenentwicklung führt, noch nicht geklärt.

List of tables

Contents	Description	Page number
	Introduction	
Table 1	Sources of resistance genes/QTLs to CCN *Heterodera avenae* in wheat	8
	Identification and characterization of resistance to the cereal cyst nematode *Heterodera filipjevi* in winter wheat	
Table 1	Selected winter wheat accessions and their response to *Heterodera filipjevi* development and reproduction	25
Table S1	List of 291 winter wheat accessions with accession number, common name, selection history, origin and host response to *Heterodera filipjevi*	90
	Genome-wide association study in wheat identifies resistance to the cereal cyst nematode *Heterodera filipjevi*	
Table 1	A genetic linkage map, SNP marker and linkage disequilibrium decay in wheat 161 wheat accessions	35
Table 2	Significant marker associated with QTLs resistant to *Heterodera filipjevi* in 161 winter wheat accessions	35
Table 3	*In-silico* annotation of significant markers and identified contigs	37
Table 4	Functional annotation of putative genes linked to *Heterodera filipjevi* resistance	38
Table 5	Selection of wheat accessions based on significant markers frequency, host status and origin	39
Table S1	List of 161 winter wheat accessions with accession number, common name, selection history, origin, and host response to *Heterodera filipjevi*	111
Table S2	*In-silico* annotation of SNP marker flanking sequence against protein coding sequence (CDS) and protein of Brachypodium, rice and sorghum	116
	GWAS in wheat identifies an amino acid transporter gene orthologue in Arabidopsis promoting nematode susceptibility	
Table 1	Primers used for polymerase chain reaction (PCR) and reverse transcriptase polymerase chain reaction (qPCR)	50
Table 2	Reverse transcription (RT) reaction	51
Table S1	List of Arabidopsis accessions compared for AtAAP6 amino acid sequence	121
Table S2	List of wheat accessions used for TaAAT expression analysis	122

List of figures

Contents	Description	Page number
	Introduction	
Fig. 1	Distribution of cereal cyst nematodes: A Distribution of *Heterodera avenae* (Ha), *H. filipjevi* (Hf) and *H. latipons* (Hl), and B Distribution of represents *H. filipjevi.* Countries in color indicate the presence of nematode species.	3
Fig. 2	The cereal cyst nematodes life cycle in wheat. *J2 second stage juvenile, J3 third stage juvenile, and J4 fourth stage juvenile.	4
Fig. 3	Structure of syncytia induced by *Heterodera avene* in susceptible wheat cultivar Prins. *A : Transverse section (TS) of syncytium associated with J2 at 4 days post infection (dpi); B : TS of syncytium with extensive arc of necrosis between J2 and its syncytium at 8 dpi; C : TS of syncytium showing large vacuoles in close contact with xylem and phloem; D : Longitudinal section of syncytium showing small vacuoles, multiple lateral meristems, and disorganized vascular tissue at 15 dpi; E: TS of syncytium with many small vacuoles, and larger vacuoles filled with amorphous material at 19 dpi; F: TS of syncytium with few large vacuoles, nuclei are hypertrophied nuclei, and cytoplasm strains intensely. Necrosis is evident around nematode head. S, SI, S2 = syncytia, N = nematode, nec = necrosis, LV = large vacuole, SV = small vacuole, x = xylem, p = phloem. (Bar equivalents.' A, F =50µm, B =100µm; C =20µm; D= 200µm; and E =25µm, (Williams and Fisher, 1993).	6
Fig. 4	Scheme of association mapping for plant-nematode interaction.	14
	Identification and characterization of resistance to the cereal cyst nematode *Heterodera filipjevi* in winter wheat	
Fig. 1	Nematode infection in different selected wheat accessions at 2, 5 and 10 days post inoculation (dpi). Columns with different letters are significantly different based on one way ANOVA (Holm-Sidak) analysis at ($P \leq 0.05$, n = 18) and a > b. Bar indicates the standard error of the mean.	24
Fig. 2	Nematode development in selected wheat accessions at (a) 10 days post inoculation and (b) 15 days post inoculation. Columns with different letters are significantly different based on one way ANOVA (Holm-Sidak) analysis at ($P \leq 0.05$, n = 18) and a > b. Bar indicates the standard error of the mean.	24
Fig. 3	Regression coefficient (R^2) for relation between cyst sizes (mm^2) to total number of eggs and juveniles in five wheat accessions at ($P \leq 0.05$, n =67).	25
Fig. 4	Effect of *Heterodera filipjevi* on wheat growth determined as cumulative root length (A), root volume (B) and root weight (C) on different wheat accessions. Columns with different letters are significantly different based on one way ANOVA (Holm-Sidak) analysis at (P 0.05, n = 18) and a > b. Bar indicates the standard error of the mean.	26

List of figures

Fig. S1 Polymerase chain reaction restriction fragment length polymorphism patterns of *Heterodera filipjevi* (Hf) and *Heterodera schachtii* (Hs) based on restriction with *Hinf*I and *Rsa*I. 89

Genome-wide association study in wheat identifies resistance to the cereal cyst nematode *Heterodera filipjevi*

Fig. 1 Distribution of polymorphic single nucleotide (SNP) markers across wheat chromosomes. 33

Fig. 2 Estimation of number of sub-populations (K) in winter wheat based on 961 SNP markers: A, Estimation of number of sub-populations (K) in winter wheat using ΔK values, B, The log probability of data as a function of K for 961 SNP markers. Means log probability of data LnP(D) for each value of K were calculated from 20 independent runs of structure, and C, Two subgroups inferred from STRUCTURE analysis. The vertical coordinate of each subgroup indicates the membership coefficients for each individual represented by a colored line where each color reflects the cultivar in one of the K clusters. The proportion of the colored segment red and green indicates the proportion of the genome drawn from the K clusters and represents the geographic eco-type information of wheat accessions. 33

Fig. 3 Principal components analysis based on correlation matrix recorded on 161 winter wheat accessions. A scatter plot of principal component 1 (PC1) plotted against principal component 2 (PC2). Each symbol represents a wheat accession in mapping panel. *BL=Breeding line; C=Cultivar, LRACE=Landrace. 34

Fig. 4 Manhattan plots of *P* values indicating genomic regions associated to cereal cyst nematode *Heterodera filipjevi* resistance in winter wheat using MLM (P+K). x-axis shows SNP markers along each wheat chromosome; *y*-axis is the $-\log_{10}$ (*P*-value). 35

Fig. 5 The genetic map of significant SNP markers associated with resistance to cereal cyst nematode based on ITMI consensus map, A genetic map of significant markers on chromosomes 1A, 2A and 3A; B genetic map of significant markers on chromosomes 4A, 2B and 3B; and C genetic map of significant markers on chromosomes 5 and 7B. A and B indicates wheat chromosomes while *indicate significant marker in marker trait association. Map drawn using MapChart 2.2 (Voorrips, 2002). 36

Fig. 6 Effect of quantitative trait loci (QTL) combinations on *Heterodera filipjevi* reduction in wheat accessions. Number of abscissa indicates the number of QTL allele combinations. 39

Fig. S1 LD decay estimated using wheat chromosomes (1-6A, 7A-5B, 6B-4D, and 5-7D) of 161 winter wheat accessions based on polymorphic single nucleotide polymorphism (SNP) markers. Decay of r^2(0-1) as a function of genetic distance between SNP markers estimated for A, B, and D genomes of winter wheat accessions. 102

Fig. S2 Heat maps of linkage disequilibrium for significant markers on chromosome 2A (S21A), 2B (S2B), 3A (S2C), 3B (S2D), 4A (S2E), 4A (S2F) and 5B (S2G). Color represents a rough measure of significance (red or black) better than pink, gray, and white. r^2 =0 (white color), $0<r^2<1$(shades of grey) and r^2=1 bright red). 107

GWAS in wheat identifies an amino acid transporter gene orthologue in Arabidopsis promoting nematode susceptibility

Fig. 1 Sequence alignment of TaAAT and AtAAP6 including conserved domain Aa_trans and SLC5-6 like_sbd superfamily protein. The underlined bold letters indicate the Aa_trans domain in wheat and Arabidopsis encoded by amino acids, *e-value = expected value. 54

Fig. 2 Average numbers of nematode in AtAAP6 gene knockout mutant compared with wild-type Arabidopsis Col-0 at 14 days post infection with *H. schachtii*. Data were analyzed using one way ANOVA at ($P\leq$ 0.05, n = 30) and Holm-Sidak test. Asterisks represent statistically significant difference to corresponding Col-0. **P<0.01. Bar indicates the standard error of the mean. 55

Fig. 3 Average size of female and syncytium produced by female of *H. schachtii* (mm^2) in *AtAAP6* knockout mutant compared with wild-type Arabidopsis Col-0 at 15 days post-infection. Data were analyzed using one way ANOVA at ($P\leq$ 0.05, n = 60) and Holm-Sidak test. Asterisks represent statistically significant difference to corresponding Col-0. **P< 0.01. Bar indicates the standard error of the mean. 56

Fig. 4 qPCR analysis of AtAAP6 gene regulation in syncytia compared to uninfected root at different time points. Data were analyzed using one way ANOVA at ($P\leq$ 0.05, n = 3) and Holm-Sidak test. Asterisks represent statistically significant difference to corresponding Col-0. **P<0.01 and ***P<0.001. Bar indicates the standard error of the mean. The experiment was repeated three times. The gene expression levels were normalized to the endogenous control gene UBQ and the fold change values were calculated by using the ΔΔCt method. 57

Fig. 5 Nematode development in various Arabidopsis accessions compared with Col-0 at 14 days post infection with *H. schachtii*. Data were analyzed using one way ANOVA at ($P\leq$ 0.05, n = 20) and Holm-Sidak test. Asterisks (*) represent statistically significant difference to corresponding Col-0. *P<0.05, **P<0.01, and ***P<0.001. Bar indicates the standard error of the mean. Uk-1, Ty-0 and Ta-0 represent lowly susceptibility accessions, and Mc-0, Zdr-1 and Jm-0 represent highly susceptible accessions. Col-0 is used as control. 58

List of figures

Fig. 6	Comparison of AtAAP6 ORF amino acid sequences in various Arabidopsis accessions. Vertical line indicates the position of the amino acid substitution, where glycine "G" is replaced by alanine "A" at position 207. *Pink box: exon, black line: intron, HS: highly susceptible accessions, LS: lowly susceptible accessions, and (....): similar amino acid sequences. Uk-1, Ty-0 and Ta-0 represent lowly susceptibility accessions, and Mc-0, Zdr-1 and Jm-0 represent highly susceptible accessions. Col-0 is used as control.	59
Fig. 7	qPCR analysis of TaAAT gene regulation in infected root of susceptible and resistant wheat accessions compared to uninfected root at 10 days post infection. Data were analyzed using one way ANOVA at ($P \leq 0.05$, $n = 3$) and Holm-Sidak test. Asterisks represent statistically significant difference to corresponding Bezostaya 1 control. *$P < 0.05$ and **$P<0.01$, ***$P<0.001$. Bar indicates the standard error of the mean. The experiment was repeated three times. The gene expression levels were normalized to the endogenous control gene q-Tubulin and the fold change values were calculated by using the $\Delta\Delta$Ct method.	63
Fig. S1	Characterization of AtAAP6 by Arabidopsis mutant. A-a graphic gene model for AtAAP6 with intron/exon structure and location of T-DNA insertions; B-T-DNA insertion was confirmed by PCR using gene-specific primers and the T-DNA primer; and C expression analysis of AtAAP6 confirmed the homozygosity of the mutant SALK_013231. *1-5 represents individual plants used for genotyping from same batch of mutant seed. Specific primers for the Arabidopsis ubiquitin (UBQ) were used as control.	118
Fig. S2	Comparison of AtAAP6 ORF amino acid sequences in various Arabidopsis accessions. Uk-1, Ty-0 and Ta-0 represent lowly susceptibility accessions, and Mc-0, Zdr-1 and Jm-0 represent highly susceptible accessions. Col-0 is used as control. ***G** glycine and **A** alanine.	119
Fig. S3	Comparison of AtAAP6 promotor region sequences in various Arabidopsis accessions. Uk-1, Ty-0 and Ta-0 represent lowly susceptibility accessions, and Mc-0, Zdr-1 and Jm-0 represent highly susceptible accessions. Col-0 is used as control. *A adenine, C cytosine, T thymine, and G guanine.	120

AA Amino acid

AAP Amino acid permeases

AAT Amino acid transporter

AFLP Amplified fragment length polymorphism

AM Association mapping

ANOVA Analysis of variance

ANTs Aromatic and neutral amino acids

APC Amino acid-polyamine-choline

ATF Amino acid transporter family

bp Basepairs

CAPS Cleaved amplified polymorphism sequences

CCN Cereal cyst nematode

cDNA Complementary deoxyribonucleic acid

CIM Composite interval mapping

CIMMYT International Maize and Wheat Improvement Centre

cM centiMorgan

Col-0 Columbia 0

Cre Cereal root eelworm

CTAB Cetyl trimethyl ammonium bromide

DArT Diversity array technology

df Degrees of freedom

DH Doubled haploid

DNA Deoxyribonucleic acid

DPI Days post infection

eg. Example

ESTs Expressed sequence tags

ExPASy Expert protein analysis system

FAO Food and agriculture organization

FAOSTA Food and agriculture organization statistics

FDR False discovery rate

GABA Amino butyric acid

GLM General linear model

GO Gene ontology

GWAS Genome-wide association studies

HR Hypersensitive reaction

HS Highly susceptible

INRES Institute of Crop Science and Resource Conservation

ISC Initial syncytial cell

ITMI International Triticeae Mapping Initiative

IWGSC International wheat genome sequencing consortium

IWWIP International Winter Wheat Improvement Program

J2 Second stage juvenile

LATs L-type amino acids

LBb1.3 Left border primer

LD Linkage disequilibrium

LHTs Lysine-histidine transporters

LS Lowly susceptible

MAF Minor allele frequency

MAS Marker-assisted selection

MCMC Markov Chain Monte Carlo

MIM Multiple interval mapping

MLM Mixed linear model

MMt Million metric tons

MR Moderately resistant

MS Moderately susceptible

NCBI National Center for Biotechnology Information

NI Non-inoculated

No. Number

OD Optical density

ORF Open reading frame

PCA Principle component analysis

PCR Polymerase chain reaction

ProTs Proline transporters

qPCR Quantitative polymerase chain reaction

QTL Quantitative-trait locus/loci

R Resistant

R^2 Phenotypic variance explained by QTL

RAPD Random amplified polymorphic DNA

RFLP Restriction fragment length polymorphism

RNA Ribonucleic acid

RTPCR Real time polymerase chain reaction

S Susceptible

SCAR Sequence characterized amplified region

SE Sieve element

SNP Single nucleotide polymorphism

SSR Simple sequence repeat

STS Sequence tag sites

TAIR Arabidopsis Information Resource

TIGR Institute for Genomic Research

UBQ Ubiquitin

WT wild type

1.1. Wheat

Wheat (*Triticum aestivum* L.) is one of the most ancient of domesticated cereals originating in the Fertile Crescent around 9,600 B.C (Piperno et al., 2004). Bread wheat is hexaploid with an estimated ~17 Gbp genome size (Hussain and Rivandi, 2007). The hexaploid genome (AABBDD) is composed of three closely-related and independently maintained genomes formed by multiple hybridization events among the three different progenitor species. The ancestral progenitor genomes are considered to be *Triticum urartu* (AA) and most probably *Aegilops speltoides* (BB) hybridized to produce tetraploid emmer wheat *Triticum turgidum* ssp. *dicoccoides* (AABB, 2n = 28), which further hybridized with goat grass *A. tauschii* (DD, 2n = 14) to produce modern bread wheat (Huang et al., 2002). Bread wheat is a major staple food for the world's population. It provides 19% of global dietary energy, which is second to rice, and grown on more land than any other commercial crop (Reynolds et al., 2009). The population of the world is estimated to be 9.15 billion by 2050 and demand for wheat is expected to be increased from 720 million metric tons (MMt) in 2014-2015 to 760 MMt in 2020, around 813 MMt in 2030, and more than 880 MMt by 2050 (Smale and McBride, 1996; FAO, 2009). Though, wheat production needs to be doubled by 2050, its production has fallen behind rice and maize (FAOSTAT, 2012). In fact, wheat production has not been improved fast enough to fulfil the projected demands (Ray et al., 2013). Further, the production of wheat is limited due to the effect of many biotic and abiotic factors. Wheat diseases, nematodes and pests cause significant yield loss. Cereal cyst nematodes (CCN) are one of major factors in rainfed wheat production system that cause significant yield loss (Dababat et al., 2015).

1.2. Cereal cyst nematode

Nematodes are tiny unsegmented roundworms that are anatomically differentiated into feeding, digestion, locomotion, and reproduction. CCNs comprise a number of closely related species which cause severe yield losses in cereals in several parts of the world including North Africa, West Asia, China, India, Australia, the US, and Europe (Nicol and Rivoal, 2008b). Ten nematode species *Heterodera avenae*, *H. latipons*, *H. filipjevi*, *H. hordecalis*, *H. zeae*, *H. mani*, *H. bifenestra*, *H. pakistanensis*, *H. arenaria* and *H. pratensis* are believed to form the CCN complex (Nicol et al., 2003). *H. filipjevi, H. avenae*, and *H. latipons* are the three important *Heterodera* species reported to cause significant economic loss in small grain crops (Rivoal and Cook, 1993). *H. avenae* is distributed all over the continents, while *H. latipons* is reported for Asia, Europe and Africa and North America (Fig. 1) (Scholz and Sikora, 2004; Sikora, 1988). *H. filipjevi* is identified throughout Europe and considered to be of East European origin (Rumpenhorst et al., 1996b). *H. filipjevi* was widely recorded in many parts of the world such as Tadzhikistan, Russia, Morocco, Tunisia, Pakistan, Libya, Turkey (Nicol et al., 2011; Rumpenhorst et al., 1996a) Estonia, Sweden, India (Nicol *et al.*, 2009), Norway (Holgado *et al.,* 2004), Iran, China, United kingdom (Mitchinson et al., 2009), and USA (Smiley et al., 2008). *H. filipjevi* was recorded to infect 87% of the several rain-fed winter locations in the Central Anatolian Plateau in Turkey with an estimated yield loss up to 50% (Nicol *et al.,* 2006). The increasing distribution of *H. filipjevi* in many parts of world ranks it one of the most important nematode parasites in wheat (Fig. 1). *H. filipjevi* has relatively narrow host ranges and, is limited to cereals such as wheat, barley and oat. Infected mature plants are stunted, have a reduced number of tillers and a “bushy-knotted” appearance (Nicol et al., 2011). Young infected plants are retarded and their lower leaves are often chlorotic thus forming pale green patches in the field. Continuous cultivation of susceptible crops in the infected field may result these patches into larger.

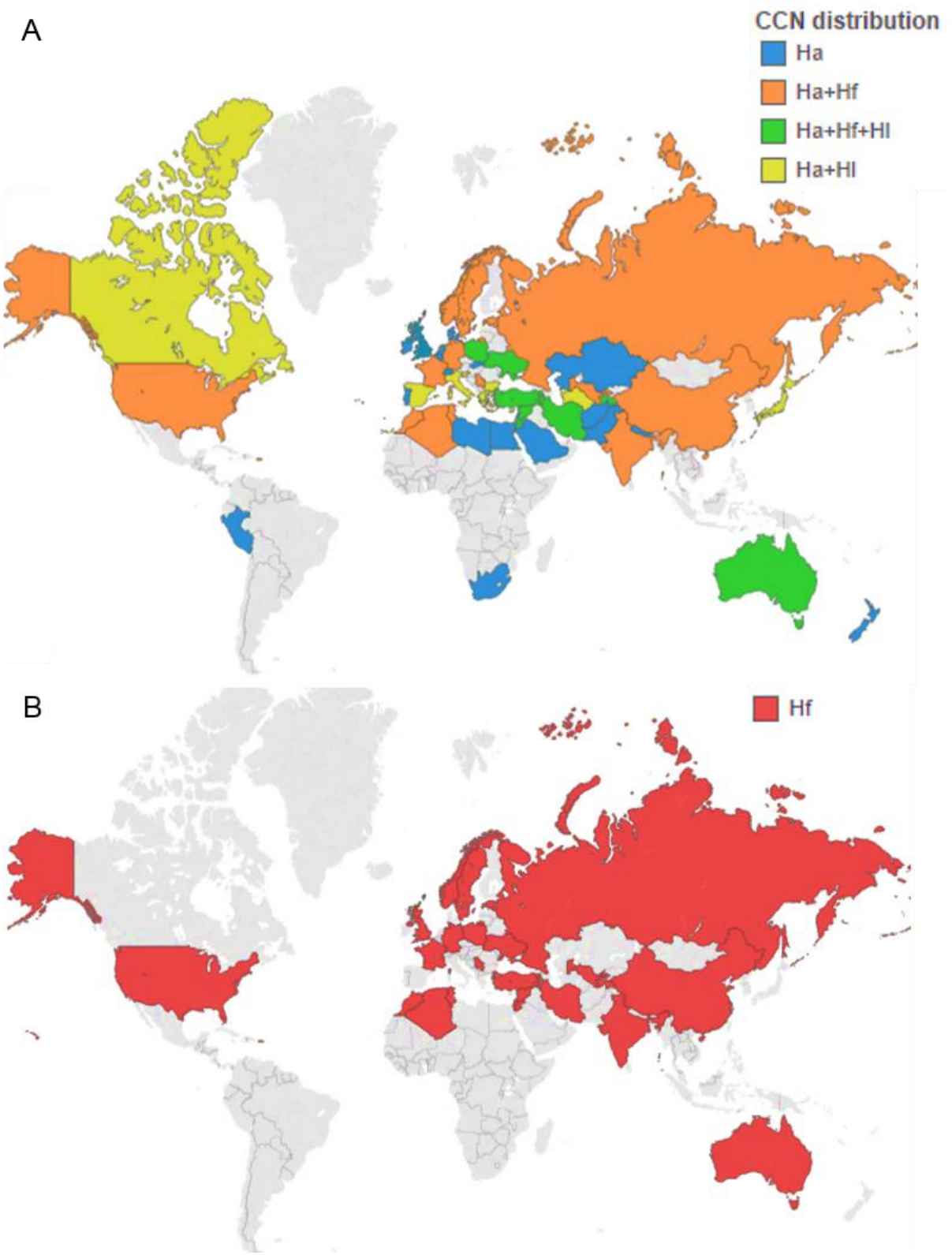

Fig. 1. Distribution of cereal cyst nematodes: A Distribution of *Heterodera avenae* (Ha), *H. filipjevi* (Hf) and *H. latipons* (Hl), and B Distribution of *H. filipjevi*. Countries in color indicate the presence of nematode species.

1.3. Biology of *Heterodera filipjevi*

H. filipjevi is a sedentary nematode and completes only one generation during each crop season (Hajihasani and Maafi, 2009; Seifi et al., 2013). The life cycle of *H. filipjevi* is similar to other CCN species such as *H. avenae* and *H. latipons* (Fig. 2). Morphologically, *H. filipjevi* is distinguished by the presence of bifenestrate vulval cones, a distinct underbridge, a robust stylet with anterior concave knobs, and a hyaline terminal tail in second stage juveniles (J2) (Abdollahi, 2008; Smiley et al., 2008).

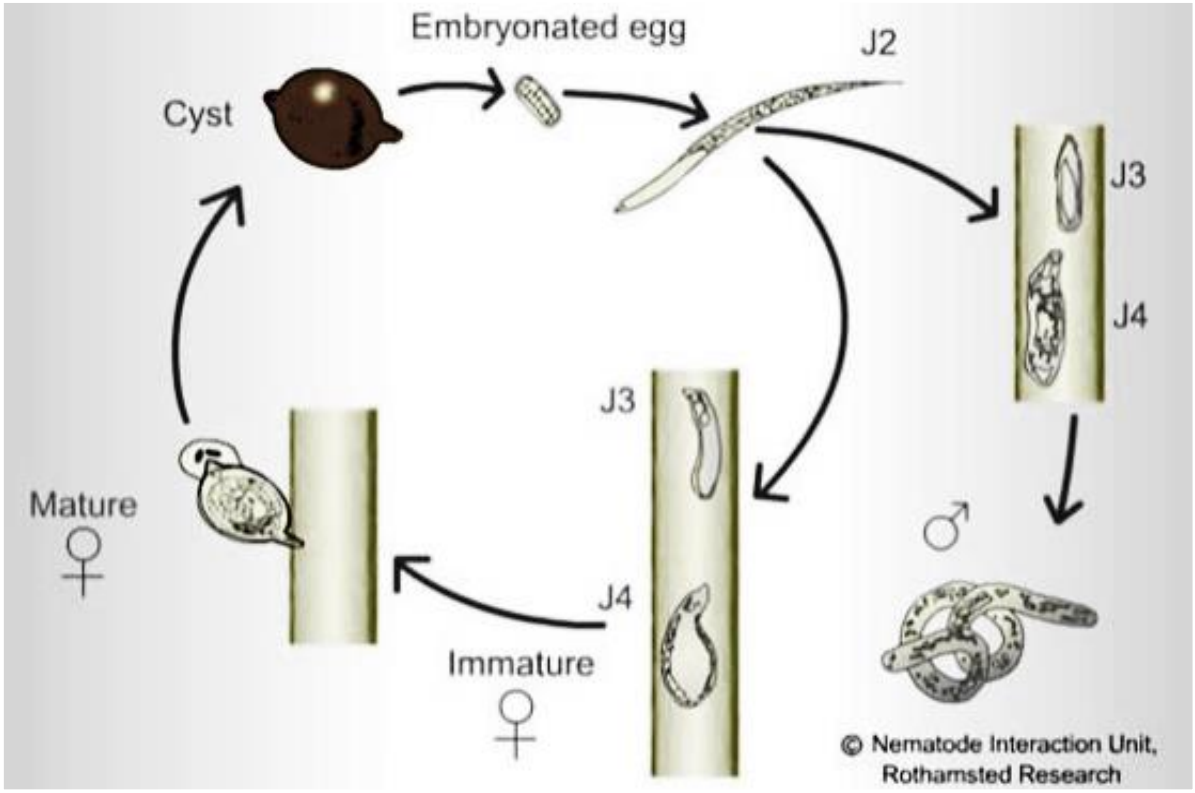

Fig. 2. The cereal cyst nematodes life cycle in wheat. *J2 second stage juvenile, J3 third stage juvenile, and J4 fourth stage juvenile.

The mechanisms by which cyst nematodes invade roots were investigated in several plant species (Sobczak and Golinowski, 2011). In general, the infective vermiform J2 invade epidermal and cortical cells behind the tips of young roots, migrate intracellularly towards the vascular cylinder and select a single cell (initial syncytial cell, ISC) in the stele into which they inject effector molecules, thereby inducing the formation of enlarged syncytial feeding structures in the roots (Wyss and Grundler, 1992). Syncytia are the only nutrient source for

the entire nematode life cycle. After feeding has commenced, the juveniles become sessile and molt consecutively into the third stage juvenile (J3), fourth stage juvenile (J4) and eventually into the adult female or male. *H. filipjevi* is sexually dimorphic and sex becomes morphologically apparent during J3 stage. Adult males regain mobility to find females for mating, whereas females remain embedded in the root tissue and continue to feed from the syncytium. After mating, the females produce several hundred eggs and then die. Their cuticles harden during a tanning process, and the body turns into a resistant brown cyst that protects the eggs in the soil for many years. In favorable conditions with sufficient nutrient supply, the majority of the J2s likely to develop into females while under adverse conditions such as resistant plants mostly males develop (Trudgill, 1967).

During nematode invasion, syncytia undergo dramatic changes in anatomy, physiology, and gene expression. Their nuclei become hypertrophied and the cytoplasm condenses with increasing numbers of mitochondria, plastids, ribosomes and endoplasmic reticulum (Sobczak et al., 2011). In wheat, a zone of necrotic cells was often observed between nematode *H. avenae* and its syncytium, where endodermis cells were broken down and become dense (Williams and Fisher, 1993). In syncytia, the level of sucrose was remarkably high. A decreased size of vacuoles and increased a volume of cytoplasm with numerous organelles such as plastids, mitochondria, and endoplasmic reticulum were observed (Fig. 3). Similar ultrastructural changes in syncytia induced by *H. schachtii* in Arabidopsis root were reported (Golinowski et al., 1996). In order to meet nutrient requirements of the nematodes, syncytia not only undergo morphological alteration, but also elevate metabolic activity (Mazarei et al., 2003; Williamson and Hussey, 1996).

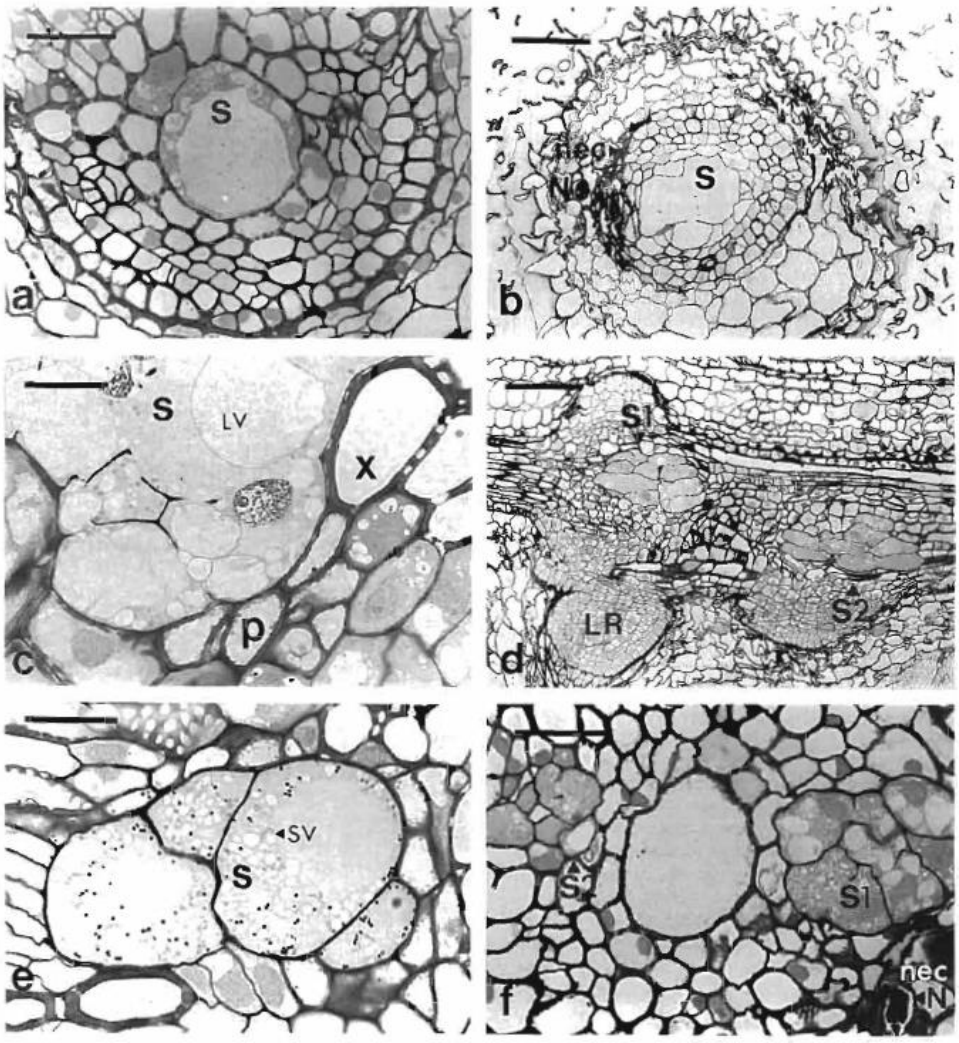

Fig. 3. Structure of syncytia induced by *Heterodera avenae* in susceptible wheat cultivar Prins. * A : Transverse section (TS) of syncytium associated with J2 at 4 days post infection (dpi); B : TS of syncytium with extensive arc of necrosis between J2 and its syncytium at 8 dpi; C : TS of syncytium showing large vacuoles in close contact with xylem and phloem; D : Longitudinal section of syncytium showing small vacuoles, multiple lateral meristems, and disorganized vascular tissue at 15 dpi; E: TS of syncytium with many small vacuoles, and larger vacuoles filled with amorphous material at 19 dpi; F: TS of syncytium with few large vacuoles, nuclei are hypertrophied nuclei, and cytoplasm strains intensely. Necrosis is evident around nematode head. S, SI, S2 = syncytia, N = nematode, nec = necrosis, LV = large vacuole, SV = small vacuole, x = xylem, p = phloem. (Bar equivalents.' A, F =50µm, B =100µm; C =20µm; D= 200µm; and E =25µm, (Williams and Fisher, 1993).

1.4. Host resistance to cereal cyst nematode

Host resistance is the most effective and preferred method to manage nematodes in wheat (McIntosh, 1997). Resistance is defined as the ability of the host to inhibit nematode multiplication. Resistance to CCN *H. avenae* has first been described in barley (Holm Nielsen, 1966; Nilsox-Ehle, 1920). Breeding for resistance to CCN in wheat was started in the early 1970s (Brown and Ellis, 1976), and later, Kimber and Feldman (1987) identified novel resistance sources in cultivated and wild wheat relatives. To date, sixteen different *H. avenae* resistance genes, including twelve *Cre1 to Cre8, CreR*, *CreX*, *CreY*, *CreZ* in wheat and its relatives (Table 1), and *Ha1* to *Ha4* genes in barley were reported (Bakker et al., 2006; Smiley and Nicol, 2009; Zhai et al., 2008). Eight of the *Cre* genes (*Cre2-7*, *CreX* and *CreY*) were originated from *Aegilops spp.* (Barloy et al., 2007; Delibes et al., 1993; Eastwood et al., 1991; Jahier et al., 1996; Ogbonnaya et al., 2001a; Romero et al., 1998). These resistance genes were identified via traditional map-based cloning and some of them were introgressed into hexaploid wheat (Ogbonnaya et al., 2001a). Three resistance genes *Cre2, CreX* and *CreY* have been introgressed into bread wheat and provide resistance against *H. avenae* (Barloy et al., 2007). The other two resistance genes *Cre8* on chromosome 6BL and *CreZ* in bread wheat are described, however, their application in wheat breeding has not reported (Jayatilake et al., 2015). The resistance gene *Crel* gene characterized in Aus 10894/Loros and was extensively used in commercial wheat breeding programs (O'Brien et al., 1980; Slootmaker et al., 1974).

It has been shown earlier that variation exists in wild and cultivated wheat for resistance against many different diseases (van Slageren, 1994). Thus, the ancient wheat populations are potential sources to explore CCN resistance. Research to identify resistance sources and their molecular characterization in host nematode parasitism is underway. However, only few effective resistance sources were identified. So far, *Cre1* is only gene reported to confer resistance against *H. filipjevi* in wheat derivative Thinopyrum (wheat grass) and wheat

landrace Sardari (Akar et al., 2009; Li et al., 2012). In fact, there is no variety providing strong and sustainable resistance against *H. filipjevi* in wheat, barley and oat.

Table 1. Sources of resistance genes/QTLs to CCN *Heterodera avenae* in wheat

Genotype	Accessions	Gene/QTL	References
Triticum aestivum ssp. *aestivum* L.	Loros, AUS10894	*Cre1* (2B)	(Slootmaker et al., 1974)
	AUS4930	*Cre1* (2BL)	(Bekal et al., 1998)
	Festiguay	*Cre8* (6B)	(Paull et al., 1998)
	SHWs	1D/4D/5B/5D/7D	(Mulki et al., 2013)
Triticum aestivum ssp. *vulgare* L.	Molineux	1B	(Williams et al., 2006)
Triticum monococcum	14087	1AS/2AS	(Singh et al., 2010)
Tritico secale	T701-4-6	*CreR*	(Dundas et al., 2001)
			(Asiedu et al., 1990)
Secale cereale	R173 family	*CreR*	(Taylor et al., 1998)
Aegilops tauschii	CPI 110813	*Cre4*	(Eastwood et al., 1994)
Aegilops variabilis		*CreX* (2AS), *CreY*(3BL)	(Barloy et al., 2007)
Aegilops tauschii	AUS18913	*Cre3* (2DL)	(Eastwood et al., 1994)
Aegilops peregrine		*Cre3* (2DL), *Rkn2*	(Barloy et al., 2007)
			(Jahier et al., 1996)
			(Rivoal et al., 2001)
Aegilops triuncialis	TR-353	*Cre7*	(Romero et al., 1996)
Aegilops ventricosa	VPM1	*Cre5* (2AS)	(Jahier et al., 2001)
			(Ogbonnaya et al., 2001b)
	11, AP-1, H-93-8	*Cre2*	(Delibes et al., 1993)
			(Andres et al., 2001)
			(Rivoal et al., 2001)
	11, AP-1, H-93-8, H-93-35	*Cre6* (5A)	(Ogbonnaya et al., 2001a)

1.5. Molecular markers

Molecular markers has revolutionized the pace and precision of plant genetic analysis and crop molecular breeding. The development of molecular markers to detect polymorphism in deoxyribonucleic acid (DNA) is one of the most significant outcomes in last three and half decades. A genetic marker is a short DNA sequence with a known chromosomal location linked to a gene or trait that describes a variation, which may occur due to mutation or

alteration in the genomic loci (Xu and Crouch, 2008). Markers are easily identifiable at specific genome location, and can be transmitted from generation to generation (Semagn et al., 2006). Based on implementation techniques, molecular markers are divided into hybridization based, polymerase chain reaction (PCR)-based, restriction enzyme-based, and DNA chip based markers. Restriction fragment length polymorphism (RFLP) markers were the first DNA-hybridization based genetic markers (Botstein et al., 1980). The random amplified polymorphic DNA (RAPD) markers were the first PCR based markers mostly used in forest trees (Williams et al., 1990). The other PCR based markers such as sequence tag sites (STS), simple sequence repeat (SSR), sequence characterized amplified region (SCAR) used in crop plants (Morgante and Olivieri, 1993). Amplified fragment length polymorphism (AFLP) and cleaved amplified polymorphism sequences (CAPS) are the restriction based markers and widely used in crops (Shavrukov, 2014; Vos et al., 1995). Diversity arrays technology (DArT) was the first DNA chip based marker used in whole genome analysis in rice (Jaccoud et al., 2001). Markers such as RFLP, AFLP, SSR, DArT and STS were deployed to detect QTLs for yield, grain quality and resistance to *Stagonospora nodorum* blotch in wheat (Akbari et al., 2006; Tadesse et al., 2015; Tommasini et al., 2007; Yao et al., 2009). In recent years, DArT markers were extensively used to identify QTLs linked to CCN and root lesion nematode resistance in wheat (Linsell et al., 2014; Mulki et al., 2013; Ogbonnaya et al., 2008; Sharma et al., 2011; Zwart et al., 2008). However, an uneven distribution and low reproducibility of DArT markers across the wheat genome has limited its application. To overcome the problem associated with previous genetic markers, a high density chip based Single nucleotide polymorphic (SNPs) markers were introduced recently (Wang et al., 2014a). SNP markers are widely used in molecular genetics due to their high resolution and abundance in genome, co-dominance, reproducibility, and amenability to high-throughput detection formats and platforms (Landegren et al., 1998; Wang et al., 2014a). The dense coverage of SNP markers has facilitated the analysis of genetic diversity, inferring

ancestral relationships between individuals in populations and marker-trait associations (Abdurakhmonov and Abdukarimov, 2008; Xu and Crouch, 2008). The high adaptation to multiplex detection systems of recent 90,000 gene-associated SNPs iSelect bead chip has become an efficient tool to uncover multiple targets in wheat (Wang et al., 2014a).

1.6. Single nucleotide polymorphic marker

A single nucleotide polymorphic (SNP) marker is a single base pair mutation at a specific locus and usually consists of two alleles. It is a position in a DNA sequence containing allelic variations within a population. SNP represents a difference in a single DNA building block, called a nucleotide. It is one of the most common and abundant genetic variation and each SNP close to particular gene can be used as a marker (Rafalski, 2002). The high frequency and diallelic nature of SNPs reduces errors of allele calling and facilitates the development of high-density marker maps. SNP genotyping measures the genetic variations between members of a species and detects the genes contributing to the particular trait such as disease susceptibility or resistance. High-density SNP markers are used to analyze genomic patterns, inferring ancestral relationships, and marker-trait associations, positional cloning and gene introgression studies in many crops (Ganal et al., 2009; Zhu et al., 2008). The 9K and 90 K iSelect SNP array have been employed to identify QTLs linked to *Fusarium* head blight, leaf rust, stripe rust and powdery mildew resistance in wheat (Buckler and Thornsberry, 2002; Gurung et al., 2014; Lagudah et al., 2006; Wang et al., 2014a). SNP markers are successfully used to characterize genetic variation in wheat, recently. The high-density 90K markers will advance the genetic studies of complex trait in wheat and facilitate genomics-assisted breeding.

1.7. Quantitative trait loci

Most of the agronomic traits in crops are quantitatively inherited and controlled by several genes (Falconer et al., 1996). The region in which these genes are located is called a quantitative trait locus (QTL). QTL is a location in a chromosomal region that controls the performance of a certain trait. Mapping markers linked to QTL identifies regions in the genome which may contain genes responsible for expression of the particular trait. The development of dense marker and genome sequences has greatly facilitated the analysis of these complex traits in wheat. Historically, QTL detection in wheat has started with linkage mapping in bi-parental populations to exploit unadapted germplasm (Würschum, 2012). In general, large samples of individuals are phenotype, genotyped with genetic marker, and detect QTLs by associating phenotype to genotype using statistical analysis.

There are many methods used to map QTLs. The three most commonly used methods are single-marker analysis, simple interval mapping and composite interval mapping. The first single-marker analysis includes t-test, analysis of variance (ANOVA), and regression which analyses the association between traits and genotypes at each marker locus (Edwards et al., 1987). However, in single-marker analysis the relative position of QTL in a marker linkage map cannot be established because each marker locus is considered separately in analysis. The second method of QTL mapping is the simple interval mapping based on maximum likelihood methods or multiple regressions (Lander and Botstein, 1989). It analyzes the association between trait and positions within marker intervals in the linkage map. However, QTL detection can be misled due to the interference of other markers. A composite interval mapping was deployed to control the markers outside the test interval (background markers) which serves as covariates (Zeng, 1994). This method is known to be precise and effective especially when linked markers are involved. However, this method cannot measure the epistatic effects and genotype*environment interactions (Collard et al., 2005). Thus, a simplified composite interval mapping approach was developed to enable the analysis of

QTL*environment interaction using large set of multiple environments data (Tinker and Mather, 1995). Further, to investigate QTL*QTL interaction, a multiple interval mapping (MIM) method was developed not only for infinitive but also for conditional effects. This method fits multiple putative QTLs directly in the Cockerham model to reveal individual effects, and interprets the maximum likelihood for estimating genetic parameters (Kao et al., 1999). MIM model is used to estimate the epistasis effect between QTLs, genotypic values of individuals, and heritability of quantitative traits. MIM is an improved method used to map multiple QTLs and identify interactions between them. In wheat, many QTLs has previously been mapped for different agronomic traits such as plant growth, grain yield, fungal and nematode resistant (Gurung et al., 2014; Mengistu, 2010; Ogbonnaya et al., 2001a; Singh et al., 2010; Williams et al., 2006; Zwart et al., 2010). Few CCN resistant sources are genetically mapped in bread wheat (Mokabli et al., 2002; Rivoal et al., 2001). Among sixteen CCN resistance genes (Table 1), only three genes (*Cre1*, *Cre3* and *Cre6*) were reported to co-segregate with markers (Eastwood et al., 1994; Lagudah et al., 1997; Ogbonnaya et al., 2001a; Williams et al., 1994). Analysis of QTL will provide a basis for analyzing genome-wide variation of complex traits. Understanding the genetic background of complex traits will facilitate the identification of candidate genes. These identified genes can be explored as genetic markers in marker assisted wheat breeding.

1.8. Association mapping

Association mapping (AM) is a population-based survey used to identify marker–trait relationships between molecular markers and functional loci based on linkage disequilibrium (LD) (Flint-Garcia et al., 2003). AM detects the causal polymorphisms within a gene that are responsible for phenotypes. LD tends to maintain over many generations between loci that are genetically linked to each other, and the closely linked alleles in LD creates a high resolution of QTLs (Ingvarsson and Street, 2011). The high resolution in AM measures the historical

recombination in accumulated natural populations, landraces, breeding materials and varieties (Soto-Cerda and Cloutier, 2012). In AM, a large set of unrelated accessions are phenotyped, genotyped, and subsequently correlated to identify the alleles at gene regions (Flint-Garcia et al., 2003; Rafalski, 2002). The association of a trait with specific molecular markers (gene tagging) not only allows localizing the genes or QTLs but also facilitates gene cloning. The major steps involved in AM are: selection of the diverse germplasm, trait measurement, genotyping with molecular markers, quantification of LD, evaluation of population structure and kinship, and finally determination of association of phenotypic and genotypic data using statistical analysis illustrated in Fig. 4 (Abdurakhmonov and Abdukarimov, 2008). Previously, two different approaches of association mapping were reported. First, it can be performed by scanning markers across the entire genome for statistical significant associations between a set of molecular markers and trait, known as a genome-wide association studies (GWAS). GWAS approach requires enough markers to cover the genome based on the expected rate of LD decay. GWAS identifies the candidate genomic regions and helps to undertake fine mapping. GWAS detects the causative polymorphism and candidate genes. The other approach is candidate gene association mapping that is carried out for restricted genomic regions. This approach requires prior knowledge of candidate gene associated with trait of interest. Candidate gene approach is particularly relevant when genome wide LD is limited (Hall et al., 2010). AM was used to identify QTLs and characterize candidate genes in several crops including small cereal grains such as rice, maize, barley, and wheat (Agrama et al., 2007; Cockram et al., 2010; Kump et al., 2011; Massman et al., 2011; Mulki et al., 2013; Neumann et al., 2011; Tommasini et al., 2007; Wang et al., 2001; William et al., 2003; Zou et al., 2000). GWAS uses the molecular markers to detect DNA polymorphisms in complex traits thus; it enhances our ability to develop resistant crop varieties.

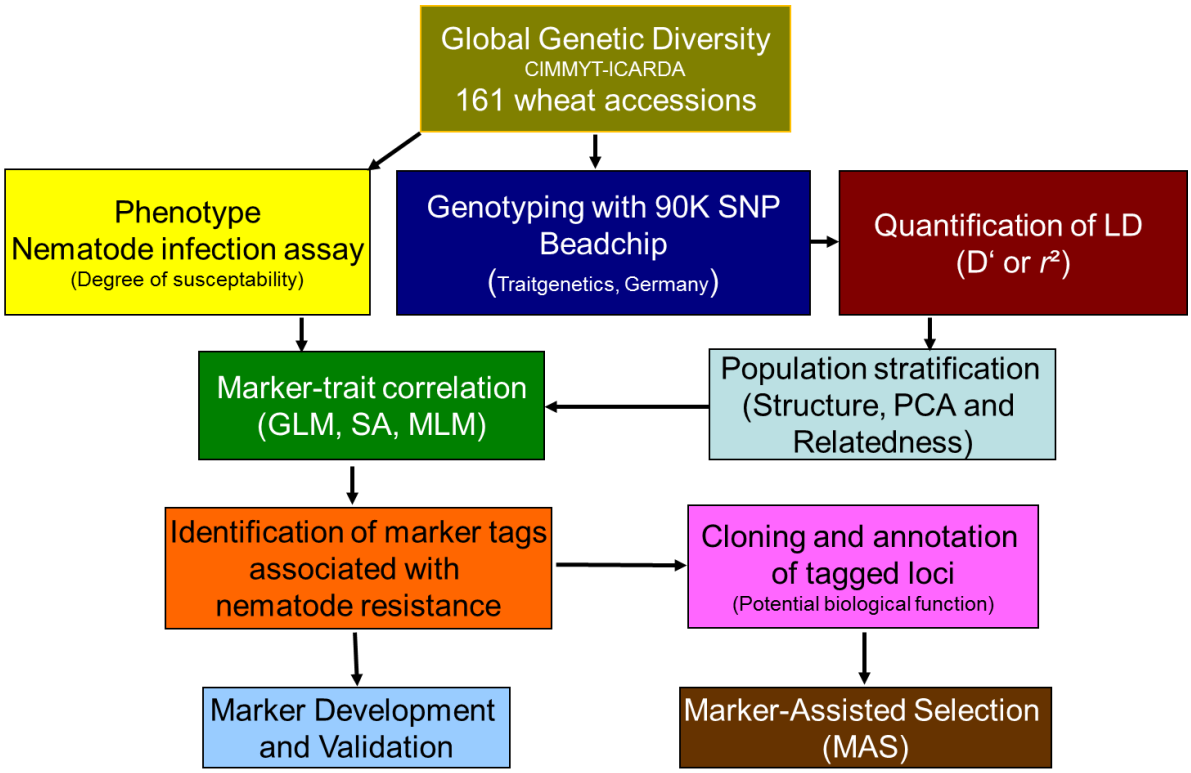

Fig. 4. Scheme of association mapping for plant-nematode interaction

1.9. Linkage disequilibrium

LD is the nonrandom association of alleles at different loci, either increased or reduced frequency of the haplotypes in a population at random combination of alleles (Flint-Garcia et al., 2003). Alleles at two or more loci are said to be in LD if their allele frequencies (individual and joint allele) are non-randomly co-inherited (Slatkin, 2008). LD determines the resolution of association mapping (Kim et al., 2007). LD, genetic diversity and population structure provides the basic information for association mapping. The resolution of AM often facilitates QTL detection with less number of markers with high LD, while high number of markers requires to achieve a high mapping resolution with low LD. LD is population-specific and depends on allele frequencies (Waugh et al., 2009). The extent of LD differs between each crop species and can vary among the mapping populations with in same crop species. LD depends on multiple factors such as local recombination rate, non-random mating, mutation rate, genetic drift and population structure (Cavanagh et al., 2013; Chao et

al., 2009; Flint-Garcia et al., 2003; Wright and Gaut, 2005). A relatively high LD (0.5-50cM) in a self-pollinated species was reported due to low recombination frequency with a rapid rate of inbreeding compared to maize (200-2000bp) and Arabidopsis (less than 10 kb) (Breseghello and Sorrells, 2006; Chao et al., 2009; Kim et al., 2007; Maccaferri et al., 2006; Tenaillon et al., 2001). LD may vary among chromosomal regions across wheat genomes (A, B, or D) in a population (Breseghello and Sorrells, 2006; Chao et al., 2009). The fine-scale mapping and inclusion of historical recombination of LD has proven to be very effective in precision and accuracy in dissecting complex traits in GWAS (Lynch and Walsh, 1998). However, population structure and alleles occurring at very low frequencies in the initial population can create false LD between unlinked loci (physically not linked), and can cause spurious associations between markers and traits (Breseghello and Sorrells, 2006; Jannink and Walsh, 2002). Thus, separating true LD from population structure is critical in association analyses (Crossa et al., 2007).

1.10. Population structure

Population structure and kinship both represent genetic relatedness between samples at different scales. The presence of population structure or familial relatedness in diverse mapping population can create false marker-trait associations (Wright and Gaut, 2005; Yu et al., 2014). Population structure is formed by non-random mating within a species and can cause changes in allele frequencies (Ersoz et al., 2007). The most commonly used approaches in crops are: a) general linear model (GLM) integrated with a structure matrix (Q), based on co-ancestry coefficients (referred as structured association) or on principal components (a referred as PCA), and b) a mixed linear model (MLM) integrating a kinship matrix (K) (Price et al., 2006). Structured association model used random unlinked markers to calculate and assign individuals into population substructures in STRUCTURE analysis (Pritchard et al., 2000). It uses a Markov Chain Monte Carlo (MCMC) Bayesian algorithm to calculate the

proportion of an individual's genome that originated from different inferred populations. Then, these individuals are clustered into different groups based on their genome characterization. The program assumes all individuals are unrelated and to be in Hardy-Weinberg equilibrium and allows to calculate the degree of population admixture of each individual. In GLM, Q matrix of sub-population for each individual from STRUCTURE uses as a covariate in a regression model, and then correlates the genotype with phenotype (Thornsberry et al., 2001). Principle component analysis (PCA) is another program used to calculate population structure. It is suggested to be effective and faster than STRUCTURE analysis (Price et al., 2006). PCA summarizes the variation in a correlated multi-attribute to a set of uncorrelated components, each of which is a particular linear combination of the original variables. The extracted uncorrelated components are called principal components (PC) which are estimated from the eigenvectors of the covariance or correlation matrix of the original variables.

MLM is the other approach which used to analyze both population structure (Q matrix) and familial relatedness (K kinship matrix). It has proven to be useful in controlling population structure and relatedness in GWAS. In MLM analysis, population structure is used as a fixed effect, whereas kinship among individuals is incorporated as the variance-covariance structure of the random effect for the individuals (Zhang et al., 2010b). Many studies have demonstrated the MLM (Q+K) model is more effective over other methods such as GLM (Q model). MLM corrects and controls the false associations in association studies. Control of false QTLs linked to flowering time in Arabidopsis using MLM (Q+K) model was reported (Zhao et al., 2007). Both STRUCTURE and PCA program have implemented in GWAS to analyze population structure for cereals such as barley, wheat and rice (Agrama et al., 2007; Breseghello and Sorrells, 2006; Crossa et al., 2007; Rostoks et al., 2006; Adhikari et al., 2012; Tommasini et al., 2007).

1.11. *In-silico* identification of genes linked to QTLs

Many QTLs linked to CCN resistance have identified by association analysis, however, the genes underlying most of the QTLs and their molecular characterization in host nematode parasitism remains unknown. The analysis of expressed sequence tags (ESTs) has proven to be major tools for gene discovery, molecular transcripts, SNPs analysis and functional annotation of putative gene products (Chaduvula et al., 2015). Genes associated with QTLs can be identified by targeting an expressed part of the wheat genome, and can be mapped with high-density markers (Cabral et al., 2014). The analyses of QTLs, co-localized with functional markers enable to identify potential candidate genes underlying a phenotypic trait. A QTL may contain one or more genes to explain the phenotypic variation. Many studies have revealed resistance genes co-segregate with or are closely linked to resistant QTLs (Yahiaoui et al., 2004; Yan et al., 2003; Zhang et al., 2010a). Many of these resistance genes were cloned and characterized in different plant species (Hulbert et al., 2001).

In-silico approach has previously been used to identify candidate gene in many crops. Genes such as oxalate oxidase, peroxidase, superoxide dismutase, chitinase and thaumatin underlying resistance QTLs were reported in wheat (Faris et al., 1999). Other studies revealed genes such as *H34 against* Hessian fly (*Mayetiola destructor*) and a receptor like protein kinase resistant to powdery mildew *Blumeria graminis* f. sp. Tritici in wheat (Li et al., 2013; Marone et al., 2013). Further, an actin-depolymerizing factor family gene *TaADF7* reported to be resistant against *Puccinia striiformis* f. sp. tritici stripe rust in wheat (Fu et al., 2014). However, many resistant genes in wheat were reported, only few nematode resistance genes were functionally characterized (Guo et al., 2013). The first cloned resistance gene in wheat against CCN *H. avenae* is *Cre3* (Lagudah et al., 1997). More recently, Xu et al., (2015) described structures and characterized the molecular mechanisms of CCN *H. avenae* resistance in *A. variabilis* (Xu et al., 2015).

To our knowledge, no resistance gene against *H. filipjevi* is functionally characterized in wheat. The wheat genome sequence and 90K gene associated SNPs iSelect Bead genotyping enabled us to a faster detection of a small genetic interval linked to the nematode resistance. It has provided a foundation for gene identification and comparative analyses of nematode-related genes. Current wheat cultivars limit the annotation process for large number of genes and proteins. Model organisms such as *Arabidopsis thaliana* and *Oryza sativa* has been used for functional annotation of genes for newly sequenced or incomplete sequence plant species (Clarke et al., 2003; Peng et al., 2015; Proietti et al., 2010). The flanking sequence of marker linked to QTLs are annotated to locate co-linear regions in Brachypodium, barley, oat, rice and Arabidopsis using several computational tools such as BLASTN, IWGSC, TIGR, TIGR, sequence alignment, phylogenetic analysis, and domain and motif analysis. Our analysis identified 11novel QTLs linked to *H. filipjevi* resistant. *In-silico* analysis revealed a genomic region of 74.9cM on chromosome 2BL containing an orthologue to amino acid transporter transmembrane family protein (*AtAAP6)* in *Arabidopsis thaliana*. A possible role of *AtAAP6* in *H. schachtii* parasitism in Arabidopsis has previously reported. It is upregulated in syncytia as shown in genechip analysis, *in situ* rtPCR, and infection assays with promoter::GUS lines (Elashry et al., 2013; Szakasits et al., 2009). Here, we characterize the role of *AtAAP6* in nematode parasitism using a T-DNA mutant line and expression analysis since the functions of *AtAAP6* is well supported statistically and biologically. Finding orthologues and characterizing them in a model plant can be an initial step towards understanding the molecular mechanism of gene function in wheat. Thus, comparative genomic approaches will facilitate to identify and characterize candidate genes, and increase our knowledge in understanding nematode resistance in wheat.

1.2. Objectives

Many resistance QTLs were reported to CCN *H. avenae* in wheat, however, no durable resistant cultivars are available. Majority of these QTLs were identified via traditional bi-parental populations using a low resolution mapping. So far, no resistance source to *H. filipjevi* in wheat has been found. We tested a diverse collection of wheat accessions to identify resistance sources, and found differences in host response to nematode from very low to very high number of females and cysts per accessions. We therefore hypothesize that the relevant genomic region (QTLs/genes) controlling nematode resistance can be identified by GWAS, and by *in-silico* annotation, the putative genes/protein underlying resistance QTLs will be predicted. We further hypothesize that wheat orthologues can be found via model plant Arabidopsis through comparative genomic approaches, and their possible role in nematode parasitism can be characterized.

The main objective of this study was to identify the QTLs/genes conferring resistance to *H. filipjevi* in 161 modern winter wheat accessions using GWAS.

The specific objectives of the research are:

1. To screen the 290 different winter wheat accessions to *H. filipjevi* and characterize the resistance response in candidate wheat accessions
2. To genotype the 161 selected winter wheat accessions by 90K iSelect SNP Bead chip array
3. To identify the QTLs linked to *H. filipjevi* resistance by GWAS
4. To identify the putative genes underlying significant QTLs
5. To identify an orthologue of wheat amino acid transporter transmembrane gene in Arabidopsis and characterize the possible role in nematode parasitism

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Identification and characterization of resistance to the cereal cyst nematode *Heterodera filipjevi* in winter wheat

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Identification and characterisation of resistance to the cereal cyst nematode *Heterodera filipjevi* in winter wheat

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Summary – The aim of this study was to search for new sources of resistance against the cereal cyst nematode, *Heterodera filipjevi*, in a collection of 290 wheat accessions. The plants were inoculated with juveniles and assessed for the number of females and cysts. One percent of the wheat accessions were ranked as resistant, 16% as moderately resistant, 41% as moderately susceptible, 26% as susceptible and 15% as highly susceptible. The infection rate and the number of females and cysts per plant were significantly lower in the resistant accession Nudakota and three moderately resistant accessions Ekonomka, Katea and Lantian 12 compared with susceptible cv. Bezostaya 1. Nematode development was reduced in resistant and moderately resistant accessions. The size of females and the total number of eggs and second-stage juveniles were reduced only in Ekonomka. No significant difference in plant height, plant weight, root length, root weight and root volume were recorded for inoculated plants compared to non-inoculated plants. This study has identified four resistant wheat accessions offering new material for breeding the resistance to *H. filipjevi*.

Keywords – breeding, host-nematode interaction, infection, susceptibility, *Triticum aestivum*.

Plant-parasitic nematodes significantly limit food production worldwide with at least 17 important nematode species in three major genera (*Meloidogyne*, *Heterodera* and *Pratylenchus*). Cereal cyst nematodes (CCN) are an important group of plant-parasitic nematodes attacking cereals. CCN comprise a number of closely related species that cause severe yield loss in cereals in many parts of the world including North Africa, West Asia, China, India, Australia, USA and Europe (Nicol & Rivoal, 2008). *Heterodera avenae*, *H. filipjevi* and *H. latipons* are frequently reported species in wheat and each species consists of various pathotypes (Rivoal & Cook, 1993; Holgado *et al*., 2005; Toktay *et al*., 2013). *Heterodera filipjevi* has been described in China, Estonia, India, Iran, Libya, Morocco, Norway, Pakistan, Russia, Sweden, Tadzhikistan, Tunisia, Turkey and USA (Rumpenhorst *et al*., 1996; Holgado *et al*., 2004; Smiley *et al*., 2008; Riley *et al*., 2009; Nicol *et al*., 2011). In Turkey, *H. filipjevi* has been found in 87% of the wheat-growing area in the Central Anatolian Plateau with an estimate of yield loss up to 50% in several rain-fed winter wheat locations (Nicol *et al*., 2006). Infected mature plants are stunted, have a reduced number of tillers and the roots are bushy and knotted (Nicol *et al*., 2011). Growth of the plants was retarded and their lower leaves are often chlorotic thus forming pale green patches in the field.

The life cycle of *H. filipjevi* has not been studied in detail but it is suggested to be similar to other CCN species such as *H. avenae* and *H. latipons* (Hajihasani *et al*., 2010). Morphologically, *H. filipjevi* can be distinguished by the presence of bifenestrate vulval cones, a distinct underbridge and a robust stylet with anterior concave knobs, and a hyaline terminal tail in second-stage juveniles (J2) (Abdollahi, 2008; Smiley *et al*., 2008). *Heterodera filipjevi* is a sedentary nematode and completes only one generation during each crop season (Hajihasani *et al*., 2010; Seifi *et al*., 2013). The mechanisms by which cyst nematodes invade roots have been investigated in several

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plant species (Sobczak & Golinowski, 2011). In general, the vermiform J2 of cyst nematodes invade epidermal and cortical cells behind the tips of young roots, migrate intracellularly towards the vascular cylinder and select a single cell (initial syncytial cell) in the stele into which they inject effector molecules, thereby inducing the formation of enlarged syncytial feeding structures in roots (Wyss & Grundler, 1992; Hewezi & Baum, 2013). After feeding has commenced, the juveniles become sessile and moult consecutively into the third-stage juvenile (J3), fourth-stage juvenile (J4) and eventually into the adult female or male. *Heterodera filipjevi* is sexually dimorphic and sex becomes morphologically apparent during the J3 stage. Adult males regain mobility to find females for mating, whereas females remain embedded in the root tissue and continue to feed from the syncytium. After mating, the females produce several hundred eggs and then die. Their cuticles harden during a tanning process, and the body turns into a resistant brown cyst that protects the eggs in the soil for many years.

Plant resistance is currently the most effective method to control cyst nematodes in cereals (McIntosh, 1997). Nematode resistance against cyst nematodes in plants is characterised by failure or limitation to produce functional feeding sites and female development (Williamson & Kumar, 2006). It has been described for a number of cyst nematodes that juveniles develop into females under favourable conditions in a susceptible host, whereas the number of males increases in resistant hosts (Trudgill, 1967). Accordingly, reduced numbers of females or cysts are the most common traits in nematode resistance. Reduced attraction towards roots and root structural barriers may also be important factors preventing invasion and syncytium induction. Analysis of nematode invasion, nematode development and nematode reproduction provides a detailed understanding of the active resistance mechanisms. Within CCN, most studies have been focused on *H. avenae*. More than nine single dominant genes, known as '*Cre*' have been reported in wild relatives of wheat and barley (Riley *et al*., 2009; Dababat *et al*., 2015). New sources of resistance to *H. filipjevi* were found in wheat, *Thinopyrum* (wheat grass), derivatives (Li *et al*., 2012) and the wheat landrace, Sardari, which is also a source of *Cre1* (Akar *et al*., 2009). Although the exploitation of plant resistance to CCN has great potential, only limited efforts have been made to identify new and effective sources of resistance in wheat. In fact, currently, there are no varieties providing strong and sustainable resistance to *H. filipjevi* in wheat, barley and oat.

Here, we present results of screening a large collection of wheat populations against *H. filipjevi*, which we assume could lead to the identification of new sources of resistance. In addition, detailed studies on the different stages of interaction between host and nematode during invasion, nematode development and reproduction can help us to understand the underlying mechanism of resistance.

Materials and methods

Wheat accessions

Two hundred and ninety-one winter wheat accessions including breeding lines, cultivars and landraces were tested (Table S1). The wheat accessions originated from Afghanistan (1), Azerbaijan (1), Bulgaria (2), Canada (2), China (7), Hungary (1), Iran (20), the International Winter Wheat Improvement Program Turkey-CIMMYT-ICARDA (95), Moldova (3), Mexico (29), Romania (1), Russia (28), Serbia (1), South Africa (26), Syria (1), Turkey (24), Ukraine (12) and the USA (36). The material was provided by the International Winter Wheat Improvement Program (http://www.iwwip.org/Nursery).

Nematode inoculum

A pure growth room culture of *H. filipjevi* from Central Anatolian Plateau, Eskisehir (39.76665°N, 30.40552°E), was collected and cysts were extracted by Cobb's decanting and sieving method (Cobb, 1918). Cysts were picked by hand and sterilised with 0.5% NaOCl for 10 min and rinsed several times with sterile distilled water. The surface-sterilised cysts were transferred into a funnel and stored at 4°C for hatching. Freshly hatched juveniles after 2 days ($\leqslant$48 h) were used as inoculum. We performed a polymerase chain reaction-restriction fragment length polymorphism analysis to confirm the species identification shown in Figure S1 (Yan *et al*., 2010).

Screening assay of wheat accessions

Six spikes of each of the 290 wheat accessions were picked by hand and one representative spike was selected from each accessions. A susceptible wheat cv. Bezostaya 1 was used as control. Seven seeds from each spike were germinated in moistened tissue in Petri dishes for 3 days at 22°C. After germination, five seedlings of a similar phenotype were selected. A sterilised potting mixture of sand, field soil and organic matter (70:29:1, v/v/v) was filled in RLC4-pine tubes (25 $\times$ 160 mm, Ray

Leach Cone-tainer™; Stuewe & Sons). One germinated seed was planted per tube in a 200 tube rack (RL200; Ray Leach Cone-tainer™) and plants were organised in a randomised block design. Each plant was inoculated with 250 freshly hatched J2 of *H. filipjevi* in 1 ml water into three holes around the shoot base 7 days after transplanting. Plants were grown in a growth room at 26°C and 65% RH. Twenty-five days after planting, plants were fertilised with water-soluble Nitrophoska® Solub/Hakaphos® (20:19:19 NPK including micro elements such as P_2O_5, K_2O, B, Cu, Fe, Mn, Mo and Zn; COMPO) at 1 g l^{-1}. Plants were harvested at 63 days post infection (dpi) to collect the cyst from the soil and the roots. The soil from each tube was collected in a 2 l beaker filled with water and the soil mixture was stirred, then left for about 30 s to allow the heavy sand and soil debris to settle down. Roots were washed very gently on the upper sieve to free any females and cysts left attached to the root system. The soil mixture was poured through 850 and 250 μm sieves. This process was repeated three times to ensure all females and cysts were collected. Females and cysts from both roots and soil were captured on a 250 μm sieve and counted under a dissecting microscope. The roots were further checked for females and cysts that had not been dislodged during the washing process. The host status of the tested wheat accessions was determined and categorised into five groups based on mean number of females and cysts present per plant (Dababat *et al*., 2014). The following ranking was used on a per plant basis: resistant (R) = <5 females and cysts; moderately resistant (MR) = 5-10 females and cysts; moderately susceptible (MS) = 11-15 females and cysts; susceptible (S) = 16-19 females and cysts; and highly susceptible (HS) = >20 females and cysts. The widely grown winter wheat cv. Bezostaya 1 in Turkey was used as the susceptible control.

Resistance assay

To identify potential mechanisms of resistance, nematode invasion, development and reproduction was monitored in the resistant accession Nudakota and three moderately resistant accessions Ekonomka, Katea and Lantian 12, and compared to the susceptible cv. Bezostaya 1. The experimental method described above for the screening assay of wheat accessions was used for the resistance assay. For plant growth measurements, non-inoculated (NI) and inoculated (I) plants were analysed. All treatments were repeated 18 times and performed in a completely randomised design. To monitor nematode infection and nematode development, roots were stained with acid fuchsin at 2, 5, 10 and 15 dpi (Byrd *et al*., 1983). To determine nematode reproduction, plants were harvested at 63 dpi, female and cysts were extracted from roots and soil, and the numbers of eggs and J2 determined after gently crushing the cysts. To measure cyst size, cysts were transferred to 2% water agar and photographed with a DM2000 dissection microscope (Leica Microsystems). The largest optical section of the cysts area was calculated using LAS software (Leica Microsystems). To assess growth parameters, the plants were washed gently, remaining soil particles were removed, and the root surface was dried with soft paper towel. Immediately after drying, the fresh plant weight and root weight was recorded. Plant height was assessed as the distance from the base of the stem to the base of the spike. Root length was determined by using WinRHIZO™ software (Regent Instruments Canada); root volume was measured volumetrically (Harrington *et al*., 1994).

Data analysis

In the first round of wheat accession screening, mean, standard deviation and standard error of number of cysts were determined. In the following resistance assay, the data were analysed using Sigma Plot 11.0. Statistical analysis included one-way analysis of variance and post-hoc analysis by the Holm-Sidak method. Statistical differences were accepted as significant at $P \leqslant 0.05$. Regression analysis was used to relate the size of cyst to the total number of eggs and J2 developed on the different wheat accessions. A polynomial regression analysis was used to calculate the best fitting equation.

Results

Screening assay of wheat accessions

The screening of 290 winter wheat accessions resulted in identifying 1% as resistant, 16% as moderately resistant, 41% as moderately susceptible, 26% as susceptible and 15% as highly susceptible to *H. filipjevi* (Table S1).

Resistance assay

Nematode invasion

The time-course of juvenile infection in selected wheat accessions Nudakota Katea, Ekonomka, Lantian 12 and cv. Bezostaya 1 at 2, 5 and 10 dpi is shown in Figure 1. No significant difference in nematode penetration among

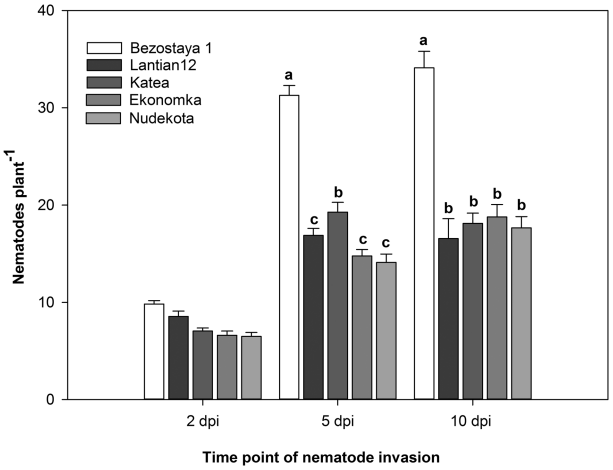

Fig. 1. Nematode infection in different selected wheat accessions at 2, 5 and 10 days post inoculation (dpi). Columns with different letters are significantly different based on one way analysis of variance (Holm-Sidak) analysis at ($P \leqslant 0.05$, n = 18) and a > b. Bar indicates the standard error of the mean.

the tested wheat accessions was found at 2 dpi. The number of J2 in the root was generally low, around 5-10. However at 5 dpi, nematode penetration was significantly greater, about 32 J2 in cv. Bezostaya 1 and up to about, 19 J2 in Ekonomka, Katea, Lantian 12 and Nudakota. The highest nematode infection was observed at 10 dpi in all accessions, with a significantly lower number in resistant and moderately resistant accessions compared to the susceptible cv. Bezostaya 1.

Nematode development

In Ekonomka, Katea, Lantian 12 and Nudakota, the number of J3 and J4 juveniles was found to be much lower at 10 dpi than in cv. Bezostaya 1 (Fig. 2A), reflecting the lower number of invading J2. At 15 dpi, the number of J3 in Katea, Ekonomka, Nudakota and Lantian 12 were significantly lower than in cv. Bezostaya 1 (Fig. 2B). At the same time point, the number of J4 females resulted from the development of J3. It was high in cv. Bezostaya 1, but significantly lower in all four resistant accessions. In addition, the number of males in Katea and Nudakota was significantly higher than in cv. Bezostaya 1.

Nematode reproduction

In addition to reduced and delayed nematode invasion and development in Ekonomka, Katea, Lantian 12 and Nudakota, the numbers of mature cysts were also significantly lower when counted at 63 dpi (Table 1). In this experiment, Lantian 12 was regarded as MS, due to slightly higher number of females per plant (Table 1).

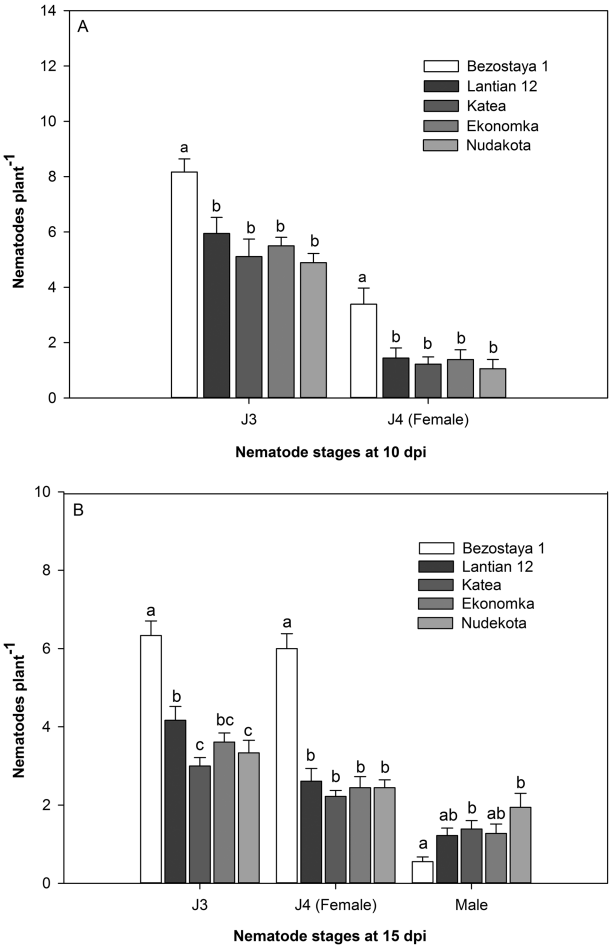

Fig. 2. Nematode development in selected wheat accessions at: A: 10 days post inoculation; B: 15 days post inoculation. Columns with different letters are significantly different based on one way ANOVA (Holm-Sidak) analysis at ($P \leqslant 0.05$, n = 18) and a > b. Bar indicates the standard error of the mean.

However, the mean number of cysts per plant was significantly less than in cv. Bezostaya 1. Cyst sizes in Katea and Ekonomka were significantly smaller, whilst there were no significant difference in Lantian 12 and Nudakota compared with cv. Bezostaya 1. Ekonomka and Nudakota contained significantly fewer eggs and juveniles per cyst compared with cv. Bezostaya 1. The regression analysis revealed no correlation between total number of eggs and juveniles to the cyst size in all wheat accessions (Fig. 3).

Plant growth

For analyses of basic plant growth parameters, we monitored plant height, plant fresh weight, root length, and root fresh weight at 63 dpi of inoculated and non-

Table 1. Selected winter wheat accessions and their response to *Heterodera filipjevi* development and reproduction.

Wheat genotype	Pedigree	Origin	Cysts per plant	SD	SE	Cyst size (mm^2)	Eggs per cyst	J2 per cyst	Total (eggs+J2)	Host status
Bezostaya 1	LUT17/SRS2	Russia	25.47^{a}	4.7	1.1	0.232^{a}	164	23	187^{a}	HS
Lantian 12	Qingnong-4/ Xiannong-4	China	10.45^{b}	3.3	0.8	0.198^{ab}	145	11	156^{ab}	MS
Katea	Hebros/Bez-1	Bulgaria	7.98^{bc}	2.5	0.6	0.178^{b}	140	16	156^{ab}	MR
Ekonomka	–	Ukraine	6.39^{bc}	1.8	0.4	0.183^{b}	96	10	106^{b}	MR
Nudakota	Jagger/ Romanian	USA	3.52^{c}	1.2	0.3	0.200^{ab}	128	11	139^{b}	R

Columns with different letters are significantly different based on one way ANOVA (Holm-Sidak) analysis at ($P \leqslant 0.05$, n = 18) and a > b > c.
Abbreviations: HS: highly susceptible; R: resistant: MR: moderately resistant; J2: second-stage juveniles; SD: standard deviation; SE: standard error.

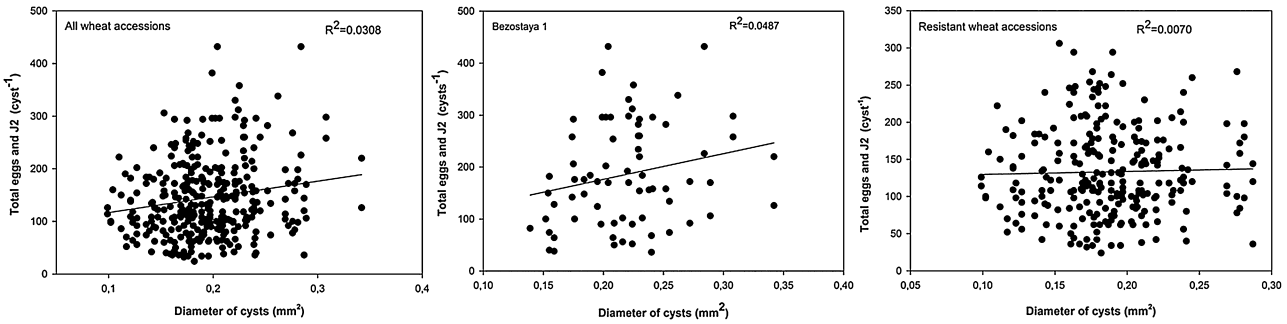

Fig. 3. Regression coefficient (R^2) for relation between cyst sizes (mm) to total number of eggs and juveniles in five wheat accessions ($P \leqslant 0.05$, n = 67).

inoculated plants. No significant differences were found in plant height, plant weight, root length, root weight and root volume between inoculated and non-inoculated Katea, Ekonomka and Nudakota (Fig. 4).

Discussion

Resistant wheat cultivars can be very effective in controlling cyst nematodes. Research to identify resistance sources and to characterise molecular markers for resistant phenotypes is ongoing in wheat and its wild relatives. However, there are very few studies focusing on the mechanism of resistance in wheat-nematode interactions. Wheat landraces and domesticated genotypes possess genetic variation including resistance to biotic and abiotic stresses (Kimber & Feldman, 1987). Here, we analysed wheat populations with wide geographical distribution and diverse genetic background to identify a new sources of resistance that can be introduced into wheat breeding. Two hundred and ninety-one wheat accessions used in this study responded differentially to *H. filipjevi* infection and damage. Seventeen percent of the wheat accessions led to significant reduction in nematode numbers compared to Bezostaza 1 and were therefore classified as R (1%) and MR (16%) (Table S1). In these wheat accessions, nematode infection and development was suppressed and relatively few females developed to maturity. The frequency of resistant accessions observed in this study varied significantly among the different geographical origin (Table 1). Two winter wheat cvs Silverstar (source of *Cre1*) and Frame (source of *Cre8*) were reported to confer moderate resistance to *H. filipjevi* (Imren *et al*., 2012). The Iranian bread wheat landrace Sardari carrying the *Cre1* gene was reported to confer moderate resistance to *H. filipjevi* (Akar *et al.*, 2009). However, the Sardari (accession number (ACCNO) 951009, Table S1) was only moderately susceptible in our study. The experimental method used by Akar *et al.* was different and the inoculum density (20 J2) was low compared to our study. A screening, performed in a glasshouse, and field

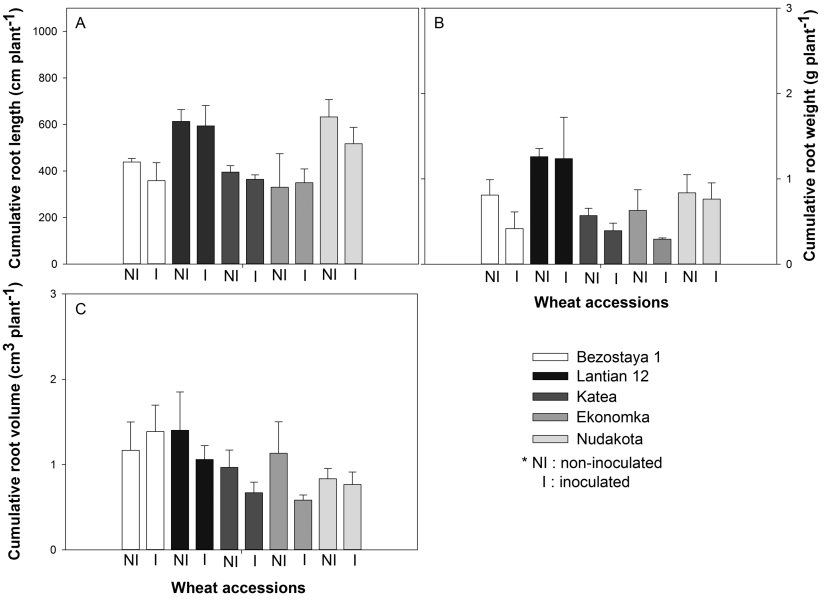

Fig. 4. Effect of *Heterodera filipjevi* on wheat growth determined as cumulative root length (A), root volume (B) and root weight (C) on different wheat accessions. Columns with different letters are significantly different based on one way ANOVA (Holm-Sidak) analysis at ($P \leqslant 0.05$, n = 18) and a > b. Bar indicates the standard error of the mean.

trials revealed one resistant wheat germplasm (6R(6D)) and two moderately resistant wheat germplasm (Mackller and CROC_1/AE.SQUARROSA(224)//OPATA) (Yuan *et al*., 2011). The two wheat accessions ES 84.24/GRK and Suzen 97 (ACCNO 000374 and 950283, Table S1) tested in this study (Yuan *et al*., 2011) were found to be highly susceptible, thus confirming our results.

Several mechanisms of resistance to cyst nematodes have been reported in host plants, including prevention of nematode infection and interruption of nematode development (Montes *et al*., 2004; Reynolds *et al*., 2011). Our data suggest that in resistant accessions nematode development is impaired at three phases: early invasion, nematode development, and reproduction, *i.e.*, the number of eggs and J2. Nematode invasion was very low in all four lines at the infection stage (2 dpi), gradually but moderately increased at 5 and 10 dpi due to juveniles that needed more time to find and invade the roots. We therefore conclude that in these accessions resistance is at least partially based on reduced invasion. This is consistent with other studies in wheat with *H. filipjevi* and *H. avenae* (Sağlam *et al*., 2009; Seifi *et al*., 2013). Other authors also found low invasion of *H. avenae* in resistant wheat cultivars Raj MR 1, CCNRV 4 and AUS 15854 (Pankaj *et al*., 2008). J2 are attracted to host plants by root exudates. Differences in the composition of root exudates might explain lower or higher attraction to roots and may alter nematode behaviour (Zhao *et al*., 2000; Robinson, 2002). J2 use their stylets as tools to pierce cell walls mechanically (Wyss & Zunke, 1986; Wyss, 2002) and to release secretions containing cell wall modifying enzymes facilitate ingress to roots (De Boer *et al*., 1996; Davis *et al*., 2000, 2008; Long *et al*., 2013). The composition of cell walls, therefore, may also determine invasion success by forming a more or less strong physical or physiological obstacle (reviewed by Bohlmann & Sobczak, 2014). Syncytia developed by *H. avenae* in susceptible wheat cv. Meering were metabolically active, while the syncytium of resistant wheat (*Triticum aestivum* cv. AUS10894) remained extensively vacuolated and less active at 13 dpi (Seah *et al*., 2000). A similar report revealed *H. avenae* female development was arrested in resistant wheat near isogenic line AUS10894 × Prins and metabolically active syncytia in the susceptible cv. Prins was reported (Williams & Fisher, 1993). At this stage, we cannot state whether the studied wheat accessions differ in chemical composition of root exudates and cell wall. Our results, however, show that the development of J3, J4 female and male does not differ between susceptible and resistant wheat accessions. We therefore conclude that the resistant accessions do not suppress growth and development of invaded nematodes by limitation or failure of the function of

the induced syncytia. This mechanism has often been observed in other host-nematode interactions *e.g.*, in tomato containing *Hero A* gene conferring resistance to potato cyst nematode *Globodera rostochiensis* (Sobczak *et al*., 2005), potato containing *Gpa2* gene to *G. pallida* (Koropacka, 2010) and sugar beet containing *HS1^{pro1}* gene to *H. schachtii* (Holtmann *et al*., 2000). Deterioration of the syncytia in these cases prevents successful completion of the nematode life cycle.

In this study, we examined cyst size as a possible indicator of resistance expecting the size of cysts to be related to the number of eggs and J2 they contain. In fact, cyst size was reduced in two resistant accessions (Katea and Ekonomka). However, counting the number of eggs and J2 revealed no clear correlation between these two traits (Fig. 3). Whereas in Katea, cyst size was reduced, the number of eggs and J2 was not significantly different compared to cv. Bezostaya 1. By contrast, both traits showed significantly reduced values in Ekonomka. Since there is no clear correlation between cyst size and number of eggs and J2, we conclude that cyst size is not a reliable trait to determine nematode resistance. Further studies are needed to verify whether and which plant factors determine these nematode traits.

Among all results achieved by analysing plant growth parameters, only root length and root weight in Lantian 12 and root weight in cv. Bezostaya 1 showed reduction after nematode infection. The fact that in most accessions none of the parameters was changed after inoculation indicates that the plants are tolerant to low nematode infection. The question rises why the susceptible cv. Bezostaya 1 obviously also shows this type of response. Since Bezostaya 1 is a cultivar which is grown extensively in Turkey, it might well be that is has been selected unintentionally by the farmers to maintain or improve wheat production under nematode infestation. Our data, however, do not imply that the studied accessions would show tolerance under field conditions. This trait is much more complex and can finally only be measured through monitoring yield under different conditions. However, here we focused on those parameters that can easily be monitored in a growth room trial. Extensive trials currently in progress will show how the selected accessions perform under field conditions. The challenge will then be to differentiate between effects that can be attributed to resistance from those that are based on tolerance.

From our results, we confirmed that wheat accessions Nudakota, Katea, Ekonomka and Lantian 12 possess resistance and can subsequently be crossed with high-yielding cultivars improving their genetic resistance to CCNs. Currently, we are working on the identification of markers and QTLs that are related to nematode resistance. Therefore, 161 wheat accessions have been included in a genome-wide association study to identify loci/genes conferring resistance to *H. filipjevi* (Pariyar *et al*., 2015). Marker-assisted selection will further improve the development of resistant cultivars. Isolation of candidate genes associated with specific markers will greatly facilitate this process.

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Genome-wide association study in wheat identifies resistance to the cereal cyst nematode *Heterodera filipjevi*

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Genome-Wide Association Study in Wheat Identifies Resistance to the Cereal Cyst Nematode *Heterodera filipjevi*

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ABSTRACT

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The cyst nematode *Heterodera filipjevi* is a plant parasite causing substantial yield loss in wheat. Resistant cultivars are the preferred method of controlling cyst nematodes. Association mapping is a powerful approach to detect associations between phenotypic variation and genetic polymorphisms; in this way favorable traits such as resistance to pathogens can be located. Therefore, a genome-wide association study of 161 winter wheat accessions was performed with a 90K iSelect single nucleotide polymorphism (SNP) chip. Population structure analysis grouped into two major subgroups and first principal component accounted 6.16% for phenotypic diversity. The genome-wide linkage disequilibrium across wheat was 3 cM. Eleven quantitative trait loci (QTLs) on chromosomes 1AL, 2AS, 2BL, 3AL, 3BL, 4AS, 4AL, 5BL, and 7BL were identified using a mixed linear model false discovery rate of $P < 0.01$ that explained 43% of total genetic variation. This is the first report of QTLs conferring resistance to *H. filipjevi* in wheat. Eight QTLs on chromosomes 1AL, 2AS, 2BL, 3AL, 4AL, and 5BL were linked to putative genes known to be involved in plant–pathogen interactions. Two other QTLs on 3BL and one QTL on 7BL linked to putative genes known to be involved in abiotic stress.

Bread wheat (*Triticum aestivum* L.) is a major staple food for the world's population. It was one of the first domesticated cereals composed of three closely related and independently maintained genomes. The hexaploid genome (AABBDD) was formed by multiple hybridization events among the three different progenitor species. The ancestral progenitor genomes of *Triticum urartu* (AA) and most probably *Aegilops speltoides* (BB) hybridized to produce tetraploid emmer wheat *Triticum turgidum* ssp. *dicoccoides* (AABB, 2n = 28), which again hybridized with goat grass *A. tauschii* (DD, 2n = 14) to produce modern bread wheat (Huang et al. 2002). Bread wheat production is limited by many biotic and abiotic factors, especially in rain-fed regions (Dababat et al. 2015). Cereal cyst nematodes (CCN) are an important group of plant-parasitic nematodes attacking cereals. CCN consist of several closely related nematode species such as *Heterodera avenae*, *H. latipons*, *H. filipjevi*, *H. hordecalis*, *H. zeae*, *H. mani*, *H. bifenestra*, *H. pakistanensis*, *H. arenaria*, and *H. pratensis* are referred as CCN complex (Nicol and Rivoal 2008). *H. filipjevi*, *H. avenae*, and *H. latipons* are the three important *Heterodera* spp. reported to cause economic losses in small grain crops (Rivoal and Cook 1993). *H. avenae* is reported from all over the continents, while *H. latipons* is reported from Asia, Europe, Africa, and North America (Scholz and Sikora 2004; Sikora 1988). *H. filipjevi* is one of the most damaging species with yield losses that locally may reach 50% (Rivoal and Nicol 2009).

Cyst nematodes of the genus *Heterodera* are biotrophic sedentary endoparasites that rely on the formation of specific hypertrophic and hypermetabolic nurse cell system (syncytium) close to the vasculature of the root. For development and reproduction, a female has to feed from a single syncytium for several weeks (Hussey and Grundler 1998). Nematode resistance in plants is characterized by limiting or preventing female development (Williamson and Kumar 2006), so the nematode population density eventually is decreased. Reduced female development may be caused by diminished nematode attraction, pronounced formation of structural barriers, increase of defense responses to nematode invasion, and failure or limitation of feeding site formation and therefore nematode development. Wheat landraces and domesticated accessions possess genetic variation including resistance to biotic and abiotic stresses (Kimber and Feldman 1987). Resistance sources to CCN include germplasm of *A. tauschii* (Coss.) (syn. *Triticum tauschii* (Coss.) Schmal; syn. *A. squarrosa* auct. non L.) and *T. turgidum* ssp. *dicoccoides* (Loureiro et al. 2009; McDonald et al. 2005; Nicol and Rivoal 2008; Rivoal et al. 2001; van Slageren 1994). Resistance to *H. avenae* was described in barley and in bread wheat (Holm Nielsen 1966; Nilsox-Ehle 1920). Nine single dominant genes known as *Cre* were found in wild relatives of wheat and successfully used to manage *H. avenae*, e.g., in Australia, France, India and Sweden (Ogbonnaya et al. 2009).

Host resistance is preferred to manage nematodes in wheat since it is effective and safe (McIntosh 1997). However, only a few resistance genes to CCN have been genetically mapped in wheat (Mokabli et al. 2002; Rivoal et al. 2001). Understanding the genetic background through mapping traits provides a baseline for breeding and gene cloning. Historically, quantitative trait loci (QTL) detection started

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with linkage mapping in biparental populations to exploit unadapted germplasm (Würschum 2012). However, the detection of closely linked markers in parental populations are limited by relatively few recombination events in linkage mapping (Riedelsheimer et al. 2012). Association mapping (AM) is an approach based on linkage disequilibrium (LD) between molecular markers and functional loci. Its high resolution makes it possible to detect historical recombinations in accumulated natural populations, landraces, breeding materials and varieties (Soto-Cerda and Cloutier 2012). Phenotype and genotype of a large set of unrelated accessions are determined and correlated to identify the alleles at gene regions (Flint-Garcia et al. 2003; Rafalski 2002). AM was used to identify QTLs and characterize candidate genes in several crops, such as rice, maize, barley, and wheat (Agrama et al. 2007; Cockram et al. 2010; Kump et al. 2011; Massman et al. 2011; Mulki et al. 2013; Neumann et al. 2011; Tommasini et al. 2007; Wang et al. 2001; William et al. 2003; Zou et al. 2000). Molecular markers such as restriction fragment length polymorphism, amplified fragment length polymorphisms, simple sequence repeats, diversity arrays technology or genome-specific sequence tagged sites were utilized in wheat for identifying QTLs, including yield, grain quality (Tadesse et al. 2015), and resistance to Stagonospora nodorum blotch (Tommasini et al. 2007; Yao et al. 2009) and *H. avenae* (Mulki et al. 2013). However, an uneven distribution and low reproducibility across the wheat genome has limited the application of these markers. The availability of a dense single nucleotide polymorphism (SNP) marker chip allows an efficient detection of target genes to uncover multiple targets in wheat because of its wide distribution in genomes and adaptation to high multiplex detection systems (Akhunov et al. 2009; Wang et al. 2014; Xu and Crouch 2008). As we tested wheat accessions for resistance to *H. filipjevi* and found differences in the resistance response, we hypothesized that the relevant genes/QTLs can be detected by association mapping. Therefore, the main objective of this study was to investigate the QTLs/genes conferring resistance to *H. filipjevi* in 161 diverse winter wheat accessions using genome-wide association study (GWAS).

MATERIALS AND METHODS

Wheat accessions screening assay. One hundred sixty-one winter wheat accessions (101 breeding lines, 58 cultivars, and 2 landraces) from the International Winter Wheat Improvement Program (IWWIP) were tested for CCN *H. filipjevi* levels (Supplementary Table S1). The accessions were selected based on diverse genetic background. Seven seeds from each spike were germinated on moistened tissue placed in Petri dishes for 3 days at 22°C. After germination, five seedlings of similar size and development were selected. A sterilized potting mixture of sand, field soil and organic matter (70:29:1, vol/vol/vol) was filled in RLC4-pine tubes (25 mm in diameter by 160 mm in height, Ray Leach Cone-tainer, Stuewe & Sons, Inc.). Each plant was inoculated with 250 freshly hatched J2s of *H. filipjevi* 7 days after transplanting and set in completely randomized design. The nematode inoculum used in these experiments was obtained from a pure culture (Pariyar et al. 2016). Plants were grown at 23 ± 2°C, 16 h of artificial light and 65% relative humidity. Twenty-five days after planting, plants were fertilized with water-soluble Nitrophoska Solub/Hakaphos (20:19:19 NPK including micro-elements such as P_2O_5, K_2O, B, Cu, Fe, Mn, Mo, and Zn, COMPO GmbH and Co. KG, Germany) at 1 g liter^{-1} of tap water. Plants were harvested at 63 days postinfection and cyst from both the soil and the roots were extracted. The soil from each tube was poured into a 2-liter beaker filled with water and the soil mixture was stirred, and then left for about 30 s to allow the heavy sand and soil debris to settle down. Roots were washed very gently on the upper sieve to free any females and cysts left attached to the root system. The soil mixture was poured through 850 and 250 µm sieves. This process was repeated three times to ensure all females and cysts were successfully collected. Females and cysts from both roots and soil were captured on the 250 µm sieve and counted under a dissecting microscope. The roots were further checked for females and cysts that had not been dislodged during the washing process. The response of the tested wheat accessions was determined and categorized into five groups based on mean number of females and cysts recorded per plant. The experiment was repeated twice. The following ranking was used: resistant (R) = <5 females and cysts/plant; moderately resistant (MR) = 5 to 10 females and cysts/plant; moderately susceptible (MS) = 11 to 15 females and cysts/plant; susceptible (S) = 16 to 19 females and cysts/plant; and highly susceptible (HS) = >20 females and cysts/plants (Pariyar et al. 2016). The widely grown winter wheat cultivar Bezostaya 1 in Turkey was used as the susceptible control. The phenotypic data were analyzed using a mixed linear model implemented in Proc mixed procedure in SAS 9.2. Variance components were estimated according to the following model:

$$Y_{ijk} = \mu + \text{year}_i + \text{block}_k\,(\text{year}_i) + \text{accession}_j + \text{accession}_j \text{ by } \text{year}_i + \varepsilon_{ijk}$$

where Y_{ij} is response variable, µ is overall mean, year is the random effect of year, accessions$_j$ is the fixed effect of the accession, block$_k$ is the random effect of the block with year, and ε_{ijk} is the random error. The data were analyzed by restricted maximum likelihood to fit a mixed model. To analyze total genetic variance to total phenotypic variance, heritability (H^2) was estimated by using PROC VARCOMP in SAS 9.2 (SAS Institute Inc., Cary, NC). Broad-sense heritability was calculated using $H^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_e/n)$, where σ^2_g is the genotypic variance, σ^2_e is the environment variance, and n is the number of environments (Holland et al. 2003).

SNP Genotyping and molecular data. Genomic DNA was isolated from 7-day-old wheat seedling leaf tissue using the cetyl trimethyl ammonium bromide (CTAB) method (Sharp et al. 1988). The quality of DNA was evaluated on a 0.8% agarose gel and normalized to 50 ng/µl. A DNA aliquot of 2 µl from each sample was used for genotyping by 90K Illumina iSelect Wheat Bead Chip, TraitGenetics GmbH, Gatersleben, Germany. To avoid monomorphic and low-quality SNPs, data were analyzed by Genome Studio software and transcribed into binary matrix software (Cavanagh et al. 2013). We defined minor allele (less frequent) as zero, major allele (more frequent) as one, and heterozygous scores as missing data. All monomorphic markers, the number of missing data greater than 5% and SNP markers with a minor allele frequency (MAF) less than 5% were removed to reduce false positive QTLs (Myles et al. 2009).

Linkage disequilibrium. Pair-wise measures of LD were performed to analyze the squared correlation coefficient (r^2) between two loci and summarize both mutational and recombination history. The extent of LD across the wheat genome was estimated by 11,680 markers with known chromosomal positions based on the International Triticeae Mapping Initiative map using a SAS 9.2 (Cavanagh et al. 2013). The values were plotted for each linkage group by genetic distance using a SAS/LD heatmap (Breseghello and Sorrells 2006). The LD decay to 0.1 was considered as the critical distance up to which a QTL region extends. LD heat maps for significant markers were created by using Haploview software (Barrett et al. 2005).

Population structure and kinship. Genetic subpopulations were analyzed using a model-based Bayesian clustering method implemented software STRUCTURE 2.3.4 with 961 polymorphic markers. The 961 markers were selected based on their 5 centimorgan (cM) grid distances from the total 11,680 markers with known chromosomal positions. Twenty independent runs were performed setting the hypothetical number of expected populations (K) range from 1 to 20. The data were processed by an admixture model using a burn-in period of 10,000 and run length of 100,000 (Evanno et al. 2005). K value was determined by estimated normal logarithm of the probability of fit (LnP(D)) provided in the STRUCTURE output and an ad hoc statistic △K based on the rate of change in LnP(D) between successive K values (Earl 2012). The estimated log probability

LnP(D) increased continuously with increasing K and △K, and was plotted against the number of subpopulations K (Evanno et al. 2005). Results were analyzed to interpret the origin and geographic distribution of the populations. Further, the principal components (PC) were estimated using the Princomp procedure in SAS 9.2 to clarify the population structure in the populations (Price et al. 2006). Principal component analysis (PCA) was analyzed by TASSEL v.3.0 (http://www.maizegenetics.net) with a total of 22,364 polymorphic SNP markers using as covariance matrix and analyzed for GWAS (Bradbury et al. 2007). The data were further analyzed by (P matrix and P+K) (Price et al. 2006). PCs were treated as fixed effects and kinship (K matrix) was used to analyze the variance and covariance structure of random individual effects (Yu and Buckler 2006).

Marker-trait associations. To identify significant QTLs conferring resistance to *H. filipjevi*, a multiple QTL model that corrected for both population structure and familial relatedness was developed using a mixed linear model (MLM) approach (promixed, SAS 9.2) (Bauer et al. 2009). The phenotypic variation (R^2) was calculated using a simple regression equation implemented in the program. Association between SNPs and nematode resistance was considered significant at $P \leq 0.01$ (Malosetti et al. 2007). The threshold LOD scores were calculated using 1,000 permutations. The least squares means of significant marker alleles were estimated and a stepwise regression forward/backward diministic process SAS promixed procedure was used to estimate the combined variation explained by the markers. A single-locus analysis was conducted and

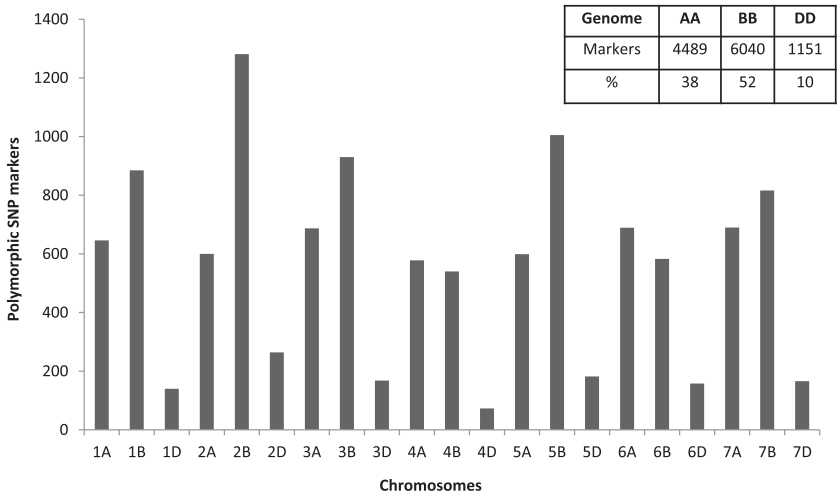

Fig. 1. Distribution of single nucleotide polymorphism (SNP) markers across wheat chromosomes.

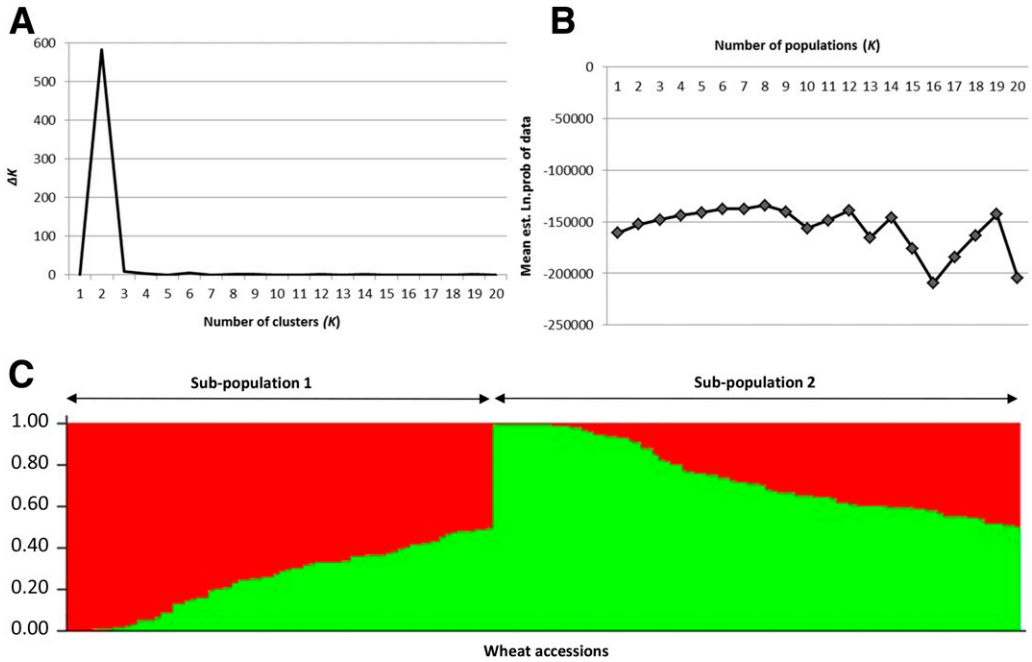

Fig. 2. Estimation of number of subpopulations (K) in winter wheat based on 961 single nucleotide polymorphism (SNP) markers. **A,** Estimation of number of subpopulations (K) in winter wheat using ΔK values. **B,** The log probability of data as a function of K for 961 SNP markers. Means log probability of data LnP(D) for each value of K were calculated from 20 independent runs of structure. **C,** Two subgroups inferred from STRUCTURE analysis. The vertical coordinate of each subgroup indicates the membership coefficients for each individual represented by a colored line where each color reflects the cultivar in one of the K clusters. The proportion of the colored segment red and green indicates the proportion of the genome drawn from the K clusters and represents the geographic eco-type information of wheat accessions.

the most significant marker based on *P* value was chosen as fixed cofactor in the model. In addition, a multilocus analysis with cross validation was used to reduce false discovery rate of QTL by controlling FDR at $P \leq 0.05$ (Bauer et al. 2009; Benjamini and Yekutieli 2005).

Functional annotation of putative genes linked to SNP marker associated to CCN resistance. To analyze the putative biological functions of genes associated to CCN resistance, we performed an in-silico functional annotation of significant SNP markers. The flanking sequences of the significant SNP markers were blasted against gene models of *Brachypodium distachyon*, *Oryza sativa*, and *Sorghum bicolor* available in the International wheat genome sequencing consortium (IWGSC), the Institute for Genomic Research (TIGR) Wheat genome annotation, and National Center for Biotechnology Information (NCBI). The genes/proteins were selected based on significant hit and lowest expected (e)-value, and putative functions were analyzed. To identify the full open reading frame (ORF) at significant marker locations, we downloaded the available wheat transcriptome assemblies from the MAS Wheat dataset (Krasileva et al. 2013). All datasets were imported in the CLC genomic workbench and blast database sequences lower than e-value 0.0e-15 were considered positive. Further, the putative genes were blasted in The Arabidopsis Information Resource (TAIR), and similar predicted proteins/genes homologs were described as annotated functions. If the significant marker was in a coding region, the substitution was designated as synonymous (no change in amino acid) or nonsynonymous substitution (change in amino acid).

RESULTS

Wheat accessions screening assay. Screening of 161 modern winter wheat accessions revealed variability in response to nematode infection. The results revealed 1% of the studied wheat accessions to be resistant, 26% moderately resistant, 26% moderately susceptible, 22% susceptible, and 24% highly susceptible to *H. filipjevi*.

Density of polymorphic SNP markers differs between the A, B, and D genomes. 23,364 polymorphic SNPs were obtained after removing all monomorphic markers, markers with a MAF <5% and missing data >5%. Fifty percent (11,680) of SNPs with known chromosomal locations were recorded and used for LD analysis. A total genomic size of 2956.5 cM was measured with the 11,680 markers across 21 chromosomes of the 161 wheat accessions (Fig. 1). The results

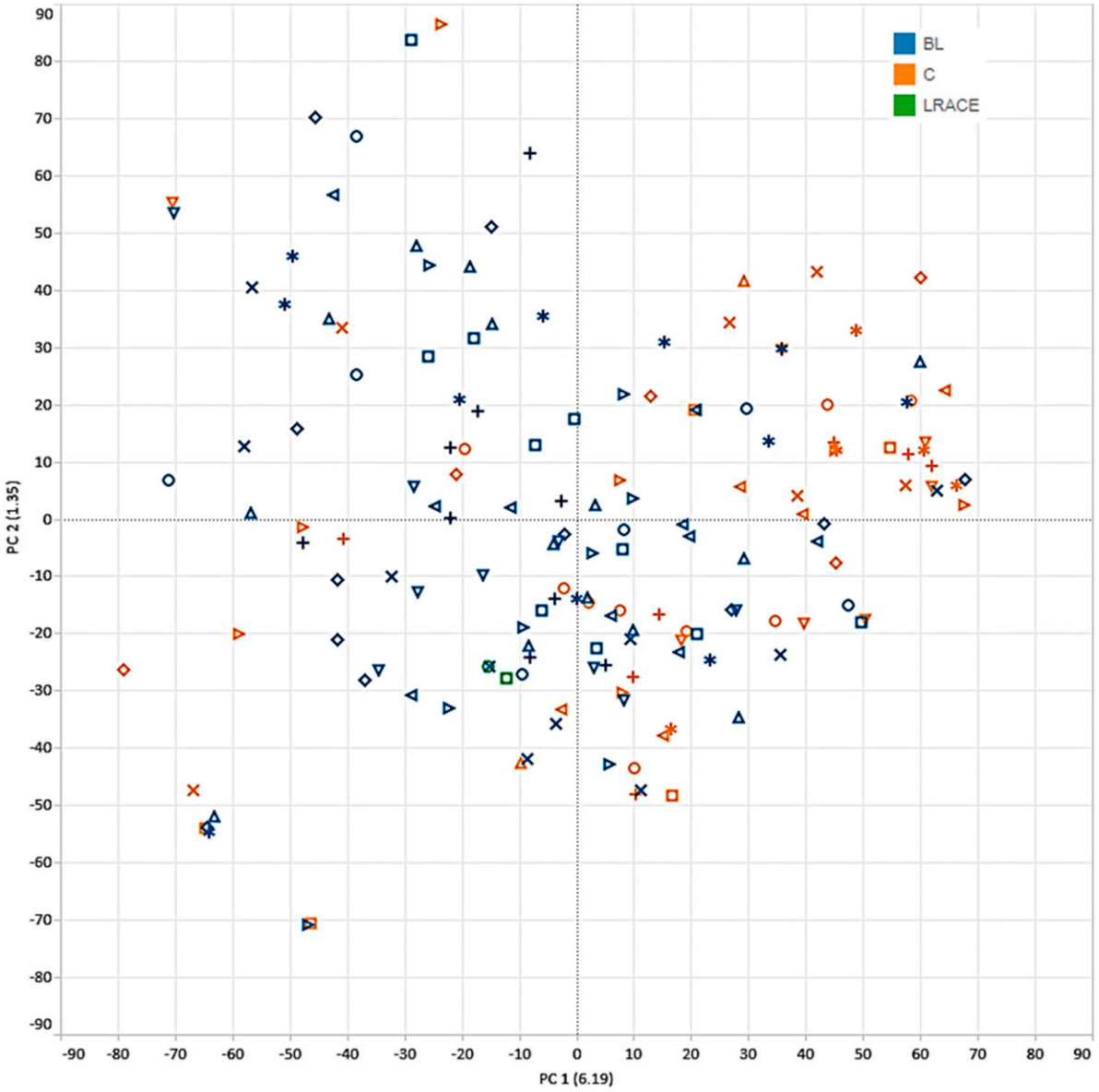

Fig. 3. Principal components analysis based on correlation matrix recorded on 161 winter wheat accessions. A scatter plot of principal component 1 (PC1) plotted against principal component 2 (PC2). Each symbol represents a wheat accession in mapping panel. BL = breeding line; C = cultivar, LRACE = landrace.

showed an uneven distribution of mapped markers across wheat genomes A, B, and D. A high density of SNPs was located on genomes A (38.43%) and B (51.71%), while a relatively low number of SNPs were detected on genome D (9.85%). The highest number of polymorphic markers was located on chromosome 2B (1,281 SNPs, 11%) with a SNP marker each 0.11 cM on average. The lowest number of polymorphic markers was distributed on chromosome 4D (73 SNPs, 0.63%) with a SNP marker each 1.55 cM. On average, one marker was mapped each 0.25 cM over all chromosomes.

Population structure and kinship. The ad hoc quantity based on the second order rate of change in the log probability (ΔK) showed a clear peak at K= 2 (Fig. 2A), indicating two genetic subpopulations. The logarithm of the data likelihood (LnP(D)) on average continued to increase with increasing numbers of assumed subpopulations (K) from 2 to 20 with exception of the depression at K10, K13 and K16 (Fig. 2B). The first group consisted of 89 winter wheat accessions mostly originating from South Africa (5), and Iran (2), including the United States (1), whereas the second group was composed of 72 wheat accessions mainly originated from Russia (8), Bulgaria (2), Moldova (2), including Turkey-CIMMYT ICARDA (3) (Fig. 2C). Population structure was further analyzed by conventional *F*-statistic (*Fst*) analysis. The average fixation index between subpopulations

TABLE 1. A genetic linkage map, single nucleotide polymorphism (SNP) marker, and linkage disequilibrium decay in wheat 161 wheat accessions[a]

Chromosome	Number of SNP	SNP %	Length (cM)	cM/SNP	LD decay r^2/(cM)
1A	646	5.53	111.7	0.17	4
2A	600	5.14	120.5	0.20	1
3A	687	5.88	169.6	0.25	2
4A	578	4.95	161.8	0.28	4
5A	599	5.13	117.7	0.20	2
6A	689	5.90	122.2	0.18	3
7A	690	5.91	167.5	0.24	1
1B	885	7.58	117.7	0.13	1
2B	1,281	10.97	145.0	0.11	2
3B	930	7.96	137.0	0.15	2
4B	540	4.62	107.0	0.20	2
5B	1,005	8.60	179.0	0.18	3
6B	583	4.99	110.4	0.19	2
7B	816	6.99	142.0	0.17	2
1D	140	1.20	143.6	1.03	10
2D	264	2.26	141.6	0.54	2
3D	168	1.44	164.1	0.98	6
4D	73	0.63	112.9	1.55	4
5D	182	1.56	167.8	0.92	2
6D	158	1.35	122.3	0.77	2
7D	166	1.42	195.1	1.18	4
Total	11,680	100	2,956.5	0.25	2.9

[a] cM, centimorgan; LD, linkage disequilibrium; and r^2, pair-wise measures of LD.

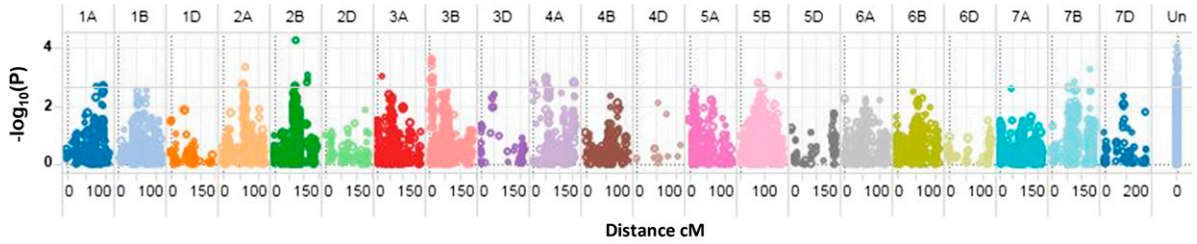

Fig. 4. Manhattan plots of *P* values indicating genomic regions associated with cereal cyst nematode *Heterodera filipjevi* resistance in winter wheat using the mixed linear model (MLM) (P+K). *x* axis shows single nucleotide polymorphism markers along each wheat chromosome; *y* axis is the $-\log_{10}$ (*P* value).

TABLE 2. Significant markers associated with QTLs conferring resistance to *Heterodera filipjevi* in 161 winter wheat accessions[a]

SN	Marker	CHR	POS (cM)	Genetic interval	P-LOD	P-FDR	*P* value	Allele	Allelic effect	R^2 (%)
1	wsnp_BE443588A_Ta_2_1	1AL	97.10	94.0–97.1	4.24	0.00055	0.00092	A/C	–4.30	10.29
2	RAC875_c13116_943	2AS	64.30	64.30	3.35	0.00164	0.00045	A/C	–3.04	8.50
3	Excalibur_c18966_804	2BL	113.60	113.60	2.91	0.00164	0.00122	G/A	–2.80	6.90
4	wsnp_BE426418A_Ta_2_1	3AL	20.00	20.0–26.4	3.01	0.00164	0.00096	T/C	–4.36	8.00
5	Bobwhite_rep_c66630_331	7BL	79.10	68.6–79.1	2.81	0.00164	0.00154	T/C	–3.31	7.50
6	Tdurum_contig10380_87	2BL	74.90	74.90	4.26	0.00056	0.00006	G/A	4.36	12.10
7	Tdurum_contig12008_803	3BL	6.14	0.0–6.14	3.61	0.00024	0.00025	T/C	3.11	8.90
8	Excalibur_c20277_483	3BL	6.14	0.0–6.15	3.64	0.00164	0.00100	G/A	3.40	7.40
9	wsnp_Ex_c55245_57821389	4AS	53.30	53.30	2.98	0.00164	0.00104	T/C	2.77	7.50
10	Tdurum_contig82236_117	4AL	155.50	155.50	2.82	0.00164	0.00152	G/A	3.35	7.60
11	Excalibur_c78724_434	5BL	158.70	158.70	3.06	0.00164	0.00087	G/A	3.65	8.10

[a] CHR, chromosome; cM, centimorgan; FDR, false discovery rate; P, probability; POS, position; LOD, maximum likelihood; QTL, quantitative trait loci; R^2, effect due to genetic variation; and SNP, single nucleotide polymorphism.

ranged from 0.011 and 0.155, while mean 0.034 *Fst* revealed a very low population differentiation due to genetic structure. In addition, PCA was used to visualize the genotyping data (Fig. 3). The first, second, and third principal components explained 6.19, 1.35, and 1.0% of the variation, respectively. The low genetic variation exhibited by first PC (6.19%) indicated no significant principal components in the mapping population (Franklin et al. 1995).

Linkage disequilibrium. The analysis of LD decay with 11,680 markers showed that the genome-wide LD across wheat was 3 cM (Table 1). The r^2 values were plotted against the genetic distance. Beyond 3cM, LD became constant at a value of $r^2 = 0.1$, which allowed a precise genetic analysis. The low intrachromosomal linkages of all chromosomes were calculated (Table 1; Supplementary Fig. S1). The lowest LD decay was observed in chromosome 1D at

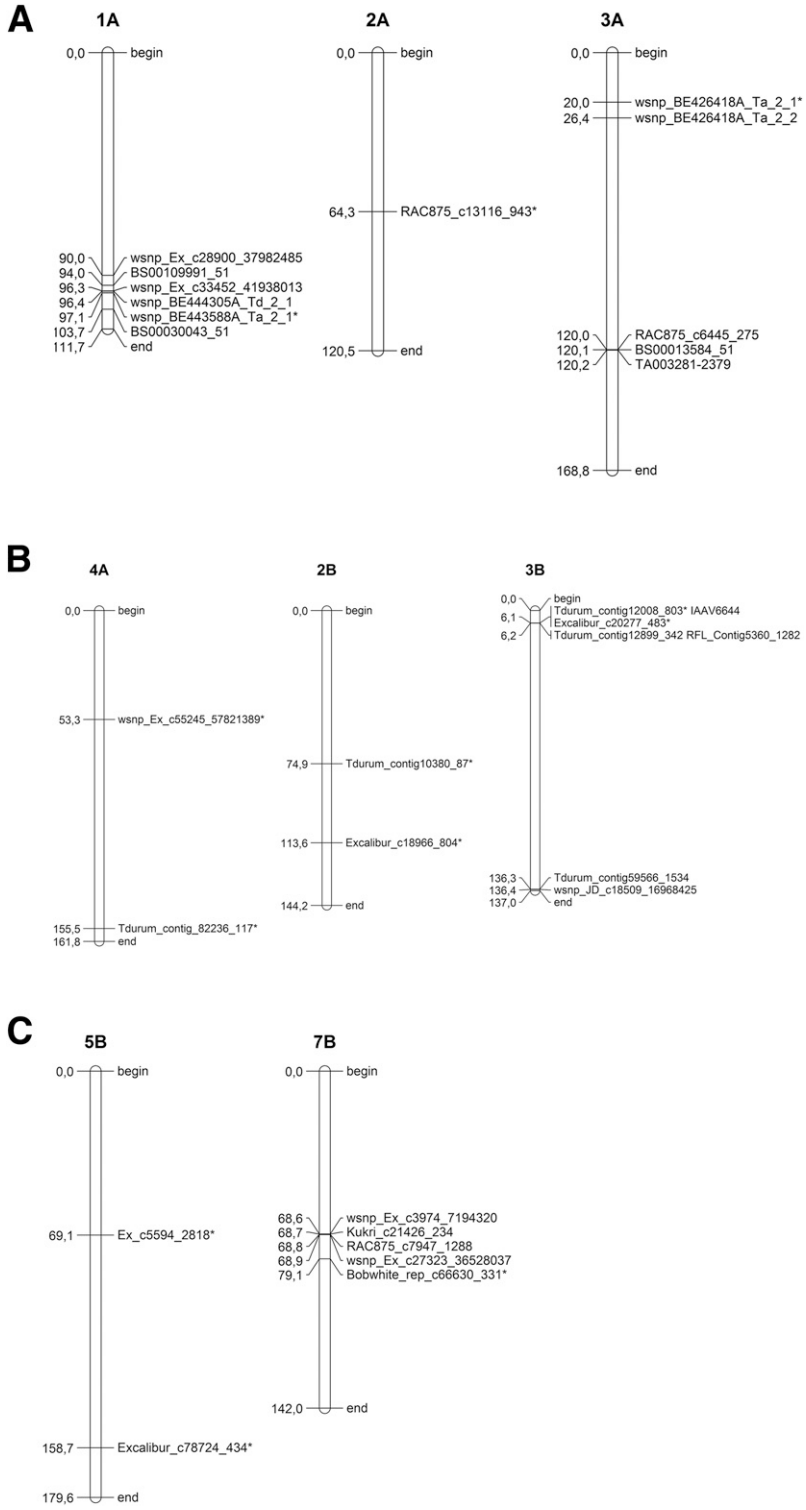

Fig. 5. The genetic map of significant single nucleotide polymorphism (SNP) markers associated with resistance to cereal cyst nematode based on International Triticeae Mapping Initiative consensus map. **A,** Genetic map of significant markers on chromosomes 1A, 2A, and 3A; **B,** genetic map of significant markers on chromosomes 4A, 2B, and 3B; and **C,** genetic map of significant markers on chromosomes 5 and 7B. **A** and **B** indicate wheat chromosomes, while * indicates significant marker in marker trait association. Map drawn using MapChart 2.2 (Voorrips 2002).

10 cM. The LD heat maps for 11 significant markers were created using Haploview software (Supplementary Fig. S2A to G).

Marker-trait associations. Eleven SNP markers were significantly associated with response to *H. filipjevi* detected on chromosomes 1A, 2A, 3A, 4A, 2B, 3B, 5B, and 7B (Fig. 4). The phenotypic variation (R^2) of individual SNPs ranged from 6.9 to 12.1% (Table 2). The total genetic variation explained by all significant markers was 43%. In five cases of the 11 SNP markers (wsnp_BE443588A_Ta_2_1 (1A, 97.1 cM), RAC875_c13116_943 (2A, 64.3 cM), Excalibur_c18966_804 (2B, 113.6 cM), wsnp_BE426418A_Ta_2_1 (3A, 20 cM), and Bobwhite_rep_c66630_331 (7B, 79.1 cM)), the alleles with higher frequency in the panel were related to resistance. In the six remaining cases the alleles with higher frequency (Tdurum_contig10380_87 (2B, 74.9 cM), Tdurum_contig1208_803 (3B, 74.9 cM), Excalibur_c20277_483 (3B, 6.14 cM), wsnp_Ex_c55245_57821389 (4A, 53.3 cM), Tdurum_contig82236_117 (4A, 155.5 cM), and Excalibur_c78724_434 (5B, 158.7 cM) were related to susceptibility (Table 2). The genetic maps of significant markers were generated according to Voorrips (2002) and shown in Figure 5A to C. The highest effect was recorded by the marker Tdurum_contig10380_87 (2B, 74.9 cM) while the lowest recorded for Excalibur_c18966_804 (2B, 113.6 cM).

Functional annotation of genes linked to SNP markers associated with CCN resistance. Based on the eleven significant markers, we were able to identify full ORFs associated to the QTLs (Table 3). To analyze their putative biological functions, we performed an in-silico annotation which led to the identification of intrachromosomal locations of SNPs co-localized with genes that could be involved in biotic and abiotic stress (Table 4). Further, the flanking sequences of significant SNPs were blasted against amino acid sequences from rice, sorghum, and *Brachypodium* (Supplementary Table S2). Eight QTLs on chromosome 1AL, 2AS, 2BL, 3AL, 4AL, and 5BL were linked to putative genes known to be involved in plant pathogen interactions (Table 4). The QTL on chromosome 1AL linked to a putative methyl transferase 1-associated protein 1 (DMAP1) gene, which contains the SANT/Myb domain (IPR032563) known to be involved in cell death and disease resistance. The QTL on chromosome 2AS linked to a putative a RING/FYVE/PHD-type Zinc finger gene known to regulate a superoxide-dependent signal and involved in cell death and disease resistance. The QTL on chromosome 2BL linked to a putative aarF domain-containing protein kinase 3 known to be involved in phosphorylating respiratory chain plastoquinone-NADPH and oxidative stress. The QTL on chromosome 3AL linked to a putative NADPH-quinone oxidoreductase subunit 0 genes, suggested to be involved in NADPH oxidation-reduction process. Another QTL on chromosome 2BL linked to a putative amino acid transporter (AAT) gene. AATs have been reported to be involved in active amino acid transport across cellular membranes in higher plants during various processes of plant growth, development, and nematode parasitism. The QTL on chromosome 4AL was associated with a putative Calmodulin-binding protein-like gene (BCL-2-associated athanogene 7) known to be involved in cell proliferation, growth arrest, and cell death. The QTL on chromosome 4AS linked to a putative gene coding for a retinoblastoma-binding protein 5 known to be involved in stress response. The QTL on chromosome 7BL linked to a putative elongation factor EF-2-like protein known to be induced in response to cold stress. The QTL on chromosome 5BL linked to a putative gene coding for a cell division protein; however, it is not yet functionally annotated. The other two QTLs on chromosome 3BL linked to a putative RNA polymerase II-associated and Paf1 superfamily, respectively, known to be involved in early flowering.

DISCUSSION

Breeding for resistance to CCN in wheat was initiated in the early 1970s (Brown and Ellis 1976). Later, Kimber and Feldman (1987) identified novel resistance sources in cultivated and wild wheat relatives. However, broad-spectrum resistance to CCNs in wheat is still limited.

In general, high density SNP genotyping and genome mapping enabled the performance of association studies to identify putative QTLs linked to disease resistance (Cavanagh et al. 2013). However, false associations caused by heterogeneous populations hamper the analyses (Matthies et al. 2012; Pritchard et al. 2000). A multiple QTL model which corrected for both population structure and relatedness MLM (P+K) was used in this study and helped in separating the true functional signal from false positives. Further, a multilocus analysis with cross validation controlled the high bias of explained variance and ensures reproducible results (Bauer et al. 2009; Benjamini and Yekutieli 2005). Five QTLs identified in this study on chromosomes 1AS, 2AS, 2BL, 3BL, and 5BL were previously linked to *H. avenae* resistance. Candidate genes underlying resistance to some of these QTLs, such as *Cre5/CreX* on chromosome 2A, *Cre1* on chromosome 2BL, and *CreY* on chromosome 3B were described previously (Barloy et al. 2007; Bekal et al. 1998; Jahier et al. 2001). However, genes associated to other QTLs on chromosomes 1AL and 5BL have not been reported before (Mulki et al. 2013; Singh et al. 2010). A recent GWAS study identified a QTL IWB66494 linked to marker Tdurum_contig10380_87 on chromosome 2BL conferring resistance to *Fusarium graminearum* (head blight) in spring wheat thus indicating the chromosomal region 2BL to be a hotspot for resistance alleles linked to multiple wheat diseases (Jansen 2015). However, at this stage, we cannot state if this QTL is linked to one or more resistance genes. The variation in chromosome and chromosomal location of significant QTLs identified different novel alleles at

TABLE 3. In silico annotation of significant markers and identified contigs[a]

90K iSelect SNP bead chip index	SNP ID	Marker name	NCBI			IWGSC		
			E-value	Accession	Identity %	E-value	Identity %	Wheat contigs ID
75423	IWA135	wsnp_BE443588A_Ta_2_1	7.96E-43	AK332961	100	3.5E-45	100	UCW_Tu-k25_contig_5434
53663	IWB53663	RAC875_c13116_943	6.98E-31	XM_003559795	92.31	2.37E-44	99.01	UCW_Tu-k35_contig_173; tuk21_contig_105
23232	IWB23232	Excalibur_c18966_804	9.69E-42	AK360630	99.01	2.89E-43	100	UCW_Tu-k51_contig_8215
75392	IWA94	wsnp_BE426418A_Ta_2_1	1.35E-52	HQ389836	99.17	9.82E-19	100	UCW_Tu-k55_contig_20701
5616	IWB5616	BobWhite_rep_c66630_331	6.12E-38	AK250157	96.04	1.49E-40	100	UCW_Tu-5_contig_768
66494	IWB66494	Tdurum_contig10380_87	3.17E-16	XM_004963496	100	5.57E-46	100	UCW_Tu-k31_contig_15495
67389	IWB67389	Tdurum_contig12008_803	9.69E-42	AK375083	100	2.37E-44	100	UCW_Tu-k21_contig_14859
23457	IWB23457	Excalibur_c20277_483	2.28E-43	FN645450	100	5.57E-46	100	UCW_Tu-k41_contig_8030
78435	IWA4260	wsnp_Ex_c55245_57821389	5.35E-83	AK363634	97.51	6.67E-100	100	UCW_Tu-k41_contig_14865
73556	IWB73556	Tdurum_contig82236_117	1.35E-33	AK331337	95.83	5.57E-46	100	UCW_Tu-k45_contig_4305; tuk35_contig_3982; tuk55_contig_3852
28883	IWB28883	Excalibur_c78724_434	1.87E-06	XM_003559125	100	2.89E-43	100	UCW_Tu-k35_contig_37987

[a] E, expected value; ID, identification; IWGSC, International wheat genome sequencing consortium; and NCBI, National Center for Biotechnology Information.

TABLE 4. Functional annotation of putative genes linked to *Heterodera filipjevi* resistance[a]

Marker	CHR	Putative gene	Allele	Amino acid change	Amino acid change	Type of change (I)	Type of change (II)	Putative function
wsnp_BE443588A_Ta_2_1	1AL	DNA methyltransferase 1-associated protein 1 (DMAP1) (IPR032563)	A/C	no_hit	no_hit	Transversion		MYB family of transcription factors suggested to code in controlling development, secondary metabolism, hormonal regulation and response to biotic and abiotic stress
RAC875_c13116_943	2AS	Zinc finger, RING/ FYVE/PHD-type (IPR013083)	A/C	N->K	N->K	Transversion	Nonsynonymous	DNA binding and zinc ion binding; involves in regulation of transcription, DNA-dependent, negative regulation of cell death and disease resistance, zinc-finger motifs- negative regulation of cell death, programmed cell death
Excalibur_c18966_804	2BL	aarF domain-containing protein kinase 3 (AK360630.1)	G/A	R->R	R->R	Transition		Phosphorylating respiratory chain, ubiquinone, plastoquinone-NADPH, electron transport associated with the plan response to oxidative stress
wsnp_BE426418A_Ta_2_1	3AL	NAD(P)H-quinone oxidoreductase subunit O (IPR020905)	T/C	no_hit	no_hit	Transition	Synonymous	NADH dehydrogenase complex (plastoquinone) assembly, oxidation reduction process, photosynthesis, light reaction, protein autophosphorylation, regulation of proton transport, oxidoreductase activity, acting on NAD(P)H, quinone
Bobwhite_rep_c66630_331	7BL	Elongation factor (EF)-2-like protein (XM_003574994)	T/C	R->R	R->R	Transition	Synonymous	Encodes a translation elongation factor 2-like protein, involves in cold-induced translation.
Tdurum_contig10380_87	2BL	Amino acid transporter (AAT) transmembrane family protein (IPR013057, IPR002422)	G/A	I->T	I->T	Transition	Nonsynonymous	AATs supposed to involve in amino acids transport across cellular membranes in higher plants, involves in plant growth and development, and response to pathogen and abiotic stress
Tdurum_contig12008_803	3BL	RNA polymerase II-associated, Paf (IPR007133)	T/C	I->T	I->T	Transition	Nonsynonymous	Encodes a PAF1 homolog which involves in the control of flowering time and vernalization
Excalibur_c20277_483	3BL	Paf1 superfamily, cl20260 (IPR007133)	G/A	R->R	R->R	Transition	Synonymous	Members of this family are components of the RNA polymerase II associated Paf1 complex, involves in the control of flowering time
wsnp_Ex_c55245_57821389	4AS	Retinoblastoma-binding protein 5-like/WD40-repeat-containing domain (IPR017986)	T/C	I->V	I->V	Transition	Nonsynonymous	Involves in tumor suppressor protein RB1 gene and WD40-repeat gene involves in stress tolerant
Tdurum_contig82236_117	4AL	Calmodulin-binding protein-like, BCL-2-associated athanogene 7 (no hit)	G/A	no_hit	no_hit	Transition		Involves in cell proliferation to growth arrest, cell death in yeast, mammals and plants, encodes calmodulin-binding proteins response to bacterial pathogens and inducers of defense responses

[a] CHR, chromosome; DNA, deoxyribonucleic acid; I, isoleucine; IPR, interpro; K, lysine; MYB, myeloblastosis; N, asparagine; PAF1, polymerase-associated factor 1; R, arginine; RB1, retinoblastoma 1; RNA, ribonucleic acid; T, threonine; and V, valine.

different loci (Table 2). Further, the moderate broad-sense heritability (46%) suggested a potential transmission of resistance alleles to successive generations. These novel QTLs could be used for pyramiding resistance through marker-assisted backcrossing.

Genotyping analysis revealed that the polymorphic SNPs on A, B, and D genomes were highly variable, indicating that recombination rates differ in the different regions of the wheat genome. Significant QTLs linked to *H. filipjevi* resistance were identified only in the A and B genome. The process of domestication might have resulted in a high number of effective recombination events in A and B genomes (Chao et al. 2009; Dubcovsky and Dvorak 2007). The low density SNPs in D genome could represent the historical development of hexaploid wheat (van Ginkel and Ogbonnaya 2007). Würschum et al. (2013) reported similar results with few SNPs located on the D genome in 172 European winter wheat cultivars.

Population structure and relatedness among individuals can lead to spurious associations between a candidate marker and a phenotype (Yu and Buckler 2006). Our analysis of both methods (structure (Q) and PCA (P)) yielded in very low genetic differentiation in the mapping population. We therefore conclude that the variability within the mapping panel is very low and therefore needed no further correction. As the result of structure (Q) and PCA (P) was nearly equivalent, the data were analyzed by PCA (P and P+K model) as most published analyses now concentrate on P and not Q to avoid the computationally demanding Q matrix in structure analysis (Price et al. 2006). In other studies two subpopulations were identified, e.g., with 96 wheat accessions and 81 diversified *A. tauschii* populations (Neumann et al. 2011; Sohail et al. 2012). Many other studies suggested that subpopulations existed within different mapping populations. Moreover, high marker allele frequency and low population structure justify the selection of our mapping population to perform GWAS (Myles et al. 2009).

LD is the basis of genetic association analysis discovering and mapping genes in natural populations (Wilson et al. 2004). Genetic association determines correlations between genetic variants and phenotypic differences within a population (Flint-Garcia et al. 2003). LD depends on the process of domestication, population subdivision, founding events, and selection (Rafalski and Morgante 2004). LD across wheat genome was <3 cM, a smaller distance compared with previously reported 5 cM (Crossa et al. 2007). Our finding supports the high LD in self-pollinating plants and could be explained by the different levels of historical recombination, effective recombination rate and recombination distance between the loci. However, it is reported that the extent of LD vary throughout the genome and introduction of new haplotypes from divergent population can increase the LD (Pritchard and Przeworski 2001). Neumann et al. (2011) reported that LD is not consistent across either whole genomes or single chromosomes. LD facilitates predicting marker density required for effective marker trait association. Dense marker coverage provides detailed insights into LD decay between two loci in close proximity and helps to identify regions influenced by a short, intense breeding history (Benson et al. 2012). The high density of SNP markers on genome A (<3 cM) and B (2 cM) implies more accuracy in genome wide and region specific LD compared with the D genome (>5 cM). High LD found in the A and B genome could be explained by various levels of historical recombination in the accessions and might result from favorable selection for phenotypes during breeding history by IWWIP. LD decay of <5 cM for 157 wheat landraces and 189 Canadian bread wheat accessions, and 5 to 10 cM for 93 Chinese bread wheat were reported (Belzile et al. 2007).

Our analysis revealed that pyramiding two or more QTLs would enhance the performance of resistance considerably (Fig. 6). The combination of two (1AL+7BL), three (1AL+3AL+7BL), four (1AL+2AS+2BL+3AL), or five (1AL+3AL+2BL+3AL+7B) QTLs in wheat accessions was predicted to increase the resistance response up to 74, 76, 85, and 86%, respectively. The three moderately resistant wheat accessions Olifants, Lantian 12, and T04/17 originating from different region possess the highest marker allele frequency (Table 5). Therefore, these lines are good candidates to be included into breeding strategies to develop durable resistance through marker-assisted backcrossing. Similarly, pyramiding resistance alleles to stripe rust has been utilized successfully in barley (Sun et al. 1997). In the same way, leaf rust resistance genes *Lr41*, *Lr42*, and *Lr43* and powdery mildew resistance gene *Pm1* and *Pm2* were successfully pyramided in wheat (Cox et al. 1994; Liu et al. 2000). Moreover, the additive effect of combining QTLs linked to resistance against *H. avenae* on chromosomes 1B and 6B in Trident/Molineux DH wheat population was reported (Williams et al. 2006).

Among the 11 identified putative ORFs, the functions of two candidates are well supported statistically and biologically. Annotation of the sequences flanking the QTL on chromosome on 2BL revealed an amino acid transporter (AAT), transmembrane family protein in rice (IPR013057, Os05g0586500, NP_001056462, Table 4). AATs are integral membrane proteins that transport amino acid across cellular membranes in higher plants. An amino acid permease (AtAAP6) ortholog in *A. thaliana* was found to be highly expressed in syncytia induced by *H. schachtii* (Szakasits et al. 2009). It was also reported to be involved in supplying amino acids to feeding structures induced by *Meloidogyne incognita* in *Arabidopsis* roots and was shown to be expressed in other sink tissues (Hammes et al. 2006; Marella et al. 2013; Puthoff et al. 2003). The other candidate is linked to the QTL on chromosome 2AS. It is a putative protein

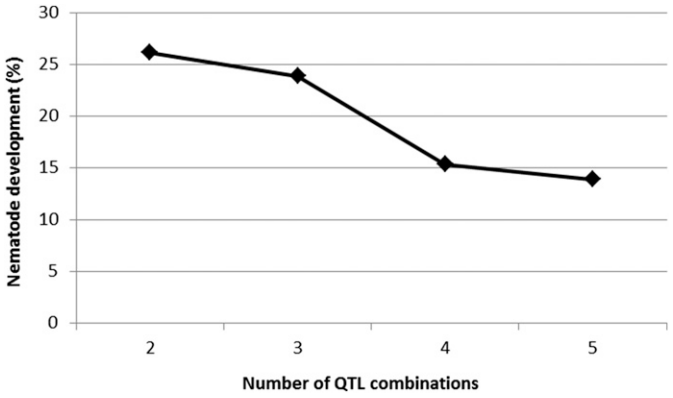

Fig. 6. Effect of quantitative trait loci (QTL) combinations on *Heterodera filipjevi* reduction in wheat accessions. Number of abscissa indicates the number of QTL allele combinations.

TABLE 5. Selection of wheat accessions based on significant markers frequency, host status, and origin[a]

Common name	Origin	Pedigree	Accessions status	Experiment 1			Experiment 2			Host status to *Heterodera filipjevi*	MAF (%)
				Cyst/plant	SD	SE	Cyst/plant	SD	SE		
Bezostaya	Russia	LUT17/SRS2	Cultivar	35.0	2.5	1.2	25.1	3.9	1.3	HS	83
T04/17	South Africa	–	Breeding	4.3	2.1	1.0	5.0	2.7	0.9	MR	92
Olifants	South Africa	–	Cultivar	5.5	4.6	2.3	8.3	2.4	0.8	MR	75
Lantian 12	China	Qingnong 4/Xiannong-4 Pedigree	Cultivar	6.0	1.5	0.8	7.5	2.3	0.8	MR	83

[a] HS, highly susceptible; MAF, marker allele frequency; MR, moderately resistance; SD, standard deviation; and SE, standard error.

(LOC100822072/*Brachypodium distachyon*: XP_003559843) with a Zinc finger, RING/FYVE/PHD-type (IPR013083) and DDT superfamily domain (IPR018501, Table 4). Zinc finger family proteins are reported to be involved in superoxide-dependent signaling and negative regulation of cell death in *A. thaliana* (Dietrich et al. 1997; Kang 2013). Currently, the functions of the identified gene candidates are analyzed experimentally to study their role in the interaction with *H. filipjevi* in more detail. However, even without knowing their detailed function, the identified QTLs can already be used for novel approaches to achieve resistance against *H. filipjevi* through marker-assisted breeding.

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GWAS in wheat identifies an amino acid transporter gene orthologue in Arabidopsis promoting nematode susceptibility

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Abstract

The cereal cyst nematode *Heterodera filipjevi* causes significant crop losses in cereals. Nematode juveniles invade epidermal and cortical cells, migrate intracellularly towards the vascular cylinder and induce enlarged syncytial feeding structures. Syncytia are the only source of nutrients throughout the nematode life cycle. A genome wide association study (GWAS) of 161 wheat accessions identified a susceptibility-related quantitative trait locus (QTL) linked to *H. filipjevi*. *In-silico* annotation of associated markers revealed a genomic region of 74.9cM on chromosome 2BL containing an orthologue (*TaAAT*) to amino acid permease 6 (*AtAAP6*) in *A. thaliana*. A possible role of *AtAAP6* in the parasitism of cyst nematode *Heterodera schachtii* was previously reported in Arabidopsis. It is upregulated in syncytia as shown in genechip analysis, *in situ* rtPCR, and infection assays with promoter::GUS lines. Here, we characterized the role of *AtAAP6* in nematode parasitism using a T-DNA mutant line and expression analysis. The nematode infection assay revealed a significant reduction in average number of females, female size, and size of female associated syncytia in the mutant line compared to wild type. Our results confirm the high abundance of

AtAAP6 in syncytia under nematode infestation at 1, 10 and 15 days post infection. Similarly, a stronger expression of *TaAAT* in infected roots of two susceptible wheat accessions were recorded compared to uninfected root. Infected and uninfected roots of two resistant wheat accessions showed no change in expression. Further, we analyzed the amino acid sequence of *AtAAP6* gene in each 3 resistant and susceptible *Arabidopsis* accessions and found a "glycine" to be substituted by "alanine" in the susceptible accessions. Our findings suggest that *AtAAP6* and *TaAAT* are important factors for nematode parasitism.

Keywords: Amino acid permeases, Arabidopsis, cyst nematode, syncytia, wheat

Introduction

The cyst nematodes *Heterodera filipjevi* and *H. schachtii* are sedentary biotrophic obligate parasites that cause significant yield losses. Nematode juveniles (J2s) invade epidermal and cortical cells, migrate intracellularly towards the vascular cylinder, and they become sedentary. These nematodes induce highly specialized feeding structure in their host root. These feeding structures accumulate nutrients to optimize the nutrient requirement for nematodes (Grundler and Hoffmann, 1994). Syncytia are the only source of nutrients throughout the nematode life cycle. In compatible interactions, the syncytium continues to develop and expand, J2 become sedentary, and undergo several molts before turning into adults. Adult males leave the roots after mating with females, while female nematodes continue feed from the syncytium until egg development is completed. During syncytium induction the affected root cell undergoes drastic morphological and physiological changes. The nuclei increase in size and smooth endoplasmic reticulum, ribosomes, mitochondria and other organelles proliferation (Golinowski et al., 1996). The levels of various amino acids, phosphorylated metabolites, sugars and organic acids in syncytia increased substantially (Hofmann et al.,2010). Syncytia are strong sinks in the plant's transport system and most of

the sinks are connected with the source tissue through the phloem (Böckenhoff et al., 1996). The composition and availability of nutrients in sinks are highly dependent on phloem transport and solute composition. The connectivity between phloem and syncytia has previously studied on syncytia induced upon *H. schachtii* on Arabidopsis roots (Grundler and Hofmann, 2011). Syncytia are symplasmically isolated in early nematode development thus facilitating cellular reorganization (Hofmann and Grundler, 2008). In later stages of nematode development, plasmodesmata are opened to the phloem elements and enhance symplasmic transport (Böckenhoff and Grundler, 1994). It is previously reported that the amino acid and sugar are transferred apoplastically from vascular tissues to the galls and root knot nematodes uptake the amino acid from gall (Marella et al., 2013). Similarly, the importance of sugar transporter genes for syncytia formation and *H. schachtii* development in Arabidopsis were shown (Hofmann et al., 2009).

Amino acids are the currency of nitrogen exchange in plants and constitute a major component used for cellular growth and differentiation (Ortiz-Lopez et al., 2000; Rentsch et al., 1998). They represent the principal long-distance transport of organic nitrogen and are distributed through xylem and phloem (Wipf et al., 2002). Response to pathogen infection leads to changes in gene expression that involve in amino acid metabolism and transport. Thus, the regulation and transport of amino acids is critical for plant adaptation, development and defense (Tegeder, 2012). Amino acid transporters (*AATs*) are integral membrane proteins that transport metabolites from source to sink tissue across cellular membranes in higher plants. The presence of PF01490 (Aa_trans) and PF00324 (AA_permease) domains distinguishes the *AAT* genes in plants (Cheng et al., 2016). The Aa_trans protein is reported to encode a vesicular amino butyric acid (GABA) transporter, while AA_permeases code for membrane permeases that transport amino acids into the cell. Arabidopsis and rice contain a

large number of different *AATs* in several gene families: more than 63 *AAT* genes were found in Arabidopsis and 85 *AAT* genes in rice (Rentsch et al., 2007; Zhao et al., 2012).

Based on the function and sequence homology, *AAT*s are grouped into two families: the amino acid/auxin permease (*AAAP*) family and the amino acid-polyamine-choline (*APC*) family (Fischer et al., 1998; Wipf et al., 2002). The *AAAP* family is further sub-divided into six sub-families: amino acid permeases (*AAPs)*, lysine-histidine transporters (*LHTs*), proline transporters (*ProTs*), γ-aminobutyric acid (*GATs*), aromatic and neutral amino acids (*ANTs*), and indole-3-acetic acid (*AUX*s) (Hunt et al., 2010; Okumoto and Pilot, 2011; Saier et al., 2009). The other *APC* superfamily consists of transporters for cationic (*CATs*), L-type amino acids (*LATs*), and GABA permeases (Tegeder, 2012). Among *AATs*, *AAP* subfamilies are the most studied amino acid transporters for nematode parasitism (Tegeder and Rentsch 2010). The *AtAAP1*, *AtAAP3*, *AtAAP6*, and *AtAAP7* are upregulated upon root knot nematode infection (Barcala et al. 2010; Hammes et al. 2005). *AtAAP6* was found to be upregulated upon cyst nematodes infection (Puthoff et al. 2003, 2007). This was further confirmed by genechip analysis, *in situ* rtPCR, and infection assays with promoter::GUS lines (Elashry et al., 2013; Szakasits et al., 2009). Though, many *AATs* in Arabidopsis (*AtAAP1-AtAAP8*) and rice (*OsAAP1-OsAAP19*) are functionally characterized, much less is known in wheat (Okumoto et al., 2002; Zhao et al., 2012).

Sequencing of the wheat genome and development of high density marker genotyping has made it possible to predict genes underlying QTLs. However, current wheat cultivars (*Triticum aestivum* L.) represent only a small fraction of the wheat gene pool and annotation for large number of genes and proteins is limited. In the past, the model organisms *Arabidopsis thaliana* and *Oryza sativa* have been used for functional annotation of genes particularly for newly sequenced plant groups (Clarke et al., 2003; Peng et al., 2015; Proietti et al., 2010). Our previous GWAS analysis revealed a QTL linked to the development of *H.*

filipjevi (Pariyar et al., 2016a). *In-silico* annotation resulted in of a significant marker revealed a genomic region of 74.9cM on chromosome 2BL associated to a putative amino acid transporter gene. Using a comparative genomic approach, we identified an orthologue gene "*AtAAP6"* in *A. thaliana*. This gene has been described to be activated in nematode induced syncytia (Szakasits et al., 2009). However, its function and regulation during nematode parasitism is still not clear. Here, we investigated the role of *AtAAP6* during the host nematode parasitism using T-DNA mutant line. The expression of *AtAAP6* gene in syncytia at different time intervals was analyzed. In an infection assay including several Arabidopsis accessions, we found variations in host susceptibility. Thus, we hypothesize this variation is based on amino acid exchange in the *AtAAP6* response.

Materials and methods

In-silico gene identification

To identify the gene linked to the QTL, we first blasted the flanking sequences of single nucleotide polymorphic marker against gene models of *Brachypodium distachyon, O. sativa*, and *Sorghum bicolor* available at International Wheat Genome Sequencing Consortium (IWGSC), the Institute for Genomic Research (TIGR) Wheat Genome Annotation, and National Center for Biotechnology Information (NCBI). We identified the full open reading frame (ORF) at significant marker location at 74.9cM on chromosome 2BL (Pariyar et al., 2016a). The computational analysis revealed *AAT*, transmembrane family protein linked to the ORF. The full-length complementary DNA (cDNA) sequence of ORF predicted a putative uncharacterized protein (NCBI accession no. AK334879). The genomic sequences were translated into amino acid sequences using ExPASy (Expert Protein Analysis System) translate tool and compared with protein sequences available in data base using Clustal Omega multiple sequence alignment tool (http://www.ebi.ac.uk/Tools/msa/clustalo).

Plant material and growth conditions

Arabidopsis plants (mutant, wild-type (WT)-Colombia-0, and accessions) were grown in petri dishes containing 0.2% knop medium supplemented with 2% sucrose (Sijmons et al., 1991). Seeds were surface-sterilized by soaking them in 5% (W/V) sodium hypochlorite for 5 min, and subsequently washed three times with sterile water. The knock out function mutant analyzed for function of *AtAAP6* in nematode parasitism was SALK_013231 (N661540) and the other six Arabidopsis accessions analyzed for amino acid sequence variation in of *AtAAP6* listed in Table S1 (Alonso et al., 2003). The T-DNA mutant seeds were obtained from Nottingham Arabidopsis Stock Centre. T-DNA insertion were confirmed by PCR using gene specific primers in combination with a T-DNA left border primer, followed by gel electrophoresis. Primer sequences were obtained from the SIGnAL website (http: //signal.salk.edu/tdnaprimers.2.html).

Nematode infection assays

Cysts of *H. schachtii* were collected from roots of mustard plants *Sinapis alba* cv. Albatros that are cultured in vitro (Sijmons et al., 1991). Hatching of J2s from the cysts were stimulated by soaking them in 3mM% of zinc chloride ($ZnCl_2$) at 25°C for 4 days. J2s were then washed three times in sterile water, and were re-suspended in 0.5% w/v GelRite to enhance the nematode infection (Duchefa). Each 10 days old plants were inoculated with freshly hatched 60 J2s. Two plants per plate were used and experiments were repeated at three times with 20 replications. The petri plates were sealed and grown in a growth chamber at 25°C under a 16 h light/8 h dark cycle. The numbers of male and female nematodes were counted at 14 days post inoculation (dpi). The average size of female nematode and associated syncytia (mm^2) in *AtAAP6* knock out-mutant was measured in longitudinal optical sections as described previously compared with wild-type at 15 dpi (Siddique et al., 2009). Around, 60

syncytia and associated female nematodes were randomly selected and photographed with a Leica DM2000 dissection microscope. The Diskus Kontour tool was used to outline the female and syncytia. The area was calculated using LAS software (Leica Microsystems). The *H. filipjevi* infection assay on wheat accessions were performed according to Pariyar et al., (2016b).

Statistical analysis

Data of the bioassay experiments were subjected to one way analysis of variance (ANOVA). Results are considered significant based on Duncan's multiple comparison tests at $P< 0.05$ using Sigma plot 12.5 Software. Regarding q-PCR data, significant differential expression between infected plants and control tissue was determined by student's *t*-test at $P \leq 0.05$ (Pfaffl, 2001).

DNA isolation and PCR

Total genomic DNA from individual leaf from both wild type and mutant was isolated using modified CTAB method (Murray and Thompson, 1980; Sambrook and Russell, 2001). Quality and quantity of the DNA was analyzed by optical density (OD) at λ=260 measured in Nano Drop 2000C spectrophotometer (Peqlab, Erlangen, Germany), and DNA purity was determined by the ratio of absorptions at A260/A280. Gene amplification for mutant was performed in 25µl reactions containing 100ng/µl of DNA, following PCR scheme of sterile deionized water: 16.9µl, 5X Buffer: 5µl, 10mM dNTP mix: 0.5µl, forward, reverse primer, and left boarder primer (pmol/ul): 0.5µl, *Taq* DNA Polymerase: 0.2µl, and DNA: 1µl. Annealing temperatures for each reaction were optimized using a C1000^{TM} Thermal Cycler (Bio-Rad 1000 Series Thermal Cycling Platform, USA). The annealing temperature with desired polymerase chain reaction (PCR) product range was selected. PCR was performed, 4

min at 94°C, followed by 35 cycles of 1 min 94°C, 1 min at 59°C, 1min at 72°C and final extension of 5 min at 72°C. The PCR products were stained on 1% agarose gels containing 100 ml of 1×TBE (Tris base, Boric acid, EDTA) buffer with 5µl of peqGreen at 80°V for 60 min. for visualization and photographed under ultraviolet light using Gel Documentation System in BIO-RAD, Gel Doc^{TM} XR. Primers sequences for the candidate genes and product size are designed by Primer3Plus software (Table 1).

Table 1. Primers used for polymerase chain reaction (PCR) and reverse transcriptase polymerase chain reaction (qPCR)

Primers	Gene	Gene locus	Product (bp)	Annealing temp.	Efficiency (%)	Plant
CACGGTTCAACAACATCCAG	UBQ-FP	At4g05320	180	59	2.1	Arabidopsis
TGAAGACCCTGACTGGGAAG	UBQ-FP					
CAACACTGACAGGAGTTACGGT	AtAAP6-FP	At5g49630	137	55	2.1	Arabidopsis
TTCGCGCTCTGGCTCTCTA	AtAAP6-RP					
TGCATGGAACTTGTGTTCTTG	Salk_013231C-RP	At5g49630	1163	60	PCR	T-DNA insertion
ATTGCATTTGCCTACGCATAC	Salk_013231C-LP					
ATTTTGCCGATTTCGGAAC	LBb1.3		568-868	60		
GTGGAACTGGCTCTGGC	qTubulin-FP		234	55	1.9	Wheat
CGCTCAATGTCAAGGGA	qTubulin-RP					
GACATCGCTCGCACAAATCT	TaAAT-FP		187	59	2.0	Wheat
GCATGCCAGCTGAATGTTCT	TaAAT-RP					
TCTTCATCTACGCCATGCTG	TaAAT_FP		1213	60	PCR	Wheat
GCCACACACAAGACACAACC	TaAAT_RP					

*LBb1.3 Left border Primer, UBQ ubiquitin, temp. Tempreture, FR forward primer, RP reverse primer, bp basepairs, TaAAT *Triticum aestivum* amino acid transporter

RNA isolation and real-time PCR

To determine abundance of *AtAAP6* gene in nematode parasitism, total RNA was isolated from 20 mg of the uninfected root section and syncytia of Arabidopsis (WT) plants 1, 10 and 15 dpi according to manufacturer's instructions and DNA digestion with DNase I (Qiagen) was performed. The uninfected root segments were used as a control. In case of wheat, total RNA was isolated from 20 mg of the uninfected root and infected root at 10 dpi. The uninfected root was used as a control. The quantity of RNA was analyzed using a Nanodrop 2000c Spectrophotometer and cDNA was synthesized using cDNA Reverse Transcription Kit

(Invitrogen). To assess RNA integrity, 6µl of the total RNA was mixed with 2µl 6x gel loading dye and loaded on a 1% agarose gel and were stained on 1% agarose gels containing 100 ml of 1×TBE (Tris base, Boric acid, EDTA) buffer with 5µl of peqGreen at 80°V for 60 min. and photographed under ultraviolet light using Gel Documentation System in BIO-RAD, Gel Doc™ XR. RNA concentration was measured photometrical at 280nm using the Nanodrop 2000C spectrophotometer (Peqlab, Erlangen, Germany). To maintain the homogeneity, 1 µg of clean and stable RNA was used to synthesis complementary DNA (cDNA) (Schmittgen and Livak, 2008). First strand cDNA was synthesized according to the "high capacity cDNA Reverse Transcription Kit" (Applied Biosystems, Darmstadt, Germany) following the manufacturer's protocol. The cDNA was synthesized using 10 x RT buffer 2µl, dNTP mix (25 mM) 2µl, Random primers (10 mM) 2 µl, RNAase inhibitor (1U/µl) 1µl, Reverse transcriptase (5 U/µl) 1 µl, RNA (1µg) 1µl, sterile distilled water, 11µl in 20 µl total volume using T gradient PCR cycler (Biometra GmbH, Göttingen, Germany). The PCR reaction for cDNA synthesis was performed following protocol (Table 2).

Table 2. Reverse transcription (RT) reaction

Stage 1	Stage 2	Stage 3	Stage 4	
Temperature (°C)	25 °C	37°C	85°C	12°C
Time (minutes)	10 min	120 min	5 min	∞

The samples were analyzed using quantitative real-time PCR (qPCR) containing 10µl of Fast SYBR Green Master Mix (Applied BioSystems), 0.5µl of each forward and reverse primers (10 mM), 1µl of complementary DNA (cDNA), and 8µl water in 20µl total volume. The Arabidopsis gene ubiquitin (UBQ: Unigene accession: At4g05320) was used as internal control (Giménez et al., 2011; Simonetti et al., 2010). The specificity of the primers was analyzed by standard PCR. The PCR amplification efficiency was determined for each primer combination by the slope of the standard curve obtained by plotting the fluorescence versus

given concentration of a mixture of all sample cDNA (ranging from 1:1 to 1:5000 dilution of the cDNA mixture sample) using the equation: $E = 10^{(-1/slope)} - 1$ (Pfaffl, 2001). The list of tested and reference gene, their respective primers sequences were shown in Table 1. The analysis was performed in a MicroAmp® fast optical 96 well reaction plate (Applied Biosynthesis, Darmstadt, Germany), with an ABI Step One Plus TM Real Time PCR System (Applied Biosynthesis, Darmstadt, Germany). The PCR reaction was conducted in 40 cycles: 95°C for 10 min, each cycle 95°C for 15s, 60°C for 60s. Changes in transcript abundance were calculated using $\Delta\Delta C_t$ method (Pfaffl, 2001; Schmittgen and Livak, 2008). Three technical replicates were used.

Analysis of amino acid sequence variation AtAAP6 gene in different Arabidopsis accessions

To analyze the variation in amino acid sequence of *AtAAP6* in ORF, three highly susceptible and lowly susceptible Arabidopsis accessions were obtained from Salk Arabidopsis 1001 genomes-SIGnAl data base (Table S1). These accessions were originated from various regions of Europe. The susceptibility reactions of these accessions to sugar beet cyst nematode *H. schachtii* were analyzed. The amino acid sequences of the *AtAAP6* gene were obtained from Arabidopsis 1,001 Genomes-SIGnAL project and then compared using Clustal Omega multiple sequence alignment tools. The amino acid sequence of *AtAAP6* gene of wild type Arabidopsis plant “Col-0” was used as control.

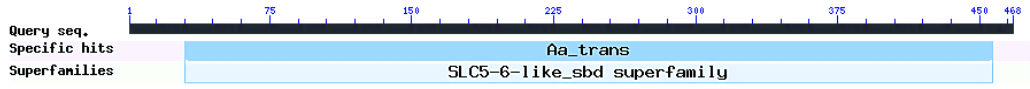
Results

In-silico identification of amino acid transporter gene

We identified the full ORF at significant marker location at 74.9cM on chromosome 2BL. The DNA coding sequences, protein sequences and Gene Ontology (GO) vocabulary for functional annotation of amino acid transporter gene from Ensembl Plants data base for *T. aestivum* and *A. thaliana* were downloaded. The ORF in wheat linked to amino acid transporter transmembrane protein in rice (IPR013057, Os05g0586500, NP_001056462). The genomic DNA sequence at significant QTL region linked to a putative *AAT* gene that has a full-length complementary DNA (cDNA) sequence AK334879.1. The alignment of the full-length cDNA sequence of AK334879.1 encodes a putative transmembrane *AAT* protein belonging to the *APC* superfamily and has a PF01490 (Aa_trans) consensus domain (Fig. 1) (http://pfam.janelia.org).

By comparing amino acid sequences and domains presence, an Arabidopsis orthologue gene "*AtAAP6*" was identified. Aa_trans domains in both plants contain SLC5-6-like_sbd super family protein. Aa_trans region has reported to be found in many amino acid transporters including UNC-47 and MTR. UNC-47 encodes a vesicular amino butyric acid (GABA) transporter, and predicted to have 10 transmembrane domains (McIntire et al., 1997). MTR is an N system amino acid transporter system protein involved in methyl tryptophan resistance. The other members of this family include proline transporters and amino acid permeases.

```
TaAAT     EKRQATYGKGAARAPEVSVEAGNGRESDWLDDDGRPRRSGTVWTASAHIITAVIGSGVLSLAWAIAQ
AtAAP6    EKKK-SF------VEQSFPEHEIGDTNKNFDEDGRDKRTG-TWTGSAHIITAVIGSGVLSLAWAIAQ
          **:: ::      . :   *   *  .. :*:*** :*:* .**.**********************
TaAAT     LGWVAGPAILLFALVIYYTSTLLAECYRSGDPETGKRNYTYDAVRSYLPGTKVKLCGVIQYANLVGV
AtAAP6    LGWVAGPAVLAFSFITYFTST-LADCYRSPDPVTGKRNYTYEVVRSYLGGRKVQLCGLAQYGNLIGI
          ********:* *::: *:*** **:**** ** ********:.***** * **:***: **.**:*:
TaAAT     AIGYTIAASISRAIGRADCFHYHDVRGRSGKDECKSSSNPYIVFGVVQILFSQIPDFDQIWWLSIVA
AtAAP6    TIGYTITASISVAVKRSNCFHKNG-----HNVKCATSNTPFIIFAIIQIILSQIPNFHNLSWLSILA
          :*****:**** *: *::*** .       : :* :*..*:*:*.::**::****:*.:: ****:*
TaAAT     AVSFTYSTIGLGLGIAQTVANGI--QGSLTGLSVGPGVT-SQKVWRSLQAFGNIAFAYSYSIILIEI
AtAAP6    AVSFCYASIGVGLSIAKAAGGGEHVRTTLTGVTVGIDVSGAEKIWRTFQAIGDIAFAYAYSTVLIEI
          **** *::**:**.**::.. *   : :***::**  *:::*:**::**:*:*****:** :*****
TaAAT     QDTVKAPPPSEAKVQKATGISVATTTVFYLCGC-GYAAFGDAAPDNLLTGFGFYEPFWLLDVANAAI
AtAAP6    QDTLKAGPPSENKAKRASLVGVSTTTFFYLCGCVGYAAFGNDAPGNFLTGFGFYEPFWLIDFANVCI
          **:** **** *.::*: :.*:***.******* ******: ** *:************:*.**..*
TaAAT     VLHLVGAYQVFCQPLFAFVEKWAAARWPDSAFIARELR-----VGPFAISVFRLTWRTAFVCLTTVV
AtAAP6    AVHLIGAYQVFCQPIFQFVESQSAKRWPDNKFITGEYKIHVPCCGDFSINFLRLVWRTSYVVVTAVV
          .:**:**********:* ***. :* ****. **: * :      * *:*..:**.***::* :*:**
TaAAT     SLLPFFGDVVGLLGAVAFWPLTVYFPVEYIVQRGVRRGSTRWVCLQ-LSAACLVVSVAAAAGSIADV
AtAAP6    AIFPFFNDFLGLIGAASFWPLTVYFPIEHIAQKKIPKFSFTWTWLKILSWTCFIVSLVAAAGSVQGL
          :::*** *.:**:**.:*********:*:*.*: : : *  *. *: ** :*::**:.*****:  :
TaAAT     IGELKEYRPFSG-
AtAAP6    IQSLKDFKPFQAP
```



TaAAT: domain interval: 38-460, e-value: 2.40e- 990

AtAAP6: domain interval: 38-460, e-value: 1.23e-111

Fig. 1. Sequence alignment of TaAAT and AtAAP6 including conserved domain Aa_trans and SLC5-6 like_sbd superfamily protein. The underlined bold letters indicate the Aa_trans domain in wheat and Arabidopsis encoded by amino acids, *e-value = expected value.

Identification of AtAAP6 mutants and expression analysis

To characterize the role of *AtAAP6* in nematode parasitism, we first screened for *AtAAP6* knock-out mutant (SALK_013231) from Arabidopsis info data base and selected. The T-DNA insertion mutant was confirmed using PCR gene-specific primers (Fig. S1B). Expression analysis of *AtAAP6* mutant and wild-type plant using qPCR revealed that the SALK_013231 mutant is *AtAAP6* knockout homozygous line (Fig. S1C).

AtAAP6 is important for syncytium and nematode development

To characterize the role of *AtAAP6* in cyst nematode parasitism, we screened *AtAAP6* knock-out mutant in nematode infection assays. We found significantly decreased number of female in mutant plants compared to Col-0 (Fig. 2). We also found a significant decrease in average size of female nematodes, and associated syncytia in mutant (Fig. 3).

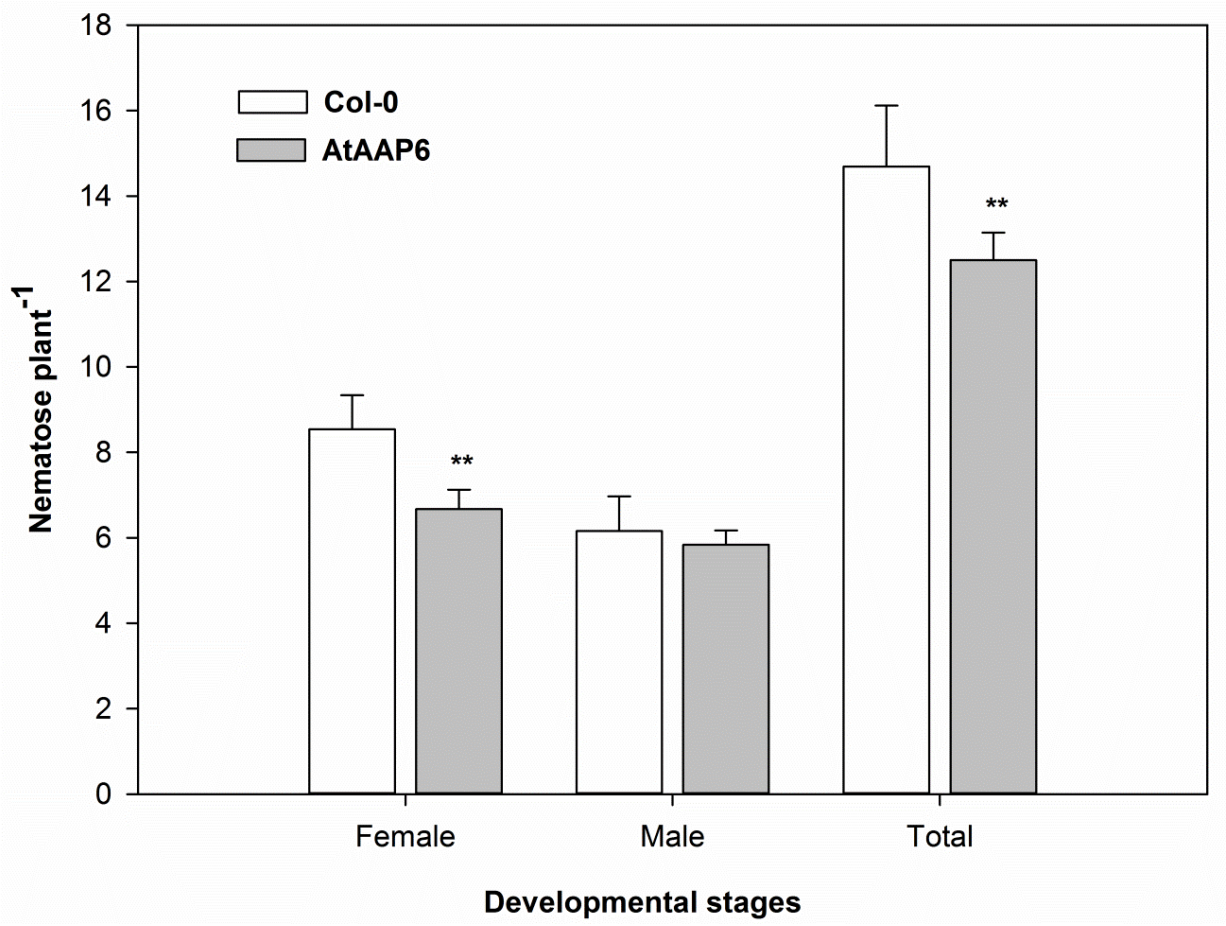


Fig. 2. Average numbers of nematode in AtAAP6 gene knockout mutant compared with wild-type Arabidopsis Col-0 at 14 days post infection with *H. schachtii*. Data were analyzed using one way ANOVA at ($P \leq 0.05$, n = 30) and Holm-Sidak test. Asterisks represent statistically significant difference to corresponding Col-0. **P<0.01. Bar indicates the standard error of the mean.

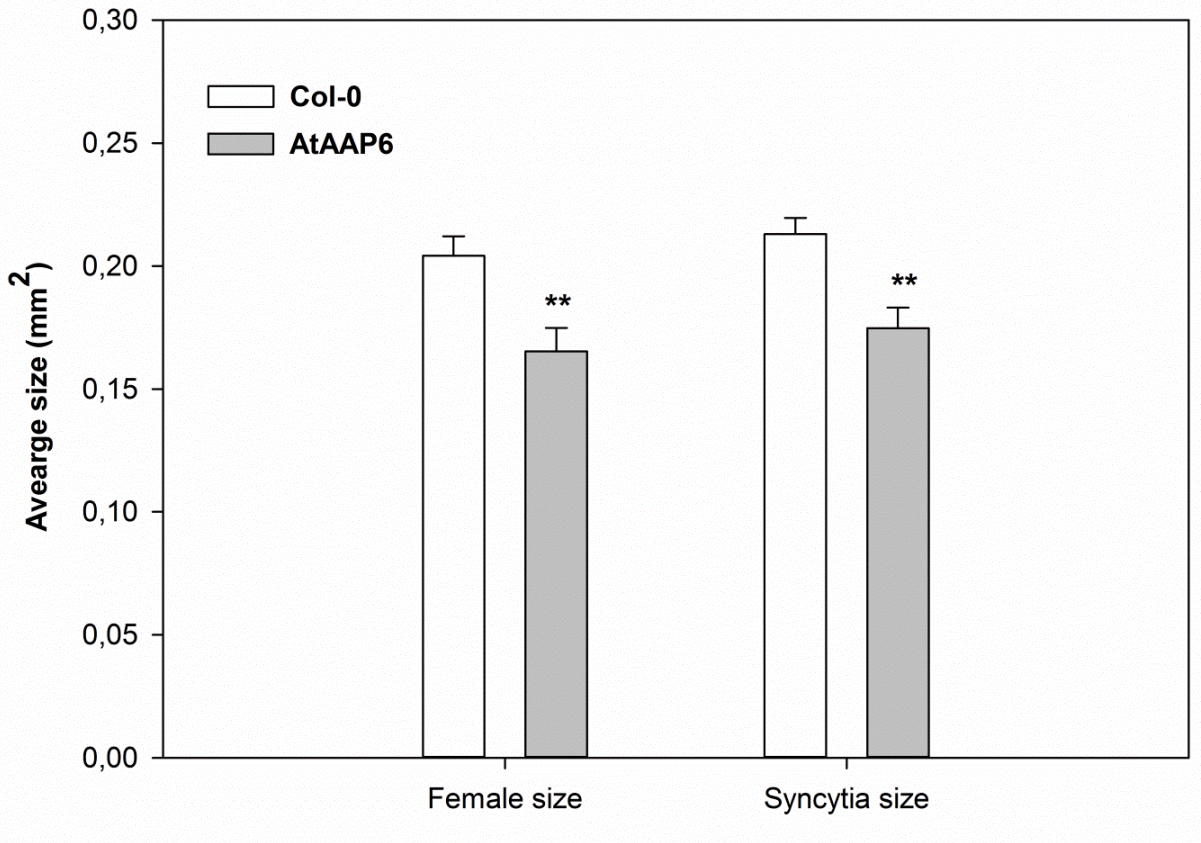


Fig. 3. Average size of female and syncytium produced by female of *H. schachtii* (mm^2) in *AtAAP6* knockout mutant compared with wild-type Arabidopsis Col-0 at 15 days post-infection. Data were analyzed using one way ANOVA at ($P \leq 0.05$, n = 60) and Holm-Sidak test. Asterisks represent statistically significant difference to corresponding Col-0. **P< 0.01. Bar indicates the standard error of the mean.

Expression of AtAAP6 genes in syncytia

The results of a qPCR using RNA isolated from syncytia showed that *AtAAP6* gene was significantly up-regulated in the syncytial samples at 1, 10, and 15 days post *H. schachtii* infection compared to uninfected root (Fig. 4). The results indicated that the expression of *AtAAP6* gradually increased with nematode infection and significantly progress with nematode development and saturated at 10 dpi. The results indicated that the expression of *AtAAP6* gene is initiated on the nematode early infestation and progress with nematode

development. A strong expression of *AtAAP6* gene in syncytia during nematode development suggested that it is an important source of amino acid transporter for nematode infection and development.

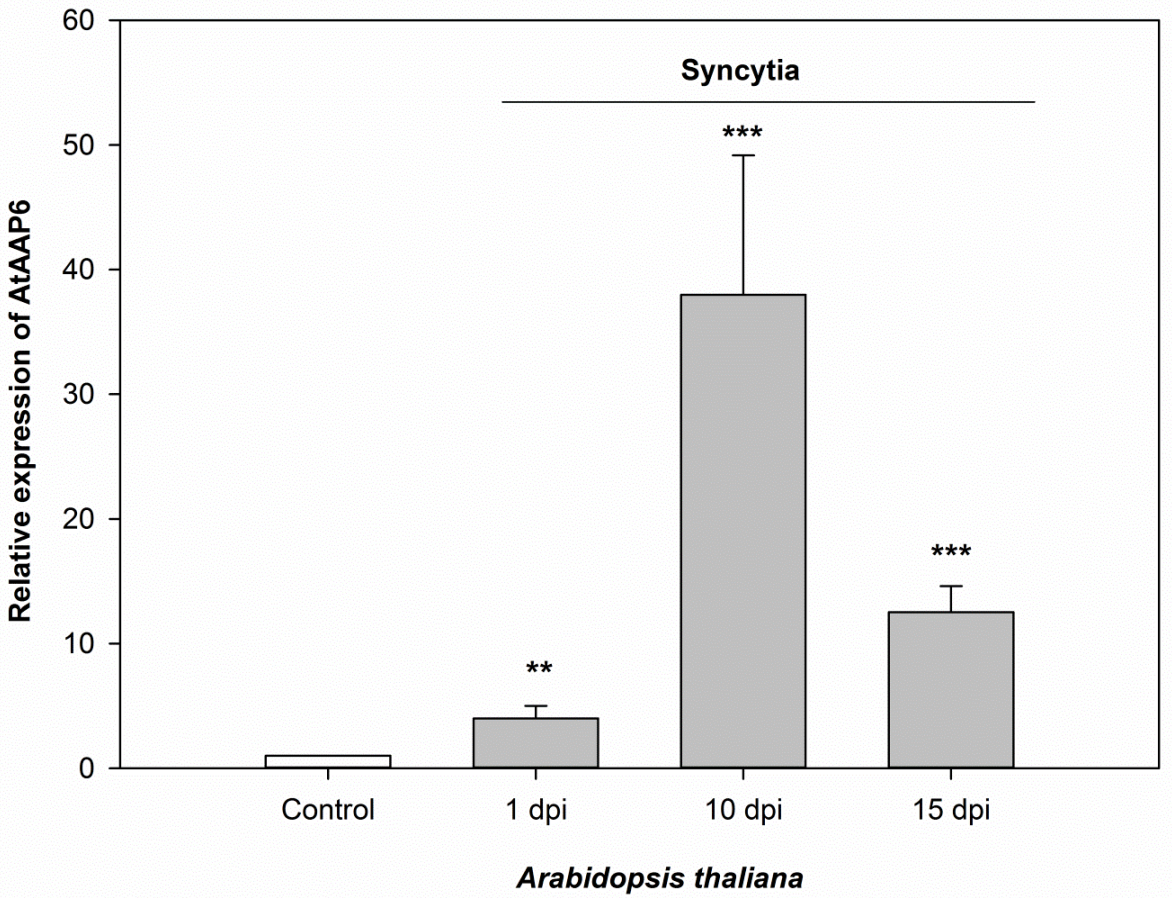


Fig. 4. qPCR analysis of AtAAP6 gene regulation in syncytia compared to uninfected root at different time points. Data were analyzed using one way ANOVA at ($P \leq 0.05$, n = 3) and Holm-Sidak test. Asterisks represent statistically significant difference to corresponding Col-0. **P<0.01 and ***P<0.001. Bar indicates the standard error of the mean. The experiment was repeated three times. The gene expression levels were normalized to the endogenous control gene UBQ and the fold change values were calculated by using the $\Delta\Delta C_t$ method.

Analysis of variation among AtAAP6 gene in different Arabidopsis accessions

The results of the infection assay revealed a significant higher number of males in the lowly susceptible accessions Uk-1, Ty-0 and Ta-0 compared to highly susceptible accessions Jm-0, Mc-0 and Zdr-1, and control line Col-0 (Fig. 5). However, no significant differences in sizes of female and female associated syncytia in all accessions were found. These accessions were obtained from Salk Arabidopsis 1001 genomes-SIGnAl data base.

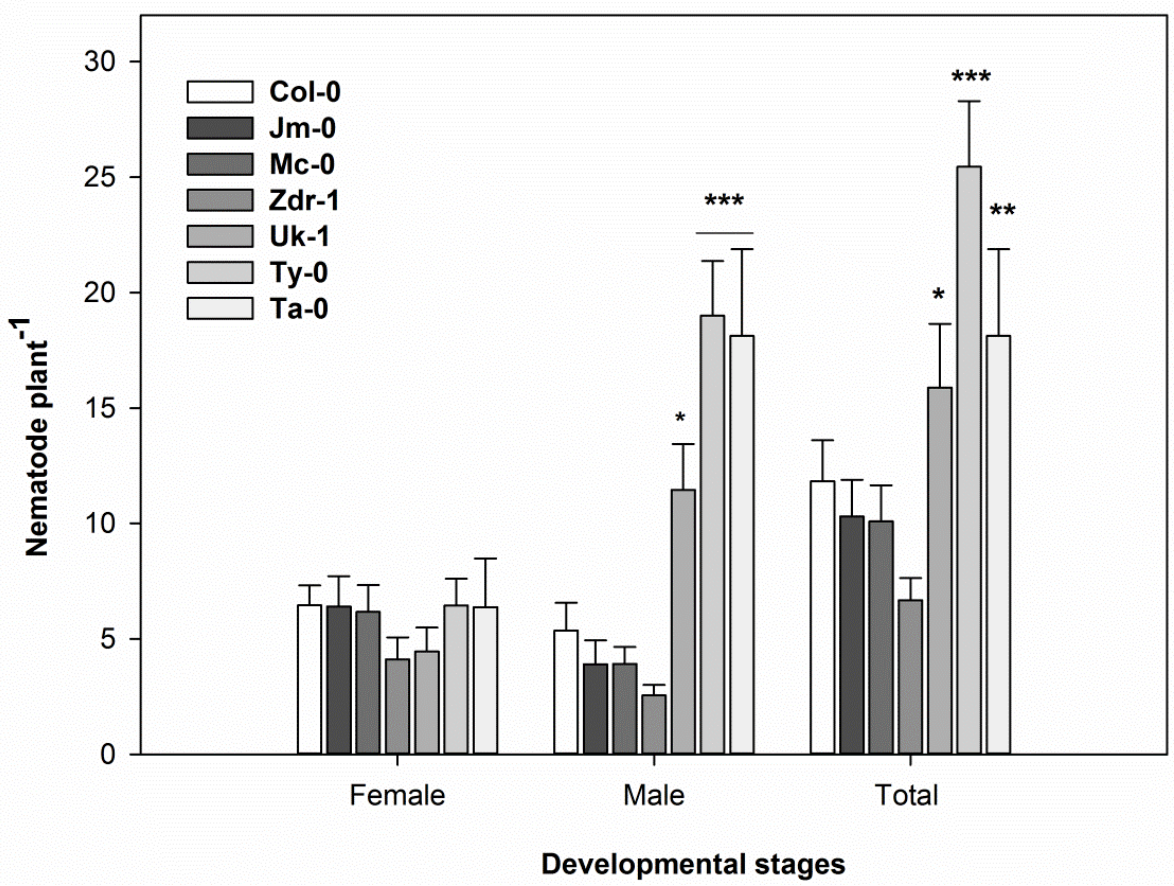


Fig. 5. Nematode development in various Arabidopsis accessions compared with Col-0 at 14 days post infection with *H. schachtii*. Data were analyzed using one way ANOVA at ($P \leq$ 0.05, n = 20) and Holm-Sidak test. Asterisks (*) represent statistically significant difference to corresponding Col-0. *P<0.05, **P<0.01, and ***P<0.001. Bar indicates the standard error of the mean. Uk-1, Ty-0 and Ta-0 represent lowly susceptibility accessions, and Mc-0, Zdr-1 and Jm-0 represent highly susceptible accessions. Col-0 is used as control.

We further analyzed the amino acid sequences of *AtAAP6* ORF in these accessions for variation. We found a single amino acid glycine "G" is substituted by alanine "A" at position of 207 in ORF region (Fig. 6, Fig. S2). Further, we compare the nucleotide sequence of the promotor region of *AtAAP6* between lowly and highly susceptible accessions and found no clear mutation (Fig. S3). However, there were random changes in nucleotide sequence of promotor regions at position 27, cytosine replaced by adenine, at position 204, adenine replaced by cytosine, and at position 291, thymine replaced by adenine among lowly susceptible and highly susceptible Arabidopsis accessions respectively.

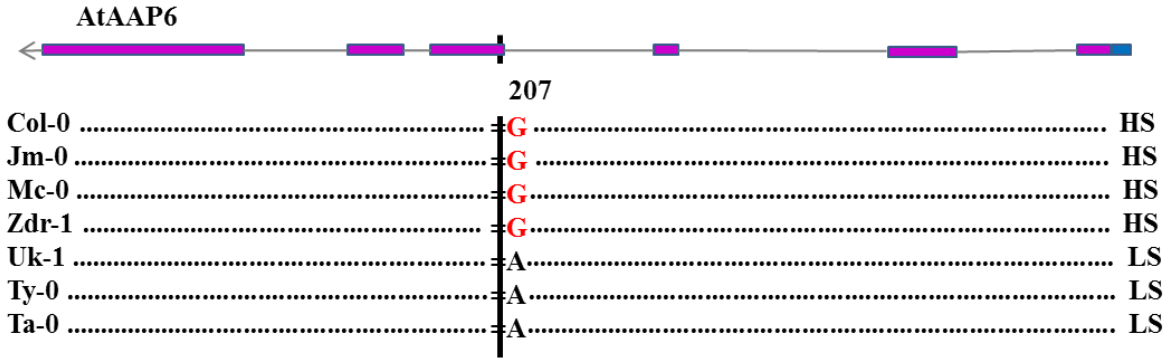


Fig. 6. Comparison of AtAAP6 ORF amino acid sequences in various Arabidopsis accessions. Vertical line indicates the position of the amino acid substitution, where glycine "G" is replaced by alanine "A" at position 207. *Pink box: exon, black line: intron, HS: highly susceptible accessions, LS: lowly susceptible accessions, and (**....**): similar amino acid sequences. Uk-1, Ty-0 and Ta-0 represent lowly susceptibility accessions, and Mc-0, Zdr-1 and Jm-0 represent highly susceptible accessions. Col-0 is used as control.

Discussion

AATs play a crucial role in transporting amino acid across cellular membranes. Many *AATs* were identified and characterized in plant species, however, very little is known about *AATs* in wheat. In fact, current wheat cultivars represent only a small fraction of the wheat gene pool and large scale annotation of genes and proteins is still limited. However, the recent

release of the wheat genome sequence provides an opportunity to compare genes and their functions by assessment of sequence similarities with genes in model plants. A large number of potential *AATs* from many different plants were identified via functional analysis and sequence homology in model plants such as Arabidopsis and rice (Rentsch et al., 1998; Wipf et al., 2002; Zhao et al., 2012). Both genomes are used to find orthologue in wheat (Clarke et al., 2003; Kim et al., 2014; Tulpan et al., 2015). We therefore used a GWAS approach to identify putative genes linked to nematode development in wheat. By using comparative genome analysis, we were able to identify an orthologue of *AAT* transmembrane protein in Arabidopsis. The identified *AAT* in both wheat and Arabidopsis share Aa_trans (PF01490) domain (Fig. 1). Many cereal genes are reported to have low sequence similarities to Arabidopsis genes but share a higher degree of sequence conservation within protein functional domains (Blümel et al., 2015). In fact, certain regions are highly conserved among diverse plant groups. Thus, domain analysis may play an important role in finding orthologous proteins in wheat.

As obligate parasites, cyst nematodes take up required amino acids from the host plant. During nematode infestation, cyst nematodes alter the expression of several amino acid transporters in syncytia (Szakasits et al., 2009). The importance of *AATs* in nematode parasitism was determined using loss-of-function mutants (Hofmann et al., 2009). Accordingly, we tested a loss-of-function mutant of *AtAAP6* and found a reduced number of female nematodes, decreased in female size, and reduced size of associated syncytia (Fig. 2 and 3). Similar results of reduced numbers of *Meloidygyne incognita* infection, development and egg mass production were reported by Marella et al. (2013). Elashry et al. (2013) found a noticeable decrease but no significant reduction of infection of *H. schachtii* in *AtAAP6* mutant. Compared to our study, the authors used a different infection assay and less number of plants per experiment which explains the different results. The reason why no constant

effects were observed in knockout mutants could also be redundancy among the large number of *AATs*, as suggested by Hofmann et al., (2010). In fact, *AAP* genes such as *AtAAP1*, *AtAAP2*, *AtAAP4* and *AtAAP6* were reported to share similar patterns of substrate specificity (Fisher et al., 1995; Rentsch et al., 1996). Moreover, it is unknown, if the loss of function of certain *AATs* has an effect on amino acid content of syncytia. However, our study revealed a loss of function mutant to significantly reduce nematode susceptibility and indicated that *AtAAP6* plays a role in nematode development.

We found that *AtAAP6* is highly expressed in syncytia at 1, 10 and 15 dpi with a peak at 10 dpi. The high expression in syncytia supports the importance of *AtAAP6* for nematode parasitism. These results are in line with the results obtained from a syncytium gene chip analysis that showed an upregulation of *AtAAP6* in syncytia (Szakasits et al., 2009). The localization of *AtAAP6* gene expression in and around the syncytium at 15 dpi was shown by using promoter::GUS lines and in situ RTPCR (Elashry et al., 2013). A high level of *AtAAP6* transcripts was clearly detected in syncytia. In uninfected root, expression *AtAAP6* was detected in pericyclic cells but not in cells of the stele.

In plants, most of the sink tissues are connected to the source tissue via phloem. Nematode induced syncytia are symplastically isolated in the early phase of nematode development. Only at later stages, a direct connection to the phloem via plasmodesmata is established (Böckenhoff and Grundler, 1994; Hoth et al., 2008). Accordingly, the apoplastic pathway still plays an important role for the translocation of nutrients into syncytia. As an indication, several sugar transporters genes are induced in syncytia and were shown to play an important role for nematode development (Hoffmann et al., 2007). Besides sugar, many transport amino acids such as glutamic acid, aspartic acid and glutamine were increased significantly in syncytia (Hofmann et al., 2010). Similarly, several *AAT* genes are strongly upregulated in syncytia (Szakasits et al., 2009). *AtAAP6* is expressed in the xylem

parenchyma cells, regulates phloem sap amino acid composition and plays a role in xylem-phloem transfer (Okumoto et al., 2002). Amino acid concentration in xylem is lower than in phloem sap, therefore a high affinity transporter (*AtAAP6*) is needed to enhance the transfer of amino acids into the sieve element (SE) (Bi et al., 2007). Accordingly, total amino acid concentration in SE was reduced by 30% in the *AtAAP6* mutant (Hunt et al., 2010; Tsay and Hsu, 2011).

Our qPCR analyses showed that the *TaAAT* gene was also significantly upregulated in susceptible wheat accessions Bezostaya 1 and Trapbow at 10 dpi compared to uninfected control roots, whereas expression in the resistant accessions Lantian 12 and BC380 remained low (Fig. 7). The expression pattern of the *TaAAT* in susceptible wheat accessions are in line with the results of Arabidopsis and *H. schachtii* parasitism. Our findings suggest that nematode infestation can increase the level of *AATs* in susceptible plants, while this mechanism is not initiated in resistant accessions. However, to study this mechanism in detail, a *TaAAT* mutant in wheat would be necessary.

Comparing the amino acid sequence of *AtAAP6* in three lowly and highly susceptible Arabidopsis accessions revealed a substitution of amino acid “G” at 207 of *AtAAP6* ORF in all highly susceptible accessions by “A” in low susceptible. “A” at position 207 of *AtAAP6* ORF was conserved in all lowly susceptible accessions while “G” in all highly susceptible accessions. The results suggest that in lowly susceptible accessions a defect in *AtAAP6* may lead to an altered host response to nematodes. The molecular mechanism by which the exchange of a single amino acid leads to the change of nematode development needs further investigation. Similar phenomena have been reported from resistance to other pathogens. A single amino acid “A” at position 918 of leucine-rich domain of the *Pi-ta* protein was shown to enhance resistant to rice blast *Magnaporthe oryzae,* while substitution of “A” by serine (S) promotes susceptibility (Bryan et al., 2000). Similarly, a change in amino acid at position 441

of *Pi-d2* gene can alter the resistance and to susceptibility of rice to rice blast (Chen et al., 2006).

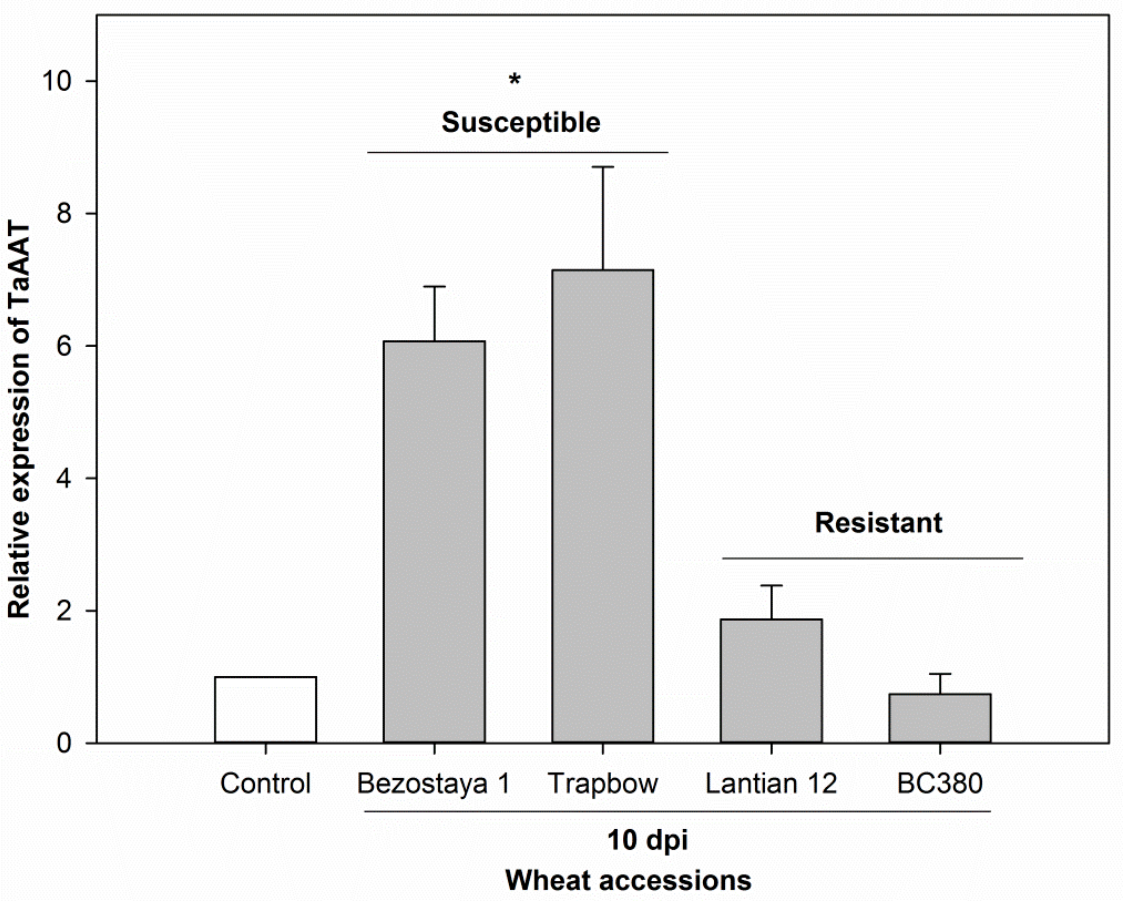


Fig. 7. qPCR analysis of TaAAT gene regulation in infected root of susceptible and resistant wheat accessions compared to uninfected root at 10 days post infection. Data were analyzed using one way ANOVA at ($P \leq 0.05$, n = 3) and Holm-Sidak test. Asterisks represent statistically significant difference to corresponding Bezostaya 1 control. *P< 0.05 and **P<0.01, ***P<0.001. Bar indicates the standard error of the mean. The experiment was repeated three times. The gene expression levels were normalized to the endogenous control gene q-Tubulin and the fold change values were calculated by using the $\Delta\Delta C_t$ method.

We conclude that *AtAAP6* and *TaAAT* are important factors for syncytium function and nematode development. We show that genomic approaches lead successfully to the identification and functional characterizations of genes in crops that are newly or not

completely sequenced. Therefore, understanding the molecular basis of nematode parasitism will greatly facilitate breeding for durable resistance or lowered susceptibility.

Acknowledgments

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The use of resistant cultivars is the most successful and preferable method to manage nematodes in cereals. Genetic variation in landraces and domesticated wheat has shown to provide resistance against a wide variety of both biotic and abiotic stresses. Wild and domesticated wheat accessions have been used to identify QTLs linked to many important agronomical traits (Kimber and Feldman, 1987; van Slageren, 1994). Although considerable research has been performed to identify resistance sources in wheat, no durable resistant cultivar to CCN is available today. Therefore, there is an urgent need to identify new resistance sources and to pyramid, and incorporate them into high yielding cultivars. In the past, resistance sources were investigated via traditional bi-parental methods which are, however, time-consuming (Kong et al., 2015). Recently, high density SNP markers have been introduced for improved mapping and screening of germplasm (Wang et al., 2014).

In this study, 290 wheat accessions analyzed responded differentially to *H. filipjevi* infection and development and 17% of the wheat accessions led to significant reduction in nematode development. We therefore classified 1% wheat accessions as resistant and 16% as moderately resistance (Table S1, page 90). These results indicated genetic variation to exist within the current IWWIP breeding materials. This can be used for a novel resistance breeding approach. Several mechanisms of resistance to cyst nematodes have been reported in host plants including prevention of nematode infection and interruption of nematode development (Montes et al., 2004; Reynolds et al., 2011). Our analysis clearly suggested that nematode development is impaired at three phases: early invasion, nematode development, and reproduction i.e. total number of eggs and J2 in resistant accessions (Fig. 1 and 2, page 24). Resistance in these accessions is at least partially based on reduced invasion which is in consistence with other studies in wheat to *H. filipjevi* and *H. avenae* (Li et al., 2015; Sağlam et al., 2009; Seifi et al., 2013). Other authors also revealed low invasion of *H. avenae* in resistant wheat cultivars Raj MR 1, CCNRV 4, and AUS 15854 (Singh et al., 2008).

Reduced invasion can be based on two factors: either reduced attractively of the roots,

or increased capability to prevent or hinder nematode invasion. J2s are attracted by host root exudates and differences in exudates composition are reported to induce nematode attraction or repellence to roots (Curtis, 2007; Grundler et al., 1991; Robinson, 2004; Zhao et al., 2000). J2s use their stylets to mechanically pierce cell walls (Wyss & Zunke, 1986; Wyss, 2002), through the stylet they release secretions containing cell wall modifying enzymes, thus facilitating ingress to roots (De Boer *et al.,* 1996; Davis *et al.,* 2008; Long *et al.,* 2013). Post invasion host defense can be activated and may induce the degradation of the syncytia, restrict the development of syncytia and thus inhibit nematode development. The syncytial cell undergoes drastic morphological and physiological changes (Fig. 3, page 6). In resistant wheat, a zone of necrotic cells was often reported between *H. avenae* J2 and its syncytium, where endodermal cells were disrupted (Williams and Fisher, 1993). The metabolic activity was higher in syncytia induced by *H. avenae* in susceptible wheat cv. Meering compared to resistant wheat cv. *T. aestivum* cv. AUS10894 (Seah et al., 2000). The development of syncytia in the resistant barley cv. Chebec upon *H. avenae* infection progressed faster compared to susceptible cv. Skiff (Aditya et al., 2015). Furthermore, it is also known that the plant cell wall provides a barrier for many pathogens (for review see Bohlmann and Sobczak, 2015). The content and composition of cell wall such as cellulose, hemicellulose, lignin and suberin may contribute to determine invasion success (for review see Wieczorek, 2015). However, at this stage, we cannot evaluate whether these wheat accessions found to be more resistant in our study differ in chemical composition of root exudates and cell wall components. Our results showed that the development of J3, J4 and adult nematodes does not differ between the susceptible and the resistant wheat accessions. We therefore conclude that the resistant accessions do not suppress growth and development of invaded nematodes by limitation or failure of the function of the induced syncytia. This mechanism has often been observed in host-nematode interactions e.g., in *Cre2* gene conferring resistance to *H. avenae* in wheat line H-93-8 (Tucker, 2015), *Hero A* gene conferring resistance to potato cyst

nematode *Globodera rostochiensis* in tomato (Sobczak et al., 2005), potato containing *Gpa2* gene resistance to *Globodera pallida* (Koropacka, 2010), and sugar beet containing $HS1^{pro1}$ gene to *H. schachtii* (Holtmann et al., 2000). In these cases, resistance genes were involved in syncytium deterioration and prevent the successful completion of nematode life cycle. Host plants respond to nematode infection and development in different ways. Intolerant plants show typical nematode damage symptoms whereas tolerant plants may hardly show any effect on growth and development (Trudgill, 1991). However, nematode resistance may be associated with plant phenotypes that negatively affect plant performance regardless nematode infection. At the given inoculum level, none of the plant growth parameters lead to negative changes indicating that plants are tolerant. We were not able to detect any disadvantage of resistant accessions compared to susceptible cv. Bezostaya 1. Our data, however, do not implicate that the studied accessions would show tolerance under field conditions. Nematode tolerance is a complex trait and can only be measured by monitoring yield under different environmental conditions. However, we found the four wheat accessions Nudakota, Katea, Ekonomka and Lantian 12 possess resistance sources and can subsequently be crossed with high-yielding cultivars improving their genetic resistance to CCNs.

Various responses of wheat accessions to *H. filipjevi* found in this study indicated that wheat accessions possess genetic variation that could provide a basis for potential resistance gene identification. Historically, QTL mapping is performed on structured panels using recombinant lines (Cui et al., 2014). Würschum (2012) reported a marker linked to CCN resistance in bi-parental populations obtained by crossing resistant and susceptible wheat genotypes. However, recent advances in genomic technologies and statistical methods such as GWAS allow analyzing the panels of unrelated individuals (Yu et al., 2006; Zhang et al., 2010; Zhou and Stephens, 2012). GWAS has been used to detect functional alleles and allelic variations in natural populations (Xu and Crouch, 2008). High density molecular markers have been used to analyze QTLs efficiently in marker-assisted selection (Xu et al., 2012). We

therefore investigated the loci conferring CCN resistance in 161 wheat accessions (Table S1, page 111). These 161 accessions were selected from 290 accessions based on their performance to *H. filipjevi* in two successive individual growth room trials. We identified eleven novel QTLs on chromosomes 1AL, 2AS, 2BL, 3AL, 3BL, 4AS, 4AL, 5BL and 7BL (Table 2, page 35). Our study revealed significant QTLs only in genome A and B were detected. Five out of 11 QTLs on chromosomes 1AL, 2AS, 2BL, 3BL, and 5BL were previously reported to CCN *H. avenae* resistance (Mulki et al., 2013; Ogbonnaya et al., 2001). The other four QTLs linked to CCN resistant on chromosome 1AL, 4AL, 4AS, and 7BL were reported for the first time in this study. CCN resistance genes such as *Cre5/CreX* on chromosome 2A, *Cre1* gene on chromosome 2BL and *CreY* gene on chromosome 3B were previously reported (Barloy et al., 2007; Bekal et al., 1998; Jahier et al., 2001; Slootmaker et al., 1974). However, genes associated to two other QTLs on chromosomes 1AL and 5BL have not yet reported. A QTL IWB66494 linked to marker Tdurum_contig10380_87 on chromosome 2BL confer resistance to *Fusarium graminearum* (head blight) was identified recently in spring wheat thus indicating the chromosomal region 2BL to be an allelic hotspot for resistance to multiple wheat diseases (Jansen, 2015). Other authors have reported the QTLs linked to Fusarium head blight, leaf rust, stripe rust, powdery mildew and cyst and root lesion nematodes resistance in wheat (Bernardo et al., 2012; Gurung et al., 2014; Lagudah et al., 2009; Linsell et al., 2014; Mulki et al., 2013; Würschum et al., 2013). We cannot confirm if these QTLs carry one or more resistance genes at this stage, however, we conclude the identified QTLs are unique and could already be used in breeding resistance not only against CCN but also to multiple pathogens in wheat.

GWAS with high density SNP markers are currently used to identify and pyramid major resistance genes in wheat and barley (Cavanagh et al., 2013). However, false associations related to heterogeneous populations often hindered successful association analysis (Matthies et al., 2012; Pritchard et al., 2000). To control the false signal, we used multiple linear models

(P+K), taking into consideration of principle component analysis (P) and relatedness (K). The corrected model separates the true functional signal from false positives. We further applied a multi-locus analysis with cross validation to control the high bias of explained variance that ensures reproducible results (Bauer et al., 2009; Benjamini and Yekutieli, 2005).

Our analysis revealed a high variation in polymorphic SNPs on A, B, and D genomes, which indicated different recombination rates in different genomic regions of wheat (Table 1, page 35). The distribution of polymorphic markers on genome A and B were significantly higher compared to D genome. The variation in effective recombination events in wheat genomes could result from the historical development of hexaploid wheat and its domestication process (Chao et al., 2009; Dubcovsky and Dvorak, 2007; van Ginkel and Ogbonnaya, 2007). Würschum et al. (2013) reported similar finding of few SNPs located on the D genome of 172 European winter wheat cultivars which is in line with our findings. The number of mapped markers in D genome is usually 3 to 5 fold lower compared to the A and B genomes (Allen et al., 2011; Cavanagh et al., 2013). In fact, the low genetic diversity in D genome in wheat is very common.

Our analysis of LD revealed <3 cM in 161 wheat accessions. The LD found in this study is a smaller distance compared to previous analysis of 5 cM (Crossa et al., 2007). In fact, LD found in this study supports the LD in self-pollinated plants that is normally smaller. The selection for favorable phenotypes during breeding history by IWWIP might be the reason of the smaller LD in this mapping population. High density of SNP markers provide more accuracy in genome wide and region specific LD on genome A (<3 cM) and B (2 cM) compared to D genome (>5 cM). The presence of dense markers facilitate the identification of the regions influenced by a short, intense breeding history (Benson et al., 2012). Other authors have revealed similar LD decay of <5 cM in 157 wheat landraces and 189 Canadian bread wheat accession, and 5-10 cM for 93 Chinese bread wheat (Belzile et al., 2007; Hao et al., 2011). However, LD depends on many factors such as the process of domestication,

population subdivision, founding events, and selection (Rafalski and Morgante, 2004). Neumann et al. (2011) reported the inconsistency of LD across the whole genome or single chromosome.

Population structure and relatedness among individuals can cause the false association between a candidate marker and a phenotype (Price et al., 2010; Yu et al., 2006). Analysis of both Structure (Q) and PCA (P) revealed a very low genetic differentiation in 161 wheat accessions that needed no further correction (Fig. 2, page 33, and Fig. 3, page 34). As the result of Q and PCA were nearly equivalent, we used (P and P+K) model to identify QTLs as most of published analyses used P instead Q to avoid the high computational demand associated with Q matrix in structure analysis (Price et al. 2006). Similar results were reported about two sub-populations of 81 diversified *A. tauschii* and 96 wheat populations (Neumann et al., 2011; Sohail et al., 2012). However, many studies suggested that sub-populations exist within different mapping populations; and population structure varies from population to population. High marker allele frequency and low population structure justify the selection of our mapping population to perform GWAS (Myles et al., 2009).

Our analysis predicted that pyramiding two or more QTLs can enhance the performance of resistance considerably (Fig. 6, page 39). Combining QTLs two, three, four or five in wheat accessions predicted to increase resistance up to 74, 76, 85, and 86%, respectively. The three moderately resistant wheat accessions Olifants, Lantian 12 and T04/17 possess the highest marker allele frequency (Table 5, page 39). We therefore considered these lines to be good candidates for breeding strategies to develop durable resistance through marker-assisted backcrossing. Pyramiding resistant genes have increased resistance levels of *A. variabili* against *H. avenae* which is line with our findings (Barloy et al. 2007). Pyramiding resistance alleles to stripe rust has been utilized successfully in barley (Sun et al. 1997). Other studies revealed pyramiding leaf rust resistance genes *Lr41, Lr42 and Lr43* and powdery mildew resistance genes *Pm1* and *Pm2* enhancing the resistance response in wheat (Cox et al.

1994; Liu et al. 2000). Williams et al., (2006) reported the additive effect of combining resistance QTLs to *H. avenae* in Trident/Molineux DH wheat population. In addition, heritability analysis revealed 46% phenotyping variation is due to genetic factors and thus suggested a potential transmission of alleles to successive generations.

Our study demonstrated that GWAS can detect QTLs linked to CCN resistance in wheat. *In-silico* analysis identified the intra-chromosomal location of SNPs co-localized with genes and proteins that are involved in responses to biotic and abiotic stress (Table 4, page 38). We further identified the full putative ORF at the significant marker location (Table 3, page 37), however the challenge is to identify and characterize the candidate gene involved in nematode parasitism. In fact, the current wheat cultivars represent only a small fraction of gene pool thus hindering the annotation process for a large number of genes and proteins (Tulpan et al., 2015). We therefore used model plants such as Brachypodium, sorghum, rice and Arabidopsis to identify candidate genes linked to putative ORF. Among the 11 ORFs, the function of an ORF linked to chromosome 2BL that contains an orthologue to *AtAAP6* gene in *A. thaliana* is well supported statistically and biologically. A large number of *AATs* from many different plants were identified via functional analysis and sequence homology in model plants such as Arabidopsis and rice (Rentsch et al., 1998; Wipf et al., 2002; Zhao et al., 2012). Both genomes have been used to find similar genes in wheat (Clarke et al., 2003; Kim et al., 2014; Tulpan et al., 2015). Many cereal genes are reported to have low sequence similarities to the Arabidopsis genes but share a higher degree of conserved sequences within functional domains of proteins (Blümel et al., 2015). A possible role of *AtAAP6* in *H. schachtii* parasitism was previously reported in Arabidopsis. It is upregulated in syncytia as shown in genechip analysis, *in situ* rtPCR, and infection assays with promoter::GUS lines (Elashry et al., 2013, Szakasits et al., 2009). We therefore characterized the role of *AtAAP6* in nematode parasitism using a T-DNA mutant line and expression analysis. We found a significant reduction in nematode females, female size, and female associated syncytia in mutant line

compared to wild type (Fig. 2, page 55, and 3, page 56). Our results confirmed that *AtAAP6* is highly expressed in syncytia at 1, 10 and 15 days post nematode infestation (Fig. 4, page 57). The expression of *AtAAP6* was highly induced by upon *M. incognita* and reduction of female has previously reported which is in line to our results (Marella et al., 2013). Similarly, Puthoff et al. (2003) reported a high expression of *AtAAP6* in syncytia induced by *H. schachtii*, and in other sink tissues. Similarly, an *AtCAT6* gene reported to be involved in supplying amino acids to feeding structures induced by *M. incognita* (Hammes et al., 2006). Our finding revealed a strong expression of *TaAAT* in infected roots of two susceptible wheat accessions compared to uninfected roots while the expression of *TaAAT* in infected and uninfected roots of two resistant wheat accessions remains unchanged (Fig. 7, page 63).

Comparing the amino acid sequence among the *AtAAP6* in resistant and susceptible Arabidopsis accessions revealed a substitution in amino acid "G" at 207 of *AtAAP6* ORF in all susceptible accessions by "A" in resistant accessions (Fig. 6, page 59). The results suggest resistant accessions may have a defect in components of *AtAAP6* pathways compared to susceptible accessions which may alter nematode response. A single amino acid "A" at position 918 of leucine-rich domain of the *Pi-ta* protein was shown to enhance resistant to rice blast *Magnaporthe oryzae,* while substitution of "A" by serine (S) promotes susceptibility (Bryan et al., 2000). Similarly, Chen et al. (2006) reported a change in amino acid at position 441 of *Pi-d2* gene can alter the resistant and susceptible reaction of rice to blast which is in line to our results. The molecular mechanism by which change in *AtAAP6* leads to change the nematode behavior in resistant accessions needs further investigation.

Genetic diversity and durability are the two most important features for resistance breeding. Genetic variability provides the raw material for breeding new cultivars. Scientists have developed high density molecular markers to dissect the complex traits in plants. By using 90K SNP genotyping platforms, we identified 11 novel QTLs and seven wheat accessions offering new material for breeding resistance to multiple wheat pathogens. A

challenge for future research is to validate these QTLs by using bi-parental populations or near-isogenic lines (NILs) and their efficiency test in multi-location field trials. Furthermore, it will be also a critical to have knock-out mutant for each candidate gene in wheat and characterizing their role in nematode parasitism. However, the prediction of putative genes linked to the QTLs provided an opportunity to identify and characterize orthologue gene in Arabidopsis. Thus, functional characterization of gene in model plant can trigger the gene identification process in wheat. This study not only provides novel resistant sources for breeding wheat cultivars against CCN but also gives access to new insights into nematode parasitism. Thus, understanding the molecular basis of nematode parasitism will greatly facilitate the nematode-resistant breeding.

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6. 1. Supplementary materials for:

Identification and characterization of resistance to the cereal cyst nematode *Heterodera filipjevi* in winter wheat

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Fig. S1. Polymerase chain reaction restriction fragment length polymorphism patterns of *Heterodera filipjevi* (*Hf*) and *H.schachtii* (*Hs*) based on restriction with *Hinf*I or *Rsa*I.

Table S1. List of 290 winter wheat accessions with accession number, common name, selection history, origin, and host response to *Heterodera filipjevi*

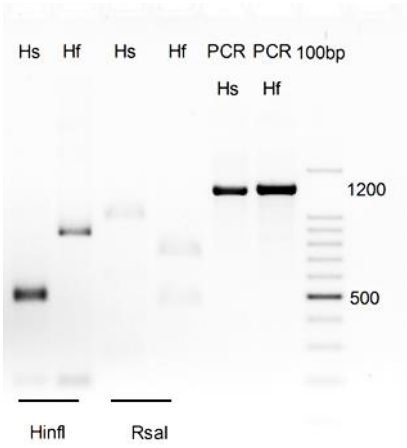

Fig. S1. Polymerase chain reaction restriction fragment length polymorphism patterns of *Heterodera filipjevi* (*Hf*) and *H.schachtii* (*Hs*) based on restriction with *Hinf*I or *Rsa*I.

Table S1. List of 290 winter wheat accessions with accession number, common name, selection history, origin, and host response to *Heterodera filipjevi*

Entry	Nursery	ACCNO	CName	SELHX	CID	Origin	Female/5 rep	SD	SE	Host status
1	11CBWF	951327	KINACI97	-7M-0M-8M-1M-3WM-0WM-4WM-2WM-0WM	SWM12289	MX-BD	24,0	4,25245	2,12623	HS
2	11CBWF	010580	KROSHKA			RUS	18,0	5,04975	2,52488	S
3	11CBWF	030689	POBEDA 50			RUS	21,3	4,90465	2,45232	HS
4	11CBWF	080951	OBRII/DNESTREANCA25//ILICIOVCA/OD.CRASNOCOLOS			MOL	19,5	2,04124	1,02062	HS
5	11CBWF	080932	DRAGANA			SERBIA	15,8	4,08928	2,04464	S
6	11CBWF	020966	L 4224 K 12			KR-RUS	10,3	1,88562	0,94281	MS
7	11CBWF	000017	8023.16.1.1/KAUZ	-0SE-0YC-1YE-0YC-2YC-0YC	C3W92WM00378S	MX-TCI	10,0	1,77951	0,88976	MR
8	11CBWF	070404	CUPRA-1/3/CROC1/AE.SQUARROSA (224)//2*OPATA/4/PANTHEON	-030YE-0E-2E-0E-2E-0E	TCI992280	TCI	24,8	5,07171	2,53585	HS
9	11CBWF	070028				TCI	24,3	10,1434	5,07171	HS
10	11CBWF	050150	KAUZ//ALTAR 84/AOS/3/F10S-1	-0SE-0YC-0YE-7YE-0YE-2YE-0YE	TCI971281	TCI	15,8	1,31233	0,65617	S
11	11CBWF	050179	BONITO//KAREE/TUGELA	-0AP-0AP-0YE-5YE-0YE-3YE-0YE	TCI97AP 039	TCI	19,8	4,49691	2,24846	HS
12	11CBWF	010634	CETINEL 2000	-6H-0YC-0R-1YC-0YC-0E	OWC852672	TR-ESK	18,2	4,08928	2,04464	S
13	11CBWF	040569	OR941611			USA-OR	14,7	1,92931	0,96465	MS
14	11CBWF	030164	KS82W409/SPN//TAM106/TX78V3630	-0SE-0YC-0E-1YE-0YE-2YM-0YM	TCI951385	TCI	16,8	1,43372	0,71686	S
15	11CBWF	040347	MAHON DEMIAS/3/HIM/CNDR//CA8055	-0AP-0YC-4E-0E-3K-0YK	TCI960471	TCI	21,3	4,36527	2,18263	HS
16	11CBWF	020135	AGRI/NAC//KAUZ/3/1D13.1/MLT	-0SE-0YC-*-1YE-1YC-0YC-3YM-0YM	CIT937146	TCI	15,7	2,65623	1,32811	S
17	11CBWF	000008	KOLLEGA			RUS	20,2	6,40746	3,20373	HS
18	11CBWF	090730	LANTIAN 22			PRC	16,2	5,94886	2,97443	S
19	11CBWF		MOSKVICH			RUS-KRAS	11,3	3,39935	1,69967	MS
20	11CBWF		POSTROCK			US-AGRIPRO	12,5	1,08012	0,54006	MS
21	11CBWF	090874	Alamoot/3/Alvd//Aldan"s"/IAS58/4/Alamoot/Gaspard			IR-KARAJ	20,3	2,35702	1,17851	HS
22	11CBWF	100019	KS2016/Trego		ARS05-1034	US-ARS-NC	6,3	3,39935	1,69967	MR
23	11CBWF	100733	MV-TOLDI			HUN	18,0	2,27303	1,13652	S
24	11CBWF	100668	LCR/SERI/3/MEX-DW/BACA//VONA/4/TAM200/JI5418	-0AP-DH16	ICWH99018	SYR	12,3	5,90668	2,95334	MS
25	11CBWF	100676	LCR/SERI/3/MEX-DW/BACA//VONA/4/TAM200/JI5418	-0AP-DH16	ICWH99018	SYR	21,0	5,11534	2,55767	HS
26	11CBWF	980135	VICTORYA			UKR	22,3	7,02772	3,51386	HS
27	11CBWF	060050	ATTILA/3/AGRI/NAC//MLT	-0E-0E-2E-0E-1E-0E	TCI981026	TCI	17,3	2,24846	1,12423	S

28	11CBWF	060074	TX69A509.2//BBY/FOX/3/GRK//NO64/PEX/4/CER/5/CHIL/2*STAR	-0E-0E-5E-0E-2E-0E	TCI981148	TCI	17,7	1,64992	0,82496	S
29	11CBWF	000261	VORONA/KAUZ//1D13.1/MLT	-0SE-0YC-*-3YE-3YC-0YC	CIT937111	TCI	19,3	5,57275	2,78638	S
30	11CBWF	090068	RSK/CA8055//CHAM6/4/NWT/3/TAST/SPRW//TAW12399.75	-0AP-0AP-25AP-0AP-4AP-0AP	TCI-02-47	TCI	17,3	2,01384	1,00692	S
31	11CBWF	090270	TAM200/KAUZ//YU MAI30	-030YE-30E-5E-0E-4AP-0AP	TCI011017	TCI	18,3	1,0274	0,5137	S
32	11CBWF	090350	HBA142A/HBZ621A//ABILENE/3/CAMPION/4/F6038W12.1	-030YE-30E-3E-0E-1E-0E	TCI012144	TCI	12,0	1,87083	0,93541	MS
33	11CBWF	090432	4WON-IR-257/5/YMH/HYS//HYS/TUR3055/3/DGA/4/VPM/MOS	-0AP-0AP-46AP-0AP-1AP-0AP	TCI-02-80	TCI	11,3	1,92931	0,96465	MS
34	11CBWF	090495	PYN/BAU/3/KAUZ//KAUZ/STAR	-030YE-30E-6E-0E-1E-0E	C3W01WM00586S	MX-TCI	21,5	4,44175	2,22088	HS
35	11CBWF		VEE#8//JUP/BJY/3/F3.71/TRM/4/BCN/5/KAUZ/6/163	-030YE-0E-1E-0E-2E-0E	TCI992192	TCI	25,7	2,01384	1,00692	HS
36	11CBWF		DORADE-5/3/BOW"S"/GEN//SHAHI	-0AP-0AP-6AP-0AP-3AP-0AP	TCI-02-522	TCI	20,0	9,09212	4,54606	HS
37	11CBWF	010004	494J6.11//TRAP#1/BOW	-0YC-0YC-0YC-8YC-0YC-1SE-0YC-2YC-0YC	C3W90M200	MX-CIT	24,0	2,25462	1,12731	HS
38	11CBWF	020321	SAULESKU #44/TR810200	-03Y-0B-0SE-3YE-0YC-2YM-0YM	C3W94WM00586S	MX-TCI	20,2	3,00925	1,50462	HS
39	11CBWF	950513	GUN91	-1A-1A-1A-0A	SWM7155	MX-YA	14,2	0,62361	0,3118	MS
40	11CBWF	070158	CHEN/AE.SQUARROSA (TAUS)//BCN/4/RAN/NE701136//CI13449/CTK/3/CUPE/5/130L1.11/GUN91//KINACI97	-030YE-0E-1E-0E-1E-0E	TCI992198	TCI	14,5	5,30723	2,65361	MS
41	11CBWF	950377	DOGU88			TR-ERZ	19,0	1,77951	0,88976	S
42	11CBWF	070676	KS92H363-2/COUGAR SIB(=NE85707/TBIRD) X NE94632(=ABILENE/NORKAN//RAWHIDE)			US-UNL	20,2	5,10446	2,55223	HS
43	11CBWF	090779	SAR-30			IR-DARI	18,3	3,32499	1,66249	S
44	11CBWF	090781	RASAD			IR-DARI	17,5	1,47196	0,73598	S
45	11CBWF	050117	KS82142/PASTOR	-0P-0YC-0YE-3YE-0YE-1YE-0YE	C3W97WM00399S	OR-CIT	13,5	7,11805	3,55903	MS
46	11CBWF	990857	BURBOT-6	-9H-0YC-1YC-0YC-0YC-2YC-0YC-3YC-0YC	WXD880137A	OR-CIT	17,0	1,87083	0,93541	S
47	11CBWF	000374	ES84.24/GRK	-0SE-0YC-1YE-0YC-2YC-0YC	CIT932135	TCI	24,7	0,84984	0,42492	HS
48	11CBWF	950283	SUZEN 97	-7E-1E-0E	YE2957	TR-ESK	21,0	1,87083	0,93541	HS
49	11CBWF	050696	TAM 105/3/NE70654/BBY//BOW"S"/4/Century*3/TA2450		AP01T1112	US-AgriPro South	17,8	3,68179	1,84089	S
50	11CBWF	050751	MILLENNIUM/NE93613		SD00258	US-SDSU	5,8	3,92287	1,96143	MR

51	11CBWF	010027	TAM200/KAUZ	-0SE-0YC-1YC-0YC-3YC-0YC-1YC-0YC	C3W91M00414S	MX-CIT	18,8	7,26101	3,6305	S
52	11CBWF	040237	PYN/BAU/3/AGRI/BJY//VEE	-0SE-0YC-17E-0E-1K -0YK	TCI961547	TCI	19,0	3,24037	1,62019	S
53	11CBWF	950369	DAGDAS94	-10A-0A	YA15662	YA-BD	16,2	2,35702	1,17851	S
54	11CBWF	050728	TREGO/BTY SIB		KS01HW152-6	Kansas State - Hays	16,3	3,47211	1,73606	S
55	11CBWF	070676	NE04424	HRW		US-UNL	22,8	4,17	2,085	HS
56	11CBWF	090783	KOHDASHT			IR-DARI	15,5	1,77951	0,88976	S
57	11CBWF	070603	ICDW-21122	BW		AFG	6,8	1,43372	0,71686	MR
58	11CBWF	951009	SARDARI			IR-DARI	14,7	1,31233	0,65617	MS
59	11CBWF	030243	SABALAN/GRK//PYN/BAU	-0YC-0E-1YE-0YE-3YM-0YM	TCI952089	TCI	23,3	3,42377	1,71189	HS
60	11CBWF	030323	CA8055/4/ROMTAST/BON/3/DIBO//SU92/CI 13645/5/AGRI/BJY//VEES	-0SE-0YC-0E-7YE-0YE-1YM-0YM	TCI951084	TCI	20,3	1,88562	0,94281	HS
61	11CBWF	000330	BILINMIYEN96.7	-0SE-3YA-3YC-0YC	F2.96.7	TCI	20,2	5,94886	2,97443	HS
62	11CBWF	000029	RIPPER			US-COL	16,0	1,08012	0,54006	S
63	11CBWF	000031	SNOWMASS			US-COL	16,5	2,85774	1,42887	S
64	11CBWF	070671	2180*K/2163//?/3/W1062A*HVA114/W3416		KS980554-12-~9	USA	11,5	2,82843	1,41421	MS
65	11CBWF	040320	BUL EVREDIKA/STOZHER/4/TAST/SPRW//CA8055/3/CSM	-0AP-0YC-2E-0E-2K-0YK	TCI96T151	TCI	13,2	9,56847	4,78423	MS
66	11CBWF	090169	TAM200/KAUZ/3/SPN/NAC//ATTILA/4/F885 K1.1/SXL	-030YE-30E-3E-0E-1E-0E	TCI012021	TCI	18,5	2,48328	1,24164	S
67	11CBWF	090194	ZCL/3/PGFN//CNO67/SON64(ES86-8)/4/KA../4/BEZ/NAD//KZM (ES85.24)/3/F900K	-030YE-30E-6E-0E-2E-0E	TCI011392	TCI	16,2	6,32895	3,16447	S
68	11CBWF	080893	CDC FALCON			CAN	16,7	7,71722	3,85861	S
69	11CBWF	080991	Bulava			RUS	15,3	2,49444	1,24722	MS
70	11CBWF	090748	MIRONIVSKA RANNOSTYGLA			UKR-MIR	20,7	6,84755	3,42377	HS
71	11CBWF	100701	PEREGRINE			CAN	16,0	3,08221	1,5411	S
72	11CBWF	090079	GRECUM 84//PYN/BAU	-0AP-0AP-18AP-0AP -1E-0E	TCI-02-726	TCI	14,5	2,44949	1,22474	MS
73	11ELITE-IRR	980825	AGRI/NAC//ATTILA	C3W92WM00232S	-0SE-0YC-4YE-0YC	MX-TCI	14,7	4,24918	2,12459	MS
74	11ELITE-IRR	980960	TAM200/JI5418	CIT930099	-0SE-0YC-2YE-0YC	TCI	18,8	8,02427	4,01213	S
75	11ELITE-IRR	950055	BESKOPRU			TR	22,3	0,62361	0,3118	HS
76	11ELITE-IRR	990149	885K4.1//MNG/SDV1/3/1D13.1/MLT	CIT925099	-0SE-0YC-3YC-0YC-3YC-0YC	TCI	17,8	3,51979	1,75989	S
77	11ELITE-IRR	990932	STAR/BWD	C3W93WM0137	-0AP-0YC-11YE-0YC	MX-TCI	13,3	2,09497	1,04748	MS
78	11ELITE-IRR	990414	FRTL//AGRI/NAC	C3W93WM0071	-0AP-0YC-29YE-0YC	MX-TCI	14,8	4,00694	2,00347	MS
79	11ELITE-IRR	232	SW89-3218//AGRI/NAC	C3W93WM0184	-0AP-0YC-*-3YE-3YC-0YC	MX-TCI	14,0	2,27303	1,13652	MS
80	11ELITE-IRR	10831	ID800994.W/MO88	CMWS92Y00272S	-030WM-1WM-05WM-015WM-7WM-0WM	MX-TCI	20,7	4,10961	2,0548	HS
81	11ELITE-IRR	991101	VORONA/HD2402	SWM17702	-0SE-9YC-0YC-1YC-0YC-4YC-0YC-34YC-0YC	MX-CIT	16,2	4,49691	2,24846	S

82	11ELITE-IRR	33	AGRI/NAC//KAUZ	C3W92WM00231S	-0SE-0YC-0YC-*-5YE-5YC-0YC	MX-TCI	15,3	2,95334	1,47667	MS
83	11ELITE-IRR	10246	ESKINA-8	CIT925080	-0SE-0YC-7YC-0YC-2YC-0YC-3YC-0YC	CIT	13,3	3,85861	1,92931	MS
84	11ELITE-IRR	30158	AGRI/BJY//VEE/3/KS82142/CUPE	TCI951027	-0SE-0YC-0E-1YE-0YE	TCI	20,5	4,02078	2,01039	HS
85	11ELITE-IRR	40007	F130-L-1-12/MV12(ATILLA-12)	TCI961246	-0SE-0YC-0E-1YE-0YE-2YM-0YM	TCI	17,7	4,49691	2,24846	S
86	11ELITE-IRR	50073	AGRI/BJY//VEE/3/AKULA/4/F10S-1	TCI972515	-0SE-0YC-0YE-4YE-0YE-1YE-0YE	TCI	13,5	1,22474	0,61237	MS
87	11ELITE-IRR	50111	SHI#4414/CROWS"//GK SAGVARI/CA8055	TCI97AP 539	-0SE-0YC-0YE-26YE-0YE-1YE-0YE	TCI	16,7	5,79272	2,89636	S
88	11ELITE-IRR	60119	VORONA/HD2402//ALBATROSS ODESSKIY	TCI960735	-0AP-0AP-0YE-5YE-0YE-1YE-0YE	TCI	17,0	4,60072	2,30036	S
89	11ELITE-IRR	60074	TX69A509.2//BBY/FOX/3/GRK//NO64/PEX/4/CER/5/CHIL/2*STAR	TCI981148	-0E-0E-5E-0E-2E-0E	TCI	11,5	1,87083	0,93541	MS
90	11ELITE-SA	950412	KARAHAN			TR	20,0	2,85774	1,42887	HS
91	11ELITE-SA		MUFFITBEY			TR	17,0	2,27303	1,13652	S
92	11ELITE-SA	980671	LFN/VOGAF//LIRA/5/K134(60)/4/TOB/BMAN//BB/3/CAL/6/F339P1.2	CIT935039	-0SE-0YC-5YE-0YC	TCI	7,8	3,85861	1,92931	MR
93	11ELITE-SA	980639	FLAMURA85//F134.71/NAC	CIT930037	-0SE-0YC-1YE-0YC	TCI	16,2	1,31233	0,65617	S
94	11ELITE-SA	990276	ORKINOS-1	.	-0YA-0YA-5YC-0YC	YA-TCI	21,3	2,4608	1,2304	HS
95	11ELITE-SA	990277	ORKINOS-2	.	-0YA-0YA-6YC-0YC	YA-TCI	7,2	1,69967	0,84984	MR
96	11ELITE-SA	990818	PMF/MAYA//YACO/3/CO693591/CTK	CIT90095T	-0YC-0YC-0YC-3YC-0YC-1YC-0YC	CIT	22,8	3,39935	1,69967	HS
97	11ELITE-SA	990125	777TWWON87/3/F12.71/SKA//CA8055	CIT922247	-0SE-0YC-3YC-0YC-3YC-0YC	CIT	10,8	0,62361	0,3118	MS
98	11ELITE-SA	990084	1D13.1/MLT//TUI	C3W90M398	-0YC-0YC-0YC-1YC-0YC-6YC-0YC	MX-CIT	12,8	0,94281	0,4714	MS
99	11ELITE-SA	990593	KVZ/HB2009/5/CNN/KHARKOV//KC66/3/SKP35/4/VEE	ICWH87046	-0YC-0R-2YC-0YC-1YA-0YC	CIT	10,8	5,03874	2,51937	MS
100	11ELITE-SA	010027	TAM200/KAUZ	C3W91M00414S	-0SE-0YC-1YC-0YC-3YC-0YC-1YC-0YC	MX-CIT	14,0	2,04124	1,02062	MS
101	11ELITE-SA	010037	JI5418/MARAS	CIT922142	-0SE-0YC-3YC-0YC-6YC-0YC-1YC-0YC	CIT	20,8	3,29983	1,64992	HS
102	11ELITE-SA	020323	SAULESKU #44/TR810200	C3W94WM00586S	-03Y-0B-0SE-3YE-0YC-4YM-0YM	MX-TCI	24,2	3,42377	1,71189	HS
103	11ELITE-SA	020319	SAULESKU #44/TR810200	C3W94WM00586S	-03Y-0B-0SE-1YE-0YC-1YM-0YM	MX-TCI	17,8	5,4365	2,71825	S
104	11ELITE-SA	020226	BILINMIYEN96.27	F2.96.27	-0SE-0YC-1YE-0YC-2YM-0YM	TCI	19,2	1,84089	0,92045	S
105	11ELITE-SA	020293	TAST/SPRW/4/ROM-TAST/BON/3/DIBO//SU92/CI13645/5/F130L1.12	CIT932182	-0SE-0YC-7YE-0YC-1YM-0YM	CIT	18,7	2,77889	1,38944	S
106	11ELITE-SA	030311	GUN91/POBEDA//F900K	CIT945243	-030SE-0YC-2YE-0YC-2YM-0YM	TCI	20,7	1,24722	0,62361	HS
107	11ELITE-SA	030418	CA8055//KS82W409/STEPHENS	TCI950547	-0SE-0YC-0E-4YE-0YE-1YM-0YM	TCI	13,8	1,43372	0,71686	MS

108	11ELITE-SA	030423	YE2453//PPBB68/CHRC	TCI950019	-3AP-0AP-0E-2YE-0YE-3YM-0YM	TCI	15,3	2,3214	1,1607	MS
109	11ELITE-SA	060161	TX69A509.2//BBY/FOX/3/GRK//NO64/PEX/4/CER/5/KAUZ//ALTAR 84/AOS	TCI981143	-0E-0E-6E-0E-1E-0E	TCI	12,5	0,70711	0,35355	MS
110	11ELITE-SA	060287	BOW/NKT//KATIA1/3/AGRI/BJY//VEE	TCI982234	-030YE-0E-3E-0E-1E-0E	TCI	19,5	7,17635	3,58818	HS
111	11ELITE-SA	060417	TIRCHMIR1//71ST2959/CROW/4/NWT/3/TAST/SPRW//TAW12399.75	TCI98-IC-0097	-0AP-0AP-4E-0E-2E-0E	TCI	21,8	2,09497	1,04748	HS
112	11ELITE-SA	991540	YILDIZ			TR	13,2	1,64992	0,82496	MS
113	18FAWWON-IRR	080009	DORADE-5/CAMPION	TCI 001049	-030YE-030YE-2E-0E -4E-0E	TCI	10,2	0,4714	0,2357	MS
114	18FAWWON-IRR	080056	T 98-9//VORONA/HD2402	TCI 001530	-030YE-030YE-11E-0E -3E-0E	TCI	23,5	6,01387	3,00694	HS
115	18FAWWON-IRR	080533	SHARK-1/GK.PINKA	TCI 001359	-030YE-030YE-10E-0E-3AP-0AP	TCI	18,5	0,70711	0,35355	S
116	18FAWWON-IRR	080684	BOW/CROW/3RSH//KAL/BB/3/GUN91	TCI011508	-030YE-30E-0YK	TCI	16,8	1,69967	0,84984	S
117	18FAWWON-IRR	070256	HK1/6/NVSR3/5/BEZ/TVR/5/CFN/BEZ//SU92/CI13645/3NAI60	ICWH99158	-0AP-0AP-0AP-4YE-0YE	TCI	15,5	2,16025	1,08012	S
118	18FAWWON-IRR		TAST/SPRW//LT176.73/7/SOTY/SUT//LER/4/2*RFN/3/FR//KAD/GB/5/TMP64../8/BOUHOUTH6	TCI97AP 212		TCI	15,8	4,87055	2,43527	S
119	18FAWWON-IRR		SN64//SKE/2*ANE/3/SX/4/BEZ/5/SERI/6/CHERVONA/7/KLEIBER/2*FL80//DONSK.POLUK.	TCI962126		TCI	16,7	1,43372	0,71686	S
120	18FAWWON-IRR	070250	HK1/4/TAST/SPRW//CA8055/3/CSN	ICWH99157	-0AP-1AP-2AP-0AP-2AP-0AP	TCI	13,5	0,70711	0,35355	MS
121	18FAWWON-IRR	080031	TX71A1039.V1*3/AMI/3/BEZ/NAD//KZM(ES85-24)/4/SHARK-1	TCI 001213	-030YE-030YE-7E-0E -3E-0E	TCI	13,7	4,92161	2,4608	MS
122	18FAWWON-IRR	080052	GANSU-1//VORONA/HD2402	TCI 001499	-030YE-030YE-5E-0E -2E-0E	TCI	14,3	7,72802	3,86401	MS
123	18FAWWON-IRR	080595	T 98-9//VORONA/HD2402	TCI 001530	-030YE-030YE-22E-0E-4AP-0AP	TCI	13,5	3,34166	1,67083	MS
124	18FAWWON-IRR	080702	CRR/ATTILA/4/WA476/391/3/NUM//W22/TAM200	TCI-01-419	-0AP-0AP-25AP-0AP-4AP-0AP	TCI	11,5	6,33772	3,16886	MS
125	18FAWWON-IRR	080660	DANA/3/SPN/NAC//ATTILA/4/SHARK-1	TCI 002097	-030YE-030YE-1E-0E -1E-0E	TCI	17,5	2,67706	1,33853	S
126	18FAWWON-IRR	100671	LCR/SERI/3/MEX-DW/BACA//VONA/4/TAM200/JI5418	ICWH99018	-0AP-DH16	TCI	13,0	5,88784	2,94392	MS
127	18FAWWON-IRR	100673	HK92/L 3676 K 11-20	ICWH99019	-0AP-DH14	TCI	14,3	0,84984	0,42492	MS
128	18FAWWON-IRR	100664	SHAHRIAR			IR-ARD	5,3	2,59272	1,29636	MR
129	18FAWWON-IRR	090861	Alamoot/4/Bloudan/3/Bb/7c*2//Y50E/Kal*3			IR-KARAJ	14,0	2,54951	1,27475	MS
130	18FAWWON-IRR	081138	Owl//Ombul/Alamo			IR-KARAJ	12,8	4,78423	2,39212	MS
131	18FAWWON-IRR	081161	Alvd//Aldan/Ias58/3/Col.No.3193/4/Zarrin			IR-KARAJ	16,8	7,70642	3,85321	S
132	18FAWWON-IRR	090902	Bow”s”/Crow”s”//Kie”s”/Vee”s”/3/MV17			IR-MIANDOAB	14,8	3,32499	1,66249	MS
133	18FAWWON-IRR	090914	Owl/Soissons//Zarrin			IR-MIANDOAB	11,7	1,0274	0,5137	MS
134	18FAWWON-IRR	090920	Spb”s”//K134(60)/Vee”s”/3/Druchamps/4/Alvand			IR-MIANDOAB	6,3	1,24722	0,62361	MR

135	18FAWWON-IRR	090911	PODOIMA			MOL	13,5	5,01664	2,50832	MS
136	18FAWWON-IRR	090915	AVANTAJ			MOL	16,8	4,78423	2,39212	S
137	18FAWWON-IRR	080189	TX71A1039.V1*3/AMI//TRAP#1/4/KAUZ//ALTAR 84/AOS/3/KAUZ	C3W00WM440	-030YE-030YE-6E-0E -5E-0E	MX	13,2	3,32499	1,66249	MS
138	18FAWWON-IRR	080840	SIRKKU/PRINIA	C3S97Y00247S	-040Y-050M-020Y-030M-41Y-1M-0Y	MX	15,3	2,35702	1,17851	MS
139	18FAWWON-IRR	080829	CHIL/PRL//BAV92/3/MILAN/KAUZ	C3S97M03230T	-040Y-020Y-030M-040SY-020M-24Y-0M-0SY	MX	13,3	1,69967	0,84984	MS
140	18FAWWON-IRR	100710	NUDELA			RO	18,8	3,39935	1,69967	S
141	18FAWWON-IRR	100704	CH-111.14098			UN	14,5	2,16025	1,08012	MS
142	18FAWWON-IRR	070632	MIT/TX93V5722//W95-301	TX04M410164		USA	11,3	6,54896	3,27448	MS
143	18FAWWON-IRR	100006	TX98D1170*2/TTCC365	WX02ARS171-3-14	ARS05-0043	US-ARS-NC	9,7	3,06413	1,53206	MR
144	18FAWWON-IRR	100031	IN97395B1-4-3-8/AWD99*5725		ARS07-0723	US-ARS-NC	9,5	1,08012	0,54006	MR
145	18FAWWON-IRR	080486	ORACLE/PEHLIVAN	TCI 00125703	-030YE-030YE-2E-0E-4AP-0AP	TCI	14,0	3,34166	1,67083	MS
146	18FAWWON-SA	080710	BUC/PVN//MILAN/3/TX96V2427	TCI-01-436	-0AP-0AP-28AP-0AP-3AP-0AP	TCI	7,0	0,8165	0,40825	MR
147	18FAWWON-SA	080217	SST44//K4500.2/SAPSUCKER/3/ALTAY	TCI 001581	-030YE-030YE-2E -0E -5E-0E	TCI	17,5	0,8165	0,40825	S
148	18FAWWON-SA	080229	KAROUS-4/7/NE COMP1/5/BEZ//TOB/8156/4/ON/3/TH*6/KF//LEE*6/K/6/TAST/SPRW..	TCI 001744	-030YE-030YE-2E -0E -3E-0E	TCI	7,0	2,85774	1,42887	MR
149	18FAWWON-SA	080271	TAM200/KAUZ//Ltg-164	TCI-01-135	-0AP-0AP-18AP-0AP-3AP-0AP	TCI	11,2	2,86744	1,43372	MS
150	18FAWWON-SA	080335	VORONA/HD2402/3/RSK/CA8055//CHAM6	TCI-01-61	-0AP-0AP-14AP-0AP-4AP-0AP	TCI	15,8	3,47211	1,73606	S
151	18FAWWON-SA	080364	DOGU88//TX71A374.4/TX71A1039.V1/3/1502 W9.1/4/MIRLEBEN	TCI991247	-0YE-0YK-0YO-0YK	TCI	16,2	3,29983	1,64992	S
152	18FAWWON-SA	080221	HBA142A/HBZ621A//ABILENE/3/BURBOT-6	TCI 001619	-030YE-030YE-1E-0E -4E-0E	TCI	12,2	0,4714	0,2357	MS
153	18FAWWON-SA	080157	80 30 VERSAILLES/EDCH//CD/3/[SAULESKU 17]	C3W00WM540	-030YE-030YE-3E-0E -2E-0E	MX-TCI	11,5	9,35414	4,67707	MS
154	18FAWWON-SA	080402	CM98-79/3/T67/X84W063-9-45//K92	X990439	-0E-030YE-2E -0E -3E-0E	KSU-TCI	11,5	4,24264	2,12132	MS
155	18FAWWON-SA	080403	CM98-112/4/HAWK/81PYI9641//MESA MOTHER LINE/3/KS82W418/SPN	X990457	-0E-030YE-1E-0E -1E-0E	KSU-TCI	6,7	0,62361	0,3118	MR
156	18FAWWON-SA	070653	HBK0935-29-15/KS90W077-2-2/VBF0589-1	AP06T3832		USA	8,5	2,85774	1,42887	MR
157	18FAWWON-SA	070671	2180*K/2163//?/3/W1062A*HVA114/W3416	KS980554-12-~9		USA	7,7	1,84089	0,92045	MR
158	18FAWWON-SA	080218	ARLIN/ALTAY	TCI 001606	-030YE-030YE-1E -0E -3E-0E	TCI	14,7	4,08928	2,04464	MS
159	18FAWWON-SA	080298	YE2453/KA//1D13.1/MLT/3/VORONA/TR810 200	TCI-01-422	-0AP-0AP-27AP-0AP-1AP-0AP	TCI	9,3	3,29983	1,64992	MR
160	18FAWWON-SA	080313	DORADE-5/4/HK96/3/CHAM6//1D13.1/MLT	TCI-01-505	-0AP-0AP-13AP-0AP-1AP-0AP	TCI	10,3	4,24918	2,12459	MS
161	18FAWWON-SA	080400	CM98-64/4/HAWK/81PYI9641//MESA MOTHER LINE/3/KS82W418/SPN	X990434	-0E-030YE-2E -0E -2E-0E	KSU-TCI	8,3	4,6428	2,3214	MR

162	18FAWWON-SA	080833	RL6043/4*NAC//PASTOR/3/BABAX	C3S97M03173T	-040Y-030M-040SY-030M-040SY-11M-0Y-0SY	MX	16,5	1,77951	0,88976	S
163	18FAWWON-SA	080398	JUP/4/CLLF/3/II14.53/ODIN//CI13431/WA00477/5/GK Aron/AgSeco 7846//2180	OCW00S436S	-0YA-2E -0E -2E-0E	OK-TCI	13,7	1,64992	0,82496	MS
164	18FAWWON-SA	070668	HBK1064-3/KS84063-9-39-3-4W//X960103	KS970093-8-9-#1		USA	6,0	0,40825	0,20412	MR
165	18FAWWON-SA	090713	JAGGER/ALLIANCE	NE02558		USA	7,3	3,68179	1,84089	MR
166	C19FAWWON-INT		LANTIAN 12			PRC	6,0	1,5456	0,7728	MR
167	C19FAWWON-INT		LANTIAN 14			PRC	10,5	3,89444	1,94722	MS
168	C19FAWWON-INT		LANTIAN 15			PRC	14,3	0,84984	0,42492	MS
169	C19FAWWON-INT		LANTIAN 17			PRC	13,0	2,67706	1,33853	MS
170	C19FAWWON-INT		LANTIAN 00-30			PRC	24,0	4,08928	2,04464	HS
171	C19FAWWON-INT		BOGDANA			UKR-MIR	15,3	0,94281	0,4714	MS
172	C19FAWWON-INT		VESTA			UKR-MIR	12,7	3,47211	1,73606	MS
173	C19FAWWON-INT		VOLODARKA			UKR-MIR	21,5	5,71548	2,85774	HS
174	C19FAWWON-INT		ECONOMKA			UKR-MIR	5,2	3,92287	1,96143	MR
175	C19FAWWON-INT		KRYZHYNKA			UKR-MIR	10,7	6,73713	3,36856	MS
176	C19FAWWON-INT		KOLOS MYRONIVSCHYNY			UKR-MIR	14,0	6,37704	3,18852	MS
177	C19FAWWON-INT		KALINOVA			UKR-MIR	13,8	4,32692	2,16346	MS
178	C19FAWWON-INT		SNIZHANA			UKR-MIR	10,0	3,89444	1,94722	MR
179	C19FAWWON-INT		KATIA			BUL	8,3	2,01384	1,00692	MR
180	C19FAWWON-INT		Gariep			SA	15,0	3,18852	1,59426	MS
181	C19FAWWON-INT		Komati			SA	16,0	4,81318	2,40659	S
182	C19FAWWON-INT		Limpopo			SA	10,7	4,02768	2,01384	MS
183	C19FAWWON-INT		T06/11			SA	13,5	1,22474	0,61237	MS
184	C19FAWWON-INT		T07/09			SA	16,3	5,8642	2,9321	S
185	C19FAWWON-INT		T08/03			SA	10,8	4,92161	2,4608	MS
186	C19FAWWON-INT		SONMEZ			TR	7,3	2,77889	1,38944	MR
187	C19FAWWON-INT		T03/17			SA	8,2	2,49444	1,24722	MR
188	C19FAWWON-INT		KS98HW518(93HW91/93HW255)//KS98H245(IKE/TA2460//*3T200)/TREGO	KS05HW136-3	KSU-HAYS	SA	11,7	7,19182	3,59591	MS
189	C19FAWWON-INT		T03/01			SA	13,5	6,68331	3,34166	MS
190	C19FAWWON-INT		T04/25			SA	16,3	4,08928	2,04464	S
191	C19FAWWON-INT		T04/17			SA	4,3	2,09497	1,04748	R
192	C19FAWWON-INT		EC - P			SA	8,0	0,70711	0,35355	MR
193	C19FAWWON-INT		Kariega			SA	8,8	3,68179	1,84089	MR
194	C19FAWWON-INT		Olifants			SA	5,5	4,63681	2,3184	MR
195	C19FAWWON-INT		KONYA			TCI	11,0	4,63681	2,3184	MS
196	C19FAWWON-INT		BSP01/19 (Krokodil)			SA	13,0	8,19553	4,09776	MS
197	C19FAWWON-INT		BSP01/18 (Duzi)			SA	9,5	2,94392	1,47196	MR
198	C19FAWWON-INT		BSP06/06			SA	10,3	6,53622	3,26811	MS
199	C19FAWWON-INT		BSP06/08			SA	8,8	2,24846	1,12423	MR
200	C19FAWWON-INT		BSP06/17			SA	7,2	2,0548	1,0274	MR
201	C19FAWWON-INT		BSP07/11			SA	18,8	2,86744	1,43372	S
202	C19FAWWON-INT		BSP08/02			SA	15,5	0,40825	0,20412	S
203	C19FAWWON-INT		BSP08/06			SA	12,2	2,65623	1,32811	MS
204	C19FAWWON-INT		BSP08/10			SA	13,2	2,49444	1,24722	MS
205	C19FAWWON-INT		BSP08/11			SA	13,0	2,44949	1,22474	MS

206	C19FAWWON-INT		BSP08/12			SA	13,8	1,92931	0,96465	MS
207	C19FAWWON-INT		BSP08/13			SA	17,7	1,92931	0,96465	S
208	C19FAWWON-INT		BSP08/17			SA	11,7	4,36527	2,18263	MS
209	C19FAWWON-INT		BC01138-S			US-AGRIPRO	13,8	5,03874	2,51937	MS
210	C19FAWWON-INT		NUDAKOTA			US-AGRIPRO	4,0	1,92931	0,96465	R
211	C19FAWWON-INT		ART			US-AGRIPRO	9,8	1,31233	0,65617	MR
212	C19FAWWON-INT		JAGARENE			US-AGRIPRO	9,0	2,27303	1,13652	MR
213	C19FAWWON-INT		HAWKEN			US-AGRIPRO	10,3	1,31233	0,65617	MS
214	C19FAWWON-INT		SARATOVSKAYA90			RUS-SAR	18,3	6,11465	3,05732	S
215	C19FAWWON-INT		SARATOVSKAYA OSTISTAYA			RUS-SAR	22,0	5,01664	2,50832	HS
216	C19FAWWON-INT		SARATOVSKAYA17			RUS-SAR	15,3	4,58863	2,29432	MS
217	C19FAWWON-INT		ZHEMCHUZHINA POVOLZHJYA			RUS-SAR	12,7	2,4608	1,2304	MS
218	C19FAWWON-INT		M808/BRIGANTINA	23		RUS-SAR	15,3	3,27448	1,63724	MS
219	C19FAWWON-INT		SARATOVSKAYA90/UKRAINA	30		RUS-SAR	10,2	3,96513	1,98256	MS
220	C19FAWWON-INT		LUTESCENS329/UROZHAINAYA	33		RUS-SAR	21,2	2,01384	1,00692	HS
221	C19FAWWON-INT		GUBERNIYA/SARATOVSKAYA17	15		RUS-SAR	11,8	0,94281	0,4714	MS
222	C19FAWWON-INT		GUBERNIYA/SARATOVSKAYA18	16		RUS-SAR	13,8	9,10433	4,55217	MS
223	C19FAWWON-INT		BEZENCHUKSKAYA616			RUS-SAM	17,8	4,02768	2,01384	S
224	C19FAWWON-INT		BIRYUZA			RUS-SAM	17,3	2,39212	1,19606	S
225	C19FAWWON-INT		MALAHIT			RUS-SAM	11,5	1,87083	0,93541	MS
226	C19FAWWON-INT		BEZENCHUKSKAYA380			RUS-SAM	10,0	1,41421	0,70711	MR
227	C19FAWWON-INT		TANYA			RUS-KRAS	10,2	6,78642	3,39321	MS
228	C19FAWWON-INT		KUMA			RUS-KRAS	13,0	4,24264	2,12132	MS
229	C19FAWWON-INT		PAMYAT			RUS-KRAS	7,7	1,64992	0,82496	MR
230	C19FAWWON-INT		MOSKVICH			RUS-KRAS	9,2	5,66176	2,83088	MR
231	C19FAWWON-INT		KRASNODAR99			RUS-KRAS	11,2	4,98888	2,49444	MS
232	C19FAWWON-INT		STARSHINA			RUS-KRAS	12,8	7,36357	3,68179	MS
233	C19FAWWON-INT		MASCOT			UK	6,3	0,2357	0,11785	MR
234	C19FAWWON-INT		LINE 39			UKR	16,3	8,33	4,165	S
235	C19FAWWON-INT		Prost/Unk95-3	TE 5644	-1T-1T-1T-1T-0T	TE-TR	9,3	3,56682	1,78341	MR
236	C19FAWWON-INT		Vorona/Parus//Hatusha/3/Lut112/4/Pehl//Rpb8-68//Chrc	TE 6035	-1T-1T-4T-0T	TE-TR	16,5	1,63299	0,8165	S
237	C19FAWWON-INT		Srz95/Gyaurs1//Sana	TE 5720	-3T-1T-2T-1T-1T-0T	TE-TR	13,2	7,35225	3,67612	MS
238	C19FAWWON-INT		Bez//Bez/Tvr/3/Krmn/Lov29/4/Kate/5/Mom	TE5446-	5T-1T-3T-1T-0T	TE-TR	9,7	4,47834	2,23917	MR
239	C19FAWWON-INT		Mex65/Momt/4/Cor71-11460/3/Pkg/Lov13//Jsw3/5/Bul5052-1	TE 5542	-1T-3T-1T-2T-0T	TE-TR	15,2	3,85861	1,92931	MS
240	C19FAWWON-INT		8272-1-1/4/Temu39.76/Chat//Cupe/3/M1223.3D.1D/Ald	TE 5694	-4T-3T-1T-1T-0T	TE-TR	15,2	2,0548	1,0274	MS
241	C19FAWWON-INT		AHMETAGA			TR	10,7	1,0274	0,5137	MS
242	C19FAWWON-INT		Zarrin/Shiroodi/6/Zarrin/5/Omid/4/Bb/Kal//Ald/3/Y50E/Kal*3//Emu"s"			Karadj	12,7	2,3214	1,1607	MS
243	C19FAWWON-INT		1-68-120/1-68-22//Mirtos/3/1-68-120/1-68-22			Karadj	15,0	0,70711	0,35355	MS
244	C19FAWWON-INT		Alamoot/Sids8			Mashhad	10,0	1,87083	0,93541	MR
245	C19FAWWON-INT		Zarrin*2/Shiroodi/3/Zarrin//Vee/Nac			Miandoab	8,7	4,02768	2,01384	MR
246	C19FAWWON-INT		Owl/Shiroodi/3/Owl//Opata*2/Wulp			Miandoab	8,5	2,67706	1,33853	MR
247	C19FAWWON-INT		1-68-120/1-68-22/4/Kal/Bb//Cj"s"/3/Hork"s"			Ardebil	11,0	0,8165	0,40825	MS

248	C19FAWWON-INT		OVERLEY*3/AMADINA	KS06O3A~57-1		KSU-Man	18,2	2,09497	1,04748	S
249	C19FAWWON-INT		2145/X940786-6-7	TX05A001822		Texas A&M	10,5	1,63299	0,8165	MS
250	C19FAWWON-INT		NE93407/TX86V1115/4/T107//TX78V3620/Ctk78/3/TX87V1233	TX05A001398		Texas A&M	15,5	3,89444	1,94722	S
251	C19FAWWON-INT		NE96644(=ODESSKAYA P./CODY)//PAVON/*3SCOUT66/3/WAHOO SIB	NI04420		UNL	17,3	3,00925	1,50462	S
252	C19FAWWON-INT		Wesley/NE93613	SD05118-1		SDSU	12,2	3,09121	1,5456	MS
253	C19FAWWON-INT		BEZOSTAYA			RUS	14,0	2,85774	1,42887	MS
254	C19FAWWON-TCI	090008	F12.71/SKA//FKG15/3/F483/4/CTK/VEE/5/SHARK/F4105W2.1	-030YE-30E-2E-0E-1E-0E	TCI011134	TCI	17,2	3,79327	1,89663	S
255	C19FAWWON-TCI	090015	55.1744/MEX67.1//NO57/3/KAUZ/4/SHARK/F4105W2.1/5/TX96V2427	-030YE-30E-3E-0E-2AP-0AP	TCI012335	TCI	7,5	1,47196	0,73598	MR
256	C19FAWWON-TCI	090019	ZANDER-6/5/YE2453/4/KS831024/3/AUR/LANC//NE7060	-0AP-0AP-16AP-0AP -1E-0E	TCI-02-257	TCI	12,2	2,35702	1,17851	MS
257	C19FAWWON-TCI	090057	GRECUM 84//PYN/BAU	-0AP-0AP-18AP-0AP -1E-0E	TCI-02-726	TCI	12,5	2,54951	1,27475	MS
258	C19FAWWON-TCI	090049	SWON98-124/3/AGRI/NAC//ATTILA	-0AP-0AP-0AP-2E-0E-3E-0E-1E-0E	ICWH99353	TCI	17,8	0,62361	0,3118	S
259	C19FAWWON-TCI	090051	VORONA/HD2402/4/TAST/SPRW//BLL/3/NWT	-030YE-30E-8E-0E-1E-0E	TCI011030	TCI	13,5	3,34166	1,67083	MS
260	C19FAWWON-TCI		PYN/PARUS/3/VPM/MOS83-11-4-8//PEW/4/Bluegil	-030YE-30E-2E-0E-1E-0E	TCI011322	TCI	17,2	1,17851	0,58926	S
261	C19FAWWON-TCI	90295	JI5418/MARAS//SHARK/F4105W2.1	-030YE-30E-7E-0E-1E-0E	TCI011194	TCI	16,5	6,16441	3,08221	S
262	C19FAWWON-TCI	90350	HBA142A/HBZ621A//ABILENE/3/CAMPION/4/F6038W12.1	-030YE-30E-3E-0E-1E-0E	TCI012144	TCI	9,7	3,51979	1,75989	MR
263	C19FAWWON-TCI	90353	BLUEGIL-2/BUCUR//SIRENA	-030YE-30E-3E-0E-1E-0E	TCI012159	TCI	15,5	0,40825	0,20412	S
264	C19FAWWON-TCI	90493	PYN/BAU/3/KAUZ//KAUZ/STAR	-030YE-30E-4E-0E-1E-0E	C3W01WM00586S	MX-TCI	12,8	1,64992	0,82496	MS
265	C19FAWWON-TCI	90532	BR1284//BH114686/ALD/3/CAZO/4/KS940786-6-7	-30E-1E-0E-2E-0E	X011602	KS-TCI	13,7	3,42377	1,71189	MS
266	C19FAWWON-TCI	90572	LOV26/LFN/SDY(ES84-24)/3/SERI/4/FDL49../5/LAGOS-6	-030YE-30E-1E-0E-1E-0E	TCI011046	TCI	19,3	4,40328	2,20164	S
267	C19FAWWON-TCI	90590	ADMIS//MILAN/DUCULA	-030YE-30E-1E-0E-1E-0E	C3W01WM00331S	MX-TCI	19,2	3,32499	1,66249	S
268	C19FAWWON-TCI	90614	AGRI/BJY//VEE/3/BUCUR/4/DOGU88//TX71A374.4/TX71A1039.V1/3/1502W9.1	-030YE-30E-1E-0E-2E-0E	TCI012082	TCI	20,0	3,62859	1,8143	HS
269	C19FAWWON-TCI	50852	VO1225			TCI	11,8	1,31233	0,65617	MS
270	C19FAWWON-TCI	108	FRTL/NEMURA	-0AP-0YC-*-1YE-1YC-0YC	C3W93WM0073	MX-TCI	11,8	1,0274	0,5137	MS
271	C19FAWWON-TCI	60585	338-K1-1//ANB/BUC/3/GS50A	-0SE-0YC-0YE-4YE-0YE-4YE-0YE	TCI971351	TCI	16,7	2,95334	1,47667	S
272	C19FAWWON-TCI	90216	BEZ/NAD//KZM (ES85.24)/3/MILAN/4/SPN/NAC//ATTILA	-030YE-30E-1E-0E-1E-0E	TCI011486	TCI	11,3	5,2015	2,60075	MS

273	C19FAWWON-TCI	90240	RAN/NE701136//CI13449/CTK/3/CUPE/4/TAM200/KAUZ/5/BWD	-030YE-30E-3E-0E-1E-0E	TCI012234	TCI	21,2	7,48703	3,74351	HS
274	C19FAWWON-TCI	90181	CTY*3/TA2460//LAGOS-6	-030YE-30E-1E-0E-2E-0E	TCI011059	TCI	9,3	4,47834	2,23917	MR
275	C19FAWWON-TCI		TOSUNBEY			TR	12,8	1,84089	0,92045	MS
276	11CBWF	950590	KATIA1			BUL	10,5	2,12132	1,06066	MS
277	11CBWF	050670	STARSHINA			RUS	14,0	4,49073	2,24537	MS
278	11CBWF	000033	AGRI/NAC//KAUZ	-0SE-0YC-0YC-*-5YE-5YC-0YC	C3W92WM00231S	MX-TCI	15,3	1,5456	0,7728	MS
279	11CBWF	060075	TX69A509.2//BBY/FOX/3/GRK//NO64/PEX/4/CER/5/CHIL/2*STAR	-0E-0E-5E-0E-3E-0E	TCI981148	TCI	13,7	5,83571	2,91786	MS
280	11CBWF	060074	TX69A509.2//BBY/FOX/3/GRK//NO64/PEX/4/CER/5/CHIL/2*STAR	-0E-0E-5E-0E-2E-0E	TCI981148	TCI	8,7	5,31246	2,65623	MR
281	11CBWF	090552	ES14/SITTA//AGRI/NAC/3/BURBOT-4	-030YE-30E-2E-0E-4AP-0AP	TCI011118	TCI	15,2	1,69967	0,84984	MS
282	11CBWF	991760	Caledon			TCI	11,2	3,96513	1,98256	MS
283	C19FAWWON-INT		Alamoot/Sids8			Mashhad	12,7	3,00925	1,50462	MS
284	C19FAWWON-INT		TOSUNBEY			TR	7,7	3,29983	1,64992	MR
285	C19FAWWON-INT		KARAHAN			TR	11,7	3,47211	1,73606	MS
286	C19FAWWON-INT		Alamoot/Shiroodi			Mashhad	15,5	4,54606	2,27303	S
287	C19FAWWON-INT		Alamoot/Sids8			Mashhad	11,8	1,24722	0,62361	MS
288	C19FAWWON-TCI	090028	RSK/CA8055//CHAM6/4/NWT/3/TAST/SPRW//TAW12399.75	-0AP-0AP-25AP-0AP-4AP-0AP	TCI-02-47	TCI	15,5	3,18852	1,59426	S
289	C19FAWWON-TCI		EXCALIBUR/WBLL1	-0P0Y-040M-040SY-030M-8ZLM-0ZTY	C3A00Y00600S	MX	22,3	7,26101	3,6305	HS
290	C19FAWWON-INT		Bezostaya 1		LUT17/SRS2	Rus	23,2	2,4608	1,2304	HS
291	C19FAWWON-INT		Katea		Hebros/Bez-1	Bul	5,3	1,24722	0,62361	MR

*ACCNO accession number, CBWF Cross block winter facultative, CName common name, CID cross identification, ELITE semi arid, FAWWON Facultative and winter wheat observation nursery, HS highly susceptible, IRR Irrigated, INT International, MR moderately resistance, MS moderately resistant, SELHX selection history, SD standard deviation, SE standard error, R resistant, S susceptible and , SA South Afrika, TCI Turkey-CIMMYT-ICARDA

Host status	**Number of wheat accessions**	**% of wheat accessions**
R	2	1
MR	47	16
MS	120	41
S	77	26
HS	44	15
Total	290	100

6.2. Supplementary materials for:

Genome-wide association study in wheat identifies resistance to the cereal cyst nematode *Heterodera filipjevi*

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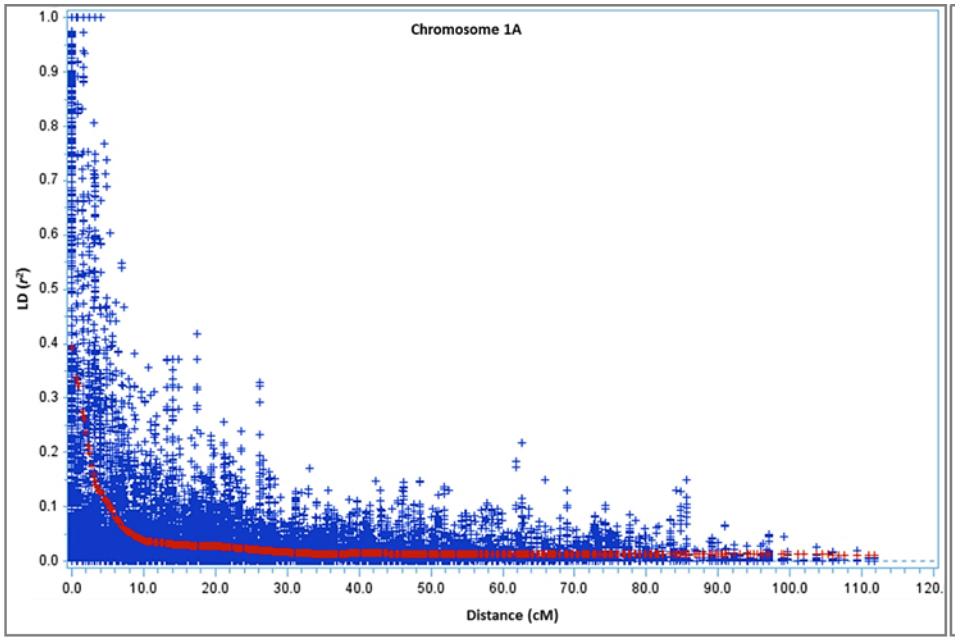
Pdf file includes:

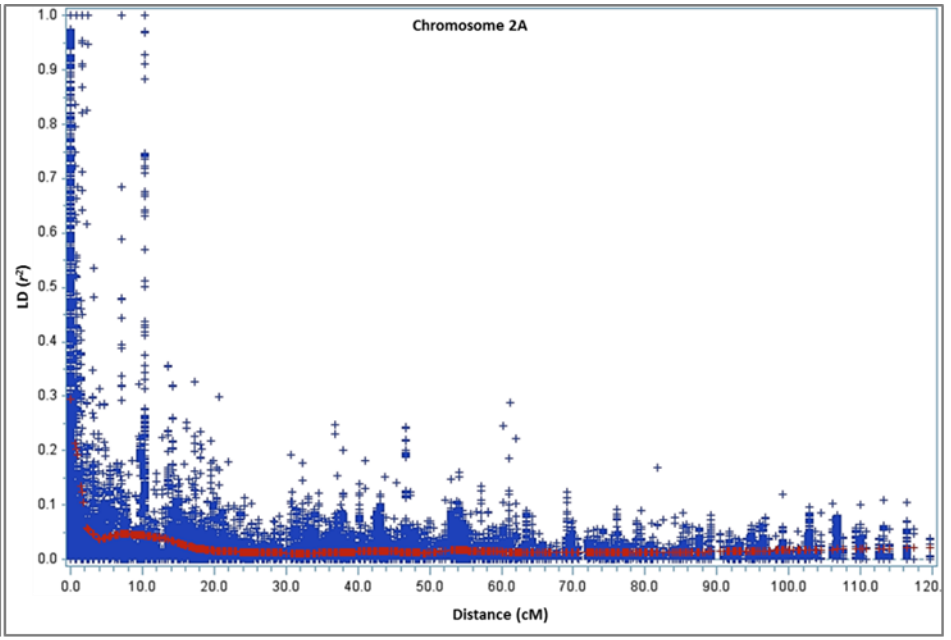
Fig. S1. LD decay estimated using wheat chromosomes (1-6A, 7A-5B, 6B-4D, and 5-7D) of 161 winter wheat accessions based on polymorphic single nucleotide polymorphism (SNP) markers. Decay of r^2(0-1) as a function of genetic distance between SNP markers estimated for A, B, and D genomes of winter wheat accessions.

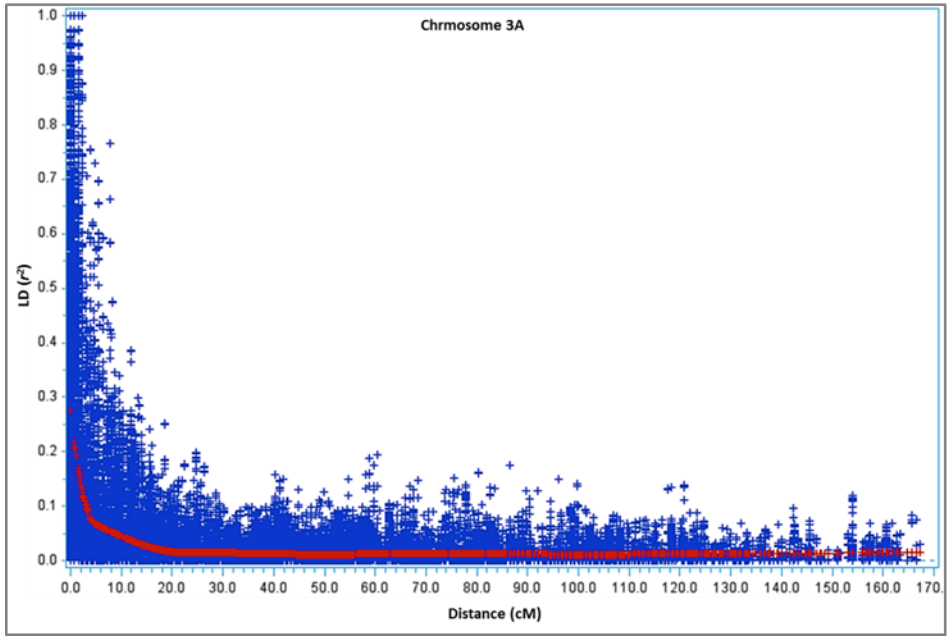
Fig. S2. Heat maps of linkage disequilibrium for significant markers on chromosome 2A (S21A), 2B (S2B), 3A (S2C), 3B (S2D), 4A (S2E), 4A (S2F) and 5B (S2G). Color represents a rough measure of significance (red or black) better than pink, gray, white. r^2 =0 (white color), $0<r^2<1$(shades of grey) and r^2=1 bright red)

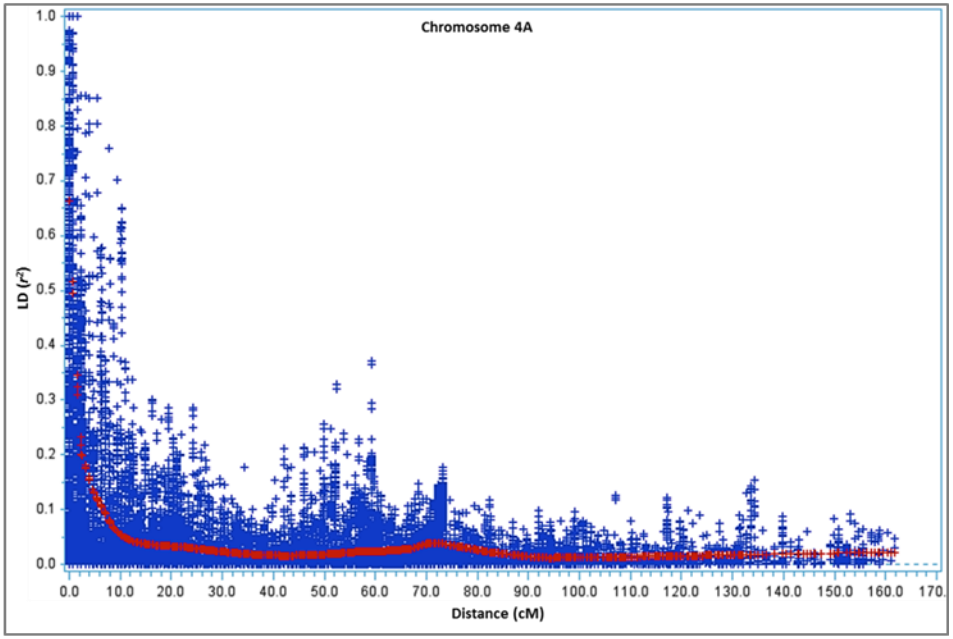
Table S1. List of 161 winter wheat accessions with accession number, common name, selection history, origin, and host response to *Heterodera filipjevi*

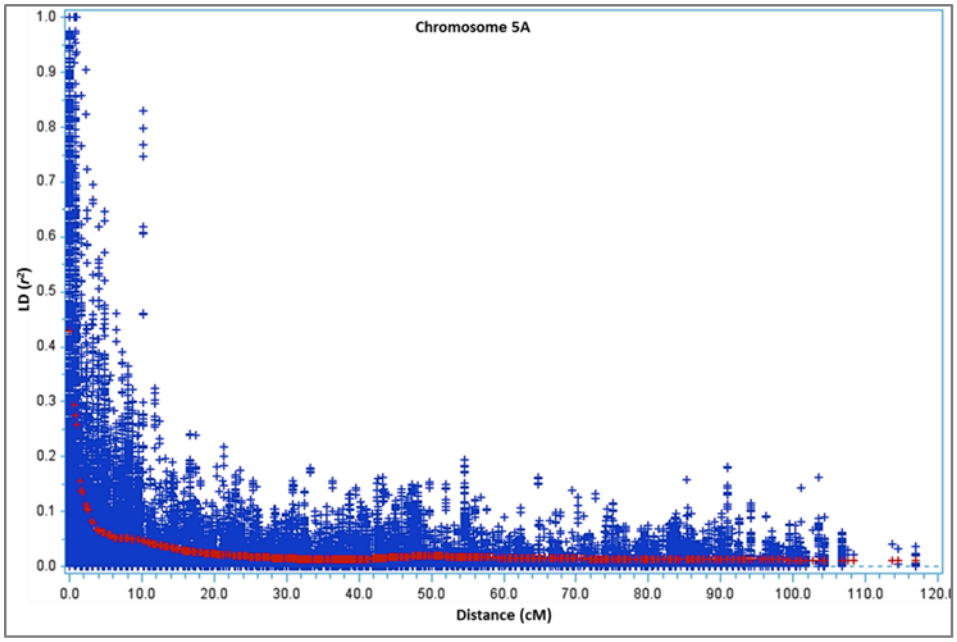
Table S2. *In-silico* annotation of SNP marker flanking sequence against protein coding sequence (CDS) and protein of Brachypodium, rice and sorghum

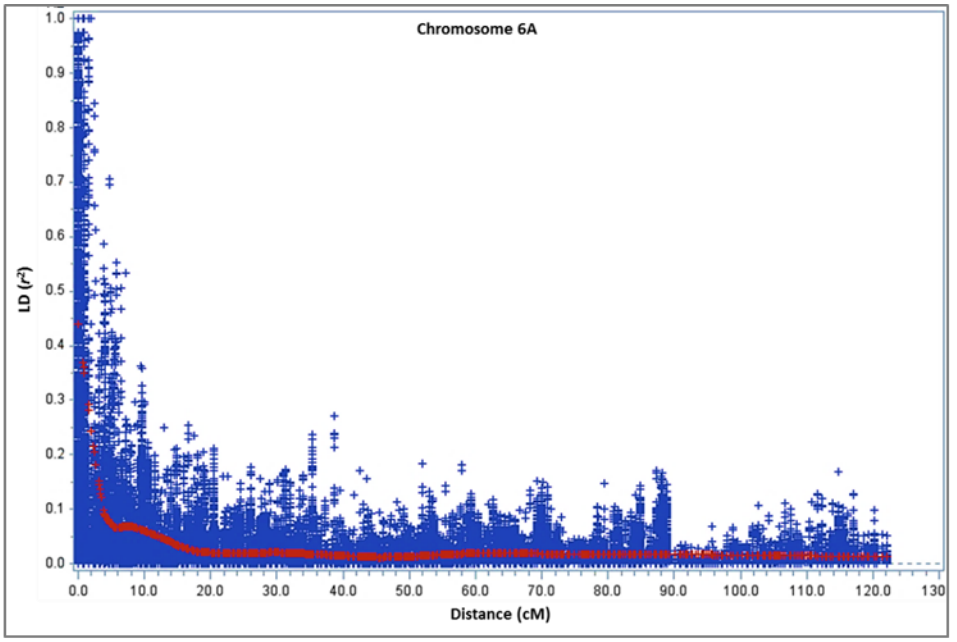

Chromosome 1A
LD (r^2)
Distance (cM)

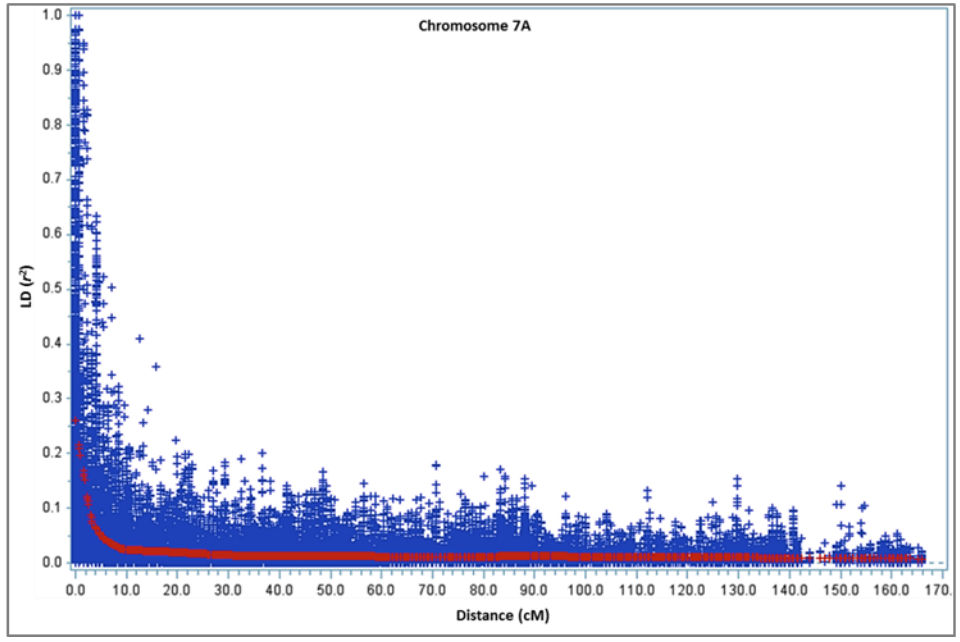

Chromosome 2A
LD (r^2)
Distance (cM)

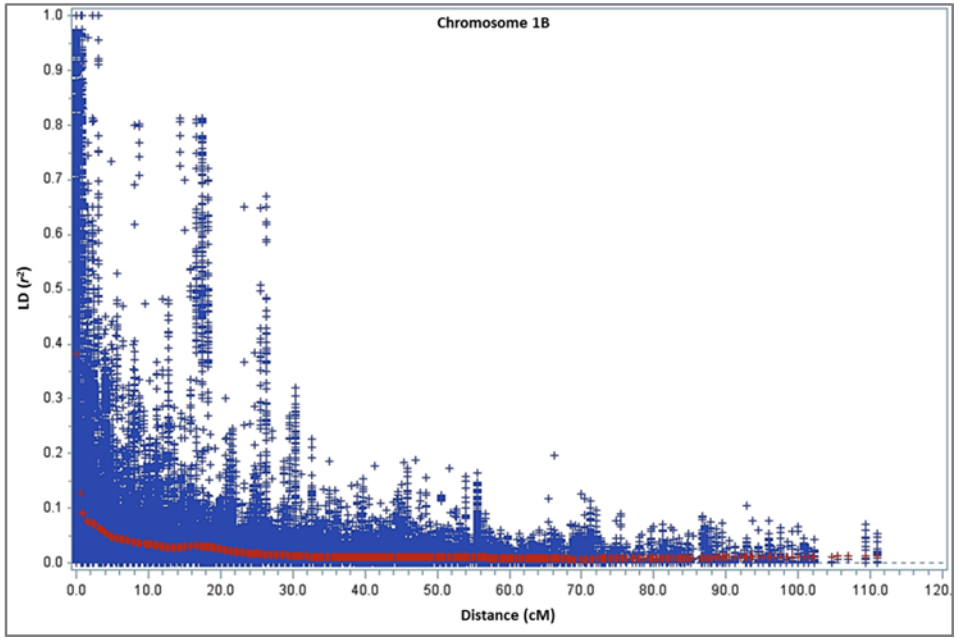

Chrmosome 3A
LD (r^2)
Distance (cM)

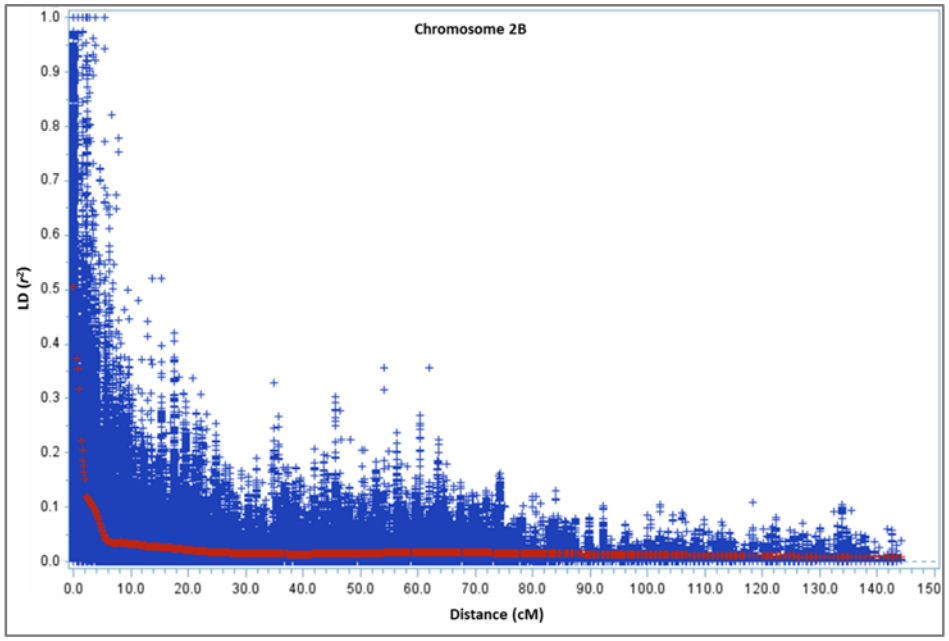

Chromosome 4A
LD (r^2)
Distance (cM)

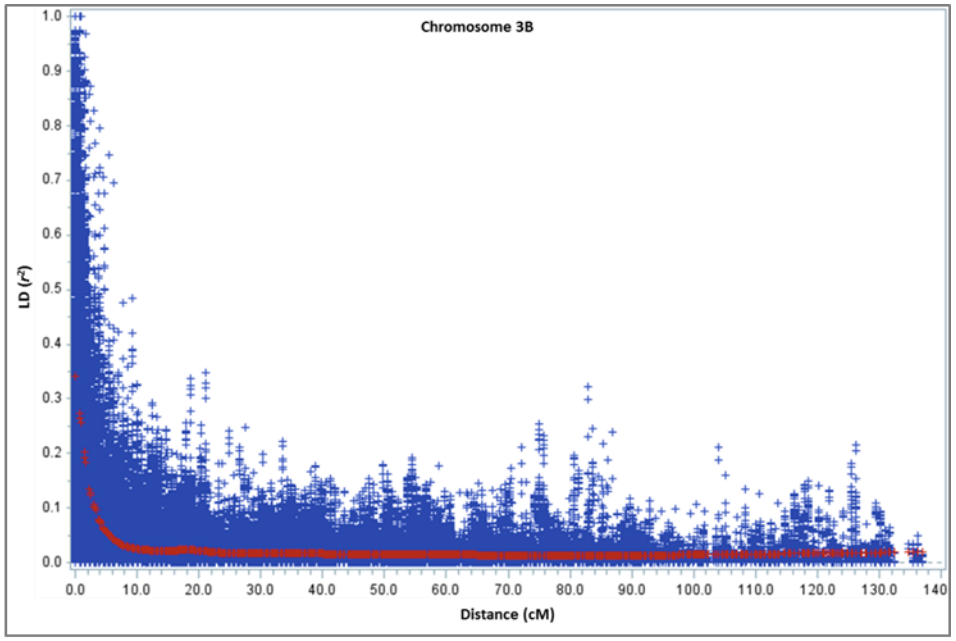

Chromosome 5A
LD (r^2)
Distance (cM)

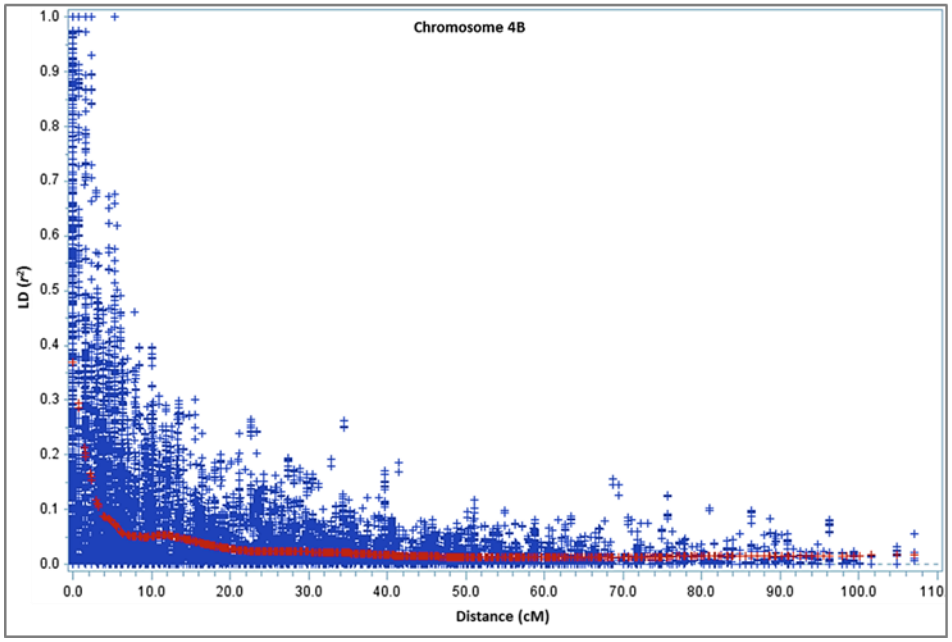

Chromosome 6A
LD (r^2)
Distance (cM)

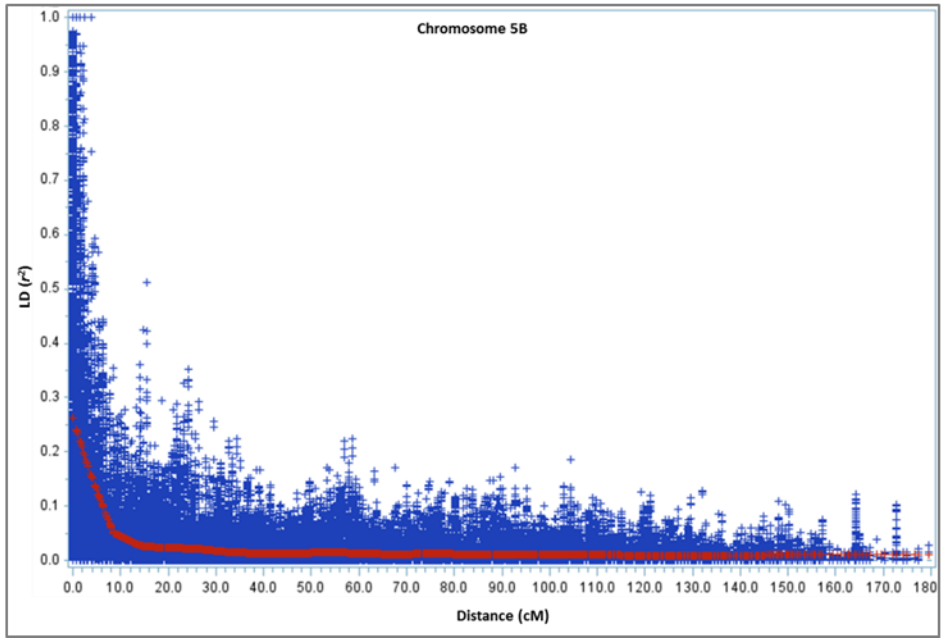

Chromosome 7A
LD (r^2)
Distance (cM)

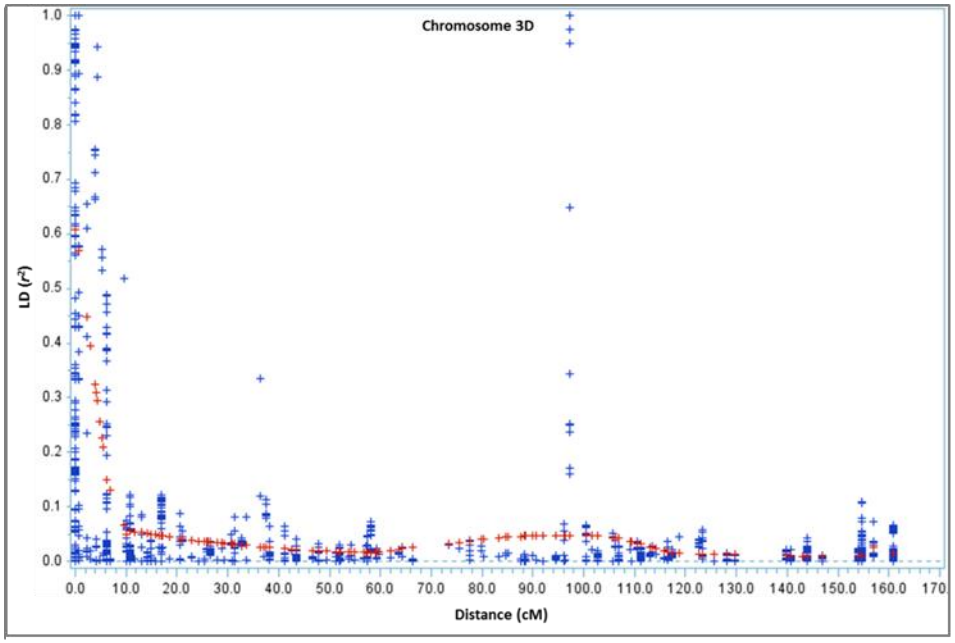

Chromosome 1B
LD (r^2)
Distance (cM)

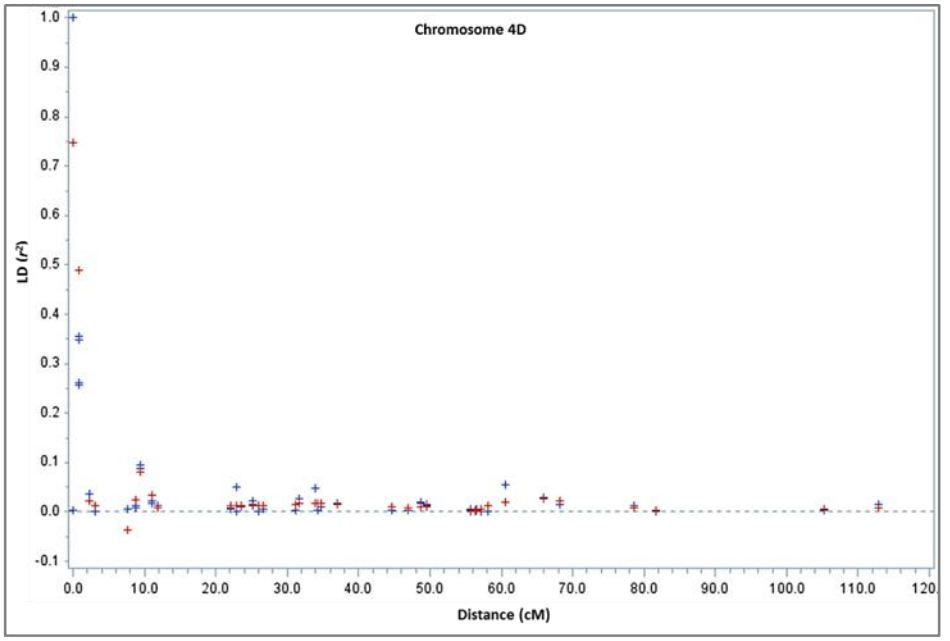

Chromosome 2B
LD (r^2)
Distance (cM)

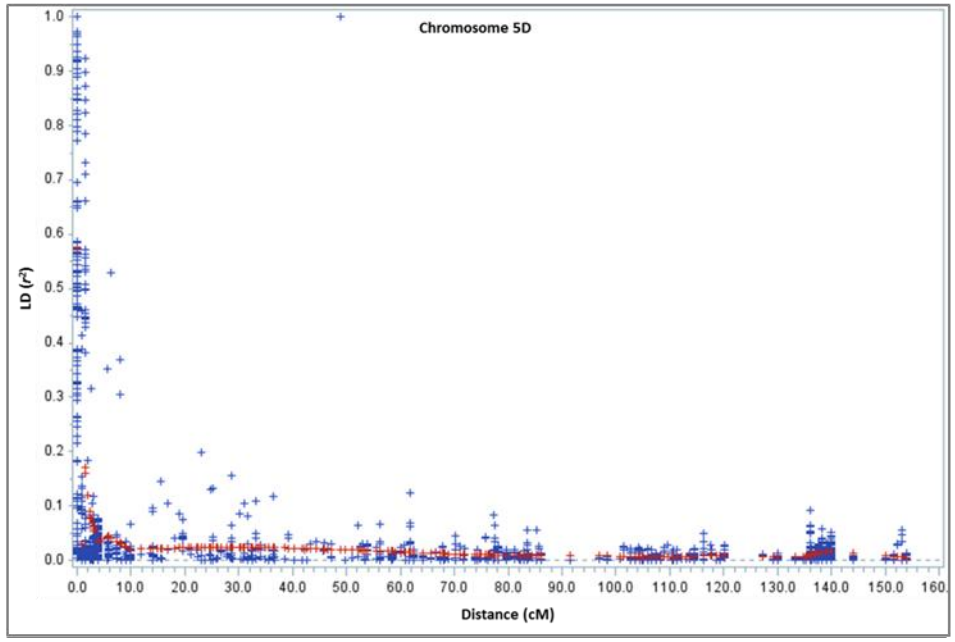

Chromosome 3B
LD (r^2)
Distance (cM)

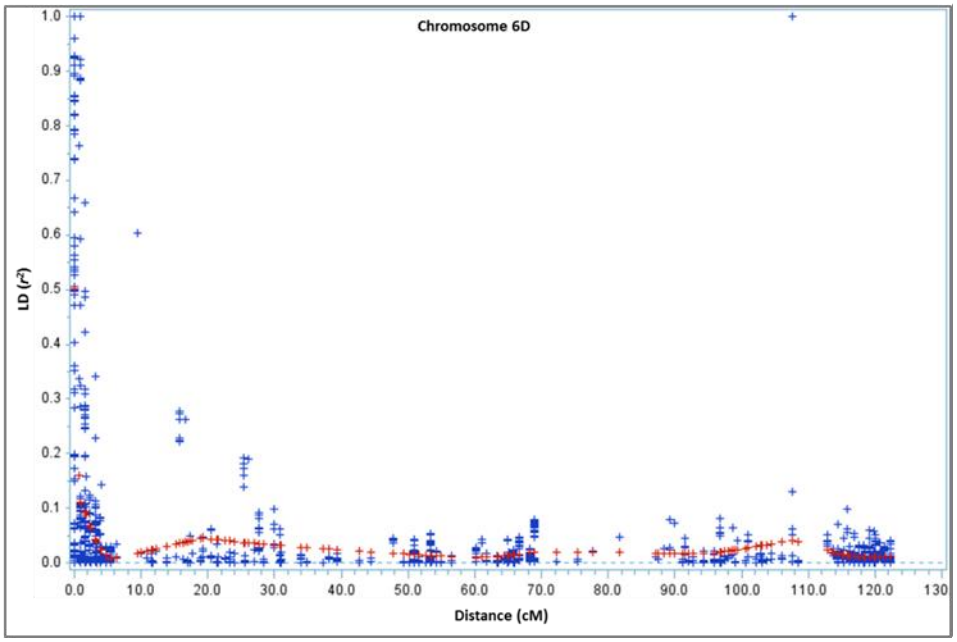

Chromosome 4B
LD (r^2)
Distance (cM)


Chromosome 5B
LD (r^2)
Distance (cM)


Chromosome 3D
LD (r^2)
Distance (cM)


Chromosome 4D
LD (r^2)
Distance (cM)


Chromosome 5D
LD (r^2)
Distance (cM)


Chromosome 6D
LD (r^2)
Distance (cM)

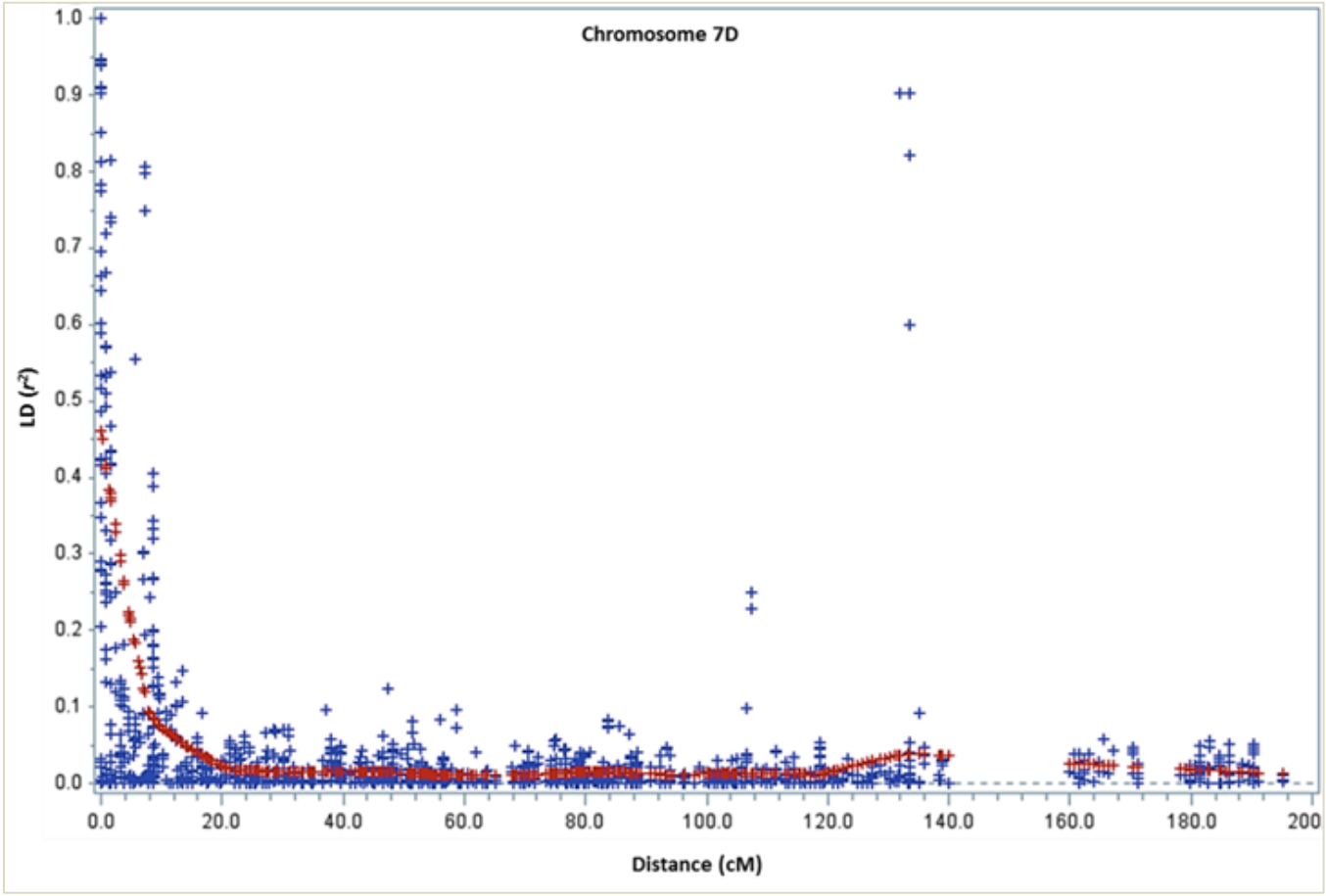
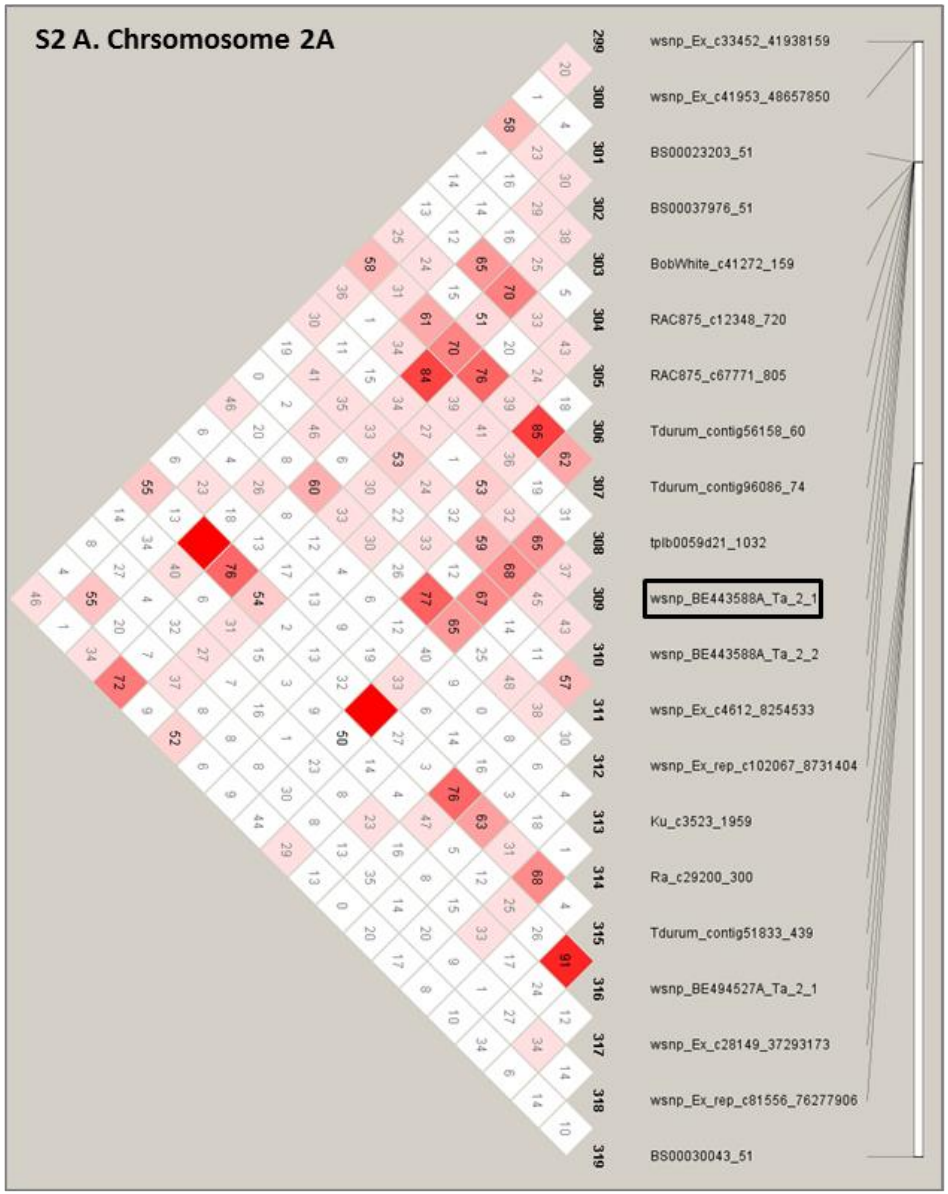


Fig. S1. LD decay estimated using wheat chromosomes (1-6A, 7A-5B, 6B-4D, and 5-7D) of 161 winter wheat accessions based on polymorphic single nucleotide polymorphism (SNP) markers. Decay of r^2(0-1) as a function of genetic distance between SNP markers estimated for A, B, and D genomes of winter wheat accessions


S2 A. Chrsomosome 2A
wsnp_Ex_c33452_41938159
wsnp_Ex_c41953_48657850
BS00023203_51
BS00037976_51
BobWhite_c41272_159
RAC875_c12348_720
RAC875_c67771_805
Tdurum_contig56158_60
Tdurum_contig96086_74
tplb0059d21_1032
wsnp_BE443588A_Ta_2_1
wsnp_BE443588A_Ta_2_2
wsnp_Ex_c4612_8254533
wsnp_Ex_rep_c102067_8731404
Ku_c3523_1959
Ra_c29200_300
Tdurum_contig51833_439
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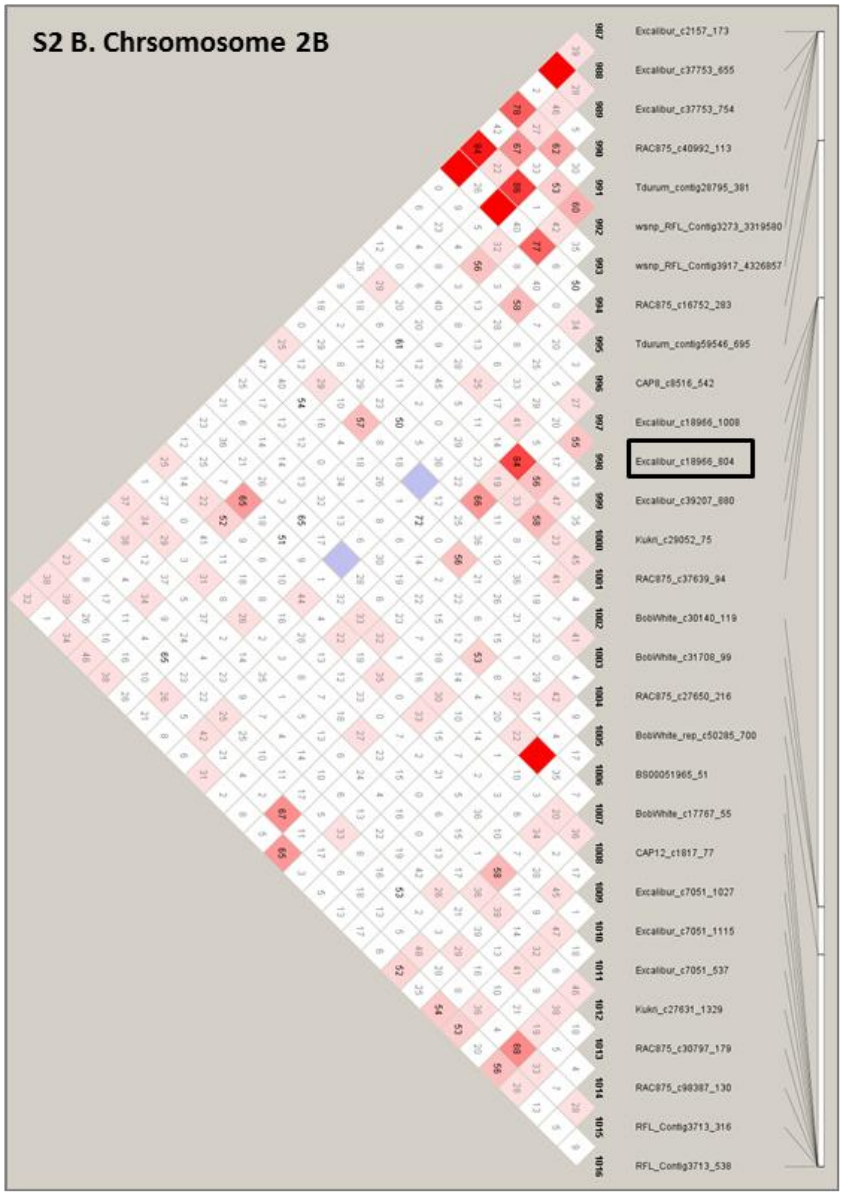
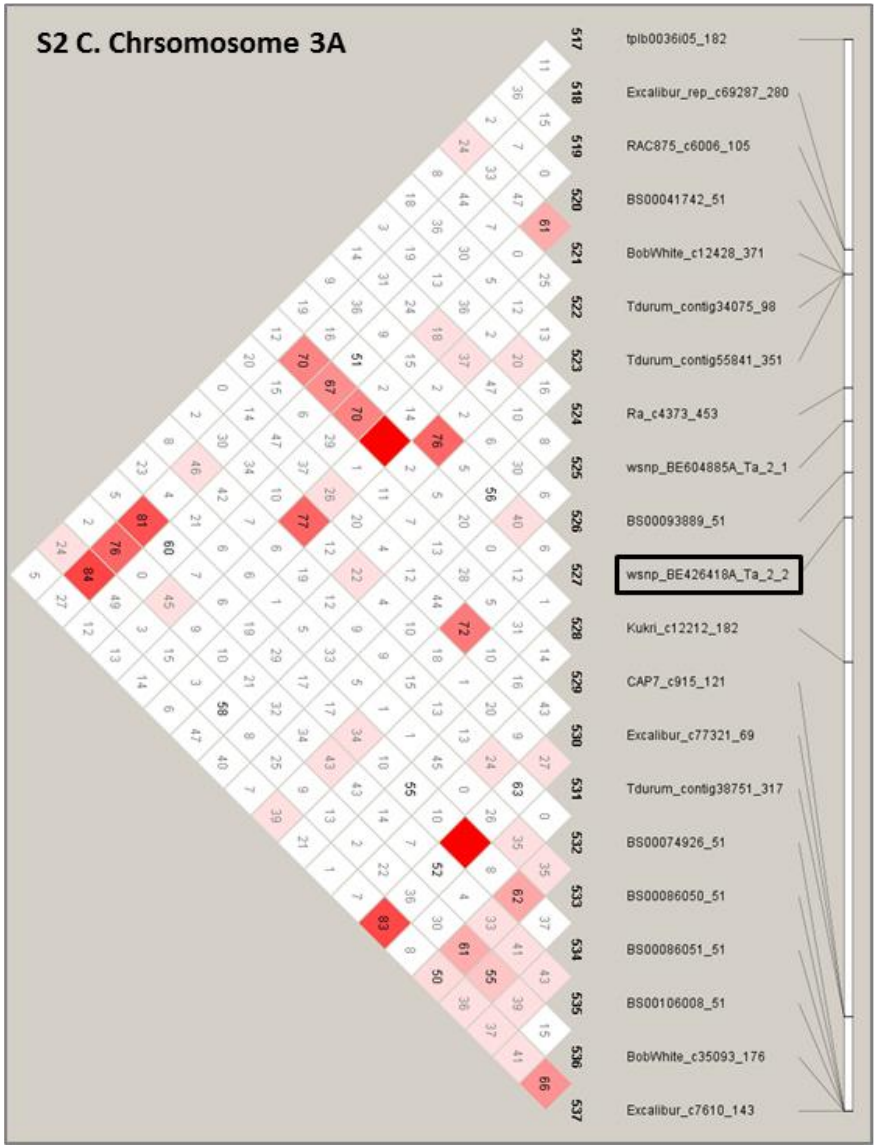
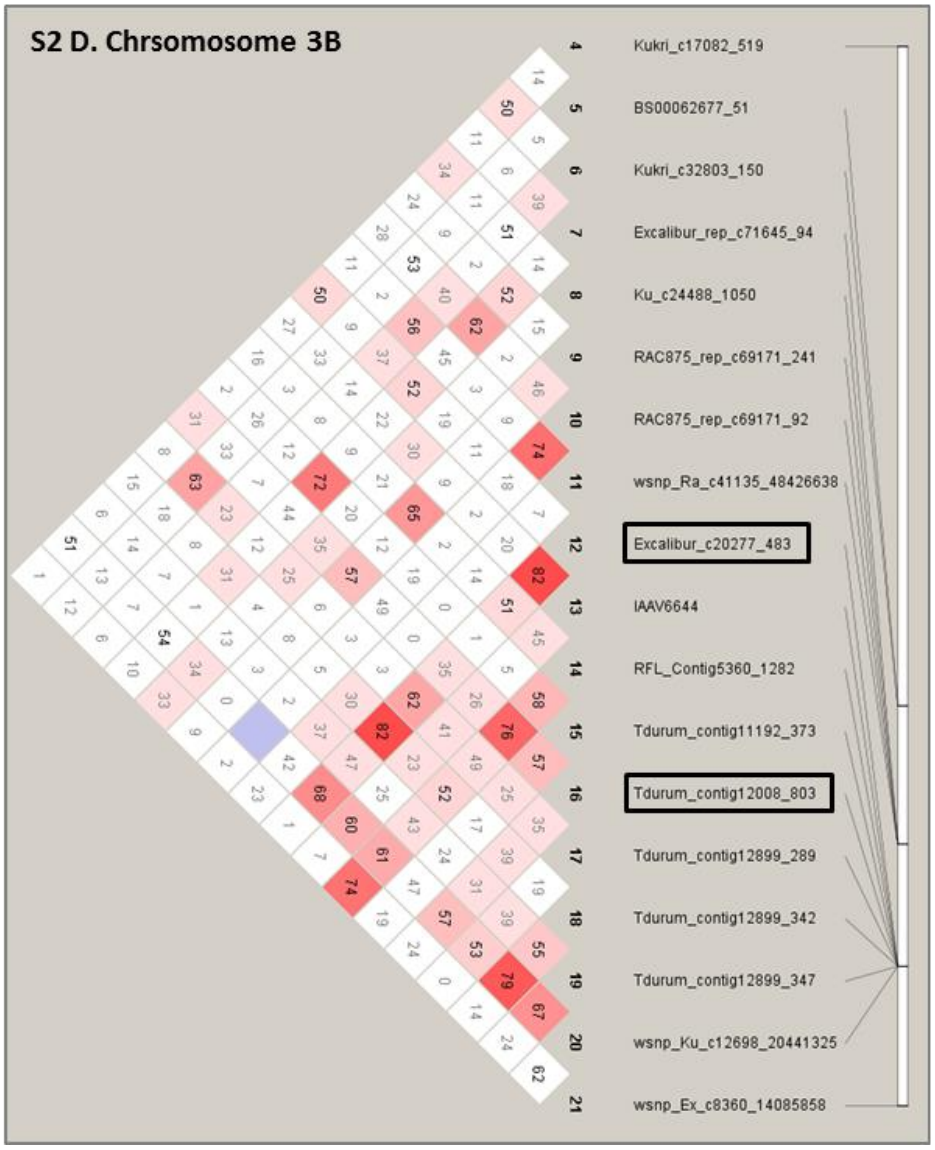
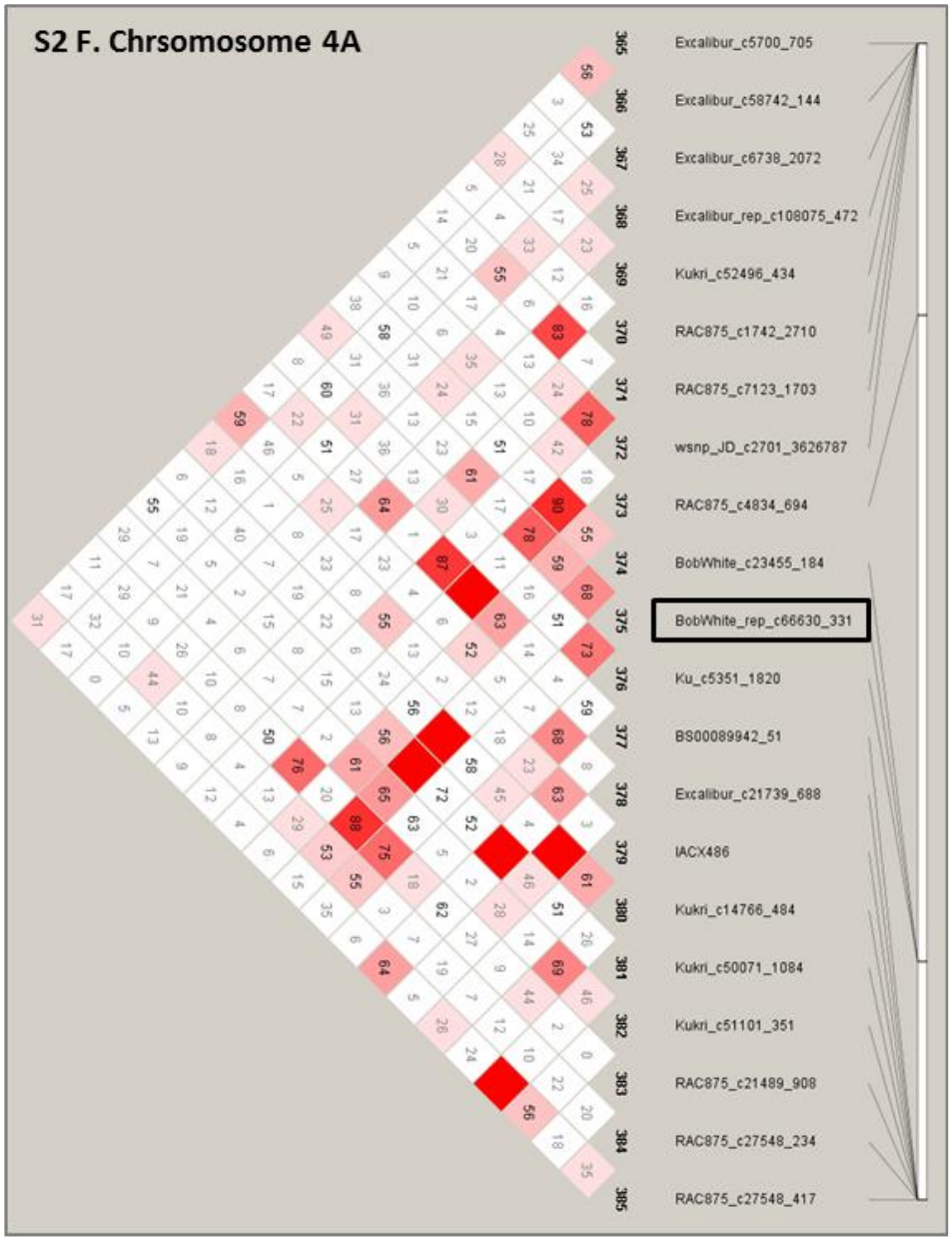
wsnp_Ex_c28149_37293173
wsnp_Ex_rep_c81556_76277906
BS00030043_51


S2 B. Chrsomosome 2B
Excalibur_c2157_173
Excalibur_c37753_655
Excalibur_c37753_754
RAC875_c40992_113
Tdurum_contig28795_381
wsnp_RFL_Contig3273_3319580
wsnp_RFL_Contig3917_4326857
RAC875_c16752_283
Tdurum_contig59546_695
CAP8_c8516_542
Excalibur_c18966_1008
Excalibur_c18966_804
Excalibur_c39207_880
Kukri_c29052_75
RAC875_c37639_94
BobWhite_c30140_119
BobWhite_c31708_99
RAC875_c27650_216
BobWhite_rep_c50285_700
BS00051965_51
BobWhite_c17767_55
CAP12_c1817_77
Excalibur_c7051_1027
Excalibur_c7051_1115
Excalibur_c7051_537
Kukri_c27631_1329
RAC875_c30797_179
RAC875_c98387_130
RFL_Contig3713_316
RFL_Contig3713_538


S2 C. Chrsomosome 3A
517 tplb0036i05_182
518 Excalibur_rep_c69287_280
519 RAC875_c6006_105
520 BS00041742_51
521 BobWhite_c12428_371
522 Tdurum_contig34075_98
523 Tdurum_contig55841_351
524 Ra_c4373_453
525 wsnp_BE604885A_Ta_2_1
526 BS00093889_51
527 wsnp_BE426418A_Ta_2_2
528 Kukri_c12212_182
529 CAP7_c915_121
530 Excalibur_c77321_69
531 Tdurum_contig38751_317
532 BS00074926_51
533 BS00086050_51
534 BS00086051_51
535 BS00106008_51
536 BobWhite_c35093_176
537 Excalibur_c7610_143


S2 D. Chrsomosome 3B
4 Kukri_c17082_519
5 BS00062677_51
6 Kukri_c32803_150
7 Excalibur_rep_c71645_94
8 Ku_c24488_1050
9 RAC875_rep_c69171_241
10 RAC875_rep_c69171_92
11 wsnp_Ra_c41135_48426638
12 Excalibur_c20277_483
13 IAAV6644
14 RFL_Contig5360_1282
15 Tdurum_contig11192_373
16 Tdurum_contig12008_803
17 Tdurum_contig12899_289
18 Tdurum_contig12899_342
19 Tdurum_contig12899_347
20 wsnp_Ku_c12698_20441325
21 wsnp_Ex_c8360_14085858


S2 E. Chrsomosome 4A


S2 F. Chrsomosome 4A
Excalibur_c5700_705
Excalibur_c58742_144
Excalibur_c6738_2072
Excalibur_rep_c108075_472
Kukri_c52496_434
RAC875_c1742_2710
RAC875_c7123_1703
wsnp_JD_c2701_3626787
RAC875_c4834_694
BobWhite_c23455_184
BobWhite_rep_c66630_331
Ku_c5351_1820
BS00089942_51
Excalibur_c21739_688
IACX486
Kukri_c14766_484
Kukri_c50071_1084
Kukri_c51101_351
RAC875_c21489_908
RAC875_c27548_234
RAC875_c27548_417

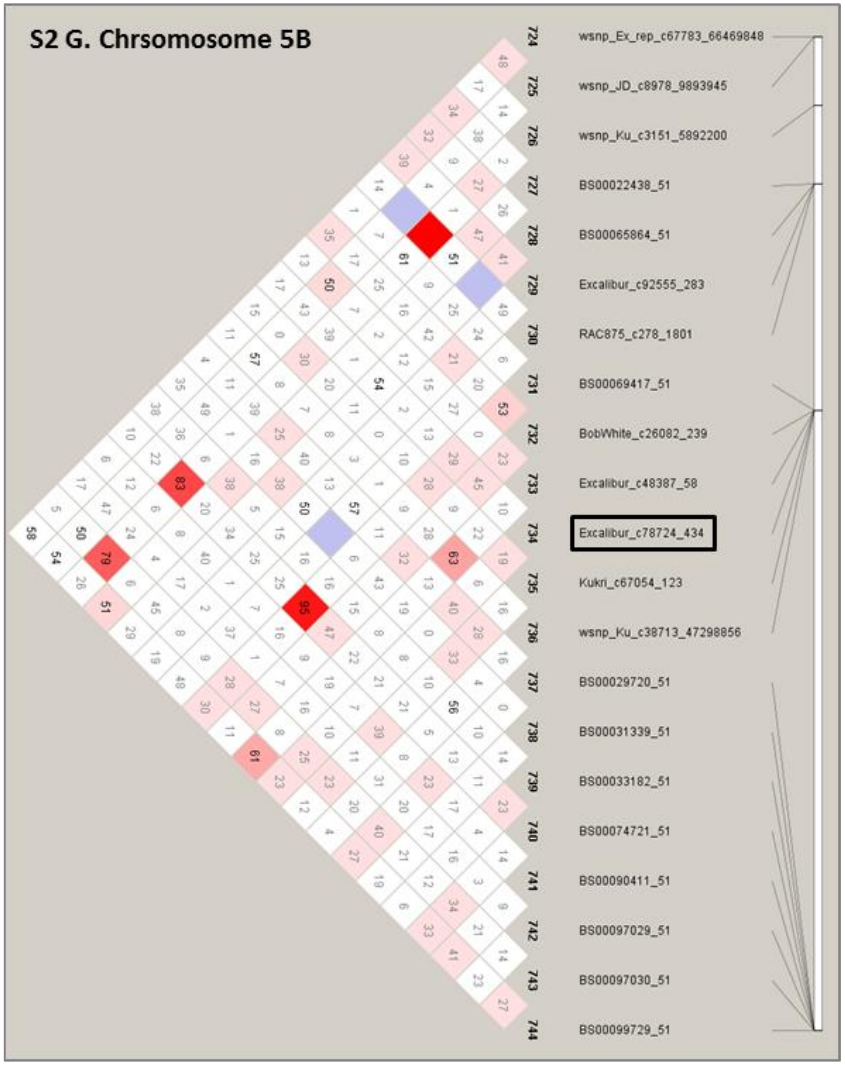


Fig. S2. Heat maps of linkage disequilibrium for significant markers on chromosome 2A (S21A), 2B (S2B), 3A (S2C), 3B (S2D), 4A (S2E), 4A (S2F) and 5B (S2G). Color represents a rough measure of significance (red or black) better than pink, gray, white. r^2 =0 (white color), $0<r^2<1$(shades of grey) and r^2=1 bright red).

TABLE S1. List of 161 winter wheat accessions with accession number, common name, selection history, origin, and host response to *Heterodera filipjevi*

Entry	Nursery	ACCNO	CName	SELHX	CID	Origin	Status	Female/5 rep	SD	SE	Host status	PC 1 (6.19)	PC 2 (1.35)	PC 3 (1.00)	Sub-population
1	11CBWF	951327	KINACI97	-7M-0M-8M-1M-3WM-0WM-4WM-2WM-0WM	SWM12289	MX-BD	C	24,0	4,252450274	2,126225137	HS	7,59066	-16,08062708	4,573162859	Admixed
2	11CBWF	010580	KROSHKA			RUS	C	18,0	5,049752469	2,524876235	S	54,6316	12,44912194	-21,94877324	G2
3	11CBWF	030689	POBEDA 50			RUS	C	21,3	4,904646323	2,452323162	HS	57,9463	11,17571755	-12,15377543	Admixed
4	11CBWF	080951	OBRII/DNESTREANCA25//ILICIOVCA/OD.CRASNOCOLOS			Moldova	BL	19,5	2,041241452	1,020620726	HS	62,9486	4,992214373	-8,049258065	G2
6	11CBWF	020966	L 4224 K 12			KR-RUS	BL	10,3	1,885618083	0,942809042	MS	35,8027	29,60273736	39,10248793	Admixed
7	11CBWF	000017	8023.16.1.1/KAUZ	-0SE-0YC-1YE-0YC-2YC-0YC	C3W92WM00378S	MX-TCI	BL	10,0	1,779513042	0,889756521	MR	-45,585	70,04309398	28,66292804	Admixed
8	11CBWF	070404	CUPRA-1/3/CROC1/AE.SQUARROSA (224)//2*OPATA/4/PANTHEON	-030YE-0E-2E-0E-2E-0E	TCI992280	TCI	BL	24,8	5,071708018	2,535854009	HS	-8,2213	-22,29836104	19,16934876	Admixed
9	11CBWF	070028				TCI	C	24,3	10,14341604	5,071708018	HS	35,7452	29,50314159	38,82943586	Admixed
11	11CBWF	050179	BONITO//KAREE/TUGELA	-0AP-0AP-0YE-5YE-0YE-3YE-0YE	TCI97AP 039	TCI	BL	19,8	4,496912521	2,248456261	HS	-24,609	2,158500679	-13,9211958	Admixed
12	11CBWF	010634	CETINEL 2000	-6H-0YC-0R-1YC-0YC-0E	OWC852672	TR-ESK	C	18,2	4,089281382	2,044640691	S	-23,546	86,42109084	-17,77572154	Admixed
13	11CBWF	040569	OR941611			USA-OR	C	14,7	1,92930615	0,964653075	MS	-2,0804	-12,28109996	4,081589313	Admixed
14	11CBWF	030164	KS82W409/SPN//TAM106/TX78V3630	-0SE-0YC-0E-1YE-0YE-2YM-0YM	TCI951385	TCI	BL	16,8	1,433720878	0,716860439	S	3,52461	-22,74445691	14,40160857	Admixed
15	11CBWF	040347	MAHON DEMIAS/3/HIM/CNDR//CA8055	-0AP-0YC-4E-0E-3K-0YK	TCI960471	TCI	BL	21,3	4,365266951	2,182633476	HS	5,12256	-25,60456649	14,88143676	Admixed
17	11CBWF	000008	KOLLEGA			RUS	C	20,2	6,407460929	3,203730464	HS	41,9002	43,21468309	35,39894601	Admixed
19	11CBWF		MOSKVICH			RUS-KRAS	C	11,3	3,399346342	1,699673171	MS	60,6722	12,02965089	-19,23758627	G2
20	11CBWF		POSTROCK			US-AGRIPRO	C	12,5	1,08012345	0,540061725	MS	-78,9	-26,43792196	8,755707469	G1
21	11CBWF	090874	Alamoot/3/Alvd//Aldan"s"/IAS58/4/Alamoot/Gaspard			IR-KARAJ	BL	20,3	2,357022604	1,178511302	HS	-3,9616	-4,500638056	-5,658558096	Admixed
22	11CBWF	100019	KS2016/Trego		ARS05-1034	US-ARS-NC	BL	6,3	3,399346342	1,699673171	MR	8,36327	-31,689575	32,07418981	Admixed
23	11CBWF	100733	MV-TOLDI			HUN	C	18,0	2,273030283	1,136515141	S	28,4582	5,477227803	18,56435976	Admixed
25	11CBWF	100676	LCR/SERI/3/MEX-DW/BACA//VONA/4/TAM200/JI5418	-0AP-DH16	ICWH99018	SYR	BL	21,0	5,115336418	2,557668209	HS	-25,547	44,23964175	64,75253206	Admixed
26	11CBWF	980135	VICTORYA			UKR	C	22,3	7,027722881	3,51386144	HS	43,8769	19,8990658	-13,94147485	Admixed
29	11CBWF	000261	VORONA/KAUZ//1D13.1/MLT	-0SE-0YC-*-3YE-3YC-0YC	CIT937111	TCI	BL	19,3	5,572751166	2,786375583	S	-28,684	83,69481746	14,64622791	Admixed
30	11CBWF	090068	RSK/CA8055//CHAM6/4/NWT/3/TAST/SPRW//TAW12399.75	-0AP-0AP-25AP-0AP-4AP-0AP	TCI-02-47	TCI	BL	17,3	2,013840996	1,006920498	S	-3,6566	-13,99638566	-3,665282948	Admixed
34	11CBWF	090495	PYN/BAU/3/KAUZ//KAUZ/STAR	-030YE-30E-6E-0E-1E-0E	C3W01WM00586S	MX-TCI	BL	21,5	4,441752657	2,220876329	HS	11,3531	-47,52238288	20,77122301	Admixed
35	11CBWF		VEE#8//JUP/BJY/3/F3.71/TRM/4/BCN/5/KAUZ/6/163	-030YE-0E-1E-0E-2E-0E	TCI992192	TCI	BL	25,7	2,013840996	1,006920498	HS	-49,456	45,78473686	13,65109452	Admixed
36	11CBWF		DORADE-5/3/BOW"S"/GEN//SHAHI	-0AP-0AP-6AP-0AP-3AP-0AP	TCI-02-522	TCI	BL	20,0	9,092121131	4,546060566	HS	-41,658	-10,75068994	-24,93319299	Admixed
37	11CBWF	010004	494J6.11//TRAP#1/BOW	-0YC-0YC-0YC-8YC-0YC-1SE-0YC-2YC-0YC	C3W90M200	MX-CIT	BL	24,0	2,254624876	1,127312438	HS	3,3539	2,305481559	-21,47251576	Admixed
38	11CBWF	020321	SAULESKU #44/TR810200	-03Y-0B-0SE-3YE-0YC-2YM-0YM	C3W94WM00586S	MX-TCI	BL	20,2	3,009245014	1,504622507	HS	-27,612	-12,90047823	-23,40590816	Admixed
39	11CBWF	950513	GUN91	-1A-1A-1A-0A	SWM7155	MX-YA	C	14,2	0,623609564	0,311804782	MS	15,0248	-37,90068751	23,83683422	Admixed
41	11CBWF	950377	DOGU88			TR-ERZ	C	19,0	1,779513042	0,889756521	S	8,02105	-30,31713102	0,547540311	Admixed
42	11CBWF	070676	KS92H363-2/COUGAR SIB(=NE85707/TBIRD) X NE94632(=ABILENE/NORKAN//RAWHIDE)			US-UNL	BL	20,2	5,104464277	2,552232139	HS	8,30682	-1,853960318	7,553830245	Admixed
43	11CBWF	090779	SAR-30			IR-DARI	LRACE	18,3	3,324989557	1,662494779	S	-12,198	-27,93144014	6,561560877	Admixed
46	11CBWF	990857	BURBOT-6	-9H-0YC-1YC-0YC-0YC-2YC-0YC-3YC-0YC	WXD880137A	OR-CIT	C	17,0	1,870828693	0,935414347	S	14,5313	-16,65583839	23,29692894	Admixed
47	11CBWF	000374	ES84.24/GRK	-0SE-0YC-1YE-0YC-2YC-0YC	CIT932135	TCI	BL	24,7	0,849836586	0,424918293	HS	-15,206	-25,79285134	26,36523446	Admixed

49	11CBWF	050696	TAM 105/3/NE70654/BBY//BOW"S"/4/Century*3/TA2450		AP01T1112	US-AgriPro South	BL	17,8	3,681787006	1,840893503	S	0,13148	-13,99848613	12,75257074	Admixed
50	11CBWF	050751	MILLENNIUM/NE93613		SD00258	US-SDSU	BL	5,8	3,922867432	1,961433716	MR	27,0186	-15,96606718	13,81966509	Admixed
52	11CBWF	040237	PYN/BAU/3/AGRI/BJY//VEE	-0SE-0YC-17E-0E-1K -0YK	TCI961547	TCI	BL	19,0	3,240370349	1,620185175	S	-43,224	35,03442039	-39,76078837	Admixed
53	11CBWF	950369	DAGDAS94	-10A-0A	YA15662	YA-BD	C	16,2	2,357022604	1,178511302	S	39,6386	-18,38315442	-30,79256875	Admixed
55	11CBWF	070676	NE04424	HRW		US-UNL	BL	22,8	4,169998668	2,084999334	HS	20,9059	19,05817367	47,813989	Admixed
56	11CBWF	090783	KOHDASHT			IR-DARI	C	15,5	1,779513042	0,889756521	S	-58,749	-20,14905755	-19,17376527	Admixed
57	11CBWF	070603	ICDW-21122	BW		AFG	LRACE	6,8	1,433720878	0,716860439	MR	-15,457	-25,84560793	-6,958557949	Admixed
59	11CBWF	030243	SABALAN/GRK//PYN/BAU	-0YC-0E-1YE-0YE-3YM-0YM	TCI952089	TCI	BL	23,3	3,423773097	1,711886549	HS	-25,906	28,22178164	-33,81014083	Admixed
60	11CBWF	030323	CA8055/4/ROMTAST/BON/3/DIBO//SU92/CI13645/5/AGRI/BJY//VEES	-0SE-0YC-0E-7YE-0YE-1YM-0YM	TCI951084	TCI	BL	20,3	1,885618083	0,942809042	HS	-17,27	18,76918733	-40,94508134	Admixed
61	11CBWF	000330	BILINMIYEN96.7	-0SE-3YA-3YC-0YC	F2.96.7	TCI	BL	20,2	5,948856099	2,97442805	HS	35,6965	-23,72701734	-23,01999374	Admixed
62	11CBWF	000029	RIPPER			US-COL	C	16,0	1,08012345	0,540061725	S	16,4104	-36,74636075	25,36600444	Admixed
66	11CBWF	090169	TAM200/KAUZ/3/SPN/NAC//ATTILA/4/F885K1.1/SXL	-030YE-30E-3E-0E-1E-0E	TCI012021	TCI	BL	18,5	2,483277404	1,241638702	S	-2,1212	-2,842673764	-27,6482104	Admixed
70	11CBWF	090748	MIRONIVSKA RANNOSTYGLA			UKR-MIR	C	20,7	6,847546195	3,423773097	HS	29,3346	41,45694928	37,99893696	Admixed
71	11CBWF	100701	PEREGRINE			CAN	C	16,0	3,082207001	1,541103501	S	18,3438	-21,19376404	14,21157297	Admixed
72	11CBWF	090079	GRECUM 84//PYN/BAU	-0AP-0AP-18AP-0AP -1E-0E	TCI-02-726	TCI	BL	14,5	2,449489743	1,224744871	MS	-42,416	56,64726514	15,72182206	Admixed
74	11ELITE-IRR	980960	TAM200/JI5418	CIT930099	-0SE-0YC-2YE-0YC	TCI	BL	18,8	8,024268745	4,012134372	S	8,38726	21,65197376	49,27634586	Admixed
75	11ELITE-IRR	950055	BESKOPRU			TR	C	22,3	0,623609564	0,311804782	HS	-19,462	12,10926916	-8,341935825	Admixed
76	11ELITE-IRR	990149	885K4.1//MNG/SDV1/3/1D13.1/MLT	CIT925099	-0SE-0YC-3YC-0YC-3YC-0YC	TCI	BL	17,8	3,519785347	1,759892673	S	8,04478	-5,321878703	-24,92922914	Admixed
80	11ELITE-IRR	10831	ID800994.W/MO88	CMWS92Y00272S	-030WM-1WM-05WM-015WM-7WM-0WM	MX-TCI	BL	20,7	4,109609335	2,054804668	HS	-8,1318	63,81643044	18,6230556	Admixed
82	11ELITE-IRR	33	AGRI/NAC//KAUZ	C3W92WM00231S	-0SE-0YC-0YC-*-5YE-5YC-0YC	MX-TCI	BL	15,3	2,953340858	1,476670429	MS	-56,494	40,31995567	-36,75114838	Admixed
84	11ELITE-IRR	30158	AGRI/BJY//VEE/3/KS82142/CUPE	TCI951027	-0SE-0YC-0E-1YE-0YE	TCI	BL	20,5	4,020779361	2,01038968	HS	-5,849	35,32592633	-35,94850511	Admixed
85	11ELITE-IRR	40007	F130-L-1-12/MV12(ATILLA-12)	TCI961246	-0SE-0YC-0E-1YE-0YE-2YM-0YM	TCI	BL	17,7	4,496912521	2,248456261	S	43,1458	-0,954346752	-0,188688866	Admixed
89	11ELITE-IRR	60074	TX69A509.2//BBY/FOX/3/GRK//NO64/PEX/4/CER/5/CHIL/2*STAR	TCI981148	-0E-0E-5E-0E-2E-0E	TCI	BL	11,5	1,870828693	0,935414347	MS	-27,841	47,72577068	-50,97537754	Admixed
91	11ELITE-SA		MUFFITBEY			TR	C	17,0	2,273030283	1,136515141	S	50,3068	-17,5844255	-30,24772545	Admixed
92	11ELITE-SA	980671	LFN/VOGAF//LIRA/5/K134(60)/4/TOB/BMAN//BB/3/CAL/6/F339P1.2	CIT935039	-0SE-0YC-5YE-0YC	TCI	BL	7,8	3,858612301	1,92930615	MR	19,7721	-3,132834151	-32,71296052	Admixed
93	11ELITE-SA	980639	FLAMURA85//F134.71/NAC	CIT930037	-0SE-0YC-1YE-0YC	TCI	BL	16,2	1,312334646	0,656167323	S	9,99452	3,623955763	-30,37975881	Admixed
94	11ELITE-SA	990276	ORKINOS-1	.	-0YA-0YA-5YC-0YC	YA-TCI	BL	21,3	2,460803843	1,230401922	HS	47,4934	-15,02552704	-32,9668754	Admixed
95	11ELITE-SA	990277	ORKINOS-2	.	-0YA-0YA-6YC-0YC	YA-TCI	BL	7,2	1,699673171	0,849836586	MR	49,8335	-18,09541837	-28,33257196	Admixed
96	11ELITE-SA	990818	PMF/MAYA//YACO/3/CO693591/CTK	CIT90095T	-0YC-0YC-0YC-3YC-0YC-1YC-0YC	CIT	BL	22,8	3,399346342	1,699673171	HS	-22,006	12,44893147	-32,37189964	Admixed
97	11ELITE-SA	990125	777TWWON87/3/F12.71/SKA//CA8055	CIT922247	-0SE-0YC-3YC-0YC-3YC-0YC	CIT	BL	10,8	0,623609564	0,311804782	MS	9,46737	-21,03468107	19,89964235	Admixed
98	11ELITE-SA	990084	1D13.1/MLT//TUI	C3W90M398	-0YC-0YC-0YC-1YC-0YC-6YC-0YC	MX-CIT	BL	12,8	0,942809042	0,471404521	MS	-20,398	20,7081371	-38,65952136	Admixed
100	11ELITE-SA	010027	TAM200/KAUZ	C3W91M00414S	-0SE-0YC-1YC-0YC-3YC-0YC-1YC-0YC	MX-CIT	BL	14,0	2,041241452	1,020620726	MS	-41,674	-21,10625006	-10,41682752	Admixed
101	11ELITE-SA	010037	JI5418/MARAS	CIT922142	-0SE-0YC-3YC-0YC-6YC-0YC-1YC-0YC	CIT	BL	20,8	3,299831646	1,649915823	HS	29,2312	-6,903742818	-15,18708841	Admixed
103	11ELITE-SA	020319	SAULESKU #44/TR810200	C3W94WM00586S	-03Y-0B-0SE-1YE-0YC-1YM-0YM	MX-TCI	BL	17,8	5,436502143	2,718251072	S	-16,357	-9,852052687	-25,3748182	Admixed
105	11ELITE-SA	020293	TAST/SPRW/4/ROM-TAST/BON/3/DIBO//SU92/CI13645/5/F130L1.12	CIT932182	-0SE-0YC-7YE-0YC-1YM-0YM	CIT	BL	18,7	2,778888667	1,389444333	S	6,00265	-17,04581884	-4,471209262	Admixed
108	11ELITE-SA	030423	YE2453//PPBB68/CHRC	TCI950019	-3AP-0AP-0E-2YE-0YE-3YM-0YM	TCI	BL	15,3	2,321398046	1,160699023	MS	-9,1342	-19,06226352	19,15820848	Admixed
109	11ELITE-SA	060161	TX69A509.2//BBY/FOX/3/GRK//NO64/PEX/4/CER/5/KAUZ//ALTAR 84/AOS	TCI981143	-0E-0E-6E-0E-1E-0E	TCI	BL	12,5	0,707106781	0,353553391	MS	-38,264	66,77405435	38,01540222	Admixed

110	11ELITE-SA	060287	BOW/NKT//KATIA1/3/AGRI/BJY//VEE	TCI982234	-030YE-0E-3E-0E-1E-0E	TCI	BL	19,5	7,176350047	3,588175024	HS	-0,2747	17,30404189	-36,50796796	Admixed
111	11ELITE-SA	060417	TIRCHMIR1//71ST2959/CROW/4/NWT/3/TAST/SPRW//TAW12399.75	TCI98-IC-0097	-0AP-0AP-4E-0E-2E-0E	TCI	BL	21,8	2,094967515	1,047483757	HS	-2,7151	3,178676837	-16,75298688	Admixed
112	11ELITE-SA	991540	YILDIZ			TR	C	13,2	1,649915823	0,824957911	MS	-40,931	33,34819436	-46,73351885	Admixed
113	18FAWWON-IRR	080009	DORADE-5/CAMPION	TCI 001049	-030YE-030YE-2E-0E -4E-0E	TCI	BL	10,2	0,471404521	0,23570226	MS	33,472	13,54556775	-15,93481309	Admixed
114	18FAWWON-IRR	080056	T 98-9//VORONA/HD2402	TCI 001530	-030YE-030YE-11E-0E -3E-0E	TCI	BL	23,5	6,013872851	3,006936425	HS	-48,747	15,78087327	-21,16204987	Admixed
116	18FAWWON-IRR	080684	BOW/CROW/3RSH//KAL/BB/3/GUN91	TCI011508	-030YE-30E-0YK	TCI	BL	16,8	1,699673171	0,849836586	S	9,98339	-19,40534362	-1,685745718	Admixed
119	18FAWWON-IRR		SN64//SKE/2*ANE/3/SX/4/BEZ/5/SERI/6/CHERVONA/7/KLEIBER/2*FL80//DONSK.POLUK.	TCI962126		TCI	BL	16,7	1,433720878	0,716860439	S	-28,441	5,667198017	-39,77354137	Admixed
125	18FAWWON-IRR	080660	DANA/3/SPN/NAC//ATTILA/4/SHARK-1	TCI 002097	-030YE-030YE-1E-0E -1E-0E	TCI	BL	17,5	2,677063067	1,338531534	S	-11,42	1,828254306	-16,03038795	Admixed
128	18FAWWON-IRR	100664	SHAHRIAR			IR-ARD	C	5,3	2,592724864	1,296362432	MR	-47,663	-1,420921959	20,45929369	Admixed
129	18FAWWON-IRR	090861	Alamoot/4/Bloudan/3/Bb/7c*2//Y50E/Kal*3			IR-KARAJ	BL	14,0	2,549509757	1,274754878	MS	-71,208	6,808910377	-5,999645641	Admixed
130	18FAWWON-IRR	081138	Owl//Ombul/Alamo			IR-KARAJ	BL	12,8	4,784233365	2,392116682	MS	-17,881	31,56418769	55,68290046	Admixed
133	18FAWWON-IRR	090914	Owl/Soissons//Zarrin			IR-MIANDOAB	BL	11,7	1,027402334	0,513701167	MS	-47,622	-4,218115805	28,06957458	Admixed
134	18FAWWON-IRR	090920	Spb"s"//K134(60)/Vee"s"/3/Druchamps/4/Alvand			IR-MIANDOAB	BL	6,3	1,247219129	0,623609564	MR	-57,955	12,73224588	5,174403228	Admixed
135	18FAWWON-IRR	090911	PODOIMA			Moldova	C	13,5	5,016638981	2,508319491	MS	66,4373	5,711386863	-22,05215405	G2
140	18FAWWON-IRR	100710	NUDELA			RO	C	18,8	3,399346342	1,699673171	S	45,2293	-7,836195752	-28,87911773	Admixed
141	18FAWWON-IRR	100704	CH-111.14098			UN	BL	14,5	2,160246899	1,08012345	MS	28,2489	-34,63089618	43,80430328	Admixed
144	18FAWWON-IRR	100031	IN97395B1-4-3-8/AWD99*5725		ARS07-0723	US-ARS-NC	BL	9,5	1,08012345	0,540061725	MR	3,11287	-25,96326085	22,07755534	Admixed
145	18FAWWON-IRR	080486	ORACLE/PEHLIVAN	TCI 00125703	-030YE-030YE-2E-0E-4AP-0AP	TCI	BL	14,0	3,341656276	1,670828138	MS	42,0187	-3,905484704	-0,432780859	Admixed
146	18FAWWON-SA	080710	BUC/PVN//MILAN/3/TX96V2427	TCI-01-436	-0AP-0AP-28AP-0AP-3AP-0AP	TCI	BL	7,0	0,816496581	0,40824829	MR	-22,267	-33,02256869	27,29814297	Admixed
148	18FAWWON-SA	080229	KAROUS-4/7/NE COMP1/5/BEZ//TOB/8156/4/ON/3/TH*6/KF//LEE*6/K/6/TAST/SPRW..	TCI 001744	-030YE-030YE-2E -0E -3E-0E	TCI	BL	7,0	2,857738033	1,428869017	MR	-9,4493	-27,26357505	14,65882031	Admixed
152	18FAWWON-SA	080221	HBA142A/HBZ621A//ABILENE/3/BURBOT-6	TCI 001619	-030YE-030YE-1E-0E -4E-0E	TCI	BL	12,2	0,471404521	0,23570226	MS	20,9726	-20,12601409	21,97469147	Admixed
155	18FAWWON-SA	080403	CM98-112/4/HAWK/81PYI9641//MESA MOTHER LINE/3/KS82W418/SPN	X990457	-0E-030YE-1E-0E -1E-0E	KSU-TCI	BL	6,7	0,623609564	0,311804782	MR	-8,086	-24,2012357	13,7288714	Admixed
156	18FAWWON-SA	070653	HBK0935-29-15/KS90W077-2-2/VBF0589-1	AP06T3832		USA	BL	8,5	2,857738033	1,428869017	MR	-3,4738	-35,86042653	25,97108013	Admixed
157	18FAWWON-SA	070671	2180*K/2163//?/3/W1062A*HVA114/W3416	KS980554-12-~9		USA	BL	7,7	1,840893503	0,920446751	MR	23,357	-24,59250208	4,340384975	Admixed
159	18FAWWON-SA	080298	YE2453/KA//1D13.1/MLT/3/VORONA/TR810200	TCI-01-422	-0AP-0AP-27AP-0AP-1AP-0AP	TCI	BL	9,3	3,299831646	1,649915823	MR	-36,965	-28,14852482	17,34374382	Admixed
161	18FAWWON-SA	080400	CM98-64/4/HAWK/81PYI9641//MESA MOTHER LINE/3/KS82W418/SPN	X990434	-0E-030YE-2E -0E -2E-0E	KSU-TCI	BL	8,3	4,642796092	2,321398046	MR	2,02975	-13,65978849	15,48225082	Admixed
163	18FAWWON-SA	080398	JUP/4/CLLF/3/II14.53/ODIN//CI13431/WA00477//5/GK Aron/AgSeco 7846//2180	OCW00S436S	-0YA-2E -0E -2E-0E	OK-TCI	BL	13,7	1,649915823	0,824957911	MS	-3,1244	-4,042580521	-14,34590865	Admixed
164	18FAWWON-SA	070668	HBK1064-3/KS84063-9-39-3-4W//X960103	KS970093-8-9-#1		USA	BL	6,0	0,40824829	0,204124145	MR	17,8207	-23,29571582	2,868032966	Admixed
165	18FAWWON-SA	090713	JAGGER/ALLIANCE	NE02558		USA	BL	7,3	3,681787006	1,840893503	MR	5,75498	-42,82768174	-3,059250135	Admixed
166	C19FAWWON-INT		LANTIAN 12			PRC	C	6,0	1,545603083	0,772801541	MR	10,1274	-43,47355849	59,04220141	Admixed
168	C19FAWWON-INT		LANTIAN 15			PRC	C	14,3	0,849836586	0,424918293	MS	20,522	18,97834724	53,19477902	Admixed
169	C19FAWWON-INT		LANTIAN 17			PRC	C	13,0	2,677063067	1,338531534	MS	9,88642	-27,74670449	15,68692443	Admixed
170	C19FAWWON-INT		LANTIAN 00-30			PRC	C	46,0	4,089281382	2,044640691	HS	26,6365	34,25080839	45,60952979	Admixed
173	C19FAWWON-INT		VOLODARKA			UKR-MIR	C	21,5	5,715476066	2,857738033	HS	48,9219	32,79322038	34,82104236	Admixed
174	C19FAWWON-INT		ECONOMKA			UKR-MIR	C	5,2	3,922867432	1,961433716	MR	60,1753	42,20339718	17,24729503	Admixed
183	C19FAWWON-INT		T06/11			SA	BL	13,5	1,224744871	0,612372436	MS	-18,602	43,99892941	35,49771828	Admixed
186	C19FAWWON-INT		SONMEZ			TR	C	7,3	2,778888667	1,389444333	MR	60,9133	13,37630554	-40,19604234	G2
187	C19FAWWON-INT		T03/17			SA	BL	8,2	2,494438258	1,247219129	MR	-28,799	-30,83505063	17,47467582	Admixed
191	C19FAWWON-INT		T04/17			SA	BL	4,3	2,094967515	1,047483757	R	2,94532	-5,996251135	-16,4429675	Admixed
192	C19FAWWON-INT		EC - P			SA	C	8,0	0,707106781	0,353553391	MR	19,1154	-19,58390698	4,499603321	Admixed
193	C19FAWWON-INT		Kariega			SA	C	8,8	3,681787006	1,840893503	MR	-64,659	-54,00692592	-34,89729441	G1
194	C19FAWWON-INT		Olifants			SA	C	5,5	4,636809248	2,318404624	MR	-40,531	-3,492297071	-12,55548057	Admixed
197	C19FAWWON-INT		BSP01/18 (Duzi)			SA	C	9,5	2,943920289	1,471960144	MR	-66,865	-47,37180179	-29,01947928	G1

199	C19FAWWON-INT		BSP06/08			SA	BL	8,8	2,248456261	1,12422813	MR	-64,004	-54,68926039	-35,12981124	G1
200	C19FAWWON-INT		BSP06/17			SA	BL	7,2	2,054804668	1,027402334	MR	-64,433	-54,04509982	-34,23678347	G1
201	C19FAWWON-INT		BSP07/11			SA	BL	18,8	2,867441756	1,433720878	S	-63,225	-51,94732734	-35,03773664	G1
202	C19FAWWON-INT		BSP08/02			SA	BL	15,5	0,40824829	0,204124145	S	-34,453	-26,46827389	19,55940506	Admixed
210	C19FAWWON-INT		NUDAKOTA			US-AGRIPRO	C	4,0	1,92930615	0,964653075	R	-2,6432	-33,31262746	28,7587197	Admixed
211	C19FAWWON-INT		ART			US-AGRIPRO	C	9,8	1,312334646	0,656167323	MR	7,70659	6,725232985	72,54165699	Admixed
212	C19FAWWON-INT		JAGARENE			US-AGRIPRO	C	9,0	2,273030283	1,136515141	MR	2,0888	-14,7769152	-13,74048125	Admixed
213	C19FAWWON-INT		HAWKEN			US-AGRIPRO	C	10,3	1,312334646	0,656167323	MS	16,6762	-48,35721458	30,18927148	Admixed
214	C19FAWWON-INT		SARATOVSKAYA90			RUS-SAR	C	18,3	6,114645443	3,057322721	S	62,0578	9,29231097	-3,778723293	G2
215	C19FAWWON-INT		SARATOVSKAYA OSTISTAYA			RUS-SAR	C	22,0	5,016638981	2,508319491	HS	57,3614	5,724020015	-12,30793458	Admixed
217	C19FAWWON-INT		ZHEMCHUZHINA POVOLZHJYA			RUS-SAR	C	12,7	2,460803843	1,230401922	MS	45,4343	11,99112978	-43,95041271	G2
220	C19FAWWON-INT		LUTESCENS329/UROZHAINAYA	33		RUS-SAR	BL	21,2	2,013840996	1,006920498	HS	67,9264	6,837394084	-3,21447292	Admixed
221	C19FAWWON-INT		GUBERNIYA/SARATOVSKAYA17	15		RUS-SAR	BL	11,8	0,942809042	0,471404521	MS	59,9975	27,49362422	24,09037215	G2
223	C19FAWWON-INT		BEZENCHUKSKAYA616			RUS-SAM	C	17,8	4,027681991	2,013840996	S	61,9818	5,492239923	3,486414892	G2
224	C19FAWWON-INT		BIRYUZA			RUS-SAM	C	17,3	2,392116682	1,196058341	S	39,4441	0,721970899	0,265791794	Admixed
226	C19FAWWON-INT		BEZENCHUKSKAYA380			RUS-SAM	C	10,0	1,414213562	0,707106781	MR	67,6962	2,321277526	12,4026366	G2
228	C19FAWWON-INT		KUMA			RUS-KRAS	C	13,0	4,242640687	2,121320344	MS	34,6272	-17,87557009	20,81593011	Admixed
229	C19FAWWON-INT		PAMYAT			RUS-KRAS	C	7,7	1,649915823	0,824957911	MR	-46,345	-70,6415442	-8,843893554	Admixed
233	C19FAWWON-INT		MASCOT			UK	C	6,3	0,23570226	0,11785113	MR	10,2591	-48,10538969	57,65896987	Admixed
235	C19FAWWON-INT		Prost/Unk95-3	TE 5644	-1T-1T-1T-1T-0T	TE-TR	BL	9,3	3,566822427	1,783411213	MR	-8,5435	-41,88857512	37,74698428	Admixed
236	C19FAWWON-INT		Vorona/Parus//Hatusha/3/Lut112/4/Pehl//Rpb8-68//Chrc	TE 6035	-1T-1T-4T-0T	TE-TR	BL	16,5	1,632993162	0,816496581	S	15,4554	30,92069509	18,96558247	Admixed
241	C19FAWWON-INT		AHMETAGA			TR	C	10,7	1,027402334	0,513701167	MS	12,8774	21,47120109	-56,85305857	Admixed
243	C19FAWWON-INT		1-68-120/1-68-22//Mirtos/3/1-68-120/1-68-22			Karadj-IR	BL	15,0	0,707106781	0,353553391	MS	-56,858	0,981980327	-5,513007177	Admixed
244	C19FAWWON-INT		Alamoot/Sids8			Mashhad-IR	C	10,0	1,870828693	0,935414347	MR	-70,326	55,08854391	49,85126888	Admixed
245	C19FAWWON-INT		Zarrin*2/Shiroodi/3/Zarrin//Vee/Nac			Miandoab-IR	BL	8,7	4,027681991	2,013840996	MR	-99,722	34,14238865	-10,16660868	Admixed
246	C19FAWWON-INT		Owl/Shiroodi/3/Owl//Opata*2/Wulp			Miandoab-IR	BL	8,5	2,677063067	1,338531534	MR	-97,568	34,15185573	-9,842950428	G1
247	C19FAWWON-INT		1-68-120/1-68-22/4/Kal/Bb//Cj"s"/3/Hork"s"			Ardebil-IR	BL	11,0	0,816496581	0,40824829	MS	-38,428	25,09669317	1,180505585	G1
248	C19FAWWON-INT		OVERLEY*3/AMADINA	KS06O3A~57-1		KSU-Man	BL	18,2	2,094967515	1,047483757	S	-6,144	-15,94661134	11,99834156	Admixed
249	C19FAWWON-INT		2145/X940786-6-7	TX05A001822		Texas A&M	BL	10,5	1,632993162	0,816496581	MS	-21,99	0,200371799	-37,30887444	Admixed
255	C19FAWWON-TCI	090015	55.1744/MEX67.1//NO57/3/KAUZ/4/SHARK/F4105W2.1/5/TX96V2427	-030YE-30E-3E-0E-2AP-0AP	TCI012335	TCI	BL	7,5	1,471960144	0,735980072	MR	-32,158	-10,10806203	11,79806714	Admixed
262	C19FAWWON-TCI	90350	HBA142A/HBZ621A//ABILENE/3/CAMPION/4/F6038W12.1	-030YE-30E-3E-0E-1E-0E	TCI012144	TCI	BL	9,7	3,519785347	1,759892673	MR	57,7135	20,43918391	-47,18717203	G2
265	C19FAWWON-TCI	90532	BR1284//BH114686/ALD/3/CAZO/4/KS940786-6-7	-30E-1E-0E-2E-0E	X011602	KS-TCI	BL	13,7	3,423773097	1,711886549	MS	-14,746	51,01842555	33,28432881	Admixed
266	C19FAWWON-TCI	90572	LOV26/LFN/SDY(ES84-24)/3/SERI/4/FDL49../5/LAGOS-6	-030YE-30E-1E-0E-1E-0E	TCI011046	TCI	BL	19,3	4,403281605	2,201640802	S	-14,612	34,10319489	-48,63710627	Admixed
267	C19FAWWON-TCI	90590	ADMIS//MILAN/DUCULA	-030YE-30E-1E-0E-1E-0E	C3W01WM00331S	MX-TCI	BL	19,2	3,324989557	1,662494779	S	27,8474	-15,9937142	-10,40541724	Admixed
268	C19FAWWON-TCI	90614	AGRI/BJY//VEE/3/BUCUR/4/DOGU88//TX71A374.4/TX71A1039.V1/3/1502W9.1	-030YE-30E-1E-0E-2E-0E	TCI012082	TCI	BL	20,0	3,628590176	1,814295088	HS	18,6523	-1,090112789	-26,65021788	Admixed
269	C19FAWWON-TCI	50852	VO1225			TCI	BL	11,8	1,312334646	0,656167323	MS	-46,661	-70,95741371	-8,034859871	Admixed
270	C19FAWWON-TCI	108	FRTL/NEMURA	-0AP-0YC-*-1YE-1YC-0YC	C3W93WM0073	MX-TCI	BL	11,8	1,027402334	0,513701167	MS	29,8041	19,30461109	22,54239931	Admixed
273	C19FAWWON-TCI	90240	RAN/NE701136//CI13449/CTK/3/CUPE/4/TAM200/KAUZ/5/BWD	-030YE-30E-3E-0E-1E-0E	TCI012234	TCI	BL	21,2	7,487025815	3,743512908	HS	-7,151	12,76637808	-26,76866766	Admixed
276	11CBWF	950590	KATIA1			BUL	C	10,5	2,121320344	1,060660172	MS	45,015	13,42314668	-44,09706761	G2
277	11CBWF	050670	STARSHINA			RUS	C	14,0	4,490731195	2,245365598	MS	38,6128	3,985046788	-2,977017507	Admixed
283	C19FAWWON-INT		Alamoot/Sids8			Mashhad-IR	BL	12,7	3,009245014	1,504622507	MS	-50,776	37,44652686	5,870324173	Admixed
284	C19FAWWON-INT		TOSUNBEY			TR	C	7,7	3,299831646	1,649915823	MR	-20,966	7,747796838	-31,71492259	Admixed
285	C19FAWWON-INT		KARAHAN			TR	C	11,7	3,472111109	1,736055555	MS	-9,6274	-42,68647092	39,35532047	Admixed
287	C19FAWWON-INT		Alamoot/Sids8			Mashhad-IR	BL	11,8	1,247219129	0,623609564	MS	-70,296	53,31918677	50,68781284	Admixed
290	C19FAWWON-INT		Bezostaya 1		LUT17/SRS2	RUS	C	35,0	2,460803843	1,230401922	HS	64,2189	22,52279899	-31,4800534	G2
291	C19FAWWON-INT		Katea		Hebros/Bez-1	BUL	C	5,3	1,247219129	0,623609564	MR	45,2689	11,99168785	-43,43761144	G2
292	C19FAWWON-INT		Sonmez			TCI	C	6,5	2,248456261	1,12422813	MR	58,2953	20,57346672	-47,88885993	G2

*ACCNO accession number, BL breeding lines, CBWF Cross block winter facultative, CName common name, CID cross identification, C cultivars, ELITE semi arid, FAWWON Facultative and winter wheat observation nursery, G1 group 1, G2 group two, HS highly susceptible, IRR Irrigated, INT International, LRACE Landraces, MR moderately resistance, MS moderately resistant, SELHX selection history, SD standard deviation, SE standard error, R resistant, S susceptible, SA South Afrika, and TCI Turkey-CIMMYT-ICARDA

Host status	Number of wheat accessions	% of wheat accessions

R	2	1
MR	42	26
MS	42	26
S	36	22
HS	39	24
Total	161	100

Breeding status	Number of wheat accessions	% of wheat accessions
BL	101	62,73291925
C	58	36,02484472
LRACE	2	1,242236025
Total	161	100

TABLE S2. *In-silico* annotation of SNP marker flanking sequence against protein coding sequence (CDS) and protein of Brachypodium, rice and sorghum

90K iSelect SNP bead chip index	SNP id	Name	Brachypodium CDS				Brachypodium Protein				Rice CDS				Rice Protein				Sorghum CDS				Sorghum Protein			
			Top BLAST Hit	e-value	identity %	length	Top BLAST Hit	e-value	identity %	length	Top BLAST Hit	e-value	identity %	length	Top BLAST Hit	e-value	identity %	length	Top BLAST Hit	e-value	identity %	length	Top BLAST Hit	e-value	identity %	length
75423	IWA135	wsnp_BE443588A_Ta_2_1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
53663	IWB53663	RAC875_c13116_943	Bradi1g18770.1	0	85,6	1326	Bradi1g18770.1	0	63,83	47	LOC_Os07g46690.1	7,00E-13	81,77	872	LOC_Os07g46690.1	0	53,33	45	Sb02g042000.1_PACid_1959731	4,00E-11	80,67	869	Sb02g042000.1_PACid_1959731	2,00E-16	65,26	449
23232	IWB23232	Excalibur_c18966_804	Bradi5g23450.1	0	93,09	1259	Bradi5g23450.1	0	94,12	51	LOC_Os04g54790.1	0	89,3	1262	LOC_Os04g54790.1	0	94,12	51	Sb06g030250.1_PACid_1974077	0	87,71	1204	Sb06g030250.1_PACid_1974077	0	92,16	51
75392	IWA94	wsnp_BE426418A_Ta_2_1	Bradi2g61410.1	2,00E-07	89,36	47	.	.	.	.	.	.	.	.	.	.	.	.	Sb03g046510.1_PACid_1964704	2,00E-10	93,18	44	.	.	.	.
5616	IWB5616	BobWhite_rep_c66630_331	Bradi3g44480.1	2,00E-38	94,95	99	Bradi3g44160.1	4,00E-12	96,97	33	LOC_Os02g32030.1	4,00E-32	92,78	97	LOC_Os01g52470.1	2,00E-11	93,94	33	Sb01g002040.1_PACid_1949534	4,00E-36	93,94	99	Sb01g002040.1_PACid_1949534	9,00E-12	93,94	33
66494	IWB66494	Tdurum_contig10380_87	Bradi5g20787.1	9,00E-25	89,58	96	Bradi5g20787.1	5,00E-12	84,85	33	.	.	.	.	.	.	.	.	Sb06g027600.1_PACid_1973759	7,00E-13	89,06	64	.	.	.	.
67389	IWB67389	Tdurum_contig12008_803	Bradi3g16550.1	3,00E-37	94,06	101	Bradi3g16550.1	1,00E-13	93,94	33	LOC_Os08g06070.1	7,00E-18	87,64	89	LOC_Os08g06070.1	4,00E-12	81,82	33	.	.	.	.	Sb07g003820.1_PACid_1975049	9,00E-11	75,76	33
23457	IWB23457	Excalibur_c20277_483	Bradi3g16550.1	0	90,39	1176	Bradi3g16550.1	0	82,61	46	LOC_Os08g06070.1	0	85,41	1206	LOC_Os08g06070.1	0	82,98	47	Sb07g003820.1_PACid_1975049	0	84,42	1155	Sb07g003820.1_PACid_1975049	0	78,72	47
78435	IWA4260	wsnp_Ex_c55245_57821389	Bradi1g70657.1	2,00E-14	84,99	673	Bradi1g70657.1	4,00E-10	71,43	189	LOC_Os03g10990.1	6,00E-83	90,98	244	LOC_Os03g10990.1	1,00E-92	60,85	189	Sb01g043410.1_PACid_1954348	5,00E-60	81,04	517	Sb01g043410.1_PACid_1954348	2,00E-89	62,9	186
73556	IWB73556	Tdurum_contig82236_117	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
28883	IWB28883	Excalibur_c78724_434	Bradi1g02240.1	2,00E-15	79,41	238	Bradi1g02240.1	7,00E-52	63,86	166	.	.	.	.	LOC_Os01g39250.1	3,00E-45	54,89	184	Sb03g025820.1_PACid_1962186	3,00E-07	96,88	32	Sb09g030660.1_PACid_1982112	2,00E-48	62,42	165

*Bradi Brachypodium, e Expect value, id Identification, LOC Locus, OS Oryza sativa, Sb Sorghum bicolor, SNP Sincle nucleotide polymorphism

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6. 3. Supplementary materials for:

GWAS in wheat identifies an amino acid transporter gene orthologue in Arabidopsis promoting nematode susceptibility

Shree R. Pariyar[1], Jenish Nakarmi[1], Muhammad Arslan Anwer[1], Abdelnaser Elashry[1], Abdelfattah A. Dababat[2], Jens Leon[3] and Florian M. W. Grundler[1]

[1]Institute of Crop Science and Resource Conservation (INRES), Molecular Phytomedicine, Karlrobert- Kreiten Strasse 13, D-53115 Bonn, Germany,

[2]International Maize and Wheat Improvement Centre (CIMMYT), P.K. 39 06511, Emek, Ankara, Turkey,

[3]INRES, Plant Breeding, Katzenburgweg

Pdf file includes:

Fig. S1. Characterization of AtAAP6 by Arabidopsis mutant. A-a graphic gene model for AtAAP6 with intron/exon structure and location of T-DNA insertions; B-T-DNA insertion was confirmed by PCR using gene-specific primers and the T-DNA primer; and C expression analysis of AtAAP6 confirmed the homozygosity of the mutant SALK_013231. *1-5 represents individual plants used for genotyping from same batch of mutant seed. Specific primers for the Arabidopsis ubiquitin (UBQ) were used as control.

Fig. S2. Comparison of AtAAP6 ORF amino acid sequences in various Arabidopsis accessions. Uk-1, Ty-0 and Ta-0 represent lowly susceptibility accessions, and Mc-0, Zdr-1 and Jm-0 represent highly susceptible accessions. Col-0 is used as control. ***G** glycine and **A** alanine.

Fig. S3. Comparison of AtAAP6 promotor region sequences in various Arabidopsis accessions. Uk-1, Ty-0 and Ta-0 represent lowly susceptibility accessions, and Mc-0, Zdr-1 and Jm-0 represent highly susceptible accessions. Col-0 is used as control. *A adenine, C cytosine, T thymine, and G guanine.

Table S1. List of Arabidopsis accessions compared for AtAAP6 amino acid sequence

Table S2. List of wheat accessions used for TaAAT expression analysis

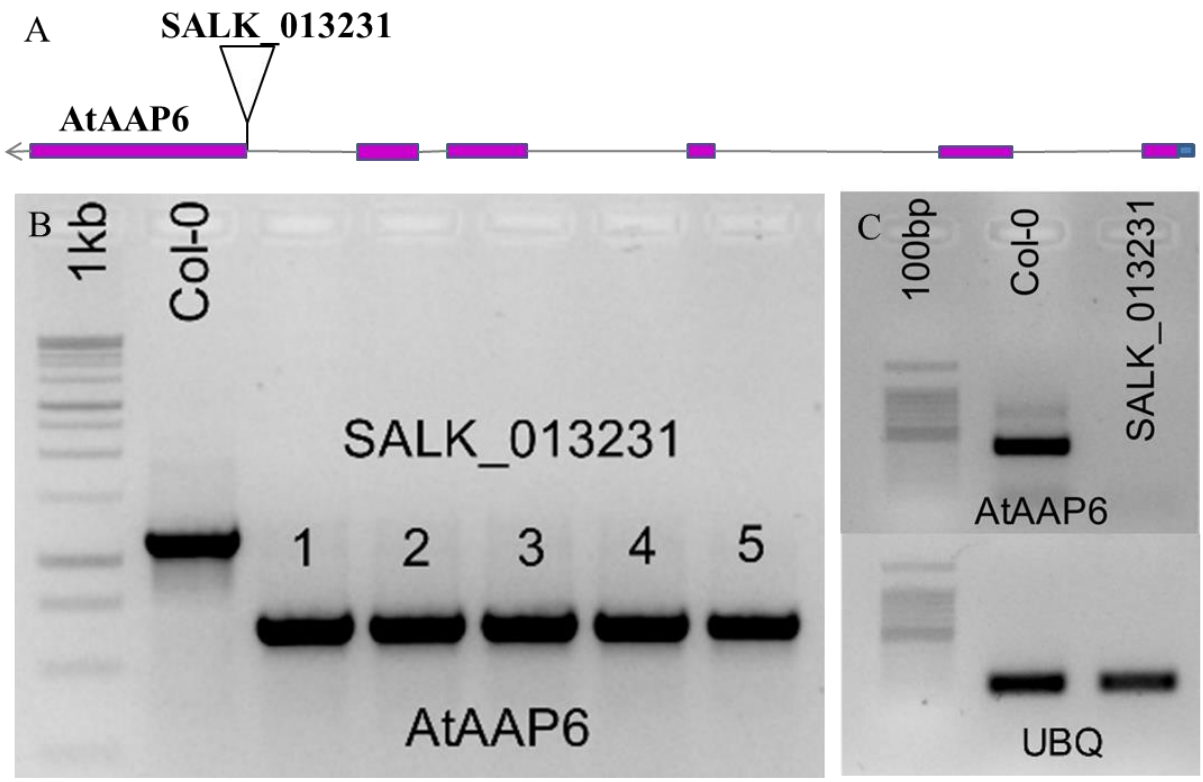

Fig. S1. Characterization of AtAAP6 by Arabidopsis mutant. A- a graphic gene model for AtAAP6 with intron/exon structure and location of T-DNA insertions; B-T-DNA insertion was confirmed by PCR using gene-specific primers and the T-DNA primer; and C expression analysis of AtAAP6 confirmed the homozygosity of the mutant SALK_013231. *1-5 represents individual plants used for genotyping from same batch of mutant seed. Specific primers for the Arabidopsis ubiquitin (UBQ) were used as control.

```
Uk-1    EKKKSFVEQSFPEHEIGDTNKNFDEDGRDKRTGTWTGSAHIITAVIGSGVLSLAWAIAQLGWVAGPAVLAFSFITYFTSTL
Ty-0    EKKKSFVEQSFPEHEIGDTNKNFDEDGRDKRTGTWTGSAHIITAVIGSGVLSLAWAIAQLGWVAGPAVLAFSFITYFTSTL
Ta-0    EKKKSFVEQSFPEHEIGDTNKNFDEDGRDKRTGTWTGSAHIITAVIGSGVLSLAWAIAQLGWVAGPAVLAFSFITYFTSTL
Col-0   EKKKSFVEQSFPEHEIGDTNKNFDEDGRDKRTGTWTGSAHIITAVIGSGVLSLAWAIAQLGWVAGPAVLAFSFITYFTSTL
Mc-0    EKKKSFVEQSFPEHEIGDTNKNFDEDGRDKRTGTWTGSAHIITAVIGSGVLSLAWAIAQLGWVAGPAVLAFSFITYFTSTL
Zdr-1   EKKKSFVEQSFPEHEIGDTNKNFDEDGRDKRTGTWTGSAHIITAVIGSGVLSLAWAIAQLGWVAGPAVLAFSFITYFTSTL
Jm-0    EKKKSFVEQSFPEHEIGDTNKNFDEDGRDKRTGTWTGSAHIITAVIGSGVLSLAWAIAQLGWVAGPAVLAFSFITYFTSTL
        ********************************************************************************
Uk-1    ADCYRSPDPVTGKRNYTYEVVRSYLGGRKVQLCGLAQYGNLIGITIGYTITASISVAVKRSNCFHKNGHNVKCATSNTPFI
Ty-0    ADCYRSPDPVTGKRNYTYEVVRSYLGGRKVQLCGLAQYGNLIGITIGYTITASISVAVKRSNCFHKNGHNVKCATSNTPFI
Ta-0    ADCYRSPDPVTGKRNYTYEVVRSYLGGRKVQLCGLAQYGNLIGITIGYTITASISVAVKRSNCFHKNGHNVKCATSNTPFI
Col-0   ADCYRSPDPVTGKRNYTYEVVRSYLGGRKVQLCGLAQYGNLIGITIGYTITASISVAVKRSNCFHKNGHNVKCATSNTPFI
Mc-0    ADCYRSPDPVTGKRNYTYEVVRSYLGGRKVQLCGLAQYGNLIGITIGYTITASISVAVKRSNCFHKNGHNVKCATSNTPFI
Zdr-1   ADCYRSPDPVTGKRNYTYEVVRSYLGGRKVQLCGLAQYGNLIGITIGYTITASISVAVKRSNCFHKNGHNVKCATSNTPFI
Jm-0    ADCYRSPDPVTGKRNYTYEVVRSYLGGRKVQLCGLAQYGNLIGITIGYTITASISVAVKRSNCFHKNGHNVKCATSNTPFI
        ********************************************************************************
Uk-1    IQIILSQIPNFHNLSWLSILAAVSFCYASIGVGLSIAKAAAGGEHVRTTLTGVTVGIDVSGAEKIWRTFQAIGDIAFAYAY
Ty-0    IQIILSQIPNFHNLSWLSILAAVSFCYASIGVGLSIAKAAAGGEHVRTTLTGVTVGIDVSGAEKIWRTFQAIGDIAFAYAY
Ta-0    IQIILSQIPNFHNLSWLSILAAVSFCYASIGVGLSIAKAAAGGEHVRTTLTGVTVGIDVSGAEKIWRTFQAIGDIAFAYAY
Col-0   IQIILSQIPNFHNLSWLSILAAVSFCYASIGVGLSIAKAAGGGEHVRTTLTGVTVGIDVSGAEKIWRTFQAIGDIAFAYAY
Mc-0    IQIILSQIPNFHNLSWLSILAAVSFCYASIGVGLSIAKAAGGGEHVRTTLTGVTVGIDVSGAEKIWRTFQAIGDIAFAYAY
Zdr-1   IQIILSQIPNFHNLSWLSILAAVSFCYASIGVGLSIAKAAGGGEHVRTTLTGVTVGIDVSGAEKIWRTFQAIGDIAFAYAY
Jm-0    IQIILSQIPNFHNLSWLSILAAVSFCYASIGVGLSIAKAAGGGEHVRTTLTGVTVGIDVSGAEKIWRTFQAIGDIAFAYAY
        ****************************************.****************************************
Uk-1    STVLIEIQDTLKAGPPSENKAKRASLVGVSTTTFFYLCGCVGYAAFGNDAPGNFLTGFGFYEPFWLIDFANVCIAVHLIGA
Ty-0    STVLIEIQDTLKAGPPSENKAKRASLVGVSTTTFFYLCGCVGYAAFGNDAPGNFLTGFGFYEPFWLIDFANVCIAVHLIGA
Ta-0    STVLIEIQDTLKAGPPSENKAKRASLVGVSTTTFFYLCGCVGYAAFGNDAPGNFLTGFGFYEPFWLIDFANVCIAVHLIGA
Col-0   STVLIEIQDTLKAGPPSENKAKRASLVGVSTTTFFYLCGCVGYAAFGNDAPGNFLTGFGFYEPFWLIDFANVCIAVHLIGA
Mc-0    STVLIEIQDTLKAGPPSENKAKRASLVGVSTTTFFYLCGCVGYAAFGNDAPGNFLTGFGFYEPFWLIDFANVCIAVHLIGA
Zdr-1   STVLIEIQDTLKAGPPSENKAKRASLVGVSTTTFFYLCGCVGYAAFGNDAPGNFLTGFGFYEPFWLIDFANVCIAVHLIGA
Jm-0    STVLIEIQDTLKAGPPSENKAKRASLVGVSTTTFFYLCGCVGYAAFGNDAPGNFLTGFGFYEPFWLIDFANVCIAVHLIGA
        ********************************************************************************
Uk-1    YQVFCQPIFQFVESQSAKRWPDNKFITGEYKIHVPCCGDFSINFLRLVWRTSYVVVTAVVAIFPFFNDFLGLIGAASFWPL
Ty-0    YQVFCQPIFQFVESQSAKRWPDNKFITGEYKIHVPCCGDFSINFLRLVWRTSYVVVTAVVAIFPFFNDFLGLIGAASFWPL
Ta-0    YQVFCQPIFQFVESQSAKRWPDNKFITGEYKIHVPCCGDFSINFLRLVWRTSYVVVTAVVAIFPFFNDFLGLIGAASFWPL
Col-0   YQVFCQPIFQFVESQSAKRWPDNKFITGEYKIHVPCCGDFSINFLRLVWRTSYVVVTAVVAIFPFFNDFLGLIGAASFWPL
Mc-0    YQVFCQPIFQFVESQSAKRWPDNKFITGEYKIHVPCCGDFSINFLRLVWRTSYVVVTAVVAIFPFFNDFLGLIGAASFWPL
Zdr-1   YQVFCQPIFQFVESQSAKRWPDNKFITGEYKIHVPCCGDFSINFLRLVWRTSYVVVTAVVAIFPFFNDFLGLIGAASFWPL
Jm-0    YQVFCQPIFQFVESQSAKRWPDNKFITGEYKIHVPCCGDFSINFLRLVWRTSYVVVTAVVAIFPFFNDFLGLIGAASFWPL
        ********************************************************************************
Uk-1    TVYFPIEHIAQKKIPKFSFTWTWLKILSWTCFIVSLVAAAGSVQGLIQSLKDFKPFQAP
Ty-0    TVYFPIEHIAQKKIPKFSFTWTWLKILSWTCFIVSLVAAAGSVQGLIQSLKDFKPFQAP
Ta-0    TVYFPIEHIAQKKIPKFSFTWTWLKILSWTCFIVSLVAAAGSVQGLIQSLKDFKPFQAP
Col-0   TVYFPIEHIAQKKIPKFSFTWTWLKILSWTCFIVSLVAAAGSVQGLIQSLKDFKPFQAP
Mc-0    TVYFPIEHIAQKKIPKFSFTWTWLKILSWTCFIVSLVAAAGSVQGLIQSLKDFKPFQAP
Zdr-1   TVYFPIEHIAQKKIPKFSFTWTWLKILSWTCFIVSLVAAAGSVQGLIQSLKDFKPFQAP
Jm-0    TVYFPIEHIAQKKTPKFSFTWTWLKILSWTCFIVSLVAAAGSVQGLIQSLKDFKPFQAP
        ************* *********************************************
```

Fig. S2. Comparison of AtAAP6 ORF amino acid sequences in various Arabidopsis accessions. Uk-1, Ty-0 and Ta-0 represent lowly susceptibility accessions, and Mc-0, Zdr-1 and Jm-0 represent highly susceptible accessions. Col-0 is used as control. ***G** glycine and **A** alanine.

```
Uk_1        TAGTTGCGTTTGCCGGTAACAGGGTCCGGGGAACGGTAACAATCGGCAAGCATGGTTGATGTAAAATATGTTATGAAAGAAAAA
Ty_0        TAGTTGCGTTTGCCGGTAACAGGGTCAGGGGAACGGTAACAATCGGCAAGCATGGTTGATGTAAAATATGTTATGAAAGAAAAA
Ta_0        TAGTTGCGTTTGCCGGTAACAGGGTCCGGGGAACGGTAACAATCGGCAAGCATGGTTGATGTAAAATATGTTATGAAAGAAAAA
Col-0       TAGTTGCGTTTGCCGGTAACAGGGTCAGGGGAACGGTAACAATCGGCAAGCATGGTTGATGTAAAATATGTTATGAAAGAAAAA
Mc_0        TAGTTGCGTTTGCCGGTAACAGGGTCAGGGGAACGGTAACAATCGGCAAGCATGGTTGATGTAAAATATGTTATGAAAGAAAAA
Zdr_1       TAGTTGCGTTTGCCGGTAACAGGGTCAGGGGAACGGTAACAATCGGCAAGCATGGTTGATGTAAAATATGTTATGAAAGAAAAA
Jm_0        TAGTTGCGTTTGCCGGTAACAGGGTCCGGGGAACGGTAACAATCGGCAAGCATGGTTGATGTAAAATATGTTATGAAAGAAAAA
            **************************.************************************************************
Uk_1        GCCATTAGTACGGCGGGTCCTGCCACCCATCCAAGTTGTGCGATTGCCCATGCCAACGACAACACTCCCGACCCTATCACGGCC
Ty_0        GCCATTAGTACGGCGGGTCCTGCCACCCATCCAAGTTGTGCGATTGCCCACGCCAAAGACAACACTCCCGACCCTATCACGGCC
Ta_0        GCCATTAGTACGGCGGGTCCTGCCACCCATCCAAGTTGTGCGATTGCCCATGCCAACGACAACACTCCCGACCCTATCACGGCC
Col-0       GCCATTAGTACGGCGGGTCCTGCCACCCATCCAAGTTGTGCGATTGCCCACGCCAAAGACAACACTCCCGACCCTATCACGGCC
Mc_0        GCCATTAGTACGGCGGGTCCTGCCACCCATCCAAGTTGTGCGATTGCCCATGCCAACGACAACACTCCCGACCCTATCACGGCC
Zdr_1       GCCATTAGTACGGCGGGTCCTGCCACCCATCCAAGTTGTGCGATTGCCCATGCCAACGACAACACTCCCGACCCTATCACGGCC
Mc_0        GCCATTAGTACGGCGGGTCCTGCCACCCATCCAAGTTGTGCGATTGCCCATGCCAACGACAACACTCCCGACCCTATCACGGCC
            ************************************************** *****.**************************
Uk_1        GTTATTATGTGTGCACTCCCGGTCATCCATGTCCCTGTTCTTTTGTCGCGGCCATCCTCGTCAAAGTTTTTGTTAGTATCGCCA
Ty_0        GTTATTATGTGTGCACTCCCGGTCATCCATGTCCCAGTTCTTTTGTCGCGGCCATCCTCGTCAAAGTTTTTGTTAGTATCGCCA
Ta_0        GTTATTATGTGTGCACTCCCGGTCATCCATGTCCCTGTTCTTTTGTCGCGGCCATCCTCGTCAAAGTTTTTGTTAGTATCGCCA
Col-0       GTTATTATGTGTGCACTCCCGGTCATCCATGTCCCAGTTCTTTTGTCGCGGCCATCCTCGTCAAAGTTTTTGTTAGTATCGCCA
Mc_0        GTTATTATGTGTGCACTCCCGGTCATCCATGTCCCAGTTCTTTTGTCGCGGCCATCCTCGTCAAAGTTTTTGTTAGTATCGCCA
Zdr_1       GTTATTATGTGTGCACTCCCGGTCATCCATGTCCCAGTTCTTTTGTCGCGGCCATCCTCGTCAAAGTTTTTGTTAGTATCGCCA
Jm_0        GTTATTATGTGTGCACTCCCGGTCATCCATGTCCCTGTTCTTTTGTCGCGGCCATCCTCGTCAAAGTTTTTGTTAGTATCGCCA
            ***********************************:************************************************
Uk_1        ATTTCATGCTCCGGGAAGCTCTGTTCAACGAACATGCTTTTCTTCTTCTCCAT
Ty_0        ATTTCATGCTCCGGGAAGCTCTGTTCAACGAACATGCTCTTCTTCTTCTCCAT
Ta_0        ATTTCATGCTCCGGGAAGCTCTGTTCAACGAACATGCTTTTCTTCTTCTCCAT
Col-0       ATTTCATGCTCCGGGAAGCTCTGTTCAACGAACATGCTCTTCTTCTTCTCCAT
Mc_0        ATTTCATGCTCCGGGAAGCTCTGTTCAACGAACATGCTCTTCTTCTTCTCCAT
Zdr_1       ATTTCATGCTCCGGGAAGCTCTGTTCAACGAACATGCTCTTCTTCTTCTCCAT
Jm_0        ATTTCATGCTCCGGGAAGCTCTGTTCAACGAACATGCTTTTCTTCTTCTCCAT
            ************************************ **************
```

Fig. S3. Comparison of AtAAP6 promotor region sequences in various Arabidopsis accessions. Uk-1, Ty-0 and Ta-0 represent lowly susceptibility accessions, and Mc-0, Zdr-1 and Jm-0 represent highly susceptible accessions. Col-0 is used as control. *A adenine, C cytosine, T thymine, and G guanine.

Table S1. List of Arabidopsis accessions compared for AtAAP6 amino acid sequence

Name	Stock ID	Site	Region	Origin	Ecotype number	Host status
Col-0						HS
Jm-0	CS6748	Jm	E. Europe	CZE	8313	HS
Mc-0	CS1362	Mc	The Pennines	UK	7252	HS
Zdr-1	CS22588	Zdr	Moravia	CZE	6984	HS
Uk-1	CS1574	Uk	W. Europe	GER	7378	MR
Ty-0	CS1572	Ty	N. Europe	UK	7351	MR
Ta-0	CS6867	Ta	E. Europe	CZE	8389	MR

*HS Highly susceptible and MR Moderately resistance

Table S2. List of wheat accessions used for TaAAT expression analysis

Common name	Accessions status	Pedigree	Asia	Origin	Cyst/plant	Host status
Bezostaya 1	Cultivar	LUT17/SRS2	Europe	Russia	35,0	HS
T04/17	Breeding line	-	Africa	South Africa	4,3	MR
Olifants	Cultivar	-	Africa	South Africa	5,5	MR
Lantian 12	Cultivar	Qingnong-4/Xiannong-4	Asia	China	6,0	MR

*HS Highly susceptible and MR Moderately resistance

6.4. Acknowledgements

I would like to express my sincere gratitude to my supervisor Prof. Dr. Florian Grundler, Molecular Phytomedicine-INRES, University of Bonn for his remarkable mentorship and excellent guidance throughout the PhD project. His enormous support on and off the university is a constant source of my inspiration. I would like to express my greatest appreciation to Prof. Dr. Jens Léon, Professor for Plant Breeding- INRES, University of Bonn, for co-supervising this study. His generous support and endless knowledge of statistics remains a key to the success of this study.

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Finally, I am indebted to my beloved family (Fischer, Osthues and Pariyar) for their unconditional love and support throughout this journey. Without them, this would have not been possible.

Name : Shree Ram Pariyar

Work experience

10.2011- Present: Graduate student Researcher - Molecular Phytomedicine, University of Bonn, Bonn, Germany

- *Duties*: Analysis of wheat resistance to cereal cyst nematode

04.2010-08.2011: Research Assistant - Department of Horticulture, University of Bonn, Bonn, Germany.

- *Duties*: Assigning lab, green house and field experiment of Paulownia tree and its tissue culture

10.2009-07.2010: Research Student - CIMMYT, Turkey

- *Duties*: Assessments of soil borne pathogens on wheat growth, yield and potential control options with major emphasis on host resistance

Publications

Pariyar, S. R., Dababat, A. A., Sannemann, W., Erginbas-Orakci, G., Elashry, A., Siddique, S., Morgounov, A., Leon, J., & Grundler, F. M. W. 2016. Genome-wide association study in wheat identifies resistance to the cereal cyst nematode *Heterodera filipjevi*. *Phytopathology* 106:1128-1138, Focus Issue.

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